The biological activity of subspecies *Trichoderma harzianum* against *Fusarium oxysporum*, the causative agent of fusarium wilt cucumber in vitro

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Abstract. The study of the effect of the strains of the fungus *Trichoderma*: *Trichoderma atrobrunneum* VKPM F-1434, *Trichoderma harzianum* 5/14, *Trichoderma Lixii* T4/14 on the number of micromycetes populations of the pathogenic fungi *Fusarium oxysporum* isolate B/14, *Fusarium oxysporum* isolate MOS509, *Fysarium oxysporum* isolate IMI58289 *in vitro*. It was found that the *Trichoderma atrobrunneum* fungus strain VKPM F-1434 showed the highest degree of inhibition on 10 days of cultivation with phytopathogenic microorganisms, which was 100 %. The study revealed that all *Trichoderma* species are capable of producing lytic enzymes. *Trichoderma atrobrunneum* strain VKPM F-1434 exhibits strong lipase and chitinase activity and average proteinase activity. In addition, *Trichoderma atrobrunneum* strain VKPM F-1434 has a growth-promoting ability, which was reflected in the germination of seeds of cucumber "*German F1*". The maximum values of indicators of germination energy were noted - 98.4 % and germination – 100 %.

1 Introduction

Fusarium is one of the most harmful and widespread diseases of agricultural plants in the world caused by soil phytopathogenic fungi, including representatives of the genus *Fusarium*. Infection can occur at any stage of crop growth and seriously reduce yield and degrade fruit quality, especially in protected ground [1]. It is economically feasible to use biological control methods that are environmentally friendly in the integrated fight against fusarium [2].

The biological protection of plants from pathogens of Fusarium infections is becoming increasingly important in the production of greenhouse cucumber (*Cucumis sativus*). Fusarium wilt of a cucumber (tracheomycosis) according to many authors, the disease causes death on average up to 10-15 %, and in some years up to 65 % of cucumber plants. It is possible to successfully reduce the rate of wilting of plants caused by fungi of the genus *Fusarium*, possibly using antagonistic microorganisms such as *Bacillus*,

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Enterobacter, and *Pseudomonas*, which are the main root colonizers and can stimulate plant protection [3].

Many researchers have shown that in the biological control *Fusarium* successfully use fungi of the genus *Trichoderma* spp. [4] five main mechanisms of combating phytopathogens, including mycoparasitism through the secretion of hydrolytic enzymes, competition for nutrition, antibiosis in the production of secondary metabolites, stimulating plant growth and stimulating systemic resistance to diseases in plants [5].

To date, more than 340 species of *Trichoderma* have been described [6, 7], which have potential biological activity against phytopathogenic fungi. The most commonly used species are *T. asperellum, T. atroviride, T. harzianum*, and *T. polysporum* [8].

Thus, based on the foregoing, the relevance of the search for effective biological control agents for fungi of the genus *Fusarium*, the causative agent of fusarium wilt of cucumber, from the genus *Trichoderma*, is shown.

The aim of this study was to assess the effect of *Trichoderma atrobrunneum* exemetabolites VKPM F-1434 and other related strains of microorganisms against fungi of the Fusarium genus, followed by assessment of their effect on the growth and development of cucumber seeds in vitro and in vivo.

2 Materials and methods

2.1 Object of research

For the experiment, we used live cultures of *Trichoderma* fungi: *Trichoderma atrobrunneum* VKPM F-1434, *Trichoderma harzianum* 5/14, *Trichoderma Lixii* T4/14; phytopathogenic fungi of the genus *Fusarium*: *Fusarium oxysporum* B/14, *Fusarium oxysporum* MOS509, *Fusarium fujikuroi* IMI 58289 from the academic collection of the department of biotechnology of the Oryol state agrarian University, for a long time stored in the refrigerator at a temperature of +4°. All represented fungi of the genus *Trichoderma* before 2015 belonged to the same species *Trichoderma harzianum* [9]. Cucumber seeds of the "*German F1*"variety were used as objects of research. The cucumber variety " *German F1*" is a hybrid universal variety that is suitable for growing in greenhouses and farms.

