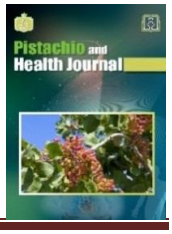

The Effects of Biocontrol Bacillus and Pseudomonas Strains on Plant Growth and Biochemical Defense Mechanisms in Pistachio Seedlings Inoculated with Phytophthora Drechsleri

Fariba Fathi (MSc)[1], Roohallah Saberi-Riseh (PhD)[1*], Mohammad Moradi (PhD)[2]

[1]Department of Plant Protection, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Iran
[2]Pistachio Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rafsanjan, Iran

Information

Article Type:

Original Article

Article History:

Received: 13 Aug. 2018
Accepted: 17 Sep. 2018

DOI:10.22123/phj.2018.144287.1010

Keywords:

Enzymatic activity
PGPR
Pistachios
Phenol content
Phytophthora drechsleri

Corresponding Author:

Roohallah Saberi-Riseh
Email: r.saberi@vru.ac.ir
Tel: +98-913-1932624

Abstract

Introduction: Plant growth promoting rhizobacteria (PGPR) are widely used in protecting plants against pathogens in horticultural crops.

Materials and Methods: In the current study, the effects of two bacterial strains, i.e. *Pseudomonas fluorescens* VUPF5 and *Bacillus subtilis* Bs96 were evaluated on plant growth and antioxidant enzymatic activities, such as GPX, PPO, and PAL, either inoculated with *Phytophthora drechsleri* or alone, in two pistachio cultivars of Sarakhs and Badami-Rize-Zarand.

Results: The two bacterial strains reduced the mortality rate of pistachio seedlings and increased growth parameters, such as dry and fresh weights of plants' roots, shoots, and height. The enzymatic activities and levels of phenolic compounds increased in seedlings after inoculation with the two bacterial strains and *P. drechsleri*. The maximum enzymatic activities were observed on the 6th day after co-inoculation with the bacterial strains and *P. drechsleri*.

Conclusions: *Pseudomonas fluorescens* VUPF5 resulted in the highest levels of enzymatic activities and phenolic compounds. The highest levels of enzymatic activities and phenol contents were found in Badami-Rize-Zarand cultivar, with a highest level of resistance to *P. drechsleri*.

►Please cite this article as follows:

Fathi F, Saberi-Riseh R, Moradi M. The Effects of Biocontrol *Bacillus* and *Pseudomonas* Strains on Plant Growth and Biochemical Defense Mechanisms in Pistachio Seedlings Inoculated with *Phytophthora Drechsleri*. Pistachio and Health Journal. 2018; 1 (3): 15-26

1. Introduction

Root and crown rot (gummosis) of pistachio trees (*Pistacia vera*) are from among the major diseases in pistachio orchards caused by several *Phytophthora* spp [1]. The most common species include *P. megasperma* [2] and *P. drechsleri* [3]. Several approaches have been used to manage root and crown rot of pistachios, including cultural, chemical, and biological controls [4, 5].

Plant growth-promoting bacteria (PGPB) are soil-borne and facilitate plant growth by several direct and indirect mechanisms. The direct mechanisms include the production of phytohormones, N2 fixation, phosphate solubilization, and siderophores production, while indirect mechanisms include fighting against phytopathogens [6] and stimulating the host plant defense system by the colonization of roots [7].

Induced resistance is a phenomenon that enhances the ability of the plant defense system using biotic and abiotic agents [8]. The induced resistance could be stimulated by siderophores, outer membrane lipopolysaccharide (LPS), bacterial flagella, or microbe-associated molecular patterns (PAMPs or MAMPs), during the root colonization process by rhizobacteria [9]. The role of lipopolysaccharides in the outer membrane of Gram-negative bacteria, especially o-antigen, siderophores, and flagella [10] has been verified in induced resistance.

During ISR, several defense-enhancing enzymes, such as chitinase, β-1,3-glucanase, peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), ascorate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), lipoxygenase (LOX), and proteinase inhibitors are released; besides, phytoalexins and phenolic compounds are accumulated [11].

There is limited information on the role of ISR induced by PGPR bacteria to control root and crown rot in pistachios; therefore, this study was conducted aimed at investigating the effects of the plant defense mechanism, measured as enzyme and phenol compounds, to control root and crown rot in pistachios, caused by *P. drechsleri*.

2. Materials and Methods

2.1. Plant growth

Two pistachio cultivars (Sarakhs and Badami-Rize-Zarand) were used in this study. To establish the cultivars in soil, a method introduced by Moradi [12] was used, with some modifications. In brief, the seeds were kept in sterile distilled water for 24h, before being soaked in a fungicide (Iprodione+ Carbendazim, of the commercial grade, 2g L^{-1}) for 5min and then placed in the mixture of sand-perlite (1-1, V/V) for germination at 28°C for 5 days. Seven germinated seeds were sown in 4kg of plastic pots containing sand soil, pH 7.2, and EC 2.3ds.m^{-1}.

