



**Full Length Article**

## Evaluating Role of Plant Growth Promoting Rhizobacteria for Improving Phosphorus use Efficiency and Productivity in Sunflower (*Helianthus annuus*)

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### Abstract

Intensive use of chemical fertilizers has produced environmental, health and agricultural hazards. Microbial inoculation as substitute to chemical fertilizers could be an effective way for sustainable sunflower production. Therefore, a field study was performed using RCBD with three replications for two consecutive years (2012 and 2013) at the Agronomic Research Area, University of Agriculture, Faisalabad, to evaluate role of plant growth promoting rhizobacteria (PGPR) for improving phosphorus use efficiency and productivity in sunflower. Sunflower hybrid (Hysun-33) was subjected to eleven treatments with different combinations of PGPR seed inoculation and phosphorus fertilizer levels viz. Control, Recommended P (100%), 100% P + *Bacillus* inoculation, 75% P + *Bacillus* inoculation, 50% P + *Bacillus* inoculation, 100% P + *Pseudomonas* inoculation, 75% P + *Pseudomonas* inoculation, 50% P + *Pseudomonas* inoculation, 100% P + Dual inoculation, 75% P + Dual inoculation, 50% P + Dual inoculation. The results depicted that both inoculants and their mixture with different phosphorus rates showed an increase in yield attributes, nutrients uptake, BCR and phosphorus use efficiency. Highest head diameter, number of achenes per head, 1000-achene weight, achene yield, achene and stover nitrogen content, achene and stover phosphorus content and BCR was obtained with 100% P + dual inoculation. However, 50% phosphorus + dual inoculation produced statistically same results as with recommended phosphorus (100%) while, phosphorus use efficiency and phosphorus recovery efficiency was found maximum, where 50% phosphorus was applied in combination with dual inoculation. The study suggested that plant growth promoting rhizobacteria are budding component for improving phosphorus use efficiency and productivity of sunflower. © 2016 Friends Science Publishers

**Keywords:** PGPR; Spring sunflower; Phosphorus use efficiency; Productivity

### Introduction

In Pakistan sunflower is one of the major oilseed crop grown for production of edible oils. It ranks second as a source of domestic oil production after cotton contributing about 16%. The area under cultivation is 0.485 million acres with the total seed production of 0.244 million tons and edible oil production of 0.095 million tons (GOP, 2013). Average yield of sunflower in Pakistan is far below than the genetic potential of various varieties under cultivation. Crop yield and its contributing factors are stressed by variety of factors, such as imbalance nutrition, irrigation water insufficiency, pitiable soil fertility, saline soils, attack of diseases and insects, delay and conventional sowing methods. The unfair plant nutrient use confines the soil production potential and do not allow farmers to utilize full prospective of sunflower crop in Pakistan. So improved management of plant nutrition is, therefore, necessary for obtaining complete prospective of sunflower crop (Gehl *et al.*, 2005).

Phosphorus is second most key macronutrient after nitrogen required by the plants. But it is provided to plants chiefly in form of chemical fertilizers. Heavy use of chemical fertilizers to enhance soil fertility and crop productivity has possessed environmental, health and agricultural hazards beside their higher cost of application (Steinshamn *et al.*, 2004). Phosphorus fertilizers when applied to the soil becomes inert due to precipitation effect with highly reactive  $Al^{3+}$  and  $Fe^{3+}$  in acidic, and  $Ca^{2+}$  in normal or calcareous soils (Gyaneshwar *et al.*, 2002). Due to which efficiency of fertilizer phosphorus is often as low as 25% (Isherword, 1998). The challenge, therefore, is to continue agricultural productivity in a way that minimizes harmful environmental effects of fertilizers, besides from lowering the production cost and harvesting more yields. Microbial (PGPR) inoculation as substitute to chemical fertilizers is a low cost and environmental affable technology that can trim down the reliance on synthetic resources and can improve crop yield (Canbolat *et al.*, 2006).

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that colonize roots of plant actively and increase plant growth and development (Wu *et al.*, 2005). Different bacteria that have been reported as PGPR belong to the genera *Pseudomonas*, *Bacillus*, *Azospirillum*, *Agrobacterium*, *Azotobacter*, *Rhizobium*, *Enterobacter* and *Phyllobacterium*. Among these *Pseudomonas*, *Azospirillum*, and *Bacillus* are the most widely reported PGPR, and considerably enhances growth and yield of agronomical crops (Bashan *et al.*, 2004). The inoculated bacteria have induced beneficial effects on plant growth and yield by the production of growth promoting substances, improving water and nutrients uptake, which have limited availability in soil such as P, N and micronutrients, producing B-group vitamins and antibiotic metabolites that promote rooting capacity and control the population of soil born pathogenic microbial community (Mostafa and Abo-Baker, 2010). Keeping this in view, selection of effective plant growth promoting rhizobacteria for specific oilseed crop and site is a critical aspect in Pakistan. Hence a study was conducted on the potential use of PGPR for improving phosphorus use efficiency and sunflower productivity under environmental conditions of Faisalabad.

## Materials and Methods

The research study was conducted for two consecutive years (2012 and 2013) at the Agronomic Research Area, University of Agriculture, Faisalabad. The experimental area is located at 73° East longitude, 31° North latitude and at an altitude of 135 meters above sea level. Soil samples were taken before sowing of the crop in each season for its physico-chemical analysis (Table 1).

