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Prospects of using fungi of genus *Trichoderma* as agents of biocontrol for fungal diseases of potatoes and cucumbers in kazakhstan

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Abstract.

Indigenous strains of species *Trichoderma asperellum* and *T. album* isolated from the rhizosphere soil of vegetable crops in Kazakhstan were assessed for antagonism in vitro against pathogenic fungi isolated from the affected samples of potatoes and cucumbers. It has been found that isolate *T. asperellum* 1K is superior in its antifungal activity to isolate *T. album* 2K, and has both antibiotic and hyperparasitic activity. It has been found that the composition of the cultural fluid of *Trichoderma* antagonist strains mainly contains polar volatile and semi-volatile compounds. In the cultural fluid of isolate *T. asperellum* 1K, 1H-1,2,3-Triazole, 4-(4-methoxyphenyl) and 1H-1,2,3-Triazole, 4-(4-methoxyphenyl) have been detected, which are natural fungicides. Additionally, hydrocarbon cetene, an ester of 3-Fluorobenzoic acid, 3-methylbut-2-enyl ester and 2-Cyclopenten-1-one, 3-ethyl-2-hydroxy- have been found in this isolate. In the cultural fluid of isolate *T. album* 2K, 2(3H)-Benzofuranone, 3-methyl-; Anisole, 2,3,4,5,6-pentachloro-; Benzo[e]isobenzofuran-1,4-dione,1,3,4,5,5 a,6,7,8,9,9 a-decahydro-6,6,9 a-trimethyl, and oleic acid have been found. The studied strains of fungi of genus *Trichoderma* may later be used as the basis for creating environmentally safe fungicides for protecting potatoes and cucumbers from phytopathogenic fungi.

Keywords: phytopathogens of potatoes and cucumbers, antagonism, hyperparasitism, *Trichoderma*, composition.

INTRODUCTION

For protecting plants from diseases caused by pathogens, out of the biological means of protection, the most promising are fungi of genus *Trichoderma*, which feature high physiological activity and inhibit the growth of a number of pathogenic fungi [1-3].

For instance, fungi of genus *Trichoderma* are widely used against one of the most common diseases of vegetable crops, fusarial head blight [4]. Ommati and Mackins [5] found the inhibitory activity of strains *T. brevicompactum*, *T. asperellum*, and *T. longibrachiatum* on the growth of potato fusarial head blight pathogen through the method of dual culture and obtaining of volatile and nonvolatile compounds. El Komy et al [6] reported the high antagonistic ability of new isolates of *Trichoderma* vs *Fusarium oxysporum f. sp. lycopersici* (FOL), which the authors associated with the production of antagonists of lytic enzymes.

Sarfraz et al [7] performed research on using fungi of genus *Trichoderma* together with fungicides, and recommended the obtained results for the comprehensive treatment of potatoes' early blight.

Despite the wide use of the fungi of genus *Trichoderma* in various countries as biocontrol agents for phytopathogens [8-13], in Kazakhstan there are virtually no developments based on them for protecting vegetable crops, particularly potatoes and cucumbers.

In this regard, this work was aimed at studying the antifungal activity of indigenous strains of the fungi of genus *Trichoderma*, which were interesting as agents of biocontrol over the spread of pathogens of fungal diseases in potatoes and cucumbers in Kazakhstan, and analyzing the composition of the promising strains of antagonists.

MATERIALS AND METHODS

Samples of infected plants. Potato tubers of cultivar "Gala" and cucumber fruit and leaves of cultivar "Buyan F1" with signs of the disease. The affected samples were obtained in 2017 at the collective farm Galym in the Sarkand district of the Almaty region.

