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P-SOLUBILIZING BACTERIA AND PHOSPHORUS FERTILIZER APPLICATIONS TO SUNFLOWER IMPROVES SEED SET, SEED FILLING EFFICIENCY AND CONCENTRATION OF MACRO-AND MICRO-NUTRIENTS OF SEEDS

Zehra EKİN

University of Yüzüncü Yıl, Faculty of Agriculture, Department of Field Crops, Turkey.
Corresponding author's email: zehraekin@yyu.edu.tr

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ABSTRACT

Phosphate solubilizing bacteria (PSB) have an ability converting insoluble forms of phosphorus to an accessible form. The study was conducted to investigate how bacterial inoculation to unfertilized sunflower (*Helianthus annuus* L.) or fertilized with phosphorus affected seed set and filling efficiency, kernel yield and chemical composition of seed under field conditions. Study plots were treated with two levels of P-solubilizing *Bacillus* M-13 bacterium (Control and PSB) and three levels of phosphorus fertilizer (0, 50 and 100 kg P_2O_5 ha^{-1}) with three replications. Applications of PSB significantly increased seed set (8.2-20.0%), seed filling efficiency (1.3-1.9%), kernel yield (37.3-67.0%) and quality by reducing hull percentage (15.2- 24.3%) and by improving nutrient concentrations of seeds. It was concluded that application of 50 kg ha^{-1} dose of P fertilizer with PSB can give similar seed set and kernel yield as with 100 kg ha^{-1} dose of P fertilizer. Besides, PSB application caused a substantial increase in all macro-and micro-nutrients in seeds. In conclusion, application of P-solubilizing bacteria with or without P fertilizer leads to increase in seed set and kernel yield and alteration in nutrient concentrations in the seeds of sunflower plant.

Key words: *Helianthus annuus* L., mineral content, PSB and phosphorus

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is grown mainly for the oil-rich seeds in different agro-climatic zones of Turkey. Phosphorus (P) is one of the most important macronutrients required for optimum plant growth and yield as well as a good seed formation. Phosphorus positively affects sunflower growth and productivity by increasing photosynthetic rate and the radiation use efficiency and consequently the availability of assimilates (Rodriguez et al., 1998). The potential for improving seed set and filling in sunflower also seems to be mainly associated with increased assimilates ratio (Karadogan et al., 2009) and higher phosphorus uptake can increase seed sets and improve yields (Zubillaga et al., 2002). Seed composition is also affected by phosphorus supply, which particularly increased oil content in the seed (Blamey and Chapman, 1981). Thus, the general findings of researchers show that the most effective method to increase seed or oil yield of sunflower is adequate P supply (Zubillaga et al., 2002; Gunes and Inal, 2009; Amanullah and Khan, 2010).

Although most agricultural soils have large amounts of inorganic and organic P, these are immobilized and mostly unavailable. Therefore, only a very low concentration of P is available to plants and many soils are actually P deficient (Adesemoye and Kloepper, 2009). In technologically advanced and wealthy countries, the need for this element can be satisfied by the use of chemical fertilizers. Even in these regions, however, fertilizers are expensive and the intensive use of chemicals has produced environmental problems that threaten the sustainability of agricultural systems (Vance, 1997). Hence, alternative strategies to increasing P availability and crop yield are required. The application of soil microorganisms is regarded to be a promising approach in this context. Among the whole microbial population in soil, bacteria are also more effective in phosphorus solubilization (Khan et al., 2009). Therefore, studies have been focused on the inoculation of P-solubilizing bacteria (PSB) into the soil so as to increase the availability of native fixed P and applied phosphates as well as nutrients such as Fe and Zn through production of plant growth promoting substances and to reduce the use of fertilizers (Adesemoye and Kloepper, 2009). In recent years, rhizosphere associated P-solubilizing bacteria have been increasingly used in non-legume crop species such as maize, sugar cane and wheat (Sundara et al., 2002; Kumar et al., 2001; Wu et al., 2005; Jilani et al., 2007). Studies with P-solubilizing *Bacillus* species indicated yield increases in sugar beet, barley (Sahin et al., 2004), wheat and spinach (Cakmakci et al., 2007). Also, Wu et al. (2005) stated that phosphorus biofertilizers can improve plant uptake of nutrients and thereby increase the use efficiency of applied chemical fertilizers or manures. Considering in all these aspects, use of biofertilizers containing PSB is an important environmental friendly way to help plant growth and development through the enhancement of P availability.