Meteorological data as mean monthly temperature, rainfall and humidity for the both cropping seasons (2012 and 2013) were collected from the meteorological observatory of the Department of Crop Physiology, University of Agriculture, Faisalabad and are shown in Fig 1.

Experiment was comprised of eleven treatments viz.

T<sub>1</sub> = Control (without P)

T<sub>2</sub> = Recommended P (100%) (57 kg ha<sup>-1</sup>)

T<sub>3</sub> = 100% of recommended P + (*Bacillus*) seed inoculation

T<sub>4</sub> = 75% of recommended P + (*Bacillus*) seed inoculation

T<sub>5</sub> = 50% of recommended P + (*Bacillus*) seed inoculation

T<sub>6</sub> = 100% of recommended P + (*Pseudomonas*) seed inoculation

T<sub>7</sub> = 75% of recommended P + (*Pseudomonas*) seed inoculation

T<sub>8</sub> = 50% of recommended P + (*Pseudomonas*) seed inoculation

T<sub>9</sub> = 100% of recommended P + \*(*Bacillus* + *Pseudomonas*) seed inoculation

T<sub>10</sub> = 75% of recommended P + \*(*Bacillus* + *Pseudomonas*) seed inoculation

T<sub>11</sub> = 50% of recommended P + \*(*Bacillus* + *Pseudomonas*) seed inoculation

\*(*Bacillus* + *Pseudomonas*) = Dual inoculation

Treatments were carried out in randomized complete block design (RCBD) with three replications. Sunflower hybrid (Hysun-33) was sown in 2<sup>nd</sup> week of February, during both the years as a test crop using seed rate of 6 kg ha<sup>-1</sup> in the pattern of 75 cm apart single rows with the help of dibbler maintaining plant to plant distance of 20 cm. For inoculation prepared inocula of respective rhizobacterial species were added into sterilized peat of an appropriate proportion (100 mL kg<sup>-1</sup> peat) and seed to peat ratio was kept 5:1 (w/w). 50 mL sticking agent in the form of 10% sterilized sugar solution was also used. Inoculated peat was thoroughly mixed to carry out procedural inoculation for sunflower seeds. Control remained untreated as the seeds were coated with cementing suspension but without inocula. Nitrogen and potassium were applied at the rate of 110 kg ha<sup>-1</sup> and 62 kg ha<sup>-1</sup> in the form of urea and SOP respectively. While, phosphorus was applied in the form of SSP according to the treatments. Whole of the phosphorous, potash and 1/2 of the nitrogen was applied at sowing while remaining dose of nitrogen was applied in two splits, 1/4 at 1<sup>st</sup> irrigation and 1/4 at flowering. Similarly, under good crop management, crop was kept free of weeds by hand hoeing as required to avoid competition between weeds and sunflower crop.

Standard procedures were followed to collect the data for yield parameters. Diameter of 10 randomly picked heads were measured in cm with the help of a measuring tape and then averaged and then from the same 10 heads, number of achenes were counted and averaged per head after manual threshing. Similarly, from the seed lot of every plot, five samples, each of 1000-achenes were randomly taken, recorded their weight and then mean 1000-achene weight was computed. The plot yield for each treatment was calculated at 10% seed moisture content accordingly and averaged and then converted to kg ha<sup>-1</sup>. N content was determined by the Kjeldahl method (Bremner, 1965). For P determination (Method 61, Salinity Laboratory Staff, 1954) was followed and phosphorus use efficiency (PUE) was calculated as suggested by Montemurro and Giorgio, (2005).

$$PUE (kg kg^{-1}) = (G_f - G_u / N_a)$$

Where, G<sub>f</sub> is the grain yield of the fertilized plot (kg), G<sub>u</sub> is the grain yield of the unfertilized plot (kg) and N<sub>a</sub> is the quantity of nutrient applied (kg).

Phosphorus recovery efficiency was computed by using the formulae given by Fageria *et al.* (2008).

$$PRE (\%) = (N_f - N_u / N_a) \times 100$$

Where, N<sub>f</sub> is the nutrient uptake of the fertilized plot (kg), N<sub>u</sub> is the nutrient uptake of the unfertilized plot (kg) and N<sub>a</sub> is the quantity of nutrient applied (kg).

Economic analysis was carried out by using the methodology described in CIMMYT (1988). Net benefit and benefit cost ratio were calculated. Net benefits were calculated by subtracting the total variable cost from the gross income for each treatment combination. Benefit cost ratio was calculated for each treatment by the following formula:

$$\text{BCR} = \text{Gross income} / \text{Total cost}$$

Data recorded for both years were statistically analyzed by using the Fisher's analysis of variance technique and LSD test at 5% probability was used to compare the differences among treatments' means (Steel *et al.*, 1997).

## Results

Data (Table 2) displays significant effect of different PGPR inoculation and chemical Phosphorus rates on head diameter. In the first year (2012) the largest head diameter (19.30 cm) was noted in T<sub>9</sub> (100% P + dual inoculation) which was significantly different as compared to other treatments, and was followed by T<sub>10</sub> (75% P + dual inoculation) with head diameter of 18.83 cm. However smallest heads in diameter (12.20 cm) were noted in T<sub>1</sub> (control) treatment where no inoculation and chemical phosphorus was added. Similar trend regarding head diameter was noted in second year (2013). Year effect was also significant and head diameter was larger in 2012 than 2013.

