Stimulatory effect of phosphorus solubilizing bacteria and phosphorus management on P uptake, phosphorus use efficiency and crude protein of wheat

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Abstract

Phosphorus is the important essential nutrient for vigorous growth and consequently increase crop yield. But its availability is very minute for plant uptake because it is fixed and precipitated in soil and goes to unavailable pool. The current study was therefore, designed to enhance P-uptake and phosphorus use efficiency (PUE) by phosphorus solubilizing bacteria (PSB) from various combinations of organic and inorganic P sources i.e. farmyard manure (FYM), poultry manure (PM) and single superphosphate (SSP) at the Agronomy Research Farm, The University of Agriculture, Peshawar, during 2015-16 and 2016-17. The experiment was laid out in randomized complete block design (RCBD) having four replications. Phosphorus was managed at the rate of 75 kg ha⁻¹ for all plots. Among the P management ratios, significantly higher crude protein (12.82 %), grain P concentration (0.38%), straw P concentration (0.17%), grain P uptake (16.5 kg ha⁻¹), straw P uptake (13.7 kg ha⁻¹), total P uptake (30.2 kg ha⁻¹) and PUE (25.3%) was achieved by the application of 50% P from PM + 50% from SSP. Phosphorus solubilizing bacteria significantly improved crude protein (11.15 %), grain P concentration (0.36%), straw P concentration (0.16 %), grain P uptake (14.6 kg ha⁻¹), straw P uptake (12.5 kg ha⁻¹), total P uptake (27.1 kg ha⁻¹) and PUE (22.0 %). It is concluded that PSB in combination with 50% P from PM + 50% from SSP proved superiority for quality wheat production, P uptake and PUE in the agro climatic condition of Peshawar-Pakistan.

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Introduction

Wheat (Triticum aestivum L.) fulfills 95% of the food requirements (Malik et al., 2006). It provides both carbohydrates and protein to human and livestock nutrition (Shewry, 2009). But unfortunately wheat in Pakistan is very than the major wheat producing countries (Kakar et al., 2015). Nutrient management is one the yield limiting factors because in the absence of essential nutrients plants are unable to complete their life cycle. Among them, phosphorus is needed for the basic metabolic processes such as energy transport (ADP and ATP), cell division. photosynthesis and respiration which affects the overall plant growth and development (Khan et al., 2014). It is also needed for root growth, strength of cereal's straw and early maturity (Gupta, 2003). But its availability to plants is a serious issue because the soils contain very little amount of phosphorus (0.02-0.5%) of which approximately 0.1% is available to plants (Zhou et al., 1992). Moreover, phosphorus is available in very minute quantity for plant uptake because it is fixed and precipitated in soil and goes to unavailable pool (Memonet al., 1992; Shenoy and Kalagudi, 2005; Khan et al., 2009; Wang et al., 2009). About 80% of added phosphate fertilizers become unavailable to plants due to precipitation reactions in soil. That is why phosphorus recovery efficiency does not exceed 20% globally (Qureshi et al., 2012). Plants are unable to absorb these insoluble and precipitated forms of phosphorus (Rengel and Marschner 2005). The unavailable forms of P can be transformed to soluble forms with the help of phosphorus solubilizing organisms (Gupta et al., 2007; Song et al., 2008; Sharma et al., 2013).

In Pakistan animal wastes like cattle manure, sheep manure and poultry manure are produced in large quantities every year and applied to cultivated lands. These manures are slow in nutrients release and can stay in soil for longer time and ensure long residual impact (Hidayatullah *et al.*, 2013; Iqbal *et al.*, 2015). Poultry manure and farmyard manures are the substitutes of commercial fertilizers and widely used for crop production (Abbas *et al.*, 2012). The depleted soil fertility by intensive cropping practices can be restored with organic fertilization in desirable quantities. Similarly, poultry manure also contains larger amounts of primary essential nutrients (nitrogen, phosphorus and potassium) in available forms to plants (Izunobi, 2002). Farming community extensively apply commercial fertilizers instead of organic manures due to their easy availability. But high cost, timely availability and continuous release of chemicals to environment are the major limitations of chemical fertilizers (Hamuda and Patko, 2013). Thus, applying chemical fertilizers, not only contaminating the environment but cause land degradation as well. To solve these problems it is needed to adopt environment friendly and sustainable agriculture which is only possible by organic and biofertilizers use. The application of phosphorus solubilizing bacteria as biofertilizers enhance P availability from unavailable pool through mineralization of organic and solubilization of inorganic P (Abd-Alla, 1994). They are very important due to their ability of mineralizing complex compounds (Toro, 2007). Phosphorus solubilization is the process in which these microorganisms release various organic acids like carboxylic acid, gluconic acid, 2-kitogluconic acid, glycoxylic acid which acidify microenvironments and consequently through their carboxyl and hydroxyl groups dissociate the bound forms of phosphate like $Ca_3(PO_4)_2$ in calcareous soils (Maliha et al., 2004; Mullen, 2005; Deubel and Merbach, 2005; Chen et al., 2006). To mobilize the phosphorus that is unavailable to plants, recently microbial inoculants are globally introduced which increase the capability of plants for P uptake (Trolove et al., 2003). The current study was therefore, designed to enhance phosphorus uptake and phosphorus use efficiency for quality wheat production PSB from various combinations of organic and inorganic P sources.

