

Full Length Research Paper

Performance of phosphate solubilizing bacteria for improving growth and yield of sunflower (*Helianthus annuus* L.) in the presence of phosphorus fertilizer

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Accepted 27 May, 2010

The ability of phosphate solubilizing bacteria (PSB) to convert insoluble forms of phosphorus to an accessible form is an important trait in sustainable farming for increasing plant yields. The beneficial effects of PSB on crop productivity have been widely described, but the use of PSB as biofertilizer is scarcely documented in sunflower. The objective of this study was to evaluate the effect of application of PSB, *Bacillus* M-13, with and without varying amounts of phosphorus (P) fertilizer on growth and yield of sunflower under field conditions. The PSB application was able to mobilize P efficiently in the sunflower and improved seed quality and oil yield. It also enhanced the head diameter, 1000 seed weight, kernel ratio and oil content and led to seed and oil yield increases of 15.0 and 24.7% over no application, respectively. However, when PSB was used in conjunction with P fertilizers, a much greater effect was observed. It was found that the highest seed yield of sunflower possible with 100 kg P₂O₅ ha⁻¹ fertilizer was achieved with about 50 kg P₂O₅ ha⁻¹ when used in conjunction with PSB. But the important effect of PSB on oil yield was noted at 100 kg P₂O₅ ha⁻¹, possibly indicating increased PSB activity in the rhizosphere following PSB application and consequently by enhanced P solubilization as evidenced by the higher levels of P content of seed.

Key words: Phosphate solubilization, *Bacillus*, sunflower.

INTRODUCTION

Phosphorus (P) is an essential plant nutrient required for higher and sustained productivity of oil from sunflower. Its influence on seed yield, oil yield and oil quality has been well established (Blamey and Chapman, 1981; Loubser and Human, 1993; Bahl and Toor, 1999; Chandrashekhara et al., 1995; Zubillaga et al., 2002) and application of phosphorus has become an essential part of sunflower fertilizer program. In general, phosphorus is added to soil as inorganic phosphates, because the free inorganic P in soil solution plays a central role in P-cycling and plant nutrition (Peix et al., 2001a). However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is immobilized rapidly after application due to phosphate fixation by aluminum, calcium, iron, magnesium

and soil colloids (Rodriguez and Fraga, 1999) and becomes unavailable to plants (Singh and Kapoor, 1994). Therefore, P is often a limiting nutrient in agricultural soils. Micro-organisms are also involved in a range of process that affect the transformation of soil P and thus an integral part of the soil P cycle (Chen et al., 2006). In particular, P-solubilizing micro-organisms (bacteria or fungi) are able to solubilize unavailable soil P and increase the yield of crops (Adesemoye and Kloepper, 2009).

Plant growth-promoting rhizobacteria (PGPR) and rhizosphere bacteria are free-living soil organisms that can benefit plant growth by different mechanisms (Glick, 1995). P-solubilization ability of micro-organisms is considered to be one of the most important traits associated with plant P nutrition (Chen et al., 2006). Several bacterial species, in association with plant rhizosphere, are capable of increasing availability of Phosphorus to plants either by mineralization of organic phosphate or by solubilization of inorganic phosphate by production of

Abbreviations: PSB, Phosphate solubilizing bacteria; PGPR, plant growth-promoting rhizobacteria.

Table 1. Monthly meteorological parameters during the crop seasons (growing periods: May to September).

