Turkish Journal of Agriculture and Forestry

Volume 47 | Number 4 Article 5

8-28-2023

An investigation of biological control possibilities against Fusarium proliferatum on red pepper

ENES ŞAHİNOĞLU

ELİF TOZLU

Follow this and additional works at: https://journals.tubitak.gov.tr/agriculture

 Part of the Agriculture Commons, and the Forest Sciences Commons

Recommended Citation

ŞAHİNOĞLU, ENES and TOZLU, ELİF (2023) "An investigation of biological control possibilities against Fusarium proliferatum on red pepper," *Turkish Journal of Agriculture and Forestry*: Vol. 47: No. 4, Article 5. https://doi.org/10.55730/1300-011X.3102
Available at: https://journals.tubitak.gov.tr/agriculture/vol47/iss4/5

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Agriculture and Forestry by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2023) 47: 467-478
© TÜBİTAK
doi:10.55730/1300-011X.3102

An investigation of biological control possibilities against *Fusarium proliferatum* on red pepper*

Enes ŞAHİNOĞLU[1], **Elif TOZLU**[2,**]

[1]Aşkale District Directorate of Provincial Agriculture and Forestry, Republic of Türkiye Ministry of Agriculture and Forestry, Erzurum, Turkey
[2]Department of Plant Protection, Faculty of Agriculture, Atatürk University, Erzurum, Turkey

Received: 28.01.2022 • **Accepted/Published Online:** 04.01.2023 • **Final Version:** 28.08.2023

Abstract: *Fusarium proliferatum* causes yield loss in many agricultural crops. The chemicals used to control it bring problems due to phytotoxicity, residues, environmental pollution and harmful effects on human health. In recent years, the use of environmentally friendly control practices has increased due to the development of environmental and human health awareness. This study was carried out to determine antifungal activities of 52 candidate bioagent bacterial strains and two fungal isolates against *F. proliferatum* isolated from red pepper on petri plate assay. The activities of five bacterial strains and 2 fungal isolates that showed the highest inhibitory effect on *F. proliferatum* in in vitro were also determined in the dark at 25 ± 2 °C. In pot assay, the experiment was established with the most effective five bacterial strains (FDG-37, 70.63%; PM-18, 69.44%; Jumbo A, 66.07%; TV-13B, 64.00%; TV-15B, 60.71%) and two fungal isolates (ET 4 and ET 14, 74.35%). As a result, it was determined that *Bacillus subtilis, Pseudomonas chlororaphis, Bacillus* sp. and *T. harzianum* can be candidate in the biological control of *F. proliferatum* on red peppers.

Key words: Bioagent bacteria, bioagent fungi, red pepper, *Fusarium proliferatum*, root rot, sustainable agriculture

1. Introduction

Significant phytopathological problems lead to reduced production and loss of quality in pepper. In particular, fungal diseases are one of the main factors limiting pepper production, which has an important economic value (Abed et al., 2016). They cause significant losses in production among cultivated areas around the world. *Fusarium proliferatum* causes disease at all stages of plant and affects almost every parts of the plant. Furthermore, *F. proliferatum* is an important pathogen under storage conditions. (Southwood, 2012).

Even though the fungicides application 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 fungicides in a short time and sporulation rapidly occurs, in addition to its permanent negative effects on human and environmental health (Uygun et al., 2010). Therefore, it is necessary either to develop an alternative to chemical control or to implement practices that will increase the effectiveness of this control method (Ramamoorthy et al., 2002). Among these alternatives, biological control is environment-friendly, sustainable, and one of the promising method and there is an increase in studies on biological control of plant diseases. Recently, important studies on biological control against many plant diseases have been carried out and successful results have been obtained (He et al., 2021). It is obvious that such studies will gain much more importance in the context of integrated pathogen control, especially considering the increasing tendency of producers and consumers towards organic products (Uygun et al., 2010).

In this study, it was aimed to determine the availability of fungal isolates and bacterial strains which are eco-friendly and harmless to health of human and other organisms as biological control agents against *F. proliferatum*.

2. Materials and methods

2.1. Material

In this study, red pepper cultivar Postal was used as the test plant in pot trials. Pathogenic fungus was isolated from red

* This study is a part of Enes Şahinoğlu's master thesis accepted by Atatürk University Graduate School of Natural and Applied Sciences and submitted as an abstract in the 2nd International Conference on Food, Agriculture and Animal Sciences (ICOFAAS), 8–11 November 2019, Antalya, Türkiye.

** Correspondence: elifalpertozlu@atauni.edu.tr

This work is licensed under a Creative Commons Attribution 4.0 International License.

pepper plants showing root rot (collapsing) symptoms in Erzurum (Türkiye). The pieces cut from the diseased plants were disinfected in 1% sodium hypochlorite (NaOCl) for 2 min and incubated in potato dextrose agar (PDA, Difco) medium at 20–25 °C for 4–5 days. Fungal colonies grown on PDA were purified. Pure cultures were transferred to the test tubes containing PDA, and were preserved at +4 °C for the later stages of the study.

For the pathogenicity tests, pepper fruits and seedlings were inoculated with the pathogen isolate. Pepper 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-h dark and 12-h light cycles. After the pepper 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 mycelial 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 h to obtain high humidity at 25 °C. The seedlings were taken from the moisture chamber one day after the inoculation. Fourteen days after the inoculation, the isolates were recovered through the reisolation from the parts demonstrating disease symptoms, and thus Koch postulates were completed (Figure 1). In the same way, after the pepper fruits were sterilized, the pathogenicity test was performed on the fruit by applying the mycelial disc of the pathogen isolate, and the Koch postulates were completed by reisolating the frungus from the symptomatic fruit (Figure 1). ET 89 isolate, which was determined to be the most virulent among the fungal isolates, was used as the material of the study. Since the morphological diagnosis of the fungi alone is not very reliable, molecular identification was also carried out. Molecular characterization was performed by targeting the 18S-ITS1-5.8SITS2-28S region. ET 89 pathogenic isolate was identified as *Fusarium proliferatum* with 99% similarity and deposited with the GenBank database under the accession numbers MN193534.