Isolation of phytopathogens. The phytopathogenic fungi were isolated from the affected fruit using the following method: the infected samples were washed with distilled water, the surface was sterilized with sodium hypochlorite (10 %), after which the samples cut into small pieces (5 mm) were transferred to Petri dishes onto the nutrient medium with the following composition (g/l): sucrose — 20.0; $NaNO_3$ — 2.0; KH_2PO_4 — 1.0; $MgSO_4 . 7H_2O$ — 0.5; KCl — 0.5; $FeSO_4 . H_2O$ — 0.01; and agar — 20.0. The petri dishes with the samples were incubated at 28 ± 1°C for 48 hours. After 48 hours, colonies of pathogens were transferred to slant agar of the nutrient medium of the same composition into test tubes. The pure culture of the fungus (strain) was obtained after several passages.

The morphological and microscopic studies of the isolated strains were performed on the Czapek's medium. The fungi were identified according to cultural characteristics described by Prell and Day [14].

Strains of fungi of genus *Trichoderma.* The study used strains of *T. asperellum* 1K and *T. album* 2K isolated in 2017 from the rhizosphere of potatoes growing at the farm Galym in the Sarkand district of Almaty region [15].

Antifungal activity was established by the method of diffusion in agar [16].

Analysis of the component composition of fungi of the genus Trichoderma was made by the method of gas chromatography-mass spectrometry (GC-MS).

Strains of *Trichoderma* were grown for 5 days on the Saburo medium with the following composition (g/l): peptone - 10, glucose – 40 (in two repetitions), after which the samples were extracted with ethyl acetate and hexane (10 ml). The extracts were analyzed by GC-MS, analytes were separated in capillary column DB-WAXetr.

Chromatography conditions were as follows: sample volume — 0.5 µl, temperature of sample introduction — 240°C, flow division — 200:1. Separation was performed with the use of a 30 m long chromatographic capillary column DB-WAXetr with the internal diameter of 0.25 mm and the film thickness of 0.25 µm at the constant flow rate of carrier gas (helium) of 1 ml/min. Chromatography temperature was programmed from 40°C (5 min exposure) to 260 °C with the heating rate of 10 °C/min (10 min exposure). Detection was performed in mode SCAN m/z 10-800.

For managing the gas chromatography system, registering and processing the obtained results and data, the authors used the Agilent MSD ChemStation software (version 1701EA). Data processing included determination of exposure time, areas of peaks, and processing the spectral information

obtained from the mass-spectrometric detector. To decrypt the received mass spectra, the authors used libraries Wiley 7th edition and NIST'02 (the total number of spectra in the libraries was over 550 thousand). The GC-MS analysis was performed in two repetitions for each isolate. The probability of identification of each compound was not less than 80 %.

RESULTS AND DISCUSSION

The morphological and microscopic studies of phytopathogenic fungi

Fungi isolates from the affected potato tubers of variety "Gala", by their cultural characteristics described by Prell and Day [14], were identified as *Phytophthora infestans, Alternaria alternata, Fusarium oxysporum.* Isolates from the affected cucumbers of variety "Buyan F1" were identified as *Fusarium oxysporum, Sclerotinia sclerotiorum, Botrytis cinerea* and *Fusarium oxysporum*.

Study of the antifungal activity

During the research it has been found that both strains of fungus *Trichoderma* feature antifungal activity; with that, the activity of strain *T. asperellum* 1K significantly exceeds the activity of strain *T. album* 2K. In addition, it has been noted that strain *T. album* 2K did not have antifungal activity against *B. cinerea.*

The most significant action of *T. asperellum* 1K has been noted against *P. infestans* (the diameter of the pathogen growth inhibition zone was 38 ± 2.0 mm (Table 1).

In studying the antifungal activity, it has been found that strain *T. asperellum* 1K, in addition to the antibiotic effect on pathogens, showed hyperparasitism, partly grew on the pathogen, colonizing it.