Month	2006					2007				
	Monthly mean air temperature (°C)			Monthly rainfall (mm)	Monthly BSS ^a (day)	Monthly mean air temperature (°C)			Monthly rainfall (mm)	Monthly BSS ^a (day)
	Max.	Min.	Mean			Max.	Min.	Mean		
May	16.8	6.7	11.8	56.5	14	18.2	9.4	13.8	30.6	18
June	26.4	12.2	19.3	-	23	23.1	12.1	17.6	35.3	24
July	27.4	15.2	21.3	29.1	26	25.7	15.0	20.4	20.7	27
August	29.9	16.8	23.4	3.2	24	26.8	15.4	21.1	8.3	22
September	24.0	11.6	17.8	3.0	27	25.0	12.6	18.8	14.1	27
Total/mean			18.7	91.8				18.3	109.0	

^a BSS : Bright Sun Shine days.

acids (Rodriguez and Fraga, 1999). These bacteria are referred to as phosphate solubilizing bacteria (PSB) and have been considered to have potential use as inoculants biofertilizer to improve the plant growth and yield (Yadav and Dadarwal, 1997; Rodriguez and Fraga, 1999; Vessey, 2003; Chen et al., 2006). Consequently, many researchers have isolated P-solubilizing bacteria from different soil and the inoculations of these bacteria to increase P-availability of plants have been intensively studied (Freitas et al., 1997; Peix et al., 2001a, b; Chen et al., 2006). At the present, bacilli, rhizobia and pseudo-nomads are the best studied P-solubilizer bacteria (Rodriguez and Fraga, 1999). Currently, researchers have described the phosphate solubilization by *Bacillus* species because of their rapid colonization in the rhizosphere and stimulation of plant growth (Sindhu et al., 2002) and also because they offer unique characteristics (e.g. stress-resistant spores) which may be appropriate for seed inoculants (Freitas et al., 1997). Several studies have shown that *Bacillus* spp. inoculation to seed and soil can solubilize fixed soil P and applied P, resulting in higher crop yields and growth promoting agent providing varied contributions to the enhancement of growth and productivity in different crops (Çakmakçı et al., 2006; Orhan et al., 2006; Canbolat et al., 2006). According to Gaind and Gaur (1991), *Bacillus subtilis* inoculant increased biomass, grain yield and P- and N- uptake of mung bean. Freitas et al. (1997) also reported that P-solubilizing *Bacillus* strains significantly increased growth and yield but not P-uptake of canola and to have potential use as inoculants for canola. Sunflower is also characterized by high plasticity under different nutrient availabilities. Phosphorus is commonly a limiting factor in sunflower growth and yield because P deficiencies reduce the accumulation of crop biomass (Zubillaga et al., 2002). This is attributable to (i) a reduction in the partitioning of assimilates to the formation of leaf area, or (ii) a decrease of the efficiency with which the intercepted radiation is used for the production of above-ground biomass (Colomb et al., 1995). Rodriguez et al. (1998) reported that under P deficiencies sunflower showed a reduction in the rate of

leaf expansion and in photosynthetic rate per unit of leaf area. However, P application produced greater and more consistent effects on crop performance as P fertilization allowed more efficient use of supplied N (soil + fertilizer). Loubser and Human (1993) noted also that the response of seed and oil yield of sunflower was in agreement with the P absorption by the plants.

P fertilizers are expensive in developing countries like Turkey. Therefore, the main purpose in managing soil phosphorus is to optimize crop production and minimize P loss from soils. Considering the importance of P nutrition in sunflower and the need for economizing P fertilizer use, microbial P-mobilization would be the only possible way to increase plant-available P (Illmer and Schinner, 1992; Peix et al., 2001a). Therefore, the beneficial effects of PSB on crop productivity have been widely described, but the use of PSB as biofertilizer is scarcely documented in sunflower. Thus, the aim of this study was to determine the effect of inoculation with *Bacillus* M-13 on growth, yield and quality of sunflower at various levels of Phosphorus fertilization under field conditions and to test the hypothesis if *Bacillus* M-13 can use an important potential as biofertilizer for sunflower production.