Materials and methods

The stimulatory effect of phosphorus solubilizing bacteria and phosphorus management on phosphorus uptake, phosphorus use efficiency and crude protein of wheat was studied in field experiment during 2015-16 and 2016-17 at Agronomy Research Farm, The

University of agriculture, Peshawar-Pakistan. The experiment was laid out in randomized complete block design with 4 replications. Two factors were included in the experiment i.e., Phosphorus solubilizing bacteria (with and without) and phosphorus management form organic and inorganic P sources i.e. farmyard manure (FYM), poultry manure (PM) and single superphosphate (SSP). Phosphorus solubilizing bacteria and organic and inorganic sources of phosphorus were integrated in various ratios (control, 100% P from SSP, 100% P from FYM, 100% P from PM, 75% P from FYM + 25% from SSP, 75% P from PM + 25% from SSP, 50% P from FYM + 50% from SSP, 50% P from PM + 50% from SSP, 25% P from FYM + 75% from SSP and 25% P from PM + 75% from SSP). Pre sowing soil analysis are given in Table 1, while physico-chemical properties of FYM and PM are given in Table 2. Phosphorus was maintained at the rate 75 kg ha⁻¹ for all treatments. Wheat variety Atta-Habib was sown at the seed rate of 120 kg ha⁻¹. Phosphorus solubilizing bacteria were applied through seed inoculation at sowing time. Farmyard manure and PM were applied before a month of sowing, whereas SSP was applied at sowing time. Parameters studied were crude protein in grain (%), grain P concentration in (%), straw P concentration in straw (%), grain P uptake (kg ha-1), straw P uptake (kg ha-1), total P uptake (kg ha-1) and phosphorus use efficiency (%). Crude protein in wheat grains was obtained by multiplying the determined percent grain N concentration by Kjeldahl method with 6.25 (AOAC, 1990). Grain P content in wheat was determined after wet digestion spectrophoto-meterically, using Lambda-35 (Perkin Elmer, Lmbda-35). Straw P was determined by spectrophotometer (Richardson, 1994).

Grain P uptake and straw P uptake was determined as: grain P uptake (kg ha⁻¹) = [grain P concentration (%) x grain yield (kg ha⁻¹)]/100 and straw P uptake (kg ha⁻¹) = [straw P concentration (%) x straw yield (kg ha⁻¹)]/100. Total P uptake was calculated by adding grain P uptake and straw P uptake. Phosphorus use efficiency was calculated according to the formula described by Fageria *et al.* (1997).

Statistical analysis

The data were statistically analyzed by the relevant procedure of analysis of variance for randomized complete block design. Least significant difference (LSD) test at 5% level of significance was carried out for mean comparison among the treatments in the instance of significance (Steel and Torrie, 1980).

Results

Crude protein (%)

Table 3 showed that production years, P management ratios and PSB significantly affected crude protein of wheat, while none of interactions was found significant. Maximum crude protein (11.10%) was recorded in 2016-17 than that of 10.91 (%) in 2015-16.

Among P management ratios, 50% P from PM + 50% from SSP and 50% P from FYM + 50% from SSP resulted in maximum and statistically similar values (12.82 and 12.77% respectively) for crude protein in wheat grain. While minimum crude protein (9.71%) was recorded in treatment which received 100% P from SSP. Phosphorus solubilizing bacterial inoculation yielded significantly higher crude protein (11.15%) as compared to (10.86%) in non PSB inoculated plots.

