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Original article

ANTAGONISTIC POTENTIAL OF NATURAL ISOLATES OF *TRICHODERMA* SP. REGARDING NEW ECONOMICALLY SIGNIFICANT MICROPATHOGENES OF GRAPES IN WESTERN CISCAUCASIA

E.G. Yurchenko, N.V. Savchuk, M.V. Burovinskaya

Studies conducted worldwide on different crops, including the grape, show the expansion of species variety in micropathogens through the emergence of new species in Alternaria Nees, 1817, and Fusarium Link, 1809 genera. The Fusarium pathogenic species attack grape buds and bunches and cause an average mass reduction of 12.8% to 60.3% and a bunch length reduction of 7.7% to 39.4%. This negatively affects the quality of grape products and the tradability of table grapes in particular. The Alternaria Nees, 1817 fungi are a part of grapes necrotic spot pathocomplex and can cause a photosynthetic potential decrease, consequently reducing the yield per bush by 18% to 27 % and the sugar content by 6.1% to 9.1%. The fungi genus Trichoderma Pers., 1801 can be efficient against such biopathogenes. This study aims to examine the Trichoderma spp. culture obtained from native ampelocenoses of the Western Ciscaucasia in vitro in the context of Fusarium generative organs wilt (Fusarium proliferatum F-41/1, F. oxysporum F-117) and necrotic spot (Alternaria sp. 425-3) pathogenes to identify the strains showing the greatest antagonistic potential. We established that the Trichoderma spp. T-441/1 strain has the most active antagonizing effect against Fusarium spp., whereas the T-404-1 strain is the most effective against Alternaria sp.

Keywords: *antimycotic activity; antagonistic strains; new diseases; ampelocenoses; fusarium wilt; mecrotic spot disease*

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Научная статья

АНТАГОНИСТИЧЕСКИЙ ПОТЕНЦИАЛ ПРИРОДНЫХ ИЗОЛЯТОВ *TRICHODERMA* SP. В ОТНОШЕНИИ НОВЫХ ЭКОНОМИЧЕСКИ ЗНАЧИМЫХ МИКОПАТОГЕНОВ ВИНОГРАДА В ЗАПАДНОМ ПРЕДКАВКАЗЬЕ

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По данным мировых исследований на многих сельскохозяйственных культурах, в том числе винограде, отмечается расширение видового состава микопатогенов за счет появления новых вредоносных видов из родов Alternaria Nees, 1817 и Fusarium Link., 1809. Патогенные виды Fusarium Link., 1809, поражающие соцветия и грозди винограда, вызывают снижение средней массы грозди на 12,8-60,3 %, уменьшение длины главной оси грозди на 7,7-39,4 %, что негативно сказывается качестве виноградной продукции, и особенно товарности столового винограда. Грибы рода Alternaria Nees, 1817, входящие в патокомплекс некротической листвой пятнистости винограда, вызывают снижение фотосинтетического потенциала виноградного растения, что может снизить урожай с куста на 18-27 % и сахар в ягодах на 6,1-9,1 %. Эффективными агентами биоконтроля данных микопатогенов могут выступать грибы рода Trichoderma Pers., 1801. Целью исследований было протестировать in vitro культуры Trichoderma spp., выделенные из естественных условий ампелоценозов Западного Предкавказья в отношении возбудителей фузариозного усыхания генеративных органов (Fusarium proliferatum F-41/1, F. oxysporum F-117) и некротической пятнистости листьев (Alternaria sp. 425-3) и выбрать штаммы с наибольшей антагонистической активностью. В качестве наиболее активных антагонистов установлены следующие штаммы Trichoderma spp.: T-441/1 в отношении Fusarium spp. и T-404-1 в отношении Alternaria sp.

Ключевые слова: *антимикотическая активность; штаммы-антагонисты; новые заболевания; ампелоценозы; фузариозное усыхание; некротическая листовая пятнистость*

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Introduction

The global economic relevance of crop diseases caused by pathogenic fungi from *Alternaria* Nees, 1817 и *Fusarium* Link., 1809 genera and manifesting in cereals, oilseed crops, many vegetables and fruits [4; 5; 19; 22] is steadily trending upwards. That fact attracts the attention of the scientific community. The above-mentioned micropathogens are new to many crops, including grapes [8; 27; 28]. In recent years, vineyards of the Western Ciscaucasia have been widely affected by necrotic leaf spot (*Alternaria* sp. pathocomplex) and infectious generative organs wilt (*Fusarium proliferatum*, F. *oxysporum*). Both diseases are mostly seen in inter-specific hybrids and cause notable harm to plants. Uncontrolled spread of the Alternarium necrotic spot disease can bring down the yield per bush rate by 18% to 27 % and decrease the sugar content by 6.1% to 9.1 %. The *Fusarium* wilt disease manifests in the average bunch mass loss of 12.8% to 60.3% and the shortening of the bunch by 7.7% - 39.4 %. This affects the grape products' tradability, which is especially harmful to table grapes [3]. Apart from the high-profit losses due to the decrease in yield, the Alternarium and *Fusarium* fungi produce mycotoxins in plants thus making them dangerous to consume [2; 25]. Therefore, the need for efficient measures against such plant diseases is even more prominent.

