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Characteristics of Antagonistic Activity of Two *Trichoderma* Species New to Kazakhstan Against Soil Pathogens

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Abstract: The high efficiency of representatives of the *Trichoderma* genus in suppressing a wide range of plant pathogens contributes to the conduct of large-scale research on the search for local strains. The purpose of this study is to characterize the antagonistic activity of two *Trichoderma* species new to Kazakhstan (*T. pararogersonii* and *T. rossicum*) isolated from the rhizosphere of *Picea schrenkiana* Fisch. et C.A. Mey. and *Malus sieversii* (Ledeb.) M. Roem. against soil phytopathogens. Identification was carried out based on analysis of its region sequences and determination of antagonistic activity was performed by the dual culture method. In the course of our study, we obtained descriptions of colonies of *T. pararogersonii* and *T. rossicum* based on isolated pure cultures, morphological data of both species, the sequence of nucleotides, information about inhibitory activity against some phytopathogens and the effect on the growth of some legumes. *T. pararogersonii* isolate is characterized by a weak level of inhibitory activity. When simultaneously seeded with most test objects, the radius of the colonies of the latter is the same or greater than the radius of the colonies of the *Trichoderma*. When using the paper disk method, a significant zone of suppression of the growth of phytopathogenic fungi by *T. pararogersonii* extract was noted in the variant with *Alternaria* spoon day 4 (23.55±0.72 mm) and the most insignificant one was noted with *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson. *T. rossicum* was characterized by a higher level of inhibitory activity. It was found that with simultaneous seeding of *Trichoderma* and test objects on Petri dishes, the rapid growth of *T. rossicum* was observed in all variants of the experiment, except for the variant with *Aspergillus niger* Tiegh, where suppression of *Trichoderma* sporulation was also observed.

Keywords: Dual Culture, Inhibitory Activity, Pathogenic Fungus, Rhizosphere

Introduction

The genus *Trichoderma* Pers. (*Hypocrea* Fr.) is widely distributed in various climatic zones of the world and is of great importance (Jaklitsch and Voglmayer, 2015; Chen and Zhuang, 2017). The popularity of fungi of the genus *Trichoderma* is due not only to the ability to produce several hundred secondary metabolites, some of which are antimicrobial. It is also able to induce plant resistance to pests and pathogens (Lorito *et al*., 2010), increase the efficiency of the use of nutrients (especially nitrogen), stimulate plant growth, and impart resistance to abiotic stresses (Mendoza-Mendoza *et al*., 2018).

Individual strains of the genus *Trichoderma* can control various phytopathogens that cause the oppression and death of many agricultural plants, such as *Botrytis cinerea*, *Sclerotium cepivorum*, *Rhizoctonia solani*, and others (Alvarado-Marchena and Rivera-Méndez, 2016; You *et al*., 2016; Abbas *et al*., 2017).

Currently, significant factual material has been accumulated in various countries on physiological, morphological, biochemical, and genetic studies of species of the genus *Trichoderma*, as well as on the

technology of obtaining biological products and their use (Nicot *et al*., 2016). A small number of isolates with a high antagonistic ability against a wide range of plant pathogens are used as bioagents (Medeiros *et al*., 2017). The mechanisms of action of *Trichoderma* include parasitism, competition for nutrients, and antibiotic synthesis (Sood *et al*., 2020). Depending on the strain, the use of *Trichoderma* can promote plant growth, as well as induce resistance (Naher *et al*., 2014; Kumar and Ashraf, 2017).

Trichoderma species and strains as agents of biological plant protection are a promising and safe alternative to chemical means of combating phytopathogenic fungi since they not only effectively restrain their development and spread but also stimulate plant growth and have a beneficial effect on the microbiological community in the soil (Kumar and Ashraf, 2017; Hassan *et al*., 2019).

The long-term and successful use of representatives of the genus *Trichoderma* in agricultural production, as well as their high efficiency in suppressing a wide range of plant pathogens, contribute to the conduct of large-scale studies on the search for strains in Kazakhstan.

