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Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Biological Control of Cotton Seedling Diseases by Fluorescent *Pseudomonas* spp.

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ARTICLE INFO

Research Article
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Received: 17 December 2014, Received in Revised Form: 03 March 2015, Accepted: 06 March 2015

ABSTRACT

Seedling root rot seen in many plants including cotton is an important disease that leads to large economic losses. Human health and the environment are negatively affected as a result of using fungicides for disease control. The goal of this study was to determine the effects of fluorescent *Pseudomonas* (FP) bacteria against seedling root rot pathogens both *in vitro* and *in vivo* conditions. 59 FP isolates obtained from the rhizosphere of cotton and weeds on the field were tested by dual-culture assays *in vitro*. After applying effective FP isolates on the seeds, antagonistic effects against the seedling root rot pathogens were investigated in a climate chamber. Resulting of dual-culture tests, FP40 had maximum effect (49.60%) against *Rhizoctonia solani*. Besides, FP51, FP48 and FP35 had highest impact as 43.80%, 43.50%, and 43.10% against *Fusarium* sp., respectively. *Pythium deliense* was mostly effected by FP57 (59.80%), FP52 (57.80%) and FP56 (57.60%). While isolates FP35 and FP57 provided protection over 70% against all three pathogens in a climate chamber, they were as effective as commercial fungicides (Vitavax and Maxim) and biofungicide (Subtilex) and shown promising results.

Keywords: Biocontrol; Biofungicide; Seedling disease; Fluorescent *pseudomonas*

Fluoresan *Pseudomonas* spp. ile Pamuk Fide Kök Çürüklüğü Hastalıklarının Biyolojik Mücadelesi

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Geliş Tarihi: 17 Aralık 2014, Düzeltmelerin Gelişi: 03 Mart 2015, Kabul: 06 Mart 2015

ÖZET

Fide kök çürüklüğü pamuk dahil pek çok bitkide görülen ve ekonomik kayıplara yol açan önemli bir hastalıktır. Fungisitler hastalığa karşı mücadelede kullanılması sonucu, çevre ve insan sağlığı olumsuz yönde etkilenmektedir. Çalışmada, fluoresan pseudomonas (FP) bakterilerinin fide kök çürüklüğü hastalık etmenlerine karşı *in-vitro* ve *in-vivo* koşullarda

etkilerinin belirlenmesi amaçlanmıştır. Pamuk ve tarladaki yabancı otların rizosferinden izole edilen 59 adet FP izolatı ile *in vitro*'da ikili kültür testleri yürütülmüştür. Daha sonra etkili bulunan FP izolatları tohuma uygulanarak fide kök çürüklüğü etmenlerine karşı antagonistik etkileri iklim odasında araştırılmıştır. İkili kültür testlerinde, *Rhizoctonia solani*'ye karşı en yüksek etkiyi FP40 (% 49.60); *Fusarium* sp.'ye karşı en yüksek etkiyi FP51 (% 43.80), FP48 (% 43.50) ve FP35 (% 43.10); *Pythium deliense*'ye karşı en yüksek etkiyi FP57 (% 59.80), FP52 (% 57.80) ve FP56 (% 57.60) izolatları göstermiştir. İklim odasında, FP35 ve FP57 izolatları her üç patojene karşı % 70'in üzerinde koruma sağlarken, ticari fungisitler (Vitavax, Maxim) ve biyofungisit (Subtilex) kadar etkili bulunmuş ve ümitvar sonuçlar elde edilmiştir.

Anahtar Kelimeler: Biyolojik mücadele; Biyofungisit; Fide hastalığı; Fluoresan *pseudomonas*

1. Introduction

Crop losses in agricultural production occurred in 9.1% by disease, 11.2% by pests and 14.7% by the weeds in the world. This amount is equivalent to one-third of the world agricultural production potential. Annual monetary value of these losses is about 550 billion dollars and the costs incurred to protect the product are about 455 billion dollars annually (Agrios 2005). The diseases caused yield losses with average 3.1% per year and 27% reduction in fiber production for 10 years study in the USA (Devay 2001). *Rhizoctonia* spp., *Pythium* spp. and *Thielaviopsis* spp. are seen as the most common and destructive damping off disease agents have been reported in the world (Agrios 1998).