The wide distribution of the fungi species under review is commonly attributed to an overall weakening of plants due to increasing abiotic influence caused by a climatic shift noticed in many agricultural regions worldwide [6; 23]. In the context of plant protection against Alternarium and *Fusarium* diseases, this urges agrarians to account for the fact that chemical treatment can lead to the further weakening of plants through phototoxicity [12; 24]. Furthermore, exclusive use of synthetic fungicides leads to increased risks of resistance in plants. For example, iprodione, tebuconazole, and mancozeb excessive treatment lead to the evolution of fungicide-resistant *Alternaria* Nees, 1817 strains [26]. The use of biological substances in the course of plant treatment against diseases can be an effective and ecologically safe alternative as it reduces the amount of pesticides the agrocenoses are subjected to. An ecology-aware approach is key in the insurance of sustainable agriculture [9].

A global tendency to discover and utilize the *Trichoderma* Pers., 1801 fungi strains is relevant to a successful implementation of the discussed approach. Numerous studies on the biology, genetic diversity, and antagonistic properties of these species are proving this point even further [7; 13; 15; 18]. The main mechanisms of intermicrobial interactions in *Trichoderma* species used in biological plant disease control are antibiosis, mycoparasitism, and dietary competition [17]. The research of *Trichoderma* spp. biological activity showed that these fungi can suppress a wide variety of mycophytopathogenes [10; 20; 29], including *Alternaria* and *Fusarium* species [14; 16; 30].

The search for new efficient biocontrol fungi species among the *Trichoderma* spp. presents great interest due to the need for the production of biosubstances against new diseases in grapes.

Materials and Methods

This research aimed to test the *Trichoderma* spp. culture extracted from native conditions of the Western Ciscaucasia in vitro. The samples with the highest antagonistic potential were to be chosen as the most efficient biosubstance producents. The research objectives included an assessment of antimycotic properties demonstrated by native strains of the *Trichoderma* genus. The said properties were tested with the following species: (1) *Alternaria* sp. selected as the most common and virulent pathogen from the grapes necrotic spot pathocomlex; (2) *Fusarium proliferatum* and F. *oxysporum*, the causative agents of the grapes generative organs wilt. The final research objective was to analyze the obtained data and select the most efficient antagonistic strains.

Laboratory tests for the activity against the target mycopathogenes are a necessary stage in the multistage screening process of the antagonistic strains aimed at the selection of the most efficient bioproducers. For this research, a confronting cell culture research method was chosen (the procedure description will follow). This approach allows us to give the most thorough and simultaneous assessment of the different interaction patterns between fungi in vitro. We assessed the antimycotic activity in five *Trichoderma* spp. strains obtained from the ampelocenoses of the Anapa-Taman and Central regions of the Krasnodar Krai (Western Ciscaucasia). These strains included T-213, T-338, T-404/1, T-441/1, T-503. We assume that the native *Trichoderma* spp. species are more adjusted to the conditions of ampelocenoses and therefore can be more efficient in biosystems as a biosubstance base component (Table 1).

We selected the pathogenic species causing the Fusarium generative organ wilt for this research: *Fusarium* proliferatum F-41/1 and F. oxysporum F-117. From the necrotic spot pathocomplex, we selected *Alternaria* sp. 425-3.

We disinfected the grape plant samples with 70% alcohol for 1 minute and with 1% sodium hypochlorite solution for 5 minutes, then rinsed them with sterile distilled water [11]. These samples later served as a source of pathogenic and antagonistic strains. We put fragments of each sample in Petri dishes with sweet potato sucrose agar. The causative agent cultures and antagonistic cultures were preliminarily grown separately in the same conditions. We cultivated the *Fusarium* spp. strains during 14 days, the *Alternaria* spp. strains during 7 days, and the antagonistic strains during 14 days. To assess the antagonistic activity of the *Trichoderma* spp. strains, we cut out blocks of antagonistic mycelium and pathogenic mycelium with separate sterile 6 mm drills. Then, we put the obtained blocks into a Petri dish 6 cm apart using a sterile inoculation loop. The Petri dish was positioned above an alcohol burner. The fungi pathogenic and antagonistic cultures grown separately served as a control experiment. We analyzed the colonies' growth on the 10th day of cultivation (measured in cm^2 and % of the Petri dish area). Additionally, we assessed the nature of the occurring interactions [1]. We conducted the experiment four times.