Biocontrol bacterial strains obtained from Atatürk University Faculty of Agriculture Plant Protection Department Culture Collection were used in this study (Table 1). Fatty acid methyl esters (FAME) analysis (Sherlock Microbial Identification System (MIS, Microbial ID, Newark, DE, USA)) (Sasser, 1990) was used for the diagnosis of bacterial strains and molecular diagnosis was used for the most effective bacterial strains and fungal isolates (Table 1). The bacterial strains were tested for hypersensitivity on tobacco plants (*Nicotina tabacum* L. var. Samsun) as described before (Klement, 1968) (Tablo 1). Bacterial strains grown on Nutrient Agar (NA, Difco) were stored in Nutrient Broth (NB, Difco) containing 15% glycerol at –80 °C, pathogen isolate ET 89 and fungal biocontrol agents on PDA at 4 °C until used.

2.2. Antifungal activity assays of the bacterial strains and fungal isolates

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

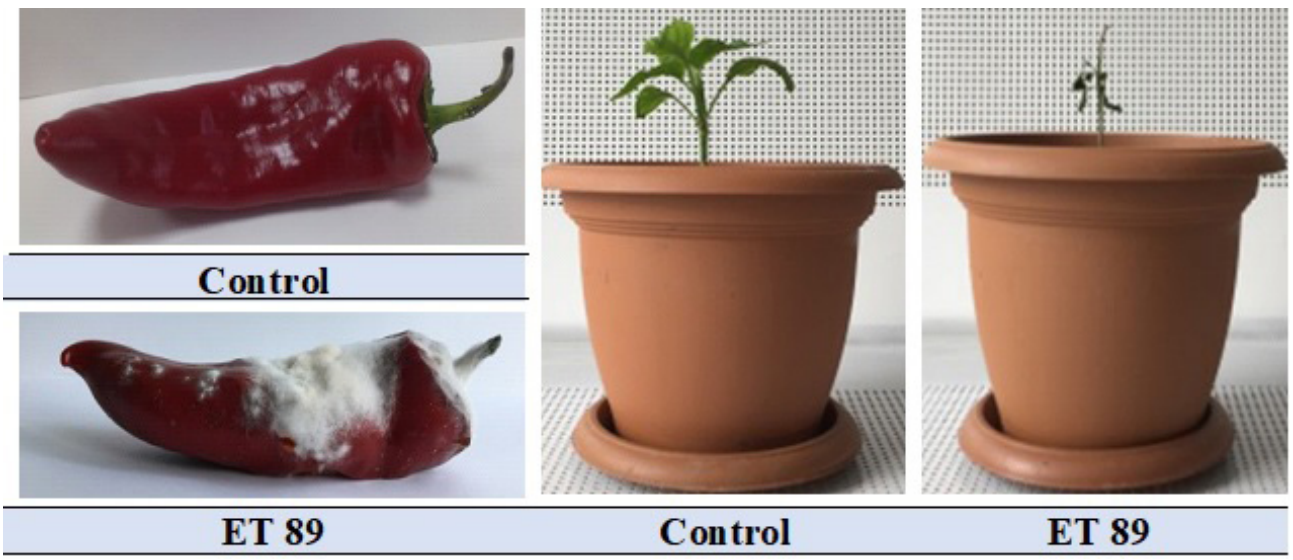

Figure 1. *Fusarium proliferaturm* isolate ET 89.

Table 1. Identification results of biocontrol bacterial strains and fungal isolates, hypersensitivity (HR) test results and pathogenicity (P).

Bacterial strains		Isolated from	MIS	S*	ITS results	Accessed number**	I (%)	HR	Reference
1	EK-7	*Rosa canina*	*Bacillus subtilis*	0.624	-	-	-	-	Mohammadi et al., 2017
2	TV-12H	*Triticum aestivum*	*Bacillus subtilis*	0.53	-	-	-	-	Çakmakcı et al., 2010
3	TV-13B	*Rubus idaeus*	*Bacillus subtilis*	0.687	*Bacillus* sp.	-	-	-	Gökçe, 2016
4	TV-16F	-	*Bacillus subtilis*	0.831	-	-	-	-	Çakmakçı et al., 2010
5	TV-17C	*Rubus idaeus*	*Bacillus subtilis*	0.677	-	-	-	-	Çakmakçı et al., 2010
6	TV-6F	-	*Bacillus subtilis*	0.831	-	-	-	-	Çakmakcı et al., 2010
7	BRTB	-	*Bacillus subtilis*	0.663	-	-	-	-	In this study
8	KBA-10	*Prunus armeniaca*	*Bacillus megaterium*	0.490	-	-	-	-	Esringü et al., 2016
9	M-3	-	*Bacillus megaterium*	0.741	-	-	-	-	Güneş et al., 2013
10	TV-13C	*Rubus idaeus*	*Bacillus megaterium*	0.595	-	-	-	-	Gökçe, 2016
11	TV-20E	-	*Bacillus megaterium*	0.519	-	-	-	-	Gökçe, 2016
12	TV-3D	*Secale cereale*	*Bacillus megaterium*	0.563	-	-	-	-	Esringü et al., 2016
13	TV-87A	-	*Bacillus megaterium*	0.467	-	-	-	-	Erman et al., 2010
14	FDP40	-	*Bacillus megaterium*	0.400	-	-	-	-	In this study
15	TV-6D	-	*Bacillus megaterium*	0.750	-	-	-	-	Esringü et al., 2016
16	TV-49A	*Triticum* sp.	*Bacillus megaterium*	0.577	-	-	-	-	Mohammadi, 2018
17	Jumbo B	fertilizer	*Bacillus atrophaeus*	0.465	-	-	-	-	In this study
18	TV-15B	Sedum	*Bacillus atrophaeus*	0.468	-	-	-	-	Mohammadi, 2018
19	TV-30D	-	*Bacillus cereus*	0.220	-	-	-	-	Aktaş, 2015
20	TV-85D	*Beta vulgaris*	*Bacillus cereus*	0.114	-	-	-	-	Mohammadi, 2018
21	IA1	-	*Bacillus cereus*	0.080	-	-	-	-	In this study
22	TV-67C	*Rubus idaeus*	*Bacillus pumilus*	0.630	-	-	-	-	Erman et al., 2010
23	TV-73A	-	*Bacillus pumilus*	0.650	-	-	-	-	Gökçe, 2016
24	FD48	-	*Bacillus sphaericus*	0.587	-	-	-	-	In this study
25	Jumbo A	Fertilizer	*Bacillus licheniformis*	0.792	*Bacillus subtilis*	MN493121	99	-	In this study
26	FDP-8	*Hypera postica*	*Bacillus thuringiensis subsp. kenyae*	0.32	-	-	-	-	Tozlu et al., 2011
27	FDG-37	Soil	*Pseudomonas fluorescens*	0.222	-	-	-	-	Karagöz et al., 2016
28	TV-11D	*Beta vulgaris*	*Pseudomonas flourescens*	0.711	-	-	-	-	Esringü et al., 2016
29	IK-37	-	*Pseudomonas chlororaphis*	0.89	-	-	-	-	Dadaşoğlu and Şahin, 2010
30	PM-18	-	*Pseudomonas chlororaphis*	0.205	*Pseudomonas chlororaphis*	MN493122	100	-	Mohammadi, 2018
31	RK-142	*Pyrus communis*	*Pseudomonas putida*	0.277	-	-	-	-	Kotan, 2002

Table 1. (Continued).

