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Effect of some Probiotic Bacteria as Biocontrol Agents of *Meloidogyne incognita* and Evaluation of Biochemical Changes of Plant Defense Enzymes on Two Cultivars of Pistachio

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ABSTRACT

Root-knot nematodes are the most economically important plant pathogens in pistachio. The ability of *Pseudomonas fluorescens* strains VUPF5, VUPF52, *Bacillus cereus* strain PRC95 and *Bacillus subtilis* strain PRC96 were tested as biocontrol agents for *Meloidogyne incognita* on the pistachio cultivars Sarakhs and Badami. The effect of these bacterial strains on defense-related enzymes activity in pistachio was also investigated. Pistachio seedlings of both cultivars were treated with bacterial strains and then were inoculated with 2000 second-stage juveniles of nematode after two days. Evaluations were made for changes of Peroxidase (POX), PolyPhenolOxidase (PPO), Phenylalanine Ammonia lyase (PAL) and Total Phenolic Content (TPC) determined at 2, 4, 7, and 10 Days After nematode Inoculation (DAI). Results showed improved activity of POX, PAL and PPO in both cultivars. The most significant result for POX activity in the treated seedlings belonged to *Pseudomonas* strain VUPF5 at 7 DAI for Sarakhs and 10 DAI for Badami. However, this strain displayed an increase in PAL activity at 2 and 4 DAI in Badami and Sarakhs, respectively. Seedlings treated by the *Pseudomonas* strain VUPF52 at 10 DAI had the highest PPO activity among cultivars. TPC concentration was slightly higher, by 8.4% at 4 DAI, in Sarakhs seedlings treated with VUPF5, but no significant increase could be seen in the Badami cultivar compared with the control. In another experiment, 4 months after nematode inoculation in seedlings of both cultivars treated by bacterial strains, numbers of galls, egg masses, and second juveniles decreased compared with the non-treated seedlings.

Keywords*: Bacillus subtilis*, *Pistachia vera* cvs. Badami and Sarakhs, , Root knot nematode.

INTRODUCTIONM

Root Knot Nematodes (RKN) are major pests affecting pistachio trees. They cause decreased yield in almost all pistachio-producing areas of Iran (Fatemy, 2009). The susceptibility of most rootstocks to RKN, limitations on the use of nematicides, the wide range of hosts, and poor control measures have increased economic losses to the pistachio industry. The most prevalent species in pistachio orchards are those of *Meloidogyne javanica* (Treub, 1885)

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Chitwood, 1949 and *M. incognita* (Kofoid and White, 1919) Chitwood, 1949 (Fatemy, 2009). Levels of resistance of pistachio cultivars to RKN decreases in the following order, Khanjari-Rafsanjan, Ghazvini, Sarakhs, Fandoghi, and Badami (Madani *et al*., 2012).

RKN populations are naturally affected by a number of soil organisms, including predators, nematophagous fungi, endophytic fungi, and bacteria. Endospore-forming bacteria (mainly *Bacillus* spp.) and fluorescent Pseudomonads are predominant rhizosphere bacteria that antagonize nematodes (Viaene *et al*., 2006). Rhizobacteria can protect plants from infection by direct mechanisms such as production of toxins, enzymes, and other metabolites or by inducing systemic resistance (Bakker *et al*., 2007). Studies on different crops have shown that *Bacillus* and *Pseudomonas* species are able to control nematodes by decreasing penetration of Second-stage Juveniles (J2s), decreasing migration of J2 towards the bacteria-treated roots, and decreasing the percentage of eggs that hatch (Huang *et al*., 2010; Siddiqui and Shaukat, 2002). *Bacillus cereus* strain S2 could induce reactive oxygen species accumulation and destroy the genital tract in *M. incognita* as well inhibits the nematode multiplication (Gao *et al*., 2016). *Bacillus megaterium* decreased penetration and gall formation in rice against *Meloidogyne graminicola* (Padgham and Sikora, 2007).

The use of plant growth promoting rhizobacteria (PGPR) increases defense enzymes of Phenylalanine Ammonia Lyase (PAL), PolyPhenolOxidase (PPO), and Peroxidase (POX) so that primed plants show decrease in disease intensity compared to untreated plants (Chen *et al*., 2000; Gao *et al*., 2016; Niu *et al*., 2011). PAL catalyzes deamination of L-phenylalanine through formation of trans-cinnamic acid, which is a precursor of several types of phenolic compounds in the phenylpropanoid pathway. PAL and POX are involved in biosynthesis of lignin in plants (Fortunato *et al*., 2014). PPO has the ability to convert phenolic compounds to quinones that are extremely toxic to pathogens (Boeckx *et al*., 2015; Peng and Kuc, 1992). These enzymes, which are related to induce systemic resistance (ISR), can be increased in plants through PGPR. Biocontrol agents that have different biocontrol mechanisms can enhance their capacity to suppress a broad spectrum of pathogens (Bakker *et al*., 2007; Chen *et al*., 2000; Silva *et al*., 2010).

