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Investigation of the Biocontrol Effectiveness of Some Bacterial Strains on Eggplant Gray Mold Disease (*Botrytis cinerea*) in in vitro and in vivo conditions

Alican AKÇA[1], Elif TOZLU[2]✉
[1,2] Department of Plant Protection, Faculty of Agriculture, Atatürk University, Erzurum, Türkiye
[1]https://orcid.org/0000-0003-0553-3482, [2]https://orcid.org/0000-0002-0016-9696
✉: elifalpertozlu@atauni.edu.tr

ABSTRACT

Gray mold agent *Botrytis cinerea* (teleomorph: *Botryotinia fuckliana* (de Barry) Whetzel) causes significant yield losses in many economically important vegetables. Chemicals are used in the control to reduce yield loss. As a result of the intensive use of chemicals in the control of plant diseases, human health and the environment are adversely affected. Therefore, interest in environmentally friendly control practices has increased recently. One such application is the use of benefical bacteria in the biological control of diseases. In this study; biocontrol potentials of 12 bioagent bacteria strains (1 *Bacillus cereus,* 2 *Bacillus megaterium,* 2 *Bacillus pumilus,* 2 *Bacillus subtilis,* 1 *Bacillus thuringiensis* subsp. *kurstaki,* 1 *Paenibacillus polymyxa,* 2 *Pantoea agglomerans* and 1 *Pseudomonas fluorescens*) have been determined against gray mold disease agent *B. cinerea* on eggplant *in vitro* and *in vivo*. *Pseudomonas chlororaphis* supsp. *aurofaciens* and *Bacillus amyloliquefaciens*, which were the most efficient strains found *in vitro* conditions, were also effective against *B. cinerea in vivo*. In conclusion, it was determined that two most effective bacterial strains could be used as a biocontrol agent in the biological control of *B. cinerea* in eggplant growing.

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Patlıcanda Kurşuni Küf Hastalığı (*Botrytis cinerea*) Üzerine Bazı Bakteriyel İzolatların *in vitro* ve *in vivo* Koşullarda Biyolojik Mücadele Etkinliklerinin Araştırılması

ÖZET

Kurşuni küf etmeni *Botrytis cinerea* (teleomorph: *Botryotinia fuckliana* (de Barry) Whetzel) ekonomik öneme sahip birçok sebzede önemli verim kayıplarına neden olmaktadır. Verim kaybını azaltmak için mücadelede kimyasallar kullanılmaktadır. Kimyasalların bitki hastalıkları mücadelesinde yoğun şekilde kullanılması sonucu, insan sağlığı ve çevre olumsuz etkilenmektedir. Bundan dolayı son zamanlarda çevre dostu mücadele uygulamalarına ilgi giderek artmıştır. Bu uygulamalardan birisi de faydalı bakterilerin hastalıkların biyolojik mücadelesinde kullanılmasıdır. Bu çalışmada; 12 biyoajan bakteri izolatının (1 *Bacillus cereus,* 2 *Bacillus megaterium,* 2 *Bacillus pumilus,* 2 *Bacillus subtilis,* 1 *Bacillus thuringiensis* subsp. *kurstaki,* 1 *Paenibacillus polymyxa,* 2 *Pantoea agglomerans* ve 1 *Pseudomonas fluorescens*) patlıcanda kurşuni küf hastalığına neden olan *B. cinerea* üzerine etkisi *in vitro* ve *in vivo* şartlarda belirlenmeye çalışılmıştır. *In vitro* şartlarda en etkili izolatlardan olan *Pseudomonas chlroraphis* subsp. *aureofaciens* ve *Bacillus amyloliquefaciens*'in *B. cinerea*'ya karşı *in vivo* şartlarda da etkili olduğu tespit edilmiştir. Sonuç olarak; en etkili bulunan 2 bakteri izolatının patlıcan yetiştiriciliğinde *B. cinerea*'nın biyolojik mücadelesinde biyokontrol ajanı olarak kullanılabileceği belirlenmiştir.