32	TV-42A	*Triticum* sp.	*Pseudomonas putida*	0.113	-	-	-	-	Aktaş, 2015
33	RK-154	*Pyrus communis*	*Pantoea agglomerans*	0.591	-	-	-	-	Kotan, 2002
34	RK-84	*Malus sylvestris*	*Pantoea agglomerans*	0.718	-	-	-	-	Karagöz and Kotan, 2010
35	RK-92	*Pyrus communis*	*Pantoea agglomerans*	0.889	-	-	-	-	Karagöz and Kotan, 2010
36	OSU-142	-	*Paenibacillus macerans*	0.450	-	-	-	-	Orhan et al., 2006
37	T26	-	*Paenibacillus macerans*	0.665	-	-	-	-	In this study
38	R-2/2	-	*Paenibacillus polmyxa*	-	-	-	-	-	Çakmakcı et al., 2010
39	FD-1	*Malacosoma neustria*	*Brevibacillus brevis*	0.625	-	-	-	-	Tozlu et al., 2011
40	FDG27	-	*Brevibacterium brevis*	0.560	-	-	-	-	In this study
41	TV-53D	*Taraxacum* sp.	*Brevibacillus choshinensis*	0.689	-	-	-	-	Gökçe, 2016
42	FDG152	-	*Brevibacillus agri*	0.480	-	-	-	-	In this study
43	TV-14C	*Fragaria* sp.	*Kocuria rosea*	0.695	-	-	-	-	Mohammadi, 2018
44	FDG66	-	*Kocuria rosea*	0.540	-	-	-	-	In this study
45	A-16	-	*Ochrobactrum anthropi*	0.318	-	-	-	-	Mohammadi et al., 2017
46	RK-102	*Malus sylvestris*	*Serratia liquefaciens*	0.572	-	-	-	-	Karakurt et al., 2010
47	RK-105	*Malus sylvestris*	*Alcaligenes piechaudii*	0.420	-	-	-	-	Kotan, 2002
48	RK-134	*Malus sylvestris*	*Enterobacter agglomerans*	0.342	-	-	-	-	Karagöz and Kotan, 2010
49	RK-175	*Prunus avium*	*Klebsiella pneumoniae*	0.673	-	-	-	-	Kotan, 2002
50	RK-346	Water	*Hydrogenophaga pseudoflava*	0.124	-	-	-	-	Dikbaş et al., 2009
51	TV-113C	*Allium* sp.	*Kluyvera cryocrescens*	0.688	-	-	-	-	Gökçe, 2016
52	FDG48	-	*Agrobacterium radiobacter*	0.320	-	-	-	-	In this study
Fungal isolates									
1	ET 4	*Aesculus hippocastanum*	-		*Trichoderma harzianum*	KY806127	87	-	Tozlu et al., 2018a
2	ET 14	*Pinus sylvestris*	-		*Trichoderma harzianum*	KY806128	97	-	Tozlu et al., 2018a

***S:** Similarity index, ** GenBank, **I:** Identification, **+:** Positive reaction, **-:** Negative reaction, **HR:** Hypersensitivity tes

dark at 25 ± 2 °C (Tozlu et al., 2018a; Mohammadi et al., 2014, 2017). 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. 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).

In order to determine the antifungal activity of ET 4 and ET 14 fungal bioagent isolates against ET 89 pathogenic fungus, mycelial disks of fungal isolates and pathogenic isolate were placed in petri dishes with a distance of about 5 cm. As the controls, only pathogen and bioagent fungal isolates were planted in petri dishes that contained PDA. The percentage interference rate was calculated (Skidmore and Dickinson, 1976).

The antifungal activity assays were conducted by using a completely randomized design with 3 replications per treatment. The experiment was performed twice.

2.3. Pot assay

The most effective bacterial strains and fungal isolates against *F. proliferatum* was tested using red pepper on Pot assays. Potential biocontrol agent fungal isolates were developed in PDA medium for about a week. The spores were mixed with sterile water to obtain spore suspension. Spore suspension was adjusted to 1×10^6 conidia/mL spore concentration using hemocytometer and 30 µL of the fungal suspension was applied to the incision 4 cm from soil surface on stem using a micropipette and 4 mm diameter disc of *F. proliferatum* growen on PDA at 25 °C for 1 week was placed on the injury site and inoculation point was wrapped with parafilm.

In order to test biocontrol bacterial strains, bacterial strains were grown on NA, then were reinoculated into 50 mL of NB on a rotary shaker at 27 °C for 24 h. Absorbance of the bacterial suspensions was measured spectrophotometrically at 600 nm and adjusted to 1×10^8 CFU/mL. Bacterial strains were applied such as bioagent fungal suspension application. Control plants were inoculated with only bioagent bacterial strains, only bioagent fungal isolate, the pathogen, NB and sdH_2O. Plants were kept at room temperature under high humidity until development of disease symptoms. The study was carried out in 3 repetitions.

2.4. Data analysis

Disease symptoms of plants were examined daily and evaluated on a modified 0–5 scale. The obtained data were converted to the percentage of disease severity using the disease severity (DS) formula (Viriyasuthee et al., 2019).

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

DS: disease severity,

S: rating score,

L: number of plant in rating,

M: total number of sampled plants,

S_{max}: highest rating.

Data analysis was made via the utilization of JMP IN (SAS Institute, Cary, NC, 5.0.1 software program) statistics software. The difference between the treatments was determined using the "LSMeans Differences Student's" test at a significance level $p < 0.01$.

