ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 17(4) 2018, 93–107

www.acta.media.pl ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2018.4.9

ORIGINAL PAPER

Accepted: 9.01.2018

THE ROLE OF *Pseudomonas* STRAINS AND ARBUSCULAR MYCORRHIZA FUNGI AS ORGANIC PHOSPHATE–SOLUBILIZING IN THE YIELD AND QUALITY IMPROVEMENT OF STRAWBERRY (*Fragaria* × *ananassa* Duch., cv. Selva) FRUIT

Mohammad Hossein Ansari[1]✉, Davood Hashemabadi[2], Maryam Mahdavi[2], Behzad Kaviani[2]

[1]Department of Agronomy and Plant Breeding, Rasht Branch, Islamic Azad University, Rasht, Iran

[2]Department of Horticulture Science, Rasht Branch, Islamic Azad University, Rasht, Iran

ABSTRACT

This study evaluated the effect of *Pseudomonas* strains and arbuscular mycorrhiza fungi (AMF) in enhancing strawberry yield and phenolic and antioxidant capacity on a phosphorus (P) deficient calcareous soil. The experiments were conducted in three replicates with six treatments (four *Pseudomonas* strains, AMF and control) and three rates of P-fertilizer (0, 75, 150 kg P ha^{-1}). Application of higher phosphate rates decreased total antioxidant capacity, total phenolic and flavonols content, whereas AMF and *Pseudomonas* strains increased quality and P concentration of fruit. The use of AMF and *Pseudomonas* strains resulted in better quality when used along with 75 kg P ha^{-1}. These results demonstrated that the rhizospheric microorganisms improved the quality of fruit, especially when they applied in combination with lower rates of chemical fertilizers. Therefore, application of these microorganisms in sustainable agriculture is recommended.

Key words: antioxidant capacity, fruit yield, phenolic content, rhizospheric microorganisms

INTRODUCTION

Fruit of strawberry is rich in phenolic compounds and natural antioxidants which the amounts depend on various factors including type of species and cultivars [Milivojević et al. 2011], environmental conditions, and the supply of plant nutrient requirements [Pešaković et al. 2016]. The application of chemical fertilizers in high quantity not only reduces fruit quality but also increase the production and transportation costs and environmental pollution [Bona et al. 2015]. Therefore, modern farmers have no choice but partial replacement of chemical fertilizers with biological fertilizers [Sharma et al. 2013].

Phosphorus (P) is the second most important element after nitrogen as a mineral nutrient. Although soils are rick source of both organic and inorganic forms, its availability is limited since it occurs mostly in insoluble form [Khan and Joergensen 2009]. Phosphate solubilizing microorganisms (PSM) are a heterogeneous group of microorganisms that, as complementary components of phosphorous cycle, can release phosphorus from insoluble sources by different sources [Walpola and Yoon 2012]. They include two groups of bacteria and fungi [Khan and Joergensen 2009]. Phosphate solubilizing microorganisms can

✉ ansary330@yahoo.com, ansari@iaurasht.ac.ir

constitute as high as 88% of all PSMs [Khan and Joergensen 2009]. Microorganisms enhance P availability for plants via mineralization of soil organic P by solubilizing the precipitated P [Chen et al. 2006]. Phosphate solubilizing bacteria include the genera of *Pseudomonas*, *Bacillus*, *Rhizobium*, *Achromobacter*, *Agrobacterium*, *Micrococcus* and *Aerobacter* [Khan and Joergensen 2009, Sharma et al. 2013]. Although there are various P solubilizing bacteria in soil, they are outnumbered by other common bacteria deployed in plant rhizosphere. So, the quantity of phosphorous released by these bacteria does not usually suffice to make a considerable increase in plant growth. Therefore, it is necessary to inoculate plants with specific bacteria with a population much higher than what is found in soil in order to exploit the advantages of the bacterium's solubilizing features in improving crop growth and quality [Glick 1995, Smith and Read 2008]. The release of P from its insoluble and fixed forms by phosphate solubilizing bacteria is associated with soil P availability and its transfer to plants [Khan and Joergensen 2009]. In addition to solubilizing soil P, these bacteria influence on plant growth and yield by producing considerable amounts of growth regulators like auxins, gibberellins and cytokinins [Glick 1995]. Some fungi play a significant role in soil phosphorous solubility and have a beneficial co-existence with plants known as mycorrhiza coexistence [Lingua et al. 2013, Baslam et al. 2013]. This coexistence allows plants to use soil nutrients because plants are not able to uptake lowly soluble elements by themselves [Gianinazzi et al. 2010]. Mycorrhiza promotes the uptake of water and P by plants [Gryndler et al. 2002]. Mycorrhizal roots are more able to uptake and mobilize nutrients than non-mycorrhizal roots. Therefore, they make lowly mobilized nutrients like P available to plants [Khaligh and Sandres 2000]. Mycorrhiza has the biochemical capability to increase available P and other nutrients, which is likely to be related to the increased activity of root phosphatase, more chelating compounds and rhizosphere acidification [Habte and Fox 1993]. In fact, the effect of mycorrhiza in the alleviation of P deficiency is mainly associated with the increase in soluble P availability [Mukerji et al. 2002].

During the last decade, strawberry fruit production has spread through almost all parts of Iran. Production starts in September with final harvest in January. In Iran more than 20,000 t of strawberries are produced each year. The largest of this annual production comes from small family farms, mainly located in Iran's calcareous areas. Production of strawberry in the area might be expanded if P deficiency of the soils could be addressed economically. Thus, the present study was aimed to evaluate the effect of novel microorganism's inoculation on the reduction of P fertilizer application, fruit yield, vitamin C, total phenolic content and total antioxidant capacity of strawberry (*Fragaria* × *ananassa*, Duch. cv. Selva).

MATERIALS AND METHODS

Experimental conditions

A pot experiment was carried out in greenhouse of Research Center of Rasht, Islamic Azad University. All farming operations were practiced in an enclosed greenhouse with uniform radiation, temperature and moisture conditions for all treatments. Day/night temperature was set to 25 ±2/18 ±2°C and the light intensity was 8,000–12,000 lx. The greenhouse moisture was supplied with a bed irrigation system and was controlled by opening the lateral valves. The relative humidity of the greenhouse varied in the range of 60–70%.

Treatments and experimental design

Experiment was conducted in a factorial randomized complete block design with three replications. Experimental factors include phosphate fertilizer at three rates (P0 = 0, P1 = 75 and P2 = 150 kg P ha^{-1} equivalent to 0, 1.30 and 2.60 g triple superphosphate kg^{-1} soil, respectively) and PSM (*P. fluorescens* strain R8, *P. fluorescens* strain R48, *P. putida* strain R108, *P. putida* strain) and AMF species *Rhizofagus intraradices.*

Preparation and characterization of Pseudomonas strains

These Pseudomonas strains were isolated from soil samples collected from several fields in Ardebil prov-

ince, Iran. The strains were evaluated for their siderophores, P-solubilizing capability, acid and alkaline pH, and produces the phytohormone indole-3-acetic acid (IAA) [Alishahi et al. 2013]. Pseudomonas strains were characterized as described below:

Siderophore production by Pseudomonas strains were tested on universal Chrome Azurol S (CAS) agar [Schwyn and Neilands 1987]. The bacterial strains were inoculated at the center of the plate and incubated at 28°C for 3 days. Siderophores production was detected by a halo of color change from blue to orange on the CAS medium and measured in triplicate as the ratio between the two diameters of the halo and the two colony diameters.