These pathogens are seen in many plants including cotton and causes large economic losses. Cotton seedling root rot inducing factors can cause the seedling root and root collar to decay and let them to die especially in infected and heavy soils during wet and cool seasons, thus leading major destruction in cotton fields and sometimes requiring replanting of the field (Agrios 2005). Despite their promise in prevention of the damping off diseases, fungicides have some problems because of phytotoxicity, environmental pollution and human health effects (Ramamoorthy et al 2002). Moreover, when disease effect is in the level of requiring replanting, there will be space in the field due to less seedlings and the farmers generally use more seeds than necessary to compensate for this risk. In replanting, the expenses for seed, seedbed preparation and soil cultivation cost increase and the yield decreases because of late planting.

Hoitink (1986) reported that chemical application is generally not successful for soil-borne pathogens whereas bio-control agents are well colonized in the rhizosphere and don't have the toxic effects on the leaves like chemicals, and they have not only control the disease but also have positive effects on plant development. On the other hand, *R. solani* was suppressed about 60% due to "chitinase enzyme" with the inoculated cotton seeds with isolates of antagonistic bacteria of *Bacillus cereus*, *B. subtilis* and *B. pumilus* in the greenhouse (Pleban et al 1995). After the treatment of cotton seeds with *Pseudomonas fluorescens* race 89B-61, antagonist bacteria colonized the roots of cotton well and protected the plant against soil-borne diseases (Quadt et al 1997). Wang et al (2004) reported that *Pseudomonas fluorescens* CS85 was previously isolated from the rhizosphere of cotton seedlings as a plant growth-promoting bacterium and biocontrol agent against *R. solani*, *Colletotrichum gossypii*, *F. oxysporum* f.sp. *vasinfectum*. Various biocontrol agents such as *Burkholderia cepacia* and *B. subtilis* have effective results throughout the world. Isolate GBO3 of *B. subtilis* and Dagger-G biofungicide of *P. fluorescens* were tested *in vitro* against *Pythium ultimum* and *Fusarium* spp. in cotton seeds and found that antagonists reduce the damage of bacterial diseases in cottonseed (Agile & Batson 1999). Nowadays, commercially named biofungicides Deny®, Subtilex® and Kodiak® are suggested against the seedling diseases (Gardener & Fravel 2002).

Works conducted against important damping off diseases using non-pathogenic *Pseudomonas* in tomato nurseries in the greenhouse and field showed that the isolates 10, 11, 23, 32 and 44 had an effect over 50% against *P. deliense* and *R. solani* in pot trials while isolates 23 and 44 were also effective against *Sclerotinia minor* and *Alternaria solani* in the field (Aşkın & Katırcıoğlu 2008). In another experiment directed by Mahmood Janlou et al (2008) conducted in the field during 2002-2003 in Iran with three isolates of *P. fluorescens* and two isolates of *Bacillus* spp. using two different fungicides (carbendazim and carboxin+thiram) and two different cotton varieties (Sahel and Siokra). Resulting that one isolate of *P. fluorescens* and two isolates of *Bacillus* spp. were effective against seedling root rot disease as fungicides in both years. They reported that it is possible to use combination of both, fungicides and antagonist bacteria against diseases in the field.

In the present study, the effects of fluorescent *Pseudomonas* strains against cotton seedling diseases were tested both *in vitro* and *in vivo* conditions.

2. Material and Methods

2.1. Plant material, pathogens and fluorescent Pseudomonas spp. strains

Delinted seeds of cotton (*Gossypium hirsutum* L.) cultivar Carmen were the plant materials. Fifty-nine fluorescent *Pseudomonas* strains isolated from cotton and weeds were used in this study that effective in the root zone of cotton as well as summer and winter weeds (Erdoğan & Benlioğlu 2010). Three pathogenic isolates of *Rhizoctonia solani* AG4, *Fusarium* sp. and *Pythium deliense* used in the experiment were originally isolated from the roots of cotton and tomato seedlings infected with damping-off disease. Isolation, purification and identification of these fungi were carried out at Adnan Menderes University, Mustafa Kemal University and Süleyman Demirel University, Faculty of Agriculture, Department of Plant Protection. Fungicides, active substances and application doses are given in Table 1.

