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Effect of phosphate solubilizing bacteria and triple superphosphate on the growth, physiological parameters and phosphorus uptake of pistachio seedlings

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ABSTRACT

Purpose: Considering the role of nutrient supply in plant growth, the effect of single and simultaneous application of triple superphosphate (TSP) and phosphate solubilizing bacteria (PSB) on the growth and phosphorus uptake of pistachio seedlings were investigated. **Research method:** A study was conducted as a factorial experiment based on a completely randomized design with three replications under greenhouse conditions. The treatments include three levels of PSB [Control (PSB_0), *Pseudomonsa sp.* (PSB_1) and *Pseudomonas fluoresens* (PSB_2)] and three levels of P [Control (P_0), 15 mg P kg^{-1} soil (P_1) and 30 mg P kg^{-1} soil (P_2) as triple superphosphate]. **Findings:** the results indicated that application of TSP and PSB increased dry weight, plant height, total chlorophyll and carotenoids content and uptake of P in pistachio seedlings. Inoculation with PSB increased shoot dry weight (36%), root dry weight (44%), total chlorophyll (31%), carotenoids (30%), shoot P uptake (62%) and root P uptake (84%) in pistachio seedlings. **Research limitations:** By measuring other traits such as nutrient concentration, the response of pistachio seedlings to microbial inoculation can be more clearly interpreted. **Originality/Value:** Using of new insecticides with new and widespread mode of action can be recommended against postharvest pest in the practical entomology.

INTRODUCTION

Pistachio (*Pistacia vera* L.) is one of the most important exporting horticultural products of Iran and Iran is the third largest producer of pistachios in the world after the United States of America and Turkey (FAOSTAT, 2020). Despite the increase in the area under pistachio cultivation in Iran, the yield of this product is still low. The yield o pistachio in Iran is very low due to soil high pH value and $CaCO_3$ content, low organic matter in the soil, salinity of soil and irrigation water and poor fertility of soil (Azarmi et al., 2016a).

Phosphorus (P) is an essential nutrient for growth and yield of plant. Phosphorus plays a critical role in various cellular processes such as membrane structures maintenance, synthesis of biomolecules and it also helps in carbohydrate metabolism, cell division and enzyme activation (Razaq et al., 2017). The concentration of P in plants varied between 0.05% to 0.5% of total plant dry weight (Malhotra et al., 2018). Although P is an essential element for the plant, only a small amount of it is absorbed by the plant. Phosphate fertilizers such as sol are soluble in water but due to the interaction with other nutrients and clay particles in the soil, its availability to the plant is reduced. The amount of P in the soil is several times that of the plant, but its reaction with aluminum and iron oxides in acidic soils and calcium in calcareous soils causes the deposition of P in the soil and reduces its availability to the plant. The content of P in soil solution is typically low (Zhu et al., 2011). Thus, plants in most soils suffer from P deficiency. The use of chemical fertilizers is the most common way to supply the phosphorus needed by the plant. However, most of the phosphate fertilizers added to the soil to compensate for the lack of available P are deposited in the soil and therefore the efficiency of phosphate fertilizer application is very low. Also, excessive consumption of phosphate fertilizers, in addition to economic costs, leads to several environmental problems such as waterway eutrophication and groundwater contamination (Kang et al., 2011).

One of the new strategies to increase the availability of P in the soil and supply it to the plant is the use of phosphate solubilizing bacteria (PSB). The PSB solubilizes the insoluble form of phosphates via production of siderophores, various organic acids, carboxyl and hydroxyl groups, and chelating them to the bound phosphates and the available calcium (Sharma et al., 2013). The main mechanism for solubilization of P in soil is reducing of soil pH by release of protons and/or organic acids and mineralization by production of acid phosphatases finally resulting in P availability in soil (Alori et al., 2017).

The application of PSB can decrease P-fertilizer application by 50% without any significant reduction in plant yield (Jilani et al., 2007). Inoculation of plants with PSB can increase the available P form in the soil. Khosravi et al. (2018) indicated that simultaneous application of PSB and phosphate sources increased shoot dry matter and nutrient uptake in lettuce. Co-inoculation with PSB and nitrogen-fixing bacteria (NFB) promoted the growth parameters and uptake of N and P in walnut seedlings (Yu et al., 2012). Kurek et al. (2013) reported that application of *Pseudomonas luteola* BN0834 strain increased total shoot length and amount of P, K and Ca in the leaves of young apple trees as well as available P content in non-rhizosphere soil.