Phosphate solubilization by Pseudomonas strains were assayed according to Goldstein [1986]. The strains were inoculated at the center of the plate and incubated at 28°C for 15 days. DCP solubilization was indicated by a clarification halo around the colony; TCP solubilization was identified by a colony growth on the media.

Indole-3-acetic acid (IAA) production by Pseudomonas strains were quantified according to De Brito Alvarez et al. [1995]. The bacterial strain was inoculated on a nitrocellulose disk placed on trypticase soy agar (TSA) with 10% added to L-tryptophan (5 mM) and incubated at 28°C for 3 days. The membrane was then stained with the Salkowskys reagent ($FeCl_3$ 2% in percloric acid 35%), the presence of a red/pink halo around the colony indicated a positive reaction.

The 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme activity was assayed based on method of Penrose and Glick [2003] which measures the amount of α-ketobutyrate produced when the enzyme ACC deaminase disintegrate ACC. The µmole quantity of α-ketobutyrate produced by this reaction was determined by comparing the absorbance of a sample to a standard curve of α-keto-butyrate ranging between 0.1 and 1.0 nmol at 540 nm. A stock solution of α-ketobutyrate was prepared in 0.1 M Tris-HCl (pH 8.5) and stored at 40°C. In order to measure the specific activity of the cultures, protein estimation was carried out according to Lowry et al. [1951]. Pseudomonas strains characterized are presented in Table 2. The bacterial strains were kept in nutrient broth with 15% glycerol at –80°C for long-term storage. For this experiment,
a single colony was transferred to 500 mL flasks containing NBRIP and grown aerobically on a rotating shaker for 48 h at 27°C and 150 rpm. The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^8 colony forming units (CFU) mL^{-1}, and the resulting suspensions were used for the experiment.

Table 1. Characteristics of *Pseudomonas* strains

Pseudomonas strains	ACC-deaminase production (µmoles mg^{-1} h^{-1})	Phosphorus solubilizing activity	IAA production (mg l^{-1})	Siderophore production (halo diameter/colony diameter)
P. fluorescens strain R8	4.61	+	6.1	1.62
P. fluorescens strain R48	4.45	+	5.8	1.50
P. putida strain R108	7.34	+	8.9	1.66
P. putida strain R156	8.17	+	9.6	1.73

Table 2. Physical and chemical characteristics of the pots soils

Mn	Zn	Fe	Available K	Available P	Total N (%)	OC (%)	pH	Ec (dS m^{-1})
		(mg kg^{-1})						
3.88	1.04	4.91	193	10.34	0.41	2.28	7.67	1.23

Pot experiment. The plastic pots with diameter of 18 cm and depth of 20 cm were filled with local soil and sand in the ration 2 : 1. The used soil was autoclaved for 120 min at 121°C with 15 psi followed by cultivation of plants. The pot capacity was around 2 kg soil, which was mixed with different rates of P-fertilizer in the respective treatments. Table 2 shows results of soil analysis. Before the transfer of the plantlets of strawberry, the roots were rinsed with distilled water. Then, they were placed in the solution containing *Pseudomonas* strains for 30 minutes. Afterwards, they were planted. In mycorrhizal treatments, 5 g mycelium spores of AMF (400 fungus spores g^{-1}) were added to the soil spaced one centimeter from root terminal. Then, the seedlings were planted.

Data collection and analysis. At fruit maturity stage, the fruits were harvested. To measure of fruit P content, the fruit tissue was digested in 15 mL $HClO_4$ and 5 mL HNO_3 then P content was obtained using spectrophotometric vanado-molybdate method [Jackson 1958].

Fruit number, fruit yield, and total soluble solids (TSS) of fruit on the basis of Brix degree were measured with refractrometer after fruit harvest. Finally, shoot and root dry weight was estimated after the samples were oven-dried at 85°C for 72 hours. To measure root volume, the roots were cut from shoot and were rinsed with water to clean all external particles. Water in a particular volume was poured in a graduated cylander and plant roots to the collar were placed inside the container. Difference between the measured volumes of water represents the root volume.

Vitamin C was quantified with a refractometer set of Merck Co. (Merck Rqflex) as described by Pantelidis et al. [2007]. Results were expressed as mg 100 g^{-1} FW.

Total phenolic content was determined using a modified Folin–Ciocalteu colorimetric method [Liu et al. 2002]. Results were expressed as milligrams of gallic acid equivalents (GAE) 100 g^{-1} fresh weight.

Total antioxidant capacity was determined using the DPPH method reported by Brand-Williams et al. [1995] with modifications [Sanchez-Moreno et al. 1998]. Results were expressed as Trolox equivalent antioxidant capacity (μmol TE 100 g^{-1} FW).

Individual phenolic compounds were identified and quantified using an Agilent 1260 series HPLC (Agilent Technologies, Santa Clara, USA) linked to a ChemStation data handling system, using a ZORBAX Eclipse Plus C18 column (4.6 × 150 mm, 3.5 μm particles). Samples were prepared according to the method of Hertog et al. [1992] before they were analyzed. Injection volume was 5 μl and the temperature was set at 30°C. Solvent A was 1% formic acid and solvent B was acetonitrile. The gradient used was as follows: 0–10 min, 10% of B in A; 10–25 min, 15–50% of B in A; 25–30 min, 50–80% of B in A; 30–32 min, 10% of B in A. By using this gradient (flow rate of 0.5 ml min^{-1}), a good level of purity and separation was achieved in fruit samples. The HPLC equipment was used with a diode array detector (DAD). Phenolic compounds were detected at 260 nm (rhamnetin, protocatechuic acid, ellagic acid), 280 nm (gallic acid, *p*-coumaric acid), 360 nm (quercetin, kaempferol), and 520 nm (cyanidin, pelargonidin). Phenolic compounds were identified according to peak retention time (RT) and UV/Vis spectra by comparing them with those of the standards. The quantities of the different phenolic compounds were based on peak areas, and expressed as mg 100 g^{-1} FW.

Data analysis. Experimental data were analyzed statistically using ANOVA. Significance of the effect of treatment was determined by the magnitude of the F-value ($P < 0.05$). When a significant F-test was obtained for the treatments, separation of means was accomplished by Fisher's protected LSD. Statistical analysis of the results was performed using general linear model (GLM) in SAS software version 9.2.

RESULTS AND DISCUSSION

Root volume. Analysis of variance showed that the interaction effect of P-fertilizer × PSM on root volume was significant ($P < 0.01$) (tab. 3). The mean comparison showed that the R156 strain along with all P-fertilizer rates induced the highest root volume.