2.5. Molecular identification of most effective bacterial strains in vivo test

Molecular characterization of the most effective strains PM-18, Jumbo A and TV-13B was performed by targeting the 16Sr region. Genomic DNA of bacterial strains was 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).

3. Results and discussion

Antifungal test results of bioagent bacterial strains and fungal isolates are provided in Table 2. In terms of in vitro tests, all of bacterial strains have more or less inhibitory activity against *F. proliferatum* ET 89 isolate (percentage interference rate F: 39.5946; $P < 0.01$). While the FDG-37 bacterial strain showed the highest inhibitory effect with 70.63%, TV-11D in the same group with the control showed the lowest inhibitory effect with 35.71% (Table 2). Percentage interference rate (%) (PIRG) of the tested fungal biocontrol agents are presented in Table 3. PIRG was found 74.35% (high) in ET 4 and in ET 14 isolates.

PM-18, Jumbo A, TV-13B, TV-15B, FDG-37, ET 4 and ET 14 which are highly effective under in vitro conditions (Figure 2) were tested under in vivo conditions in pot assays, and their symptoms were assessed (Table 4). The difference between treatments was statistically significant in pot assays (disease severity F: 171.6140; $p < 0.01$). The results are given in Table 4 and Figure 3.

Molecular diagnosis was performed from pure cultures of 3 bacterial strains that showed the highest inhibitory effect in both in vitro and in vivo tests. PM-18, Jumbo A and TV-13B were identified as *Pseudomonas chlororaphis* (similarity: 100%; accession number: MN493122), *Bacillus subtilis* (similarity: 99%; accession number: MN493121) *Bacillus* sp., respectively.

Nowadays, fungal and bacterial preparations made from biocontrol agents are becoming increasingly common in the fight against pathogens. Many studies have shown that *T. harzianum* is the most commonly used fungal biocontrol agent in this genus against plant pathogenic fungi, and also is the most promising due to

Table 2. Antifungal activities of the bacterial strains and fungal isolates against pathogen fungi on petri plate assays.

Applications	Percentage interference rate (%)		Applications	Percentage interference rate (%)	
FDG-37	70.63	A	**M-3**	51.79	I-O
PM-18	69.44	AB	**TV-30D**	51.79	I-O
Jumbo A	66.07	BC	**TV-14C**	51.59	J-O
TV-13B	64.00	CD	**TV-3D**	51.19	J-P
TV-15B	60.71	DE	**RK-346**	50.40	K-Q
TV-53D	58.33	EF	**IK-37**	50.00	K-Q
R-2/2	57.73	E-G	**TV-13C**	49.60	L-Q
RK-105	56.54	F-H	**TV-49A**	49.60	L-Q
BRTB	55.95	F-I	**TV-67C**	49.60	L-Q
RK-84	55.36	F-J	**TV-17C**	49.60	L-Q
TV-16F	55.36	F-J	**FDP-40**	49.40	L-Q
TV-6D	54.76	F-J	**IA-1**	49.40	L-Q
A-16	54.76	F-J	**RK-102**	49.40	L-Q
RK-92	54.76	F-J	**T-26**	49.40	L-Q
TV-113C	54.17	F-K	**FDG-48**	49.21	M-Q
KBA-10	53.57	G-L	**RK-134**	48.81	N-R
RK-175	53.57	G-L	**TV-85D**	48.02	O-R
TV-20E	53.57	G-L	**FDG-66**	47.62	O-R
TV-42A	53.57	G-L	**TV-87A**	47.62	O-R
TV-12H	53.17	H-M	**TV-6F**	47.22	P-R
FDG-152	53.98	H-N	**FDP-8**	47.02	P-R
FDG-27	52.98	H-N	**OSU-142**	46.83	QR
Jumbo B	52.98	H-N	**FD-1**	44.64	RS
RK-142	52.98	H-N	**TV-73A**	42.07	ST
RK-154	52.98	H-N	**FD-48**	38.69	TU
EK 7	52.38	H-N	**TV-11D**	35.71	V
Control	0	V			
CV			**5.03**		
LSD			**4.20**		

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

its antagonistic activity (Dubey et al., 2007; Hajieghrari et al., 2008; Sharma, 2011; Sundaramoorthy and Balabaskar, 2013; Ali and Nadarajah, 2014; Altınok and Erdoğan, 2015; Valenzuela et al., 2015; Parmar et al., 2015; Akrami, 2015; Basumataray et al., 2015; Barari, 2016; Bae et al., 2016; Saravanakumar et al., 2016; Chen et al., 2021). *T. harzianum* has effect mechanisms such as chi-tinase, β-1-3 glucanase and β-1-4 glucanase pro-duction, antibiotics, competitions, inorganic plant nutrient resolution, disabling pathogen enzymes, and encouraging endurance (Harman, 2006).

In a study, *T. harzianum* KUEN isolate was used against *F. oxysporum* f.sp. *cepae* and they found an antagonistic effect of 73.3% in dual culture tests (Coşkuntuna and Özer 2008). In a study on pepper, among five different *Trichoderma* species, *T. viride* and then *T. harzianum* were found to be effective agents in vitro (Sahi, 2012). The production of volatile and nonvolatile metabolites was examined in a dual culture study to evaluate the antagonistic activities of *T. harzianum* isolate T100, *T. viride* and *T. hamatum* against *Fusarium oxysporum* and *F. proliferatum*, and it was determined that *T. viride*, *T. hamatum* and T100 were

Table 3. Percentage interference rate (%) of *Trichoderma harzianum* isolates tested against ET 89 isolate in in vitro conditions.

Isolates	**PIRG (%)**	
ET 4	74.35	+++
ET 14	74.35	+++

PIRG: Percentage interference rate (%).
60% < PIRG ≤ 75% : High.

effective against *Fusarium*, while T100 was the only fungus being able to lyse the confronting mycelia (Ghanbarzadeh et al., 2014).

