

# Partial substitution of exogenously applied phosphatic fertilizers by phosphate solubilizing bacteria in maize under calcareous soil

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## ABSTRACT

Phosphorus (P) availability is the major constrain in obtaining optimum crop yield in calcareous soils due to its precipitation as dicalcium and octacalcium phosphate by reacting with  $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$ . Therefore, we explored the role of phosphate solubilizing bacteria (without and with PSB @  $2 \text{ kg ha}^{-1}$ ) in optimizing maize yield and P availability from soluble and insoluble P sources applied @ of  $100 \text{ kg P}_2\text{O}_5$  into calcareous soil. PSB inoculation significantly improved maize plant height (5.6%), 1,000 grain weight (11%), dry matter (7.5%), stover (10.8%) and grain yield (6.8%), plant P concentration (10.1%) and uptake (18.6%), extractable P (3.1%), agronomic (48%) and uptake (53%) P use efficiency over un-inoculated plots. Phosphorus application significantly improved maize yield, soil health and agronomic P use efficiency (4.84 times over control); however, its impact was more pronounced when applied as 50% P each from farmyard manure (FYM) and single super phosphate (SSP). On the basis of overall performance, the sources were ranked as 50% FYM + 50% SSP > 50% rock phosphate (RP) + 50% SSP > 100% SSP > 75% FYM + 25% SSP > 75% RP + 25% SSP > 100% FYM > 100 RP > control. Interactively, a significant and maximum increased over absolute control in most of the soil and plant tested characteristics were observed when  $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  was supplemented 50% each as FYM and SSP along with PSB inoculation which was followed by 50% P each as FYM and SSP demonstrating that PSB were effective in enhancing RP solubilization under calcareous soil. Maximum value cost ratio of 3.1 was observed for 50% P each as FYM and SSP + PSB which was similar

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to 100% P as FYM + PSB and 75% FYM+ 25% SSP + PSB. Therefore, in calcareous soil P shall be amended 50% each as organic and mineral sources with PSB for its efficient utilization and obtaining optimum yield of maize.

**Subjects** Agricultural Science, Microbiology, Plant Science, Soil Science

**Keywords** Calcareous soil, Farmyard manure, Maize, Phosphorus use efficiency, Rock phosphate

## INTRODUCTION

Maize is an important cereal crop after wheat and rice in Pakistan. Many countries have adopted its growing both in spring and summer seasons to fulfill the daily food requirements of fast growing population (*PARC, 2007*). According to United States Department of Agriculture (USDA), Pakistan's maize production in 2021-22 was 7.9 million tons. In spite of its growth over a large area, the average yield of maize is very low in Pakistan especially in Khyber Pakhtunkhwa (*Mann, Jehangir & Masih, 2004*). The nature of Pakistani soils is alkaline calcareous, low in organic matter and essential nutrients. Thus, improvement of maize yield in such soils typically requires intensive use of chemical fertilizers, especially for supplementation of primary macro nutrients (N, P & K).

Phosphorus is an essential nutrient that performs a major role in several activities like growth, development and metabolism. It is a main component of nucleic acids, nucleotides, phospholipids, phytins, enzymes and many co-enzymes (*Hadgu et al., 2014*). It is the second most growth limiting nutrient after N in plant productivity (*Salimpour et al., 2010*). A significant portion of applied P become precipitated and enter into the immobile pools by reacting with  $\text{Ca}^{+2}/\text{Mg}^{+2}$  in calcareous and  $\text{Fe}^{+3}/\text{Al}^{+3}$  in acidic soil conditions (*Gyaneshwar et al., 2002a; Gyaneshwar et al., 2002b*). Globally, the efficiency of applied phosphatic fertilizers is approximately 10–25% (*Isherword, 1998*), consequently, the available P in soil is very low as  $1.0 \text{ mg kg}^{-1}$  (*Goldstein, 1994*). Therefore, it is necessary to enhance P availability for optimum plant production under calcareous soils.

Phosphate solubilizing bacteria (PSB) could be used as a potential remedy for improving PUE, crop growth and soil health. Using PSB is economical and eco-friendly practice. In the plant rhizosphere, these bacteria secrete various organic acids (*Deubel & Merbach, 2005*), acidify rhizosphere soil (*Zhang et al., 2016*) thus have potential to dissolve the precipitated  $\text{Ca}_3(\text{PO}_4)_2$  in calcareous soil. The application of PSB together with PGPR could reduce the application of expensive P fertilizers by 50% without compromising crop yield (*Khan et al., 2009*). It indicates that utilizing PSB based inoculants in improving crop production and promoting organic farming in sustainable manner. The PSB has been reported to efficiently increase the yield of rice (*Tiwari, Lehri & Pathak, 1989*), maize (*Adnan et al., 2021*) and wheat (*Afzal & Bano, 2008*) crops. In contrast, the continuous and injudicious application/use of huge amount of P fertilizers in alkaline soil not only disturb the soil fertility (*Gyaneshwar et al., 2002a; Gyaneshwar et al., 2002b*), but also affect the microbial population/diversity as well as crop yield to a great extent. This demands sound, eco-friendly and economically viable approaches under the current scenario when the phosphatic fertilizers are being replaced by organic manure (*Shenoy & Kalagudi, 2005*).

We believe that changing agricultural management practices makes it both timely and imperative to recognize the role of PSB in soil for improving soil fertility and crop yield. However, their potential benefits have not been efficiently understood because of their poor role at different conditions of soil and climate. We hypothesized that integrated application of organic (FYM), natural (RP) and chemical (SSP) P sources along with PSB may be more efficient than their sole application without PSB inoculation. In this scenario, this experiment was conducted to explore the ability of PSB as part of an integrated agronomic management strategy for improving maize yield and P availability from organic and mineral P sources under alkaline calcareous soils.

## MATERIAL AND METHODS

### Experimental site

This study was executed at the agronomic research farm (34.02071349555321, 71.48139963048956) of The University of Agriculture Peshawar (UAP) in 2021-22. The soil of the experimental site before the experiment was non saline ( $0.56 \text{ d Sm}^{-1}$ ), alkaline (7.86) and calcareous (15.4% lime) in nature, and silt loam in texture (clay 11%, silt 57.6% and sand 30.9%), low in organic matter (0.91%) and AB-DTPA extractable P ( $3.63 \text{ mg kg}^{-1}$ ).