Bacillus M-13 has been recently reported as P-solubilizing and auxin (IAA-like substances) producing bacterium (Sahin et al., 2004; Cakmakci et al., 2007). The objective of current study was to investigate the effects of *Bacillus* M-13 and phosphorus fertilizer, either alone or in combination, on seed set and filling efficiency, kernel yield and changes in accumulation of macro- (K, Ca, N, Mg and Na) and micro-nutrients (Zn, Mn, Fe, Cu) in the seeds of sunflower.

MATERIALS AND METHODS

Bacterial strain and culture conditions

The bacterial strain *Bacillus* M-13 used in current study was already present with the authors and was reported for P-solubilizing ability earlier (Sahin et al. 2004). This bacterial strain was originally isolated from pepper plants at Atatürk University and phenotypic properties were Gram (+),catalase (+), oxidase (-), pigment (-), nitrate reduction (+), starch hydrolysis (+), P solubilization (+) and growth in N-free basal medium (+) (Sahin et al., 2004). For this experiment, bacterial strain saving in nutrient broth with 15% glycerol at -80°C was incubated at 28°C for 24 h on nutrient agar. A single colony was inoculated 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 units (CFU) ml^{-1} according to MacFarland Standards and final suspensions were used for the experiment. Sunflower seeds were treated with 70% ethanol for 2 min, then 1.2% sodium hypochloride for 10 min, and rinsed ten times in sterile tap water. Sterilized seeds were then treated with the bacterial suspension for 30 min.

Experimental setup and plant analysis procedures

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. All meteorological data were collected at an official meteorological station located in the proximity of the experimental field. Long term average temperature and total precipitation in the experimental area were 9.0 °C and 545.9 mm, respectively. The average temperature during the crop cycle (from May to September) was 18.7°C in 2006 and 18.3°C in 2007, and similar to the long term average (1975-2007), which was 18.2°C (Fig. 1). Total precipitation during the crop cycle was also 91.8 and 109.0 mm in 2006 and 2007, respectively. These values were lower than the long term average, which was 118.2 mm. The soil chosen for experiment was a silt-clay-loam with pH= 7.6, high in organic matter (2.42%) and total nitrogen (0.15%), moderate in available Olsen phosphorus (7.10 mg kg^{-1}), low in $CaCO_3$ content (4.4%) and salinity (0.17%). The results were submitted as average of two years.

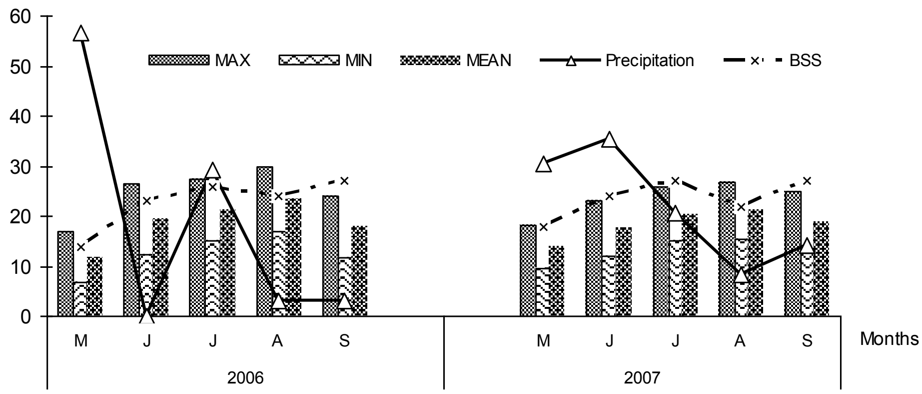

Figure 1. Monthly maximum, minimum and mean air temperatures (°C), precipitation (mm) and bright sun shine days (BSS) at Ahlat district, Turkey in 2006 and 2007.

