Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2021, 11(10), 66-69, doi: 10.15421/2021_320

ORIGINAL ARTICLE

Antaqonistic activity of tester strains of *Trichoderma* and *Penicillium* against tomato pathogens

J.T. Aghayev*, K.G. Huseynov

Scientific Institute of Plant Protection and Industrial Crops, Baku, Azerbaijan

**Corresponding author E-mail: cabrayil@yandex.ru*

Received: 13.11.2021. Accepted: 17.12.2021.

During our investigations on the tomato (*Licopersicum esculentum* L.) 36 pathogens have been found and identified. *Fusarium solani* (Mart.) Sacc., *Alternaria solani* Sorauer, *Verticillium albo-atrum* Reinke and Berthold, cause high yield damage by spreading in root system and xylem tubes of plants. Numerous investigations have been carried out in order to obtain natural antagonists against pathogens, to reinstate the ecosystem partially and to minimize use of chemical agents. *Trichoderma lignorum* (Tode.) Harz., (T/A-1) and *Penicillium sp.* (P/A-1) antagonists have been obtained from natural rhizosphere. These antagonists showed higher efficiency for antagonistic ability against pathogens among 28 various microbiological colonies. Optimum substrate was determined in order to increase and produce antagonists in laboratory conditions. The strains of *T/A-1 and P/A-1* tested on the plants grown under condition of artifical infection with cultural substances of antagonists showed high antagonistic features against mentioned pathogens.

Keywords: *Solanum lycopersicum*, Pathogens, *Fusarium solani*, *Alternaria solani, Verticillium albo-atrum,* Biological protection.

Introduction

Low infection of plants with diseases and high quality of crops grown in natural conditions in the soils with high humus content indicate the richness of microflora in these soils (Tabolin, 2010). By infection of root, root neck and xylem tubes of tomato seedlings with soil-born tracheomycosis, the plant organs of tomato grown in natural soils are damaged and high risk of yield loss appears (Abdallah Rania Aydi et al., 2016). In order to prevent prevalence of diseases trichodermin, boverin, gliocladium and pencillin preparations are used in farms against pathogens.

The efficiency of use of preparations obtained from fungal-antagonists, such as *Tr.lygnorum*, *Tr.harzianum,* against Tracheomycosis pathogens in xylem tubes has been widely studied (Aghayev, 2011; Bodnya, 2011; Borovaya, 2013).

The antagonists of the genus *Trichoderma* and *Penicillium* are distinguished by their importance in this sphere. A number of scientists have investigated the antagonistic activity of isolates of the genus *Penicillium* and *Trichoderma* against pathogenic bacteria and fungi and high outcomes have been obtained from the strains of *Trichoderma harsianum* Rifai and *Penicillium decumbens Thom* (Santamarina et al., 2002). Currently, researches on obtaining of bacteria and fungi distinguished by the antagonistic features are expanding. (Xiaomeng, et al., 2019).

Materials and Methods

Separation of antagonists from rhizosphere

For finding biological agents to control *F.solani*, *A.solani* and *V. albo-atrum* pathogens of tomato plant, soil samples taken from the rhizosphere of tomato plant have been analysed by methods adopted by laboratory studies (Tabolin, 2010; Tarunina, 1981).

As local aborigen microflora is more effective against the diseases, which infect the plants in fields, research studies on purification and application of antagonistic microorganisms, by analyzing rhizospheric soils of cultivars, have been carried out by following methods (Titov, 2011).

To this end, 10 grams of each soil samples, taken from the rhizosphere of tomato plants grown in different regions, after grinding in the porcelain cup were mixed in 100 ml of sterile water. In order to be selected and used for antagonistic abilities, soil samples taken from the rhizosphere of the tomato plant were dissolved in the ratios of 1:100, 1:1000, and 1:10000.

Four samples taken from each mixture were cultured in Petri dishes in barley dextrose agar by using smear, spread and lining techniques. Cultivated samples were placed at 25°C temperature in thermostat. After 7-10 days, appeared colonies were analyzed by systematic microscopy method and species were identified. During the analyses, antagonistic colonies were re-cultured on solid agar substrate in Petri dishes and test tubes and recultivated in the thermostate at 25°C. After 7-10 days, in order to study antagonistic abilities of the obtained strains 10 mm diameter cut blocks of strains were cultured in the center of pure medium of pathogen fungi in Petri dishes. Records of microbes distinguished by antagonistic activity were made daily. Microorganisms, of which antagonistic activity was identified by block method and pure medium was obtained, have been identified mainly in *Trichoderma, Ampelomyces and Penicillium* samples. Correlation of higher antagonistic *Trichoderma sp.,* and *Penicillium sp., with Fusarium*

solani.and *Alternaria solani* and *Verticillium albo-atrum* fungi were studied at a temperature of 25°C, under ambient reaction pH-6. The experiment was repeated 10 times.