MATERIALS AND METHODS

Field experiments were conducted on a farm located in Ahlat district (38°46'N and 42°30'E with an altitude of 1722 m), Eastern Anatolia region of Turkey in 2006 and 2007. The soil of the experimental site was a silt-clay-loam, with approximately 2.4% organic matter content, pH 7.6 and 4.4% lime content. The 0 - 60 cm soil layers contained 7.10 ppm available P₂O₅ (Spectrophotometrically), 2066.71 ppm available K₂O (Ammonium acetate method) and 0.15% total nitrogen (Kjeldahl method), respectively. In 2006 - 2007 and 2007 - 2008, during the crop season (growing periods: May to September), monthly mean air temperatures, total rainfall and bright sun shine day values were collected from experimental meteorological station near the research farm and are presented in Table 1. Total rainfall during growing season (from May to September) was 91.8 and 109.0 mm, respectively, and the average temperature for the same period was 18.7 and 18.3°C in 2006 and 2007, respectively.

These values indicated that hot and dry conditions prevailed in

2006. The bacterial strain used in the study was *Bacillus* M-13 (Phosphate-solubilizing bacterium) that kindly obtained from Atatürk University, Turkey. *Bacillus* M-13 was reported as plant growth promoting bacteria and it may well be suited alone or in combination to achieve sustainable and ecological agricultural production (Çakmakçı et al., 2006, Orhan et al., 2006). The bacterial strain *Bacillus* M-13 was originally isolated from pepper plants at Atatürk University (Şahin et al., 2004) and was gram (+), P solubilization (+), catalase (+), oxidase (-), pigment (-), nitrate reduction (+), starch hydrolysis (+), growth at 36°C and in N-free basal medium (+) (Canbolat et al., 2006). This strain was also capable of dissolving insoluble P in the previous field experiments with barley, sugar beet and raspberry etc (Çakmakçı et al., 2006; Orhan et al., 2006; Canbolat et al., 2006). The bacterial strain was kept in nutrient broth (NB) with 15% glycerol at -80°C. For this study, the bacterial strain was incubated on nutrient agar. A single colony was transferred to 1000 ml flask containing NB and incubated aerobically, on a rotating shaker (150 rpm) overnight at 28°C. The bacterial suspension was then diluted with sterile distilled water to a final concentration of 10^9 colony forming units (CFU) ml^{-1} according to MacFarland Standards. Sunflower seeds were sterilized with 70% ethanol for 2 min, then 1.2% sodium hypochloride for 10 min and rinsed ten times in sterile tap water. Seeds were then treated with the bacterial suspensions for 30 min.

In the study, "TARSAN-1018" commercial hybrid of oilseed sunflower, which had early maturing, high yield potential, responsive to higher inputs, more tolerant to diseases and pests, higher drought tolerance, more self fertile, superior in their seed filling ability and higher adaptation ability, was used as plant material. The experiments were carried out in a factorial design, using two different inoculations (control, *Bacillus* M-13) and three levels of P fertilization (0, 50 or 100 kg P_2O_5 ha^{-1}) with three replicates. The plots were 4.2 m wide and 4 m long and consisted of 6 rows spaced 0.7 m apart. The experimental crop was sown on 10th May 2006 and 12th May 2007. The plots were hand-thinned to 7.1 plants per m^2 when the plants were at the 4 to 6-leaf stage. Phosphorus levels of 0, 50 and 100 kg P_2O_5 ha^{-1} were tested and it was applied as triple super Phosphate at sowing in both years. All plots received nitrogen at 120 kg N ha^{-1} as ammonium Sulphate $[(\text{NH}_4)_2\text{SO}_4]$ form. Nitrogen was applied as split into two applications, half with sowing and the remaining half at the beginning of stem elongation. Weeds were controlled by hand-hoeing. The experiment was conducted in irrigated conditions and it was sprinkler irrigation system irrigated regularly to avoid drought stress. A total of 4 - 5 irrigations each year were applied.