Grain P concentration (%)

Phosphorus management ratios and PSB significantly affected grain P concentration of wheat in two consecutive growing years (Table 3). All possible interactions were found significant except Y x PSB. Mean values of the data showed maximum P concentration in wheat grain (0.35%) in production year 2016-17 than that of 0.34 (%) in 2015-16. In P management ratios, the application of 50% P from PM + 50% from SSP and 50% P from FYM + 50% from SSP yielded maximum grain P concentration (0.38%), whereas minimum grain P concentration (0.35%) was recorded in both 100 % P from FYM and 100% P from PM. The application of PSB considerably increased P concentration in wheat grain.

Soil properties	Soil properties Unit	
Clay	%	12.7
Silt	%	50.0
Sand	%	37.3
Textural class	-	Slit loam
pH	-	7.67
Organic matter	%	0.81
Phosphorus	mg kg-1	2.31
Potassium	mg kg-1	105.9

Table 1. Pre-sowing soil physico-chemical properties (0-30 cm depth).

Maximum grain P concentration (0.36%) was recorded in PSB inoculated treated plots as compared to 0.35 (%) in non PSB treated plots. In case of PSB x R, increase in grain P concentration was observed in all ratios in the presence of PSB (Fig. 1).

Straw P concentration (%)

Phosphorus concentration in wheat straw varied considerably by production years, P management ratios and PSB (Table 3). Among interactions, Y x R and Y x PSB showed significant variation in P concentration of wheat straw. Significantly higher straw P concentration (0.15%) was measured in production year 2016-17 than that of 0.14 (%) in 2015-16. Regarding P management ratios the application of 50% P from PM + 50% from SSP and 50% P from FYM + 50% from SSP resulted in higher and statistically similar value (0.17%) for wheat straw P concentration. Lower and similar straw P concentration (0.15%) was recorded in treatments which got 100% P from SSP, 100% P from FYM, 100% P from PM, 25% P from FYM + 75% from SSP and 25% P from PM + 75% from SSP. In case of PSB application, maximum straw P concentration of 0.16 (%) was noted in PSB treated plots than that of 0.14 (%) in non PSB treated plots.

Table 2. Physico-chemical properties of FYM and PM used in the experiment.

Quality parameters	FYM	PM
Total Phosphorus (%)	0.41	1.87
Total Nitrogen (%)	0.87	2.34
Total Potassium (%)	0.65	1.45

Grain P uptake (kg ha-1)

A prominent variation in grain P uptake was observed by P management ratios and PSB two production years (Table 4). Among interactions, Y x R and PSB x R were found significant. Higher grain P uptake (14.1 kg ha⁻¹) was recorded in 2016-17 than that of 13.2 (kg ha⁻¹) in 2015-16. Among P management ratios, higher grain P uptake (16.5 kg ha⁻¹) was noted by the application of 50% P from PM + 50% from SSP which was statistically at equivalence to 16.0 (kg ha⁻¹) in 50% P from FYM + 50% from SSP. While lower P uptake by wheat grain (12.7 and 12.9 kg ha⁻¹) was noted in treatments received 100% P from FYM and 100% P from PM respectively. Wheat grain P uptake was also increased by seed inoculation with PSB.

Significantly maximum grain P uptake (14.6 kg ha⁻¹) was noted in PSB inoculated plots as compared to 12.7 (kg ha⁻¹) in the absence of PSB.

The interactive effect of PSB and R indicated that increase in grain P uptake was observed in all ratios with PSB application as compared to without PSB and higher grain P was noted in 50% P from PM + 50% from SSP plots sown with PSB inoculated seed (Fig.2).

Treatments	Crude protein	Grain P	Straw P	
	(%)	concentration (%)	concentration (%)	
Phosphorus Solubilizing Bacteria (PSB)				
Without PSB	10.86 b	0.35 b	0.14 b	
with PSB	11.15 a	0.36 a	0.16 a	
Significance	**	**	**	
Phosphorus management ratios (R)				
100% P from single superphosphate (SSP)	9.71 d	0.36 c	0.15 c	
100% P from farmyard manure (FYM)	10.50 c	0.35 d	0.15 c	
100% P from poultry manure (PM)	10.65 c	0.35 d	0.15 c	
75% P from FYM + 25% from SSP	11.89 b	0.36 c 0.16		
75% P from PM + 25% from SSP	11.91 b	0.36 c 0.16 b		
50% P from FYM + 50% from SSP	12.77 a	0.38 a	0.17 a	
50% P from PM + 50% from SSP	12.82 a	0.38 a	0.17 a	
25% P from FYM + 75% from SSP	10.52 c	0.36 c 0.15 c		
25% P from PM + 75% from SSP	10.52 c	0.37 b	0.15 c	
LSD (P≤0.05)	0.218	0.003 0.01		
Years				
2015-16	10.91 b	0.34 b 0.14 b		
2016-17	11.10 a	0.35 a 0.15 a		
Significance	**	**	*	
Interactions				
PSB x R	ns	** ns		
Y x PSB	ns	ns **		
Y x R	ns	* **		
Y x R x PSB	ns	**	ns	

Table 3. Crude protein, grain P, straw P and grain P uptake by wheat as affected by phosphorus solubilizing bacteria and phosphorus management ratios.