2.2. The preparation of P. drechsleri inoculum

A virulent isolate of *phytophthora drechsleri* taken from infected trees was retrieved from the culture collection of the Pistachio Research Center (PRC), where it had been maintained on corn meal agar (CMA). The inoculum was produced on the rice substrate as described by Holmes and Benson [13]. In brief, 25g of rice grains and 18ml of deionized water were added to a flask and autoclaved twice for 30min on two

consecutive days. The autoclaved rice was inoculated with six plugs of 3-day old mycelium of *P. drechsleri* and then incubated for two weeks at 28°C.

2.3. The inoculation of P. drechsleri on pistachio seedlings

Two-month-old pistachio seedlings were inoculated by 5g of P. drechsleri of colonized rice around seedling roots and then were covered with soil. Control plants were inoculated with 5g of sterile rice [13]

2.4. The inoculation of bacterial inoculum

Pseudomonas fluorescens VUPF5 and *B. subtilis* Bs96 were retrieved from the PGPR collection center of Vali-e-Asr University of Rafsanjan, Iran. The inoculum of the two strains was prepared on nutrient agar (NA) at 28°C for 24h. The suspension of bacterial strains was prepared in sterile distilled water, and the concentration was adjusted at 40×10^{10} CFU mL^{-1} (OD 0.5 at 540nm $=10^{10}$), using a spectrophotometer (U-2000, Hitachi Instruments, Tokyo, Japan). The 40ml suspension of each bacterial strain was added to each pot for soil drenching purposes (equal to 10^8 CFU g^{-1}).

2.5. The determination of shoot and root dry weight

Forty five days after inoculation, the seedlings' height from the soil surface to the terminal bud were measured using a ruler, the plants were uprooted from the soil and separated into shoots and roots, and were then weighed. The dry weights were then measured after oven-drying at 70ºC for 72h [14].

2.6. The determination of the mortality rate

The mortality rate was obtained using the following equation [15]:

X= 100– (100× A)/B

X= Mortality rate, A= The number of infected plants in each treatment;

B= The number of infected plants in control plants;

2.7. The enzyme extraction and activity determination

Fresh samples were collected on days 0 (before), 3, 6, 9, and 12 after the inoculation of bacterial strains, P. drechsleri alone, or their combination. Five hundred milligrams of the fresh leaf tissue of pistachios were homogenized in 5ml of the 50mM potassium phosphate buffer, pH 7.2, containing 1mM EDTA and 1% (w/v) soluble PVP. After centrifugation (4000rpm, 20min at 4°C), the supernatant was used for the determination of the activities of Guaiacol peroxidase (GPX), polyphenol oxidase (PPO), and Phenylalanin ammonia lyase (PAL) [16].

2.7.1. The guaiacol peroxidase (GPX) activity assay

The GPX activity was done using the method introduced by Plewa et al. [17] The assay mixture contained 2.77ml of the 50mM potassium phosphate buffer (pH 7), 100µl of 4% guaiacol, 100µl of 1% H_2O_2, and 100µl of the diluted enzyme extract. To quantify GPX, the concentration of tetraguaiacol was measured spectrophotometrically by absorbance at 470nm, using the extinction coefficient of 25.5mM $^{-1}$ cm^{-1}.

2.7.2. The polyphenol oxidase (PPO) activity assay

The PPO activity was done using the method introduced by Nicoli et al. [18], with some modifications. The assay mixture contained 200µl of 0.02M pyrogallol, 2.5ml of the 50mM potassium phosphate buffer (pH 7), and 100µl of the diluted enzyme extract. The absorbance rate was measured in 420nm of the assay mixture based on the measurement of the pyrogallol oxidation by oxidative enzymes.

2.7.3. The phenylalanine ammonia lyase (PAL) activity assay

The PAL activity was done by incubating the assay mixture containing 1mM of the extraction buffer, 0.5ml of 10mM L-phenylalanine, 0.4ml of deionized water, and 0.1ml of the enzyme extract for 1h at 37°C. The reaction was stopped by the addition of 0.5ml of 6M HCl and was measured by absorbance at 260nm. One unit of the PAL activity was equal to 1µmol of the cinnamic acid produced per min [19].

2.7.4. The determination of total phenolic compounds

The total phenolic contents were measured according to the Folin-Ciocalteaumethod [20]. In brief, the fresh leaf tissue of pistachio seedlings (0.5g) was extracted by 5ml of 95% ethanol and was kept in the dark for 48h. Next, 0.5ml of the supernatant was mixed with 0.5ml of ethanol and 1.5ml of distillation water. Afterwards, 0.25ml of 50% folin regent and 0.5ml of 5% carbonate sodium were added to the samples. The samples were kept for 1h, and the absorbance rate was measured at 725nm. Gallic acid was used to produce the standard curve.