The sunflower plants were harvested by hand middle two rows excluding side rows and 1 m from each end of plots at the stage of physiological maturation when the back of the head had turned from green to yellow and the bracts were turning brown (in the second week of September in both years). Plant height, stem diameter and head diameter were determined at physiological maturity, by harvesting 10 plants of sunflower per experimental unit. Heads were separated from vegetative parts. Head diameter was estimated for distance in cm across the apical head at its widest point. The head samples for yield were also dried to constant weight and threshed mechanically. Seed yield was adjusted to a 10% moisture basis. Filled grain and empty hulls were separated by hand. Hereafter, grain number per head refers to filled grains only. Seed oil and N contents were determined using the Soxhlet and Kjeldahl method in seed kernel (dehulled). Seed kernel ratio (%) was calculated by seed kernel weight, using the conversion method where seed kernel weight in g of 10 g hull-seeds (with 4 replicates) taken at random from the dried seeds in each plot was divided by seed hull weight. Seed kernel yield was estimated for each plot using the formula: Seed kernel ratio x seed yield / 100. Measurements of oil yield were calculated from oil content, using the conversion method where oil content is multiplied by seed kernel yield to calculate its oil yield equivalent to seed kernel. All data in the present study

were evaluated by analysis of variance (ANOVA) using SAS software (SAS Institute, Cary, NC, USA) and means were separated by Duncan's Multiple Range Test using MSTAT-C software. Since there was no year x treatment interaction ($p > 0.05$) for the variables (Tables 2 and 3), only average results for both years are presented.

RESULTS AND DISCUSSION

Analysis of variance showed statistically significant differences between years for the characteristics investigated (Tables 2 and 3). More favorable climatic conditions increased growth and yield of sunflower in 2007 (Table 1). Especially, a higher number of sun shine days during 2007 season coincided with the establishment of the crop canopy. Sunflower is a C_3 crop. Crop growth depends on the ability of leaves or roots to capture and use solar radiation, CO_2 , water and nutrients, with solar radiation providing the energy to drive both CO_2 assimilation and water transpiration processes (Albrizio and Steduto, 2005). The growth of canopy is also known to be hastened by a high amount of irradiance which is primarily due to the effects on above-ground biomass (Salisbury and Ross, 1992). Thus, favorable conditions during plant growth in 2007 - 2008 resulted in the higher plant height, stem diameter and head diameter and maintained plant growth at a higher rate for a longer time period leading to higher yield.

PSB and P fertilizer application on yield components and yield

The results of PSB and P fertilizer treatments on plant height, stem diameter, head diameter, number of filled grains per head, 1000 seed weight and seed yield are shown in Table 2. The PSB application significantly increased the parameters (except plant height) over control. The results showed that the inoculation with *Bacillus* M-13 without P fertilizer increased stem diameter by 11.2%, head diameter by 13.5%, number of filled grains per head by 10.1%, 1000 seed weight by 14.1% and seed yield by 30.1%, compared to the Control + P_0 application (Table 2). The high response of plant to the PSB inoculation might be due to mobilization of available P by the native soil microflora, or attributed of increased PSB activity in the rhizosphere following PSB application and consequently by enhanced P solubilization. For these reason, its enhanced P uptake by the crops following PSB additions has led to an increase in stem and head diameter and thus number of filled grains per head and 1000 seed weight growth ultimately leading to higher seed yields. Several studies have reported that seed or soil inoculation with phosphate-solubilizing bacteria such as *Bacillus* spp. can solubilize fixed soil P and applied phosphates, resulting in higher crop yields (Yadav and Dadarwal 1997; Pal, 1998; Sindhu et al.,

Table 2. The effect of PSB and P fertilizer treatments on yield components and yield of sunflower¹.