Table 1.

Origin of the *Trichoderma* Pers., 1801 strains

№	Strain	Extraction date	Extraction place	Biological sample type	Plant species variation
1	*T-213*	20.06.2018	Agricultural Enterprise [AE] Yuzhnaya, Kurchanskaya station	bunch	Levokumsky
2	*T-338*	21.05.2020	Krasnodar, individual household	leaves	Sultana
3	*T-404/1*	10.07.2020	AE Yuzhnaya, Golubaya Bukhta station	bunch	Aligote
4	*T-441/1*	6.08.2020	AE Yyzhnaya, Kurchanskaya station	bunch	Riton
5	*T-503*	28.09.2020	Krasnodar, individual household	bunch	Tana 22

Results

The *Trichoderma* spp. strains T-404/1 and T-338 demonstrated the highest hyperparasitic activity against the *Alternaria* sp. pathogen (Table 2).

Table 2.

Antagonistic activity of the native *Trichoderma* spp. strains against the grapes necrotic spot causative agent *Alternaria* sp. at a temperature of 25ºC in sweet potato sucrose agar, 10th day of cultivation

Studied strain	Coverage area				K1, mm	K2, cm^2
	Antagonistic strain		Pathogen			
	cm^2	%	cm^2	%		
Alternaria sp. (control variant)	-	-	81.0	100.0	-	-
Dietary competition+hyperparasitism+antibiosis						
T-404/1	63.6	100.0	7.1	11.1	6.0	7.1
Dietary competition+hyperparasitism						
T-441/1	45.8	71.9	16.6	26.1	0	8.3

T-338	63.6	100.0	10.2	16.0	0	10.2
T-213	51.9	81.5	11.3	17.7	0	6.0
T-503	45.5	71.4	13.9	21.8	0	3.7

*Note**: K1 – sterile zone area; K2 – hyperparasitic zone area.

The antibiotic properties were only found in the *Trichoderma* sp. T-404/1 strain. The sterile zone around its mycelium was 6.0 cm (Fig. 1).

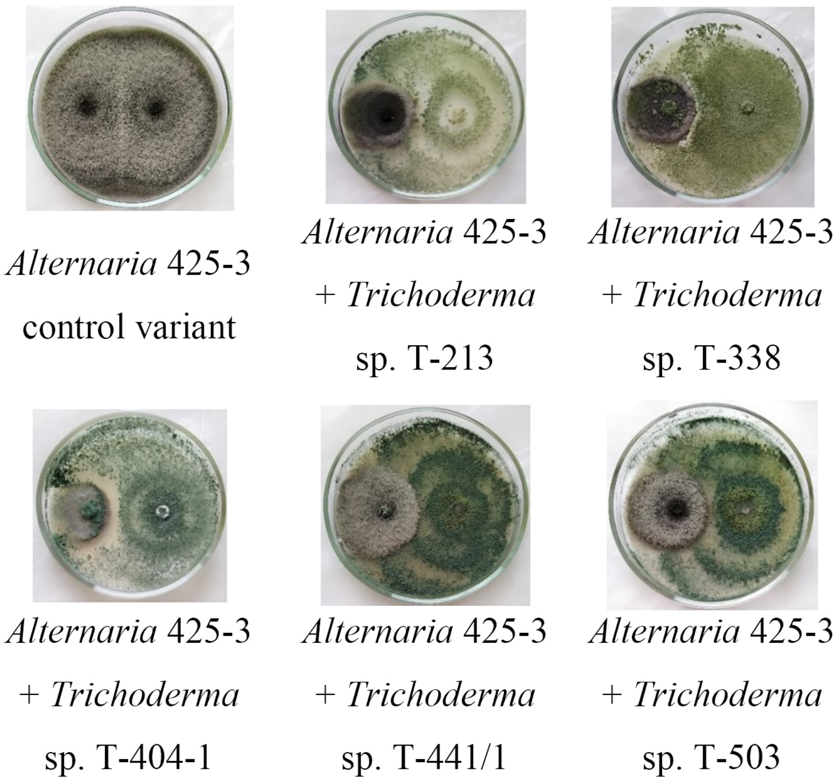


Fig. 1. Antagonistic activity of the *Trichoderma* fungi strains against the grapes necrotic spot causative agent in sweet potato sucrose agar, 10th day of cultivation: the pathogen is on the left, the antagonist is on the right.