In the study, TARSAN-1018 cultivar was selected based on its adaptation in the Eastern Anatolia region of Turkey. The experiment was arranged as a factorial design with two factors: Two levels of P-solubilizing bacteria (no application and 10^9 CFU ml^{-1}) and three levels of phosphorus (0, 50 and 100 kg P_2O_5 ha^{-1}) on the basis of a randomized complete block design in three replications. Each plot consisted of 6 rows, 4 m long with 70 cm spaced between rows. The experimental crop was sown in the second week of May in both years. Nitrogen fertilizer of 120 kg ha^{-1} was used in the form of ammonium sulphate. Nitrogen fertilizer was top dressed in two portions, half at the time of planting and the remaining half at the beginning of stem elongation. Phosphorus fertilizer was applied in the sowing as triple superphosphate. Cultural practices and irrigation were given as needed during the growth season according to the local recommendations in both years. The sunflower plants were harvested at the stage of physiological maturity 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). The middle rows excluding side rows were harvested and plot size was 8.4 m^2 at harvest. Ten randomly tagged heads from each plot were evaluated for seed setting (measured as percentage of filled seed / non-functional florets + empty seeds + filled seed according to Cantagallo et al., 2004), seed filling efficiency (measured as percentage of filled seed weight (g) / filled + empty seed weight (g) according to Killi and Altunbay, 2005) and hull percentage (measured as mass of hull / mass of seed sample x 100 according to Killi and Altunbay, 2005). Filled and empty seeds were determined on a one-eighth section of the head. Later, it was counted, weighed and dried in air-ventilated oven at 60°C to constant weight for dry mass determination. In order to determine the average size of the seeds, a sample of 30 seeds was randomly selected. For each individual seed, the two principal dimensions, namely length and width all in mm, were measured using a micrometer (least count 0.01 mm). Hereafter, the hull content was determined by manual dehulling from a sample of 10 g (with 4 replicates) taken at random from the dried seeds in each plot, expressed as percentage. The hull and kernel of seeds were individually weighed with 0.001 g accuracy. Seed kernel yield was also estimated for each plot using the formula: Seed kernel percentage x seed yield /100. The seed samples were ground with an electric coffee mill. After extraction, macro and micro mineral element concentrations were analyzed with Atomic Absorption Spectrometers (Thermo Solar AA series instrument). Chemical analyses were performed in duplicate and expressed as mg kg^{-1} on dry weight basis.

Statistical analysis

Data were evaluated by analysis of variance (ANOVA) using SAS software (SAS Institute, Cary, NC, USA). Differences among treatments were subjected to Duncan's Multiple Range test ($p<0.05$). The significantly different means were denoted by different letter. Since there was no year x treatment interaction ($p> 0.05$) for the variables (Table 1, 2 and 3), only combined analysis of variance over two years are presented.

RESULTS

Applications of PSB and P fertilizer have a significant positive effect on seed set and filling efficiency, kernel yield and concentrations of macro- (P, K, Mg, Ca and Na) and micro-nutrients (Fe, Zn, Mn and Cu) in the seeds of sunflower. Statistical analysis revealed highly significant differences for bacteria (PSB) and P fertilizer (P) terms, whereas PSB×P interaction term was non-significant for seed set and filling efficiency recorded in this study (Table 1). It was observed that seed set and filling efficiency increased consistently with increase in P fertilizer levels, while PSB application the most effective in causing increase in these parameters. The PSB+P_{100} plots showed significantly increases in seed set by 20.0% and seed filling efficiency by 1.9% as compared to that in the Control+P_0 plot. Year significantly affected seed set and filling efficiency and the second study year had higher values than the first year of the experiment (Table 1).