The productive potential of a head is measured in terms of number of achenes per head. It is one of the most important yield components and has direct bearing on final achene yield of sunflower. In 2012 (Table 2) significantly greater number of achenes (954) per head was counted in sunflower from the treatment (T<sub>9</sub>), where 100% phosphorus was applied with dual inoculation. This was followed by T<sub>10</sub> (75% P + dual inoculation) and T<sub>6</sub> (100% P + *Pseudomonas* inoculation) which however, were statistically same with each by giving (915 and 888) number of achenes, respectively. The lowest number of achenes per head (610) was counted in plots where no inoculation and chemical phosphorus was added (T<sub>1</sub>). Parallel data fashion was observed in 2013. While the comparison of both years' values depicts that year had non-significant influence on achene numbers per head.

A 1000-achene weight has a key role in defining the yield potential of a seed crop, as it expresses the extent of seed development. Data (Table 2) shows that the treatments under study made significant effect on the parameter under discussion during both growing seasons. In each seasons, the heaviest 1000 achene in weight (54.01 and 52.62 g) was recorded in treatment T<sub>9</sub> (100% P + dual inoculation). It was followed by T<sub>10</sub> (75% P + dual inoculation) and T<sub>6</sub> (100% P + *Pseudomonas* inoculation) respectively. However, the difference among these two (T<sub>10</sub> and T<sub>6</sub>) was also

significant. Whereas, 1000 achene in T<sub>1</sub> (control) was found least in weight (39.13 and 38.00 g) during both seasons. Whereas, year effect was also found significant with heavier 1000 achenes in first year (2012) than the following year (2013).

Results pertaining to achene yield in response to different PGPR inoculation and chemical phosphorus rates are presented in table (2). During both cropping periods achene yield was affected significantly by the applied treatments. The application of 100% P with dual inoculation (T<sub>9</sub>) in both cropping periods (2012 and 2013) produced the maximum achene yield (3190 and 3027 kg ha<sup>-1</sup>), lagging behind T<sub>10</sub> (75% P + dual inoculation) which was also significantly different against rest of the treatments by producing (2970 and 2830 kg ha<sup>-1</sup>) achene yield. While the plots where no inoculation and chemical phosphorus was applied (T<sub>1</sub>), provided the minimum achene yield (1618 and 1570 kg ha<sup>-1</sup>) in each cropping period. Among different inoculation sources, dual inoculation with different phosphorus rates significantly increased achene yield over all single inoculation and phosphorus rates. However, among single inoculation treatments, *Pseudomonas* performed better than *Bacillus*, but they did not show significant difference. However, 2012 was recorded significantly more productive by giving higher yield than 2013.

Achene nitrogen content is given in Table (3), which shows that for the growing season of both years (2011 and 2012) the influence of various PGPR inoculation and chemical phosphorus rates on achene nitrogen content was remained significant. In the first year of trial (2012), the highest nitrogen content in achene (3.15%) was accumulated in plants of the treatment T<sub>9</sub> (100% P + dual inoculation) which were found statistically at par with T<sub>10</sub> (75% P + dual inoculation) with achene nitrogen content of 3.09%. While, the least nitrogen content in achene (2.59%) was recorded in plots where no inoculation and chemical phosphorus was applied (T<sub>1</sub>). Results obtained in 2013 showed similar trend to that observed during 2012. Year effect on achene nitrogen content was non-significant. However its value was slightly greater in 2012 than in 2013.

Stover nitrogen content was significantly affected by different PGPR inoculation and chemical nitrogen rates in both years of experimentation i.e., 2012 and 2013 (Table 3). In the results of first period research (2012) maximum stover nitrogen content (0.742%) was given by treatment (T<sub>9</sub>), where recommended nitrogen was applied with dual inoculation, but it did not vary significantly with T<sub>10</sub> (75% P + dual inoculation) treatment (0.720%). However, minimum stover nitrogen content (0.590%) was produced by (T<sub>1</sub>) treatment in which neither inoculation nor chemical nitrogen was applied. Same trend was also noted in the results of second period research (2013). The year also had considerable influence by recording greater stover nitrogen content in first season (2012) than in second season (2013).

**Table 1:** Physico-chemical analysis of soil

Characteristics	Saturation (%)	pH	EC (dSm-1)	Organic matter (%)	Total nitrogen (%)	Available P (ppm)	Available K (ppm)
2012	39	7.8	1.37	0.83	0.042	7.9	140
2013	37	7.7	1.3	0.80	0.046	8.4	142