The present study was done to assess the efficacy of the probiotic strains (*Pseudomonas fluorescens* strains VUPF5, VUPF52, *Bacillus subtilis* strain PRC96 and *Bacillus cereus* strain PRC95) against *Meloidogyne incognita* in perennial pistachio plants and to evaluate the induction of plant-defense enzymes activity 2, 4, 7, and 10 DAI in pistachio plants. Also assessed was the relationship of susceptibility of Sarakhs and Badami cultivars to biochemical changes.

MATERIALS AND METHODS

Bacterial Inoculum

All experiments used single colonies of *Psuedomonas fluorescens* strains VUPF5 and VUPF52 (maintained at the bacteriology collection of Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran) and *Bacillus cereus* strain PRC95 and *Bacillus subtilis* strain PRC96 (maintained at the bacteriology collection of Pistachio Research Center (PRC), Rafsanjan, Iran). Bacterial inoculum was prepared by inoculating nutrient broth with fresh single colonies for 72 hours at 28°C. Absorbance of the bacterial suspension was measured spectrophotometrically and adjusted to

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1×10^8 CFU mL^{-1} by sterile distilled water. Ten mL of this suspension were introduced to the soil around the pistachio seedling in each pot.

Preparation of Nematode Inoculum

Soil and root samples were collected from pistachio orchards in Rafsanjan (Kerman province, Iran). To produce pure nematode cultures, single egg masses were used to establish a population on *Solanum lycopersicum* L. cv. Earlyurbana. The species was identified using perineal patterns (Eisenback and Triantaphyllou, 1991; Jepson, 1987) and molecular assays (Soleymanzadeh, *et al*. 2016), as *Meloidogyne incognita* (rDNA sequence of *M. incognita* is deposit at the Gen Bank as KU380337 for the D2-D3 region).

To obtain nematode inoculum, tomato roots with galls were placed in 0.5% sodium hypochlorite solution and shaken for 4 min, then, promptly rinsed with sterile distilled water on a 500 mesh sieve. The suspension of eggs was poured onto the surface of the tissue paper that covered a coarse sieve in a dish containing water. The dishes were kept at 27°C and J2s were collected from dishes for up to 4 days (Hussey and Barker, 1973). All experiments used 2,000 J2s to inoculate 2-month-old pistachio seedlings.

Plant Material

Seeds of Sarakhs and Badami cultivars (*Pistacia vera* L.) were obtained from PRC. These were soaked in 0.5% sodium hypochlorite for 3 minutes, and then washed with sterile distilled water. They were then placed in a sterilized sand/perlite (50/50, v/v) substrate at 27°C for 7 days. The germinated seeds were transplanted at a rate of one seedling per pot and each pot contained 1 kg autoclaved soil (sand:clay; 2:1 v/v). These pots were maintained in a greenhouse at 28°C until harvest (Khatamidoost *et al*., 2015).

Multiplication Factors of Nematode Assay

After 4 month, the nematode-infected pistachios were uprooted, washed, and stained in Phloxine-B (150 mg L^{-1}) for 15 minutes, then rinsed with tap water to enhance egg-mass visibility (Dickson and Struble, 1965). Numbers of galls and egg masses were counted under a stereomicroscope (Carl Zeiss, Germany). J2s were extracted from 250 g of well-mixed soil from each pot with modified Whitehead tray and this number was then multiplied by the amount of soil in each pot (Whitehead and Hemming, 1965).

Enzyme Activity Assay

Evaluations were made for POX, PAL, PPO, and Total Phenol Content (TPC) on days 2, 4, 6, 9 and 12 after bacterization (same 2, 4, 7, and 10 DAI in the presence of nematode). Fresh leaves were taken from each plant per treatment and deeply frozen (–20°C). Five hundred mg of the frozen leaf material per treatment was homogenized in cool phosphate buffer (50 Mm containing 1 mM EDTA and 1% (w/v) PVP; pH 7.0) followed by centrifugation at 20,000×g for 20 minutes at 4°C (Denley BR401 UK). The supernatant was used to quantify enzyme activity (Chen *et al*., 2000).

Peroxidase Activity (POX) (EC 1.11.1.7)

Peroxidase activity was determined using the method recommended by (Zhang *et al*., 2005). The reaction mixture consisted of 30 µL of plant extract, 100 µL of 4% guaiacol, and 2.77 mL of 50 mM phosphate buffer

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(pH 7.0). The reaction was initiated by addition of 100 µL of 1% hydrogen peroxide to the mixture. The formation of tetragauiacol was measured as absorbance at 470 nm every 30 seconds for 3 minutes using an extinction coefficient of 25.5 mM^{-1} cm^{-1} by spectrophotometer (Spectronic ®, UK). One unit of POX activity was expressed as a change in absorbance at 470 nm for 1 mg fresh weight per minute**.**

Phenylalanine Ammonia Lyase Activity (PAL) (EC 4.3.1.5)

PAL activity was determined based on the rate of cinnamic acid production. One mL of the extraction buffer, 0.5 mL of 10 mM L-phenylalanine, 0.4 mL of deionized water, and 0.1mL of enzyme extract were incubated at 37°C for 1 hour. The cinnamic acid production was terminated by addition of 0.5 mL of 6M HCl. PAL activity was determined from a standard curve of cinammic acid vs. absorbance (260 nm). One unit of PAL activity equaled 1µmol of cinnamic acid produced per minute (Li *et al*., 2008).