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INTRODUCTION

Botrytis cinerea is one of the most common studied fungal pathogens, causing gray mold rot in more than 500 plant species (Williamson et al., 2007). This pathogen has adverse effects on a variety of economically important crops and is classified as the important plant fungal pathogen (Dean et al., 2012). The annual economic losses of *B. cinerea* easily exceed $ 10 billion worldwide (Weiberg et al., 2013). Jiang and Liu (2015) stated that gray mold (*Botrytis*) is a common fungal disease in eggplants, reducing the production by 20-30%.

B. cinerea has a wide host range, various attack modes, and both sexual and asexual stages to survive in suitable or unsuitable conditions (Fillinger and Elad, 2016). Conidia (asexual spores) of *B. cinerea* are easily spread by wind or water (Brandhoff et al., 2017). To date, the primary means of controlling gray mold has remained as the application of synthetic fungicides, which may account for about 8 percent of the entire global fungicide market, with annual global expenditures on *Botrytis* control often exceeding € 1 billion (Dean et al., 2012). However, the controlling effects of fungicides on *B. cinerea*, whose plastic and prone to develop chemical resistance genes, are unsatisfactory (Droby et al., 2009; Kanetis et al., 2017). Even though the utilization of chemicals seems to be simple and successful in the short-term control against the pathogen, it should not be disregarded that the fungus develops resistance against chemicals in a short time and sporulation rapidly occurs, in addition to its permanent negative effects on human and environmental health. For this reason, it is necessary to either develop an alternative control method to chemical control or to perform applications that would enhance the effectiveness of this control method. Today, with the increase of environmental awareness, an increase in studies regarding putting forth the negative effects of chemicals used in the control against pathogens and the use of environmentally friendly preparations in controlling diseases is observed (Aşkın and Katırcıoğlu, 2008; Soylu et al., 2010; Laslo and Mara, 2019). The fact that some of these environmentally friendly methods have been made into preparations and have been started to be used in many countries over the years is the best proof that studies on biological controlling have been fruitful (Uygun et al., 2010). In recent years, the use of plant growth promoting bacteria (PGPB) strains for both microbial fertilization and biological control has increased (Ji et al., 2006). Bacterial antagonists represent an important biocontrol option against diseases caused by *B. cinerea* due to their rapid development and the diversity of antifungal and defense elicitor compounds they produce (Haidar et al., 2016).

In this study, it was aimed to develop environmentally friendly bioagents that can be alternative to chemicals in the control against gray mold and do not threaten the health of humans and other organizms.

MATERIAL and METHOD

Material

Plant material, pathogenic fungus isolate and potential biogenic bacteria strains

Eggplant stems and fruit samples that were demonstrating gray mold symptom were collected during survey studies conducted in greenhouses in Serik District of Antalya. *B. cinerea* ET 33 isolate was obtained as a result of isolations from diseased samples. Also, eggplant variety (cv. Berceste F1) was used as the test plant. 12 bacteria strains were selected as bioagents from the bacterial strains tested against bacterial and fungal plant pathogens and pests in previous studies from the Microorganism Culture Collection at Atatürk University, Faculty of Agriculture, Plant Protection Department (Table 1).

Methods

Isolation of fungal disease agent

Surface disinfection was applied to the samples brought from the field in the laboratory. After, they were placed in petri dishes containing Potato Dextrose Agar (PDA) and left for incubation at 20-25 °C. Pure cultures were obtained by taking hyphal discs in a diameter of 4 mm from the tip of colonies that were developed within 7-8 days, and transferring them to petri dishes containing PDA. These isolates were then transferred to the test tubes containing PDA, and were preserved at +4 °C for the later stages of the study.