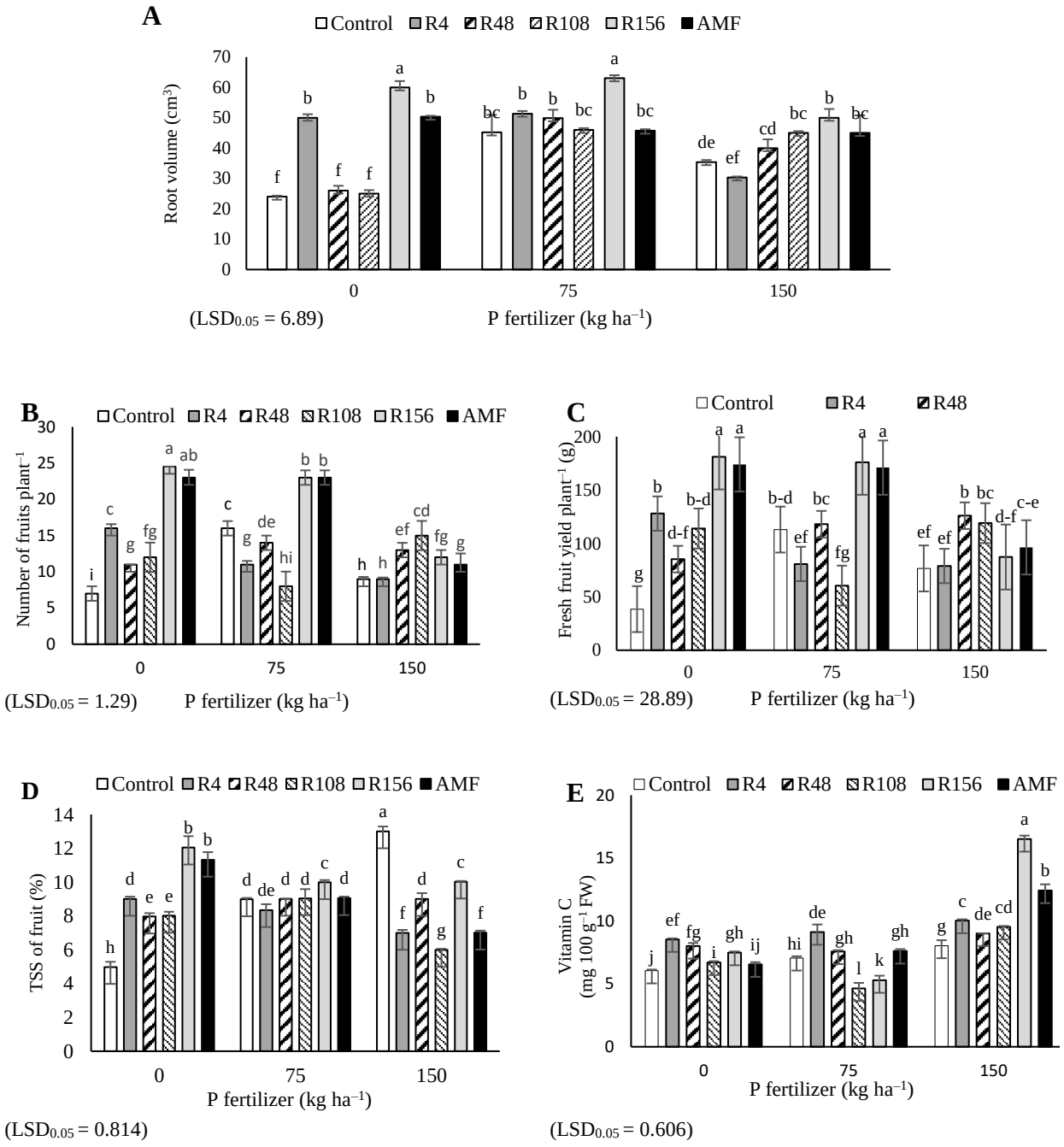

Fig. 1. Interaction effect of P-fertilizer × PSM on root volume (A), fruit number (B), fruit yield (C), TSS (D) and vitamin C (E). Means followed by different letters on the same column are significantly different according to LSD test at $p \leq 0.05$

But, there was no statistically significant difference at the rate of 150 kg P ha^{-1} along with AMF and R108 strain (fig. 1A). Similar to our result, Amanullah and Khan [2015] stated that Pseudomonas produced higher levels of formic acid, acetic acid, and propionic acid in root zone than AMF and improved root length and volume through improving the uptake of nutrients. Increased root growth is one of the most important criterions to evaluate the beneficial PGPRs effects. Rootlet expansion, either through the increased length of initial roots or through the propagation of lateral and adventitious roots, is a good strategy for young seedlings to enhance their ability for the establishment and uptake of nutrients [Patten and Glick 2002]. Bacteria can produce plant growth regulators like gibberellins, cytokinins and auxin, through which they can improve nutrients availability by expanding root system to reach water and nutrient sources [Rudresha et al. 2005]. Researchers show that the application of different strains of Pseudomonas can result in higher root volume of lettuce, canola, and tomato [Glick et al. 1997, Tomic et al. 2015]. In addition, ACC-deaminase containing bacteria stimulated root length, root volume and plant growth by reducing ethylene level [Ahmad et al. 2008]. The strains used in the present study exhibited similar capability (tab. 1).

The number of fruits. Phosphorus is the most decisive element for the number of fruits of strawberry [Esitken et al. 2010]. The interaction effect of P-fertilizer × PSM on the number of fruits per plant was significant ($P < 0.01$) (tab. 3). In Karlidag et al. [2011] study, the interaction effect of P-fertilizer × PSM was significant on the number of strawberry fruits. Means comparison revealed that at 0 and 75 kg P ha^{-1}, R156 and AMF produced the highest number of fruits, but at 150 kg P ha^{-1}, R108 strain showed the largest number of fruits (fig. 1B). In most inoculation treatments the increase in P rate not only had no impact on the increase in fruit number, but also resulted in its loss so that number of fruits was even lower in some inoculation treatments than in control. Indeed, the largest number of fruits was produced through the use of AMF and R156 strain without the use of P-fertilizer. The loss of number of fruits was reported in strawberry by Bona et al. [2015] that showed that

Table 3. *F* value and mean comparison of P-fertilizer and PSM main effect of number of fruits, root volume and fruit yield