Variable	Plant height (cm)	Stem diameter (cm)	Head diameter (cm)	No. of filled grains per head	1000 seed weight (g)	Seed yield (t ha ⁻¹)
Years (Y)						
2006	157.5 b	2.49 b	23.3 b	879.5 b	92.3 a	5.09 b
2007	170.4 a	2.90 a	25.2 a	1132.5 a	79.9 b	5.50 a
Mean	164.0	2.69	24.2	1005.9	86.1	5.29
Phosphate-solubilizing bacterium (PSB)						
Control	163.2	2.58 b	23.1 b	978.1 b	82.5 b	4.93 b
PSB (<i>Bacillus</i> M-13)	164.7	2.81 a	25.3 a	1033.9 a	89.7 a	5.67 a
P² levels						
0 kg P ha ⁻¹	161.7b	2.46 c	21.6 c	998.5	75.9 c	4.51 b
50 kg P ha ⁻¹	163.2b	2.75 b	25.1 b	998.2	89.8 b	5.59 a
100 kg P ha ⁻¹	167.0a	2.88 a	26.0 a	1021.2	92.6 a	5.79 a
Phosphate-solubilizing bacterium (PSB) x P levels						
Control + P ₀	160.9	2.33	20.2 e	950.7	70.9	3.92 e
Control + P ₅₀	162.7	2.68	24.2 c	986.7	87.5	5.36 cd
Control + P ₁₀₀	166.0	2.72	24.8 c	996.9	89.2	5.51 bc
PSB + P ₀	162.6	2.59	22.9 d	1046.5	80.9	5.10 d
PSB + P ₅₀	163.7	2.81	26.0 b	1009.7	92.2	5.82 ab
PSB + P ₁₀₀	168.0	3.04	27.1 a	1045.6	96.1	6.08 a
CV ³ (%)	2.91	5.48	3.72	5.48	3.63	6.04
Source	ANOVA					
Y	**	**	**	**	**	**
PSB	NS	**	**	**	**	**
Y x PSB	NS	NS	NS	NS	NS	NS
P	**	**	**	NS	**	**
Y x P	NS	NS	NS	NS	NS	NS
PSB x P	NS	NS	**	NS	NS	*
Y x PSB x P	NS	NS	NS	NS	NS	NS

¹ Means followed by different letter within each column are significantly different, ² Phosphorus fertilization, ³ Coefficient of variation. *: p<0.05, **: p<0.01, NS: Not significant.

2002; Dobbelaere et al., 2003; Canbolat et al., 2006) and also in increased inorganic P availability to plant by solubilization of inorganic P (Rodriguez and Fraga, 1999). The positive effects of *Bacillus* M-13 on the yield and growth of crops such as sugar beet, apple, raspberry and barley were also explained by P-dissolving ability (Çakmakçı et al., 2001, 2006; Karlıdağ et al., 2007). The results are in agreement with previous researches. According to Canbolat et al. (2006), the higher total P and N uptake of barley indicated that *Bacillus* M-13 were able to solubilize P and fix N, with consequent promotion of plant growth. Analysis of variance showed that growth and yield characters (except number of filled grains per head) were significantly affected by P levels (Table 2). In the study, the highest plant height, stem diameter, head diameter and 1000 seed weight were obtained with the highest P level, that is, 100 kg P₂O₅ ha⁻¹. P uptake is highly correlated with yield components of sunflower. It is also known that P availability in soils is important for the

uptake of N from soils and its utilization in plant (Kim et al., 2003) and thus better crop growth. In the study, increases in plant growth values with P application might be due to fact that P application allowed more efficient use of supplied N (soil + fertilizer) because of a better development of the plant when having higher amount of available P and thus it produced greater and more consistent effects on crop performance as previously reported by Colomb et al. (1995), Rodriguez et al. (1998) and Zubillaga et al. (2002). Chandrashekara et al. (1995) and Loubser and Human (1993) noted that seed yield of sunflower increased with increase in P fertilizer levels.

