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POSSIBILITIES FOR BIOLOGICAL CONTROL OF FUSARIUM ROOT ROT ON CUCUMBER WITH ANTAGONISTIC FUNGI FROM TRICHODERMA AND GLIOCLADIUM GENUS

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Abstract: The method of potential control of root and stem rot of cucumber (*Cucumis sativus*), caused by *Fusarium oxysporum* f. sp. *radicis-cucumericum* D.J. Vakalounakis (Vakalounakis, 1996), was evaluated. The efficacy of antagonistic strains of fungi of the genus *Trichoderma* spp. and *Gliocladium* spp. was studied, applied separately and combined, on the growth and immune response of plants to Fusarium root and stem rot. Comparative experiments with antagonists in greenhouse conditions were performed to establish the symbiotic properties of the strains. The biometric data on the seedlings and the plants during the vegetation were collected and analyzed. The percentage of Fusarium root rot of the varieties was calculated. It was found that combined introduction of several strains of *Trichoderma viride* Pers. (Persoon, 1794) and *Gliocladium virens* Mill., Giddens & Foste (Miller et al., 1957) in substrate improves biometric parameters of seedlings and plants, accelerates flowering and fruit formation in cucumbers and has a prophylactic effect against the development of Fusarium root rot in this crop. The symbiotic effect of soil application of these strains of antagonists is above 75 per cent. The results from this study indicate that mix from several different strains of *Trichoderma viride* and *Gliocladium virens* can be used to control Fusarium root and stem rot on greenhouse cucumber.

INTRODUCTION

Fusarium root rot and wilt of cucumbers are one of the major diseases in greenhouses. Often, the development of soil pathogens is a result of technological mistakes in growing. Changing requirements to production quality contribute to

the change and enrich the elements of the system - different composition measures and means to important diseases and pests, modernization and automation of processes. The development of diseases on root system depends on the purity of substrates and the rearing conditions - irrigation regime, temperature and plant protection measures. In certain conditions the soil pathogens can become a limiting factor for the yield (Pakhnenko, 2001). 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. The organic cultivation of vegetable crops in greenhouses is becoming more relevant and has real opportunities for practical application. Suppression or complete elimination of phytopathogens using natural mechanisms to maintain the balance in the soil biota is a basic principle in the development of technology for biological control of soil pathogens (Akhter et al., 2015; Santamarina et al., 2002; Singh et al., 1999; Hichar et al., 2015). Best known in this respect are fungi of the genus *Trichoderma* and *Gliocladium*. Microbiological fungal fungicides based on antagonistic strains of the genus *Trichoderma* and *Gliocladium* are the most biologically active substances applied to major soil-borne pathogens (Benítez, 2004; Celar & Valic, 2005; Kim & Hwang, 2007; Papavizas, 1985; Redda et al., 2018). They are effective against Fusarium wilt, Phytophthora blight, Rhizoctonia and Pythium root rot, Sclerotinia steam rot, Botrytis gray mould on vegetable and flower crops (Ezziyani et al., 2004; Huang et al., 2011; Fiume & Fiume, 2006; Kamala & Indira, 2011). Their antagonistic activity is determined by the largest reproductive ability and isolation of liquid and volatile antibiotics with harmful action on soil microorganisms, which parasite on the roots, root collar and the vascular system of the plants. There are real opportunities to combat the complex causes root rot in plants in greenhouse conditions by using several antagonists in common use (Harman et al., 2004; Hichar et al., 2015; Raupach & Kloepper, 1998).

Biological products produced on the base of indigenous strains, must be consistent with the geographical area origin of isolates (Manczinger et al., 2002). This requires study and selection of efficient for a specific geographical area strains that may actually serve to control plant diseases. The main object of the task lies on the study of the symbiotic action of local saprophytic strains of fungi *Trichoderma viride* and *Gliocladium virens* with good technological parameters for the production of a biological preparation and having a high index of antagonistic activity against Fusarium root rot on cucumbers.

