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Determination of Antagonistic Effect Between Some Fusarium Species and Root Bacteria Isolated from Eggplant Roots

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ABSTRACT

In this study, it was aimed to determine the antagonistic effect between and isolated from eggplant roots and some root bacteria conditions. 24 bacteria were isolated from soil samples taken from the rhizosphere regions of infected and non-infected plants in different eggplant production areas and 22 of them were evaluated as candidate biocontrol agents. According to the results of the research, 13 out of 22 bacteria were effective against, 14 against, and 9 against between 40% and 100%. The most effective bacteria against were 22B (100%) and 11B (74.4%). Among the bacteria tested for effectiveness against, 3B (100%), 10B (100%) and 18B (100%) completely inhibited fungal growth. These bacteria are followed by 11B (88.85%) and 13B (85.55%). 11B and 24B inhibited mycelial growth of 100%.

1. Introduction

Eggplant () is a purple-black vegetable that was first consumed in China and India. Türkiye ranks 4th in eggplant production after China, India and Egypt (Anonymous, 2018). Root rot and wilt diseases caused by soil-borne *Fusarium* spp. are factors that limit and reduce production in eggplant. They can infect the eggplant at every stage of the growing period. In the control against; In addition to the use of resistant varieties and non-infected soil and plants, alternation and the use of fungicide, biological control against and are the main methods (Agrios, 1988; Kurt, 2020).

The fungus penetrates plant tissue through wounds and natural openings. It forms lesions on the edges of the leaves and does not show a systemic distribution. There is discoloration in the leaf veins and sagging of the petioles. Root rot, wilt and death are seen in the later stages of the infection.When a longitudinal section of the plant is taken, chocolate-colored spots and rot appear on the root collar and root tissue (Miller et al., 1996). species can maintain their vitality in the soil for a long time in the form of chlamidospores (Türktaş and Koral, 2018). Biological control studies have a special importance in order to reduce the density of species such as, which has limited chemical control, in the soil. The relationship between these disease agents and various bacterial antagonists in biological control is examined (Özaktan et al., 2010).

There are bacteria in colonies in the rhizosphere region of plants, and some of them directly or indirectly affect the plant positively by taking advantage of the nutrients secreted by the roots. These bacteria are called plant growth promoting rhizobacteria (PGPR). PGPRs play an important role in improving plant health through events such as nitrogen fixation, minimizing the contamination of metal elements in the soil, phosphate solubility, phytohormone production, and antifungal activities of the ACC deaminase enzyme. PGPRs play a vital role in supporting plant growth and increasing soil fertility, as well as having a great importance in controlling plant diseases (Seyedsayamdost 2019).

Bioagent bacteria slow the growth of fungal pathogens both *in vivo* and *in vitro* with antifungal activities (Chakraborty et al., 2008). Gram-negative and gram-positive biocontrol agents protect plants from infections by phytopathogenic organisms. In a study by Saha et al. (2012), they tested 141 isolates of *Bacillus subtilis* on *Fusarium solani*, which causes *Fusarium* Wilt, *in vitro* and found that it limited fungal growth by showing an antagonistic effect.

Antimicrobial metabolites of bacterial species are low molecular weight and have proven to be lethal against other plant pathogenic microorganisms (Sahu et al., 2019). In a study, *Bacillus* and *Pseudomonas* isolates were isolated from the rhizosphere part of eggplant, and siderophore, protease and cyanide enzyme production were tested, and their cell wall destructive activities were examined and it was stated that they showed an antagonistic effect against *Fusarium oxysporum*

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Schlecht. f.sp. *melongenae* (Fomg) and reduced the severity of the disease (Jarl et al.,1999).

In addition, these biocontrol agents form a biofilm against many fungal pathogens by producing siderophores (Beneduzive et al., 2012).

Bacillus spp. has proven to be a biocontrol agent by producing secondary metabolites against some important fungal pathogens (Hanschen and Winkelmann, 2020).

It has been determined that biocontrol agents such as *Brevibacillus breves*, which secretes fengycin and iturin-A, and *Bacillus subtilis*, which secretes gramicidin, are the best biocontrol agents that inhibit the growth of fungal plant pathogens (Latorre et al., 2016).

It is found in some commercial preparations such as *Bacillus* spp., *Pseudomonas* spp., *Serratia* spp. and *Streptomyces* spp, developed for biological control against *Fusarium* wilt (Bora and Özaktan, 1998; Janisiewicz and Korsten, 2002).