2.2 Method of research

Direct confrontational analysis of *Trichoderma atrobrunneum* VKPM F-1434 against *Fusarium oxysporum* was tested by double culture method on PDA (Zhang, et al 2017) [10]; testing of the antagonistic activity of fungi of the genus *Trichoderma* was performed using the method (You, et al., 2016) [11]. Spore suspensions of antagonists for research were prepared according to the methods (Regalado et al., 2000) [12]. Determination of enzymatic activity was performed using express tests according to the method (Cruz-Quiroz, et al., 2017) [13]. Germination energy and laboratory germination were determined according to the requirements Russian Standard 12038-84. The prevalence of Fusarium infection was taken into account in the phase of active fruiting on the 40th day of vegetation by a combined method (Chumakova, 1974).

Statistical processing of results was performed using the Microsoft Office 2010 (Excel) package. All experiments were carried out in five-fold repetition.

3 Results

3.1 Determination of antagonistic activity of fungi of the genus *Trichoderma*

To assess the degree of manifestation of antagonistic activity and mechanisms of action on phytopathogens, the influence of *Trichoderma atrobrunneum* antagonists strain VKPM F-1434, *Trichoderma Lixii* isolate T4/14, *Trichoderma harzianum* isolate 5/14, on strains of phytopathogenic fungi *Fusarium oxysporum* isolate B/14, *Fusarium oxysporum* isolate MOS509, *Fusarium fujikuroi* isolate IMI58289 under in vitro conditions by double culture method.

The results of the research showed that in the control all phytopathogenic microorganisms intensively grew along the Petri dish and occupied almost the entire area of the Petri dish, while they formed a well-developed air mycelium with a bright pigment (Tabl. 1).

Table 1. Percentage of inhibition of growth of *Fusarium* fungi colonies by antagonists in double culture, %

Antagonist - strains	*F. oxysporum* isolate Б/14		*F. oxysporum* isolate MOS509		*F. fujikuroi* isolate IMI 58289	
	day		day		day	
	5	10	5	10	5	10
T. atrobrunneum strain VKPM F-1434	39,1±0,14	100	47,5±0,51	100	77,2±0,21	100
T.Lixii isolate T4/14	27,6±0,36	42,6±0,21	28,7±0,5	59,0±0,62	78,3±0,25	100
T.harzianum isolate 5/14	28,2±0,11	34,8±0,08	28,1±0,16	54,2±0,32	63,8±0,11	84,6±0,65

It was noted that all antagonists in double culture are able to inhibit the growth of phytopathogens, while the fungus *T. atrobrunneum* strain VKPM F-1434 on the 10th day of cultivation with phytopathogenic microorganisms showed the highest degree of inhibition (100 %); *T. Lixii* isolate T4/14 against *F. fujikuroi* isolate IMI 58289-100 %; *T. harzianum* isolate 5/14 against *F. fujikuroi* isolate IMI 58289 – 84 %. *T. lixie* isolate T4/14 and *T. harzianum* isolate 5/14 had a very low level of inhibition of *F. oxysporum* isolate B/14, *F. oxysporum* isolate MOS509 (<60 %).

3.2 Determination of the activity of enzymes of fungi of the genus *Trichoderma* associated with mycoparasitism

Inhibition of growth of pathogens by fungi is their generic feature and is due to the ability of mycoparasite to hydrolyze the cell walls of phytopathogenic fungi and use them as a substrate due to the produced enzymes and secreted various compounds [14]. The results of determining the enzymatic activity of the studied antagonist strains are presented in the table 2.

In our study, all *Trichoderma* species are capable of producing lytic enzymes. In *T. atrobrunneum* strain VKPM F-1434, the degree of manifestation of lipase and chitinase activity is strong, average proteinase. Strains of *T. Lixii* strain T4 / 14 and *T. harzianum* strain 5/14 have medium lipase and chitinase activity, weak - proteinase. Which confirms the data of mycoparasitic ability of strains within species [15].