MATERIALS AND METHODS

The experimental work was carried out in 2018-2019 on the territory of the institute for vegetable crops "Maritsa" in a greenhouse with a cucumber of the Tony F1 variety under artificial infectious background with *Fusarium oxysporum* (isolate №24 of cucumber plant). Tony F1 is an early high-yielding variety with a female type of flowering. The growing period from germination to the first harvest is 44-46 days. Plants were grown as a soil culture with a seeding density of 1.79 plants m2 (scheme of experiment (200 + 80/35 cm), 3 replications, 10 plants per replication). The possibilities for biological control of Fusarium root rot on cucumber with antagonistic fungi from *Trichoderma* and *Gliocladium* genus was studied. The experiment included several isolates of antagonistic fungi *Trichoderma viride* strain №1, *Trichoderma viride* strain №2, *Gliocladium virens* strains № 1, *Gliocladium virens* strains №2, *Gliocladium virens* strains №3, isolates from the rhizosphere of diseased plants of local origin. Antagonists were applied to the soil as pure microbial cultures during the transplanting of seedlings to a permanent place in 1: 1 ratio to the pathogen, calculated in accordance to the number of antagonists in any combination (Zvyagintsev, 1991). During the experiment, biometric indicators of seedlings and plants were measured: height of the stem (H), leaves number, diameter of the stem (mm), weight of the stem (g), the root mass (g), total mass of 1 plant (g) and Fusarium root and steam rot rate (%). The obtained results were subjected to mathematical processing of Duncan (Dimova and Marinkov, 1999).

RESULTS AND DISCUSSION

Impact on the growth of cucumber plants

The action of antagonists of the genus Trichoderma and Gliocladium had a positive effect on the biometric indicators of seedlings and cucumber plants. As shown in Table 1, the antagonists help to form seedlings with a strong root system and a larger diameter of the stem. In the variant with the introduction of a mixture of antagonists, the increase in mass of the root was 35 per cent, the diameter of the stem – 23 per cent, compared to the control variant.

The growth-promoting action of antagonists continued during the vegetation. Soil treatment with mix of strains Trichoderma viride and Gliocladium virens promoted the rich formation of flowers and fruits (Table 2). The combined use of the Trichoderma viride №2 + Gliocladium virens №2 strains had positive effect on the biometric parameters of the plants - stem diameter, the root length, the number of leaves and the number of fruits per plant, compared to variants where the antagonist was used separately. The stem diameter in the variant with mix of antagonists Trichoderma viride №2 and Gliocladium virens №2 was 10.75 mm, the number of leaves increased from 27 to 32, and the number of fruits from 16 to

Table 1. Biometric indicators of the seedlings of cucumber variety Tony obtained on a substrate treated with fungi of the Trichoderma viride and Gliocladium virens.

№	Antagonist	Stem height, cm	Diameter of stem, mm	Length of root, cm	Weight of root, g	Leaves number	Weight of plant, g
1	Control	15,5	3	4,42	0,22	4,58	5,91
2	Tr_1	19,33	3,9	4,11	0,29	4,55	7,36
3	Tr_2	22,37	4,5	5,79	0,27	5,42	8,87
4	$Tr_1 + Tr_2$	18,77	3,9	5,77	0,32	4,55	7,65
5	G_1	16,55	3,9	5,38	0,3	4,33	6,84
6	G_2	18,77	3,9	5,38	0,19	4,33	6,32
7	$G_1 + G_2$	16,11	3,3	3,72	0,26	4,33	5,11
8	$Tr_1 + G_1$	18,27	3,4	3,77	0,22	4,66	6,93
9	$Tr_1 + G_2$	17,42	4	5,66	0,3	4,58	6,96
10	$Tr_2 + G_1$	27,2	4,7	5,61	0,3	5,44	12,62
11	$Tr_2 + G_2$	26,88	4,5	7,22	0,34	4,99	10,18

Abbreviations: **Tr** – *Trichoderma* isolates (1, 2); **G** – *Gliocladium* isolates (1, 2).

21 compared to the control variant. Biometric indicators of plants in the variant with a mixture of four antagonist isolates are stable. Higher root length indicators affect the health of plants.

Influence to Fusarium root rot on cucumbers

The main purpose of this experiment is to follow the impact of antagonists to Fusarium root rot on cucumbers. In the two years of the experiment the development of Fusarium root rot was nearly 33 per cent in the control variant. This is a sufficiently high rate of attack on which a reliable connection for the action of the introduced strains antagonistic against soil borne pathogens can be made. The effect from antagonists on the Fusarium root rot on cucumber ranges from 25 per cent in 2018 to 17 per cent in 2019 (Table 3).