### Experimental material

The farm yard manure (FYM) containing 1.6% N, 0.54% P and 0.91% K was purchased from local dairy form. The rock phosphate containing 11.35% P was obtained from Hattar Industrial State, Haripur. The PSB inoculum having a bacterial load of  $10^9 \text{ cells g}^{-1}$  was purchased from National Agricultural Research Center (NARC) Islamabad. It was composed of *Azotobacter* (17%), *Bacillus* (22%), *endosymbiotic rhizobia* (16%), *Enterobacter* (9%), *Flavobacterium* (13%), *Pseudomonas* (9%) and *Thiobacillus* (6%) (Adnan et al., 2022) obtained from National Agricultural Research Center (NARC) Islamabad.

### Treatments detail

The experiment was consisted of two factors including two levels of PSB (with and without PSB) and eight phosphorus sources combinations (control, 100% FYM, 100% RP, 75% FYM + 25% SSP, 75% RP + 25% SSP, 50% FYM+50% SSP, 50% RP + 50% SSP and 100% SSP) added at the rate of  $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ .

### Experimental procedure

This field study was arranged in factorial (two) split plot randomized complete block design (RCBD) with three replications. PSB was applied to main plots at the rate of  $2 \text{ kg ha}^{-1}$  through seed inoculation while, different combinations of P sources into subplot with a size of  $3 \times 4 \text{ m}^2$ . The FYM and RP were analyzed for their P content and were applied a month before sowing of maize crop. The basal dose of  $140 \text{ kg N ha}^{-1}$  and  $80 \text{ kg K}_2\text{O ha}^{-1}$  (inclusive of N and K to be received from FYM) were added to each sub plot as urea and sulphate of potash (SOP) respectively. Urea was supplemented in two doses half each at sowing and knee height stage. PSB was applied at the rate of  $2 \text{ kg ha}^{-1}$  via seed inoculation

techniques. The inoculated seed of cultivar Jalal was sown at the rate of 30 kg seed ha<sup>-1</sup> while maintaining the recommended row to row (75 cm) and plant to plant (25 cm) distances. Recommended irrigation schedule was followed as per crop requirements subject to weather condition. Weeds were controlled by using recommended chemical herbicides. Uniform standard cultural practices recommended for field experiment were adopted throughout the growing season.

### Agronomic parameters

Plant height was measured from the base to the top by selecting ten plants randomly in each sub plot at maturity stage. Thousand grains were counted from grain harvested in each treatment plot and were weighed using electronic balance. The whole above the ground plants was harvested, air dried and weighed with field balance and transformed into kg ha<sup>-1</sup> as follows:

$$\text{Total dry matter (kg ha}^{-1}\text{)} = \frac{\text{Dry matter yield of a subplot in kgs}}{\text{Subplot Size m}^2} \times 10,000 \text{ m}^2.$$

For grain yield, the cobs were husked from the plants harvested from each subplot, dried and shelled, and changed to kg ha<sup>-1</sup> as follows:

$$\text{Grain yield (kg ha}^{-1}\text{)} = \frac{\text{Grain yield of a subplot in kgs}}{\text{Subplot Size m}^2} \times 10,000 \text{ m}^2.$$

### Soil analysis

The soil of the experimental plot was physico-chemically characterized (pH, EC, texture, organic matter, lime and AB-DTPA extractable P contents) before the experiment. While, the post-harvest soil analysis were performed for AB-DTPA extractable P and organic matter contents. The pH of the composite soil sample collected from experimental site was measured in 1:5 soil water suspensions by pH meter (*Mclean, 1983*). Soil EC (1:5 soil water extract) was measured by electrical conductivity meter (*Rhoades, 1982*). The texture of soil sample collected from experimental site before the experiment was measured by hydrometer method (*Koehler, Moudre & McNeal, 1984*). The soil organic matter (SOM) was quantified by adopting the procedure of *Nelson & Sommers (1983)*. Post-harvest P concentration in soil was measured by *Soltanpour & Schwab (1977)* protocol. Lime content in samples was quantified by acid neutralization method (*Leo, 1963*).

### Plant analysis

The whole above the ground plant samples randomly collected from each sub plot at maturity were examined for P concentration by spectrophotometer using the protocol of *Richards (1954)*. Phosphorus uptake by maize plant in each plot was measured by using the following expression:

$$\text{P uptake (kg ha}^{-1}\text{)} = \frac{\text{Plant P content}}{100} \times \text{Biomass in kg ha}^{-1}.$$

The agronomic phosphorus use efficiency (APUE) was measured as follows:

$$\text{APUE (kg kg}^{-1}\text{)} = \frac{(\text{Grain yield of fertilized plot} - \text{Grain yield of control plot})}{\text{Fertilizer P applied}}.$$

While, the apparent phosphorus recovery (APR) was measured by the following formula:

$$\text{APR (\%)} = \frac{(\text{P uptake in fertilized plot} - \text{P uptake in control plot})}{\text{Fertilizer P applied}} \times 100.$$

### Economic analysis

The profitability of maize crop in response to applied treatments was carried out by the procedure outlined by *CIMMYT (1988)*.

### Statistical analysis

The collected data were run for analysis of variance (ANOVA) suited for two factorial split plot RCBD using Statistix 8.1 (*Steel & Torrie, 1980*). In case of significant F test, the means were further compared by least significant difference (LSD) test ( $\alpha = 0.05$ ).

## RESULTS

### Yield and yield component of maize

Maize plant height was significantly improved by using phosphate solubilizing bacteria (PSB) and P sources ([Table 1](#)). Application of PSB was better by 5.6% over no PSB for plant height. Taller plants were observed for plots either treated with 50% P as RP or FYM with 50% P as SSP. Significantly taller plants were recorded when SSP was integrated with other P sources especially RP and FYM at 50:50. In phosphorus sources combination, plant height increased from 143.5 cm to 186.2 cm with 50 FYM + 50% SSP. The increasing trend in plant height was observed at each increment of SSP (%) with the combine application of FYM and RP. The interaction effect of PSB and P sources for plant height was non-significant. However, the increasing trend in plant height was observed at each combination of SSP with FYM. It was shown that 50% FYM + 50% SSP proved better than any other combination with PSB. Application of 50% FYM + 50% SSP with PSB was better by 32.9% over absolute control.

PSB inoculation significantly improved thousand grain weight of maize over without PSB ([Table 1](#)). The heavier thousand grains were found in plots treated with PSB (222.5 g) as compared to control (200.5 g), resulting 11.0% improvement in 1,000 grain weight in response to PSB inoculation. Similarly, significant change was found in thousand grain weight among the plots either treated with 50% FYM or RP with 50% of SSP fertilizers. The heavier thousand grain weight 236.8 g was recorded in plots treated with 50% FYM + 50% SSP which as at par to 50% RP + 50% SSP. The lighter (179.3 g) 1,000 grains were observed under control. Plots treated with 50% FYM + 50% SSP maximally increased maize 1,000 grain weight by 32.1% over P plot. The significant interaction of PSB and P sources for 1,000 grain weight indicated that 50% FYM + 50% SSP with PSB was the most appropriate combination representing a maximum increase of 49.1% over absolute control ([Fig. 1](#)).