Table 1- Fungicides, active substance and application doses

Çizelge 1- Fungisitler, etkili madde ve uygulama dozları

*Trade name**	*Company*	*Active substance and percentage*	*Formulation*	*Dose*
Vitavax 200 FF	Hektaş	Carboxin 205 g L^{-1} + Thiram 205 g L^{-1}	FF	400 mL 100 kg^{-1} seed
$MaximXL_{035FS}$	Syngenta	Fludioxonil 25 g + Mefenoxam 10 g	FS	300 mL 100 kg^{-1} seed
Subtilex Seed™	Bioglobal	*Bacillus subtilis* MBI 600	WP	18 g 100 kg^{-1} seed

*, Vitavax 200 FF and $MaximXL_{035FS}$, commercial fungicides; Subtilex Seed™, Biofungicide

2.2. Preparation of pathogen inoculums

Inoculum for seedling disease agents (*R. solani*, *Fusarium* sp. and *P. deliense*) was prepared by recommendation of Martin (2000) that is oat bran formulation (30 g Oat bran, 30 g vermiculite, and 60 mL of sterile water). Then, *R. solani* and *Fusarium* sp. cultures were developed in media of Yeast Dextrose Agar (YDA) and Potato Sucrose Agar (PSA) at 24±1 °C for one week. An agar disk from the edge of *P. deliense* culture produced on Corn Meal Agar (CMA) at 21±1 °C for one week was taken and mixed as 4-5 counts to each bag. Bags were closed tightly and incubated (24±1 °C and 21±1 °C) for three weeks and mixed at the end of the first week.

2.3. In vitro assay

In vitro inhibition zone tests for FP strains against *R. solani*, *Fusarium* sp. and *P. deliense* were performed according to the dual-culture assay in which FP strains were subjected to pre-selection against three damping-off agents (*R. solani*, *Fusarium* sp. and *P. deliense*) on Potato Dextrose Agar (PDA)

plates. Then, to determine the rate of inhibition by FP strains, *in vitro* experiment was conducted as randomized plot design with three replications on PDA plates. Each plate was inoculated with four droplets of 10 µL bacterial suspension (at 10^8 cfu mL^{-1} concentration) symmetrically placed on four sites at equal distances (2 cm) from the center of plate. As a control, 10 µL of sterile water was dropped into four separate points on PDA plate. After incubation of PDA in petri plates for one day at 24±1 °C, an agar disc of 5 mm diameter taken from the edge of ten days grown *R. solani* (AG4), *Fusarium* sp. and *P. deliense* cultures and planted into the middle point of the bacteria inoculated petri plates and the control petri plates. After seven days incubation period of media for *R. solani* (AG4) and *Fusarium* sp. at 24±1 °C, for *P. deliense* at 21±1 °C, colony diameters of *R. solani* (AG4), *Fusarium* sp. and *P. deliense* were measured separately and percent inhibition zones were determined by the Equation 1 (Weller & Cook 1986; Gamliel & Katan 1993).

Inhibition Zone (%)= C-T/C x 100 (1)

Where; C, diameter (mm) in the petri plates used as a control; T, fungi colony diameter (mm) in petri plates with bacteria.