In this study, antagonistic effects of 22 bacteria were investigated against *Fusarium oxysporum, Fusarium solani* and *Fusarium profileratum* from isolated eggplant roots.

2. Materials and Methods

2.1. Materials

2.1.1. Fungal material

The pathogens of *Fusarium oxyporum*, *Fusarium solani* and *Fusarium proliferatum* used in the study were obtained from the root parts of diseased plants from the villages of Amasya (Sevincer, Karaköprü and Karasenir).

2.1.2. Bioagent Bacteria

22 potential bioagent bacteria used in the study were isolated from the rhizosphere region of infected and non-infected plants in the eggplant production areas in Muğla province Fethiye district, Antalya province Manavgat district and Aydın, Amasya, Konya.

2.2. Methods

2.2.1. Collection and Isolation of Plant and Soil Samples

Infected and non-infected plant samples were collected by survey in the infected areas and brought to the laboratory. The plant samples to be isolated were first washed with tap water and the tissues between 0.5-1 cm from the root collar, thin roots, branches and leaves in the infected areas were cut with the help of a sterile scalpel and sterilization process was started. The sterilization process consists of 5 steps. In the first step, 1% NaClO was added to 100 ml of water. Tissue pieces were kept in this solution for 3 minutes, and then with the help of sterile forceps, they were kept in 100 ml sterile distilled water 3 times for 3 minutes and transferred to blotting paper. Afterwards, tissue pieces were inoculated in PDA (Potato Dextrose Agar) medium as 4-5 pieces per petri dish. They were placed in an incubator at 25°C for their growth. From the 2nd day, the developments in the petri dishes were checked and transferred to the PDA medium until pure growth was achieved. Macroscopic and microscopic diagnoses of isolated agents were made.

Since the soil samples taken from the infected areas were moist, they were left to dry by laying at room temperature the night before the isolation. Then, after the dried soils were sieved with the help of a sieve with a diameter of 1 mm, 10 g of soil was weighed and placed in sterile 250 ml flasks. 90 ml of sterile water was added to the soils in the flask and shaken in a water bath for 30 minutes. 1 ml of the suspension in the flask was taken with the help of a micro pipette, put into the tube containing 9 ml of sterile water, and after mixing for 30 seconds in the tube mixer, 1 ml of this mixture was taken and added to the tube containing 9 ml of sterile water for dilution. This dilution step was repeated 6 times.

From these suspensions, 100 µl of the fifth and sixth dilutions were taken and plotted on the NA medium with sterile glass baguettes. It was incubated at 25±1°C for 24 hours until bacterial colonies developed. Then, colonies growing on Nutrient Agar medium were examined and colonies showing different morphological development were selected and NA cultured until pure culture was obtained.

2.2.2. Single Spore Isolation

Fusarium grown in PDA media at 24-25°C for 7 days was taken from the tip of the preparations with the help of a sterile loop and transferred to an eppendorf tube containing 1000µl of sterile distilled water. Then, the eppendorf tube was vortexed for 30 seconds. 30µl was taken from this homogeneous tube with the help of a sterile pipette and transferred to an eppendorf tube containing 1000µl of distilled water. This tube was also vortexed for 30 seconds. After dilution twice, 30µl was taken from the last tube with the help of a sterile pipette and inoculated into 2% water agar (20.0g Agar-100ml water-30ml Streptomycine solution). The prepared preparations were placed in an incubator at 20±5°C, alternating 12 hours of dark and 12 hours of light. After 12-18 hours, the petri dishes were checked with a microscope and the spores forming the germ tube were cut with the help of a sterile scalpel and planted in PDA medium containing 40mg Streptomycine/100ml. They were placed in a 25°C incubator for growth (Leslie and Summerell, 2006).

The fungi, whose development was followed for 7-10 weeks, were stored in 3 ways for later use. In the first method, it was transferred using slanted agar and stored at +4°C. In the second procedure, *Fusarium* colonies were transferred to eppendorf tubes with 15% glycerol suspension and stored at -20°C. In the third method, *Fusarium* colonies incubated on Whatman filter papers, which is a long-term storage method, were stored at -20°C.

2.2.3. Identification of Fusarium Species and Bacterial Isolates

The characterization of 12 purified *Fusarium* and 22 bacterial isolates used in the assays was done by MALDI-TOF biotyping.