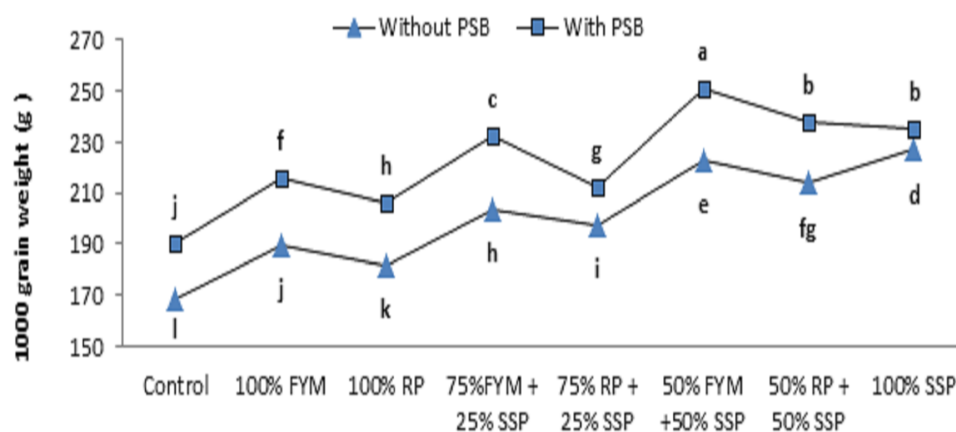
Experimental data clearly revealed that PSB inoculation and P sources considerably improved dry matter, grain yield and stover yield of maize ([Table 2](#)). The significant interactive effect was also observed for PSB and P sources on the aforesaid yield and yield components of maize. Sole PSB application significantly improved dry matter, grain yield

**Table 1** Maize plant height (cm) and 1,000 grains weight (g) as influenced by phosphorus sources with and without PSB inoculation.

Inoculation	Plant height (cm)	% increase over control	1,000 grains weight (g)	% increase over control
Without PSB	163.8 b	—	200.5 b	—
With PSB	173.0 a	5.6	222.5 a	11.0
LSD ( $\alpha = 0.05$ )	2.45	—	8.33	—
Phosphorus sources combinations				
P sources (%)	SSP (%)			
0	0	143.5 f	—	179.3 g
100 FYM	0	169.0 d	17.8	202.7 f
100 RP	0	159.8 e	11.4	193.5 g
75 FYM	25	176.2 bc	22.8	218.0 d
75 RP	25	162.0 e	12.9	204.7 e
50 FYM	50	186.2 a	29.7	236.8 a
50 RP	50	172.2 cd	20.0	225.6 b
0	100	178.0 b	24.0	231.2 c
LSD ( $\alpha = 0.05$ )		4.91	—	6.14
Interaction				
PSB * P sources		NS	—	Fig. 1
LSD ( $\alpha = 0.05$ )		—	—	16.4

**Notes.**

Means with similar letters (in each category) are statistically comparable at  $\alpha = 0.05$ . PSB, FYM, RP, SSP and NS mean phosphate solubilizing bacteria, farmyard manure, rock phosphate, single super phosphate and non-significance  $\alpha = 0.05$ , respectively.

**Figure 1** Integrated effect of PSB and P sources on 1,000 grain weight of maize. Lines marker with similar letters are statistically identical ( $\alpha = 0.05$ ). FYM, PSB, RP and SSP denote farmyard manure, phosphate solubilizing bacteria, rock phosphate and single super phosphate respectively.

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and stover yield of 7.82%, 10.51% and 6.77% respectively over PSB control (where no PSB was applied). Similarly, data revealed that P application irrespective of their sources improved dry matter, grain yield and stover yield of maize. Specifically, maximum dry matter of 28.92% was observed where P was applied 50% from FYM + 50% from SSP.

**Table 2** Effect of PSB and phosphorus sources on dry matter, grain and stover yield ( $\text{kg ha}^{-1}$ ) of maize.

Inoculation		Dry matter	Grain yield ( $\text{kg ha}^{-1}$ )	Stover yield
Without PSB		8,449 b	2,322 b	6,128 b
With PSB		9,110 a	2,566 a	6,543 a
LSD ( $\alpha = 0.05$ )		45.1	57.7	66.3
Phosphorus sources combinations				
P sources (%)	SSP (%)			
0	0	7,690 h	1,976 e	5,714 e
100 FYM	0	8,554 e	2,331 c	6,223 c
100 RP	0	7,853 g	2,197 d	5,655 e
75 FYM	25	8,833 d	2,578 b	6,256 c
75 RP	25	8,199 f	2,350 c	5,849 d
50 FYM	50	9,914 a	2,818 a	7,095 a
50 RP	50	9,698 b	2,672 b	7,026 a
0	100	9,493 c	2,628 b	6,866 b
LSD ( $\alpha = 0.05$ )		90.2	115.5	132.6
Interaction				
PSB * P sources		<a href="#">Fig. 2</a>	<a href="#">Fig. 3</a>	<a href="#">Fig. 4</a>
LSD ( $\alpha = 0.05$ )		127.6	163.3	187.6

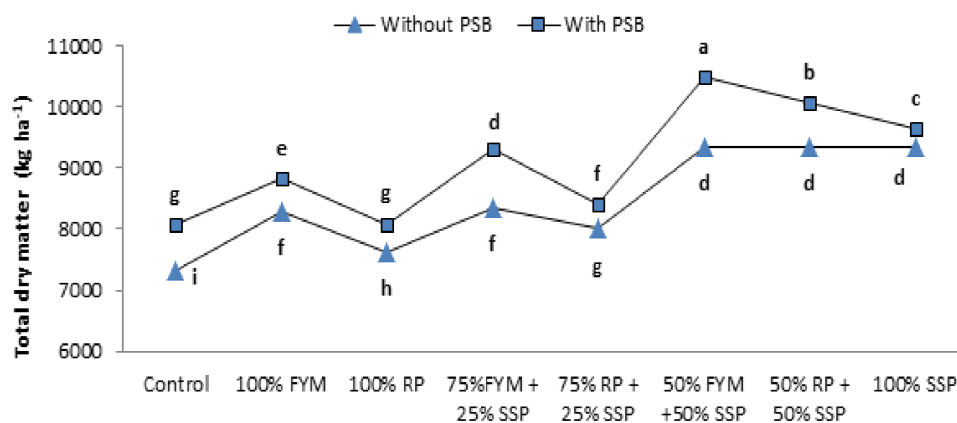
**Notes.**

Means with similar letters (in each category) are statistically comparable at  $\alpha = 0.05$ . PSB, FYM, RP and SSP denote phosphate solubilizing bacteria, farmyard manure, rock phosphate and single super phosphate respectively.