Polyphenol Oxidase Activity (PPO) (EC 1. 14. 18. 1)

PPO activity was assayed as described by Nicoli *et al*. (1991) with some modifications. The reaction mixture contained 200 µL of 0.02M freshly prepared pyrogallol in 2.5 mL of 50 mM potassium phosphate buffer (pH 7.0) and 100 µL of enzyme extract. PPO activity was measured by a change in assay mixture at 420 nm based on measurement of the disappearance of pyrogallol by enzymatic oxidation. An extinction coefficient of 6.2 mM^{-1} cm^{-1} was used and one unit of PPO activity was expressed by the change in absorbance of 1 mg fresh weight per minute.

Total Soluble Proteins

To quantify the total protein content as mg g^{-1} of fresh weight of leaves, the Bradford test (Bradford, 1976) was performed by adding 5.0 mL Bradford reagent to 0.1 mL of plant extract and absorbance was read at 595 nm. A standard curve was created with bovine serum albumin.

Total Phenolic Content

The phenolic content was determined using Gao method (Gao *et al*., 2000). Gallic acid was used for preparation of the standard curve. The results were expressed as mg of Gallic acid per mg of fresh weight.

Experimental Designs

The experiment was factorial in a completely randomized design with 5 treatments (control, VUPF5, VUPF52, PRC95, PRC96) in two sets (with and without the nematodes) with three replicates. In treatments with the presence of nematode, the second-stage juveniles were inoculated at 2 days after soil bacterization. The average enzyme levels of POX, PAL, PPO, and TPC were determined for each replication at different days after bacterial treatment (2, 4, 6, 9, and 12) in each cultivar.

In another assay, the effect of bacterial strains on multiplication factors of nematode was investigated using a completely randomized design with three replications for both cultivars. The averages of numbers of galls, egg masses per root system, and juveniles per pot were determined after 4 months. Data were subjected to Analysis Of Variance (ANOVA) using the general linear model in SAS software (version 9.1). Comparisons of means were made using

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LSD (Least Significant Difference) test (P-value≤ 0.05).

RESULTS

Enzyme Activity Assay

POX Activity

In both cultivars, POX levels were significantly higher in pistachio plants treated by bacterial strains than in the untreated controls (P< 0.05). The presence of nematode was effective on levels of enzymes activity ($F_{(1,140)}$= 901.66, P< 0.0001). In both cultivars, POX levels increased from 4 to 10 DAI compared to those without nematode (Figure 1). There were significant differences between bacterial strains for induction of POX activity ($F_{(4,140)}$= 182.14, P< 0.0001). Generally, both cultivars treated by *Pseudomonas* strain VUPF5 showed high levels of POX activity, but in Sarakhs cultivar only *Pseudomonas* strain VUPF52 and *B. cereus* strain PRC95 had good performance in presence as well as absence of the nematode (Figure 1).