2.4. In vivo experiments

A local cotton variety cv. Carmen seeds delinted with sulfuric acid were used in pot experiments (Akpınar & Benlioğlu 2008). Subsequently, these seeds were tested for germination percentage in a growth chamber (at 24±1 °C; 50-70% relative humidity; a 12 h light/12 h dark). Germinations were in plastic containers (1000 mL) and each container received forty seeds (ten seeds for each replicate) in a completely randomized plot design in four replications. Firstly, biofungicides (Table 1) were stirred in 1 mL of water at recommended doses and after cotton seeds coated, they let to dry for 12 h at room temperature. The control seeds were coated with only 1% of carboxy-methyl-cellulose (CMC). To coat cotton seeds with FP strains, a 2.5% of NaOCl surface disinfection for 1 min performed with sterile water. In the trial, cotton seeds (totally forty seeds for each treatment) were inoculated with each FP isolates using 1% CMC. For this purpose, antagonistic bacteria strains (at 10^8 cfu mL^{-1} concentration) produced at Nutrient Broth (NB) for 24 h, and were suspended with 1% of medium viscosity of CMC (2 mL) to coat cotton seeds (Quadt et al 1997). Seeds were spread on the filter paper to be dried in a sterile cabinet at room temperature and planted in one liter of plastic containers containing 100 mg of *R. solani* (AG4), *Fusarium* sp., and *P. deliense* inoculum within 24 h. Approximately, seven days after planting, germinated seeds were counted and recorded. Seeds that were not germinated or germinated but later knocked down and intact seedlings were counted and evaluated. Percentage of disease incidence (DI) was calculated using the Equation 2 (Hassanein 2012). These trials were performed as randomized plot design with four replications in a growth chamber (at 24±1 °C; 50-70% relative humidity; a 12 h light/12 h dark).

DI (%)= no. of infected plants/total no. of plants x 100 (2)

2.5. Statistical analysis

All data obtained in experiments were analyzed using JMP statistical software (PC version 5.0, SAS Institute, Cary, NC, for PC computer) with the 95% confidence level.

3. Results and Discussion

3.1. In vitro assay

In our study, the effects of fifty-nine fluorescent *Pseudomonas* strains isolated from the rhizosphere of cotton and weeds on mycelium growth of seedling root rot pathogens (*R. solani*, *Fusarium* sp. and *P. deliense*) were tested. Results of dual-culture assay *in vitro* against *R. solani* showed the highest inhibition rate (49.60%) by strain FP40. Maximum effects (43.80%, 43.50% and 43.10%) against *Fusarium* sp. were obtained from strains of FP51, FP48 and FP35, respectively. Strains of FP57, FP52 and FP47 had the highest impact against *P. deliense* as 59.80%, 57.80% and 57.60%, respectively (Table 2, 3 and 4). In a similar study, Waara et al (1993) concluded that *Pseudomonas*

species suppressed *R. solani*, *Pythium* spp., *Fusarium* spp. both *in vitro* and *in vivo*. Also, *P. fluorescens* isolated from cotton fields in Egypt was suppressed by *Pythium carolinianum in vitro* (Abdelzaher & Elnaghy 1998). The another research by Laha & Verma (1998) aiming to investigate the effects of the 58 different *P. fluorescens* isolates from cotton rhizosphere on root rot of cotton, 16 of them inhibited the development of *R. solani* by 10-36% in Pseudomonas agar in fluorescein (PAF) culture. Demir et al (1999) used 128 isolates of fluorescent pseudomonas isolated from healthy cotton seedlings and rhizosphere soils to test *in vitro* for their effect on *R. solani* and found that the application of dried xanthum gum (XG) formulations to cotton seeds were increased emergence, reduced disease incidence as compared to control seeds without bacteria. *P. fluorescens* (Gh/R 1810) was the most effective strain, resulting 16.36% greater emergence and 57.94% greater survival of cotton seedlings than the untreated control. The mycelia growth of fungi *Pythium aphanidermatum* and *R. solani* were inhibited by five of Pseudomonas isolates (Afsharmanesh et al 2006). In a research to determine antagonistic effects of six of *Pseudomonas* isolates and six of *Bacillus* isolates *in vivo* and *in vitro* against *Fusarium oxysporum* f. sp. *ciceris*, all isolates had antagonistic effect on pathogen as a result of *in vitro* dual-culture assay (Karimi et al 2012).