This was followed by 50% from RP + 50% SSP, 100% from SSP and 75% from FYM + 25% from SSP having an increase of 26.11%, 23.45% and 14.86% over control. Lower dry matter yield was observed for P control. The interaction between PSB  $\times$  P sources indicates that, greater dry matter yield was obtained when PSB and P as 50% from FYM + 50% from SSP were applied in combination ([Fig. 2](#)) exhibiting a maximum increase of 43.5% over absolute control.

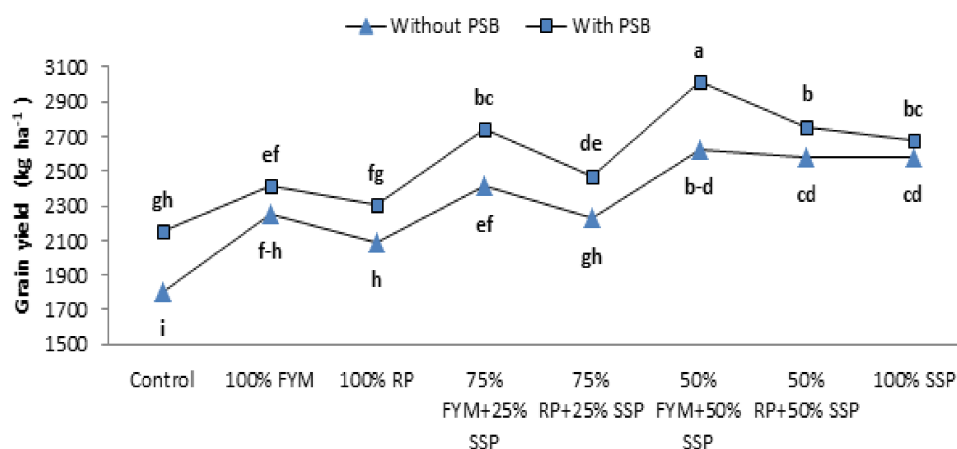
Maximum grain yield of 42.61% was observed where P was applied 50% from FYM + 50% from SSP. This was followed by 50% from RP + 50% SSP, 100% from SSP and 75% from FYM + 25% from SSP, having an increase of 35.22%, 33% and 30.47% over control. The performance of 50% P each from RP and SSP, 100% from SSP and 75% from FYM + 25% from SSP were at par however, their effect was significant when compared to remaining treatment combinations. Lower grain yield was observed for P control treatment. The interaction between PSB  $\times$  P sources indicates that, maximum grain yield was produced when PSB and P as 50% from FYM + 50% from SSP were applied in combination ([Fig. 3](#)) representing a 67.4% increase over absolute control.

The treatment receiving P as 50% from FYM + 50% from SSP recorded maximum stover yield of 24.17% over control treatment. This was followed by 50% from RP + 50% SSP, 100% from SSP and 75% from FYM + 25% from SSP, having an increase of 22.96%, 20.16% and 9.49% over control. Phosphorus application as 50% each from FYM and SSP was statistically comparable to 50% each from RP and SSP but those were



**Figure 2** Interactive effect of PSB and P sources on dry matter ( $\text{kg ha}^{-1}$ ) of maize. Lines marker with similar letters are statistically identical ( $\alpha = 0.05$ ). FYM, PSB, RP and SSP denote farmyard manure, phosphate solubilizing bacteria, rock phosphate and single super phosphate respectively.

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**Figure 3** Interactive effect of PSB and P sources on grain yield ( $\text{kg ha}^{-1}$ ) of maize. Lines marker with similar letters are statistically identical ( $\alpha = 0.05$ ). FYM, PSB, RP and SSP denote farmyard manure, phosphate solubilizing bacteria, rock phosphate and single super phosphate respectively.

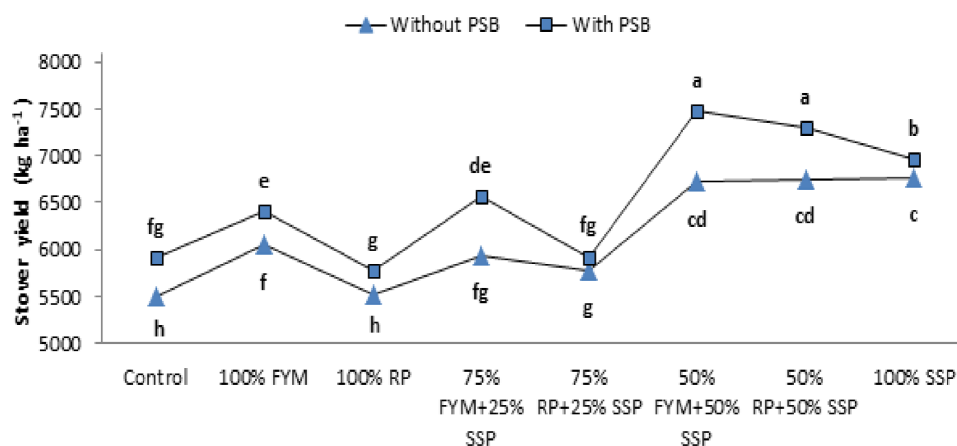
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significantly different from the rest of P sources. Lower stover yield was observed for P control treatment. The interaction between PSB  $\times$  P sources indicates that, maximum stover yield was produced when PSB and P as 50% from FYM + 50% from SSP were applied in combination (Fig. 4) resulting 35.7% increase over absolute control.

### Phosphorus concentration (%) and uptake ( $\text{kg ha}^{-1}$ )

Data regarding inoculation of PSB together with different sources of P on maize P concentration and uptake is presented in Table 3. The combined inoculation of PSB with different P sources in increasing the P concentration and uptake by maize was highly significant ( $p \leq 0.05$ ). Compared to un-inoculated PSB, the maximum plant P concentration of 0.210% having a 10.1% increase over control was observed in the PSB





**Figure 4** Interactive effect of PSB and P sources on stover yield ( $\text{kg ha}^{-1}$ ) of maize. Lines marker with similar letters are statistically identical ( $\alpha = 0.05$ ). FYM, PSB, RP and SSP denote farmyard manure, phosphate solubilizing bacteria, rock phosphate and single super phosphate respectively.

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inoculated treatments. Similarly, the high value of P ( $19.4 \text{ kg ha}^{-1}$ ) taken by maize plants with 18.6% increase over control was recorded in the PSB applied treatments in comparison to the treatments without PSB. The minimum data for both maize plants P concentration (0.191%) and uptake ( $16.4 \text{ kg ha}^{-1}$ ) was found in the treatments without PSB inoculation.