The interaction between PSB and P fertilizer were significant on only head diameter and seed yield in the study (Table 2). On the other hand, PSB application without any P fertilizer increased the all parameters. When PSB was used in conjunction with P fertilizer, however, a much greater effects were observed. All the treatments including P fertilizer and PSB improved the yield com-

ponents and yield significantly over the P fertilizer treatments without PSB. The head diameter and seed yield in treatments involving PSB applications were significantly influenced by the changes in the P fertilizer levels, and the highest values were obtained from *Bacillus* M-13 +P₁₀₀ combined plot, followed by *Bacillus* M-13 +P₅₀ combined plot (Table 2). However, PSB application achieved comparable seed yields to 50 kg P₂O₅ ha⁻¹ fertilization in the absence of any fertilizer application, although the differences between P fertilizer levels were not significant in seed yield. The positive effects of *Bacillus* M-13 on seed yield in this study may suggest that sunflower's response could be more in PSB inoculation with P fertilizer combinations than alone fertilizer usage. Previous studies indicated that inoculation with beneficial bacteria can increase the efficiency of fertilizer use at both high and low fertilization levels (Wu et al., 2005; Ekin et al., 2009). Similarly, Han et al. (2006) reported that combined together, P fertilizer and *Bacillus megaterium* var. *phosphaticum* consistently increased further mineral availability, uptake and plant growth of pepper and cucumber, suggesting its potential use as fertilizer. Kumar et al. (2001) reported that the inoculation of phosphate in solubilizing and phytohormone producing *Azotobacter chroococcum* mutants exerted a favorable influence on wheat as shown by increased grain, straw, biological yield, 1000 grain weight and root biomass over the control. Thus application of mutant strains of *A. chroococcum* may have led to more solubilization of insoluble phosphates in the soil and consequent higher uptake of phosphates. According to Sundara et al. (2002), PSB application reduced the required P dosage by 25%, when used in conjunction with P fertilizers and the PSB improved juice quality and sugar yields of sugarcane. The results of the present study are also in agreement with those of Freitas et al. (1997), Kumar et al. (2001), Peix et al. (2001a,b), Chen et al. (2006) and Adesemoye and Kloepper (2009), who reported that the microbial inoculants can be used as an economic input to increase crop productivity with chemical fertilizers.

PSB and P fertilizer effects on oil yield and seed quality components

Two years of trials under field conditions showed that treatments including PSB and P fertilizer significantly affected the oil yield and seed quality components (Table 3). In general, crude oil content, kernel ratio, P content and oil yield increased significantly in response to P fertilizer treatments, the maximum being at 100 kg P₂O₅ ha⁻¹, while the P application significantly decreased the protein content. This response further correlated with the bacterial inoculation. PSB application performed better than the control and improved seed quality and oil yield of sunflower. PSB showed significant increase in oil yield (24.7%), crude oil content (4.8%), kernel ratio (3.4%) and

P content (13.2%) over control. However, when *Bacillus* M-13 and P application were combined, a much greater effect observed. The combined effect of P fertilizer and PSB showed maximum increase in oil yield by 84.8%, crude oil content by 9.1%, kernel ratio by 7.7% and P content by 28.0% as compared to the Control+P₀ application, although the protein content (16.7%) significantly declined (Table 3).

In the study, the highest oil content and oil yield were obtained from PSB +P₁₀₀ combined plot, while the highest protein content was recorded for Control +P₀ combined plot. Blamey and Chapman (1981) noted that P fertilization tended to increase the oil concentration in the seed, while protein level in the seed was increased by N but decreased by P fertilization, the opposite effects being generally observed with respect to the oil yield. Zubillaga et al. (2002) reported that there were significant negative correlations between the protein and oil concentrations in the seed, and increases in the levels of P application significantly increased the oil yield. The results of this study indicated that improvement in oil content and kernel ratio of seed was significant upon PSB additions thus leading to higher oil yield and P-solubilizing *Bacillus* M-13 was able to mobilize P efficiently in the sunflower as the contents of P and oil in seed were increased by 14.8 and 5.0% with respect to Control +P₀, respectively. Increased plant growth parameters and seed quality in case of PSB application might be attributed to the production of higher quantities of growth promoting substances and complementary effect of enhanced phosphate availability (Kumar et al., 2001). Microbial inoculation with P-solubilizing *Bacillus* M-13 may also increase the efficiency of applied P₂O₅ by reducing phosphate fixation in soil fractions. Previous studies have showed that this P solubilizing bacteria can serve as efficient biofertilizer candidates for improving the P-nutrition of various crops (Çakmakçı et al., 2001, 2006; Şahin et al., 2004; Orhan et al., 2006; Karlıdağ et al., 2007).