Table 2- Inhibition rates of FP strains against *R. solani in vitro*

Çizelge 2- In vitro'da fluoresan pseudomonas izolatlarının R. solani'yi engelleme oranları

Strains	*Origin*	*Fungus colony diameter (mm)**	*Inhibition rate (%)*
FP6	*G. hirsutum* (Nazilli 84 S)	14.41 cde	38.50
FP20	*Solanum nigrum*	13.33 def	43.20
FP30	*G. hirsutum* (Carmen)	17.40 b	25.70
FP35	*Convolvulus arvensis*	13.00 ef	44.40
FP40	*Malva sylvestris*	11.83 f	49.60
FP42	*Raphanus* sp.	16.80 b	28.20
FP43	*Malva sylvestris*	14.25 cde	38.90
FP45	*Raphanus* sp.	15.73 bc	32.90
FP47	*G. hirsutum* (Nazilli 84 S)	13.83 de	36.40
FP48	*G. hirsutum* (Carmen)	15.03 cd	35.90
FP53	*G. hirsutum* (Giza 45)	14.67 cde	32.60
FP59	*G. hirsutum* (Carmen)	14.33 cde	34.20
Control		23.40 a	00.00
CV %		6.70	

*, in the same column means with different letters indicate the significant difference (LSD test, P<0.05)

Table 3- Inhibition rates of FP strains against *Fusarium* sp. *in vitro*

Çizelge 3- In vitro'da fluoresan pseudomonas izolatlarının Fusarium sp.'yi engelleme oranları

Strains	*Origin*	*Fungus colony diameter (mm)**	*Inhibition rate (%)*
FP9	*Datura stramonium*	18.53 bcd	36.60
FP14	*Solanum nigrum*	18.17 bcd	35.50
FP20	*Solanum nigrum*	16.75 cd	41.50
FP21	*Datura stramonium*	17.83 bcd	36.70
FP23	*Portulaca* sp.	16.75 cd	41.50
FP25	*Chenepodium album*	20.00 b	31.50
FP30	*G. hirsutum* (Carmen)	19.57 b	32.90
FP35	*Convolvulus arvensis*	16.58 d	43.10
FP48	*G. hirsutum* (Carmen)	16.50 d	43.50
FP49	*G. hirsutum* (BA 119)	17.00 cd	41.30
FP51	*G. hirsutum* (Nazilli 143)	16.41 d	43.80
FP57	*G. hirsutum* (STN-8A)	19.17 bc	32.30
Control		29.20 a	00.00
CV %		7.20	

*, in the same column means with different letters indicate the significant difference (LSD test, P<0.05)

Table 4- Inhibition rates of FP strains against *P. deliense in vitro*

Çizelge 4- In vitro'da fluoresan pseudomonas izolatlarının P. deliense'yi engelleme oranları

Strains	*Origin*	*Fungus colony diameter (mm)**	*Inhibition rate (%)*
FP5	*Portulaca* sp.	18.53 bc	40.40
FP9	*Datura stramonium*	16.33 d	47.60
FP14	*Solanum nigrum*	16.40 d	47.40
FP19	*Chenepodium album*	17.25 bcd	44.70
FP20	*Solanum nigrum*	18.97 b	39.20
FP21	*Datura stramonium*	16.06 d	48.50
FP23	*Portulaca* sp.	16.25 d	47.90
FP28	*Xanthium strumarium*	15.93 d	48.90
FP30	*G. hirsutum* (Carmen)	16.73 cd	46.30
FP35	*Convolvulus arvensis*	16.83 cd	46.00
FP48	*G. hirsutum* (Carmen)	17.25 bcd	44.70
FP49	*G. hirsutum* (BA 119)	15.63 d	49.90
FP52	*G. hirsutum* (Nazilli 84)	13.17 e	57.80
FP56	*G. hirsutum* (Nazilli 84 S)	13.23 e	57.60
FP57	*G. hirsutum* (STN-8A)	12.53 e	59.80
Control		31.18 a	00.00
CV %		6.80	

*, in the same column means with different letters indicate the significant difference (LSD test, $P<0.05$)