Methods for obtaining optimal substrate for antagonists

Increasing of microbe antagonists in bran cereal, chaff, sawdust, grape waste was studied in order to increase obtained antagonists and to obtain a substrate that is economically effective and is able to collect maximal spore mass,

The specified substrates were initially filled in glass bottles, mouth of bottles were closed tightly with corks, and substrates were sterilized. After a complete sterilization, microbes were cultured in parallel, placed at a thermostat at 25°C temperature, and appropriate records were made on the 5^{th}, 10^{th}, 15^{th}, and 20^{th} days. 1 ml of the obtained mixtures was cultured in potato dextrose agar in Petri dishes. The pH of the growth medium was raised up to 4.5-5. Each mixture sample was cultivated in 5 variants. The materials were placed in a 26°C thermostat and recultivation was performed from the appeared colonies. Fungi of the genus of *Trichoderma* and *Penicillium* determined after microscopic analysis were recultivated repeteatedly in sterile zone in order to determine pure medium for the bacteries of the genus of *Pseudomonas and Bacillus,* and the main material was obtained in test tubes.

Study of antagonistic features of active metobolites

The antagonistic ability of cultural solution of metabolites (*Trichoderma lygnorum, Penicillium sp.*) differed by activity was studied on tomato seedlings under artificial infection conditions. Artificial infection of tomato seedlings was conducted in several ways. For this purpose, cultural substance obtained from pure medium of *Fusarium solani*, *Fusarium oxysporum* f. *sp. licopersici*, *Verticillium albo-atrum*, *Verticillium sp.*, *Rhysoctonia solani* fungi which rot the roots and root neck, has been given to the substrate and the seeds have been artificially infected by pre-sowing soaking methods. Appropriate natural conditions for total occuring of infection were provided. Artificial infection was carried out applying cultural solution of pathogens onto the isolated tomato seedlings in fixator by using methods of spraying, scrubbing with brush and injection into the damaged part. Observations were made in every 4 hours and lasted for 15 days. In order to study the effectivity of obtained biological agents small-scale experiments have been conducted on seedlings which had been artificially infected with above-mentioned pathogens. The working substance prepared in different impurity and spore density, applied onto the leaves on which the treats of the disease has been observed. Records on disease development have been made everyday during 15 days. Small-scale experiments were performed in repetitions with an area of 30 m^2 per repetitions based on commonly adopted methods.

Results and Discussion

22 antagonistic microbe colonies have been investigated by systematic microscopy methods. The species of *Penicillium* and *Trichoderma* showed high antagonistic activity against pathogens during conducted experiments in laboratory conditions. Correlation of those species with relevant pathogen fungi has been studied during experiments, which conducted to determine level of antagonistic features of those species. 2 species-*Penicillium sp.* (P/A-1) and *Trichoderma* out of 5 strains were considered as high antagonistic against Fusarium solani, Alternaria solani, Verticillium albo-atrum pathogens and the experiments on determination of the substrate, which collects maximum spore mass of these species, have been conducted. The results of investigation were described in Table 1.

Table 1. Correlation of strains of P/A-1 and T/A-1 with pathogen fungi (antagonistic activity) in different growth mediums. Temperature 25°C, pH=6.

Experiment variants: Mutual cultivation schemes of active metabolites and pathogens in Petri dishes	**Size of Covered Colonies, mm**								
	Barley Dextrose Agar			**Potato Dextrose Agar**			**Water Agar**		
	5^{th} day	**10^{th} day**	**15^{th} day**	**5^{th} day**	**10^{th} day**	**15^{th} day**	**5^{th} day**	**10^{th} day**	**15^{th} day**
P/A-1+*F.solani*	8	22	38	12	28	39	6	19	33
P/A-1+*A.solani*	5	21	32	7	22	35	4	14	29
P/A-1+*V.albo-atrum*	-	3	16	5	19	28	-	3	8
T/ A-1+*F.solani*	18	27	48	14	22	53	8	19	46
T/A-1+*A.solani*	8	20	42	12	29	44	5	15	37
T/A-1+*V.albo-atrum*	14	25	45	19	25	46	12	11	24

Note: P/A-1-*Penicillium sp.* strain; T/A-1-*Trichoderma lygnorum* strain.