From the rhizosphere of wormwood (*Artemisia glabella*) and shrubby Ajania (*Ajania fruticulosa* (Ledeb.) Poljak.) cultivated in Kazakhstan on an industrial scale as raw materials for the pharmaceutical industry in the treatment of oncological diseases, four strains of fungi of the genus *Trichoderma* were obtained, differing in colony density, texture, color, and growth rate. The isolates were identified as *Trichoderma viride* Pers. (*T. lignorum* (Tode) Harz.), based on macroscopic and microscopic morphological features (Rakhimova *et al*., 2012). When studying the antibiotic activity of the isolated strains, it was found that strain 90-2 of *T.* viride exhibited antagonistic properties against fungi of the genus *Fusarium*, and strain 9(2) showed antagonistic activity against fungi of the genus *Alternaria* (Rakhimova *et al*., 2012). Antagonist strains (*Trichoderma viride* 22, *Trichoderma album* 23, *Trichoderma asperellum* 175, *Trichoderma asperellum* 1m) have been identified concerning pathogens affecting legumes (chickpeas, peas, beans) and fodder (alfalfa) crops growing in the Almaty region (Bekmakhanova *et al*., 2015).

The purpose of this study is to characterize the antagonistic activity of two *Trichoderma* species new to Kazakhstan (*T. pararogersonii* and *T. rossicum*) against soil phytopathogens.

Materials and Methods

Soil samples were collected in the rhizosphere of various woody plants on the territory of two ridges of the Northern Tien Shan: Dzungar and Kungei Alatau in Kazakhstan. Soil samples were taken during the growing season of 2020-2021 in a soil horizon of 5-20 cm after removing the top layer of litter in dark coniferous and small-leaved forests on mountain forest soils. 867 soil samples were selected and examined, while representatives of the genus *Trichoderma* were found in 95 samples. Two species turned out to be new to the territory of Kazakhstan: *Trichoderma pararogersonii* (Jaklitsch, 2009) Voglmayr (Kazakhstan, Almaty region, Kungei Alatau ridge, Kolsai Kolderi state national nature park, Kayyndy gorge, spruce forest, rhizosphere of spruce (*Picea schrenkiana* Fisch. et C.A. Mey.), point 249 v, 1910 m above sea level, 42°59′18.0″ N, 78°27′40.8″ VII 2020, collected by A. M. Assylbek, determined by Rakhimova *et al*. (2012) and *T. rossicum* Bissett, C. P. Kubicek et Szakács (Kazakhstan, Almaty region, Dzungar Alatau ridge, Zhongar-Alatau state national natural park, Krutoe gorge, apple forest, rhizosphere of apple trees *(Malus sieversii* (Ledeb.) M. Roem.), type 439 a, 1,445 m above sea level, 45°33′05.0″, N, 80°43′16.0″ E. 26 VII 2020, collected by A. M. Assylbek, determined by Rakhimova *et al*. (2012) (Fig. 1).

The geographical location of each sample collection site was recorded using GPS (Germin). Isolation and subsequent identification of soil fungi were carried out according to Khabirova *et al*., (2022), using the literature on fungi of the genus *Trichoderma* (Jaklitsch, 2009; 2011). The names of fungi species and authors are given following the Index Fungorum database (Fungorum 2022). For molecular genetic identification of fungi samples, 3-7 daily strains of fungi were used. The mycelium was frozen at -20°C. Then it was ground with a pestle in a 1.5 mL Eppendorf test tube to a powdery state. Deoxyribonucleic Acid (DNA) was isolated from the resulting mass using the plant/fungi DNA isolation kit from Norgen Biotek Corp. (Ontario, Canada) according to the manufacturer's protocol.

Fig. 1: Map with sample collection points of *Trichoderma pararogersonii* and *T. rossicum*

The DNA concentration in the samples was determined using a Qubit^{tm} dsDNA HS assay kit (life technologies, Oregon, USA) fluorimeter on a double-stranded Deoxyribonucleic Acid High Sensitivity (dsDNA HS) scale. The following universal primers of the Internal Transcribed Spacer (ITS) region of fungi were used in the work: ITS1 (5-TCCGTAGGTGAACCTGCGG-3) and (5-TCCTCCGCTTATTGATATGC-3). The reaction mixture for amplification consisted of: 12.5 µL Q5® Hot Start High-Fidelity 2× Master Mix, 1.25 µL Forward Primer (10 µm), 1.25 µL Reverse Primer (10 µm), 1.5 µL DNA, and 8.5 water. The total volume of the Polymerase Chain Reaction (PCR) mixture was 25 µL.