Considering the importance of P in the growth and yield of pistachio trees and the accumulation of a large part of phosphate fertilizers consumed in the soil during the past years, the aim of this study was to investigate the role of PSB and triple superphosphate on the growth, physiological parameters and P uptake of pistachio seedlings.

Table 1. Selected physical and chemical properties of the used soil.

Texture	pH	ECe ($dS.m^{-1}$)	OM (%)	P_{av} ($mg.kg^{-1}$)	K_{av} ($mg.kg^{-1}$)	Ca_s ($mg. L^{-1}$)	Mg_s ($mg. L^{-1}$)
Sandy Loam	7.5	1.4	0.57	7.12	179	112	91

EC: Electrical Conductivity, OM: Organic matter, av: Available forms of nutrient in the soil, s: Soluble forms of nutrient in the saturated soil solution.

MATERIALS AND METHODS

Phosphate solubilizing bacteria inoculum preparation

To investigate the effect of PSB on the growth of pistachio seedlings, two strains of *Pseudomonas sp.* with high ability to dissolve insoluble mineral phosphates (tricalcium phosphate) were selected from the soil biology laboratory of the Faculty of Agriculture of University of Birjand. In order to the preparation of bacteria inoculum, pure culture of bacteria was grown on tryptic soybean broth (TSB) medium for 48h at 28 ˚C and their population determined. These bacteria were able to solubilize of insoluble P ($Ca_3(PO_4)_2$) and produce siderophore and indole-3-acetic acid (IAA) in laboratory condition.

Plant materials and growth conditions

The study was conducted as a factorial experiment based on a completely randomized design with three replications under greenhouse conditions. The treatments include three levels of PSB [Control (PSB_0), *Pseudomonsa sp.* 1 (PSB_1) and *Pseudomonas sp.* 2 (PSB_2)] and three levels of P [Control (P_0), 15 mg P kg^{-1} soil (P_1) and 30 mg P kg^{-1} soil (P_2) as triple superphosphate]. The soil sample was air-dried, crushed to pass through a 2-mm sieve to remove stones and mixed thoroughly. Some physicochemical properties of the soil (Table 1) used in this study were measured based on standard methods (Sparks, 1996). The pots were filled with 2 kg of soil. The P treatments were supplied at the rate of 0, 15 and 30 mg kg^{-1} soil as triple superphosphate and then five germinated pistachios (*P. vera* L. cv. Badami) seeds were placed in each pot. For treatment, each pistachio seed was inoculated with bacterial inoculum (500 µL for each seed with CFU $=10^8$ cell mL^{-1}). Two weeks after germination, the number of seedling was decreased to 2 in each pot. Seedlings were irrigated with water to maintain the soil at field capacity for a period of three months. During the growth period, the greenhouse temperature was 28±2 ºC on day and 19±2 ºC on night, and the relative humidity was 45%.

Plant analysis

Three months after sowing, the pistachio seedlings were cut at the soil surface and roots were separated and washed to be free from soil particles. Shoot and root of plants was dried at 70 °C for 48 hours in the oven, shoot dry weight (ShDW) was recorded and then ground into powder for chemical analysis. The ground plant samples were dry-ashed at 500 °C and dissolved with 2 N HCl and made to volume with hot distilled water. Total concentrations of P in the pistachio aerial part were determined following the vanadate-molybdate method (Chapman & Pratt, 1961) using spectrophotometer. The total chlorophyll and carotenoids content were determined according to the methods proposed by (Porra, 2002) and (Lichtenthaler & Wellburn, 1983), respectively. Briefly, the leaf sample of pistachio seedling was ground in acetone, centrifuged at 3000 rpm for 10 min and then absorbance was measured in the supernatant using a spectrophotometer. Also the volume of the roots was measured through changes in the volume of water in the lab glass measuring cylinder.

Data Analysis

Experimental data were subjected to statistical analyses using ANOVA procedure. Also, all data were analyzed using PROC GLM in SAS software version 9.2. The means were compared using LSD to be significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Shoot and root dry weight

According to the variance analysis, application of TSP, PSB and interaction of TSP × PSB had a significant effect ($p \leq 0.01$) on the shoot and root dry weight of pistachio seedlings (Table 2).