ANOVA	Root volume (cm^3)	Number of fruits plant^{-1} (pcs.)	Fruit yield plant^{-1} (g)	TSS (%)	Vitamin C (mg 100 g^{-1} FW)
P	30.76**	57.67**	10.39**	2.96ns	29.00**
PSM	16.58**	54.16**	18.96**	40.06**	38.59**
P × PSM	23.43**	51.25**	20.03**	63.64**	44.61**
P (kg ha^{-1})					
0	39.69b	15.55a	120a	8.88a	7.22b
75	49.68a	15.77a	118a	9.07a	6.88b
150	40.95b	11.59b	97b	8.67a	10.92a
LSD ($P \leq 0.05$)	2.81	0.889	11.79	0.632	0.377
PSM					
Control	39.69b	15.55a	76.3c	8.98b	7.22b
R4	49.68a	15.77a	110b	8.11c	6.88b
R48	40.95b	11.59b	109b	8.66b	10.92a
R108	39.69b	15.55a	98.1b	7.68c	7.22b
R156	49.68a	15.77a	133a	10.68a	6.88b
AMF	40.95b	11.59b	147a	9.12b	10.92a
LSD ($P \leq 0.05$)	3.98	1.25	16.6	0.470	0.533

Statistically significant differences based on two ways ANOVA are reported in columns P, PSM, and P × PSM (ns, nonsignificant; * significant at $P \leq 0.05$; ** significant at $P \leq 0.01$). Means followed by different letters on the same column are significantly different according to LSD test at $p \leq 0.05$

overuse of P-fertilizer resulted in too much increase in the number of flowers whilst they would abscise if the plant lacks enough foliage. Also, the increase in soil P content results in the loss of yield due to high ratio of P to Zn or P to Fe, and the accumulation of B, Mo and Cd in plant tissues [Hamilton et al. 1993]. However, it can be suppose that the application of P-fertilizer with AMF improve the solubility of otherwise insoluble soil elements by various mechanisms. They increase root uptake zone in soil by expanding their hyphae network. Then, plants can realize their highest yield by meeting their nutrient requirements [Tomic et al. 2015]. PSM-induced increase in fruit number can be related to higher P uptake by the plant in the presence of these microorganisms. As a key element for flowering, P can induce the accumulation of more dry matter and minerals in plant leaves and stems [Panigrahi et al. 2009]. Therefore, the accumulated minerals can be mobilized to reproductive organs during reproductive phase making their way into yield increase [Sharma et al. 2013, Bona et al. 2015].

Fruit yield. At 0 and 75 kg P ha^{-1} rates, R156 and AMF produced the highest fruit yield. Application of 150 kg P ha^{-1} along with AMF and R156 increased the fruit yield but this increase was less than that of induced by R48 and R108 (fig. 1C). The yield loss at higher P-fertilizer rates by P. putida was reported by Güneş et al. [2009]. Turan et al. [2004] related strawberry yield loss to high unbalanced production of flowers and their abscission, which is demonstrated by fruit yield loss in control as P-fertilizer rate was increased from 30 to 60 mg. AMF and the strain R156 that demonstrated the highest fruit yield at the lowest phosphate levels produced the highest fruit number too. Esitken et al. [2010] reported 43% higher yield of strawberry at lower P-fertilizer rates under Pseudomonas inoculation. Many studies have shown that plants fertilized with phosphate and inoculated with PSM have exhibited an increased yield and higher fertilizer use efficiency [Chen et al. 2006, Adesemoye and Kloepper 2009]. In addition to P nutrition improvement, higher fruit yield can be related to the plant growth stimulation by these microorganisms [Esitken et al. 2010]. PSM may usually influence on plant growth through other methods like the production of hormones, siderophores, etc. [Han et al. 2006, Ghaderi et al. 2008]. Similar observations were reported by Zaidi and Khan [2006] about the cooperation of fungus Glomus fasciculatum, Bradyrhizobium japonicum and Bacillus subtilis and a 24% enhancement of yield.

Total soluble solids (TSS). Analysis of variance showed that the interaction effect of P-fertilizer × PSM on TSS content was significant ($P < 0.01$) (tab. 3). Similar result was reported by Esitken et al. [2010]. Means comparison showed that the highest TSS content under the absence of P-fertilizer was obtained from AMF and the R156 strain, while the R156 strain at 75 kg P ha^{-1} had superiority compared to others. In the presence 150 kg P ha^{-1} rate, non-inoculated (control) plants had higher TSS content than inoculated ones (fig. 1D). In non-inoculated treatments, the rate of TSS increased in line with increasing the consumption of P-fertilizer, whereas this trend was not observed in inoculation treatments. The late-season variations of TSS content in horticultural products are associated with the hydrolysis of saccharides and the loss of fruit water [Ertutk et al. 2012, Ipek et al. 2014]. Thus, the inoculation treatments did not perform well in terms of this trait, which can be caused by late–season growth induction by microorganisms.

Content of vitamin C. Results indicated that at 0 and 75 kg P ha^{-1} rates, the R4 strain showed the highest vitamin C content (8.54 and 9.11 mg 100 g^{-1} FW, respectively). At 150 kg P ha^{-1} rate, the R165 strain (16.51 g 100 g^{-1} FW) showed 47.3% higher vitamin C content compared to control, and AMF produced the second highest vitamin C content (12.42 mg 100 g^{-1} FW) (fig. 1E). Studies carried out by Bona et al. [2015] on strawberry and Karimi et al. [2013] on green beans have demonstrated the significant influence of bio-fertilizers on vitamin C concentration. Ipek et al. [2014] reported the highest vitamin C in treatments with Agrobacterium A18. Adequate P availability in root zone induces rapid root expansion and better exploitation of water and other nutrients by plants, resulted in an improved level of vitamin C [Erturk et al. 2012]. According to Bagel et al. [1989], AMF plays an important role

in activation of enzymes that is necessary for vitamin C synthesis through improving P uptake. Indeed, higher P uptake stimulated the enzymatic activities, leading to higher vitamin C content in strawberry and/or the plant use of soil trace element sources along with AMF plays a role in the enhancement of vitamin C content in strawberry. It may be said that the mycorrhiza augments water and nutrients uptake through expanding the contact area of the roots with soil using its hyphae, which finally improves fruit quality [Turan et al. 2004].

Total phenolic content and antioxidant capacity. The results indicated that as P-fertilizer rates was increased, total phenolic content and antioxidant capacity were decreased, whereas they were higher in PSM treatments compared to control (except for 156 strain) (tab. 4). Means comparison for the interaction effect of P-fertilizer × PSM on total phenolic content and antioxidant capacity revealed that at 0 and 75 kg P ha^{-1} rates, AMF and the strain R4 had the highest total phenolic content, but at 150 kg P ha^{-1} rate, AMF had even lower mean total phenolic content than control and the strain R4 had the highest total phenolic content and antioxidant capacity (figs 2A and 2B). It implies that the increase in 150 kg P ha^{-1} rate disrupts AMF and Pseudomonas functions although the decrease in total phenolic content and antioxidant capacity was more obvious in combination with AMF. Bona et al. [2015] reported the superiority of AMF over Pseudomonas in the improvement of strawberry qualitative traits under non-use of P-fertilizer condition, too. Pešaković et al. [2016] reported the highest total phenolic content and antioxidant capacity of strawberries under an integrated treatment of chemical fertilizer and Pseudomonas. Consistent with these results, Reganold et al. [2010] proved the significantly increasing effect of biological compounds on total phenol and antioxidant capacity as compared to chemical compounds. Castellanos-Morales et al. [2010] reported the influence of chemical fertilizers on the reduction of total phenol synthesis. The quality of strawberry fruits depends on environmental and genetic factors and plant nutrition plays a key role in its improvement [Karlidag et al. 2011]. Thus, the integrated fertilization can improve the quality of the fruits in addition to increasing fruit yield.