PCR was performed on an Eppendorf ProS amplifier (Hamburg, Germany) at the following amplification mode: 94°C for 30 sec; 55°C for 1 min; 72°C for 40 sec in a total of 30 cycles; and 72°C for 10 min. The amplification results were viewed in 1.2% agarose gel. The PCR products were purified with CleanSweep™ PCR Purification reagent (Applied Biosystems, USA). The sequencing reaction was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to the manufacturer's instructions (BigDye® Terminator v 3.1 Cycle Sequencing Kit Protocol, Applied Biosystems, USA), followed by fragment separation on an automatic genetic analyzer 3500 DNA Analyzer (Applied Biosystems, USA). The sequencing results were processed in the SeqA software (Applied Biosystems, USA). The obtained nucleotide sequences of the ITS region of fungal DNA were compared with the GeneBank database data (GenBank, 2022), using the BLAST software. The phylogenetic analysis was performed using MEGA6 software. The ClustalW algorithm was used to align nucleotide sequences and the Neighbor-Joining (NJ) method was used to build phylogenetic trees (Simonsen *et al*., 2008).

Antagonistic properties of isolates against the phytopathogenic fungi *Alternaria* sp., *Aspergillus niger, and Fusarium* sp. (strain 320), stored in the collection of the Institute of Botany and Phytointroduction, were determined by the method of dual cultures (Asaturova *et al*., 2012) at the optimal temperature for the growth of microorganisms 25-26°C on Potato Dextrose Agar (PDA). The size of fungal colonies was measured on days 4-6 using the linear method (mm) from the back of the dish and the percentage of inhibition was calculated (Asaturova *et al*., 2012).

The inhibitory activity was also determined by the paper disk method. A disk of filter paper moistened with an extract from the *Trichoderma* mycelium was applied to the test culture of phytopathogenic fungi evenly distributed over the surface of the solid nutrient medium in a petri dish. After incubation, the growth suppression zone of the test culture was determined on the fifth day.

Evaluation of the effect of strains of the genus *Trichoderma* on the growth of roots and seedlings of legumes was carried out on peas (varieties Nikitka and Zhigalov 112) and beans (varieties Saxa without fiber and Bemol). In the experimental version, seeds (100 seeds in three repetitions) were germinated and treated with *Trichoderma* spores by powdering until full saturation. The seeds that had not been treated with spores were used in the control variant. The seeds were sown in the Magic bed nutrient soil for seedlings (Buiskie udobreniya, Russia) in mini cassettes for seedlings with a cell size of 55×55×40. On the 7th-8th day after germination, the length of stems and roots was measured and the growth rate was calculated (Asaturova *et al*., 2012).

Statistical analysis of the results (determination of the arithmetic mean and its error) (Zverev and Zefirov, 2013) was carried out using the Excel 2010 software package.

Results

The antagonistic activity was determined for two species new to the territory of Kazakhstan: *Trichoderma pararogersonii* (Jaklitsch, 2009), Voglmayr and *T. rossicum* Bissett, C.P. Kubicek et Szakács.

Cultural and Morphological Features of Trichoderma Pararogersonii

On the PDA medium, colonies were well-growing, densely fluffy, patchy, and brownish-green, with well-defined concentric zones (Fig. 2). The substrate and aerial mycelium was well-developed. The aerial hyphae were especially numerous along the border of the colonies. The reverse was not stained.

Sporulation was detected on the second day of cultivation. The conidiophores were long, with single, or in whorls of 2-4 phialides (Fig. 3). The dimensions of the phialides were 6.5-10.5×2.5-3.5 microns (µm). The conidia were ellipsoidal, green, and smooth, measuring 4.0-4.7×3.0-3.5 µm. The chlamydospores were not numerous and appeared after 4-6 days of cultivation.

The degree of homology with the nearest strain KY750455.1:53-596 *Trichoderma pararogersonii* isolate CTCCSJ-F-KZ40688 was 100.00%, which allows us to attribute the studied sample to this species (Fig. 4).