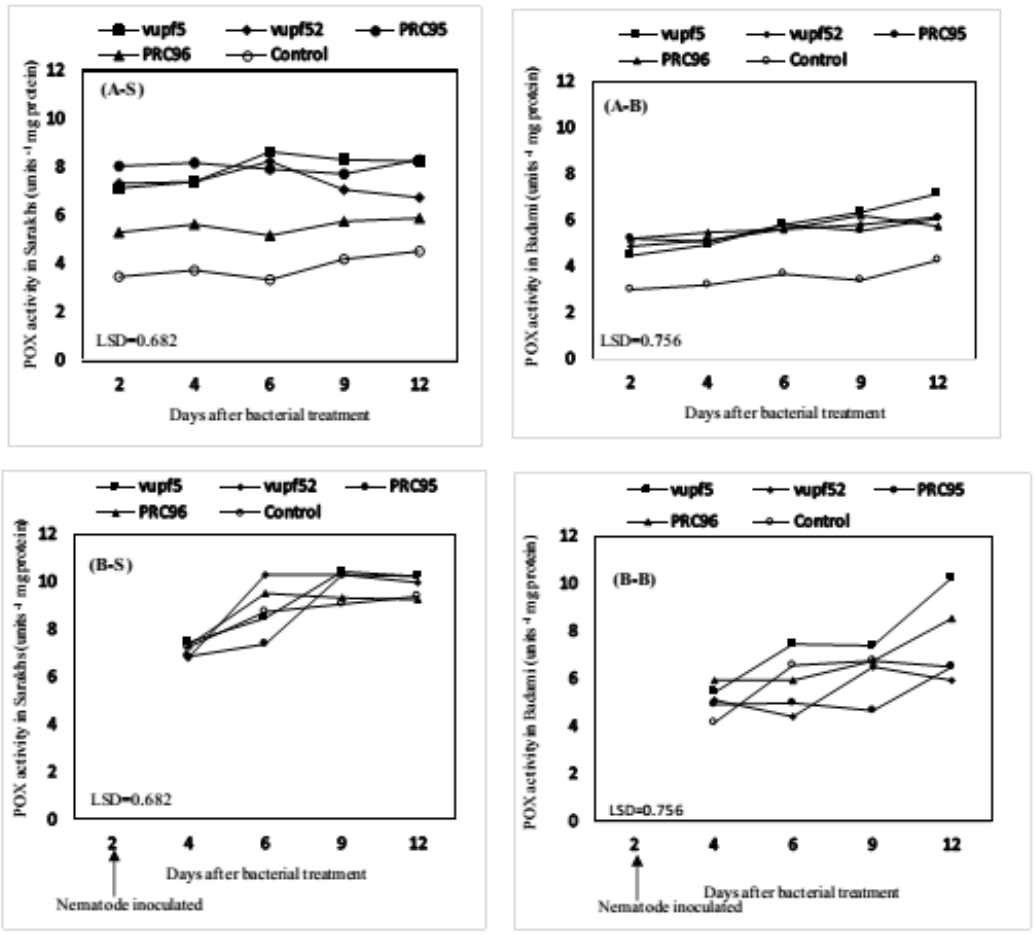

Figure 1 (A, B). POX activity in Sarakhs (S) and Badami (B) cultivars of pistachio, difference between means more than LSD are significant. (A) Pistachio roots treated with bacterial strains only, (B) treated roots were inoculated with *Meloidogyne incognita* 2 days after bacterial treatment.

[DOR: 20.1001.1.16807073.2018.20.1.12.4]

PAL Activity

Based on the results of analysis of variance, there were significant differences between two cultivars for PAL activity as well as that of other enzymes ($F_{(1,140)}$= 174.48, P< 0.0001). Sarakhs cultivar seedlings treated by the *Pseudomonas* strain VUPF52 in the absence of nematode showed high levels of PAL activity from sixth day until twelfth day and low levels of PAL activity (P< 0.05) after nematode inoculation (Figure 2). Treated seedlings of Sarakhs cultivar by *Bacillus* strains in the absence of nematode had low levels of PAL activity and increased levels of enzyme activity after nematode inoculation. However, this trait was observed in treated seedlings of Badami cultivar by only *Bacillus* strain PRC96 (Figure 2). In both cultivars treated by *Pseudomonas* strain VUPF5 at 6 and 9 days showed high levels of enzyme activity in absence as well as presence of nematode**.**

PPO Activity

PPO activity from 4 DAI was induced in both cultivars by *Pseudomonas* strain VUFP52 and reached a peak at 10 DAI (Figure 3-B). Sarakhs seedlings treated by