Pathogenicity test of fungal disease agent

Eggplant seedlings were planted in the soil that was prepared with the mixture of organic matter-rich peat and perlite in equal volume, and were kept in the plant growth cabinet at 24 °C for 12-hour dark and 12-hour light cycles. After the eggplant seedlings reached the period of 3-5 leaves, 30 µl sterile water was given to their stems via wounds opened 5 cm above the soil with the help of a micropipette, and then micellar discs of 4 mm diameter, which were taken from the fungal culture developed for 7-8 days, were placed in these wounds. For controls, sterile water was similarly given to the inflicted wounds, followed by the inoculation of sterile PDA discs. The treated plants were kept in wet polyethylene bags for 24 hours to obtain high humidity at 25°C temperature.

Table 1. Identification, hypersensitivity, nitrogen fixation, phosphate solubility and pathogenicity test results of bacterial bioagent strains used in this study.

Çizelge 1. *Çalışmada kullanılan biyoajan bakteri strainlerinin tanısı, aşırı duyarlılık testi, azot fiksasyonu, fosfat çözünürlüğü ve patojenite testi sonuçları*

Bacterial strains	*Isolated from*	*MIS Results*	*S**	*ITS results*	*Accessed number***	*I (%)*	*N*	*P*	*HR*	*Reference*
TV-87A	Sugar beet	*Bacillus megaterium*	0.467	*Bacillus amyloliquafaciens*	MN507862	95	+	-	-	Erman et al., 2010
RK-79	Apple	*Pantoea agglomerans*	0.762	-	-		+	+		Gökçe and Kotan, 2016
TV-67C	Raspberry	*Bacillus pumilus*	0.630	-	-		-	-	-	Erman et al., 2010
TV-17C	Raspberry	*Bacillus subtilis*	0.677	-	-		+	+		Çakmakçı et al., 2010
TV-6F	Wheat	*Bacillus subtilis*	0.831	-	-		+	-		Çakmakçı et al., 2010
FDG-37	Soil	*Pseudomonas fluorescens*	0.222	*Pseudomonas chlororaphis* subsp. *aureofaciens*	NR114473	98.7	+	+	-	Karagöz et al., 2016
RK-92	Pear	*Pantoea agglomerans*	0.889	-	-		+	+	-	Gökçe and Kotan, 2016
TV-91C	Wheat	*Bacillus megaterium*	0.474	-	-		+	+	-	Çığ et al., 2014
TV-12E	Wheat	*Paenibacillus polymyxa*	0.551	-	-		+	+	-	Erman et al., 2010
BAB-410	*Ricania simulans*	*Bacillus thuringiensis* subsp. *kurstaki*	0.620	-	-		*	*	-	Göktürk et al., 2018
TV-125A	Sahlep	*Bacillus cereus*	0.297	-	-		*	*	-	Çığ et al., 2014
FD-9	*Bemisia tabaci*	*Bacillus pumilus*	0.620	-	-		*	*	-	In this study

S:** Similarity index, *** GenBank, **I:** Identification, **N:** Nitrogen fixation, **P:** Phosphate solubility, **+:** Positive reaction, **-:** Negative reaction, **HR:** Hypersensitivity test

The seedlings were taken from the moisture chamber one day after the inoculation. Fourteen days after the inoculation, the isolates were recovered through the re-isolation from the parts demonstrating disease symptoms, and thus Koch postulates were completed.

Molecular identification of fungal disease agent

Morphological diagnosis of ET 33 isolate developed in PDA was made and molecular identification was made using the ITS1 (5´TCC GTA GGT GAA CCT TGC GG 3 ´) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). After the obtained sequence data were combined with the BioEdit program, they were compared with the species found in the GenBank via the program BLASTN + and sequence data entry was performed (Table 1).

Identification of bacterial strain FD-9 by microbial identification system (MIS) and hypersensitivity test

Fatty acid methyl esters of the FD-9 strain was analyzed according to the standard protocol of the Microbial Identification (= MIS, MIDI, Inc., Newark, DE) system (Paisley, 1995). These tests were repeated 3 times, and the highest diagnostic result in terms of percentage was considered as the definitive result.

The bacterial strain FD-9 was tested for hypersensitivity on tobacco plants (*Nicotina tabacum* L. var. Samsun) as described before (Klement, 1968).