In our study, we tested ET 4 and ET 14 isolates of *T. harzianum*, and in in vitro conditions. It was indicated that *T. harzianum* isolates ET 4 and ET 14 have inhibited *F. oxysporum* isolated from pepper (33.33%; 45.00%), potato (13.87%, 14.28%, 17.77%, 44.40%; 49.33%, 16.67%, 37.25%, 57.33%), cucumber (0.00%; 39.99%); *Fusarium solani* isolated from cucumber (3.33%; 2.21%), tomato (10.25%; 18.33%); *Geotrichum candidum* isolated from carrot (80.24%; 29.63%); *Sclerotinia sclerotiorum* isolated from cucumber (28.57%; 0.00%); *Alternaria alternata* isolated from tomato (54.17%; 66.63%), apple (28.87%; 64.40%) (Tozlu et al., 2018b); cucumber (73.87%; 55.85%), strawberry (83.33%; 74.44%) (Tozlu et al., 2018a); quince (67.74%; 61.29%) (Tekiner et al., 2019a); orange (70.47%; 61.91%) (Tekiner et al., 2020); *Alternaria solani* isolated from tomato (60%; 48%) (Çamlıca and Tozlu, 2019); *Colletotrichum gloesporoides* isolated from orange (82.50%; 70.00%) (Tekiner et al., 2019b); *Botrytis cinerea* isolated from orange (85.65%; 83.33%), strawberry (84.31%; 76.47%), black mulberry (87.23%; 83.08%) (Tekiner et al., 2020); *Fusarium oxysporum* isolated from potato (69.44%; 66.66%) (Çakar and Tozlu, 2022), respectively. Based on these results, it was concluded that these isolates were effective against different pathogens as well, and could effectively be used as biocontrol agents in biological control studies.

Bacteria are also important beneficial microorganisms that can be used as an alternative to chemical pesticides in agriculture. Among them, *Bacillus* species are the most frequently used biocontrol agents in biological control studies (Khan et al., 2017). The antimicrobial compound(s) produced by *Bacillus* spp. can be formulated in an affordable, stable, and effective manner revealed that these organisms can be used safely in modern agriculture (Göğüsgeren and Çolak, 2009). In addition to this, the fact that *Bacillus* species form spores can be found in almost all types of soils, and endophytically colonized plants has led them to be widely used as bioagents (Tiwari et al., 2014). It has been emphasized by different researchers that *B. subtilis* can be a decent bioagent within the genus *Bacillus* (Fira et al., 2018; Stein, 2005; Cawoy et al., 2015). Ajilogba et al. (2013) reported that species belonging to the *Bacillus* genus obtained in almost all types of soil are among the most commonly used bacteria in the control against soil-borne fungal agents. *Bacillus* spp. isolates suppress plant diseases such as *Fusarium, Alternaria, Rhizoctonia, Aspergillus, Cryphonectria* and *Phytophthora*, etc., in vitro (Mnif and Ghrib, 2015). Bjelic et al. (2017) found that *Bacillus megaterium* B8 (45%) and B9 (42%) isolates showed antifungal activity against *F. proliferatum*. In another study, *B. subtilis* Y-1 strain showed significant inhibitory effect against *F. oxysporum, F. moniliforme, F. proliferatum, F. solani* and *Rhizoctonia solani*, and its antibacterial activity against *F. proliferatum* has been recorded as 54.52% (Ju et al., 2014).

Also, *Pseudomonas* is used as a biological control agent against diseases (Erdoğan and Benlioglu, 2010; Senol, 2014). *Pseudomonas* excretes a great variety of antibiotics such as 2,4-diacetylphloroglucinol (2,4-DAPG), pyoluteorin, pyrrolnitrin, and hydrogen cyanide (Raaijmakers et al., 2002; Ramette et al., 2003). In a study, the effect of volatile compounds produced by *P. fluorescens* MGR12 on the growth of *F. proliferatum* was investigated, and it was determined that bacterial strains secreted volatiles that negatively affected mycelial growth of pathogenic fungus in both rich and minimal environments (Cordero et al., 2014).

In our previous study, it was recorded that *P. chlororapsis* PM-18 strain has inhibited *A. alternata* isolated from tomatoes (62.50%) in vitro. The fact that it also inhibits the development of another pathogen indicates that this bacterial strain can be used as a bioagent against different pathogens.

Of the 52 bacterial strains used in our study, 26 strains belong to *Bacillus* and 6 strains belong to *Pseudomonas* genus. Among these strains, the most effective results against *F. proliferatum* ET 89 isolate were obtained from Jumbo A (*B. subtilis*), PM-18 (*P. chlororaphis*) and TV-13B (*Bacillus* sp.) strains whose species could not be determined molecularly.



Figure 2. Antifungal activities of the bacterial strains and fungal isolates against *Fusarium proliferatum* ET 89 on petri plate assays.

Table 4. Antifungal effect of most effective bacterial strains and fungal isolates against *Fusarium proliferatum* ET 89 isolate in in vivo.

Applications		**DS (%)****	
*Control 1	PM-18	0	C
	Jumbo A	0	C
	TV-13B	0	C
	TV-15B	0	C
	FDG-37	0	C
	ET 4	0	C
	ET 14	0	C
Control 2	0		C
Control 3	0		C
Control 4	100.00		A
PM-18 + ET 89	0		C
ET 14 + ET 89	0		C
Jumbo A + ET 89	0		C
TV-13B + ET 89	0		C
ET 4 + ET 89	0		C
TV-15B + ET 89	18.75		B
FDG-37 + ET 89	96.67		A
CV	**32.03**		
LSD	**13.75**		

*Control 1: Only bacterial biocontrol agent, Control 2: NB, Control 3: Sterile water, Control 4: Only pathogen.
**DS: Disease severity. In the same column by the same letter are not significantly different to the test of LSMeans Differences Student's ($p < 0.01$).