2.8. The experimental design and data analysis

The average of PAL, PPO, GPX, total phenol, and growth factors were calculated in each replication. The growth factor data were analyzed by the one-way ANOVA, while for the analysis of the enzymatic activity over time and cultivars, a factorial design was used. The mean comparisons were made using the Duncan's new multiple range test at 5% probability. SAS 9.1 (SAS Institute, Inc, Cary, NC, USA) was used to analyze the data and compare the means.

3. Results

3.1. Seedling growth

Seedlings' growth was measured using root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW), and shoot dry weight (SDW). The results demonstrated that the Plants' height (H) was significantly affected by *P. drechsleri*, cultivars, bacterial strains, and their interactions (Table 1). Higher values were recorded in non-inoculated seedlings than the seedlings inoculated with *P. drechsleri*. Cultivar Badami-Rize-Zarand showed significantly higher values ($P< 0.05$) of all the estimated parameters. Bacterial strains resulted in an increase in the assessed growth factors in *P. drechsleri* inoculated and non-inoculated seedlings, compared to the seedlings not inoculated with bacterial strains. Concerning the dry weight of the roots, inoculation with bacterial strains alone or in combination with *P. drechsleri* resulted in 2.25 and 3.75 times, and concerning the dry weight of the shoots, it resulted in 1.5 and 2.75 times more than that of the control group in Badami cultivar, respectively. Similar results were observed in Sarakhs cultivar concerning the dry weight of the roots, the dry weight of the roots and the shoots after inoculation with bacterial strains alone were 2.43 and 1.37 times, and in combination with *P. drechsleri*, it was 2.5 and 3.2 times more than that of the control group. Besides, *Pseudomonas fluorescens* VUPF5 resulted in stronger growth parameters compared to *B. subtilis* Bs96 (Table 2). The effects of inoculation with bacterial strains were more significant in Sarakhs cultivar than Badami cultivar.

Table1. Analysis of variance for growth parameters in pistachio seedlings inoculated with *Phytophthoradrechsleri*and bacterial strains alone and their combinations

Source of variations	df	Mean Squares				
		H	SFW	RFW	SDW	SRW
Cultivars (C)	1	235.11**	8.20**	35.98**	2.31**	1.20**
Bacterial strains (B)	2	17.69**	0.81**	5.32**	0.24**	0.28**
***P. drechsleri* inoculation (D)**	1	25**	0.80**	3.40**	0.24**	0.11**
C× B	2	1.19^{ns}	0.01^{ns}	3.25**	0.04**	0.07**
C× D	1	5.44*	0.54**	0.81**	0.04**	0.0004^{ns}
B× D	2	1.75^{ns}	0.66**	1.19**	0.06**	0.01**
C× B× D	2	0.69^{ns}	0.21**	0.88**	0.01*	0.01**
Error	24	0.91	0.01	0.02	0.003	0.001
CV%		10.38	9.16	7.34	11.61	10.14

**–significant (P< 0.01), *significant (P< 0.05), ^{ns}-not significant

Table 2. Efficacy of *Bacillus subtilis* strain 96 and *Pseudomonas fluorescent* strain VUPF5 on growth parameters in pistachio seedlings either in inoculation with Phytophthora*drechsleri*or alone.

	Inoculation with *Phytophthoradrechsleri*						No inoculation with pathogen				
Cultivars	**Bacterial isolates**	**Plant height (cm)**	**Shoot fresh weight(g)**	**root fresh weight(g)**	**Shoot dry weight(g)**	**root dry weight(g)**	**Plant height (cm)**	**Shoot fresh weight(g)**	**root fresh weight(g)**	**Shoot dry weight(g)**	**root dry weight(g)**
Badami-Rize-Zarand	VUPF5	8^{c}	1.50^{b}	3.96^{b}	0.80^{b}	0.75^{a}	13.66^{a}	1.85^{a}	4.48^{a}	0.91^{ab}	1.06^{a}
	96	8^{c}	1.61^{b}	3.20^{c}	0.84^{b}	0.72^{a}	13.33^{a}	2^{a}	3.45^{c}	0.96^{a}	0.77^{a}
	Control	6^{e}	0.63^{d}	0.78^{e}	0.31^{c}	0.20^{e}	10.66^{b}	1.16^{b}	3^{c}	0.64^{b}	0.47^{b}
Sarakhs	VUPF5	7^{cd}	0.70^{d}	1.23^{d}	0.28^{c}	0.25^{de}	12.33^{ab}	0.75^{d}	1.65^{d}	0.35^{c}	0.39^{c}
	96	6.66^{cde}	0.80^{d}	1.13^{e}	0.32^{c}	0.23^{e}	11^{b}	0.85^{c}	1.37^{d}	0.37^{c}	0.32^{cd}
	Control	5^{e}	0.12^{f}	0.76^{e}	0.1^{d}	0.1^{f}	8.20^{c}	0.43^{e}	1.22^{d}	0.27^{c}	0.16^{e}

Means followed with different letters show significant differences at P< 0.05 (LSD)

3.2. The seedling mortality rate

The seedling mortality rate declined significantly (P< 0.05) by 50%and 75% in pistachio seedlings treated with *P. fluorescens* VUPF5 and *B. subtilis* Bs96, respectively. All pistachio seedlings inoculated with *P. drechsleri* died after 3 weeks from inoculation (Fig.1).