Determination of *in vitro* antagonistic activities of bacterial strains

For this purpose, a disc of 4 mm diameter was taken from 5 days old active growing fungal culture and was placed in the middle of the petri dishes containing PDA. Putative antagonist bacterial strains, were drawn on the edge of the petri with a swab, and were left for incubation in the dark at 25 °C. As a control, only pathogenic fungus with PDA was cultivated. Measurements were made when the petri dishes completely covered the fungal mycelium agar surface in the control petri dish (Tozlu et al., 2018). The inhibition percentage of the fungal colony growth by bacterial bio control agent was calculated via the utilization of the radial growth inhibition percentage formula described by Wang et al. (2012).

Inhibition (%) = (C−T) × 100 / (C −6)

C: the diameter of the mycelial growth in control petri plates

6: the diameter of pathogen disk

T: the diameter of mycelial growth in treated petri plates

In the study, 3 petri plates were used per bacterial isolate.

In vivo tests

To test the effectiveness of 5 bacterial strains, which were found effective *in vitro* conditions against pathogen, a pot experiment with eggplant seedlings was established in the climate chamber with 3 repetitions. Bioagent bacterial strains were streaked on Nutrient Agar (NA), incubated at 28°C for 48 hours, then were reinoculated into Nutrient Broth (NB) incubated at 28°C for 24 hour furthers. Bacterial cell was centrifuged and its concentration was adjusted to 10^{8} cfu/ mL 30 µl of the suspension prepared from bioagent bacteria was given to the wounded part inflicted on the stem of the eggplant seedlings with the help of a micropipette. And then, the mycelium disc taken from the tip of the pathogen fungus, which was developed in PDA, was placed in the suspension-applied wound, and the stem was wrapped with parafilm. The study was carried out in 3 repetitions. The following 0-4 scale was used in the evaluation.

0: No leaf lesion

1: 5% of leaf area infected

2: 25% of leaf area infected

3: 50% of leaf area infected

4: 75% or more of leaf area

The scale values obtained from *in vivo* test results were converted to percent disease severity with the help of the disease severity (DS) formula (Viriyasuthee et al., 2019). Disease severity (DS) was calculated as follows:

$DS\ (\%) = \Sigma\ (S \times L) \times 100 / (M \times S_{max})$

DS= disease severity

S: rating score

L: number of plant in rating

M: total number of sampled plants

Smax: highest rating

Molecular diagnosis of the most effective antagonist bacterial strains

Molecular characterization of the most effective strains TV-87A and FDG-37 was realized through targeting the 16Sr region.

Genomic DNA of bacterial strains were extracted from bacterial suspension of strains using the genomic DNA Purification Kit (QIAGEN) according to the manufacturer's instructions. Amplicons for the 16S rDNA sequences were generated using universal primers 27F (AGAGTTTGATCMTGGCTCAG) and 907R (CCGTCAATTCMTTTRAGTTT) using published reaction conditions (Chandler et al., 2011). After the obtained sequence data were combined with the BioEdit program, they were compared with the species found in the GenBank (www.ncbi.nlm.nih.gov) (Zhang et al., 2000) via the program BLASTN +, and sequence data entry was performed (Table 1).

Statistical Analysis

Analysis of variance was applied to the values regarding the obtained *in vitro* test results, and the differences between the averages were compared with the LSMeans Differences Student's test at a significance level of P <0.01. Data analysis was made via the utilization of JMP IN (SAS Institute, cary, NC,%.0 PC version) statistics software.

RESULTS and DISCUSSION

Following molecular diagnosis of ET 33 isolate fungal disease agent was identified as *Botrytis cinerea* with 99% similarity to those available in GenBank.

According to the results of analysis of variance in the study, the difference between applications was found to be statistically significant (mycelial growth F: 7.8012; P < 0.01 and inhibition of mycelial growth F: 7.8008; P < 0.01). Differences between groups were also determined using the LSMeans Differences Student's test. The percentage inhibition rate results of bacterial strains whose antifungal effects were tested under *in vitro* conditions against the ET 33 isolate of *B. cinerea* are presented in Table 2.