The mix of strains *Trichoderma viride* №1 and №2, *Gliocladium virens* №1 and №2, applied as spore suspension with concentration 2.1010 spore/ml in dose 100 ml per plant during the transplanting of seedlings to a permanent place, showed the increase of their antagonistic effect against the development of Fusarium root rot on cucumbers. In this variant plants, diseased from Fusarium root rot were not found. The same results were obtained in the variant with soil application of spore suspension of strain *Gliocladium virens* №2 in dose 100 ml per plant. Plants in these variants had a higher mass of the root system compared to the control. The use of antagonists accelerated the process of flowering and fruit development.

Table 2. Biometric indices of plants and Fusarium root and stem rot rate on cucumber (variety Tony), treated with Trichoderma viride and Gliocladium virens. Phase of growing: flowering - beginning of fruit formation.

№.	Antagonist	Stem height, m	Diameter of stem, mm	Length of root, cm	Number of leaves	Number of flowers, fruit sets, fruits	Fusarium root and stem rot, %
1	Control	2,23	6,15	14,16	27	15,91	33,33+
2	Tr_1	2,29	6,75	18,92	30,33	20,49	12,5
3	Tr_2	2,55	8,08	12,25	30	18,99	16,67-
4	$Tr_1 + Tr_2$	2,29	7,66	17,33	30,5	20,08	12,50-
5	G_1	2,06	7,66	15,92	29,66	16,76	8,33
6	G_2	2,35	8,58	20,42	31,5	15,83	0,00+
7	$G_1 + G_2$	2,12	6,5	14,33	25,33	13,07	12,50+
8	$Tr_1 + G_1$	2	6	12,58	23,33	11,1	8,33+
9	$Tr_1 + G_2$	2,75	6,33	15,42	29,33	17,5	16,67+
10	$Tr_2 + G_1$	2,45	7,83	20,66	28,16	15,66	8,33+
11	$Tr_2 + G_2$	2,44	10,75	13,08	32,16	20,83	4,17+
12	$Tr_1 + Tr_2 + G_1 + G_2$	2,38	6,92	21,08	30,33	18,82	0,00+

Abbreviations: **Tr** – *Trichoderma* isolates (1, 2); **G** – *Gliocladium* isolates (1, 2).

The results of our studies gave an idea of the multiple actions of strains of the collection *Trichoderma viride* and *Gliocladium virens* on the growth of cucumber plants. Strains have a high antagonistic activity to *Fusarium oxysporum*, the ability to co-develop and enhance the positive effect over time. At 33 per cent of Fusarium root rot on cucumbers of the control variant, the symbiotic effect of soil application of these antagonist strains (*Trichoderma viride* and *Gliocladium virens*) was higher than 75 per cent. The high fungicidal activity of *Gliocladium virens* strains against the pathogen Fusarium oxysporum complements the effect of *Trichoderma viride* strains on the growth and productivity of cucumber plants.

Table 3. Effect of application of *Trichoderma viride* and *Gliocladium virens* to Fusarium root and stem rot on cucumber (variety Tony).

№	Antagonist	*Fusarium* root and stem rot,%		Effect an average of two years, %
		First year	Second year	
1	Control	33,33	33,33	-
2	Tr_1	16,67	8,33	62,5
3	Tr_2	16,67	16,66	50
4	$Tr_1 + Tr_2$	16,67	8,33	62,5
5	G_1	16,67	0	62,5
6	G_2	0	0	100
7	$G_1 + G_2$	8,33	16,66	62,5
8	$Tr_1 + G_1$	0	16,66	75
9	$Tr_1 + G_2$	25	8,33	50
10	$Tr_2 + G_1$	8,33	8,33	75
11	$Tr_2 + G_2$	8,33	0	87,49
12	$Tr_1 + Tr_2 + G_1 + G_2$	0	0	100.00

Abbreviations: **Tr** – *Trichoderma* isolates (1, 2); **G** – *Gliocladium* isolates (1, 2).

CONCLUSIONS

Collection of samples strains *Trichoderma viride* and *Gliocladium virens* have multiple actions on the growth and development of cucumber plants – a high antagonistic effect on Fusarium root rot, the ability to accelerate the growth phases and improve plant biometric parameters. Antagonistic fungal strains of *Trichoderma viride* and *Gliocladium virens*, isolated from the region, exhibit significant fungicidal effect on the development of the soil infection by *Fusarium oxysporum* f. sp. *cucumericum* in a single application into the soil as a spore suspension with a concentration 2.1010 spore/ml. The effect is expressed as a suppression of disease more than 50 per cent compared to the untreated control. The results of the preliminary experiments demonstrated the symbiotic properties of collections strains *Trichoderma viride* and *Gliocladium virens* in combine use.

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