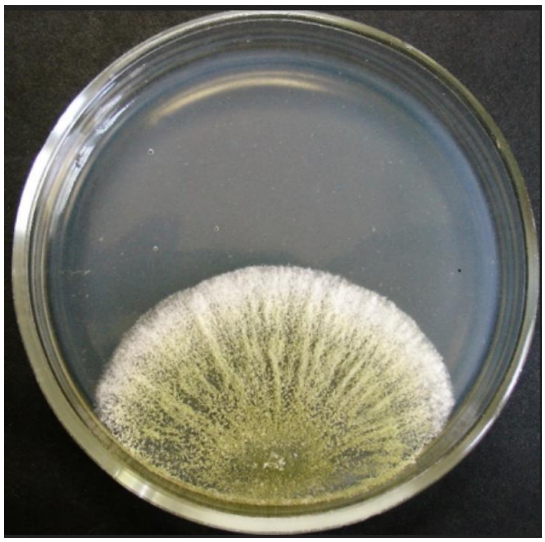

Fig. 2: Appearance of the colony of *Trichoderma pararogersonii*

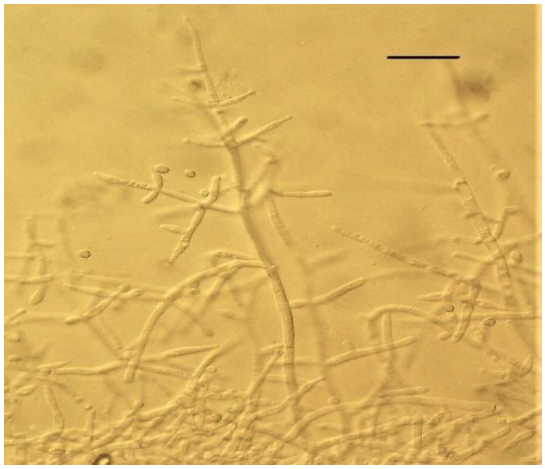

Fig. 3: Mycelium and conidiophores of Trichoderma pararogersonii. scale: 10 µm

Molecular Genetic Identification

The nucleotide sequence was obtained by sequencing the ITS region.

CAATGTGAACCGTACCAAACCGTTGCCTCGGC
GGGGTCACGCCCCGGGCGCGTCGCAGCCCCGGAA
CCAGGCGCCCGCCGGAGGGACCAACCAAACTCTC
TTTGCAGTCCCCTCGCGGACGTTATTCCTTACAGC
TCTGAGCAAAAAAAATTCAAAATGAATCAAAACT
TTCAACAACGGATCTCTTGGTTCTGGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGC
ACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCT
GTCCGAGCGTCATTTCAACCCTCGAACCCCCCCCG
GGGGGTCCGGCGTTGGGGATCGGGGACCCCTGAG
ACGGGATCCCGGCCCCGAAATACAGTGGCGGTCT
CGCCGCAGCCTCCCCTGCGCAGTAGTTTGCACAAC
TCGCACCGGGAGCGCGGCGCGCCCACGTCCGTAA
AACACCCAACCCTCTGAA.ATGTTGACCTCGGATC
AGGTAGGAATACCCGCTGAACTTAAGCATATCA.

The obtained isolate *of Trichoderma pararogersonii* was characterized by a weak level of inhibitory activity. In the conditions of simultaneous seeding with most test objects, the radius of the colonies of the latter was the same or greater than the radius of the colonies of the *Trichoderma* (Table 1, Figs. 5-6). The rapid growth *of Trichoderma pararogersonii* and suppression of the growth of the test object was observed only in the variant of the experiment with *Penicillium expansum* with simultaneous seeding with the test object (Table 1). When using the paper disk method, a significant zone of suppression of the growth of phytopathogenic fungi with *Trichoderma pararogersonii* extract (strain 249v) was noted in the variant with *Alternaria* sp. on day 4 and the most insignificant one was noted in the variant with *Purpureocillium lilacinum* (Table 2).

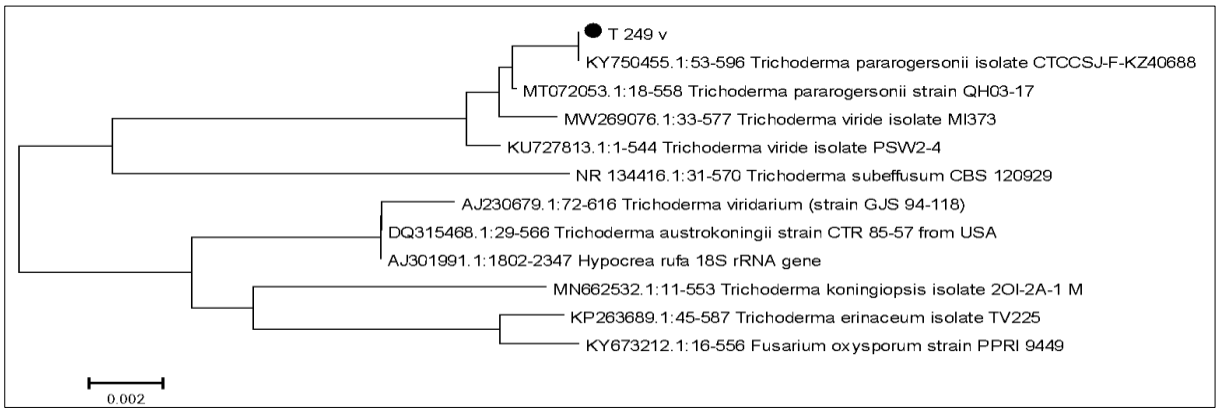

Fig. 4: A phylogenetic tree constructed by comparing the ITS region of the studied sample with the sequences of reference strains placed in the blast international database