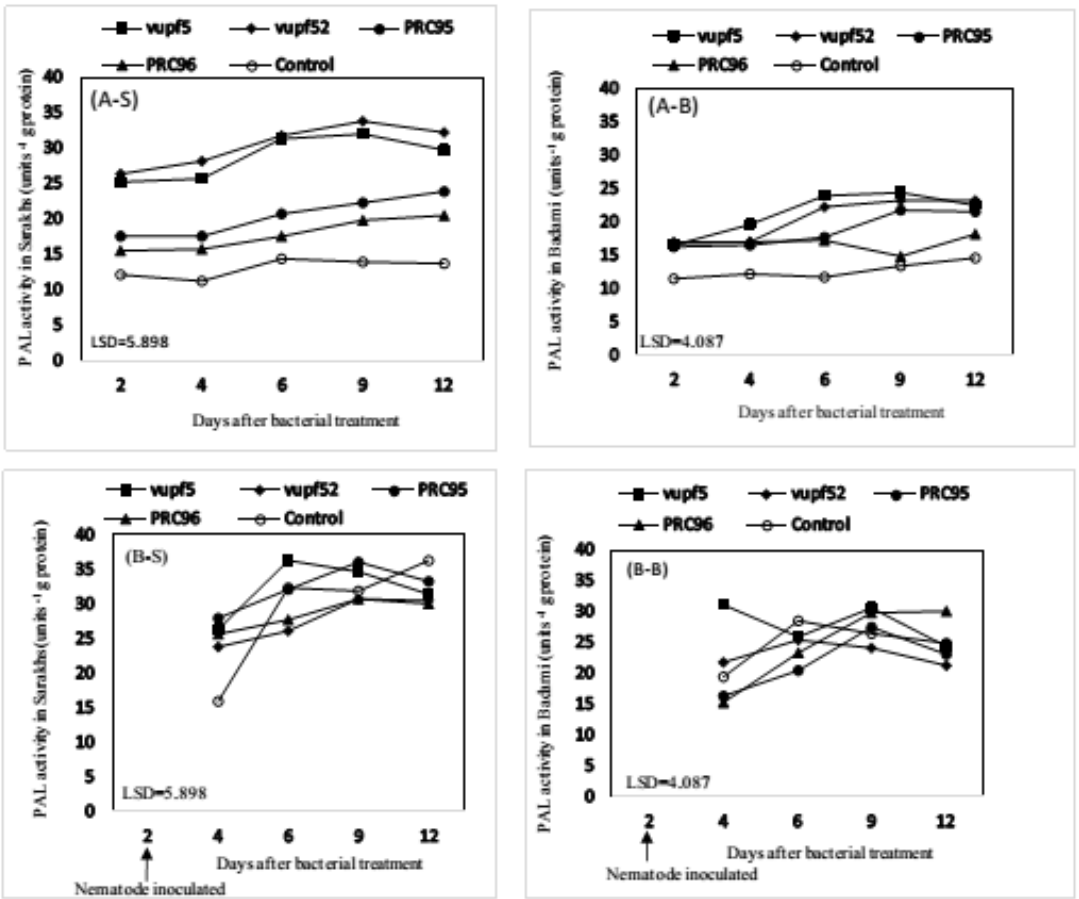

Figure 2 (A, B). PAL activity in Sarakhs (S) and Badami (B) cultivars of pistachio, difference between means more than LSD are significant. (A) Pistachio roots treated with bacterial strains only, (B) treated roots were inoculated with *Meloidogyne incognita* 2 days after bacterial treatment.

[DOR: 20.1001.1.16807073.2018.20.1.12.4]

Bacillus subtilis strain PRC96 showed a significant increase from 4 to 10 DAI and reached the higher levels of enzyme activity compared with the other treatments (10.46 unit mg^{-1} protein). In the seedlings of this cultivar treated by *Bacillus cereus* PRC95, PPO activity was significantly lower in the presence of nematode. Although in the absence of nematode, Sarakhs seedlings treated with this strain displayed the highest level of PPO activity on the twelfth day in comparison with the other treatments (Figure 3-A). Based on the results, there were significant differences between sampling times and PPO activity ($F_{(4, 140)}$= 363.33, P< 0.0001). On the twelfth day, almost all bacteria-treated plants showed higher levels of PPO activity in both experiments with and without nematode.

TPC Activity

The results showed that TPC increased significantly in seedlings of both cultivars treated with bacterial strains from day 2 to 12 in absence of nematode (Figure 4-A). Seedlings of Sarakhs cultivar treated by *Pseudomonas* strain VUPF5 in the presence of nematodes showed the highest levels of TPC in all sampling time points. Badami cultivar treated by this strain only showed an

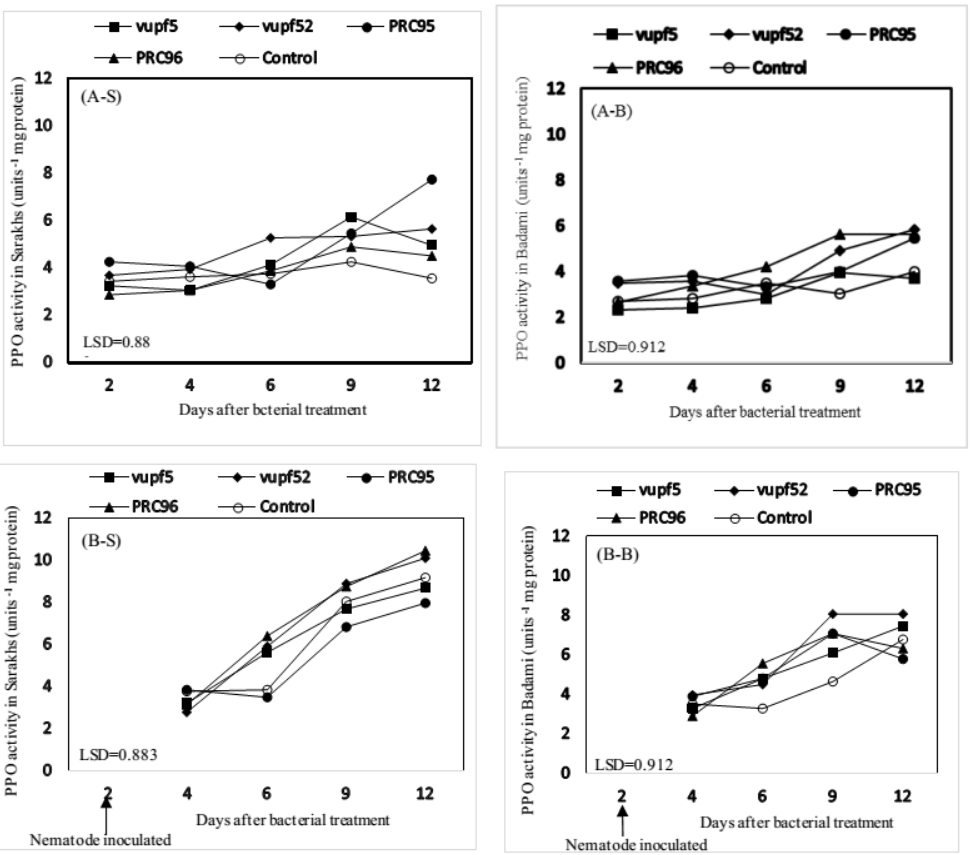

Figure 3 (A, B). PPO activity in Sarakhs (S) and Badami (B) cultivars of pistachio, difference between means more than LSD are significant. (A) Pistachio roots treated with bacterial strains only, (B) treated roots were inoculated with *Meloidogyne incognita* 2 days after bacterial treatment.