The result showed that application of TSP and inoculation with PSB increased shoot and root dry weight of pistachio seedlings. Use of TSP increased pistachio seedling shoot and root dry weight by 41% and 59% compared to the control, respectively. Phosphorus is present in the structure of phosphoproteins, phospholipids and nucleic acids and thus plays a crucial role in many processes, such as photosynthesis, respiration, nucleic acid synthesis, membrane synthesis and stability, carbohydrates transport and energy generation (Marschner, 1995). An increase in the shoot and root dry weight of pistachio seedlings with application inorganic P had been reported (Shahriaripour et al., 2011; Fekri et al., 2015). Also, inoculation with PSB_1 and PSB_2 rhizobacteria enhanced shoot dry weight by 28% and 45%, and root dry weight by 36% and 53% in comparison with the control, respectively. However, the highest value of the shoot dry weight (1.64 g $plant^{-1}$) and root dry weight (1.67 g $plant^{-1}$) was observed after the combined use of PSB_2 and TSP, and PSB_1 and TSP (Table 3). Beneficial soil bacteria can increase plant growth through the production of plant hormones (such as auxin and cytokinin), siderophore and organic acids. Pseudomonas is one of the most important microorganisms that produce plant hormones, including auxin. Auxin produced by these bacteria can directly increase cell division and growth or indirectly increase ACC-deaminase production (Patten and Glick, 2002). Shaharroona et al (2006) reported that inoculation with IAA-producing bacteria increased weight, length, sub-roots, and production of thinner roots, thereby increasing water and nutrient uptake.

Plant height and root volume

The plant height of pistachio seedlings was significantly ($p \leq 0.05$) influenced by the PSB, TSP and interaction of PSB × TSP. The root volume of pistachio seedlings was significantly ($p \leq 0.05$) affected only by the main effect of TSP and PSB application (Table 2).

Compared with the control, inoculation by PSB_1 and PSB_2 rhizobacteria significantly increased the pistachio seedlings height by 62% and 36%, respectively. Also application of TSP drastically enhanced seedling height by 50% compared to the control (Table 3). The result indicated that application of TSP and inoculation with PSB rhizobacteria significantly improvement root volume of pistachio seedlings. According to the results, application of TSP increased root volume by 13% (Fig. 1a). Phosphorus plays an essential role in root growth, modification of root anatomy and density of hair roots and thus increasing the yield of agricultural products (Elhaissoufi et al., 2020). Also, inoculation of pistachio seedlings with the PSB_1 and PSB_2 drastically increased root volume of seedlings by 2-fold and 2.5-fold compared to the control, respectively (Fig. 1b). Inoculation with PSB, in addition to increasing the availability of P in the rhizosphere, affects root function by regulating auxin-responsive genes expression and therefore plays a key role in modulating the plant's internal auxin content and phosphorus uptake (Liu et al., 2019). Also, ACC-deaminase plays a key role in stimulating root growth. It seems that auxin hormone and ACC-deaminase complement each other in increasing plant growth.

Table 2. Analysis of variance (mean square) of measured traits.

SOV	df	ShDW	RDW	Plant Height	Root Volume	Total Chlorophyll	Carotenoids	Shoot P Uptake	Root P Uptake
TSP	1	0.274^{**}	0.654^{**}	187^{*}	1.39^{*}	1.11^{**}	0.164^{**}	20.8^{**}	7.66^{**}
PSB	2	0.153^{**}	0.163^{**}	76.1^{**}	20.6^{**}	0.742^{**}	0.079^{**}	7.40^{**}	5.15^{**}
TSP × PSB	2	0.040^{**}	0.059^{**}	12.8^{*}	0.22^{ns}	0.044^{**}	0.001^{ns}	0.018^{ns}	0.297^{*}
Erorr	12	0.005	0.005	2.81	0.28	0.004	0.001	0.183	0.051
CV (%)		4.76	5.39	8.67	9.71	2.64	3.06	10.5	6.62

SOV: Source of Variation; ShDW: Shoot Dry Weight; RDW: Root Dry Weight.
*, ** and ns: significant at p≤0.01 significant at p≤ 0.05 and non-significant, respectively.

Table 3. The effect of TSP × PSB interaction on the ShDW, RDW, plant height and total chlorophyll content of pistachio seedlings.