3.3. The assessment of the enzymatic activity and the total phenol content in pistachio seedlings

Induced systemic resistance (ISR) is an important factor in plants' resistance to pathogens, being measured by enzymes, such as GPX, PPO, and PAL as well as the total phenol content. The levels of GPX, PPO, PAL, and the content of phenolic compounds in pistachio cultivars' leaves were affected by *P. drechsleri* inoculation, bacterial strains, and their combination (Table 3). Seedlings inoculated with *P. drechsleri* had significantly higher levels of GPX, PPO, PAL, and phenolic compounds than the control group. Seedlings inoculated with a combination of *P. drechsleri* and bacterial strains had higher levels of GPX, PPO, PAL, and the total phenol content than the seedlings inoculated either with the pathogen or bacterial strains. The results also indicated that the estimated parameters increased shortly after inoculation and reached their maximum activity on the 6th day, but they declined after wards. When bacterial strains and the pathogen were applied simultaneously, the activities of GPX, PPO, and PAL amounted to 1.88, 1.68, and 1.26, respectively, being 1.35 times more than when bacterial strains were applied alone.

Non-inoculated seedlings showed constant levels of GPX, PPO, PAL, and the total phenol content, during the 12 days of the assessment. Cultivar Badami–Rize–Zarand had a higher level of enzymatic activities and phenolic compound contents than Sarakhs. The activities of GPX, PPO, PAL, and the total phenol content in the leaves of Badami–Rize–Zarand cultivar were about 60%, 57%, 72%, and59% higher than those of Sarakhs cultivar, respectively. In addition, *P. fluorescens* VUPF5 was more effective than *B. subtilis* Bs96 in inducing antioxidant activities in both cultivars (Fig.2-5).

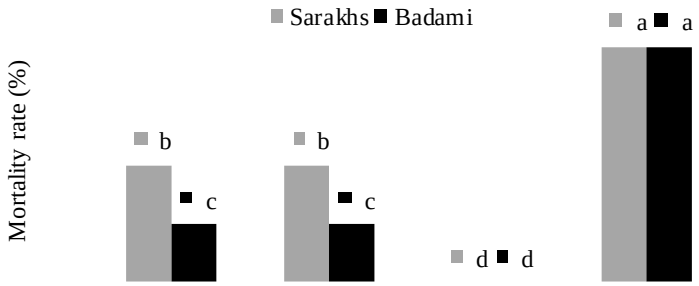

Fig. 1: Effect of pistachio seedlings inoculated with bacterial strains and *Phytophthora drechsleri* on the mortality rate under greenhouse conditions.

Table 3. Analysis of variance for biochemical defence in pistachio seedlings inoculation with *Phytophthora drechsleri* and bacterial strains alone and their combinations

Source of variations		Mean squares			
	df	GPX	PPO	PAL	Total phenol
Cultivars (C)	1	2.40**	2.81**	696.06**	925.97**
Bacterial strains (B)	2	0.34**	0.07**	7.99**	10.64**
***P. drechsleri* inoculation (D)**	1	1.56**	0.39**	32.99**	34.94**
Time (T)	4	1.06**	0.15**	57.82**	20.64**
C× B	2	0.06**	0.009**	3.37**	1.17**
C× D	1	0.33**	0.03**	32.47**	4.95**
C× T	4	0.21**	0.005**	2.40**	1.64**
B× D	2	0.08**	0.001^{ns}	0.06**	2.60**
B× T	8	0.03**	0.005**	1.5*	0.76**
D× T	4	0.21**	0.035**	3.79**	2.42**
C× B× D× T	30	0.01**	0.0008**	0.69**	0.20**
Error	120	0.001	0.0004	0.20	0.08
CV%		11.81	6.55	12.84	5.31