**Table 2:** Effect of PGPR inoculation and chemical phosphorus rates on yield attributes of sunflower

Treatments	Head diameter (cm)		Number of achenes per head		1000- achene weight (g)		Achene yield (kg ha <sup>-1</sup> )	
	2012	2013	2012	2013	2012	2013	2012	2013
T <sub>1</sub>	12.20 h	11.83 h	610 i	586 i	39.13 h	38.00 g	1618 h	1570 h
T <sub>2</sub>	17.30 e	16.76 de	810 de	786 e	48.52 e	47.23 d	2603 e	2497 e
T <sub>3</sub>	18.03 cd	17.55 c	870 c	839 cd	51.40 c	50.20 c	2793 cd	2671 cd
T <sub>4</sub>	16.66 f	16.12 f	771 fg	745 fg	47.02 f	45.56 e	2471 f	2369 f
T <sub>5</sub>	15.60 g	15.23 g	739 h	700 h	45.60 g	44.30 f	2223 g	2121 g
T <sub>6</sub>	18.30 c	17.79 bc	888 bc	859 bc	51.70 c	50.56 c	2825 c	2701 c
T <sub>7</sub>	16.83 f	16.30 ef	785 ef	757 f	47.44 f	46.16 e	2486 f	2394 f
T <sub>8</sub>	15.86 g	15.42 g	750 gh	719 gh	46.92 f	44.57 f	2240 g	2139 g
T <sub>9</sub>	19.30 a	18.82 a	954 a	916 a	54.01 a	52.62 a	3190 a	3027 a
T <sub>10</sub>	18.83 b	18.27 b	915 b	884 b	52.82 b	51.56 b	2970 b	2830 b
T <sub>11</sub>	17.60 de	17.02 d	820 d	816 d	49.51 d	48.12 d	2680 de	2580 de
LSD 5%	0.44	0.52	30.54	27.60	0.94	0.98	116.22	97.12
Year means	16.96 a	16.47 b	810	783	48.55 a	47.17 b	2555 a	2446 b
LSD 5%	0.14		NS		0.27		31.30	

Means not sharing common letters in column differ significantly at 5% probability

NS=Non-significant

**Table 3:** Effect of PGPR inoculation and chemical phosphorus rates on nutrients uptake in sunflower

Treatments	Achene N content (%)		Stover N content (%)		Achene P content (%)		Stover P content (%)	
	2012	2013	2012	2013	2012	2013	2012	2013
T <sub>1</sub>	2.59 h	2.51 h	0.590 h	0.571 g	0.362 h	0.345 h	0.220 h	0.216 g
T <sub>2</sub>	3.00 cde	2.97 cde	0.696 cde	0.684 bcd	0.596 e	0.568 e	0.301 cd	0.294 c
T <sub>3</sub>	3.06 bc	3.02 bc	0.709 bcd	0.693 bc	0.629 cd	0.600 cd	0.331 b	0.320 b
T <sub>4</sub>	2.94 ef	2.92 ef	0.679 ef	0.668 de	0.565 f	0.536 f	0.280 ef	0.266 de
T <sub>5</sub>	2.87 g	2.84 g	0.654 g	0.641 f	0.531 g	0.503 g	0.257 g	0.248 f
T <sub>6</sub>	3.08 b	3.04 b	0.715 bc	0.700 b	0.637 bc	0.608 bc	0.342 ab	0.329 ab
T <sub>7</sub>	2.97 de	2.93 de	0.686 de	0.675 cd	0.572 f	0.546 f	0.287 de	0.276 d
T <sub>8</sub>	2.89 fg	2.86 fg	0.660 fg	0.647 ef	0.538 g	0.514 g	0.269 fg	0.254 ef
T <sub>9</sub>	3.15 a	3.11 a	0.742 a	0.726 a	0.675 a	0.640 a	0.357 a	0.345 a
T <sub>10</sub>	3.09 ab	3.06 ab	0.720 ab	0.706 ab	0.658 ab	0.624 ab	0.347 ab	0.336 ab
T <sub>11</sub>	3.01 cd	2.97 cd	0.703 bcd	0.687 bcd	0.610 de	0.580 de	0.310 c	0.300 c
LSD 5%	0.06	0.05	0.0233	0.0236	0.023	0.021	0.0172	0.0166
Year means	2.97	2.93	0.687 a	0.673 b	0.580	0.551	0.300	0.289
LSD 5%	NS		0.006		NS		NS	

Means not sharing common letters in column differ significantly at 5% probability

NS=Non-significant

Regarding achene phosphorus content (Table 3) in each year, T<sub>9</sub> (100% P + dual inoculation) treatment significantly improved achene phosphorus content (0.675 and 0.640%) in comparison to all other treatments, except that of T<sub>10</sub> (75% P + dual inoculation) with which the difference was statistically non-significant in each year. The minimum phosphorus content in achene (0.362 and 0.345%) were achieved by T<sub>1</sub> (control) treatment. Regarding year effect, more phosphorus content in achene of sunflower was found in 2012 than 2013, but difference among these did not reach at significant level.

The results pertaining to stover phosphorus content (Table 3) presented that in first growing period (2012), the maximum phosphorus content (0.357%) in stover was obtained in plants of T<sub>9</sub> treatment (100% P + dual inoculation). It was trailed by T<sub>10</sub> (75% P + dual

inoculation) and T<sub>6</sub> (100% P + *Pseudomonas* inoculation) treatments which however, were statistically similar with T<sub>9</sub>. While, the minimum phosphorus content in stover (0.220%) was obtained in T<sub>1</sub> (control) treatment. Almost similar trend was revealed in second growing period (2013), while year effect on stover phosphorus was also found non-significant.

