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IN VITRO COMPREHENSIVE ASSESSMENT OF ANTAGONISTIC ACTIVITY OF *TRICHODERMA* GENUS FUNGI AGAINST *FUSARIUM* SPP.

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Abstract

As a result of researches from different substrates (peat, soil ground and mineral rock) new isolates of *Trichoderma* genus fungi were isolated with a wide variability of cultural and morphological features. Studying of new isolates and also collection *Trichoderma* genus fungus strains antagonistic activity has shown the presence of differently directed mechanism of antagonistic interaction in relation to phytopathogenic micromycetes *F. semitectum, F. verticilliodes* and *F. solani*. Inhibiting the linear pathogens growth on the 5-th day of co-culture cultivation varied in relation to *F. semitectum* from 39% to 100%, *F. verticilliodes* – from 48% to 100%, *F. solani* – from 45% to 85%. The growth coefficient of *Fusarium* spp. phytopathogens in a co-culture with the studied antagonists is fallen significantly and depending on the antagonist isolate or strain can be decreased for more than 5 times. For a majority of studied *Trichoderma* fungi a presence of a mixed type of antagonistic activity fungistatic alimental (II type) and antibiotic (IV type) antagonism is determined. The comprehensive assessment of antagonistic activity has shown that the most active antagonist is a fungus *Trichoderma* sp. D-11, effectively inhibiting the linear growth of studied phytopathogens and rendering a high antagonism level by II and IV type what allows to substantiate the perspectiveness of its use as a basis of a preparation to control *Fusarium* genus phytopathogenic micromycetes for biological control of fusarium etiology diseases.

Keywords: *Trichoderma, Fusarium, antagonistic activity, comprehensive assessment*

Introduction

To develop the biological plant protection against pathogens it is necessary to do search of active antagonists and hyperparasites with a high competition ability and a wide range of adaptation to different biological and abiotic factors (Muromtsev, 1980; Prokofiev, 1983). The microorganisms with the antagonistic peculiarities among which the representatives of the genuses *Bacillus, Pseudomonas, Trichoderma* answer these demands (Pavlyushin, 2002; Holmes, 2004; Bazhanov, 1998; Borodko, 1999). The effective biocontrol agents are the antagonist fungi of the genus *Trichoderma*, based on which a set of biological preparations for plant protection against phytopathogens and stimulation of growth and development of a wide crop assortment is developed. It is caused by high antagonistic potential of the indicated fungi, growth speed, sporulation ability at submerged cultivation (blastic type of conidiogenesis presence), technologicity of cultivation in the production (Grinko, 2000; Windham et al., 1968; Papavizas, 1985; Berestetsky and Sokornova, 2009). One should do the concrete search of antagonists to the specific pathogen species in their co-inhabitance medium, as it is more expedient to use local fungi of the genus *Trichoderma*, the most adopted to specified conditions (Golovanova, 2002; Muromtsev, 1980; Papavizas, 1985).To develop a strategy of antagonists use in the biological control practically it is necessary to know their interrelations with the phytopathogens. By antagonist population introduction into ecosystem with already formed biotic connections for getting maximum protective effect the

population should successfully colonize substrate and being interrelated with the phytopathogenic microorganisms, decrease or suppress their development with the help of biologically active substances (antibiotics, lytic enzymes and other metabolic products). So, the antagonist should differ by high competition ability necessary to establish topic and trophic connections and occupy a correspondent ecological niche (Bazhanov, 1998; Sukhovitskaya, 1998; Philipchuk, 1998). So, the main selection criteria by active strains screening are their high growth speed, sporulation and the effective pathogen suppression *in vitro* (Monte, 2001; Vey et al., 2001). Based on it, the objective of our researches was to study the antagonistic activity of fungi genus *Trichoderma* in relation to phytopathogenic micromycetes genus *Fusarium*.

Materials and methods

The antagonists genus *Trichoderma* isolation was done from greens (dill, salad and parsley) rhizosphere and rhizoplane. The fungi genus *Fusarium* isolation was accomplished from the phytopathological material of plant samples. By micromycetes isolation from soil, 0,5-1 g plates were put in flasks with sterile water. The micromycetes desorption from soil particles was done on laboratory rocker (180 rotations/min.) in the course of 10-15 minutes (Zvyagintsev, 1991). While carrying out the researches the monospore isolates of *Fusarium* sp., were used which were obtained from fungus sporodochium formed at prolonged crop storage. To stimulate the typical sporulation formation carnation-leaf agar (CLA) was used. The species identification was done using Gerlach and Nirenberg atlas (Gerlach, Nirenberg, 1982). The antagonistic action study of isolated isolates and collection strains (*Trichoderma viride* IZR 2A and *Trichoderma harzianum* IZR S-4 – strains-bases of biopreparations Trichodermin-BL and Lignorin) of fungi genus *Trichoderma* in relation to phytopathogenic micromycetes genus *Fusarium* was done *in vitro* conditions by dual culture method (Egorov, 1976). The incubation temperature – 25°C, repetitions – 4. As a control a pure phytopathogen culture was used. In the experiments a character of the fungus growth was periodically recorded (substrate or aerial mycelium), colony diameter, on the 3-rd day of co-culture cultivation the presence of sterile zones was analyzed, on the 5-th day – the antagonist zones increase on the phytopathogen colony were marked. To evaluate the antagonistic activity of the fungi genus *Trichoderma* the growth coefficient (RC) and the percentage of phytopathogen inhibition was recorded (Tarunina and Maslova, 1979).