[DOR: 20.1001.1.16807073.2018.20.1.12.4]

increase at 2 DAI by 24.52% and then showed lower levels compared to the controls. A decrease in TPC happened at 10 DAI in both cultivars for *Bacillus* strain PRC96 and *Pseudomonas* strainVUPF52 (Figure 4-B).

Multiplication Factors of Nematode

Efficiency of probiotic strains in decreasing reproduction factors of the nematode was not affected by the cultivar, for instance (F gall g^{-1} $root_{(1,\ 20)}$= 2.37, P= 0.13). Number of galls and egg masses were significantly lower on pistachio roots treated with bacterial strains than on the untreated control (P< 0.01) (Figures 1, 2). *Pseudomonas* strain VUPF52 was more efficient in reducing number of galls (58%) and egg masses (41%) than the other treatments. *Bacillus cereus* PRC95 showed similar results in terms of reducing number of galls and both *Bacillus* strains showed similar results in reducing number of egg masses. Seedlings treated by *Bacillus cereus* PRC95 decreased J2s pot^{-1} (73%) compared to the untreated control, however, all bacterial strains had a statistically similar effects on this trait (Table 1).

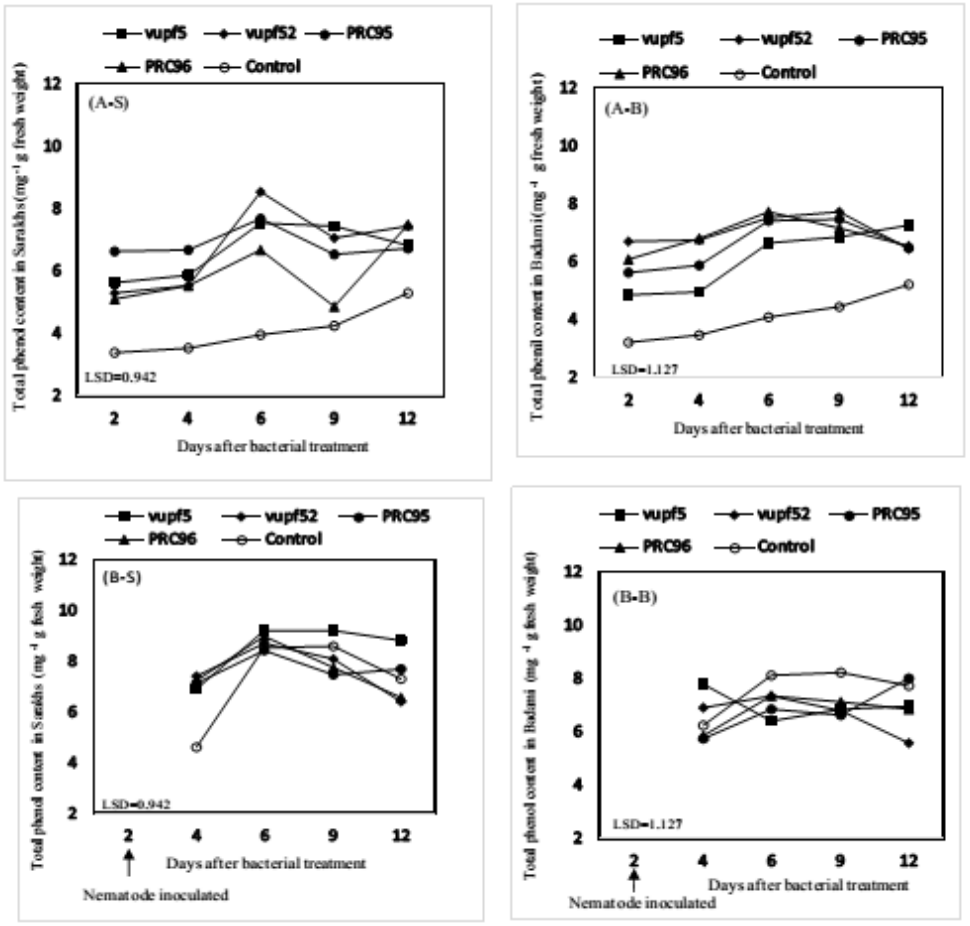

Figure 4 (A, B). Total phenol content in Sarakhs (S) and Badami (B) cultivars of pistachio, difference between means more than LSD are significant. (A) Pistachio roots treated with bacterial strains only, (B) treated roots were inoculated with *Meloidogyne incognita* 2 days after bacterial treatment.