Phosphorus use efficiency (Fig. 2) displays that for the growing season of both years (2012 and 2013), treatment T<sub>11</sub> (50% P + dual inoculation) significantly improved phosphorus use efficiency (37.26 and 35.46 kg kg<sup>-1</sup>) as related to all other treatments. This was trailed by T<sub>10</sub> (75% P + dual inoculation), which improved phosphorus use efficiency compared to remaining treatments in each cropping season with giving values (31.63 and 29.49 kg kg<sup>-1</sup>) respectively. However, the least phosphorus use efficiency (17.29 and 16.27 kg kg<sup>-1</sup>) in both cropping

**Table 4:** Effect of PGPR inoculation and chemical phosphorus rates on net income and benefit cost ratio (BCR) of sunflower

Treatments	G.I (Rs. ha <sup>-1</sup> )	T.E (Rs. ha <sup>-1</sup> )	N.I (Rs. ha <sup>-1</sup> )	BCR	G.I (Rs. ha <sup>-1</sup> )	T.E (Rs. ha <sup>-1</sup> )	N.I (Rs. ha <sup>-1</sup> )	BCR
	2012				2013			
T <sub>1</sub>	80900	57776	23124	1.39	78500	57776	20724	1.35
T <sub>2</sub>	130167	63473	66694	2.05	124883	63473	61410	1.96
T <sub>3</sub>	139667	63573	76094	2.20	133550	63573	69977	2.10
T <sub>4</sub>	123583	61881	61702	2.00	118633	61881	56752	1.92
T <sub>5</sub>	111167	60729	50438	1.83	106050	60729	45321	1.75
T <sub>6</sub>	141250	63573	77677	2.22	135083	63573	71510	2.12
T <sub>7</sub>	124333	61881	62452	2.01	119733	61881	57852	1.93
T <sub>8</sub>	112000	60729	51271	1.84	106950	60729	46221	1.76
T <sub>9</sub>	159500	63573	95927	2.51	151350	63573	87777	2.38
T <sub>10</sub>	148500	61881	86619	2.40	141533	61881	79652	2.29
T <sub>11</sub>	134000	60729	73271	2.21	129033	60729	68304	2.12

G.I= Gross income, T.E=Total expenditure, N.I=Net income, BCR=Benefit cost ratio

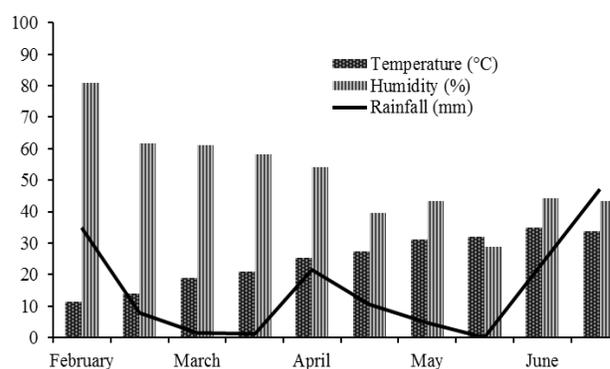
seasons was given by (T<sub>2</sub>) where recommended phosphorus was applied without any inoculation. The year also had considerable influence by recording greater phosphorus use efficiency in first season (2012) than in second season (2013).

The result presented in (Fig. 3) depicts that various PGPR inoculation and chemical use efficiency (Fig. 2) displays that for the growing season of both years rates had significant effect on phosphorus recovery efficiency during both growing periods. In first growing period (2012), the maximum phosphorus recovery (69.35%) was obtained by the plots of treatment T<sub>11</sub> (50% P + dual inoculation) against rest of the treatments. It was trailed by T<sub>10</sub> (75% P + dual inoculation) and T<sub>9</sub> (100% P + dual inoculation) which however, were statistically identical among themselves during 2012 but different in 2013. While, the minimum phosphorus recovery (35.61%) was obtained by the plots of treatment T<sub>2</sub> recommended phosphorus was applied without inoculation.

The economic analysis for sunflower production under local circumstances of Faisalabad, Pakistan is exhibited in Table 4. Different treatments of inoculation sources and chemical phosphorus rates resulted in diverse net income (Rs. ha<sup>-1</sup>). Treatment T<sub>9</sub> resulted in highest net income of Rs. 95927 and 87777 per hectare during 2012 and 2013, respectively. In 2012, the uppermost benefit cost ratio of 2.51 was also obtained in T<sub>9</sub> where 100% phosphorus + dual inoculation were applied and it ranged from 1.39 to 2.40 among other treatments. However, for growing period of 2013, T<sub>9</sub> gained relatively lesser benefit cost ratio (2.38) than in 2012 and it ranged from 1.35 to 2.29 among other treatments.

## Discussion

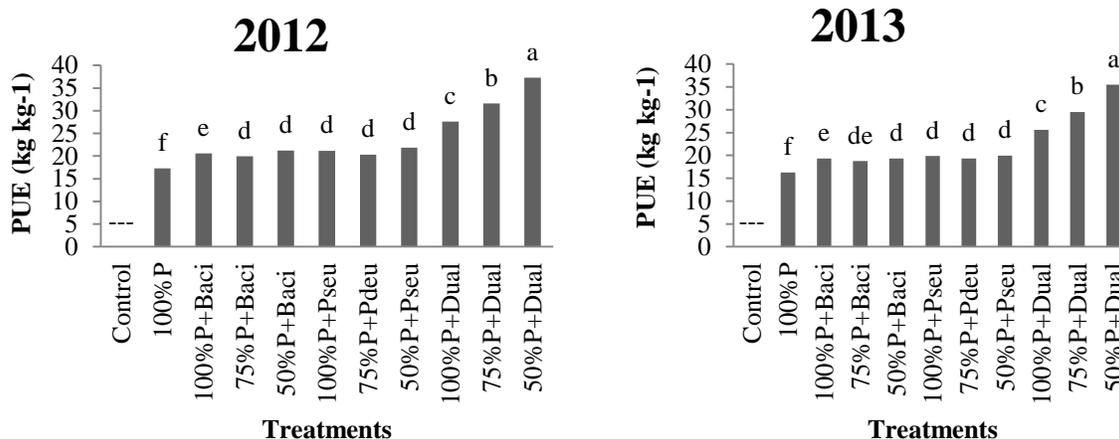
The results of spring grown sunflower designated that integrated application of nominated bacterial isolates with different chemical phosphorus fertilizer rates significantly promoted the plant growth, yield and nutrients uptake aspects. Evaluation among the single inoculation of PGPR exposed that rhizobacterial isolate *Pseudomonas*