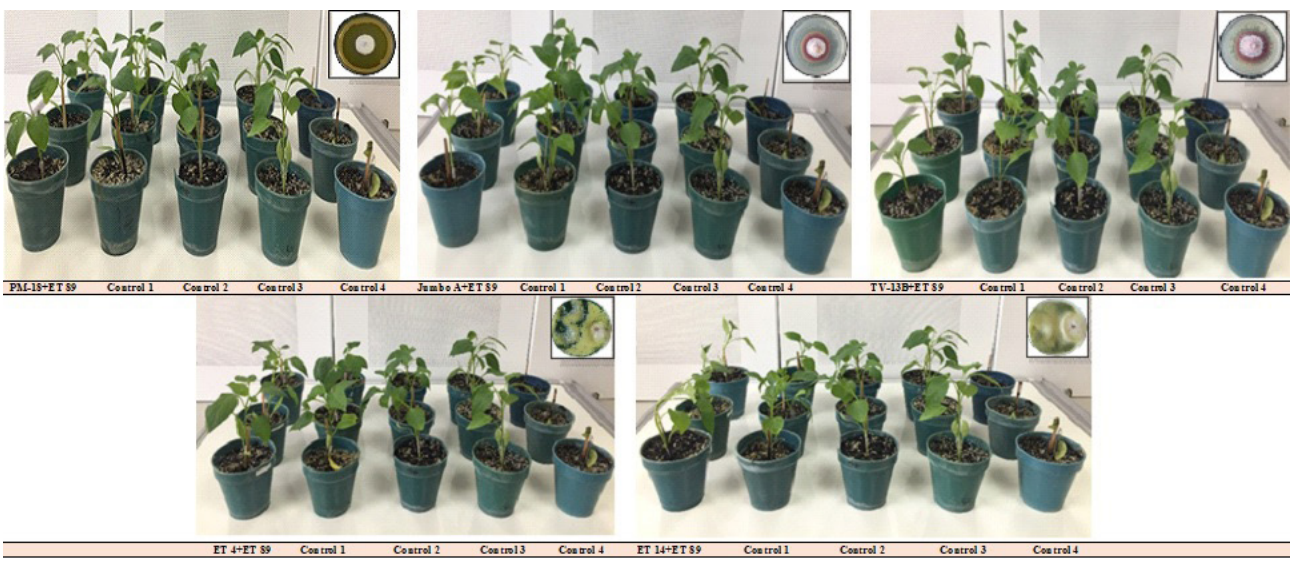

Figure 3. The most effective biocontrol bacterial strains and fungal isolates against *Fusarium proliferatum* ET 89 isolate in in vivo (Control 1: only bacterial biocontrol agent, Control 2: NB, Control 3: sterile water, Control 4: only pathogen).

4. Conclusion

In this study, in vivo and in vitro studies showed that *T. harzianum* and *P. chlororaphis, B. subtilis* and an unidentified *Bacillus* sp. strain can be used in red pepper cultivation as biocontrol agents for prevention of pepper root rot disease. In future, further studies are being planned to prepare a commercial preparation after making a good carrier consisting of organic materials with long shelf life for the most effective bacterial strains and fungal isolates.

Acknowledgment

We would like to thank Prof. Dr. Recep KOTAN for bacterial strains.

References

Abed H, Rouag N, Mouatassem D, Rouabhi A (2016). Screening for *Pseudomonas* and *Bacillus* antagonistic rhizobacteria strains for the biocontrol of *Fusarium* wilt of chickpea. Eurasian Journal of Soil Science 5 (3): 182-191.

Ajilogba CF, Babalola OO, Ahmad F (2013). Antagonistic effects of *Bacillus* species in biocontrol of tomato *Fusarium* wilt. Studies on Ethno-Medicine 7 (3): 205-216.

Akrami M (2015). Effects of *Trichoderma* spp. in Bio-controlling *Fusarium solani* and *F. oxysporum* of cucumber (*Cucumis sativus*). Journal of Applied Environmental and Biological Sciences 4 (3): 241-245.

Aktaş S (2015). Investigation of biological control possibilities of tomato pith necrosis using PGPR and bio-control bacteria in controlled condition. MSc, Atatürk University Graduate School of Natural and Applied Sciences, Erzurum, Türkiye.

Ali H, Nadarajah K (2014). Evaluating the efficacy of *Trichoderma* spp. and *Bacillus substilis* as biocontrol agents against *Magnaporthe grisea* in rice. Australian Journal of Crop Science 8 (9): 1324-1335.

Altınok HH, Erdoğan O (2015). Determination of the *in vitro* effect of *Trichoderma harzianum* on phytopathogenic strains of *Fusarium oxysporum*. Notulae Botanicae Horti Agrobotanici 43 (2): 494-500.

Bae SJ, Mohanta TK, Chung JY, Ryu M, Park G et al. (2016). *Trichoderma* metabolites as biological control agents against *Phytophthora* pathogens. Biological Control 92: 128-138.

Barari H (2016). Biocontrol of tomato *Fusarium* wilt by *Trichoderma* species under in vitro and in vivo conditions. Cercetări Agronomice în Moldova 49 (1): 91-98.

Basumataray M, Dutta BK, Singha DM (2015). Some in vitro observations on the biological control of *Sclerotium rolfsii*, a serious pathogen of various agricultural crop Plants. Journal of Agriculture and Veterinary Science 8 (2): 87-94.

Bjelıć D, Ignjatov M , Marinkovıć J, Spremo N, Karaman M et al. (2017). Antifungal activity of indigenous *Bacillus* spp. isolated from soil. Matica Srpska Journal For Natural Sciences 133: 261-269. https://doi.org/10.2298/ZMSPN1733261B

Cawoy H, Debois D, Franzil L, De Pauw E, Thonart P et al. (2015). Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis/amyloliquefaciens*. Microbial Biotechnology 8: 281-295. https://doi.org/10.1111/1751-7915.12238

Chandler JA, Lang JM, Bhatnagar S, Eisen JA, Kopp A (2011). Bacterial communities of diverse *Drosophila* species: Ecological Context of a Host-microbe Model System. Plos Genetics 7:e1002272.

Chen J, Zhou L, Din IU, Arafat Y, Li Q et al. (2021). Antagonistic activity of *Trichoderma* spp. against *Fusarium oxysporum* in rhizosphere of *Radix pseudostellariae* Triggers the expression of host defense genes and improves its growth under long-term monoculture system. Frontier in Microbiology 12 : 579920. https://doi.org/10.3389/fmicb.2021.579920

Cordero P, Príncipe A, Jofré E, Mori G, Fischer S (2014). Inhibition of the phytopathogenic fungus *Fusarium proliferatum* by volatile compounds produced by *Pseudomonas*. Archives Microbiology 196: 803-809.

Coşkuntuna A, Özer N (2008). Biological control of onion basal rot disease using *Trichoderma harzianum* and induction of antifungal compounds in onion set following seed treatment. Crop Protection 27: 330-336.

Çakar G, Tozlu E (2022). The biological control of *Fusarium oxysporum*, the causal agent of potato rot. Gesunde Pflanzen https://doi.org/10.1007/s10343-021-00610-1.