Phosphorus Source	Phosphate Solubilizing Bacteria			Phosphate Solubilizing Bacteria		
	PSB$_0$	PSB$_1$	PSB$_2$	PSB$_0$	PSB$_1$	PSB$_2$
	ShDW (g plant^{-1})			RDW (g plant^{-1})		
TSP$_0$	1.05 ± 0.06 d	1.35 ± 0.03 c	1.52 ± 0.04 ab	0.89 ± 0.04 e	1.21 ± 0.04 d	1.36 ± 0.05 c
TSP$_1$	1.48 ± 0.02 b	1.54 ± 0.05 ab	1.64 ± 0.04 a	1.42 ± 0.04 bc	1.67 ± 0.05 a	1.52 ± 0.05 b
	Plant height (cm plant^{-1})			Total chlorophyll (mg g^{-1} FW)		
TSP$_0$	12.2 ± 1.07 d	19.8 ± 1.33 b	16.4 ± 0.92 c	2.19 ± 0.03 d	2.66 ± 0.04 b	3.07 ± 0.05 a
TSP$_1$	18.3 ± 0.66 bc	23.5 ± 0.70 a	25.9 ± 0.97 a	1.88 ± 0.02 e	2.13 ± 0.04 d	2.42 ± 0.03 c

For each parameter, values having a common letter are not significantly different (p≤0.05) according to LSD test. The numbers following ± sign are the standard errors. PSB: Phosphate solubilizing bacteria, TSP: Triple superphosphate, ShDW: Shoot dry weight, RDW: Root dry weight.

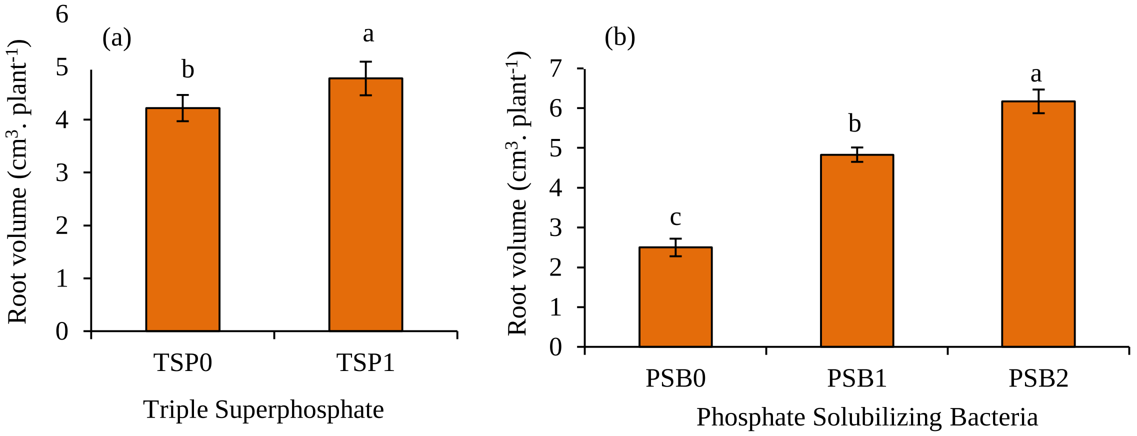

Fig. 1. Main effect of TSP (a) and PSB (b) on the root volume of pistachio seedlings. Within each graph, values followed by the same letter are not significantly different (p≤0.05) according to LSD test. The error bars in the graphs are standard errors.