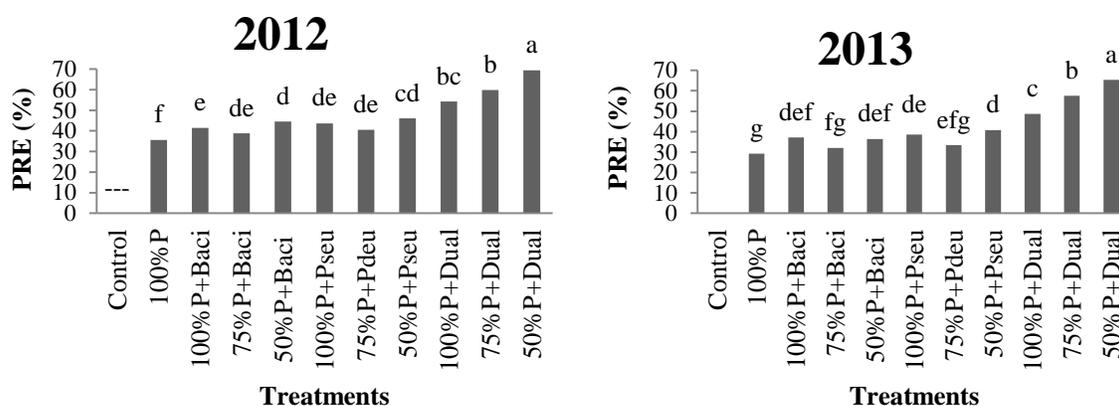
**Fig. 1:** Meteorological data showing mean monthly temperature, humidity and rainfall during growing seasons (2012 and 2013) of sunflower

performed better than *Bacillus* under field condition for respective traits through collaboration with chemical phosphorus fertilizer, though the difference among themselves was non-significant for most of the parameters. Differential specificity of the individual bacterial isolate might be expressed by numerous features like competition, capability of bacteria to colonize, solubilizing ability of insoluble soil P, soil characteristics, climatic conditions and indigenous micro flora existing in root zone (Khalid *et al.*, 2004; Kannan *et al.*, 2005; Lalfakzual *et al.*, 2008).

However all parameters were united for more pronounced improvement by mixtures of both inoculants. Increased plant growth following co-inoculation was might be due to the synergistic influence of both bacteria. Combine inoculations have a greater success rate and it has been found (Khammas and Kaiser, 1992) that these microorganisms collaborate synergistically by providing nutrients, eliminating some inhibitory products, or exciting each other by physical or biochemical mechanisms and looked like that in co-inoculation nutrition is more balanced and the adsorption of N, P and other mineral nutrients is expressively improved, yielding a better crop. It is very likely that phosphate solubilizing action of the nominated



**Fig. 2:** Effect of PGPR inoculation and chemical phosphorus rates on phosphorus use efficiency (PUE) of sunflower during 2012 and 2013



**Fig. 3:** Effect of PGPR inoculation and chemical phosphorus rates on phosphorus recovery efficiency (PRE) of sunflower during 2012 and 2013

strains improved the P discharge from complexes of insoluble P. This statement is supported by the data of P content in achene and stover as P content and uptake were also the maximum where seeds were inoculated with these dual PGPR. Joe and Sivakumaar (2009) also established that the dual inoculation was superior in positively boosting the growth (plant height, head diameter and dry matter production) and yield (no of seeds head<sup>-1</sup>, stalk yield and achene yield) parameters of sunflower crop. Several other studies (Acharya *et al.*, 1999; Selvakumari *et al.*, 2000) also proposed the optimistic influence of co-inoculation in augmenting the growth, yield and nutrients uptake of different crops.

Significant improvement in achene yield and BCR

was recorded in response to rhizobacterial inoculation along with 100% P level. Adding P enhances the photosynthetic activity in plants which might increase the ability of plant to produce higher head diameter, number of grains and their weight that eventually resulted in high grain and biological yield (Namvar *et al.*, 2012). Sharma and Prasad (2003) recorded that the phosphate-solubilizing bacteria, when inoculated in conjunction with phosphorus fertilization, effectively enriched grain, straw yield and NPK uptake of rice-wheat cropping system. Gupta *et al.* (2009) also found that grains yield, straw yield, nitrogen and phosphorus uptake were recorded highest with application of 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> along with *Bacillus polymyxa* and *B. megaterium*.