Çakmakçı R, Erman M, Kotan R, Çığ F, Karagöz K et al. (2010). Growth promotion and yield enhancement of sugar beet and wheat by application of plant growth promption rhizobacteria. In: Proceeding of the International Conference on Organic Agriculture in Scope of Environmental Problems; Famagusta, Cyprus Island. pp. 198-202.

Çamlıca E, Tozlu E (2019). Biological control of *Alternaria solani* in tomato. Fresenius Environmental Bulletin 28 (10): 7092-7100.

Dadaşoğlu F, Şahin F (2010). Use of bacteria in biological control of *Malacosoma neustria* L. (Lepidoptera: Lasiocampidae). Atatürk University Journal of Agricultural Faculty 41 (2): 97-104 (in Turkish with an abstract in English).

Dikbaş N, Kotan R, Dadaşoğlu F (2009). Identification of bacteria isolated from drinking water and determinatıon of relationship between their antibıotic susceptibilities and fatty acid methyl esters. The Journal of Food 34 (4): 225-230.

Dubey SC, Suresh M, Singh B (2007). Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. Biological Control 40: 118-127.

Erdoğan O, Benlioğlu K (2010). Biological control of *Verticillium* wilt on cotton by the use of fluorescent *Pseudomonas* spp. Biological Control 53 (1): 39-45.

Erman M, Kotan R, Çakmakçı R, Çığ F, Karagöz K et al. (2010). Effect of nitrogen fixing and phosphate-solubilizing rhizobacteria isolated from Van Lake Basin on the growth and quality properties in wheat and sugar beet. In: Proceeding of the Turkey IV. Organic Farming Symposium; Erzurum, Türkiye. pp. 326-330.

Esringü A, Kotan R, Bayram F, Ekinci M, Yıldırım E et al. (2016). The effect of different bacteria bioformulation applications on plant growth, yield and enztme levels in garlic cultivation. Nevşehir Journal of Science and Technology 5: 214-227 (in Turkish with an abstract in English).

Fira DJ, Dimkić I, Berić T, Lozo J, Stanković S (2018). Biological control of plant pathogens by *Bacillus* species. Journal of Biotechnology 285: 44-55. https://doi.org/10.1016/j.jbiotec.2018.07.044

Ghanbarzadeh B, Safaie N, Goltapeh EM (2014). Antagonistic activity and hyphal interactions of *Trichoderma* spp. against *Fusarium proliferatum* and *F. oxysporum* in vitro. Archives of Phytopathology and Plant Protection 47 (16): 1979-1987. https://doi.org/10.1080/03235408.2013.864506

Göğüsgeren N, Çolak Ö (2012). Determination strains of *Bacillus* spp. which produces an effective antibiotic against *Trichoderma* spp. and investigation of the opportunity to be in situ of these strains at micelle culture of *Ganoderma lucidum*. Çukurova University Journal of the Faculty of Engineering, 27 (3): 165-164 (in Turkish with an abstract in English).

Gökçe AY, Kotan R (2016). Investigation of biological control possibilities of wheat root rot disease caused by Bipolaris sorokiniana (Sacc.) using PGPR and bio-control bacteria in controlled condition. Plant Protection Bulletin 56 (1): 49-75.

Güneş A, Turan M, Güllüce M, Şahin F, Karaman MR (2013). Effects of Different Bacteria Application on Solubility of Rock Phosphate. Soil-Water Journal 2 (1): 53-65.

Hajieghrari B, Torabi-Giglou M, Mohammadi MR, Davari M (2008). Biological potantial of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. African Journal of Biotechnology 7: 967-972.

Harman GE (2006). Overview of mechanisms and uses of *Trichoderma* spp., Phytopathol. 96: 190-194.

He DC, He MH, Amalin DM, Alvindia DG, Zhan J (2021). Biological Control of Plant Diseases: An Evolutionary and Eco-Economic Consideration. Pathogens 10 (10): 1311.

Ju R, Zhao Y, Li J, Jiang H, Liu P et al. (2014). Identification and evaluation of a potential biocontrol agent, Bacillus subtilis, against Fusarium sp. in apple seedlings. Annals Microbiology 64: 377-383.

Karagöz K, Kotan R (2010). Effects of some plant growth promoting bacteria on growth of lettuce and Bacterial leaf spot disease. Turkish Journal of Biological Control 1 (2): 165-179 (in Turkish with an abstract in English).

Karagöz K, Kotan R, Dadaşoğlu F, Dadaşoğlu E (2016). Identification and characterisation of potential biofertilizer bacterial strains. In: Proceeding of the 1st International Conference on Advances in Natural and Applied Sciences (ICANAS); Antalya, Türkye. pp. 21-23.

Karakurt H, Kotan R, Aslantaş R, Dadaşoğlu F, Karagöz K et al. (2010). Effects of Some Plant Growth Promoting Bacterial Strains on Plant Growth of Saplings at 'Sekerpare' Apricot Cultivar. Atatürk University Journal of Agricultural Faculty 41 (1): 7-12 (in Turkish with an abstract in English).

Khan N, Maymon M, Hirsch A M (2017). Combating *Fusarium* infection using *Bacillus*-based antimicrobials. Microorganisms 5: E75. https://doi.org/10. 3390/microorganisms5040075

Klement Z (1968). Pathogenicity factors in reard to relationships of phytopathogenic bacteria. Phytopathology 58: 1218-1222.

Kotan R (2002). Isolation and identification of pathogenic and saprophytic bacterial organisms from pome fruits grown in Eastern Anatolia Region of Türkey by commercial and molecular techniques, and researches on the biological control strategies. PhD, Atatürk University Graduate School of Natural and Applied Sciences, Erzurum, Türkiye.

Mnif I, Ghribi D (2015). Potential of bacterial derived biopesticides in pest management. Crop Protection 77: 52-64.

Mohammadi P (2018). Evaluation of biological control of (*Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis *et al.*) bacterial wilt and canker of tomato by antagonisiıc bacteria. PhD, Atatürk University Graduate School of Natural and Applied Sciences, Erzurum, Türkiye.

Mohammadi P, Kotan R, Tozlu E (2014). Screening of antagonistic bacteria for biological control of green mold caused by *Penicillium digitatum* of citrus fruits. In: Proceeding of the 21st Plant Protection Congress; Urmia, Iran. p. 189.