Table 1: Determination of the inhibitory activity of *Trichoderma pararogersonii* isolate during simultaneous seeding with test objects (by the method of dual cultures)

		Mycelium growth inhibition of the test object, %		
No.	Test object	On day 2	On day 4	On day 6
1	*Alternaria* sp.	25.9	15.8	2.1
2	*Aspergillus niger*	1.9	6.6	1.9
3	*Fusarium* sp. (strain 379 zh)	44.5	21.7	0.4
4	*Penicillium expansum*	58.7	23.2	36.8

Table 2: The zone of suppression of the growth of phytopathogenic fungi by extracts of *Trichoderma pararogersonii* (strain 249v) by the method of paper disks, mm

		Growth suppression zone, mm		
No.	Test object	On day 2	On day 4	On day 6
1	*Alternaria* sp.	23.10±0.54	23.55±0.72	23.20±0.44
2	*Aspergillus niger*	16.80±0.41	16.15±0.38	16.38±0.47
3	*Fusarium* sp. (strain 379 zh)	13.75±0.35	15.29±0.29	16.05±0.40
4	*Penicillium expansum*	11.33±0.55	13.57±0.45	20.00±0.43
5	*Purpureocillium lilacinum*	10.10±0.20	11.40±0.29	11.57±0.53

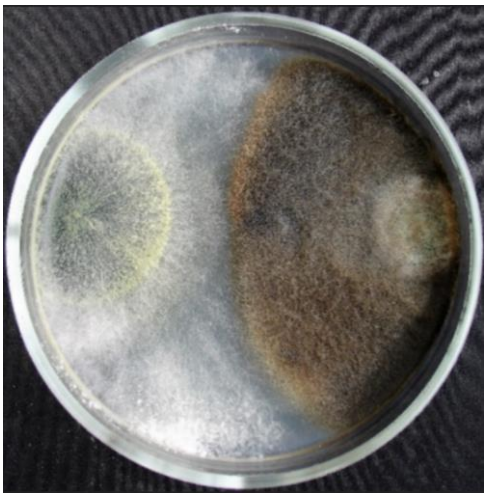

Fig. 5: Dual cultures *of Trichoderma pararogersonii* and *Alternaria* sp. Simultaneous seeding, after 4 days

Fig. 6: Dual cultures *of Trichoderma pararogersonii* and *Fusarium*sp. (strain 376zh). Simultaneous seeding, after 4 days

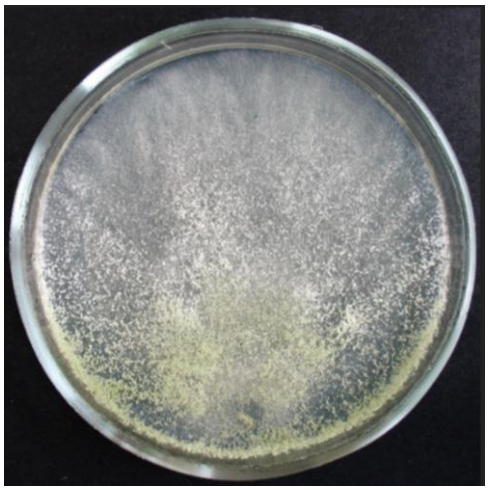

Fig. 7: Appearance of the colony *Trichoderma rossicum* strain 439a

Fig. 8: Conidia and conidiophores of *Trichoderma rossicum*. Scale: 10 µm

Table 3: Effect of the *Trichoderma pararogersonii* strain on the growth of roots and seedlings of peas and beans, on day 8, cm

Test object	Treatment method (the variant of the experiment)	Length of ground part of the test object	Growth percentage	Length of the underground part of the test	Growth percentage
Nikitka peas	Control (water)	5.76±0.19		60.42±2.92	
	T. pararogersonii	7.86±0.79	36.5	61.20±1.66	1.3
Zhigalov 112 peas	Control (water)	9.57±0.73		55.60±1.08	
	T. pararogersonii	10.66±0.29	11.4	51.90±4.97	-
Saxa beans without fiber	Control (water)	11.60±0.61		119.54±2.35	
	T. pararogersonii	12.18±0.33	5.0	128.43±3.62	7.4
Bemol Beans	Control (water)	16.13±0.67		90.38±4.28	
	T. pararogersonii	20.28±1.02	24.4	129.20±2.16	42.9