Table 2. The ability to form lytic enzymes in the studied antagonist strains

Antagonist -strains	Enzyme activity		
	chitinase	proteinases	lipase
T. atrobrunneum strain VKPM F-1434	++++	+++	++++
T.Lixii isolate T4/14	+++	++	+++
T.harzianum isolate 5/14	+++	++	+++
The degree of manifestation of enzymatic activity was determined visually: - absence; + very weak; ++ weak; +++ average; ++++ strong			

3.3 Determination of the stimulating and fungicidal effect of presowing treatment of cucumber seeds with spore suspensions of the studied antagonists *in vitro*

Many researchers have shown that microorganisms with antagonistic activity can stimulate the growth and development of various plants, as well as change the soil microbiota, thereby improving the phytosanitary state of the soil [16].

В наших исследованиях особое значение придавалось использованию аборигенных штаммов антагонистов, так как их биологическая активность непосредственно связана with habitat and with the entire soil complex as a whole [17]. Based on the results of the antagonistic, mycoparasitic, and enzymatic activity of the studied antagonist microorganisms, in vitro experiments were performed on their ability to stimulate the germination of cucumber seeds. The results are presented in Figure 1.

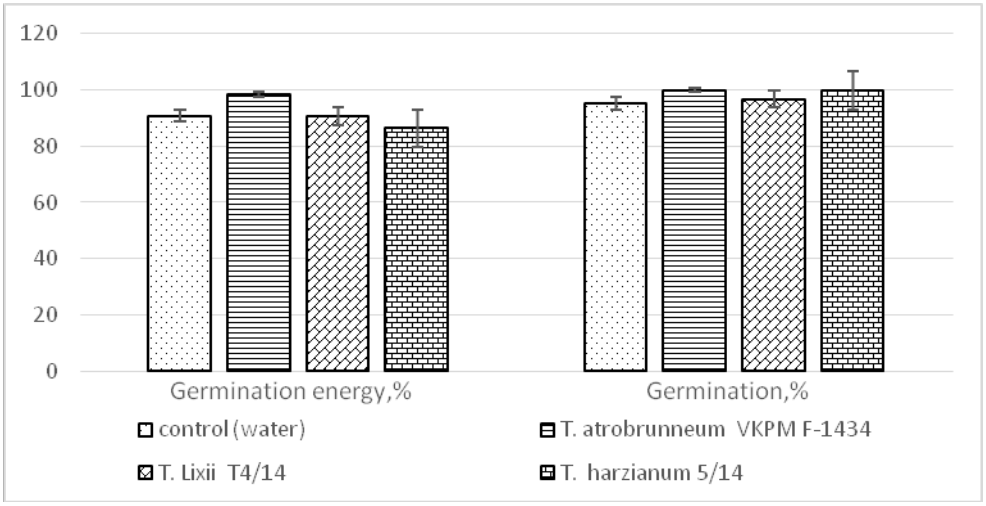

Fig. 1. Indicators of germination energy (3rd day), germination of cucumber seeds (7th day) for various processing options, %

The maximum germination energy of cucumber seeds was noted in the variant with *T. atrobrunneum* strain VKPM F-1434, *T. Lixii* isolate T4/14. The greatest number of seedlings was observed in the variant with *T. atrobrunneum* strain VKPM F-1434, *T. harzianum* strain 5/14, which exceeded the control by 5 %. Thus, the studied antagonists possessed growth-promoting ability (*in vitro*), which positively affected the germination of cucumber seeds.

3.4 Biocontrol potential of the studied fungi of the genus *Trichoderma* against fungi of the genus *Fusarium in vivo*

Trichoderma species produce a huge amount of water-soluble metabolites, including pyrenes, terpenoids, steroids and polyketides, and others [5]. Able to inhibit the growth of plant pathogens in vitro and in vivo. The biocontrol potential of the studied antagonist microorganisms was evaluated in vivo against an artificially created infectious background against cucumber microplants. 50 ml of an aqueous suspension of *Fusarium* fungi, the titer of which is higher than that of the antagonists (10^9 conidia/ml), was added to plastic cuvettes with 7-day-old seeds of cucumber treated with spore suspensions of the studied antagonist microorganisms. *T. atrobrunneum* strain VKPM F-1434 and *T. harzianum* isolate 5/14 stimulate root growth, while *T. Lixii* isolate T4/14 is characterized by stimulation of both roots and seedlings.