Means with same letter in rows/columns are statistically similar at 5% level of significance. "*" and "**" represent significant difference at 5% and 1% levels of significance respectively while "ns" denotes non-significant effect.

Straw P uptake (kg ha-1)

Production years, P management ratios and PSB significantly affected straw P uptake of wheat as shown in Table 4. In case of interactions, Y x PSB and Y x R were found significant. Maximum straw P uptake (11.9 kg ha⁻¹) was recorded in production year 2016-17 as compared to that of 11.1 (kg ha⁻¹) in 2015-16. Ratios of P management revealed that straw P uptake was maximum (13.7 kg ha⁻¹) in treatment 50% P from PM + 50% from SSP which was statistically at par with 50% P from FYM + 50% from SSP, while it was minimum (11.1 and 11.2 kg ha⁻¹) in plots supplied

with 100% P from FYM and 100% P form PM. Regarding PSB inoculation, greater value for P uptake by wheat straw (12.5 kg ha⁻¹) was observed in the in plots sown with PSB inoculated seed as compared to 10.4 (kg ha⁻¹) in case of without PSB inoculation.

Total P uptake (kg ha-1)

Total P uptake by wheat crop was significantly affected by P management ratios and PSB in two consecutive growing years (Table 4). Moreover, Y x R and Y x PSB also affected total P uptake by wheat crop significantly. Maximum total P uptake (25.9 kg ha⁻¹) was noted in production year 2016-17 than that of 24.3 (kg ha⁻¹) in 2015-16. In case of P management ratios, maximum value for total P uptake (30.2 kg ha⁻¹) was obtained with the application of 50% P from PM + 50% from SSP which was statistically similar to that of at par with 50% P from FYM + 50% from SSP,

while minimum total P uptake (23.8 and 24.1 kg ha⁻¹) was recorded in plots added with 100% P from organic sources alone (FYM and PM respectively). Regarding PSB, maximum total P uptake (27.1 kg ha⁻¹) was noted in PSB treated plots as compared to 23.1 (kg ha⁻¹) in without PSB inoculated treatments.

Table 4. Straw P uptake, total P uptake and phosphorus use efficiency by wheat as affected by phosphorus solubilizing bacteria and phosphorus management ratios.

Treatments	Grain P uptake	Straw P uptake	Total P uptake	PUE (%)
	(kg ha-1)	(kg ha-1)	(kg ha-1)	
Phosphorus Solubilizing Bacteria (PSB)				
Without PSB	12.7 b	10.4 b	23.1 b	19.1 b
with PSB	14.6 a	12.5 a	27.1 a	22.0 a
Significance	**	**	**	**
Phosphorus management ratios (R)				
100% P from single superphosphate (SSP)	14.1 b	12.2 C	26.2 b	20.0 b
100% P from farmyard manure (FYM)	12.7 c	11.1 e	23.8 c	16.8 c
100% P from poultry manure (PM)	12.9 c	11.2 de	24.1 C	17.2 C
75% P from FYM + 25% from SSP	14.1 b	12.1 cd	26.2 b	19.9 b
75% P from PM + 25% from SSP	14.4 b	12.2 C	26.6 b	20.5 b
50% P from FYM + 50% from SSP	16.0 a	13.3 ab	29.3 a	24.1 a
50% P from PM + 50% from SSP	16.5 a	13.7 a	30.2 a	25.3 a
25% P from FYM + 75% from SSP	14.2 b	12.3 c	26.6 b	20.5 b
25% P from PM + 75% from SSP	14.4 b	12.4 bc	26.8 b	20.7 b
LSD (P≤0.05)	0.58	0.91	1.17	1.68
Years				
2015-16	13.2 b	11.1 b	24.3 b	18.2 b
2016-17	14.1 a	11.9 a	25.9 a	22.9 a
Significance	**	*	**	**
Interactions				
PSB x R	*	ns	ns	Ns
Y x PSB	ns	**	*	Ns
Y x R	**	**	**	**
Y x R x PSB	ns	ns	ns	Ns

Means with same letter in rows/columns are statistically similar at 5% level of significance. "*" and "**" represent significant difference at 5% and 1% levels of significance respectively while "ns" denotes non-significant effect.