Table 4. *F* value and mean comparison of P-fertilizer and PSM main effect P fruit, total phenolic content, total antioxidant capacity

ANOVA	P fruit (mg kg^{-1})	Total phenolic content (mg 100 g^{-1} FW)	Total antioxidant capacity (Trolox, mmol 100 g^{-1} FW)
P	4.64*	35.29**	106**
PSM	10.56**	20.61**	32**
P × PSM	8.00**	2.38*	6.4**
P (kg ha^{-1})			
0	2.42b	146.5a	388a
75	2.91a	117.3b	347b
150	2.43b	90.99c	285c
LSD ($P \leq 0.05$)	0.374	13.43	14.39
PSM			
Control	2.42b	79.84d	259d
R4	2.91a	154.2a	426a
R48	2.43b	108.0bc	296c
R108	2.42b	121.8b	375b
R156	2.91a	94.77cd	273d
AMF	2.43b	151.1a	412a
LSD ($P \leq 0.05$)	0.529	18.99	20.35

Explanations as in Table 3

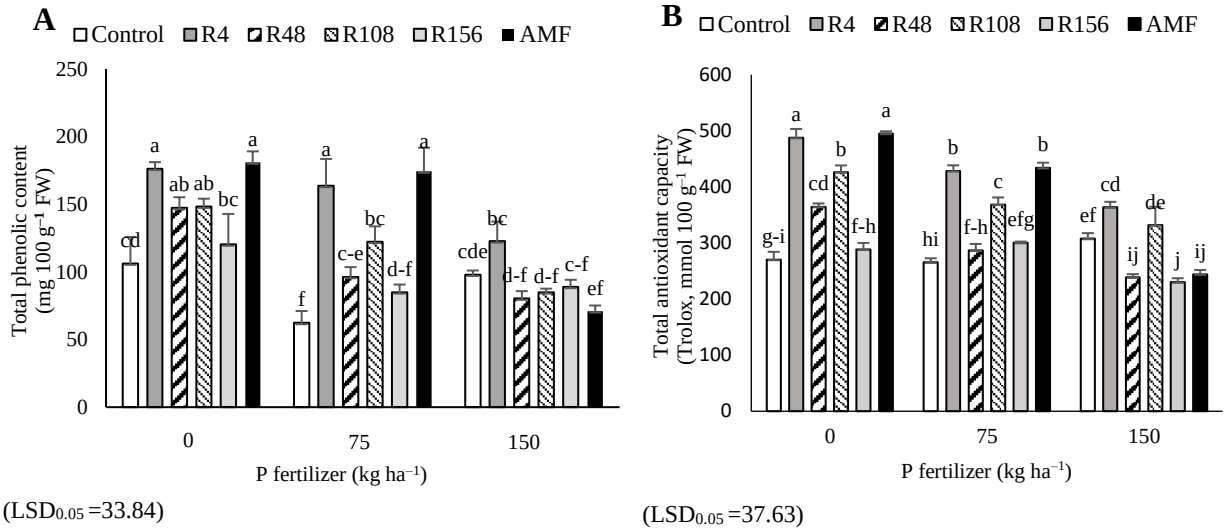

Fig. 2. Interaction effect of P fertilizer and PSMs on total phenolic content (A) and total antioxidant capacity. Means followed by different letters on the same column are significantly different according to LSD test at $p \leq 0.05$

Table 5. *F* value and mean comparison of P fertilizer and PSM main effect of flavonols content and phenolic acids content of strawberry fruits

	Flavonols content (mg 100 g^{-1} FW)			Phenolic acids content (mg 100 g^{-1} FW)			
ANOVA	Rhamnetin	Quercetin	Kaempferol	Ellagic	Gallic	p-Coumaric	Protocatechuic
P	32.49**	656**	234**	136**	113.6**	0.13ns	0.70ns
PSM	17.22**	68.77**	242**	11.56**	1.08ns	2.23ns	0.17ns
P × PSM	2.34*	6.45**	19.01**	1.69ns	1.04ns	0.74ns	0.37ns
P (kg ha^{-1})							
0	0.143a	0.406c	1.281a	14.86a	2.78a	3.13a	0.493a
75	0.115b	0.552b	1.154b	13.52b	1.66b	3.17a	0.500a
150	0.094c	0.777a	0.931c	12.05c	0.957c	3.11a	0.499a
LSD ($P \leq 0.05$)	0.0124	0.021	0.033	0.345	0.247	0.245	0.0129
PSM							
Control	0.086d	0.540c	0.833f	12.91c	1.721a	3.302a	0.495a
R4	0.149a	0.681a	1.444a	14.20a	1.772a	3.248a	0.500a
R48	0.106bc	0.670a	0.982d	13.81ab	1.650a	3.107a	0.497a
R108	0.121b	0.551bc	1.199c	13.53b	1.755a	3.303a	0.498a
R156	0.099cd	0.453d	0.902e	12.67c	1.983a	2.844a	0.493a
AMF	0.144a	0.575b	1.372b	13.75ab	1.926a	3.041a	0.499a
LSD ($P \leq 0.05$)	0.017	0.029	0.046	0.488	0.349	0.346	0.0182

Explanations as in Table 3

Phenolic acids content. The main effect of P-fertilizer rates was significant on ellagic and gallic contents, but was not significant on p-Coumaric and protocatechuic. It was found that the application of P-fertilizer reduced ellagic and gallic content significantly so that the highest ellagic and Gallic contents were obtained from non-use P-fertilizer. In addition, the impact of microorganisms was significant only on ellagic, and the strains R4, R48 and AMF had higher ellagic content than other treatments (tab. 5). Plant growth promoting bacteria may affect the production of secondary metabolites in plants. For example, it has been shown that Azospirillum sp. can reduce phenolic compounds in rice [Chamam et al. 2013] and Pseudomonas fluorescens SS101 can induce the biosynthesis of camalexing and glucosinolates in arabidopsis plant [Van De Mortel et al. 2012]. The impact of AMF on the production of secondary metabolites like the concentration of phenolic acids and flavonols has been well proven [Khaosaad et al. 2008, Castellanos-Morales et al. 2010].

Flavonols content. We found that the application of P-fertilizer reduced rhamnetin and kaemferol, but increased quercetin. As well, PSM, except R156 strain, increased rhamnetin, kaempferol, and quercetin as compared to control significantly (tab. 5). Means comparison for interaction effect of P-fertilizer × PSM showed that the highest rhamnetin and kaempferol were produced by R4 strain and AMF under non-use of P-fertilizer condition, whereas the highest quercetin was obtained from the R4 and R48 strains fertilized with 150 kg P ha^{-1} (tab. 6). The increase in rhamnetin and kaemferol by PSM seems to be associated with the impact on the improvement of soil physical, chemical and biological properties and the increase in nutrient uptake, which induced the synthesis of these compounds. Pešaković et al. [2016] reported higher rhamnetin, kaempferol and quercetin in strawberries under an integrated system, whilst Lingua et al. [2013] reported similar findings that the application of chemical fertilizers may improve fruit yield, but would reduce the fruit quality of strawberries.