Table 1 – Antagonistic activity of the fungi of genus *Trichoderma* against pathogens of potato and cucumber diseases

Phytopathogens	**Antagonists**	
	***T. asperellum* 1K**	***T. album* 2K**
Diameter of phytopathogens' growth inhibition zone (mm)		
P. infestans (potatoes)	40 ± 2.2	18 ± 1.0
A. alternata (potatoes)	32 ± 3.0	22 ± 1.8
F. oxysporum (potatoes)	hyperparasitism	21 ± 1.5
B. cinerea (cucumbers)	25 ± 2.8	20 ± 2.0
F. oxysporum (cucumbers)	hyperparasitism	0
S. sclerotiorum (cucumbers)	38 ± 2.0	15 ± 2.0

Analysis of the component composition of fungi of genus Trichoderma

The literature contains data about the relationship between the antagonistic action of the fungi of genus *Trichoderma* with the synthesis of volatile compounds. For instance, Amin et al [17] reported about volatile metabolites acting on fungal pathogens of the following plants: *Fusarium oxysporum, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum, Colletotrichum capsici, Helminthosporium oryzae, Alternaria brassicicola*. A number of authors reported that the cultural fluid of fungi *Trichoderma,* which suppressed the growth of phytopathogenic fungi, contained hydrocarbons, alcohols, ketones, aldehydes, alkanes, alkenes, esters, aromatic compounds, heterocyclic compounds, and various terpenes [18]. Khethr et al [19] noted that the terpenoid limonene was the main component in species *Trichoderma,* which had been developed as effective biological control agents. Pyrones, koninginins (complex pyrans), nitrogen heterocyclic compounds, as well as butenolides, hydroxy-lactones, diketopiperazines, peptaibols have been identified as fungal metabolites of the fungi of genus *Trichoderma* [20].

In the research, for identifying metabolites that are in the component composition of the cultural fluid of antagonist strains, chromatographic analysis with the use of gas chromatography was performed. The results of gas chromatographic analysis of the component composition of the fungi of genus *Trichoderma* showed that the samples mainly contained polar volatile and semi-volatile compounds. For instance, in the cultural fluid of isolate *T. asperellum* 1K, 1H-1,2,3-Triazole, 4-(4-methoxyphenyl) and 1H-1,2,3-Triazole, 4-(4-methoxyphenyl) have been detected, which are natural fungicides. Additionally, hydrocarbon cetene, ester of 3-Fluorobenzoic acid, 3-methylbut-2-enyl ester and 2-Cyclopenten-1-one, 3-ethyl-2-hydroxy- have been found in this isolate. In the cultural fluid of isolate *T. album* 2K, 2(3H)-Benzofuranone, 3-methyl-; Anisole, 2,3,4,5,6-pentachloro-; Benzo[e]isobenzofuran-1,4-dione,1,3,4,5,5a,6,7,8,9,9a-decahydro-6,6,9a-trimethyl, and oleic acid have been found (Table 2).

Table 2 – Results of chromatographic analysis of the component composition of the fungi of genus *Trichoderma*

Antagonists	**Exposure time, min.**	**Compounds**
T. asperellum 1K	10.3	Cetene
	13.2	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-
	15.3	3-Fluorobenzoic acid, 3-methylbut-2-enyl ester
	17.0	5H-Benzo[b]pyran-8-ol, 2,3,5,5,8a-pentamethyl-6,7,8,8a-tetrahydro-
	18.3	Decanamide-
	18.9	1H-1,2,3-Triazole, 4-(4-methoxyphenyl)-
T. album 2K	16.3	2(3H)-Benzofuranone, 3-methyl-
	17.6	Anisole, 2,3,4,5,6-pentachloro-
	24.3	Benzo[e]isobenzofuran-1,4-dione,1,3,4,5,5a,6,7,8,9,9a-decahydro-6,6,9a-trimethyl
	24.7	Oleic Acid

CONCLUSION

Thus, as a result of the experiments, it has been found that isolate *T. asperellum* 1K is superior in its antifungal activity to isolate *T. album* 2K, and has both antibiotic and hyperparasitic activity. The component composition of the cultural fluid of antagonist strains includes organic fatty acids, natural fungicides, and esters. The studied strains of the fungi of genus *Trichoderma* may later be used as the basis for creating environmentally safe fungicides for protecting potatoes and cucumbers from phytopathogenic fungi.

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