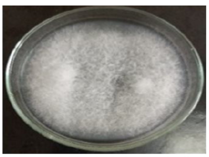
We also assessed the antagonistic activity against the *Fusarium* generative organs wilt causative agent focusing on the most aggressive isolate *Fusarium proliferatum* F-41/1 (Table 3).

Table 3.

Antagonistic activity of the native *Trichoderma* spp. strains against the grape *Fusarium* generative organs wilt causative agent *Fusarium proliferatum* F-41/1 at a temperature of 25 ºC in sweet potato sucrose agar, 10th day of cultivation

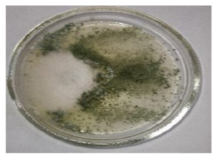
Studies strain	Coverage area				K1, mm	K2, cm^2
	Antagonistic strain		Pathogen			
	cm^2	%	cm^2	%		
F.proliferatum F-41/1 (control variant)	-	-	63.6	100.0	-	-
Dietary competition+antibiosis						
T-441/1	47.7	75.0	15.9	25.0	5.1	0
Dietary competition+hyperparasitism						
T-404/1	54.1	85.0	9.5	15.0	0	0.7
Dietary competition						
T-213	47.7	75.0	15.9	25.0	0	0
T-338	43.8	70.0	19.8	30.0	0	0
T-503	50.9	80.0	12.7	20.0	0	0

*Note**: K1 – sterile zone area; K2 – hyperparasitic zone area.

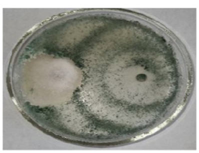


F. proliferatum F-41/1 control variant

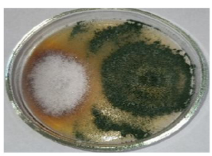
F. proliferatum F-41/1 + *Trichoderma* sp. T-213



F. proliferatum F-41/1 + *Trichoderma* sp. T-338



F. proliferatum F-41/1 + *Trichoderma* sp. T-404-1



F. proliferatum F-41/1 + *Trichoderma* sp. T-441/1

F. proliferatum F-41/1 + *Trichoderma* sp. T-503

Fig. 2. Antagonistic activity of the *Trichoderma* fungi strains against the grapes *Fusarium* generative organs wilt causative agent (*Fusarium proliferatum* isolate F-41/1) in sweet potato sucrose agar, 10th day of cultivation: the pathogen is on the left, the antagonist is on the right.

Table 4.

Antagonistic activity of the native *Trichoderma* spp. strains against the grape *Fusarium* generative organs wilt causative agent *Fusarium oxysporum* F-117 at a temperature of 25 ºC in sweet potato sucrose agar, 10th day of cultivation

Studied strain	Coverage area				K1, mm	K2, cm^2
	Antagonistic strain		Pathogen			
	cm^2	%	cm^2	%		
F. oxysporum F-117 (control variant)	-	-	63,6	100.0	-	-
Dietary competition+hyperparasitism+antibiosis						
T-503	50.9	80.1	12.7	19.9	12.0	5.7
Dietary competition+hyperparasitism						
T-338	55.9	88.0	7.7	12.0	0	2.3
T-404/1	60.5	95.2	3.1	4.8	0	2.5
T-441/1	53.5	84.1	10.1	15.9	0	4.0
Dietary competition+antibiosis						
T-213	57.2	90.0	6.4	10.0	5.2	0

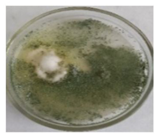
Note:* K1 – sterile zone area; K2 – hyperparasitic zone area.

F. oxysporum F-117

control variant

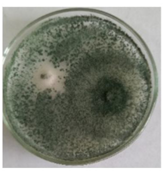
F. oxysporum F-117 +

Trichoderma sp. T-213



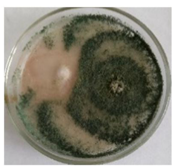
F. oxysporum F-117 +

Trichoderma sp. T-338



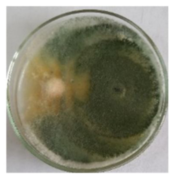
F. oxysporum F-117 +

Trichoderma sp. T-404-1



F. oxysporum F-117 +

Trichoderma sp. T-441/1



F. oxysporum F-117 +

Trichoderma sp. T-503

Fig. 3. Antagonistic activity of the Trichoderma fungi strains against the grapes Fusarium generative organs wilt causative agent (Fusarium oxysporum isolate F-117) in sweet potato sucrose agar, 10th day of cultivation: the pathogen is on the left, the antagonist is on the right.