2.2.4. Testing of Antagonistic Effects of Bacterial Isolates against Fusarium Species (Fusarium solani, F. oxysporum, F. proliferatum)

As a result of isolation studies, rhizosphere bacteria were evaluated *in vitro* against *Fusarium solani, Fusarium oxysporum* and *Fusarium proliferatum* with dual culture method to determine their antifungal activities. Fungi were

grown on Potato Dextrose Agar (PDA) medium and rhizosphere bacteria were grown on Nutrient Agar (NA, Merck) medium. Fungi were incubated at 25°C±1 for 7 days and bacteria at 25°C±1 for 24 hours. After incubation, 2 agar discs of 4 mm, taken from *Fusarium* cultures, were placed opposite each other, equidistant from the center of petri dishes with 9 cm diameter antibiotic-free PDA media was left for incubation. The experiment was set up with 2 replications for each bacterium. The control petri dish was formed by mutually planting fungi without drawing bacteria between them.

In order to understand how much bacteria and fungus inhibit mycelial growth, the inhibition zone (Zone of Inhibition, Z_1) was evaluated by measuring the distance. The percent effect of inhibition rates was calculated according to the formula given below;

Inhibition rate (%) = $(r_1-r_2/r_1) \times 100$

According to the formula r_1, radial growth of the pathogen; r_2 represents the radial evolution of the pathogen and the biological agent (Tozlu, 2003; Ghildiyal and Pandey, 2008).

3. Results and Discussion

3.1. Fusarium Species Isolated from Eggplant Roots

In our study, a total of 16 *Fusarium* isolates obtained from eggplant roots were identified. Of these isolates, 6 were identified as *Fusarium oxysporum*, 7 as *F. solani*, 1 as *F. proliferatum*, and 1 as *Fusarium* sp. According to the diagnostic results, a complete diagnosis of 1 isolate (may be *F. oxysporum* or *F. proliferatum*) could not be made (Table-1).

Table 1
Fusarium species isolated from eggplant roots

Izolate Codes	Fungi Species
Alakova KB.3	*Fusarium oxysporum*
Sevincer I.K.8	*Fusarium solani*
Alakova-2 K.B.2	*Fusarium solani*
Kayseri- 4 2	*Fusarium oxysporum*
Kayseri-1 2	*Fusarium oxysporum*
Alakova Gövde 2	*Fusarium solani*
Isa Keles Sevincer 2	*Fusarium* sp.
Aşılı küçük kok-2 3	*Fusarium solani*
Aşılı Karaköprü I.K. saf1	*Fusarium proliferatum*
Aşılı küçük kok-1 3	*Fusarium oxysporum*
Körkuyu K.B	*Fusarium solani*
Karasenir K.B H.B 1	*Fusarium oxysporum*
Anamur Kayabaşı no. 3 KB2	*Fusarium oxysporum-Fusarium proliferatum*
Anamur Kayabaşı Black no.2 10	*Fusarium oxysporum*
Aşısız Anamur I.K.2	*Fusarium solani*
Sevincer I.K 8	*Fusarium solani*

The isolates highlighted according to the table are the species used in the study.*3.2. Bacteria Species Isolated From Eggplant Roots*

Code numbers and species names of bacterial isolates obtained from eggplant rhizosphere are as given in Table 2:

Table 2
Bacteria species isolated from eggplant roots

Isolate Codes	Bacteria Species
1B	*Stenotrophomonas maltophilia*
2B	*Lysinibacillus fusiformis*
3B	*Pseudomonas putida*
5B	*Staphylococcus epidermidis*
6B	*Bacillus megaterium*
7B	*Enterobacter bugandensis*
8B	*Bacillus megaterium*
9B	*Pseudomonas chlororaphis*
10B	*Pseudomonas chlororaphis*
11B	*Bacillus subtilis*
12B	*Pseudomonas chlororaphis*
13B	*Pseudomonas chlororaphis*
14B	*Stenotrophomonas maltophilia*
16B	*Pseudomonas chlororaphis*
17B	*Acinetobacter vivianii*
18B	*Enterobacter bugandensis*
19B	*Exiguobacterium* sp.
20B	*Acinetobacter calcoaceticus*
21B	*Acinetobacter calcoaceticus*
22B	*Bacillus thuringiensis*
23B	*Pseudomonas chlororaphis*
24B	*Bacillus cereus*

These bacteria; *Stenotrophomonas maltophilia* (2), *Lysinibacillus fusiformis* (1), *Pseudomonas putida* (1), *Staphylococcus epidermidis* (1), *Bacillus megaterium* (2), *Enterobacter bugandensis* (2), *Pseudomonas chlororaphis* (6), *Bacillus subtilis* (1), *Acinetobacter vivianii* (1), *Exiguobacterium* sp. (1), *Acinetobacter calcoaceticus* (2), *Bacillus thuringiensis* (1), *Bacillus cereus* (1).