Phosphorus use efficiency (%)

A significant effect of growing years, P management ratios, PSB and Y x R on phosphorus use efficiency (PUE) of wheat is given in Table 4.

Phosphorus use efficiency was higher (22.9%) in production year 2016-17 than that of 18.2% in 2015-16. Ratios of integrated phosphorus management indicated that 50% P form PM + 50% form SSP and 50% P form FYM + 50% form SSP gave higher and statistically similar values (25.3 and 24.1% respectively) for PUE, while lower values (16.8 and 17.2%) were recorded on treatments 100% FYM and 100% PM respectively.

Regarding PSB application, maximum PUE (22.0%) was recorded in plots treated with PSB, whereas it was minimum (19.1%) in the absence of PSB.

Discussion

Crude protein

Incorporation of organic manures with inorganic P fertilizer in various ratios caused a prominent increase in crude protein in wheat grain than inorganic P fertilizer (SSP) alone. Application of 50% SSP + 50% PM and 50% SSP + 50% FYM gave higher values for grain protein content of wheat. Farhad et al. (2011) found substantial difference in protein content of maize grain by applying PM along with recommended rate of NPK. Our results are in conformity with those of Zafar et al. (2011a) that protein content of maize considerably increased by the application of both DAP and SSP in combination with PM and PSB. Organic fertilizers (FYM, Panchagavya, Jeevamrutha) vermicompost, in combination with inorganic fertilizer (60-75-75 kg NPK ha-1) significantly improved grain crude protein

of rice (Sharada and Sujathamma, 2018). The findings of Jayanthi et al. (2002) further supported our results because they observed maximum protein in oat with FYM and vermicompost in addition with 50% recommended dose of NPK fertilizer. Similarly, Kumawat (2003) stated that the addition of organic fertilizers increased grain and straw N, P and K concentrations and protein content. Combine application of 50% recommended dose + 50% vermicompost increased grain protein content (Choudhary and Kumar, 2013). Inoculation of PSB significantly increased protein content in wheat grain. Crude protein of oat also varied significantly with PSB application (Shabbir et al., 2013). Zafar et al. (2011b) also reported similar findings. Our findings are also agreement with investigations of Yanni et al. (2001) who described that phosphorus solubilizing bacteria inoculation improved crude protein of rice grain.

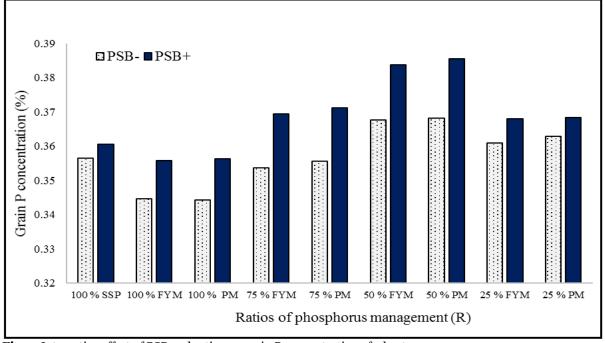


Fig. 1. Interactive effect of PSB and ratios on grain P concentration of wheat.

Phosphorus concentration

Various proportions of integrated P management caused magnificently enhanced P concentrations in wheat grain and straw. Greater P concentration in wheat grain and straw was recorded by applying 50% PM + 50 SSP and 50% FYM + 50% SSP. The combine use of FYM and DAP resulted the highest P concentration in plants (Tadesse *et al.*, 2013). Our investigations are in agreement with the results of Akhtar *et al.* (2016) who investigated that combined use of inorganic P and FYM (50% DAP + 50% FYM) improved shoot P concentration of maize compared to sole application of inorganic P (100% P from DAP). An increasing trend was observed in straw and grain P concentration of wheat with PSB inoculation than un-inoculated control. Similarly, Ram *et al.* (2015)

reported that phosphorus solubilizing fungi significantly affected grain and leaves P concentration of wheat.

Phosphorus uptake

Ratios of integrated phosphorus application indicated that more grain, straw and total P uptake was associated with the application of 50% PM + 50% SSP and 50% FYM + 50% SSP. Various factors are responsible for nutrients uptake i.e. nutrients availability, water availability, soil aeration, root growth, other soil correlated characters and climatic conditions (Leigh and Jones, 1984).