Table 2. Antifungal activities of the bacterial strains against *Botrytis cinerea* ET 33 isolate in in vitro condition.
Çizelge 2. *In vitro şartlarda Botrytis cinerea'nın ET 33 izolatına karşı biyoajan bakteri strainlerinin antifungal aktivitesi.*

Bacterial strains	Mycelial Growth(mm)	Inhibition of Mycelial Growth(%)
Bacillus pumilus FD-9	34.2 A	65.23 A
Pseudomonas chlororaphis subsp. *aureofaciens* FDG-37	34.2 A	65.23 A
Bacillus subtilis TV-6F	36.7 A	62.14 A
Bacillus amyloliquafaciens TV-87A	37.5 A	61.11 A
Bacillus pumilis TV-67C	39.0 A	59.26 A
Bacillus subtilis TV-17C	47.3 AB	48.97 AB
Bacillus thuringiensis subsp. *kurstaki* BAB-410	58.3 BC	35.39 BC
Bacillus megaterium TV-91C	58.8 BC	34.77 BC
Pantoea agglomerans RK-92	68.3 CD	23.05 CD
Pantoea agglomerans RK-79	69.3 CDE	21.81 CDE
Bacillus cereus TV-125A	70.2 CDE	20.78 CDE
Paenibacillus polymyxa TV-12E	83.8 DE	3.91 DE
Control (*Botrytis cinerea*)	87.0 E	0.00 E
CV	19.55	34.88
LSD	1.83	22.68

*Mean values given in the same column followed by the same letter are not significantly different according to the LSD Means Student's test ($P < 0.01$).

In the bacterial strains tested in the *in vitro* experiments, while the bacterial strains FD-9 and FDG-37, which have the highest efficacy, inhibited the development of pathogenic fungus by 65.23%, the TV-12E strain, which has the lowest efficacy, took place in the same group with the control with a 3.91% inhibition rate (Table 2, Fig 1).

The effectiveness of the bacterial strains of TV-87A, FDG-37, TV-6F, TV-67C and FD-9, which are highly effective under *in vitro* conditions, were further tested under *in vivo* conditions in pot trials, and their symptoms were assessed (Table 3).

In the conducted study, no symptoms were encountered neither in the controls where only NB and only bioagent applications were performed, nor in in the eggplant plants in pots where TV-87A bacterial strain and ET 33 fungal isolate were applied together, and statistically these applications were included in the same group (Table 3). These applications were followed by pots in which bacterial strains FDG-37 (5.88%), TV-6F (82.50%), TV-67C (85.83%) and FD-9 (87.50%) were applied together with the pathogen. The most severe symptoms were detected in pots (93.33%) where disease agent ET 33 was applied alone or applied together with bacterial strains TV-6F and TV-67C (Table 3). Pot assay of bacterial strains tested in *in vivo* conditions is presented in Fig 2.

Molecular diagnosis was performed from pure cultures of 2 bacterial biocontrol agents that showed the highest inhibitory effect in both *in vitro* and *in vivo* tests. FDG-37 and TV-87A were identified as *Pseudomonas chlororaphis* subsp. *aureofaciens* (similarity: %98.7; accession number: NR114473) and *Bacillus amyloliquafaciens* (similarity: %95; accession number: MN507862) respectively.

Due to the inadequacy of cultural measures alone and the damage caused by the unconscious use of pesticides to the environment, studies all over the world have turned to alternative methods that can be utilized in the control against different plant pathogens. Among these methods, biological control also has an important place.