Furthermore, significant ( $p \leq 0.05$ ) increase in plant P concentration and uptake by maize plants was also recorded in all the treatments where different P sources were applied in combination with different doses of SSP (Table 3). It is obvious from the data that compared to control the maximum value of both P concentrations (0.247%) and uptake ( $24.6 \text{ kg ha}^{-1}$ ) by maize plants was recorded in the treatments where 50% FYM was supplemented together with 50% SSP, respectively. It was followed by the treatment where 100% sole SSP was applied. Similarly, in comparison to control the lowest P concentration of 0.159% with a 16.5% increase over control by maize plants was depicted in the treatments having 100% FYM without any dose of SSP. The minimum P uptake ( $17.3 \text{ kg ha}^{-1}$ ) was observed under 75% RP together with 25% SSP. Overall, the role of PSB in inoculated treatments as well as the combination of different sources of P with SSP on P uptake and concentration by maize plants was significant in comparison to control and the treatments without PSB.

The combined effect of PSB and P supplements, on maize P uptake is summarized in Fig. 5. The inoculation of PSB together with different rates of SSP and P sources was found non-significant ( $p \leq 0.05$ ) in improving plant P concentration but was significant for plant P uptake (Fig. 5). The highest P content (101.1%) and uptake ( $188.7 \text{ kg ha}^{-1}$ ) in maize were under 50% SSP + 50% FYM along with PSB inoculation, corresponding to 95% high P content and 170% P uptake in plants over absolute control, respectively. These results were closed to the treatments where 100% SSP was applied alone and in combination with PSB. The un-inoculated PSB treatments were also improved in the presence of different P sources alone and in combination with various rates of SSP. During the interactive effects

**Table 3** Effect of PSB and phosphorus sources on maize phosphorus concentration (%) and uptake ( $\text{kg ha}^{-1}$ ).

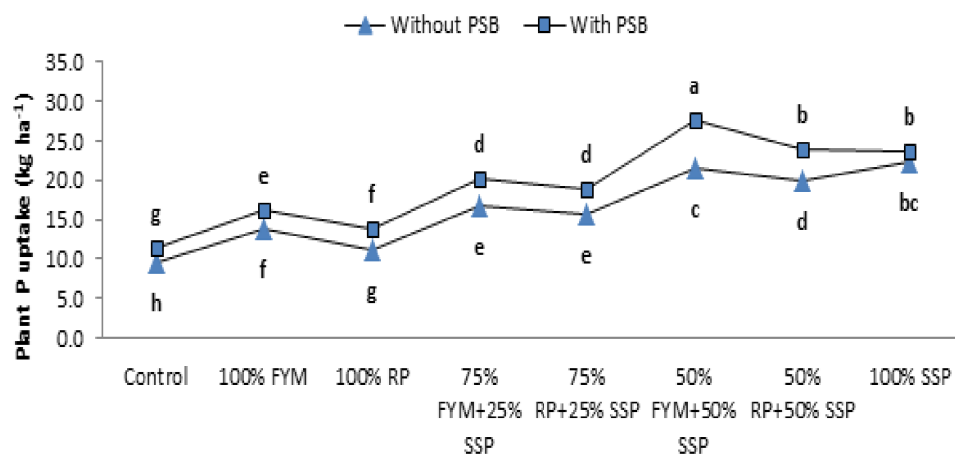
Inoculation		Plant P concentration (%)	% increase over control	Plant P uptake ( $\text{kg ha}^{-1}$ )	% increase over control
Without PSB		0.191 b	—	16.4 b	—
With PSB		0.210 a	10.1	19.4 a	18.6
LSD ( $\alpha = 0.05$ )		0.0055	—	0.501	—
<b>Phosphorus sources combinations</b>					
P sources (%)	SSP (%)				
0	0	0.136 f	—	10.5 h	—
100 FYM	0	0.174 d	27.9	14.9 f	42.2
100 RP	0	0.159 e	16.5	12.5 g	19.0
75 FYM	25	0.208 c	52.6	18.4 d	75.4
75 RP	25	0.211 c	54.9	17.3 e	65.2
50 FYM	50	0.247 a	81.5	24.6 a	134.5
50 RP	50	0.225 b	64.9	21.9 c	108.1
0	100	0.242 a	77.7	23.0 b	119.0
LSD ( $\alpha = 0.05$ )		0.110	—	1.003	—
<b>Interaction</b>					
PSB * P sources		NS	—	Fig. 5	—
LSD ( $\alpha = 0.05$ )		—	—	1.42	—

**Notes.**

Means with similar letters (in each category) are statistically comparable at  $\alpha = 0.05$ . Lines marker with similar letters are statistically identical ( $\alpha = 0.05$ ). FYM, PSB, RP and SSP denote farmyard manure, phosphate solubilizing bacteria, rock phosphate and single super phosphate respectively.

of various sources and rates of P fertilizers in combination with different rates of SSP, the minimum increase in P concentration of 7.5% and uptake of  $18.8 \text{ kg ha}^{-1}$  by maize plants was observed in control, followed by the treatments having sole 100% FYM and RP, respectively. Overall, the recorded trend of PSB inoculation in combination with P sources and SSP in enhancing P concentration and uptake was positive and significant towards maize plants.

Likewise, the statistical analysis of the interactive effects of both PSB with different P sources on maize plants uptake is demonstrated in Fig. 5. Compared to un-inoculated treatments, the interaction of PSB with various P sources and rates along with SSP was effective and significant ( $p \leq 0.05$ ) in enhancing the P uptake ( $\text{kg ha}^{-1}$ ) by maize plants. The maximum P uptake ( $26.5 \text{ kg ha}^{-1}$ ) in maize was recorded in the treatments, provided the interaction of PSB in combination with 50% SSP and 50% FYM. It was almost equivalent to the treatments having 100% supplemented SSP alone ( $23 \text{ kg ha}^{-1}$ ) and 50% RP combined with 50% SSP ( $21.5 \text{ kg ha}^{-1}$ ). The minimum P uptake and improvement in maize plants was found in all those treatments having different P sources and various rates of SSP without PSB inoculation. Overall, Compared to control and supplemented 100% FYM and/or 100% RP alone treatments, significant linear increase in P uptake by all maize plants was depicted in the treatments inoculated with and even without PSB along with various sources of organic P fertilizers and different rates of SSP.