The P/A-1 strain showed antagonisity against *Fusarium solani* and prevented development of colonies of pathogen in 33-39 mm in each three growth mediums. Activity of the P/A-1 strain against the *Alternaria solani* and *Verticillium albo-atrum* fungi was observed. It is known that the results obtained from these variants cannot meet expectations in agriculture. The results obtained from these variants are in line with expectations. Thus, use of the strain P/A-1 against *F. solani* may have promise in wide agriculture. The strain of P/A-1 against A. solani and V.albo-atrum pathogens can be considered partly useful.

The strain of T/A-1 showed the highest antagonistic ability against *F.solani* and *A.solani* in potato dextrose agar and barley dextrose agar. Thus, the development of *F.solani* colonies on a diameter of 48 mm was prevented on the 15th day in barley dextrose agar. In potato dextrose agar medium, activity zone of antagonist was 53 mm on the 15th day and 46 mm in the water agar. On the 15th day, the strain of T/A-1 stopped the development of *A.solani* in barley dextrose agar in 42 mm, in potato dextrose agar in 44 mm, and in water agar in 37 mm. The antagonistic ability of T/A-1 against *V.albo-atrum* was 45mm in barley dextrose agar on the 15th day, 46 mm in potato dextrose agar and 24mm in water agar.

The results of conducted experiments show that the strains of P/A-1 and T/A-1 can be used to control F*. solani, A. solani* and *V. albo-atrum*.

Numerous experiments have been carried out to arrange a large-scale production of the obtained antagonists and to get the substrate, which is economically effective and collects maximum spore mass for their development. To this end, the strains of P/A-1 and A-1 increased in barley dextrose agar and in potato dextrose agar have been studied in bran cereal, chaff, sawdust and grape waste. Both antagonists were cultivated in these sterilized growth substrates. On the 5th, 10th, and 15th days after cultivation, appropriate records were made and reports were prepared.

After 10 days, the substrate made of bran cereal surrounded wholly by micelles. Both antagonists (P/A-1 and T/A-1) collect spore and micelles that are more active on the substrate made of bran cereal (Table 2).

Table 2. Development of antagonists in different growth mediums (25°C in Petri dishes, pH=6).

Growth Mediums	**Development of the Colonies (mm)**						
	P/A-1			**T/A-1**			**Note**
	5th day	**10th day**	**25 th day**	**5th day**	**10th day**	**25 th day**	
Bran cereal	55	90	90	65	90	90	High growth
Chaff	4	10	32	12	28	64	Medium growth
Sawdust	-	2	11	-	10	31	Weak growth
Grape waste	22	36	60	34	51	90	High growth

In the growth medium made from grape waste, on the 20th day the 90-mm area was fully covered. From the obtained results we can conclude that strains of P/A-1 and T/A-1 highly develop in growth substrates made of bran cereal and grape waste. These nutrients can be further enriched and used in extensive production of antagonists as an effective nutritional element for them.

Effect of pre-sowing treatment of tomato seeds with strains of P/A-1 and T/A-1 on development and spread of major diseases in seedlings

Soil and seeds spread rracheomycosis. Pre-sowing treatment of seeds is one of the most important measures in preventing winter stocks and sources of diseases. Obtained strains of P-A-1 and T/A-1 pathogens: small-scale experiments have been conducted to study the efficiency of antagonistic ability against *A.solani, F.solani, V. albo-atrum*. The experiments were performed under condition of artificial infection of seeds with cultural solution of *A. Solani, F.sololani* and *V. albo-atrum*. Tomato seeds were soaked for 20 days in cultural solution of pathogens (titre 4 to 104 spores/ml). Consumption of the cultural solution was 10 ml/kg. Tomato seeds infected with pathogens were involved into experiments after drying.

Experiment variants:

1. Soaking of seeds with P/A-1 cultural solution, (Titre: 4×10^6 spore/ml), consumption-10 ml/kg;
2. Soaking of seeds with T/A-1cultural solution (Titre: 4×10^6 spore/ml), consumption-10 ml/kg;
3. Control: soaking with distilled water-consumption-10 ml/kg.