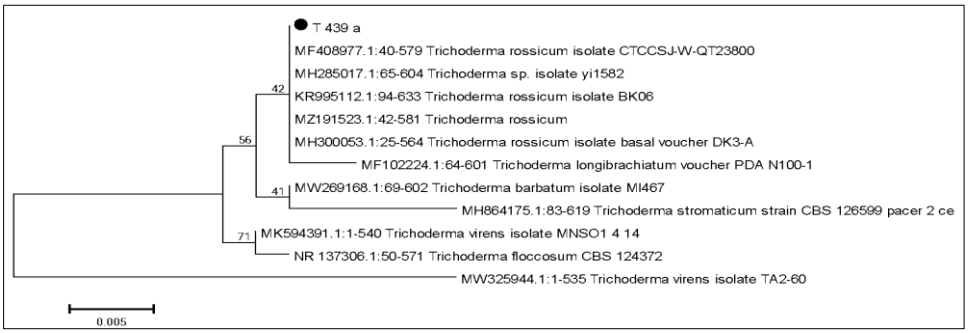

Fig. 9: A phylogenetic tree constructed by comparing the ITS region of the studied sample with the sequences of reference strains placed in the Blast International Database

When studying the effect of *Trichoderma pararogersonii* strain 249 v on the growth of legume seedlings, it was found that in all studied varieties of peas and beans, the length of the ground and underground parts on the eighth day in the experimental variants was slightly bigger than in the control variant (Table 3).

Strain 249 v of *Trichoderma pararogersonii* obtained by us had a positive effect on the growth rate of peas and beans.

Cultural and Morphological Features of Trichoderma Rossicum

The colonies were colorless, thin, usually non-zonal, and rapidly growing (Fig. 7). The aerial hyphae were quite abundant. At 25°C on the PDA, the mycelium covered the surface of the dish for a week or 10 days. Sporulation began on days 2-3, while the surface of the colony became coarse and gradually acquired a yellowish-green hue. The reverse side of the colonies was not colored.

The conidiophores developed slowly and their concentration was observed at the borders of colonies. The size of the phialides was 5-7×3.5-4.5 µm. The conidia were oval or ellipsoid, 4.5-5×2.7-3 µm in size, and smooth (Fig. 8). Chlamydospores were not observed.

Cultural and Morphological Features of Trichoderma Rossicum

AACTGTTGCCTCGGCGGGATCTCTGCCCCGGG
CGCGTCGCAGCCCCGGACCAAGGCGCCCGCCGGA
GGACCAACCCAAAACTCTTTTTGTATACCCCCTCG
CGGGTTTTTTACTTCTGAGAATTTCTCGGCGCCCC
TAGTGGGCGTTTCGAAAATGAATCAAAACTTTCA
ACAACGGATCTCTTGGTTCTGGCATCGATGAAGA
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACA
TTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTC
CGAGCGTCATTTCAACCCTCGAACCCCTCCGGGG
GGTCGGCGTTGGGGATCGGCCCTTTCACCGGGTG
CCGGCCCCTAAATACAGTGGCGGTCTCGCCGCAG
CCTCTCATGCGCAGTAGTTTGCACACTCGCACCG
GGAGCGCGGCGCGTCCACGTCCGTAAAACACCCC
AACTTCTGAAATGTTGACCTCGGATCAGGTAGGA
ATACCCGCTGAACTTAAGCATATCAATAA.

The degree of homology with the nearest strain MF408977.1:40-579 of *Trichoderma rossicum* isolate CTCCSJ-W-QT23800 was 100.00%, which made it possible to attribute the studied sample to this species (Fig. 9).

When determining the inhibitory activity of *Trichoderma rossicum* strain 439a by the method of dual cultures, it was found that with simultaneous seeding of *Trichoderma* and test objects on petri dishes, rapid growth of *T. rossicum* was observed in all variants, except for the variant with *Aspergillus niger* (Table 4, Figs. 10-12). *Aspergillus niger* suppressed the sporulation of *Trichoderma rossicum* strain 439a.

When studying the effect of *Trichoderma rossicum* strain 439a on the growth of legumes, it was found that in all studied varieties of peas and beans, the length of the ground and underground parts on the eighth day was slightly longer than in the control variant (Table 5).

Strain 439a of *Trichoderma rossicum* obtained by us had a positive effect on the growth rate of peas and beans.