**significant (P< 0.01), ^{ns}-not significant

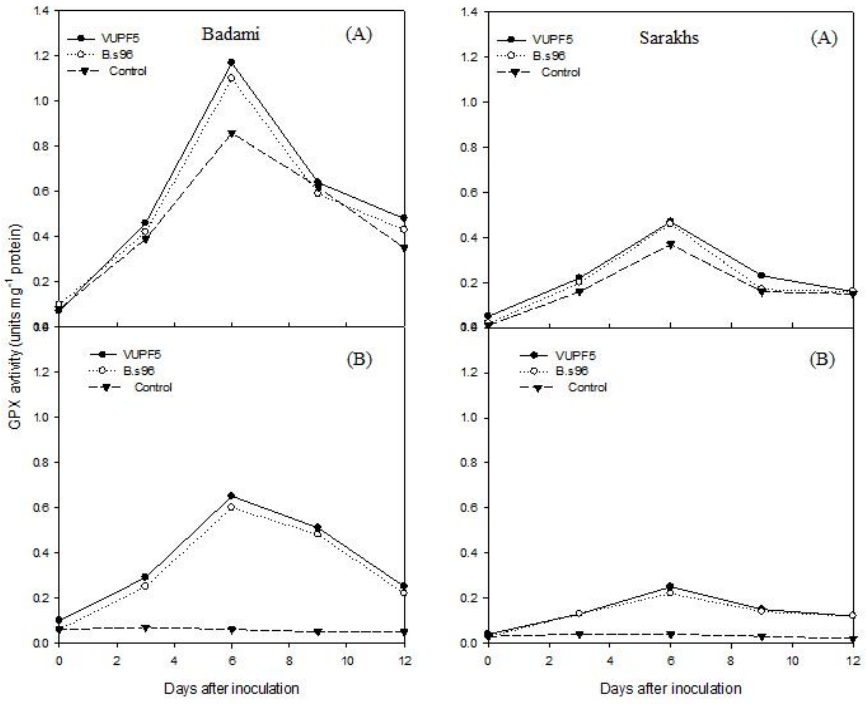

Fig. 2: Guaiacol peroxidase (GPX) activity in Badami and Sarakhs pistachio cultivars after inoculation with *Phytophthora drechsleri* in combination with two bacterial strains (A) or the bacteria strains alone (B).

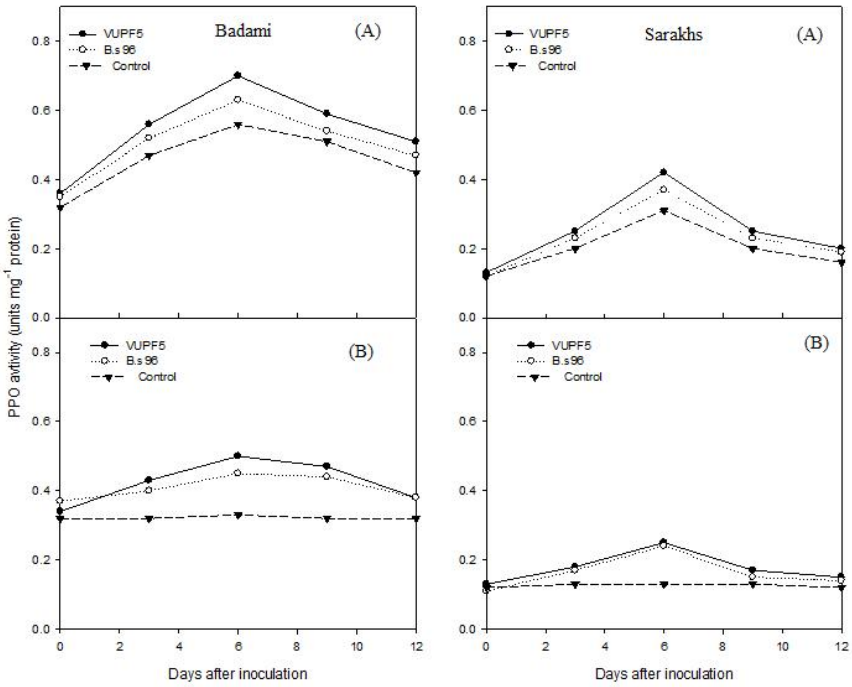

Fig.3: Polyphenoloxidase (PPO) activity in Badami and Sarakhs pistachio cultivars after inoculationwith Phytophthora drechsleriin combination with two bacterial strains (A) or the bacteria strains alone (B)