**Figure 5** Interactive effect of PSB and P sources on plant P uptake ( $\text{kg ha}^{-1}$ ) of maize. Lines marker with similar letters are statistically identical ( $\alpha = 0.05$ ). FYM, PSB, RP and SSP denote farmyard manure, phosphate solubilizing bacteria, rock phosphate and single super phosphate respectively.

Full-size DOI: [10.7717/peerj.15038/fig-5](https://doi.org/10.7717/peerj.15038/fig-5)

### Post-harvest extractable P ( $\text{mg kg}^{-1}$ ) and organic matter (%) in soil

Analysis of the data showed a non-significant effect of PSB on soil extractable P (Table 4). However, inoculation improved soil extractable P by 3.1% over control. Soil organic matter (SOM) was shown maximum in those plot to which no PSB was applied. In case of different sources of phosphorus, soil extractable P was significantly affected and maximum soil P was noticed in those plots to which 100% phosphorus was applied only from SSP source, while this was significantly at par with the combine applications of 100% FYM with no SSP supplementation followed by 50% FYM and 50% SSP source. The minimum soil P was recorded at control treatment. The application of SSP have shown maximum increase of 41.2% followed by 100% sole application of FYM 39.9% and 50% FYM+50% SSP have increase of 39.2%. In case of soil organic matter, various combined sources of P significantly affect the soil organic matter. The maximum SOM was observed in those plots to which only 100% of FYM was applied, which was followed by the combine application of 75% FYM and 25% SSP.

The interaction between bio-fertilizers with various phosphorus sources on post-harvest soil AB-DTPA extractable P are indicated in Fig. 6. Integration of PSB with phosphorus source showed significant impact on soil fertility and nutrients availability, The maximum soil extractable P ( $3.07 \text{ mg kg}^{-1}$ ) was recorded in those plots to which 100% FYM in combination with PSB was applied representing a maximum increase (46%) over absolute control.

### Phosphorous Use efficiency (PUE)

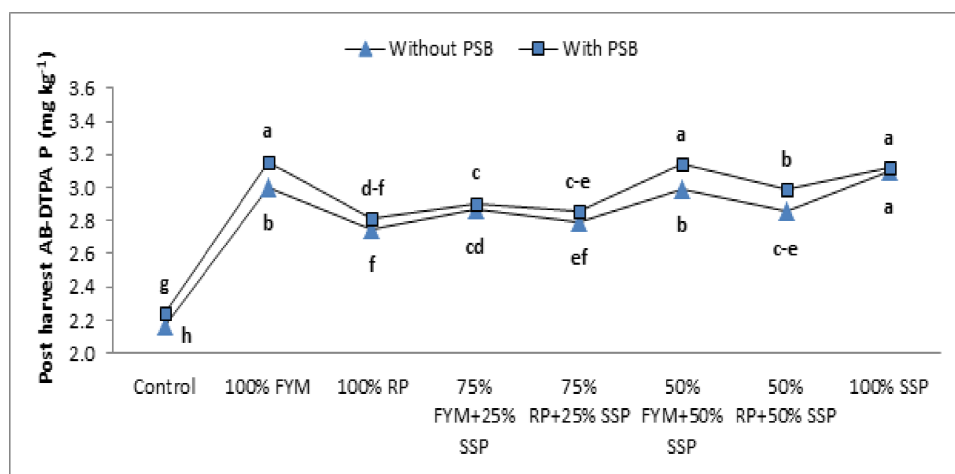
Analysis of variance exhibited a significant influence of PSB and P sources over Agronomic P efficiency (APE-Table 5) and apparent P recovery (APR-Table 6) in maize. PSB inoculation significantly improved APE from 5.2 to 7.7 ( $\text{kg kg}^{-1}$ ) and APR from 6.81 to 10.48 (%) when compared to without PSB inoculated plots. Among the P sources the maximum APE ( $10.2 \text{ kg kg}^{-1}$ ) and APR (15.79%) were observed in plots where P was supplemented 50%

**Table 4** Effect of PSB and phosphorus sources on soil AB-DTPA extractable P ( $\text{mg kg}^{-1}$ ), organic matter (%) after harvesting maize.

Inoculation	AB-DTPA extractable P ( $\text{mg kg}^{-1}$ )	% increase over control	Soil organic matter (%)	% increase over control
Without PSB	2.81	—	1.04 a	—
With PSB	2.90	3.1	1.02 b	-1.6
LSD ( $\alpha = 0.05$ )	NS	—	0.016	—
<b>Phosphorus sources (%)</b>				
P sources	SSP			
0	0	2.20 d	—	0.89 d
100 FYM	0	3.08 a	39.9	1.39 a
100 RP	0	2.78 c	26.1	0.89 d
75 FYM	25	2.89 b	31.0	1.26 b
75 RP	25	2.82 c	28.0	0.88 d
50 FYM	50	3.07 a	39.2	1.11 c
50 RP	50	2.92 b	32.6	0.89 d
0	100	3.11 a	41.2	0.90 d
LSD ( $\alpha = 0.05$ )	0.046	—	0.0322	—
<b>Interaction</b>				
PSB * P sources	Fig. 6	—	NS	—
LSD ( $\alpha = 0.05$ )	0.065	—	—	—

**Notes.**

Means with similar letters (in each category) are statistically comparable at  $\alpha = 0.05$ . PSB, FYM, RP, SSP and NS denote phosphate solubilizing bacteria, farmyard manure, rock phosphate, single super phosphate and non-significance  $\alpha = 0.05$ , respectively.

**Figure 6** Interactive effect of PSB and P sources on post-harvest soil AB-DTPA extractable P ( $\text{mg kg}^{-1}$ ). Lines marker with similar letters are statistically identical ( $\alpha = 0.05$ ). FYM, PSB, RP and SSP denote farmyard manure, phosphate solubilizing bacteria, rock phosphate and single super phosphate respectively.

Full-size DOI: 10.7717/peerj.15038/fig-6

**Table 5** Interactive effect of PSB and phosphorus sources on agronomic P efficiency ( $\text{kg kg}^{-1}$ ) of maize.

Phosphorus (%) sources	SSP (%)	Without PSB	With PSB	Mean	% increase over control
0	0	—	3.5	1.7 e	—
100 FYM	0	4.5	6.1	5.3 c	204
100 RP	0	2.9	5.0	4.0 d	127
75 FYM	25	6.1	9.4	7.8 b	345
75 RP	25	4.2	6.7	5.5 c	215
50 FYM	50	8.2	12.1	10.2 a	483
50 RP	50	7.8	9.6	8.7 b	399
0	100	7.8	8.7	8.3 b	374
Mean		5.2b	7.7a	—	—

**Notes.**

LSD value for PSB = 0.577 and P sources = 0.493 and their interaction is non-significant. Means with similar letters (in each category) are statistically comparable at  $\alpha = 0.05$ . PSB, FYM, RP and SSP denote phosphate solubilizing bacteria, farmyard manure, rock phosphate and single super phosphate respectively.