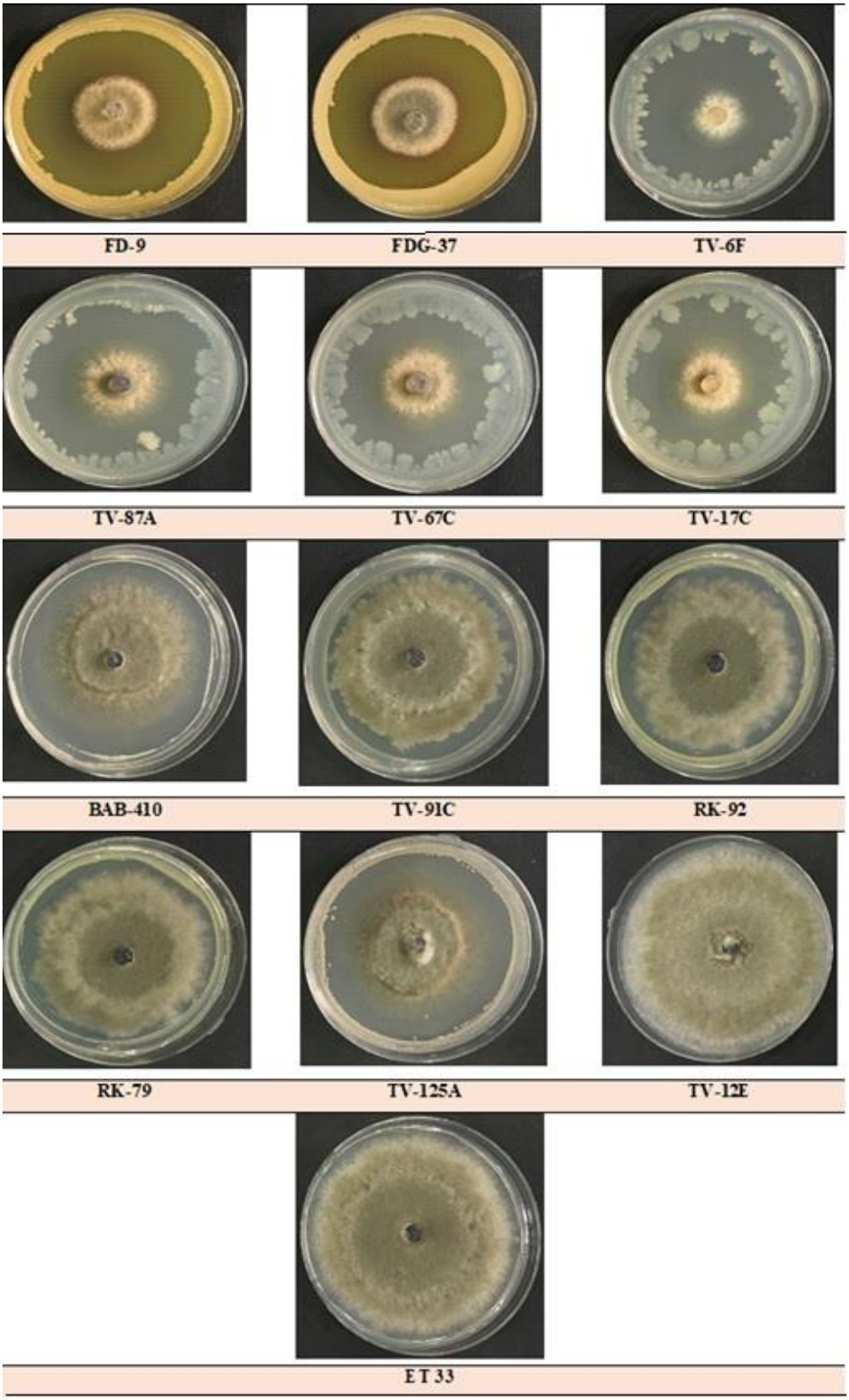

Figure 1. Antifungal activities of the bacterial strains against *Botrytis cinerea* ET 33 isolate in in vitro condition.
Şekil 1. *In vitro şartlarda Botrytis cinerea'nın ET 33 izolatına karşı biyoajan bakteri strainlerinin antifungal aktivitesi.*

Figure 2. The most effective biocontrol bacterial strains against Botrytis cinerea ET 33 isolate in in vitro and in vivo condition (Control 1: Only pathogen, Control 2: Only biocontrol agent, Control 3: Only NB).