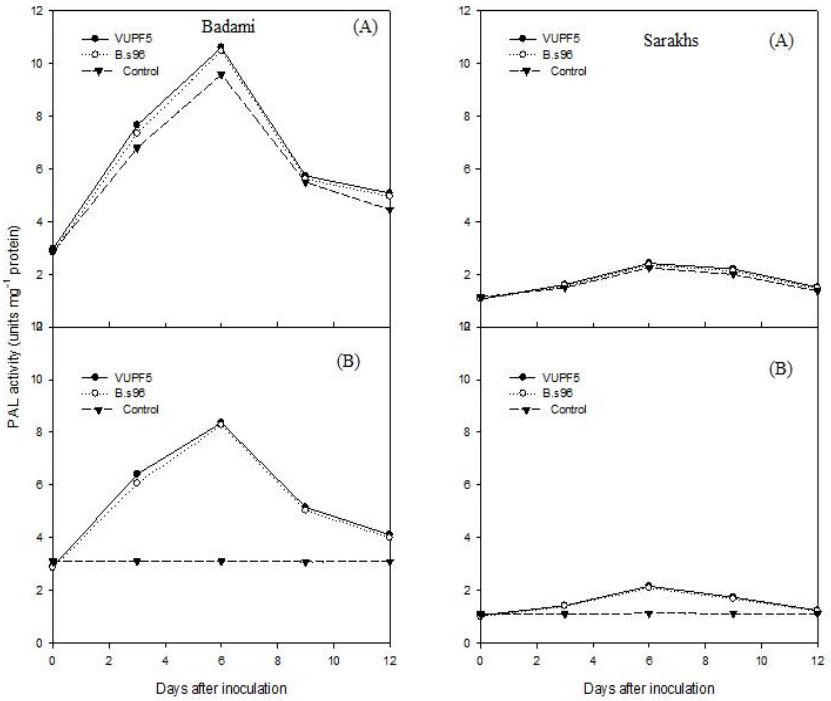

Fig. 4: Phenylalanine ammonialyase (PAL) activity in Badami and Sarakhs pistachio cultivars after inoculation with *Phytophthora drechsleri* in combination with two bacterial strains (A) or the bacteria strains alone (B)

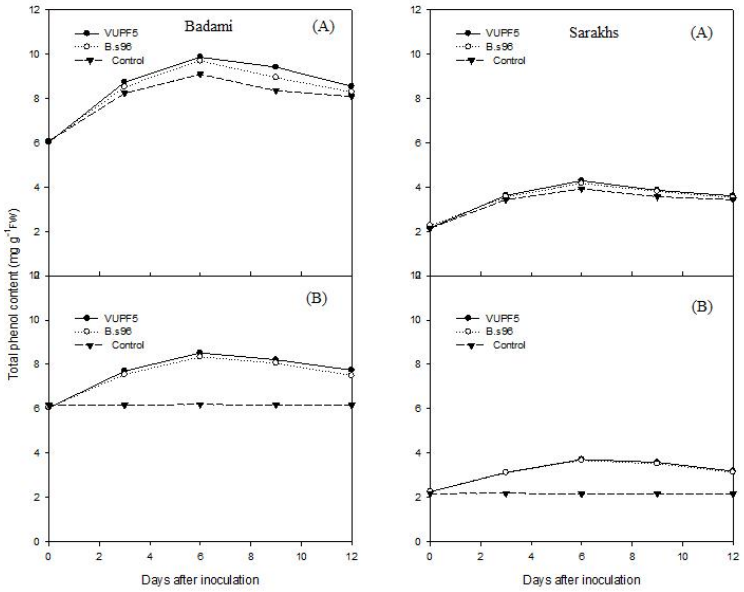

Fig. 5: Total phenol content in Badami and Sarakhs pistachio cultivars after inoculation with *Phytophthora drechsleri* in combination with two bacterial strains (A) or the bacteria strains alone (B)

4. Discussion

In recent years, considerable emphasis has been placed on the biocontrol of plant diseases. PGRP bacteria can colonize plant roots, stimulate plantgrowth, and reduce the incidence of diseases. These bacteriacause a significant increase in plant growth by producing plant hormones, providing nutrients, and colonizing plant roots. The use of PGPR bacteria has been reported to lead to an increase in several growth indicators, such as the germination rate, root growth, shoot and root weights, chlorophyll contents, the leaf area, and the biocontrol of pathogens [21]. These bacteria produce indoleacetic acid (IAA) that causes the elongation of roots and improves nutrient uptake by plants. Kidoglu et al. [22] reported that PGPR increased plant growth by increasing IAA generation in tomatoes, peppers, and cucumbers. The members of the genus *Pseudomona*s are highly competitive compared with other microorganisms due to their high affinity to the host root exudates and their faster growth rate. The inoculation of pistachio seedlings with *P. Drechsleri* had a negative effect on growth parameters (i.e. RFW, SFW, RDW, SDW, and plant height), while the responses of the seedlings inoculated with PGPR were positive. These findings are consistent with the studies by Chithrashree et al. [23] on rice, and by Dey et al. [24] on cucumbers, showing that seedlings inoculated with PGPR were of higher dry weights than seedlings inoculated with the pathogen alone.

Plants utilize defense mechanisms to counter the effects of free radicals. Several enzymes, such as peroxidase, catalase, polyphenol oxidase, and phenolic compounds accumulate in response to stress [25]. Induced systemic resistance has been reported by *Bacillus* and *Pseudomonas* against several diseases in various plants, such as banana, beans, rice, and cucumbers [26]. Antioxidant enzymes remove harmful oxygen species in cells and increase plant resistance by neutralizing the negative effects of oxygen free radicals. Current study results showed that *P. drechsleri* increased antioxidant activities in the leaves of Badami–Rize–Zarand and Sarakhs cultivars compared to non-inoculated control plants. The activities of the defense enzymes measured as GPX, PPO, and PAL

increased by the inoculation of seedlings with both antagonist agents and *P. drechsleri*. Ramamoorthy et al. [27] reported that P. *fluorescens* Pf1 induced the accumulation of PAL, PO, PPO, and phenolic compounds in tomatoes and hot pepper plants, and increased resistance against *P. aphanidermatum*.