The growth coefficient (RC) was calculated by formula:

$$RC = \frac{d \times h \times g}{t}$$

where, RC – growth coefficient; *d* – colony diameter, mm; *h* – colony height, mm; *g* – colony density, point; *t* – colony age, days.

The phytopathogen growth inhibition on the record day was determined by formula:

$$\mathrm{D} = \frac{Dk - Do}{Dk} \times 100\%$$

where *D* – phytopathogen growth inhibition, %; D_k – fungus colonies diameter in control, mm; D_o – fungus colonies diameter in the experiment, mm.

The experiment was carried out on nutritive Chapek, wart-agar, Saburo, PDA.

The type of interrelations between fungi genus *Trichoderma* was characterized by the methods mentioned Poluksenova et al. (2004):

I – indifferent interrelations (the fungus genus *Trichoderma* colonies increase on the surface of the phytopathogen colony keeping the growth speed of both fungi);

II – fungistatic alimentary (one-side) antagonism (the fungus genus *Trichoderma* colonies increase on the phytopathogen colony surface which stops the active growth in this case);
III – territorial antagonism (the accumulation of the fungus genus *Trichoderma* by the pathogen colonies, usually the pathogen drops in growth behind);
IV – antibiotic antagonism (slowing down the pathogen colonies growth in a distance from the fungus genus *Trichoderma*, zone formation in which the pathogen growth is not observed as a result of antibiotic substance secretion by the fungus genus *Trichoderma*);
V – mutual antagonism (fungus genus *Trichoderma* increase on the surface of the phytopathogen colony with the mutual inhibition of growth speed).

Results and discussion

Considering the fact that the effective antagonists screening to specific pathogens should be done in their cohabitation medium as the use of local strains of fungi genus *Trichoderma*, the most adapted to the specific conditions is the most appropriate, we have isolated from greens rhizosphere and rhizoplane the isolates of micromycetes genus *Trichoderma*: TK-2, T-3, T-1, T-4-1, C-3, K-4, PMT-1, D-11.

Cultural and macromorphological characters of the fungi *Trichoderma* spp.

The analysis of cultural and macromorphological traits has allowed to determine that all isolates when cultured on nutritive agars PDA, wart-agar, Saburo, Chapek have formed correctly rounded form colonies. The edge of the most isolates colonies was clearly limited. Nevertheless, the isolates *Trichoderma* sp. TK-2 on wart agar and Chapek , T-3 – PDA and Chapek agar, T-1, C-3, K-4, PMT-1 – on PDA, D-11 – on Chapek agar have formed irregular, refined edge of the colony.

On all the studied nutritive agars a profile of *Trichoderma* sp. isolate D-11colony was salient, the isolate *Trichoderma* sp. T-3 on PDA and Saburo agar has formed a protuberant profile, on wart agar and Chapek – even. All other isolates have formed an even colony profile regardless of the medium.

The colonies structure analysis has shown the formation of three consistency types – tomentose, velvet and woolly. Each isolate on different nutritive agars has formed 2-3 different types of colonies: on PDA – tomentose and velvet, wart-agar – velvet and woolly, on Chapek agar – tomentose and velvet, on Saburo the structure of all colonies has got a woolly texture.

The colonies color varied depending on both the isolate and culture medium – yellow, yellow-green, dark-green. The color of the colonies reverse side also varied – white, beige, pale yellow, yellow, brown.