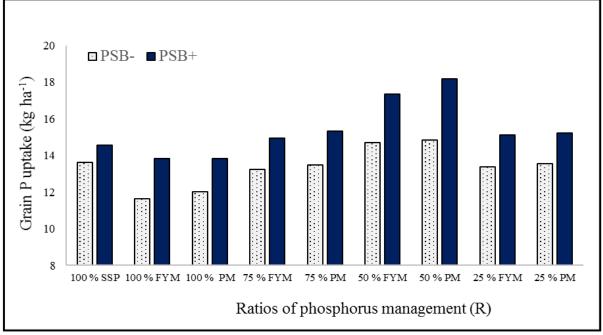


Fig. 2. Interactive effect of PSB and ratios on grain P uptake of wheat.

The presence of a nutrient in available form is very much important for plants and it has been observed that soil parameters (N, P, K and organic matter contents) considerably enhanced with the addition of organic amendments alone or in combination with inorganic fertilizers (Sarwar et al., 2008). Thus, plant nutrients uptake increased with the integrated use of chemical fertilizers and organic manures (Sarwar et al., 2009). Garg and Bahl (2008) concluded that poultry manure supplemented with mineral P fertilizer increased the available P and its uptake. Awad et al. (2014) stated that P uptake by maize significantly increased due to integrated use of organic (GM and PM) and inorganic manures. Pattanayak et al. (2001) reported that the SSP when amended with organic matter (GM) resulted in maximum P uptake. Gupta (2003) and Selvakumari et al. (2000) observed higher P uptake with integrated management of organic and inorganic

consequently increase plant P uptake (Chen *et al.*,
ed that 2006). The availability of fixed phosphates can be increased for plant growth by phosphate solubilizing microbes (Shah *et al.*, 2001; Ekin, 2010). Stimulatory

microbes (Shah et al., 2001; Ekin, 2010). Stimulatory effect of Pseudomonas on P uptake by wheat, cotton and maize crops was also reported by (Egamberdiyeva al., 2004). Phosphorus et solubilizing bacterial inoculants increase P uptake in several crops (Rodriguez and Fraga, 1999; Gulati et al. 2007). Similar findings were also reported by Hossain et al. (2004), Karpagam and Nagalakshmi (2014) and Sharif et al. (2015).

phosphorus sources. Similarly, our findings about P

uptake are also in line with the results of Yaduvanshi,

(2001). A significant increase in grain P uptake in

Phosphate solubilizing bacteria may convert insoluble

phosphorus to soluble and available form and

wheat was observed by the inoculation with PSB.

Phosphorus use efficiency

A prominent variation in PUE was observed by the integrated use of phosphorus in different proportions. Greater PUE was recorded by the combine use of organic manure (PM and FYM) and chemical P fertilizer (SSP) in 50:50 (50% PM + 50% SSP and 50% FYM + 50% SSP).Organic matters provide substrate for microbial growth, produce organic acids which dissolve fixed P, release additional plant nutrients as well as improve soil physical and biological properties which might be the probable reason for better results (Majumdar et al., 2002). Higher PUE of maize crop was observed by the combine use of PM and chemical fertilizers in 50:50 proportion (Zafar et al., 2011b; Zafar et al., 2013). Organic amendment along with inorganic P fertilizer increased the recovery of P (18-27%) by maize crop (Meena 2010). Yaseen and Malhi (2010) obtained similar findings for PUE. Comparing the relevant treatments with PSB inoculation, an increasing trend was observed in PUE by wheat crop with PSB inoculation than uninoculated treatments. This increase might be due to the increased availability of P by efficient P solubilizers in the rhizosphere by the production of aromatic and aliphatic acids, phosphatases and phospholipases (Chhonkar and Tilak, 1997). Many researchers (Zafar et al., 2011b; Zafar et al., 2013; Abbasi et al., 2015) observed a significant increase in PUE by phosphorus solubilizing microorganisms.

Conclusion

It is concluded from the results that seed inoculation with PSB significantly improved crude protein, grain and straw P concentrations, P uptake and PUE. Integrated use of organic manures and inorganic fertilizer was superior to sole application of organic manures or inorganic fertilizer.

The application of 50% P from PM + 50% from SSP and 50% P from FYM + 50% from SSP proved superiority for quality wheat production, P-uptake and PUE and thus, recommended for enhancing P availability for wheat crop in the agro climatic condition of Peshawar-Pakistan.

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