Şekil 2. *In vitro ve in vivo şartlarda Botrytis cinerea'nın ET 33 izolatına karşı en etkili biyoajan bakteri strainleri (Kontrol 1: Sadece patojen, Kontrol 2: Sadece biyoajan bakteri, Kontrol 3: Sadece NB).*

Table 3. *Antifungal effect of tested bacterial strains against Botrytis cinerea ET 33 isolate in in vivo conditions.*
Çizelge 3. In vivo şartlarda Botrytis cinerea'nın ET 33 izolatına karşı biyoajan bakteri strainlerinin antifungal aktivitesi.

Treatment		*DS (%)**
Control 2	TV-87A	0.00 A
	TV-67C	0.00 A
	FDG-37	0.00 A
	TV-6F	0.00 A
	FD-9	0.00 A
Control 3 (Only NB)		0.00 A
TV-87A+ET 33		0.00 A
FDG-37+ET 33		5.88 A
TV-6F+ET 33		82.50 B
TV-67C+ET 33		85.83 BC
FD-9+ET 33		87.50 BC
Control 1 (Only pathogen)		93.33 C
	CV	**19.29**
	LSD	**9.66**

*DS: Disease severity. Mean values in the same column by the same letter are not significantly different to the LSMeans Student's test ($P < 0.01$).

The *Bacillus* species are most frequently used biocontrol agents in biological control studies. The antimicrobial compound(s) produced by *Bacillus* spp. can be formulated in a cheap, stable, and effective manner revealed that these organisms can be used safely in modern agriculture (Göğüsgeren and Çolak, 2009). In addition, the fact that *Bacillus* species form spores can be found in almost all types of soils, and endophtically colonized plants has led them to be widely used as bioagents (Tiwari et al., 2014). As with many plant pathogens, studies were conducted where species belonging to the *Bacillus* genus were used in the biological control against *B. cinerea* and successful results were obtained. Recently, Aktan and Soylu (2020) also reported that PGPB isolates of *Pseudomonas* spp and *Enterobacter cloacae* were found highly effective for siderophore production, *S. marcescens* for solubilisation of phosphorus, *E. cloacae* for IAA production, *Bacillus* spp for ammonia production. Overall their results suggest that use of the most efficient PGPB isolates have an excellent potential to be used as biofertiliser for cultivation of economically important crops.

In a study a total of 163 bacterial strains isolated from tomato leaves were evaluated for their ability to suppress gray mold on tomatoes, and 4 strains were found to reduce the incidence of *B. cinerea* (Yıldız et al., 2007). In another study on tomato, where the effectiveness of 2 strains belonging to the *Bacillus* genus was determined in both laboratory and greenhouse conditions against the *B. cinerea*, it was reported that both strains reduced the disease severity by 67% and 66%, respectively (Sheng et al., 2016). In very recent study, antifungal activivites of several putative bacterial biocontrol agent (BCA) isolates, which were obtained from commercial vermicompost, were investigated against soilborne fungal disease agents *Sclerotinia sclerotiorum, Macrophomina phaseolina, Botrytis cinerea, Verticilium dahliae in vitro*. According to their results, 28 bacterial isolates (49.12% of total isolates) were inhibited mycelial growth of *S. sclerotiorum* by 1.72- 75.43%, *M. phaseolina* by 1.67-65.83%, *B. cinerea* by 3.44-57.18%, and *V. dahliae* by 2.28-58.74%, respectively (Soylu et al., 2020). In a study conducted on strawberries, 186 bacterial strains were tested against the gray mold disease agent, and it was determined that 36 of tested isolates suppressed the development of *B. cinerea*, and that among the *Bacillus* species, *B. lentimorbus*, *B. megaterium*, *B. pumilis*, *B. subtilis* ere the most effective species (Dönmez et al., 2011). In another study, it was reported that *Xanthomonas maltophila*, *B. pumilis, Lactobacillus* sp., *Pseudomonas* sp., and *Gliocladium catenulatum* strains (Elad et al., 1994) reduced the *B. cinerea* sporulation, whereas *Paecilomyces lilacinus*, *Bacillus firmus*, and *Actinomycetes* sp. reduced the severity of infection (Yıldız, 1990).