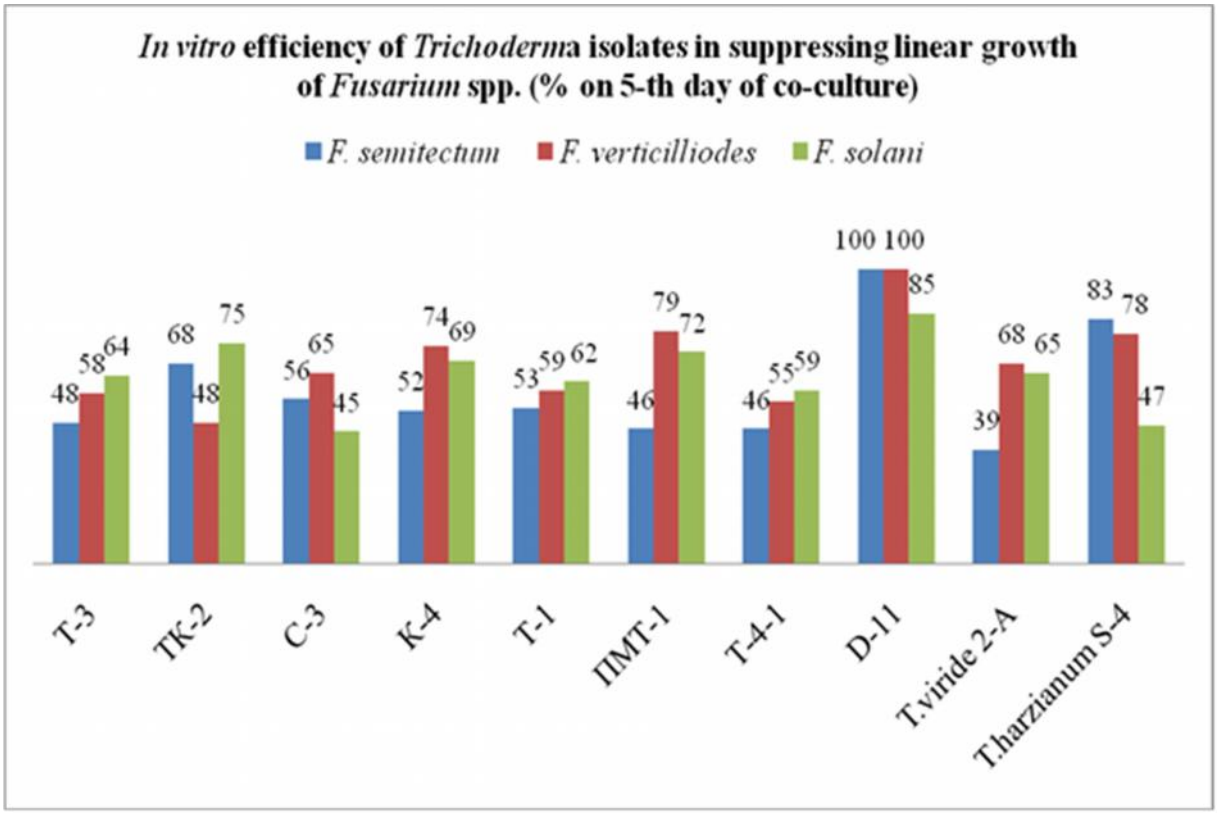
Most of the studied isolates have formed a colony without a marked centre, except the isolates of *Trichoderma* sp. TK-2 on PDA, *Trichoderma* sp. D-11 – on PDA, Saburo and Chapek agars the colony’s centre has got a crater-like appearance.

All isolates cultured on studied nutritive agars have got a typical fungal smell.

Starting of all isolates sporulation on wart-agar was noticed on the 3-rd day, apart the isolate *Trichoderma* sp. K-4 (4-th day). On PDA the isolates *Trichoderma* spp. T-3, C-3, K-4 started the sporulation on the 3-rd day, of *Trichoderma* spp. TK-2, T4-1, PMT-1 –on the 4-th day, *Trichoderma* spp. T-1 and D-11 – on the 5-th day of cultivation. On Saburo agar *Trichoderma* spp. TK-2, T-3, T-1, PMT-1 the isolates spores formation was marked on the 4-th day, *Trichoderma* spp. T4-1, C-3 and D-11 isolates – on the 5-th day. When cultured on Chapek agar *Trichoderma* spp. T-1, PMT-1, D-11 isolates have formed spores on the 4-th day, *Trichoderma* spp. TK-2, T-3, T4-1, C-3, K-4 – on the 5-th day.

The analysis of *Trichoderma* spp. isolates intensity of sporulation has shown that higher productivity was characteristic for *Trichoderma* sp. D-11 strain on wart agar (titer $9,0\times10^7$ spores/cm^2) and Chapek (titer $8,4\times10^7$ spores/cm^2).
Thus, it is shown that depending on the selected culture medium a variability of cultural-morphological peculiarities of *Trichoderma* spp. fungi strains is observed.