Mohammadi P, Kotan R, Tozlu E, Şenol Kotan M (2017). Potential of some bacteria for biological control of postharvest citrus green mold caused by *Penicillium digitatum*. Plant Protection Science 53 (3): 134-143.

Orhan E, Eşitken A, Ercişli S, Turan M, Şahin F (2006). Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. Scientia Horticulture 111: 38-43.

Parmar HJ, Bodar NP, Lakhani HN, Patel SV, Umrania VV et al. (2015). Production of lytic enzymes by *Trichoderma* strains during in vitro antagonism with *Sclerotium rolfsii*, the causal agent of stem rot of groundnut. African Journal of Microbiology Research 9 (6): 365-372.

Raaijmakers JM, Vlami M, de Souza JT (2002). Antibiotic production by bacterial biocontrol agents. *Antonie van Leeuwenhoek* 81 (1-4) : 537-547.

Ramamoorthy V, Raguchander T, Samiyappan R (2002). Enhancing resisitance of tomato and hot pepper to *Pythium* diseases by seed treatment with *fluorescent Pseudomonas*. European Journal of Plant Pathology 108: 429-441.

Ramette A, Frapolli M, Défago G, Moënne-Loccoz Y (2003). Phylogeny of HCN synthase-encoding *hcnBC* genes in biocontrol fluorescent *Pseudomonas* and its relationship with host plant species and HCN synthesis ability. *Molecular Plant-Microbe Interactions* 16 (6): 525-535.

Sahi IY (2012). In vitro biological control of *Fusarium oxysporum* causing wilt in *Capsicum annuum*. Mycopathologia 5: 85-88.

Saravanakumar K, Yu C, Dou K, Wang M, Li Y et al. (2016). Synergistic effect of *Trichoderma*-derived antifungal metabolites and cell wall degrading enzymes on enhanced biocontrol of *Fusarium oxysporum*. Biological Control 94: 37-46.

Sasser M (1990). "Tracking" a strain using the Microbial Identification System. MIDI technical note 102. MIDI, Newark, Del.

Senol M (2014). Antifungal Activity of *Pseudomonas fluorescens* and *Bacillus subtilis* against *Fusarium culmorum*, purification of chitinase enzyme obtained from bacteria and determination of some kinetic properties. MSc, Atatürk University Graduate School of Natural and Applied Sciences, Erzurum, Türkiye.

Sharma P (2011). Complexity of *Trichoderma-Fusarium* interaction and manifestation of biological control. Australian Journal of Crop Science 5 (8): 1027-1038.

Skidmore AM, Dickinson CM (1976). Colony Interactions and hyphal interference between *Sepatoria nodorum* and Phylloplane Fungi. Transaction of the British Mycological. Society 66: 57-64.

Southwood MJ (2012). Evolution and detection of *Fusarium oxysporum* f.sp. *cepae* in onion in South Africa. PhD, Stellenbosch University, Stellenbosch, South Africa. https://doi.org/10.1016/S0007-1536(76)80092-7

Stein T (2005). *Bacillus subtilis* antibiotics: Structures, synthesis and specific functions. Molecular Microbiology 56: 845-857. https://doi.org/10.1111/j.1365-2958.2005.04587.x

Sundaramoorthy S, Balabaskar P (2013). Biocontrol efficacy of *Tricoderma* spp. Against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. Journal of Applied Biology and Biotechnology 1 (3): 36-40.

Tekiner N, Kotan R, Tozlu E, Dadaşoğlu F (2020). Biological control of *Botrytis cinerea* and *Alternaria alternata* with bioagent bacteria and fungi under in vitro conditions. Fresenius Environmental Bulletin 29 (1): 640-649.

Tekiner N, Tozlu E, Kotan R (2019b). Investigation of biological control possibility of Anthracnose disease agent, *Colletotrichum gloeosporioides*, on orange. Atatürk University Journal of Agricultural Faculty 50 (3): 282-291 (in Turkish with an abstract in English).

Tekiner N, Tozlu E, Kotan R, Dadaşoğlu F (2019a). Determination of some biological control agents against Alternaria fruit rot in quince. Alinteri Journal of Agriculture Sciences 34 (1): 25-31.

Tiwari K, Thakur HK (2014). Diversity and molecular characterization of dominant *Bacillus amyloliquefaciens* (JNU-001) endophytic bacterial strains isolated from native Neem varieties of Sanganer region of Rajasthan. Journal of Biodiversity, Bioprospecting and Development 1 (1): 1-15.

Tozlu E, Dadaşoğlu F, Kotan R, Tozlu G (2011). Insecticidal Effect of Some Bacteria on *Bruchus dentipes* Baudi (Coleoptera: Bruchidae). Fresenius Environmental Bulletin 20 (4): 918-923.

Tozlu E, Tekiner N, Kotan R, Örtücü S (2018b). The investigation of the biological control of *Alternaria alternata* (Fr.) Keissl. (1912). Indian Journal of Agricultural Sciences 88 (8): 1241-1247.

Tozlu E., Tekiner N, Kotan R (2018a). Screening of *Trichoderma harzianum* Rifai (1969) isolates of domestic plant origin against different fungal plant pathogens for use as biopesticide. Fresenius Environmental Bulletin 27 (6): 4232-4238.

Uygun N, Ulusoy MR, Satar S (2010). Biological control. Turkish Journal of Biological Control 1 (1): 1-14 (in Turkish with an abstract in English).

Valenzuela NL, Angel DN, Ortiz DT, Rosas RA, Garcia Ortiz CF et al. (2015). Biological control of anthracnose by postharvest application of *Trichoderma* spp. on Maradol papaya fruit. Biological Control 91 (12): 88-93.

Viriyasuthee V, Saepaisan S, Saksirirat W, Gleason ML, Chen RS et al. (2019). Effective plant ages for screening for field resistance to alternaria leaf spot (caused by *Alternaria* spp.) under natural infection in jerusalem artichoke (*Helianthus tuberosus* L.). Agronomy 9: 754. https://doi.org/10.3390/agronomy9110754

Wang H, Yan Y, Wang J, Zhang H, Qi W (2012). Production and characterization of antifungal compounds produced by *Lactobacillus plantarum* IMAU10014. PLoS ONE 7 (1): e29452. https://doi.org/10.1371/journal.pone.0029452