Table 6. Mean comparison of P fertilizer × PSM interaction effect on flavonols content (Rhamnetin, Quercetin, Kaempferol) of strawberry fruit

P-fertilizer (kg ha^{-1})	PSM	Rhamnetin	Quercetin	Kaempferol
		(mg 100 g^{-1} FW)		
0	Control	0.114 ±0.008^{defg}	0.346 ±0.003^{hi}	0.847 ±0.008^{gh}
	R4	0.168 ±0.014^{a}	0.483 ±0.012^{f}	1.669 ±0.058^{a}
	R48	0.136 ±0.016^{bcd}	0.473 ±0.009^{f}	1.236 ±0.003^{d}
	R108	0.159 ±0.006^{ab}	0.396 ±0.003^{gh}	1.375 ±0.029^{c}
	R156	0.114 ±0.020^{defg}	0.330 ±0.012^{i}	0.920 ±0.010^{efg}
	AMF	0.168 ±0.016^{a}	0.406 ±0.007^{g}	1.642 ±0.031^{a}
75	Control	0.067 ±0.013^{h}	0.468 ±0.010^{f}	0.866 ±0.027^{gh}
	R4	0.154 ±0.018^{abc}	0.690 ±0.017^{c}	1.428 ±0.034^{bc}
	R48	0.097 ±0.007^{efgh}	0.680 ±0.010^{c}	0.911 ±0.028^{fg}
	R108	0.118 ±0.009^{def}	0.550 ±0.010^{e}	1.237 ±0.046^{d}
	R156	0.089 ±0.004^{fgh}	0.413 ±0.013^{g}	1.000 ±0.007^{e}
	AMF	0.168 ±0.007^{a}	0.510 ±0.010^{ef}	1.480 ±0.048^{b}
150	Control	0.098 ±0.003^{efgh}	0.810 ±0.036^{b}	0.995 ±0.036^{e}
	R4	0.125 ±0.016^{cde}	0.870 ±0.0203^{a}	1.236 ±0.037^{d}
	R48	0.084 ±0.006^{gh}	0.856 ±0.051^{ab}	0.799 ±0.017^{h}
	R108	0.086 ±0.003^{fgh}	0.706 ±0.007^{c}	0.985 ±0.026^{ef}
	R156	0.094 ±0.003^{efgh}	0.616 ±0.0073^{d}	0.786 ±0.027^{h}
	AMF	0.077 ±0.006^{h}	0.807 ±0.012^{b}	0.787 ±0.002^{h}
	LSD ($P < 0.05$)	0.031	0.051	0.082

Means of three replicates ± standard error. Means followed by different letters on the same column are significantly different according to LSD test at $p \leq 0.05$

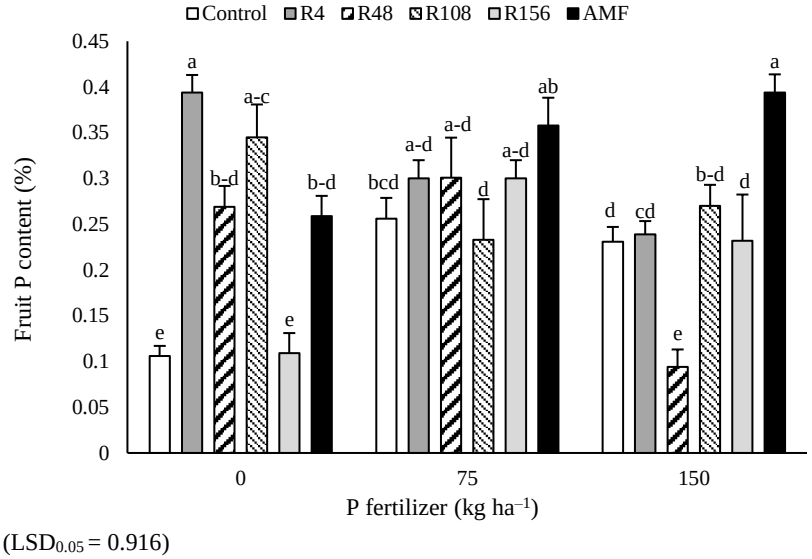

Fig. 3. Interaction effect of P fertilizer and PSM on fruit P content. Means followed by different letters on the same column are significantly different according to LSD test at $p \leq 0.05$

Fruit P content. Mean comparison for the interaction effect of P-fertilizer × PSM for fruit P content showed that under non-use of P-fertilizer condition, all microorganisms (except for R156 strain) were significantly superior compared to control and the R108 and R4 strains showed highest fruit P content. At 75 and 150 kg P ha^{-1}, AMF exhibited the highest leaf P content. There was no statistically significant difference between AMF with R4, R108 and R156 strains at 75 kg P ha^{-1} (fig. 3). According to the results, it appears that as P content increased in soil and as PSM are applied, the microorganisms secrete organic acids like oxalic acid and citric acid through which they cause the release of phosphate into soil solution via chelating and forming stable complexes with Fe, Al and K and also, gluconic acid and 2-keto-oxalic acid reduce perimeter pH and dissolve insoluble phosphates of soil by proton release [Walpola and Yoon 2012]. As a result of P release into soil, its availability for roots increases resulting in higher leaf absorption efficiency in leaves. However, the type and quantity of organic acids in each environment depends on the microorganisms generating those [Vassileva et al. 2010]. These findings are similar to results reported by Copetta et al. [2011] for tomato. Also in a study on strawberries, Esitken et al. [2010] concluded that in addition to their impact on fruit yield and fruit number per plant, PSM increased macronutrients like P in leaves. Güneş et al. [2009] observed the highest P content in rice when the roots were inoculated with bacteria and the lowest one in control. Researchers have related higher P uptake of plants that are symbiotic with PSM to carbon generation by the microorganisms and its impact on higher P uptake. Karlidag et al. [2011] revealed that the factors that increase leaf P content will increase fruit P content. They suggested that microorganisms are effective in enhancing fruit P content. Phosphorous usually competes with other elements, especially zinc and iron, on uptake and affects the concentration and total uptake of micronutrients in plants, a fact that has been confirmed by Kochain [1991] and Ohki [1984] that reported the interaction between P and other elements.