Based on the study results, the peroxidase enzyme exerts a direct effect on plant resistance through the neutralization of free radicals and hydrogen peroxide that are toxic to pathogens. The polyphenol oxidase enzyme is involved in the process of necrosis and forms a defensive barrier against pathogens. Peroxidase and polyphenol oxidase play an important role in lignin biosynthesis and the function of other oxidized phenols. Phenylalanine ammonia lyase is one of the plant defense biochemical markers against stress and is the initiator of the phenylpropanoid pathway that results in the conversion of phenylalanine into cinnamic acid through D-amination [28]. Cinnamic acid serves as a precursor of phenolic compounds, lignin, coumarin, phytoalexins, and other downstream metabolites. In contrast, phenolic compounds play a major role in reducing or inhibiting lipid oxidation and ROS scavenging. They also serve as essential antioxidants in plants [29]. High levels of PO, PPO, and phenolic compounds have been reported in plants inoculated with either biocontrol lagentsor pathogens, or a combination of them [30].

In this study, the enzyme levels were affected by two bacterial strains, with *Pseudomonas fluorescens* VUPF5 exerting a more significant effect. Saaravanakumar et al. [31] reported that the treatment of tea plants with PGPR bacteria activated defense enzymes, such as peroxidase, polyphenol oxidase, phenylalanine ammonialyase, chitinase, and β-1,3-glucanase, and that it also enhanced resistance against the blister disease.

Phenolic compounds are considered asosmolites, getting increased in *biotic stress* conditions [31]. A close correlation was observed among the phenolic compound content, PGPR, and increased resistance to plant pathogens in chickpea plants. The increase in phenolic compounds might be due to the induction of salicylic acid and gallic acid biosynthesis that playsa key role in the induced resistance [32]. Some studies have reported that the accumulation of phenolic compounds in rice [33] and cucumbers [34] increases resistance against diseasesdue to PGPR bioformulations. These results are consistent with those of the current study.

5. Conclusions

The study results demonstrated that *P. drechsleri* can be controlled in pistachios by using PGPR bacteria. *Pseudomonas fluorescens* VUPF5 and *B. subtilis* Bs96 demonstrated high efficacy in the suppression of diseases by increasing plant defense enzymes. However, more studies are required to identify the effects of ISR induced by PGPR bacteria to inhibit pistachios'root and crown rot in orchard conditions.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank Vali-e-Asr University of Rafsanjan and Iran's Pistachio Research Institute (IPRI) for providing the research materials and funds.

References

1. Mostowfizadeh-Ghalamfarsa R, Cooke DE, Banihashemi Z. *Phytophthora parsiana* sp. nov., a new high-temperature tolerant species. Mycol. Res. 2008; 112: *783-794*.
2. Mirabolfathy M, Ershad D, Hedjaroude GhA. Isolation of *Phytophthora citrophthora* from pistachio tree in Damghan. Iran J. Plant Pathol. 1989; 25: *73*.
3. Banihashemi Z, Moradi M. The frequency of isolation of *Phytophthora* spp. from crown and root of pistachio nut tree and reaction of the crown and root to the causal agent. *Iran J. Plant Pathol.* 2004; 40: *57-75*.
4. Moradi M. Assessment of Application of Systemic and Protective Fungicides for Long-term Control of Pistachio Crown and Root Rot. Final Report of Iranian Pistachio Research Institute. 2015. 2-06-06-88008. (Persian).
5. Saberi-Riseh R, Sharifi-Tehrani A. Antagonistic effects on several bacteria on *Fusarium oxysporum*, the causal agent of root and crown rot of onion under field conditions. *Agric. Appl. Biol. Sci*. 2005; 65: *657-661*.
6. Glick BR, Karaturovic DM, Newell PC. A novel procedure for rapid isolation of plan growth-promoting *pseudomonads*. *Can.* J. Microbiol. 1995; 41: *533–536*.
7. Choudhary DK, Johri BN. Interactions of *Bacillus* spp. and plants -with special reference to induced systemic resistance, Microbiol. Res. 2009; 164: *49- 513*.
8. Kloepper JW, Tuzun S Kuc JA. Proposed definitions related to induced disease resistance. Biocontrol Sci. Technol. 1992; 2: *349–351*.
9. Boller T, Felix G. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol. 2009; 60: *379–406*.
10. Van Loon LC, Bakker PAHM. Signalling in rhizobacteria-plant interactions. In: De Kroon H, Visser EJW. *Root ecology*. Berlin: 2003. p. *297–330*.
11. Van Loon LC, Bakker PAHM, Pieterse CMJ. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 1998; 36: *453-483*.
12. Moradi M. Isolation and identification of Phytophthora species from root and crown of pistachio in Kerman and Fars provinces and resistance determination of root and crown among current pistachio cultivars. M.Sc., Faculty of Agriculture, Shiraz University, Iran, 1998.
13. Holmes KA, Benson DM. Evaluation of *Phytophthora parasitica* var. *nicotianae* for biocontrol of *Phytophthora parasitica* on *Catharanthus roseus*. Plant Dis. 1994; 78: *193–199*.
14. Shahidi Bonjar GH, Fooladi MH, Mahdavi MJ, Shahghasi A. Broadspectrim, a novel antibacterial from *Streptomyces* sp. Biotechnolo. 2004; 3: *126-130*.
15. Hokeberg M, Gerhardson B, Johnsson L. Biological control of cereal seedborne diseases by seed bacterization with greenhouse- selected by bacteria. Eur. J. Plant Pathol. 1997; 103: *25-33*.
16. Nasibi F, Barand A, Manouchehri Kalantari Kh, Rezanejad F. The effect of argini pretreatment on germination, growth and physiological parameters in the increase of low temperature tolerance in *Pistacia vera* L. in vitro culture. Intel J Agri Crop Sci. 2013; 17: 1918-1925.
17. Plewa MJ, Smith SR, Wanger ED. Dieth yldithiocarbamate suppresses the plant activation of aromatic amines into mutagens by inhibiting tobacco cell peroxidase. Mutat. Res. 1991; 247: *57–64*.
18. Nicoli MC, Elizable BE, Piotti A, Lerici CR. Effect of sugar and maillard reaction products on polyphenol oxidase and peroxidase activity in food. J. Food Biochem. 1991; 15: *169-184*.
19. D´cunha GB, Satyanarayan V, Nair PM. Purification of phenylalanine ammonialyase from *Rhodotorula glutinis*. Phytochem. 1994; *42: 17*.