Table 4: Determination of the inhibitory activity of *Trichoderma rossicum* strain 439a during simultaneous seeding with test objects (by the method of dual cultures)

		Mycelium growth inhibition of the test object, %		
No.	Test object	On day 2	On day 4	On day 6
1	*Alternaria* sp.	54.5	62.6	62.9
2	*Aspergillus niger*	0.0	10.5	2.8
3	*Fusarium* sp. (strain 320)	67.6	59.3	72.5

Table 5: Effect of *Trichoderma rossicum* strain 439a on the growth of roots and seedlings of peas and beans, on day 8, cm

Test object	Treatment method (the variant of the experiment)	Length of ground part of the test object	Growth percentage	Length of the underground part of the test object	Growth percentage
Nikitka peas	Control (water)	5.76±0.19		60.42±2.92	
	T. rossicum	7.13±0.25	23.8	61.50±3.43	1.80
Zhigalov 112 peas	Control (water)	9.57±0.73		55.60±1.08	
	T. rossicum	14.98±0.27	56.5	75.53±4.49	35.8
Saxa beans without fiber	Control (water)	11.60±0.61		119.54±2.35	
	T. rossicum	14.50±0.87	25.0	213.02±4.05	78.2
Bemol Beans	Control (water)	16.13±0.67		90.38±4.28	
	T. rossicum	20.37±0.55	24.9	93.41±2.10	3.30

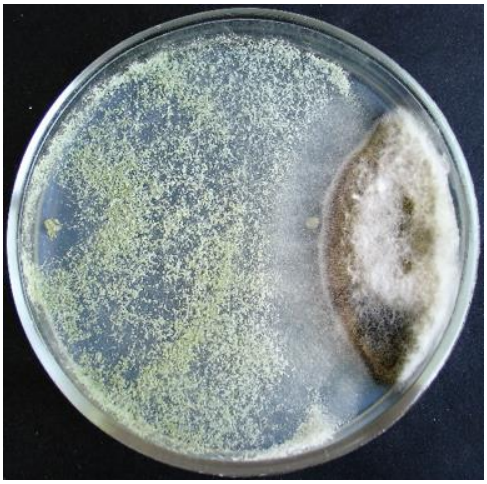

Fig. 10: Dual cultures *of Trichoderma rossicum* and *Alternaria* sp. Simultaneous seeding, after 6 days

Fig. 11: Dual cultures *of Trichoderma rossicum* and *Aspergillus niger.* Simultaneous seeding, after 6 days

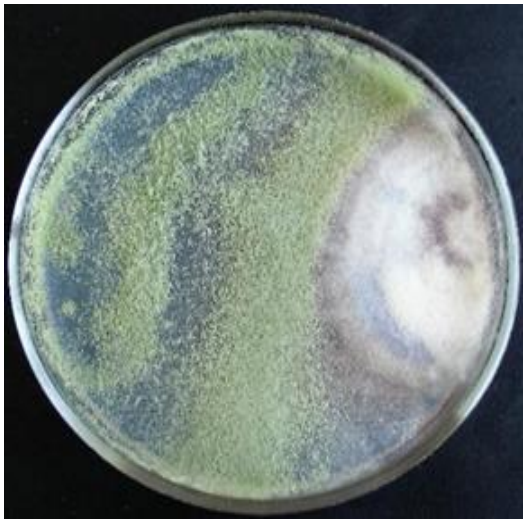

Fig. 12: Dual cultures *of Trichoderma rossicum* and *Fusarium* sp. (strain 323). Simultaneous seeding, after 4 days

Discussion

According to our study and scientific data (Rakhimova *et al*., 2012; Bekmakhanova *et al*., 2015), 18 species of the genus *Trichoderma* have been found in Kazakhstan, two of which are new: *T. pararogersonii* and *T. rossicum.* For *T. rossicum*, the most closely related species is *T. stromaticum*, known from tropical America and usually forming associations with *Theobroma cacao* L. The endogenous strains of *T. stromaticum* penetrating deeply into the vascular system of cocoa stems have been described (Samuels *et al*., 2012). The habitat of *T. rossicum* is soil, the known distribution has been found in Russia, Austria, and Peru (Jaklitsch, 2011). *T. stromaticum, T. rossicum* and the recently discovered species *T. barbatum, T. caesareum, T. floccosum, T.*

ivoriense, T. lanuginosum, and T. vermipilum form a unique section of *Trichoderma*, clade Stromaticum (Samuels *et al*., 2012). *T. pararogersonii* is characteristic of the wood and bark of broad-leaved trees and is widespread in Mediterranean Europe, in particular, in Croatia and Greece (Jaklitsch and Voglmayer, 2015). This species belongs to the section (clade) Viride, which also includes *T. viride, T. viridescens, T. asperellum, T. hamatum, T. koningii, T. koningiopsis*, etc.