It was shown that by the end of the experiment, the number of phytopathogenic populations decreased by 75-80 % compared with the initial number of phytopathogenic fungi due to the hyperparasitism of the *T. atrobrunneum* micromycete strain VKPM F-1434, which no longer affected the susceptibility of cucumber seedlings, but at the same time decreased the number of introduced antagonist by 52 % due to the decrease in substrate (phytopathogens) and the processes of restoring the structure of the microbial pool in the soil. The number of populations of micromycetes of the genus *Fusarium* decreased by 58-82 % compared with the initial number of phytopathogenic fungi due to hyperparasitism of micromycete *T. harzianum* isolate 5/14, while the number of introduced antagonist decreased by 64 %. The population of micromycetes of the genus *Fusarium* decreased by 37-39 %, *Fusarium fujikuroi* isolate IMI 58289 by 79 % compared to the initial number of phytopathogenic fungi due to the hyperparasitism of micromycete *T. Lixii* isolate T4/14, which no longer affected the susceptibility of cucumber seedlings, even at the same time, the number of introduced antagonist decreased by 35 % (Fig. 2).

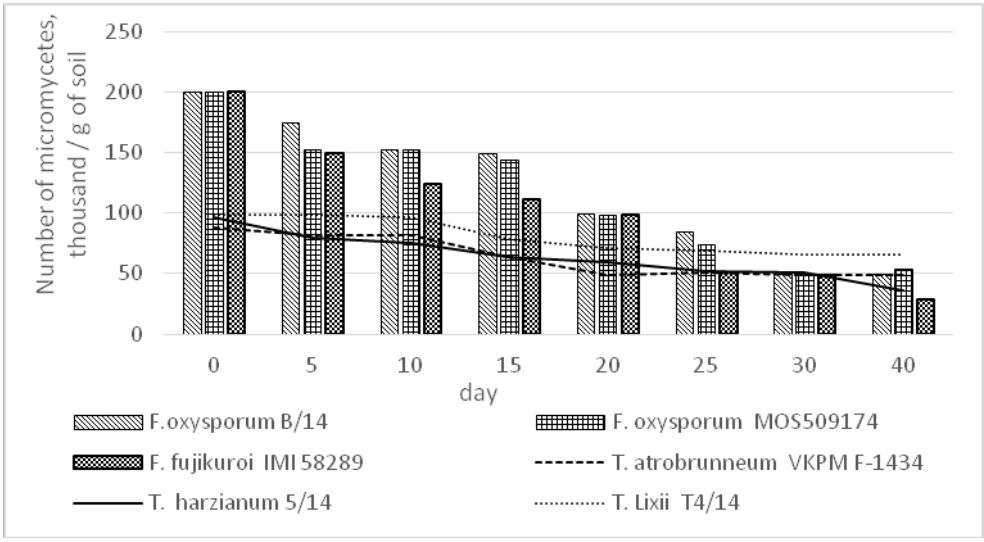

Fig. 2. Changes in the number of phytopathogenic micromycetes of the genus *Fusarium* under the influence of antagonists of the genus *T. atrobrunneum* strain VKPM F-1434, *T. harzianum* isolate 5/14, *T. Lixii* isolate T4/14

By the end of the experiment, the degree of development of a fusarium infection of 40-day-old cucumber plants was evaluated under the action of the studied antagonist

microorganisms in the root zone. The degree of damage to cucumber plants by *Fusarium* infections when using *T. atrobrunneum* strain VKPM F-1434 was 0.5 %, which is 95 % lower than the control variant (variant without treatment). Compared with the control, *T. harzianum* decreases by 55 %, *T. Lixii* isolate T4 / 14 by 28 %.

4 Conclusion

The results of tests of the biological activity of antagonist strains obtained in laboratory conditions and in the open ground can vary significantly, since microbial antagonism in the soil proceeds taking into account many natural factors, often significantly different from the antagonism of the same microbes on artificial nutrient media [18].

Therefore, the search for antagonist microorganisms should include studies of the interaction of microorganisms in controlled conditions and in a natural environment.

Given the widespread prevalence of fungal diseases of cucumber, especially protected soil, it is especially important to select indigenous strains of antagonists that are able to efficiently reduce the number of phytopathogens and at the same time stimulate plant growth and development of an environmentally friendly crop to protect the culture. The *Trichoderma atrobrunneum* VKPM F-1434 strain meets these requirements.

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