**Table 6** Interactive effect of PSB and phosphorus sources on apparent P recovery (%) of applied fertilizers in maize.

Phosphorus (%) sources	SSP (%)	Without PSB	With PSB	Mean	% increase over control
0	0	—	2.05 k	1.03 h	0
100 FYM	0	4.18 j	7.06 g	5.62 f	447
100 RP	0	1.66 k	4.91 i	3.28 g	219
75 FYM	25	7.17 g	10.98 f	9.07 d	783
75 RP	25	6.20 h	10.30 f	8.25 e	702
50 FYM	50	11.98 e	19.60 a	15.79 a	1436
50 RP	50	10.35 f	15.10 b	12.72 c	1138
0	100	12.90 d	13.86 c	13.38 b	1202
Mean		6.81 b	10.48 a	—	—

**Notes.**

LSD value for PSB = 0.247 and P sources = 0.1544 while there is = 0.697. Means followed by different letters in each category are significantly different at  $\alpha = 0.05$ . PSB, FYM, RP and SSP denote phosphate solubilizing bacteria, farmyard manure, rock phosphate and single super phosphate, respectively.

each from FYM and SSP which was followed by plots treated with 100% SSP and 50% RP + 50% SSP while, the lowest APE and APR were observed in control plots. With respect to APE the sources could be ranked as 50% each FYM and SSP ( $10.2 \text{ kg kg}^{-1}$ ) > 50% each RP and SSP ( $8.7 \text{ kg kg}^{-1}$ )  $\geq$  SSP ( $8.2 \text{ kg kg}^{-1}$ )  $\geq$  75% FYM + 25% SSP ( $7.8 \text{ kg kg}^{-1}$ ) > 75% RP + 25% SSP ( $5.5 \text{ kg kg}^{-1}$ ) > 100% FYM ( $5.3 \text{ kg kg}^{-1}$ ) > 100 RP ( $4.0 \text{ kg kg}^{-1}$ ) > control ( $1.7 \text{ kg kg}^{-1}$ ) when averaged across the PSB inoculation (Table 5). The similar trend was observed for sources in APR as well (Table 6).

The interaction effect of PSB and P sources was significant for APR (Table 6) while, non-significant for APE. The maximum APE (19.60%) was observed in plots treated with 100 kg  $\text{P}_2\text{O}_5$  as 50% each from FYM and SSP along with PSB inoculation which was followed by 50% each from RP and SSP + PSB (15.10%) while the lowest APE was

observed for plots solely treated with RP (1.66%). The performance of solely applied PSB was even superior than sole RP treated plots. Furthermore, The APE of 100% FYM with PSB was significantly better than 100% RP with and without PSB and sole application of FYM, and was at par to plots treated with 75%P as FYM and 25% as SSP. PSB were effective in enhancing APE regardless of the sources used however, its role was more pronounced when P was either supplemented as FYM or RP compared to SSP. SSP was observed as a most recoverable source without PSB inoculation.

### Economic analysis

Data concerning value cost analysis of the study (Table 7) showed a maximum yield return of PKR 150767 ha<sup>-1</sup> with a value cost ratio (VCR) of 3.1 for plots amended with 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as 50% each from FYM and SSP along with PSB. The VCR value of P applied as 50% each from FYM and SSP along with PSB was similar to those of 75% FYM + 25% SSP + PSB (Rs. 137186 ha<sup>-1</sup>) and 100% FYM (Rs. 120648 ha<sup>-1</sup>) however their net return was lower than 50% P as FYM + 50% SSP + PSB while the lowest VCR was recorded for absolute control plots. These observations have proven that under PSB inoculation the organic manure applied alone or in integration to mineral fertilizers are comparatively more economical than those of solely applied mineral fertilizers either with or without PSB inoculation. PSB inoculation improved VCR value regardless of the P sources used however, its impact was more pronounced in organic sources than chemical fertilizers. Even under control P the PSB inoculation improved VCR value from 2.54 to 2.98.

## DISCUSSION

Globally, the efficiency of applied phosphatic fertilizers is too low (10–25%) due to precipitation reaction with ions like Ca and Mg in alkaline and Fe in acidic soils. Our result demonstrated that the integration of SSP with FYM or RP and PSB were the most appropriate combinations in term of yield and yield component of maize under calcareous soils. *Achal, Savant & Reddy (2007)* and *Khan et al. (2008)* also found increased crop yield and soil fertility under integrated application of organic manures and synthetic P fertilizers with PSB. *Memon (1982)* observed that photosynthetic processes and photosynthate assimilation increased with phosphorus application. The metabolic activity and plant growth development such as plant height increase with phosphorus supplementation from different sources. Improved plant height and yield in maize under varying P sources and levels has also been reported by *Sahoo & Panda (2001)*. The same results were reported by *Singaram & Kothandaraman (1994)*, who observed quick growth and development of plants under higher application rate of P. Our findings are in conformity to *Hussain, Khan & Ahmad (2006)* who observed increase in root growth, enzymatic activities, metabolic activities and other yielding and growth parameters of maize in response to P application. Similarly, *Beigzade et al. (2013)* reported that, the application of NP fertilizers increased 1,000-grain weight of maize. The PSB like *Azotobacter chroococcum* considerably increased wheat grain and straw yield (*Vibha & NidhiKumari, 2014*). Similarly, *Reyes, Bernier & Antoun (2002)* also observed improved growth and yield of maize in response to inoculation of *Rhizobium* or/and *Penicillium*. *P. fluorescens* has been reported to optimize

**Table 7** Economic analysis of applied fertilizers.

P Sources (%)	SSP (%)	PSB inoculation	Grain yield (kg ha <sup>-1</sup> )	Yield value (PKR ha <sup>-1</sup> )	Input cost (PKR ha <sup>-1</sup> )						Value cost ratio	
					FYM	RP	SSP	Urea	SOP	PSB		Total
0	0	-	1802	90083	0	0	0	12174	23360	0	35534	2.54
100 FYM	0	-	2250	112499	32347	0	0	4720	1872	0	38939	2.89
100 RP	0	-	2092	104583	0	3463	0	12174	23360	0	38997	2.68
75 FYM	25	-	2412	120583	24260	0	5833	6584	7244	0	43921	2.75
75 RP	25	-	2225	111250	0	2597	5833	12174	23360	0	43964	2.53
50 FYM	50	-	2622	131083	16173	0	11667	8447	12616	0	48903	2.68
50 RP	50	-	2585	129228	0	1731	11667	12174	23360	0	48932	2.64
0	100	-	2583	129133	0	0	23333	12174	23360	0	58867	2.19
0	0	+	2150	107513	0	0	0	12174	23360	500	36034	2.98
100 FYM	0	+	2413	120648	32347	0	0	4720	1872	500	39439	3.06
100 RP	0	+	2303	115158	0	3463	0	12174	23360	500	39497	2.92
75 FYM	25	+	2744	137186	24260	0	5833	6584	7244	500	44421	3.07
75 RP	25	+	2476	123776	0	2597	5833	12174	23360	500	44464	2.78
50 FYM	50	+	3015	150767	16173	0	11667	8447	12616	500	49403	3.08
50 RP	50	+	2760	137983	0	1731	11667	12174	23360	500	49432	2.79
0	100	+	2673	133650	0	0	23333	12174	23360	500	59367	2.25