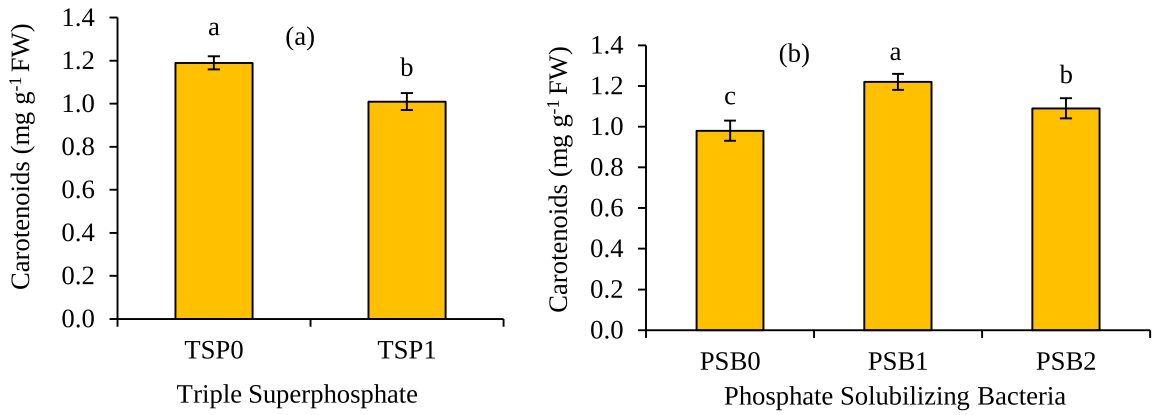

Fig. 2. Main effect of TSP (a) and PSB (b) on the carotenoids content of pistachio seedlings. Within each graph, values followed by the same letter are not significantly different ($p \leq 0.05$) according to LSD test. The error bars in the graphs are standard errors.

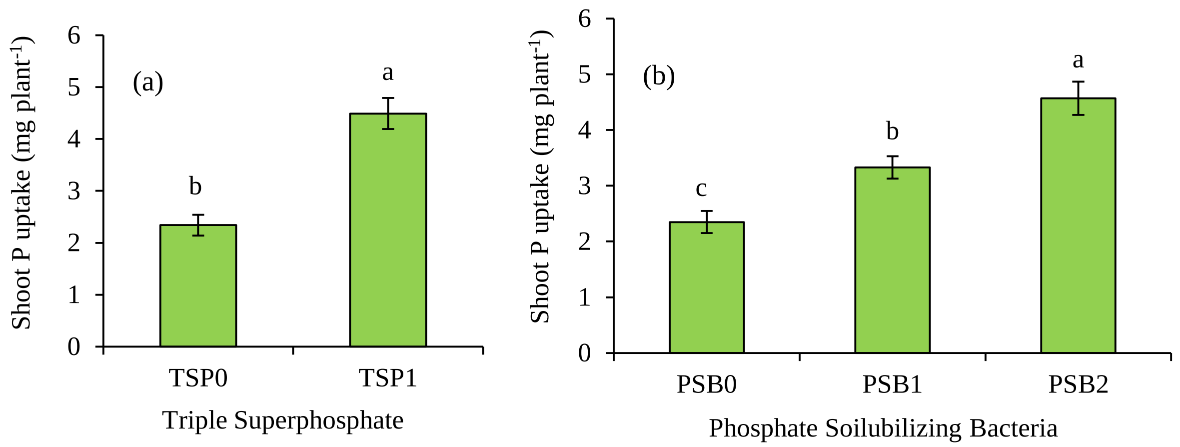

Fig. 3. Main effect of TSP (a) and PSB (b) on the shoot P uptake in pistachio seedlings. Within each graph, values followed by the same letter are not significantly different ($p \leq 0.05$) according to LSD test. The error bars in the graphs are standard errors.

Total chlorophyll and carotenoids content

The total chlorophyll content was influenced ($p \leq 0.01$) by the PSB, TSP and interaction of PSB × TSP. Also the carotenoids concentration in the leaf was affected ($p \leq 0.01$) only by the main effect of TSP and PSB application (Table 2).

The results showed that inoculation with the PSB_1 and PSB_2 rhizobacteria significantly increased total chlorophyll content in the leaves of pistachio seedling by 21% and 40% in comparison with the non-inoculated control, respectively. Also application of TSP significantly reduced total chlorophyll concentration at all the PSB levels (Table 3). According to the results, application of TSP reduced the content of carotenoids by 15% compared to the control, respectively (Fig. 2a). On the other hand, the treatment with PSB_1 and PSB_2 significantly increased carotenoids content of pistachio seedling by 24% and 11% in comparison with the control, respectively (Fig. 2b). The increase in chlorophyll content and photosynthetic activity with accumulation of P in the plant tissues has been reported (Azarmi-Atajan & Sayyari-Zohan, 2020; Elhaissoufi et al., 2020). Bacteria, in addition to increasing soil phosphorus availability, improve phosphorus uptake and chlorophyll content in plants by affecting root morphology and its development in soil. Marathe et al. (2017) reported that the use of an IAA-producing PSB strain (*Pseudomonas aeruginosa*) could increase nutrient (N, P and K) uptake and chlorophyll concentration in the plant. Elhaissoufi et al. (2020) indicated that combined application of PSB and rock phosphate (RP) increased chlorophyll a and b content in wheat.