In contrast to the Stromaticum clade, where antagonistic activity has been studied only for new species of *T. hebeiense, T. sichuanense* and *T. verticillatum* (Chen and Zhuang, 2017), antagonist strains from the viride clade have been studied quite well.

When studying the antagonistic activity of 15 *Trichoderma* isolates belonging to the species *T. harzianum, T. asperellum, T. longibrachiatum, T.* viride against *Fusarium oxysporum* f. sp. *capsici*, it was found that the value of inhibition of mycelial growth of the pathogen in vitro ranged between 35.7% and 85.8% (Hewedy *et al*., 2020). In our case, suppression by *Trossicum* of mycelial growth of *Fusarium* sp. equaled 72.5%.

Strains of antagonists of *Trichoderma* viride, *T. album*, and *T. asperellum* species have been identified concerning pathogens affecting legumes (chickpeas, peas, beans) and fodder (alfalfa) crops growing in the Almaty region. The strains showed the highest antagonistic activity and growth rate against *Fusarium solani, F. sporotrichiella* var. *poae*, and *Alternaria compacta*, with the diameter of the pathogen growth suppression zone ranging from 28-39 mm. *Trichoderma* strains were less effective on *Fusarium oxysporum, Alternaria alternate, and A. tenuis*: The diameter of the pathogen growth suppression zone ranged from 22-30 mm (Bekmakhanova *et al*., 2015). In our experiments, the zone of suppression of the growth of phytopathogenic fungi (test objects) by extracts *of Trichoderma pararogersonii* was significantly smaller and ranged from 11.57±0.53-23.20±0.44 mm on the sixth day.

Antibiotic activity against toxin-forming strains of *Fusarium sambucinum* and *F. sporotrichioides* was shown by strains MG/6, T-30, and TH-7 of *Trichoderma asperellum*. The highest antagonistic activity against phytopathogens of the genus *Fusarium* was noted for strains MG/6 and T-30 (Popova and Sadykova, 2014). Some researchers believe that *T. harzianum* and *T.* viride strains were the best antagonists for some plant pathogens with an inhibition percentage of 60-80% (Abd Elahi *et al*., 2012; Kumar *et al*., 2012). In our case, the suppression by *T. rossicum* of mycelial growth of test objects was up to 72.5%.

When studying the effect of *Trichoderma asperellum* MG-97 spores and *Fusarium sporotrichioides* Z3-06 metabolites on the physiological parameters of young wheat plants (*Triticum aestivum*), it was found that *T. asperellum* increased the laboratory germination of seeds and enhanced plant growth on the 10th and 30th days of vegetation. Therefore, the leaf area, the mass of the aboveground part of plants, and the mass of roots increased. In turn, *F. sporotrichioides* metabolites negatively affected most of the listed indicators. With the combined action of *Trichoderma* spores and *fusarium* metabolites, growth rates were close to the control variant, but seed germination and germination energy were suppressed (Golovanova *et al*., 2020). Strain 439a of *Trichoderma rossicum* obtained by us had a positive effect on the growing intensity of the ground and underground parts of legume seedlings (peas and beans). However, we did not evaluate the germination of seeds and germination energy.

Thus, the strain of *T. rossicum* isolated by us actively restrained the growth and development of pathogens and positively affected the growth rate of peas and beans, which makes it possible to use the studied strain as a biological plant protection agent, especially since local strains are more productive and also more resistant to biocontrol since they are well adapted to conditions of the local environment.

Conclusion

The paper describes the antagonistic activity against soil phytopathogens of two *Trichoderma* species new to Kazakhstan (*T. pararogersonii* (Jaklitsch, 2009), Voglmayr and *T. rossicum* Bissett, C.P. Kubicek et Szakács) isolated from the rhizosphere of *Picea schrenkiana* Fisch. et C.A. Mey. and *Malus sieversii* (Ledeb.) M. Roem.

T. pararogersonii isolate is characterized by a weak level of inhibitory activity. At the same time, *T. rossicum* is characterized by a higher level of antagonistic activity. We found that with simultaneous seeding of *Trichoderma* and test objects on Petri dishes, the rapid growth of *T. rossicum* was observed in all variants of the experiment, except for the variant with *Aspergillus niger* Tiegh, where suppression of *Trichoderma* sporulation was also observed. In addition, *T. rossicum* had a positive effect on the growth rate of peas and beans.

The level of antagonistic activity of the *T. rossicum* strain isolated by us makes it possible to use it as a biological plant protection agent, especially since local strains are more productive, as well as more stable under biocontrol since they are well adapted to the conditions of the local environment.

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Author's Contributions

All authors equally contributed to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

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