20. Roland SF, Laima SK. Phenolics and cold tolerance of *Brassica napus*. Plant Agri. 1999; 1: *1-5*.

21. Cummings SP. The application of plant growth promoting rhizobacteria in low input and organic cultivation of graminaceous crops; potential and problems. Environ. Biotechnol. 2009; 5: *43-50*.

22. Kidoglu F, Gül A, Ozaktan H, Tüzel Y. Effect of rhizobacteria on plant growth of different vegetables. International Symposium on High Technology for Greenhouse. Naples, Italy: 2007.

23. Chithrashree Udayashankar AC, Nayaka SC, Reddy MS, Srinivas C. Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *Oryzae*. Biol. Control. 2011; 59: *114-122*.

24. Dey R, Pal KK, Tilak KVBR. Plant Growth Promoting Rhizobacteria in Crop Protection and Challenges. In proceedings of Future Challenges in Crop Protection Against Fungal Pathogens. New York: 2014. P. *31-58*.

25. Elad Y, Chet I. Possible role of competition for nutrients in biocontrol of *Pythium* damping-off by bacteria. Phytopathology. 1987; 77: *190-195*.

26. Kavino M, Harish S, Kumar N, Saravanakumar D, Damodaran T, Soorianathasundaram K, Samiyappan R. Rhizosphere and endophytic bacteria for induction of systemic resistance of banana plantlets against *bunchy top virus*. Soil Biol. Biochem. 2007; 39: *1087-1098*.

27. Ramamoorthy V, Raguchander T, Samiyappan R. Induction of defense related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporum* f. sp. *Lycopersici*. Plant Soil. 2002; 239: *55-68*.

28. Solecka D. Role of phenyl propanoid compounds in plant responses to different stress factor. Acta. Physiol. Plant. 1997; 19: *257-268*.

29. He Y, Zhu ZY. Exogenous salicylic acid alleviates NaCl toxicity and increases antioxidative enzyme activity in *Lycopersicun esculentum*. Biol. Plant. 2008; 52: *792-795*.

30. Chen C, Belanger RR, Benhamou N, Paulitz TC. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria and *Pythium aphanidermatum*. Physiol. Mol. Plant Path. 2000; 56: *13-23*.

31. Saravanakumar D, Vijayakumar C, Kumar N, Samiyappan R. PGPR-induced defense responses in the tea plant against blister blight disease. Crop Prot. 2007; 26: *556–565*.

32. Singh UP, Sarma BK, Singh DP. Effect of plant growth-promoting rhizobacteria and culture filtrate of *Sclerotium rolfsii* on phenolic and salicylic acid contents in chickpea (*Cicer arietinum*). Curr. Microbiol. 2003; 46: *0131-0140*.

33. Nayar K. *Development and evaluation of a biopesticide formulation for control of foliar diseases of rice*. Ph.D Thesis, Tamil Nadu, Agriculture Uinversity, Coimbatore, 1996.

34. Liu L, Kloepper JW, Tuzun S. Induction of systemic resistance in cucumber against *Fusarium* wilt by plant growth promoting rhizobacteria. Phytopathology. 1995; 85: *695-69*.