The escalation in the yield components and achene yield in the PGPR-inoculated treatments could also be attributed to plant growth regulators (PGRs), such as auxins (Fallik *et al.*, 1989) and gibberellin (Lucangeli and Bottini, 1997) exudation by PGPRs (Martinez-Toledo *et al.*, 1988; Vessey, 2003) and the presence of microbial communities in the soil or rhizosphere which supported the plant growth through cycling and availability of nutrients, improving the roots health by competing with root pathogens during the growth stage and raising the absorption of nutrients (Roesty *et al.*, 2006). Elshanshoury (1995) stated that dual inoculation of *Azospirillum brasilense* with *Azotobacter chroococcum* in disinfected soil resulted in substantial encouragement of their populations in the rhizosphere of wheat seedlings. Additionally, he proposed that dual inoculations meaningfully amplified the plant growth, indole acetic acid (IAA) concentrations, N, P, Mg, and total soluble sugars in wheat shoots.

Both years 2012 and 2013 experiments revealed that integrated use of rhizobacterial isolates with chemical phosphorus fertilizer supplemented substantially for chemical phosphorus fertilization in sunflower. As most of the yield and nutrients uptake attributes gave relatively comparable response to 50% phosphorus application with dual inoculation as that with recommended dose of phosphorus without inoculation. It might be due to improved phosphorus use and its recovery efficiency as favorable bacterial inoculation may have exploited nutrient availability exclusively to the plants through mineralization, solubilization of phosphorus, production of phytohormones, organic acids and redox changes (Abou-Shanab *et al.*, 2003; Raghothama and Karthikeyan, 2005). Phosphorus solubilizing microbes (PSM) have high potential as bio-fertilizers particularly in soils with P deficiency to increase the growth and yield performance of crops (Miller *et al.*, 2010; Awasthi *et al.*, 2011). Yazdani *et al.* (2011) stated that application of PGPR and PSM together decreased P application by 50%. In his experiment, grain yield, biological yield, harvest index and nutrients uptake increased significantly compared to check. Similar results had also been described by researchers that dual inoculation in combination of half the recommended doses of NPK enhanced growth and yield attributes (Afifi *et al.*, 2003; El-Kholy *et al.*, 2005).

## Conclusion

The study suggested that PGPR in combination with chemical phosphorus fertilizer has a great potential to increase yield of sunflower and net benefit with improving the usage efficiency of chemical phosphorus fertilizers, as PGPR (*Bacillus* + *Pseudomonas*) with 50% phosphorus fertilizer gave the crop yield as with full dose of phosphorus fertilizer. So 50% of phosphorus fertilizer doses can be reduced by applying PGPR (*Bacillus* + *Pseudomonas*) inoculation sources for achieving sunflower achene yield

which was obtained by using 100% phosphorus fertilizers without inoculation.

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## References

- Abou-Shanab, R.I., T.A. Delorme, R.L. Chaney, K. Ghanem, H. Moawad and H.A. Ghazlan, 2003. Phenotypic characterization of microbes in the rhizosphere of *Alyssum murale*. *Int. J. Phytoremed.*, 5: 367–379
- Acharya, A., C.R. Sharma and S.P. Dev, 1999. Effect of *Azospirillum* inoculation on production of rice crop in Alfisols of Himachal Pradesh. *Ind. J. Hill Farm.*, 12: 42–46
- Afifi, M.H., F.M. Manal and A.M. Gomaa, 2003. Efficiency of applying biofertilizers to maize crop “Statistical under different levels of mineral fertilizers. *Ann. Agric. Sci. Moshtohor*, 41: 1411–1420
- Awasthi, R., R. Tewari and H. Nayyar, 2011. Synergy between Plants and P-Solubilizing Microbes in soils: Effects on Growth and Physiology of Crops. *J. Microbiol.*, 2: 484–503
- Bashan, Y., G. Holguin and E. de-Bashan, 2004. *Azospirillum* plant relationships: physiological, molecular, agricultural and environmental advances (1997–2003). *Can. J. Microbiol.*, 50: 521–577
- Bremner, J.M., 1965. Total nitrogen. In: *Methods of soil Analysis: Chemical and Microbiological Properties*, Part II, pp: 1238–1255. C.A. Black (ed.). American Society of Agronomy, Madison, Wisconsin, USA
- Canbolat, M.Y., S. Bilen, R. Çakmakçı, F. Sahin and A. Aydin, 2006. Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. *Biol. Fert. Soils*, 42: 350–357
- CIMMYT, 1988. *From Agronomic Data to Farmer Recommendation: An Economic Training Manual*, pp: 25–33. Mexico
- El-Kholy, M.A., S. El-Ashry and A.M. Gomaa, 2005. Biofertilization of maize crop and its impact on yield and grains nutrient content under low rates of mineral fertilizers. *J. Appl. Sci. Res.*, 1: 117–121
- Elshanshoury, A.R., 1995. Interactions of *Azotobacter chroococcum*, *Azospirillum brasilense* and *Streptomyces mutabilis*, in relation to their effect on wheat development. *J. Agron. Crop Sci.*, 175: 119–127
- Fageria, N.K., V.C. Baligar and Y.C. Li, 2008. The role of nutrient efficient plants in improving crop yields in the twenty first century. *J. Plant Nutr.*, 31: 1121–1157
- Fallik, F., Y. Okon, E. Epstein, A. Goldman and M. Fischer, 1989. Identification and qualification of IAA and IBA in *Azospirillum* brazilensis inoculated maize roots. *Soil Biol. Biochem.*, 21: 147–153
- Gehl, R.J., J.P. Schmidt, L.D. Maddux and W.B. Gordon, 2005. Corn yield response to nitrogen rate and timing in sandy irrigated soils. *Agron. J.*, 97: 1230–1238
- GOP, Economic survey of Pakistan 2013–2014. *Ministry of Food, Agriculture and Livestock (Federal Bureau of Statistics)*, pp: 20–33. Islamabad, Pakistan
- Gupta, S.C., R.P. Singh and V. Ravinder, 2009. Response of chickpea to P levels from different sources with various PSB species. *Legume Res.*, 32: 5359–5370
- Gyaneshwar, P., G.N. Kumar, L.J. Parekh and P.S. Poole, 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant Soil*, 245: 83–93
- Isherwood, K.F., 1998. Fertilizer use and environment. In: *Proc. Symp. Plant Nutrition Management for Sustainable Agricultural Growth*, pp: 57–76. N. Ahmed and A. Hamid (eds.). NFDC, Islamabad, Pakistan
- Joe, M.M. and P.K. Sivakumar, 2009. Long term survivability of *Azospirillum* co-aggregates: Bioinoculation effect on the growth and yield of sunflower. *Agricultura*, 6: 71–77