[DOR: 20.1001.1.16807073.2018.20.1.12.4]

Table 1. Effects of bacterial strains on mean number of root galls, egg masses per root, and second juveniles per pot.[a]

Treatment	Sarakhs cultivar			Badami cultivar		
	Gall NO±*SD*	Egg-mass NO±*SD*	J2s pot^{-1}±*SD*	Gall NO±SD	Egg-mass NO±*SD*	J2s pot^{-1}±*SD*
Nematode	136.67 ± 10.59 ^{a}	42.66 ± 4.50 ^{a}	888.3 ± 382.67 ^{a}	173.33 ± 12.34 ^{a}	56.66 ± 10.26 ^{a}	899.3 ± 403.70 ^{a}
VUPF52	68.67 ± 22.50 ^{b}	30.33 ± 6.11 ^{a}	222.7 ± 89.18 ^{b}	61.67 ± 14.74 ^{c}	28.0 ± 2.64 ^{c}	262.0 ± 83.37 ^{b}
VUPF5	94.67 ± 17.00 ^{b}	38.33 ± 15.37 ^{a}	258.0 ± 53.81 ^{b}	121.0 ± 33.04 ^{b}	42.33 ± 8.39 ^{b}	282.0 ± 58.95 ^{b}
PRC95	87.33 ± 21.54 ^{b}	36.33 ± 10.01 ^{a}	214.0 ± 70.37 ^{b}	91.67 ± 12.05 ^{bc}	36.33 ± 5.13 ^{bc}	264.0 ± 52.09 ^{b}
PRC96	100.33 ± 28.18 ^{ab}	29.66 ± 13.01 ^{a}	261.7 ± 144.11 ^{b}	98.67 ± 24.17 ^{bc}	37.33 ± 4.72 ^{bc}	322.3 ± 155.10 ^{b}

[a] Means followed by the same letter in a column are not significantly different by *LSD* (Least Significant Difference) test (P-value≤ 0.05). *SD*: Standard Deviation of means.

DISCUSSION

Many studies have shown that *P. fluorescens*, *B. subtilis*, and *B. cereus* are the most effective antagonistic bacteria in controlling various diseases and potentially useful as biocontrol agents (Bakker, 2007; Chen, 2010; Gao, 2016). The bacterial strains in the present study provided a significant protective effect on multiplication factors of *M. incognita* in pistachio seedlings of both cultivars. Furthermore, in the absence of the nematode, these bacterial strains increased POX, PAL, PPO activity and TPC in both treated cultivars, while Badami cultivar showed a more prominent increase in enzymes. Previous studies have also shown that resistance mediated by some rhizobacteria species was cultivar specific and that increased activity of these enzymes depended on factors such as inducing agent, plant genotype, and challenging pathogen (Liu *et al*., 1995; Van Wees, 2008; Zivdar *et al*., 2016). Nematode inoculation increased levels of enzymes activity in pistachio seedlings more than bacterial treatments alone. Previous studies have shown that mechanisms underlying induction of enzymes activity by the pathogen and PGPR may differ (Chen *et al*., 2000; Van Wees, 2008). Considering the significant decrease in multiplication factors of *M. incognita* in the treated seedlings, it was tempting to speculate the involvement of inducible defense responses by bacterial strains against the nematode.

The highest levels of POX activity were observed at 7 and 10 DAI on treated seedlings of both cultivars. These results are in agreement with those of previous studies that determined maximum peroxidase activity in pea plants treated with *Bacillus subtilis* at 7 DAI by *Fusarium nudum* (Podile and Laxmi, 1998). An increase in POX activity is usually associated with the

later stages of infection (Peng and Kuc, 1992; Li *et al*., 2008).

PAL activity usually begins about 24 hours after pathogen inoculation (Fortunato *et al*., 2014; Podile and Laxmi, 1998). In the Sarakhs cultivar, there was low PAL activity for nematode treatments alone at 2 DAI (Figure 2-B-S). This could be explained by a delay in nematode contact with the pistachio roots. In the Badami cultivar, at 2 DAI, some J2s may have entered the roots so that the PAL level at 4 DAI was high (Padgham and Sikora, 2007) (Figure 2-B-B). Previous studies have shown that the rhizospheres of different plants contain different nutritional sources and root exudates that support rhizobactrial strains (Liu *et al*., 1995; Bowsher *et al*., 2016). The delay in increase of activity of PAL with *Pseudomonas* strain VUPF5 in Sarakhs by 26.53 vs. 31.07 units g^{-1} protein for Badami at 2 DAI can be explained, as previously mentioned, by slower movement of nematodes toward the Sarakhs exudates or by a lag phase in bacterial strain adaptation in this cultivar. Treated roots of both cultivars by *Bacillus* strains PRC95 and PRC96 before nematode inoculation were accompanied by an increase in PAL production but at a lower level than that for *Pseudomonas* strains. These results for *Bacillus* strains are in agreement with those of a previous study on plants primed with *Bacillus cereus* AR156 to avoid activation of energy-consuming defense response prior to a pathogen attack (Niu *et al*., 2011; Van Wees *et al*., 2008).

PPO participates in oxidation of many types of phenolic compound (Li *et al*., 2008). In Badami cultivar, seedlings treated by *Bacillus* strains at 7 DAI and by *Pseudomonas* strains at 10 DAI showed higher levels of PPO activity, but, seedlings of Sarakhs cultivar treated by all bacterial strains exhibited an increasing trend of changes from the first to 10 DAI. The changes in PPO activity for *Bacillus* strains treatments in the Badami cultivar showed a wave trend (Figure 3). It is believed that this trend was associated with a proliferation of bacteria that had established themselves in sufficient density (Chen *et al*., 2010).