The experiments were repeated 4 times and records were made in every 7 days. Observations continued until the seedlings were transferred to the field (Table 3).

Table 3. Effect of pre-sowing treatment of tomato seeds on disease development in seedlings in artificial infection condition.

Experiment Variants	**Spreading, (%)**				**Biological benefit, (%)**		
	Alternaria solani		***Fuzarium solani***	***Verticillium albo-atrum***	***Alternaria solani***	***Fuzarium solani***	***Verticillium albo-atrum***
	Spread	**Spread Intensity**					
Soaking of seeds with P/A-1 CS, titre: 4×10^6 spore/ml	14.5	2.8	18.0	19.8	35.4	57.6	47.9

Soaking of seeds with T/A-1 CS, titre: $4 \cdot 10^6$ spore/ml	8.6	2.2	22.4	18.0	64.1	47.8	52.6
Control: soaking with distilled water	24.0	6.5	42.5	38.0	-	-	-

CS-cultural solution

During the treatment with the strain of P/A-1 CS, the prevalence of *A. solani* was 14.5%, *F. solani* was 18%, *V. albo-atrum* was 19.8% on experimented seedlings. During the soaking with the strain of T/A-1 CS, the prevalence of *A. solani was* 8.6%, *F. solani was* 22.4%, *V.albo-atrum* was 18% on seedlings. In control variant the prevalence of *A.solani* was 24%, *F. solani was* 42.5%, *V. solani* was 38%.

The strain of T/A-1 showed higher results than the antagonists used during the experiment. The biological benefit against pathogens was 64.1% (*A.solani*), 47.8% (*F.solani*), and 52.6% (*V.albo-atrum*) in the variant of soaking of tomato seeds with the cultural solution of strain of T/A-1.

Conclusion

According to the obtained results, the strains of P/A-1 and T/A-1 have been found to be antagonistic against *Fusarium solani*, *Alternaria solani,* and *Verticillium albo-atrum* pathogens.

Growth substrates made from grape waste and bran cereal have been considered optimal for development of P/A-1 and T/A-1 antagonistic fungi.

Compared to other strains, the strain of T/A-1 has been found to be more antagonistic against pathogens. The biological benefit against *Alternaria solani, Fuzarium solan,* and *Verticillium albo-atrum* was 64.1%, 47.8%, and 52.6% respectively, in pre-sowing soaking variant of seeds with T/A-1 CS.

References

Abdallah, R.A.B., Jabnoun-Khiareddine, H., Mokni-Tlili, S. (2016). Biocontrol of Fusarium Wilt and Growth Promotion of Tomato Plants Using Endophyt Bacteria Isolated from Solanum elaeagnifolium Stems. Journal of Phytopathology, 164:811-824.

Agayev, J.T. (2011). Biological measures against the main diseases of tomato. Azerbaijan Agrarian Science Journal, 3:96-97 (Azeri).

Bodnya, E.E. (2011). The biomethod gives a tangible effect. Plant Protection and Quarantine, 4:10-12 (Russian).

Borovaya, V.P. (2013). Vioways on winter wheat crops. Protection and Quarantine Plants. 4:30-31 (Russian).

Tabolin, S.B. (2010). Nematode-mycotic infections of the rhizosphere of berry crops and biological methods of dealing with them. Abstract of the Dissertation Ph.D., Moscow, p:132 (Russian).

Tarunina, T.A. (1981). Methods for assessing the antagonistic activity of strains of the genus *Trichoderma* Fatopathogenic fungi. Guidelines. L., VIZR, p:13 (Russian).

Titov, A.S. (2011). Environmentally friendly ways to protect strawberry plants from a complex of pests. The Dissertation, Candidate of Agricultural Sciences, Moscow, p:115 (Russian).

Xiaomeng, Ch., Yajie, W., Yu, G., Tongguo, G., Dongdong, Z. (2019). Inhibitory abilities of bacillus isolates and culture filtrates against the gray mold caused by botrytis cinerea on postharvest fruit. Plant Pathology Journal, 35:425-436.

Citation:

Aghayev, J.T., Huseynov, K.G. (2021). Antaqonistic activiti of tester strains of *Trichoderma* and *Penicillium* against tomato pathogens. *Ukrainian Journal of Ecology.* 11:66-69.