- Kannan, P., A. Saravanan, S. Krishnakumar and S.K. Natarajan, 2005. Biological properties of soil as influenced by different organic manures. *Res. J. Agric. Biol. Sci.*, 1: 181–183
- Khalid, A., M. Arshad and Z.A. Zahir, 2004a. Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbiol.*, 96: 473–480
- Khammas, K.M. and P. Kaiser, 1992. Pectin decomposition and associated nitrogen fixation by mixed cultures of *Azospirillum* and *Bacillus* species. *Can. J. Microbiol.*, 38: 794–797
- Lalfakzual, L., H. Kayang and M.S. Dkhar, 2008. The effect of fertilizers on soil and microbial components and chemical properties under leguminous cultivation. *J. Agri. Environ. Sci.*, 3: 314–324
- Lucangeli, C. and R. Bottini, 1997. Effects of *Azospirillum spp.* On endogenous gibberellin content and growth of maize (*Zea mays* L.) treated with uniconazol. *Symbiosis*, 23: 63–71
- Martínez, T.M.V., T. Rubia, J. Moreno and J. Gonzalez-Lopez, 1988. Root exudates of *Zea mays* and production of auxins, gibberellins and cytokinins by *Azotobacter chroococcum*. *Plant Soil*, 110: 149–152
- Miller, S.H., P. Browne, C. Prigent-Combaret, E. Combes-Meynet, J.P. Morrissey and F. Ogara, 2010. Biochemical and genomic comparison of inorganic phosphate solubilization in *Pseudomonas* species. *Environ. Microbiol. Rep.*, 2: 403–411
- Montemurro, F. and D.D. Giorgio, 2005. Quality and nitrogen use efficiency of sunflower grown at different nitrogen levels under Mediterranean conditions. *J. Plant Nutr.*, 28: 335–350
- Mostafa, G.G. and A.A. Abo-Baker, 2010. Effect of bio- and chemical fertilization on growth of sunflower (*Helianthus annuus* L.) at South Valley area. *Asian J. Crop Sci.*, 2: 137–146
- Namvar, A., T. Khandan and M. Shojaei, 2012. Effects of bio and chemical fertilizer on grain and oil yield of sunflower (*Helianthus annuus* L.) under different rates of plant density. *Ann. Biol. Res.*, 3: 1125–1131
- Raghothama, K.G. and A.S. Karthikeyan, 2005. Phosphate acquisition. *Plant Soil*, 274: 37–49
- Roesty, D., R. Gaur and B.N. Johri, 2006. Plant growth stage, fertilizer management and bio-inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria affect the rhizobacterial community structure in rain-fed wheat fields. *Soil Biol. Biochem.*, 38: 1111–1120
- Selvakumari, G., M. Baskar, D. Jayanthi and K.K. Mathan, 2000. Effect of irrigation of fly ash with fertilizers and organic manures on nutrient availability, yield and nutrient uptake of rice in Alfisols. *J. Ind. Soc. Soil. Sci.*, 48: 268–278
- Sharma, S.N. and R. Prasad, 2003. Yield and P uptake by rice and wheat grown in a sequence as influenced by phosphate fertilization with diammonium phosphate and Mussoorie rock phosphate with or without crop residues and phosphate solubilizing bacteria. *J. Agric. Sci.*, 141: 359–369
- Steel, R.G.D., J.H. Torrie and D.A. Dicky, 1997. *Principles and Procedures of Statistics: A Biometrical Approach*, 3<sup>rd</sup> edition, pp: 172–177. McGraw Hill Book Int. Co., New York
- Steinshamn, H., E. Thuen, M.A. Bleken, U.T. Brenoe, G. Ekerholt and C. Yri, 2004. Utilization of nitrogen (N) and phosphorus (P) in an organic dairy farming system in Norway. *Agric. Ecosyst. Environ.*, 104: 509–522
- U.S. Salinity Lab. Staff, 1954. *Diagnosis and Improvement of Saline Alkali Soil*, p 160. USDA. Handbook No. 60, Washington, DC, USA
- Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil*, 255: 571–586
- Wu, S.C., Z.H. Cao, Z.G. Li, K.C. Cheung and M.H. Wong, 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma*, 125: 155–166
- Yazdani, M., P. Rahdari and S. Motevalli, 2011. Effects of Organic Manure and Biological Fertilizers on Macronutrient Uptake of Corn (*Zea Mays* L.). *Adv. Environ. Biol.*, 5: 3761–3767

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