All the native strains (T-213, T-338, T-404/1, T-441/1, T-503) showed antagonistic activity against the F-41/1 *Fusarium proliferatum* isolate, with the *Trichoderma* sp. T-213 strain showing the highest activity rate (Fig. 2).

We studied the suppression activity of the *Trichoderma* spp. strains against *Fusarium oxysporum* isolate F-117, another species belonging to the generative organs wilt pathocomplex (Table 4).

We established that the antagonistic strains demonstrated higher activity against the *F. oxysporum* F-117 isolate compared to the *F. proliferatum* F-41/1 isolate (Fig. 3).

Discussion

The analysis of obtained data revealed that all the *Trichoderma* spp. strains featuring in this research demonstrated antimycotic activity against mycopathogenes of the *Alternaria* Nees, 1817 and *Fusarium* Link., 1809 genera. The strains were obtained from contaminated tissue of grapes native to Western Ciscaucasia. The antimycotic properties were based on different intermicrobial interaction patterns.

A great proportion of the *Trichoderma* spp. strains under review combined several types of antimycotic effect against the *Alternaria* sp. For example, we noticed hyperparasitism and dietary competition between the T-404/1, T-338, T-441/1 strains and the necrotic spot causative agent *Alternaria* sp. The T-404/1 strain demonstrated three simultaneous antimycotic mechanics: dietary competition, mycoparasitism, and antibiotic activity. We attribute prominent antibiotic features of the T-404/1 strain to it actively secreting diffusive metabolites into the growth medium and by this suppressing the spread of necrotic spot causative agent. T*richoderma* fungi are known to produce secondary metabolites and antibiotics with prominent antimycotic properties [21]. All the antagonistic strains showed high dietary competitiveness. The *Trichoderma* spp. T-338 and T-441/1 strains demonstrated the highest hyperparasitic activity.

We set forth the suppressive activity of the *Trichoderma* strains against the *Fusarium* spp. cultures. In doing so, their activity rate can vary depending on the species of a causative agent within the same pathogenic micromycetes genus (*Fusarium* spp.). The antagonistic strains demonstrated the use of one or more antimycotic mechanics in suppression of pathogen F. proliferatum 41/1 and F. oxysporum F-117.colonies. We noticed two suppression mechanics in the T-404/1 strain against F. *proliferatum* 41/1. The highest antimycotic activity rate against this isolate was noticed in T-404/1 and T-503 strains. Their growth spread area was 85% and 80% of the total area respectively. The researched *Trichoderma* strains demonstrated the highest activity rate against the F. *oxysporum* F-117 isolate. Their antimycotic mechanisms included dietary competition, hyperparasitism, and antibiosis. We found the same triple action pattern in the

T-503 strain. The combination of dietary competition and hyperparasitism was found in the T-338, T-404/1, and T-441/1 strains, while the T-213 strain showed only antibiotic activity and dietary competition. The strains T-338, T-503, and T-404/1 demonstrated the highest dietary competitiveness rate. The *Trichoderma* spp. strains T-338, T-404/1, and T-441/1 are the most active against the causative agents of *Fusarium* generative organs wilt.

Conclusion

Necrotic leaf spot (*Alternaria* spp. pathocomplex) and infectious generative organs wilt (*Fusarium* spp. complex) are new harmful diseases of grapes in the Western Caucasus region (Russia) that should be taken under control by developing measures. Biotechnology is a global trend in plant protection. Antagonist strains from the native microflora of vineyards were tested for the first time for antimycotic activity against pathogens of new diseases of grapes.

After summarizing the data obtained from antimycotic activity screening in the researched antagonizing strains against *Alternaria* sp. and *Fusarium* spp., we can conclude that the *Trichoderma* spp. T-338 and T-404/1 show distinguished mycoparasitic reactions against all the phytopathogenic isolates featured in this study. The majority of the strains under review demonstrate high competitiveness (T-338, T-441/1, T-404/1, T-503). All the strains demonstrate antagonistic activity to varying extents. However, we can select the following strains as the most active antagonists: T-441/1 (against *Fusarium* spp.) and T-404-1 (against *Alternaria* sp.).

Therefore, we consider the strains *Trichoderma* spp. T-404/1 and T-441/1 to have the highest potential in further research on the biocontrol measures against new grape biopathogenes.

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