3.3. Antagonistic Effects of Some Selected Bacteria on Fusarium Species

In this study, soil samples were taken from different regions and tested against 3 *Fusarium* species in order to investigate the diversity of rhizobacteria and to reveal their activities. In the trials, the success rate was accepted as 40% and above.

While *Bacillus thuringiensis* was 100% effective against *Fusarium solani*, *Bacillus subtilis* was 74.4% effective.

Pseudomonas putida and *Enterobacter bugandensis* were 100% effective against *Fusarium oxysporum*. It was also observed that *Staphylococcus epidermidis* inhibited mycelial growth with a rate of 77.7% and *Pseudomonas chlororaphis* with a rate of 85.5%. In a study conducted by Özaktan and Bora (2004) using *P. putida* against *F. oxysorum*, they found that the severity of the disease decreased by 80-84%.

It prevented mycelial growth of *Bacillus subtilis* and *Bacillus thuringiensis* with 100% inhibition rate against *Fusarium proliferatum*. *Pseudomonas chlororaphis* was a successful biological agent with a 72.2% inhibition rate (Table 3).

Table 3
Percentages of inhibition of *Fusarium* species by bacteria used in the antibiosis trial

BACTERIA ISOLATES	CODE	INHIBITION RATES (%)		
		F. solani	*F. oxysporum*	*F. proliferatum*
Stenotrophomonas maltophilia	1B	46,65	36,6	37,7
Lysinibacillus fusiformis	2B	36,60	25,5	38,8
Pseudomonas putida	3B	33,3	100	23,3
Staphylococcus epidermidis	5B	57,75	77,75	24,4
Bacillus megaterium	6B	47,75	42,2	38,85
Enterobacter bugandensis	7B	0	54,4	26,6
Bacillus megaterium	8B	0	0	44,4
Pseudomonas chlororaphis	9B	42,15	55,5	57,7
Pseudomonas chlororaphis	10B	42,2	100	26,6
Bacillus subtilis	11B	74,4	88,85	100
Pseudomonas chlororaphis	12B	51,05	55,5	44,4
Pseudomonas chlororaphis	13B	52,15	85,55	72,2
Stenotrophomonas maltophilia	14B	56,6	66,65	64,4
Pseudomonas chlororaphis	16B	35,5	42,2	54,4
Acinetobacter vivianii	17B	0	31,05	29,95
Enterobacter bugandensis	18B	0	100	28,85
Exiguobacterium sp[2]	19B	35,5	37,7	29,95
Acinetobacter calcoaceticus	20B	39,9	36,65	22,2
Acinetobacter calcoaceticus	21B	41,05	28,85	34,4
Bacillus thuringiensis	22B	100	42,2	35,5
Pseudomonas chlororaphis	23B	34,4	33,25	48,6
Bacillus cereus	24B	53,3	44,4	100

Many *Bacillus* species have been reported to be biocontrol agents against fungal diseases (Ongena and Jacques 2008), and biomolecules derived from them have been reported to inhibit the germination of fungal spores (Matar et al., 2009; des Grades et al., 2012). It is known that many antimicrobial peptide substances or bacteriocins are produced by *Bacillus subtilis* (Hammami et al., 2009; Umer et al., 2021).

P. aeruginosa, *P. putida*, *P. fluorescent* and *P. syringae* from *Pseudomonas* strains have the ability to control soil-borne fungal pathogens (Aksoy, 2006), in some studies, the germination of siderophores produced by *Pseudomonas putida* Trevisan and chlamidospores of *Fusarium* species. It has been observed that it suppresses the development of the pathogen by inhibiting it (Elad and Baker, 1985; Özaktan et al., 2010).

In this study, it is seen that the effect of the use of natural biopreparates and natural products used in the biological control of *Fusarium* Wilt in the eggplant plant is not to be underestimated. In addition, the results suggest that these agents will be have a potential natural fungicide. Making preparations of bioagents that are effective in biological control and applying them in combination with alternative methods will provide great benefits in control strategies.

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