**Notes.**

Price of maize = Rs, 50 kg<sup>-1</sup>; FYM = Rs. 4000 ton<sup>-1</sup>; RP = Rs. 9 kg<sup>-1</sup>; SSP = Rs. 42 kg<sup>-1</sup>; Urea = Rs. 40 kg<sup>-1</sup> and SOP = Rs. 146 kg<sup>-1</sup>; Value cost ratio (VCR) = Value of yield / Cost of fertilizer. PSB +, PSB -, FYM, RP, SOP and SSP stand for with phosphate solubilizing bacteria, without phosphate solubilizing bacteria, farmyard manure, rock phosphate, sulphate of potash and single super phosphate respectively.

peanut growth, yield, and shoot N and P contents (*Dey et al., 2004*). These findings are in conformity to *Yang et al. (2007)* who also observed positive role of their integration effect. *Cheema et al. (2010)* also observed increased maize yield under the combine use of poultry manure and urea. The combination of organic and mineral fertilizers improves soil enzymatic activity, because the added organic manures contain different enzymes that improve soil biological properties (*Melero et al., 2007*). Additionally, organic amendments improve soil physical condition (*Kaur & Reddy, 2014*). Our findings are in accordance with *Mondal et al. (1994)* they also observed positive impact of integrated P management on soil fertility and subsequently on the productivity of rice, wheat and maize.

The increase P concentration and uptake in maize under integration of both organic and mineral P sources like SSP with FYM or RP (at 50:50 ratio) along with PSB could be attributed to the improved microbial and enzymatic activity, soil physical condition like water holding capacity, porosity, aeration and bulk density as documented by *Taiwo & Ogundiya (2008)*. Our findings are also in agreement to *Ahmad et al. (1997)* who observed higher nutrients availability under organic manure supplemented with effective microbes. We observed that solely RP was the least efficient sources but when integrated with SSP and/or PSB its performance was at par to SSP which has also been verified by *Akande, Adedira & Oluwatoyinbo (2005)*. Such improvement in maize yield and plant P uptake under co-application of RP+SSP+PSB has also been documented by *Mishra & Bangar (1986)* and *Wahid et al. (2022)* as a result of improved RP solubility by PSB *via* conversion

of insoluble P in RP into plant available P. Furthermore, our results are in agreement to [Zhang et al. \(2021\)](#) who concluded that the available P concentration induces due to the activities of phosphorus mineralized bacteria, which was also confirmed by [Samad et al. \(2017\)](#). The phosphorus becomes more available at every stage due to the supplementation of PSB ([Shade et al., 2014](#)). Despite PSB are widely accepted as eco-friendly P bio-fertilizers for increasing agricultural productivity ([Tian et al., 2021](#)), they also play an essential role in soil P cycling ([Tamburini et al., 2012](#)), ensuring plant P supplies and soil quality improvement ([Bai et al., 2020](#)). Our findings declared that application of organic and synthetic P sources in integration to PSB were more efficient than either their soil or combined application without PSB inoculation ([Tables 5 & 6](#)). This could be attributed to their ability in improving labile inorganic P pools and reducing the exchangeable aluminum (Al) in soil. [Meena \(2010\)](#) also reported 18–27% P recovery by under integration of organic and inorganic P sources. Similar findings for PAE and PUE were also obtained by [Sistani, Adeli & Tewolde \(2010\)](#). PSB release  $H^+$  ion and organic acid thus lower the pH of rhizosphere soil and enhance P solubility from RP ([Adnan et al., 2020](#); [Adnan et al., 2017](#)). Furthermore PSB also play a vital role in mineralization of organic nutrients thus facilitate the provision of nutrients ([Khan & Sharif, 2012](#)).

Our results ([Table 7](#)) regarding value cost ration (VCR) are in agreement to [Sharif et al. \(2011\)](#) who observed higher VCR under integrated application of organic manure with chemical fertilizers. In addition to higher VCR, organic sources and PSB also improve soil health and are eco-friendly than synthetic fertilizers ([Khan & Sharif, 2012](#)). According to [Alam et al. \(2005\)](#) integration of DAP and organic wastes like filter cake and poultry waste gave higher value cost ration than sole DAP. Therefore, we encourage using organic manure and bio-fertilizers in integration to chemical fertilizers for obtaining good quality optimum and economical production of maize under calcareous soil condition.

## CONCLUSION

The application of PSB along with  $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  significantly improved yield and P nutrition in maize plant over control however, their impact was more pronounced when applied P was applied 50% each as FYM and SSP. With respect to performance, the sources were ranked as 50% each FYM and SSP > 50% each RP and SSP > SSP > 75% FYM + 25% SSP > 75% RP + 25% SSP > 100% FYM > 100 RP > control when averaged across the PSB inoculation. Phosphorus applied as 50% each from FYM and SSP with PSB were observed the most economical combination. Therefore, in calcareous soils, phosphorus shall be managed by adopting integrated approaches for improving farmer's net return, maize yield and soil health.

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The authors declare there are no competing interests.

### Author Contributions

- Hajira conceived and designed the experiments, performed the experiments, prepared figures and/or tables, and approved the final draft.
- Yousaf Jamal conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.
- Muhammad Adnan conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.
- Manzoor Ahmad analyzed the data, prepared figures and/or tables, and approved the final draft.
- Maria Mussarat analyzed the data, prepared figures and/or tables, and approved the final draft.
- Muhammad Hamzah Saleem analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Beena Saeed conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Fazli Wahid performed the experiments, authored or reviewed drafts of the article, co-supervised the project, and approved the final draft.
- Rafi Ullah performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Shah Fahad analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Baber Ali conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
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- Sezai Ercisli analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Kirill S. Golokhvast analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

## Data Availability

The following information was supplied regarding data availability:

The raw data are available in the [Supplemental File](#).

## Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.15038#supplemental-information>.

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