Table 1. Effect of P fertilization and PSB treatments on seed set, seed filling efficiency, seed dimensions, hull percentage and kernel yield of sunflower [1]

Variable	Seed setting (%)	Filling efficiency (%)	Seed length (mm)	Seed width (mm)	Hull percentage (%)	Kernel yield (t ha^{-1})
Years (Y)						
2006	84.2 b	97.6 b	12.55 b	6.77	26.5 a	3.76 b
2007	85.5 a	98.3 a	12.88 a	6.85	25.7 b	4.10 a
Mean	84.9	97.9	12.71	6.82	26.1	3.93
Phosphate-Solubilizing Bacteria (PSB)						
Control	81.7 b	97.4 b	12.68	6.72	27.3 a	3.64 b
PSB (*Bacillus* M-13)	88.0 a	98.5 a	12.76	6.91	24.8 b	4.22 a
P[2] levels (P)						
P_0 (0)	79.7 c	97.6 b	12.60	6.61 b	27.6 a	3.27 b
P_{50} (50 kg ha^{-1})	85.4 b	98.1 ab	12.72	6.78 ab	25.7 b	4.18 a
P_{100} (100 kg ha^{-1})	89.4 a	98.2 a	12.83	7.06 a	24.9 c	4.33 a
$LSD_{(0.05)}$	1.095	0.502		0.312	0.070	20.57
PSB x P levels						
Control + P_0	76.6	96.9	12.54	6.43	29.6 a	2.76 d
Control + P_{50}	81.7	97.5	12.80	6.77	26.9 b	4.11 b
Control + P_{100}	87.0	97.7	12.69	6.98	25.5 cd	4.05 bc
PSB + P_0	82.9	98.2	12.67	6.79	25.7 c	3.79 c
PSB + P_{50}	89.1	98.6	12.65	6.79	24.6 de	4.25 b
PSB + P_{100}	91.9	98.7	12.97	7.15	24.3 e	4.61 a
$LSD_{(0.05)}$					0.099	29.08
ANOVA						
Y	**	**	*	NS	*	**
PSB	**	**	NS	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
CV [3] (%)	1.52	0.60	2.97	5.40	3.17	6.18

[1] Means followed by different letter within each column are significantly different at $p<0.05$. [2] Phosphorus. [3] Coefficient of variation. *: Statistical difference at $p<0.05$, **: Statistical difference at $p< 0.01$, NS: No statistical difference at $p<0.05$ and $p<0.01$

PSB and P fertilizer applications have a significant effect on the seed hull percentage observed in this study. Analysis of variance of the data showed highly significant differences for both bacteria (PSB) and P fertilizer (P) terms for seed hull percentage. The interaction (PSB×P) term was also significant for this parameter (Table 1). Generally, the applications of PSB and P fertilizer significantly reduced the hull percentage of sunflower seeds. However, the maximum reduction in hull percentage was observed at PSB+P_{100} plots as compared to that of control plants. PSB application did not significantly affect the seed length and width, but seed width showed a significant increase in response to P application, the maximum being at P_{100} level. Independent of bacterial inoculation, phosphorus fertilizer application increased seed width up to 6.8% as compared to P_0.

PSB application without any P fertilizer increased seed kernel yield observed in this study. However, when PSB was used in conjunction with P fertilizer, a much greater effect was observed. All the treatments involving PSB+P fertilizer enhanced the seed kernel yield significantly over the P fertilizer applications alone (Table 1). Statistical analysis also revealed highly significant differences for bacteria (PSB) and P fertilizer (P) terms, so that PSB×P interaction term was significant for kernel yield in the sunflower. Combination of 50 kg P ha^{-1} dose with PSB increased kernel yield over all P fertilizer doses. However, the maximum increase in kernel yield was observed at the highest level of P fertilizer (100 kg ha^{-1}) along with PSB at which kernel yield increased more than 50% as compared to that of control plants.