Some rhizobacterial strains induce low levels of defense-enzymes activity but this could be accompanied by an increase in other components or activation of other defense responses (Del Río *et al*., 2003). It has been postulated that the oxidation-reduction ratio of different antioxidants can serve as a signal for modulation of ROS-scavenging mechanisms (Choudhary *et al*., 2007; Karpinski *et al*., 1997). The high level of TPC in the Badami cultivar is noteworthy because it was tempting to speculate the importance of the bacterial strains in inducing this compound. Some studies have indicated that these compounds are related to plant defense mechanisms against pathogenic attack (Del Río *et al*., 2003). On the other hand, faster oxidation of phenolic compounds by POX and PPO enzymes and production of lignin and its derivatives probably affects the lack of increase determined in TPC in the Sarakhs cultivar (Silva *et al*., 2010).

It is worthwhile to consider ability of the bacterial strains for induction of POX, PAL, PPO and TPC as one of the main factors related to reduction of multiplication factors of *M. incognita* in both cultivars. POX, PAL and PPO are important defense enzymes of plants, which are related to the plant systemic resistance against pathogens (Choudhary *et al*, 2007). Production of secondary metabolites such as siderophores, proteases and volatile metabolites has been documented previously for these bacterial strains (Khatamidoost *et al*., 2015). Previous studies have suggested that rhizobacterial strains which can induce different defense mechanisms in host plants have variable ability for controlling diseases (Liu *et al*., 1995). As *Bacillus cereus* strain S2 could produce nematicidal substances as well enhance activity of defense-related enzymes

[DOR: 20.1001.1.16807073.2018.20.1.12.4]

which has been caused severely inhibition of *M. incognita* (Gao *et al*., 2016).

Meloidogyne species produce at least five generations per year on trees in southern Iran (Fatemy, 2009). Genetic variation among populations of nematodes may happen and rhizobacterial strains can alleviate disease severity of virulence populations on pistachio cultivars. The studied bacterial strains in the present research could be used as components in an integrated approach to manage *M. incognita* on pistachio plants. The effects of these bacterial strains in combination with each other and with antagonistic fungi, arbuscular mycorrhizal fungi, and manure are yet to be investigated in field tests.

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اثر برخی از باکتریهای پروبیوتیک به عنوان عوامل کنترل بیولوژیک نماتد *Meloidigyne incognita* و ارزیابی تغییرات بیوشیمیایی آنزیمهای مرتبط با دفاع در دو رقم پسته

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چکیده

نماتدهای ریشه گرهی از نظر اقتصادی مهمترین بیمارگرهای گیاهی پسته می‌باشند. توانایی *Bacillus cereus* strain PRC95 *Pseudomonas fluorescens* strains VUPF5, VUPF52، *Bacillus subtilis* strain PRC96 در بیوکنترل نماتد *Meloidogyne incognita* در دو رقم پسته، سرخس و بادامی، ارزیابی گردید. تاثیر باکتری‌ها بر روی فعالیت آنزیمهای مرتبط با دفاع نیز ارزیابی گردید. نهالهای هر دو رقم با استرین‌های باکتریایی تیمار و بعد از دو روز با ۲۰۰۰ لارو سن دوم نماتد مایه‌زنی گردیدند. تغییرات پراکسیداز (POX)، پلی‌فنل‌اکسیداز (PPO)، فنیل‌آلانین‌آمونیالیاز (PAL) و محتوی فنل کل (TPC) در روزهای ۲، ۴، ۷ و ۱۰ بعد از مایه‌زنی نماتد تعیین گردید. نتایج نشان‌دهنده‌ی بهبود فعالیت آنزیم‌ها در هر دو رقم بود. بهترین فعالیت POX در نهال‌های تیمار شده با استرین *Pseudomonas* strain VUPF5 برای سرخس در ۷ روز بعد از مایه‌زنی و برای بادامی ۱۰ روز بعد از مایه‌زنی با نماتد بدست آمد. این استرین افزایش فعالیت آنزیم PAL را در ۲ و ۴ روز بعد از مایه‌زنی به ترتیب در رقم بادامی و سرخس نشان داد. نهالهای تیمار شده با *Pseudomonas* strain VUPF52 در ۱۰ روز بعد از مایه‌زنی بالاترین فعالیت PPO را در هر دو رقم نشان دادند. غلظت TPC ۴ روز بعد از مایه‌زنی با نماتد به میزان ۸/۴ درصد در نهالهای تیمار شده رقم سرخس توسط استرین *Pseudomonas* VUPF5 افزایش نشان داد اما در رقم بادامی افزایشی در مقایسه‌ی شاهد نشان نداد. تعداد گال، کیسه تخم و لارو سن دوم نهالهای تیمار شده دو رقم ۴ ماه بعد از مایه‌زنی با نماتد به طور معنی‌داری در مقایسه با شاهد کاهش داشتند.

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