3.2. In vivo experiments

The results of germination seven days after planting were given in Table 5, 6 and 7 for the experiment, conducted in the pots in a growth chamber in order to determine the effects of FP strains, commercial fungicides and biofungicides against seedling root rot agents in cotton. The FP strains were significantly different in pots trials. Although commercial fungicides Maxim (76.0%) and Vitavax (73.6%) as well as strains FP35 (73.2%) and FP48 (73.1%) had maximum effect against *R. solani*, they were in the same statistical group with biofungicide Subtilex (70.9%). The lowest antagonistic effect against *R. solani* was obtained from the strains FP45 (50.5%) and FP47 (51.9%). In addition, applications of Maxim, Vitavax, FP35, FP48 and Subtilex that showed highest effect against *R. solani*, also resulted the highest number of total germinated plants, 37, 36, 36, 36 and 36, respectively. Commercial fungicides Maxim (77.0%), Vitavax (73.3%) and strain FP57 (73.2%) had also maximum impact on *Fusarium* sp. and found in the same statistical grouping. In contrast, the lowest impact against *Fusarium* sp. was obtained from FP21 (35.0%). Total numbers of germinated plants were also obtained from Maxim, Vitavax and FP57 as 39, 37 and 35, respectively. *P. deliense* was affected by Maxim (79.1%) and Vitavax (76.1%) at most and followed by Subtilex (73.9%) and strains FP20 (73.9%), FP35 (73.8%) and FP57 (73.4%). The lowest impact against *P. deliense* was belonging to strain FP5 (31.6%). Moreover, the number of total germinated plants was also higher in the applications that had the highest impact on *P. deliense* (Table 5, 6 and 7). Prior to sedaxane, other fungicides, such as carboxin fungicides and several analogs, pyraccarbolid, fenfuram, methfuroxam, furrmetamid, and pyrazoles IIa and IIb were selectively effective in controlling *R. solani* in cotton in either *in vitro* or *in vivo* experiments. A seed treatment containing the active ingredient sedaxane is an innovative option for growers whose fields have a historic incidence of *R. solani* and have previously experienced soybeans losses due to the pathogen (Huppatz et al 1983). Dagger G, bioformulation of *P. fluorescens* had suppressed

both *Rhizoctonia* spp. and *Pythium* spp. in cotton (Bradow 1991). *P. flu*orescens strain BL915 was effective against *R. solani* by producing pyrrolnitrin (Hill et al 1994). In a study, Zaki & Kersten (1998) used *P. cepacia* D1 race, biofungicides such as Deny and Kodiak and fungicidal mixtures (metalaxyl, triadimenol and thiram) against *R. solani* in field trials in Arizona in 1995-1996. Study resulted that plants from the cotton seeds treated with D1 race and fungicidal mixtures were not infected with disease. Nemli & Sayar (2002) examined the effects of many fungicides and fungicide combinations against cotton seedling root rot and found that combinations of carboxin+thiram+metalaxyl and fluodioxinil+metalaxyl were more effective than other applications had. Akpınar & Benlioğlu (2008) used two of endophytic bacteria (*Burkholderia cepacia* F5), one of *Bacillus megaterium* (C5), one of biofungicide (*T. harzianum* KUEN-1565) and fungicides (Trilex, BYF 182, Vitavax 200 FF) against damping off diseases caused by *R. solani* in pot and field trials in cotton during 2006-2007. The best results of pot trials for pre-emergence damping off disease were obtained from seed treatment applications of BYF 182, Trilex, Vitavax and F5. In field trials, the lowest rate of damping off disease before emergence were obtained from fungicides Vitavax and BYF 182 in Söke and from fungicides Vitavax and Trilex in Nazilli. On the other hand, Ardakani et al (2009) reported that Bentonite-B1 application increased healthy seedlings and was more effective than applications of carboxin+thiram against *R. solani* at 15, 30, 45 and 60 days after planting in a study using *P. fluorescens* (talk and bentonite formulation) and one of fungicide (carboxin+thiram) with a cotton variety in a climate chamber in Iran.