Table 2. Effect of PSB and P fertilizer applications on concentrations of macro-nutrients in the sunflower seeds[1]

Variable	P (%)	K (%)	Mg (%)	Ca (%)	Na (%)
			Years (Y)		
2006	0.656 b	0.73 b	0.380 b	0.145 b	0.421 b
2007	0.717 a	1.42 a	0.597 a	0.438 a	0.833 a
Mean	0.686	1.08	0.489	0.291	0.627
			Phosphate-Solubilizing Bacteria (PSB)		
Control	0.644 b	1.05 b	0.486	0.276 b	0.603 b
PSB (*Bacillus* M-13)	0.729 a	1.10 a	0.492	0.306 a	0.650 a
			P[2] levels (P)		
P_0 (0)	0.647 c	0.92 c	0.449 c	0.246 c	0.601 b
P_{50} (50 kg ha^{-1})	0.684 b	1.00 b	0.477 b	0.282 b	0.627 ab
P_{100} (100 kg ha^{-1})	0.728 a	1.31 a	0.539 a	0.346 a	0.652 a
LSD$_{(0.05)}$	0.026	0.046	0.005	0.026	0.027
			PSB x P levels		
Control + P_0	0.603	0.90	0.434 e	0.235 b	0.606 b
Control + P_{50}	0.645	0.98	0.491 c	0.238 b	0.595 b
Control + P_{100}	0.683	1.26	0.531 b	0.354 a	0.609 b
PSB + P_0	0.692	0.94	0.464 d	0.256 b	0.596 b
PSB + P_{50}	0.723	1.02	0.463 d	0.326 a	0.659 a
PSB + P_{100}	0.772	1.36	0.548 a	0.337 a	0.695 a
LSD$_{(0.05)}$			0.007	0.037	0.038
			ANOVA		
Y	**	**	**	**	**
PSB	**	**	NS	**	**
Y x PSB	NS	NS	*	NS	NS
P	**	**	**	**	**
Y x P	NS	NS	NS	NS	NS
PSB x P	NS	NS	**	**	**
Y x PSB x P	NS	NS	NS	NS	NS
CV[3] (%)	5.33	4.88	2.55	9.13	4.06

[1] Means followed by different letter within each column are significantly different at $p<0.05$. [2] Phosphorus. [3] Coefficient of variation. *: Statistical difference at $p<0.05$, **: Statistical difference at $p< 0.01$, NS: No statistical difference at $p<0.05$ and $p<0.01$. P concentration is also published in Part I of this paper.

The concentrations of macro-nutrients (P, K, Mg, Ca and Na) in the seeds were also significantly affected by the applications of PSB and P fertilizer (Table 2). P fertilizer application significantly increased the concentrations of all macro-nutrients except for Na in the seeds. The PSB treatment was also significant for P, K, Ca and Na contents in the seeds, whereas, it was non-significant for Mg contents in seeds. It was observed that the concentrations of P, K, Ca and Na in the seeds increased consistently with increase in P fertilizer doses, when applied in conjunction with PSB. Although seed Mg contents were also increased markedly due to P fertilizer, it was not much affected by PSB application at lower level of P fertilizer. The interaction (PSB×P) term was significant for concentrations of Mg, Ca and Na in seeds, whereas, it was non-significant for P and K contents in the seeds (Table 2). However, the maximum increase in concentrations of K, P and Mg in the seeds was observed at the highest level of P fertilizer (100 kg ha^{-1}) along with PSB by 51.1, 28.0 and 26.3% as compared to that of control plants, respectively.

Phosphorus fertilizer and PSB applications significantly increased the concentrations of micro-nutrients (Fe, Zn, Mn and Cu) in the seeds of sunflower plant (Table 3). For P fertilizer (P) term, the increase was significant for Fe, Zn, Mn and Cu contents in the seeds, while differences between P fertilizer levels were non-significant for seed Fe concentration. The bacteria (PSB) term was significantly different for concentrations of all these micro-nutrients (Fe, Zn, Mn and Cu) in the seeds. The PSB×P interaction term was non-significant for only Zn content (Table 3). PSB application with P fertilizer increased concentrations of Zn, Mn and Cu in the seeds while a marked reduction in seed Fe contents was observed in PSB application. It was noted that maximum increase in concentrations of Zn, Mn and Cu in the seeds was observed at the highest level of P fertilizer (100 kg ha^{-1}) with PSB by 40.5, 21.7 and 24.7% as compared to that of control plants, respectively. However, the maximum increase in concentration of Fe in the seeds was observed at the 100 kg P ha^{-1} fertilized soil without PSB by 41.5% as compared to control (Table 3).