Screening of antagonistic activity of *Trichoderma* genus fungal strains activity in relation to phytopathogenic micromycetes *Fusarium* spp.
The results of our researches have shown that all the studied isolates and strains of *Trichoderma* spp. fungi possess the antagonistic activity in relation to phytopathogenic micromycetes of *Fusarium* spp. The analysis of character of *Trichoderma* spp. strains and isolates interaction with phytopathogens testify to different mechanisms of antagonistic interaction.
In relation to the studied fungi of *Fusarium* spp. all *Trichoderma* spp. isolates and strains have shown rather high effect in inhibiting a linear growth of pathogens. On the 5-th day of co-culture *F. semitectum* growth inhibition varied from 39 (*T. viride* 2A) to 100% (isolate D-11), *F. verticilliodes* – from 48 (isolateTK-2) to 100% (isolate D-11), *F. solani* – from 45 (isolate C-3) to 85% (isolate D-11) (Fig. 1).



By subsequent observations it is determined that in relation to *F. semitectum* fungus all isolates and strains of *Trichoderma* spp. have shown the antagonism by II and IV type. For all isolates and strains of *Trichoderma* spp. a sterile zone formation was observed, the size of which varied from 8,0 to 15,8 mm, and also rather significant for some antagonist isolates and strains increase on phytopathogens colony is observed (Table 1).

Table 1. The antagonistic activity of *Trichoderma* spp. isolates and strains in relation to fungi genus *Fusarium*

Trichoderma isolates (strains)	*F. semitectum*		*F. verticilliodes*		*F. solani*	
	F^1	F^2	F^1	F^2	F^1	F^2
T-3	9,0±1,29	17,5±4,6	9,5±0,9	–	–	27,5±4,5
TK-2	8,8±1,52	30,0±2,4	5,0±1,3	–	–	22,5±3,3
C-3	9,5±2,05	12,5±3,9	–	21,5±3,1	–	9,3±1,5
K-4	13,3±2,38	21,3±4,6	5,8±1,5	25,3±2,0	–	26,8±3,7
T-1	9,5±2,05	22,5±4,6	–	7,5±4,6	–	16,3±3,9
ПМТ-1	8,5±2,05	20,0±2,4	18,3±2,4	33,8±3,9	2,2±0,7	32,0±3,8
T-4-1	8,0±1,29	15,0±1,6	3,5±2,1	9,3±1,5	–	27,5±4,5
D-11	15,8±3,52	45,0±3,3	5,8±1,5	39,0±2,3	4,7±0,7	31,2±3,9
T. viride 2-A	8,5±2,05	6,3±3,9	6,0±1,3	17,5±4,6	–	27,5±4,5
T. harzianum S-4	10,8±1,52	40,0±4,6	23,5±2,1	24,8±3,3	–	9,2±1,5

Note: F^1 – sterile zone on the 3-rd day of co-cultivation, mm; F^2 – the antagonist increase zone on phytopathogen on the 5-th day of co-cultivation, mm

In relation to *Fusarium verticilliodes* the isolates C-3 and T-1 were characterized by IV type antagonist action, the isolates T-3 and K-2 – by II, the other isolates and strains were characterized by a mixed type of antagonistic action. In relation to *F. solani* all isolates and strains of *Trichoderma* spp. were formed on the pathogen colony, what testifies to the II type antagonism, moreover, the isolates PMT-1 and D-11 have provoked sterile zones formation (IV type of antagonistic activity).

The data of *Fusarium* spp. micromycetes growth coefficient have shown that the given parameter by co-cultivation with antagonists fall significantly and varies depending on antagonist isolate or strain. The most intensive growth coefficient increase is marked in the variant *F. solani* + *Trichoderma* sp. D-11 – 5,1 times decrease (Table 2).

Table 2. *In vitro Trichoderma* spp. influence on growth coefficient of *Fusarium* spp.

Trichoderma isolates (strains)	Growth coefficient on the 3-rd day of co-culture		
	F. semitectum	*F. verticilliodes*	*F. solani*
Control	80	98	154
T-3	65	31	107
TK-2	32	36	78
C-3	53	75	104
K-4	65	51	96
T-1	59	24	92
PMT-1	46	33	91
T-4-1	64	53	106
D-11	53	47	30
T. viride 2-A	71	53	93
T. harzianum S-4	61	29	100
SED_{05}	*12,3*	*15,6*	7,1

Conclusions

The analysis of components of the antagonistic activity of new isolates and strains of *Trichoderma* fungi indicates a complex mechanism of antagonistic interaction with phytopathogenic micromycetes *F. semitectum*, *F. verticilliodes* and *F. solani*. The vast majority of the studied antagonists were characterized by a mixed type of antagonistic action with the pronounced presence as fungistatically alimentary (II type) and antibiotic activity (IV type). According to the results of a comprehensive assessment of the antagonistic activity a fungus *Trichoderma* sp. D-11 was more active. The results obtained allow to justify a perspectiveness of its use as a basis for biological preparation to control phytopathogenic micromycetes genus *Fusarium*.

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