4. Conclusions

As a result, *Pseudomonas* strains 35 and 57 were as effective as commercial fungicides (Vitavax and Maxim) and biofungicides (Subtilex) against especially all three pathogens (*R. solani*, *Fusarium* sp., and *P. deliense*) and had promising results. Coating seeds with the bacteria in the study was

Table 5- Effects of FP strains, commercial fungicides and biofungicide against *R. solani* in pot trials

Çizelge 5- Saksı denemelerinde fluresan pseudomonas, biyopreparat ve fungisitlerin R. solani'ye etkileri

Treatments	*Total emerged plants*	*Average of emerged plants (%)*	*Effect against R. solani (%)**
FP6	33	17.5	60.4 abc
FP20	33	17.5	58.1 abc
FP30	33	17.5	65.6 abc
FP35	36	9.0	73.2 a
FP40	33	17.5	61.9 abc
FP42	34	15.0	62.8 abc
FP43	33	17.5	59.1 abc
FP45	30	25.0	50.5 c
FP47	31	22.5	51.9 c
FP48	36	9.0	73.1 a
FP53	33	17.5	67.4 abc
FP59	32	20.0	54.8 bc
Control (+)	23	42.5	15.7 d
Vitavax (K)	36	9.0	73.6 a
Maxim (K)	37	7.5	76.0 a
Subtilex (K)	36	9.0	70.9 ab
CV %			32.03

*, in the same column means with different letters indicate the significant difference (LSD test, $P<0.05$)

Table 6- Effects of FP strains, commercial fungicides and biofungicide against *Fusarium* sp. in pot trials

Çizelge 6- Saksı denemelerinde fluresan pseudomonas, biyopreparat ve fungisitlerin Fusarium sp.'ye etkileri

Treatments	*Total emerged plants*	*Average of emerged plants (%)*	*Effect against Fusarium sp. (%)**
FP9	31	22.5	55.3 c
FP14	33	17.5	64.8 abcd
FP20	32	20.0	57.1 bcd
FP21	27	32.5	35.0 e
FP23	34	15.0	70.8 def
FP25	30	25.0	56.3 cd
FP30	33	17.5	67.6 abcd
FP35	34	15.0	70.9 abc
FP48	34	15.0	70.5 abc
FP49	28	30.0	55.4 c
FP51	33	17.5	64.5 abcd
FP57	35	12.5	72.0 ab
Control (+)	25	37.5	18.6 f
Vitavax (K)	37	7.5	73.3 a
Maxim (K)	39	2.5	77.0 a
Subtilex (K)	34	15.0	70.1 abc
CV %			26.40

*, in the same column means with different letters indicate the significant difference (LSD test, P<0.05)

Table 7- Effects of FP strains, commercial fungicides and biofungicide against *P. deliense* in pot trials

Çizelge 7- Saksı denemelerinde fluresan pseudomonas, biyopreparat ve fungisitlerin P. deliense'ye etkileri

Treatments	*Total emerged plants*	*Average of emerged plants (%)*	*Effect against P. deliense (%)**
FP5	28	30.0	31.6 f
FP9	33	17.5	62.6 bcde
FP14	35	12.5	71.9 abcd
FP19	33	17.5	67.3 abcde
FP20	36	10.0	73.9 abc
FP21	30	25.0	56.1 e
FP23	30	25.0	58.8 de
FP28	30	25.0	60.0 cde
FP30	34	15.0	70.7 abcd
FP35	36	10.0	73.8 abc
FP48	35	12.5	71.3 abcd
FP49	33	17.5	65.9 abcde
FP52	33	17.5	69.2 abcde
FP56	30	25.0	62.7 bcde
FP57	36	10.0	73.4 abc
Control (+)	24	40.0	20.3 f
Vitavax (K)	37	7.5	76.1 ab
Maxim (K)	37	7.5	79.1 a
Subtilex (K)	37	7.5	73.9 abc
CV %			28.20

*, in the same column means with different letters indicate the significant difference (LSD test, P<0.05)

carried out with simple laboratory facilities and the method used was effective and easy to apply commercially. On the other hand, field trials with these antagonistic bacteria and more detailed studies of bio-controlling mechanisms of action are needed. Since fungicides are polluting the environment and demand for organic cotton production, biological preparations can be used as an alternative to fungicides.

Acknowledgements

This project was supported by the General Directorate of Agricultural Researches and Policies of Turkey. The authors thank to Prof. Dr. Kemal BENLİOĞLU, Prof. Dr. Şener KURT and Assoc. Prof. Dr. Hülya ÖZGÖNEN for kindly providing *R. solani* AG4, *Fusarium* sp. and *P. deliense* isolates respectively.

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