Shoot and Root P uptake

The uptake of P in the shoot of pistachio seedlings was affected ($p \leq 0.01$) only by the main effect of TSP and PSB application. Also, the root P uptake was influenced ($p \leq 0.05$) by the PSB, TSP and interaction of PSB × TSP (Table 2).

The results revealed that application of TSP significantly increased uptake of P in the pistachio seedling shoot by 49% compared to the control, respectively (Fig. 3a). Also, inoculation with PSB_1 and PSB_2 drastically increased uptake of P in the shoot by 29% and 94% in comparison with the control, respectively (Fig. 3b). According to the result, application of TSP drastically increased root P uptake by 96% compared to the control. Also, treatment of pistachio seedling significantly enhanced root P uptake by 57% and 2.3-fold in comparison with the control, respectively. The highest (1.69 mg plant^{-1}) and lowest (4.75 mg plant^{-1}) uptake of root P uptake were obtained from control and simultaneous application of TSP and PSB_2, respectively (Fig. 4). The PSB increase soil P availability by reducing of soil pH by release of protons and/or organic acids and mineralization by production of acid phosphatases (Alori et al., 2017). Numerous reports suggest that gluconic acid is the most important organic acid secreted by phosphate-soluble bacteria, including Pseudomonas (Rodriguez & Fraga, 1999). Dissolution and availability of soil P by microbial population depends on type of phosphate source (organic or inorganic), type of host plant, microbial population, pH and composition of anions and cations (Niu et al., 2010). Azarmi et al. (2016b) findings showed that inoculation with plant growth promoting rhizobacteria (with the ability to dissolve phosphate compounds) increased P uptake in pistachio seedlings under saline conditions. Also, application of *Pseudomonas luteola* BN0834 strain increased total shoot length and amount of P, K and Ca in the leaves of young apple trees as well as available P content in non-rhizosphere soil (Kurek et al., 2013). In addition to P solubilization, PSB can be able to mineralize organic P by production of phosphatase enzyme.

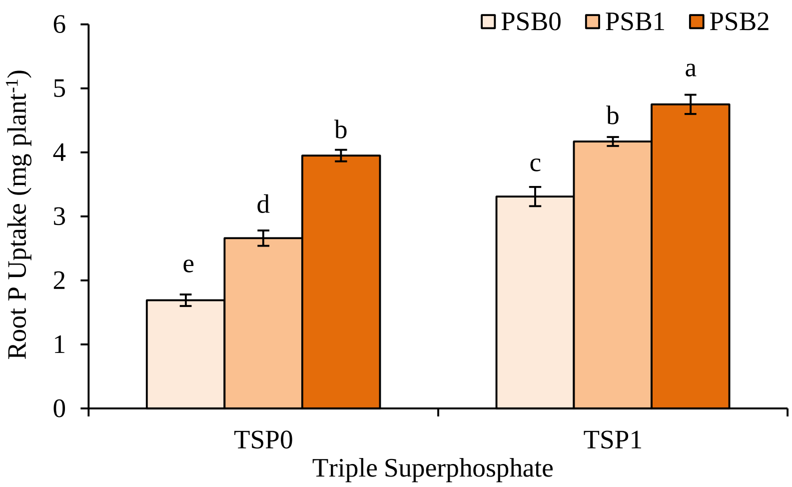

Fig. 4. Interactive effect of TSP and PSB on the root P uptake in pistachio seedlings. Within each graph, values followed by the same letter are not significantly different ($p \leq 0.05$) according to LSD test. PSB: Phosphate Solubilizing Bacteria. The error bars in the graphs are standard errors.

CONCLUSION

The result of present study revealed that application of TSP and PSB promoted growth and improvement of pistachio seedling. According to the result, inoculation with PSB increased shoot and root dry weight, chlorophyll and carotenoids content and P uptake. Due to the production of siderophore, IAA, ACC-deaminase enzyme and dissolution of tricalcium phosphate by bacteria used in this study in vitro, the increase in pistachio seedlings can be attributed to the development of root growth and thus increase the absorption of water and nutrients such as P. Our results also showed that no significant difference between TSP and PSB was observed in most of the measured traits. Therefore, due to improve shoot and root growth and P uptake by PSB, they can be used as inoculants and bio-fertilizer for increasing the establishment percentage and growth of pistachio seedlings.

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Conflict of interest
The authors declare no conflict of interest.

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