Çiftçi and Altınok (2019) found *Pseudomonas aeruginosa* as the most successful isolate against *B. cinerea* in pot experiments with a yield rate of 58.1% compared to the positive control containing only pathogen inoculum on eggplant seeds.

Toral et al. (2020) evaluated the potential use of XT1 as a plant growth promoter and biocontrol agent against *B. cinerea*, the results of this study revealed the importance of jasmonic acid (JA) and ethylene hormones.

TV-6F strain of *B. subtilis*, was previously tested under *in vitro* conditions and was found to suppress disease development successfully in the previous studies such as in red cabbage against *S. sclerotiorum* (76.46%)

(Tozlu et al., 2016), in raspberry against *B. cinerea* (95.24%) (Tekiner et al., 2018), in cucumber (77.61%), strawberry (77.11%) (Tozlu et al., 2018) and in quince (77.61%) against *Alternaria alternata* (Tekiner et al., 2019), in citrus fruits against *Penicillium digitatum* (69%) (Mohammadi et al., 2017) and in tomato against *Alternaria solani* (69%) (Çamlıca and Tozlu, 2019). In this study, the TV-6F strain inhibited the mycelial growth of *B. cinerea*, *in vitro* conditions by 62.14%. TV-87A strain of *B. megaterium*, whose effectiveness was tested in this study similarly, was tested against *A. alternata*, which was isolated from cucumber and strawberry in a previous study, and was found to inhibit the development of the pathogen at the rates of 77.61% and 77.11% respectively (Tozlu et al., 2018).

It was reported in another study that the TV-87A strain of *B. megaterium* was also effective under the control of *Bipolaris sorokiniana*, which causes wheat root rot, and caused significant increases in plant growth parameters (Gökçe and Kotan, 2016). This strain, which also inhibited the development of *B. cinerea* at the rate of 61.11% *in vitro* in this study, completely stopped the pathogen development in eggplant plants under *in vivo* conditions.

Similarly, the TV-67C strain of *B. pumilus* was tested against *A. alternata*, which was isolated from cucumber and strawberry, and was found effective at the rates of 87.63% and 65.89%, respectively (Tozlu et al., 2018). In addition, Çamlıca and Tozlu (2019) detected that TV-67C strain prevented the development of *A. solani* in tomato under *in vitro* conditions at the rate of 69%.

In several studies, some fluorescent *Pseudomonas* species asserted disease control in lettuce and tomato plants under greenhouse conditions (Lee et al., 2006). Walker et al. (2001) detected that strain of *Pseudomonas antimicrobica* prevented the conidial germination of *B. cinerea*. It was determined that FDG-37 strain of *P. fluorescens* prevented the development of *Geotrichum candidum*, which causes bitter rot disease in carrots, under *in vitro condition* at the rate of 26.85% (Tozlu, 2016), and prevented the development of *B. sorokiniana*, causing root rot in wheat, at the rate of 66.39% (Gökçe and Kotan, 2016). In this study, on the other hand, the same isolate was effective at a rate of 65.23% against the *B. cinerea*, which was isolated from eggplant.

CONCLUSION

In this study, antagonistic bacterial strains belonging to *Bacillus* (8), *Pantoea* (2), *Paenibacillus* (1), and *Pseudomonas* (1) genus were tested against *B. cinerea* ET 33 isolate under *in vitro* and *in vivo* conditions. Results indicated that antagonist bacterial strains *P. chlororaphis* subsp. *aureofaciens* FDG-37 *and B. amyloliquafaciens* TV-87A were effective against *B. cinerea*. Microbial formulations consisting of these bacteria have direct potential for the control of *B. cinerea*. In future, further studies are being planned to prepare a commercial preparation after making a good carrier consisting of organic material with long shelf life for the most effective bacterial strain.

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Author Contribution Rates

The authors declare that they contribute equally to the article.

Conflict of interest/Competing interests

The authors declare that there is no conflict of interests.

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