In the present study, higher phosphate fertilization rate resulted in lower leaf and fruit P content in some treatments. In addition to modifying plant growth, ACC deamilase containing Pseudomonas bacteria reduced ethylene synthesis through ACC hydrolysis into NH_3 and α-ketobutyrate [Glick et al. 1997]. On the other hand, the bacteria applied in the present study had the capability to dissolve soil insoluble P through generating organic acids like citric acid, oxalic acid and gluconic acid making a great deal of dissolved P available to plants [Vassileva et al. 2010]. According to Shaharoona et al. [2008], Pseudomonas fluorescens biotype F adjusted wheat growth components and increased N, P and K uptake as compared to non-inoculated plants. The results showed that PSM can induce growth and P absorption in fruits resulted in higher plant yield.

CONCLUSIONS

The present study showed that the application of 150 kg P ha^{-1} decreased the root volume, fruit number and fruit yield more than those of treated with 0 and 75 kg ha^{-1}. The maximum fruit yield was observed in AMF and R156 strain.

2. Inoculation of strawberry with AMF and PSM resulted in increased concentration of the total phenolic content and antioxidant capacity in the fruit produced by plants cultivated under conditions of reduced fertilization.

3. Overall, our results suggest that the common fertilization practice provides nutrients in excess. Lower concentration of nutrients and inoculation with soil microorganisms can result in quality of fruits, with a higher concentration of antioxidant molecules, consistent with the improved nutritional quality of crop. This paper supports the view that inoculations with PSMs have some potential to increase use efficiency of fertilizer in both organic and conventional farming.

ACKNOWLEDGMENT

This study was supported by Rasht Branch, Islamic Azad University, Rasht, Iran.

REFERENCES

Adesemoye, A.O., Kloepper, J.W. (2009). Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biotechnol., 85(1), 1–12.

Ahmad F., Ahmad, I., Khan, M.S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth-promoting activities. Microbiol. Res., 163, 173–181.

Alishahi, F., Alikhani, H.A., Heidari, A., Mohammadi, L. (2013). The study of inorganic insoluble phosphate solubilization and other plant growth promoting characteristics of indigenous *Pseudomonas fluorescens* bacteria of Kordan and Gonbad regions. Intl. J. Agron. Agric. Res., 3, 53–60.

Amanullah, A. Khan, A. (2015). Phosphorus and compost management influence maize (*Zea mays*) productivity under semiarid condition with and without phosphate solubilizing bacteria. Front Plant Sci., 6, 1083–1090.

Bagel, S.D., Shaikh, G.A., Adsule R.N. (1989). Influence of different levels of N, P and K fertilizers on the protein, ascorbic acid, sugars and mineral contents. J. Maharashtra Agric. Univ., 14(2), 153–155.

Baslam, M., Esteban, R., García-Plazaola, J.I. Goicoechea, N. (2013). Effectiveness of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of major carotenoids, chlorophylls and tocopherol in green and red leaf lettuces. Appl. Microbiol. Biotechnol., 97, 3119–3128.

Bona, E., Lingua, G., Manassero, P., Cantamessa, S., Marsano, F., Todeschini, V., Copetta, A., D'Agostino, G., Massa, N., Avidano, L., Gamalero, E. (2015). AM fungi and PGP Pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen and phosphorus levels. Mycorrhiza, 25(3), 181–193.

Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol., 28, 25−30.

Castellanos-Morales, V., Villegas, J., Wendelin, S., Vierheilig, H., Eder, R., Cardenas-Navarro, R. (2010). Root colonization by the arbuscular mycorrhizal fungus *Glomus intraradices* alters the quality of strawberry fruits (*Fragaria × ananassa* Duch) at different nitrogen levels. J. Sci. Food Agric., 90, 1774–1782.

Chamam A., Sanguin, H., Bellvert, F., Meiffren, G., Comte, G., Wisniewski-Dye, F., Bertrand, C., Prigent-

-Combaret, C. (2013). Plant secondary metabolite profiling evidences strain-dependent effect in the *Azospirillum-Oryza sativa* assotiation. Phytochemistry, 87, 65–77.

Chen, Y.P., Rekha, P.D., Arunshen, A.B., Lai, W.A., Young, C.C. (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Apple. Soil Ecol., 34, 33–41.

Copetta, A., Bardi, L., Bertolone, E., Berta, G. (2011). Fruit production and quality of tomato plants (*Solanum lycopersicum* L) are affected by green compost and arbuscular mycorrhizal fungi. Plant Biosyst., 145, 106–115.

De Brito Alvarez, M.A., Gagne, S., Antoun, H. (1995). Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. Appl. Environ. Microbiol., 61(1), 194–199.

Erturk, Y., Ercilsi, S., Cakmakci, R. (2012). Yield and growth response of strawberry to plant growth promoting rhizobacteria inoculation. J. Plant Nutr., 35, 817–826.

Esitken, A., Yildiz, H.E., Ercisli, S., Donmez, M.F., Turan, M., Gunes, A. (2010). Effects of plant growth promoting bacteria (PGPB) on yield, growth and n utrient contents of organically grown strawberry. Sci. Hortic., 124, 62–66.

Ghaderi, A., Aliasgharzad, N., Oustan, S., Olsson, P.A. (2008). Efficiency of three *Pseudomonas* isolates in releasing phosphate from an artificial variable charge mineral (iron III hydroxide). Soil Environ., 27, 71–76.

Gianinazzi, S., Gollotte, A., Binet, M., Van Tuinen, D., Redecher, D., Wipf, D. (2010). The role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza, 20, 519–530.

Glick, B.R. (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol., 41, 109–117.

Glick, B.R., Liu, C., Ghosh, S., Dumbroff, E.B. (1997). Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. Soil Biol. Biochem., 29, 1233–1239.

Goldstein, A.H. (1986). Bacterial solubilization of mineral phosphates: historical perspectives and future prospects. Am. J. Altern. Agric., 1, 51–57.

Gryndler, M., Vosatka, M., Hrselova, H., Catska, V., Chvatalova, I., Jansa, J. (2002). Effect of dual inoculation with arbuscular mycorrhizal fungi and bacteria on growth and mineral nutrition of strawberry. J. Plant Nutr., 25, 1341–1358.

Güneş, A., Ataoğlu, N., Turan, M., Eşitken, A., Ketterings, Q.M. (2009). Effects of phosphate-solubilizing microorganisms on strawberry yield and nutrient concentrations. J. Plant Nutr. Soil Sci., 172(3), 385–392.

Habte, M., Fox, R.L. (1993) Effectiveness of vam fungi in nonsterile soil before and after optimization of P in soil solution. Plant Soil, 151, 219–226.

Hamilton, M.A., Westermann, D.T., James, D.W. (1993). Factors affecting Zn uptake in cropping systems. Soil Sci. Soc. Am. J., 57, 1310–1315.

Han, H.S., Supanjani, D., Kee, K.D. (2006). Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ., 52(3),130–136.

Hertog, M.G.L., Hollman, P.C.H., Venema, D.P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J. Sci. Food Agric., 40, 1591–1598.

Ipek, M., Pirlak, L., Esitken, A., Figen Dönmez, M., Turan, M., Sahin, F. (2014). Plant Growth-Promoting Rhizobacteria (PGPR) increase yield, growth and nutrition of strawberry under high-calcareous soil conditions. J. Plant Nutr., 37(7), 990–1001.