Table 3. Effect of PSB and P fertilizer applications on concentrations of micro-nutrients in the sunflower seeds[1]

Variable	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
	Years (Y)			
2006	63.13 b	64.19 b	29.00 b	16.88 b
2007	85.52 a	84.65 a	48.60 a	36.04 a
Mean	74.33	74.42	38.80	26.46
	Phosphate-Solubilizing Bacteria (PSB)			
Control	73.13 b	71.65 b	37.19 b	25.19 b
PSB (*Bacillus* M-13)	75.52 a	77.19 a	40.42 a	27.73 a
	P[2] levels (P)			
P_0 (0)	65.29 b	66.01 c	35.72 c	25.65 b
P_{50} (50 kg ha^{-1})	78.21 a	7238 b	38.39 b	26.62 ab
P_{100} (100 kg ha^{-1})	79.48 a	84.87 a	42.30 a	27.12 a
$LSD_{(0.05)}$	2.86	3.61	1.36	1.01
	PSB x P levels			
Control + P_0	57.63 d	61.07	36.27 c	23.42 e
Control + P_{50}	80.25 ab	69.93	34.82 c	27.10 bc
Control + P_{100}	81.52 a	83.94	40.46 b	25.05 d
PSB + P_0	72.95 c	70.95	35.17 c	27.87 ab
PSB + P_{50}	76.16 bc	74.83	41.96 b	26.13 cd
PSB + P_{100}	77.44 ab	85.80	44.13 a	29.20 a
$LSD_{(0.05)}$	4.05		1.93	1.43
	ANOVA			
Y	**	**	**	**
PSB	*	**	**	**
Y x PSB	NS	NS	NS	NS
P	**	**	**	*
Y x P	NS	NS	NS	NS
PSB x P	**	NS	**	**
Y x PSB x P	NS	NS	NS	NS
CV[3] (%)	4.55	5.72	4.15	4.51

[1] Means followed by different letter within each column are significantly different at p<0.05. [2] Phosphorus. [3] Coefficient of variation. *: Statistical difference at p<0.05, **: Statistical difference at p< 0.01, NS: No statistical difference at p<0.05 and p<0.01

DISCUSSION

Biomass or seed accumulation in the sunflower is closely correlated with nutrient uptake throughout its life span. P fertilization enhances not only yields, but also affects the seed chemical composition of the sunflower plant (Gunes and Inal, 2009; Amanullah and Khan, 2010). Phosphorus fertilizer and P-solubilizing bacteria had a significant effect on seed set, seed filling efficiency and kernel yield as well as all chemical content of sunflower seed in this study. However, when P-solubilizing bacteria used in conjunction with P fertilizer, a much greater effects were observed. The mean seed set and kernel yield of the treatments involving PSB were 88.0% and 4.22 t ha^{-1}, respectively, as opposed to 81.7% and 3.64 t ha^{-1} in treatments without PSB. Thus the mean increases in seed set and kernel yield by PSB application were 7.7 and 15.9%, respectively. Important result of this study is also that PSB application increased the seed setting and kernel yield over all P fertilizer doses and thereby may be reduced the amount of applied P. This is apparent from the similar seed set and kernel yield in those treatments where the highest dose of P was applied and in treatments where the lowest dose of P was applied but PSB was supplied. This may be related to increased PSB activity in the rhizosphere following PSB application and consequently by enhanced P solubilization. Results of several studies have also demonstrated that PSB inoculation increased the efficiency of native and applied P_2O_5 by reducing phosphate fixation in soil fractions and those microbial inoculants can be used as an economic input to increase crop productivity with lower fertilizer levels (Whitelaw et al., 1997; Kumar et al., 2001; Dasci et al., 2010). Likewise, Cakmakci et al. (2007) reported that seed inoculation of phosphate solubilizing *Bacillus* M-13 increased nutrient concentration and uptake by wheat and spinach plants, which lead towards luxurious growth and better crop development. In general, effects of PSB application on investigated parameters of sunflower were lower in the first year due to relatively dry conditions prevailed than in second experimental year. Greater performance of the bacteria used in the study under relatively wetter conditions prevailed in the second year may derive from the suggestion that the phosphate solubilizing activity of free-living bacteria may strongly dependent on favorable moisture and temperature conditions (Sahin et al., 2004).