Conclusion

It is concluded that application of PSB *Bacillus* M-13 strain has beneficial effects on growth, yield and quality of sunflower. The highest seed yield of sunflower possible with P fertilizer was achieved with about 50 kg P₂O₅ ha⁻¹ when used in conjunction with PSB, while the combined effect of the 100 kg P₂O₅ ha⁻¹ fertilizer level and PSB showed maximum increase in oil yield. In situations where high oil yield is desired, such as for oil production, an adequate supply of P is essential to optimize yields. Hence, the study suggests that *Bacillus* M-13 alone, or in combination with P fertilizer, has a great potential to increase oil yield and yield components of sunflower and to improve the usage efficiency of chemical fertilizers as in many other crops previously tested. PSB *Bacillus* M-13 strain as plant growth promoting

Table 3. The effect of PSB and P fertilizer treatments on oil yield, crude oil content, protein content, kernel ratio and P content¹.

Variable	Oil yield (t ha ⁻¹)	Dehulled seed (%)			
		Crude oil content	Protein content	Kernel ratio	P content
Years (Y)					
2006	1.98 b	52.7 b	23.7 a	73.6 b	0.656 b
2007	2.20 a	53.5 a	23.1 b	74.3 a	0.717 a
Mean	2.09	53.1	23.4	73.9	0.686
Phosphate-solubilizing bacterium (PSB)					
Control	1.86 b	51.8 b	24.4 a	72.7 b	0.644 b
PSB (<i>Bacillus M-13</i>)	2.32 a	54.3 a	22.3 b	75.2 a	0.729 a
P² levels (P)					
0 kg P ha ⁻¹	1.69 c	51.7 b	24.3 a	72.4 c	0.647 c
50 kg P ha ⁻¹	2.22 b	53.5 a	23.3 b	74.3 b	0.684 b
100 kg P ha ⁻¹	2.35 a	54.0 a	22.6 c	75.1 a	0.728 a
Phosphate-solubilizing bacterium (PSB) x P levels (P)					
Control + P ₀	1.38 d	50.4	25.1	70.4 e	0.603
Control + P ₅₀	2.05 c	52.4	24.4	73.1 d	0.645
Control + P ₁₀₀	2.16 c	52.6	23.8	74.5 bc	0.683
PSB + P ₀	2.00 c	52.9	23.5	74.3 c	0.692
PSB + P ₅₀	2.39 b	54.6	22.1	75.4 ab	0.723
PSB + P ₁₀₀	2.55 a	55.0	21.5	75.8 a	0.772
CV ³ (%)	6.30	2.18	2.25	1.12	5.33
Source ANOVA					
Y	**	**	**	*	**
PSB	**	**	**	**	**
Y x PSB	NS	NS	NS	NS	NS
P	**	**	**	**	**
Y x P	NS	NS	NS	NS	NS
PSB x P	*	NS	NS	**	NS
Y x PSB x P	NS	NS	NS	NS	NS

¹Means followed by different letter within each column are significantly different², Phosphorus fertilization³ Coefficient of variation. *: p<0.05, **: p<0.01, NS: Not significant.

bacteria can be used as an economic input to increase crop productivity.

ACKNOWLEDGEMENTS

The author thanks Dr. M. Figen Dönmez from Department of Plant Protection, Atatürk University, Turkey for kindly provided the PSB strain *Bacillus M-13*. We are also grateful to Faruk Oğuz and Assist. Prof. Ismail Hakkı Ekin (Department of Microbiology) from Yüzüncü Yıl University, Turkey for their contribution in this work.

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