Karimi, K., Bolandnazar, S.A., Ashoori, S. (2013). Effect of bio-fertilizer and arbuscular mycorrhizal fungi on yield, growth characteristics and quality of green eban (*Phaseolous vulgaris* L.). J. Sustain. Agric. Prod. Sci., 23(3), 157–167 [in Persian].

Jackson, M. L. 1958. Soil Chemical Analysis. Englewood Cliffs: Prentice Hall, Inc.

Karlidag, H., Yildirim, E., Turan, M., Donmez, F. (2011). Effect of plant growth promoting bacteria on minearl-organic fertilizer use efficiency, plant growth and mineral contents of strawberry (*Faragria × ananassa* L Duch). Ind. J. Biotechnol., 9, 289–297.

Khaligh, A., Sandres, F.E. (2000). Effects of vercicular-arbuscular mycorrhizal inoculation on the yield and phosphorus uptake of field-grown barley. Soil Biol. Biochem., 32, 1641–1696.

Khan, K.S., Joergensen, R.G. (2009). Changes in microbial biomass and P fraction in biogenic bousehold waste compost amended with inorganic P fertilizers. Bioresour. Technol., 100, 303–309.

Khaosaad, T., Krenn, L., Medjakovic, S., Ranner, A., Lössl, A., Nell, M., Jungbauer, A., Vierheilig, H. (2008). Effect of mycorrhization on the isoflavone content and the phytoestrogen activity of red clover. J. Plant Physiol., 165, 1161–1167.

Kochain, L.V. (1991). Mechanisms of micronutrient uptake and translocation in plant. In: Micronutrient in agriculture, Mortvelt, J.J., Cox, F.R., Shuman, L.M., Welch, R.M. (eds). 2nd ed., Soil Science Society of America, Madison, WL, 229–285.

Lingua, G., Bona, E., Manassero, P., Marsano, F., Todeschini, V., Cantamessa, S., Copetta, A., D'Agostino, G., Gamalero, E., Berta, G. (2013). Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads increases anthocyanin concentration in strawberry fruits (*Fragaria × ananassa* var Selva) in conditions of reduced fertilization. Int. J. Mol. Sci., 14, 16207–16225.

Liu, M., Li, X.Q., Weber, C., Lee, C.Y., Brown, J., Liu, R.H. (2002). Antioxidant and antiproliferative activities of raspberries. J. Agric. Food Chem., 50, 2926–2930.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with Folin-phenol reagent. J. Biol. Chem., 193(1), 265–275.

Milivojević, J., Maksimović, V., Nikolić, M., Bogdanović, J., Maltić, R., Milatović, D. (2011). Chemical and antioxidant properties of cultivated and wild Fragaria and Rubus berries. J. Food Qual., 34, 1–9.

Mukerji, K.G., Manohara Chary, C., Chamola, B.P. (2002). Techniques in mycorrhizal studies. Kluwer Academic Publishers, Dordrecht.

Ohki, K. (1984). Manganese deficiency and toxicity effects on growth, development, and nutrient composition in wheat. Agron. J., 76, 212–218.

Panigrahi, K., Eggen, M., Maeng, J.H., Shen, Q., Berkowitz, D.B. (2009). The α, α-difluorinated phosphonate L-pSer-analogue, an accessible chemical tool for studying kinase-dependent signal transduction. Chem. Biol., 16(9), 928–936.

Pantelidis, E.G., Vasilakakis, M., Manganaris, A.G., Diamantidis, G. (2007). Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries. Food Chem., 102, 777–783.

Patten, C.L, Glick, B.R. (2002). Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol., 68(8), 3795–3801.

Penrose, D.M., Glick, B.R. (2003). Methods for isolating and characterizing ACC deaminase containing plant growth promoting rhizobacteria. Physiol. Plant., 118(1), 10–15.

Pešaković, M., Milenković, S., Đukić, D., Mandić, L., Karaklajić-Stajić, Ž., Tomić, J., Miletić, N. (2016). Phenolic composition and antioxidant capacity of integrated and conventionally grown strawberry (*Fragaria × ananassa* Duch). Hort. Sci., 43(1), 17–24.

Reganold, J.P., Andrews, P.K., Reeve, J.R., Carpenter-Boggs, L., Schadt, C.W., Alldredge, J.R., Ross, C.F., Davies, N.M. Zhou, J. (2010). Fruit and soil quality of organic and conventional strawberry agroecosystems. PLoS ONE, 5(10), 10.1371.

Rudresha, D.L., Shivaprakasha, M.K., Prasad, R.D. (2005). Effect of combined application of Rhizobium, phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer aritenium* L). Appl. Soil Eco., 28, 139–146.

Sanchez-Moreno, C., Larrauri, J.A., Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agric., 79, 270–276.

Schwyn, B., Neilands, J. (1987). Universal chemical assay for the detection and determination of siderophores. Anal. Biochem., 160(1), 47–56.

Shaharoona, B., Naveed, M., Arsahd, M., Zahir, A. (2008). Fertilizer-dependent efficiency of Pseudomonads for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L). Appl. Microbiol. Biotechnol., 79, 147–155.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A. (2013). Phosphate solubilizing microbes, sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus, 2, 587.

Smith, S., Read, D. (2008). Mycorrhizal Symbiosis, 3rd ed. Academic Press, London.

Tomic, J.M., Milivojevic, J.M., Pesakovic, M.I. (2015). The response to bacterial inoculation is cultivar-related in strawberries. Turk. J. Agric. Forest, 39(2), 332–341.

Turan, M., Ataoglu, N., Sezen, Y. (2004). Effects of phosphorus solubilizing bacteria (*Bacillus megaterium*) on yield and phosphorus contents of tomato plant (*Lycopersicon esculentum* L). Proceedings of the National Fertilizer Congress Farming-Industry-Environment, 11–13 October, Tokat, Turkey.

Van de Mortel, J., De Vos, R., Dekkers, E., Pineda, A., Guillod, L., Bouwmeester, K., Van Loon, J., Dicke, M., Raaijmakers, J. (2012). Metabolic and transcriptomic changes induced in arabidopsis by the rhizobacterium *Pseudomonas fluorescens* SS101. Plant Physiol., 160, 2173–2188.

Vassileva, M., Serrano, M., Bravo, V., Jurado, E., Nikolaeva, I., Martos, V., Vassilev, N. (2010). Multifunctional properties of phosphate-solubilizing microorganisms grown on agro-industrial wastes in fermentation and soil conditions. Appl. Microbiol. Biotechnol., 85, 1287–1299.

Walpola, B.C., Yoon, M.H. (2012). Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils, A review. Afr. J. Microbiol. Res., 6(37), 6600–6605.

Zaidi, A., Khan, M.S. (2006). Co-inoculation effects of phosphate solubilizing microorganisms and *Glomus fasciculatum* on green gram Bradyrhizobium symbiosis. Turk. J. Agric. Forest, 30, 223–230.