PSB application had no significant effect on the seed length and width, but led to a reduction in the hull content of the seed. Phosphorus fertilizer significantly also increased seed width but decreased hull content. The hull content of seed is closely related to the cultivar, seed size and oil content of kernel. In general, the varieties with high oil content have low hull percentage because of the negative relationship between the hull content and oil content of seed found in the other studies (Connor and Hall, 1997; Dagustu, 2002). It is well known that the most of the oil is accumulated in the kernel (shelled seed) and the hull decreases the oil recovery and reduces food value of the de-oiled meal (Sharma et al., 2009). For this reason, an increase in the kernel oil content by declining hull content of seed is generally desired. Positive effects of P on oil content of seed have also been proposed in sunflower (Blamey and Chapman, 1981). Zubillaga et al. (2002) reported that increased availability of phosphorus facilities better nitrogen and other nutrients utilization, which extended growth period and thus better sunflower crop growth, besides improving oil yield. The reason for lower hull content in PSB application may be related to complementary effect of enhanced phosphate availability (Cakmakci et al., 2007) and consequently increased seed oil content (detailed in the first part of this article).

The uptake or accumulation of the macronutrients like N, P and K is the direct reflection of the biomass production. In the sunflower, seeds accumulated 79% of the mature plant's P, 18–31% of its Mg and K, but only 5–7% of its Ca and Cl (Hocking and Ster, 1983). The result of this experiment revealed that combination of P fertilizer with PSB increased the concentrations of P, K and Mg nutrient elements in sunflower seeds. Phosphorus concentration was highest under PSB+P_{100} treatment, even better than control and P fertilizer alone. These results indicated that availability of P in the soil as a consequence of applying P fertilizer can be increased by inoculation with PSB. Whitelaw et al. (1997) reported that inoculation of P-solubilizing bacteria along with P fertilizers was expected to improve the P nutrition of plants. The other macro nutrients which were observed in this study showed significant fluctuations in response to the various treatments. The potassium concentration of seed was highest at high P treatments with or without PSB application. Magnesium, calcium and sodium concentrations were generally higher in PSB application than P application alone. In general, PSB application alone significantly and/or relatively increased the micro nutrient element concentrations (except for iron) of seed over half dose of P fertilizer. This may be due to nutrient availability affected by P fertilization and activity of PSB which stimulate the availability of nutrient elements. The results are supported by Gunes and Inal (2009) who stated that P supply positively also affect the uptake of nutrients (except for iron and zinc) in sunflower. Jilani et al. (2007) observed that combination of chemical fertilizers with biofertilizer increased the nutrient concentrations in maize crop. However, additional researches are needed to clarify the mechanisms involved with each of the nutrients.

In conclusion, the current study indicate that inoculation of P-solubilizing *Bacillus* M-13 and phosphorus fertilizer applications effectively promoted seed set and filling, kernel yield and seed nutrients composition of sunflower plants. Contrary, the seed hull contents decreased in PSB treated plants, which shows that this bacterial strain may play favorable role in seed quality and oil recovery. However, the performance of PSB treatments without fertilizer was more or less close to the solely P fertilizer treatments in terms of sunflower seed set, seed filling, kernel yield and concentration of P in the seed. Among all the treatments studied, the PSB treatment with 100 kg P_2O_5 ha^{-1} fertilizer maintained the highest sunflower seed kernel yield, while 50 kg P_2O_5 ha^{-1} can be used to improve seed quality if applied with PSB. It is also a sustainable crop production technology. As such, this bacterial strain may be used alone or in combination with P fertilizer for higher seed quality and yield in sunflower.

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