Agricultural Sciences Journal
Available online at http://asj.mnsuam.edu.pk/index.php
ISSN 2707-9716 Print
ISSN 2707-9724 Online
https://doi.org/10.56520/asj.v6i2.387

Research Article

PHOSPHORUS BIOFERTILIZER AS EFFICIENT FERTILIZER FOR IMPROVING NUTRIENT UPTAKES AND CORN YIELD

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Abstract

Phosphorus use efficiency in soils of Pakistan is reported less than 15%. However, phosphorus (P) bio-fertilizers have potential to improve P use efficiency (PUE) in these soils on sustainable basis. A primary study was conducted under control conditions of greenhouse to compare P availability from reduced rates of phosphorus bio-fertilizer (PSB-DAP) with P available from 100% rate of DAP recommended for maize. Based on the findings, a field study was conducted to evaluate the effects of 100% rate of uncoated DAP and 50-75% rate of PSB-DAP on corn yield and PUE. The treatment for both studies included; control (with fertilizers), 100% of recommended P_2O_5 from Commercial DAP (C-DAP_{100}), 75% of recommended P_2O_5 from PSB-DAP (PSB-DAP_{75}) and 50% of recommended P_2O_5 from PSB-DAP (PSB-DAP_{50}). Results explored that available P from PSB-DAP_{50} and C-DAP_{100} was statistically at par while PSB-DAP_{75} at 30, 45 and 60 days of incubation. The findings of field study also revealed that PBS-DAP improved cob weight by 5-10%, chlorophyll contents by 14-23%, 1000-grains weight by 8-10%, grain yield by 10%, and P uptake by 35%, PUE efficiency by 42% compared to C-DAP_{100}. This work can be used as a case study to promote P bio-fertilizers among farming community and researchers in order to for make effective use of alternate P sources for crop production

Keywords: PSB, biofertilizer, impregnated DAP, PUE, maize.
(Received: 18-Apr-2024 Accepted: 12-Jul-2024) Cite as: Ahmed. W., Ayesha. Z., Mahmood. A., Ghaffar. A., Siddiq. S., Ikram, R.M., Kamran, A., Ud-Din, A.S., Jahan, T.S., 2024 Phosphorus biofertilizer as efficient fertilizer for improving nutrient uptakes and corn yield. Agric. Sci. J. 6(2): 84-90.

1. INTRODUCTION

Maize is one of the most important crop and globally known as queen of cereals (Gong et al., 2024). There is a continuous increase in the gaps between potential yield and actual productivity of maize. Better phosphorus (P) management practices can be used to exploited through the adoption of hybrids (Ciampitti and Vyn, 2014). Phosphorus is the second most important macronutrient after nitrogen (N) (Pandey, 2018). Its sufficient supply is essential for seedling growth, early rooting and maturity, formation and quality of seed and photosynthetic water use efficiency (Memon et al., 2011). It is also required for ATP biosynthesis during respiration and photosynthesis and energy transfer (Reddy et al., 2002). Thus, soil deficient in P is a major challenge because 80% of applied P from synthetic fertilizers immediately either precipitates or converts to plant unavailable P (Fernandez et al., 2007).

Phosphorus use efficiency (PUE) is poor in soils featured with calcareousness, alkaline pH and deficient organic matter. Most of cultivated soils of Pakistan (90%) are P deficient because 75-85% of applied P is lost (Memon et al., 2011). Therefore, phosphorus management always remained among first preferences while considering the macronutrients supply to the crops (Salazar et al., 2011, Pandey, 2018). However, recent development in soil microbiology is expected to resolve the low PUE in such soils.

Soil microbes especially phosphorus solubilizing bacteria (PSB) play a significant role in phosphorus dynamics of alkaline or calcareous soils. These PSB prolong P availability to plant roots in soils by two mechanisms; 1) solubilize the fixed P through organic acids and 2) avoid precipitation of Ca^{+2} and Mg^{+2} in alkaline and calcareous soils (Afzal and Bano, 2008). Moreover, the PSB lowers pH to stop the adsorption of P on calcium carbonate surface. In this way, PSB may play critical roles in sustainable agriculture models (Estrada-Bonilla et al., 2017).

The role of P fertilizers for cereal crops throughout the world cannot be ignored. Maize is one of the key cereal crop after wheat and rice. It provides proteins, (10%), carbohydrates (72%), oils (4.8%), fiber (5.8%) and mineral. In calcareous soils, hybrid cultivars of maize fail to give their potential yield due to poor PUE and thus per acre maize yield in Pakistan is too much lower than important maize producers. Arif et al. (2018) reported a significant improvement in P physiological and recovery efficiency when they incubated seeds with PSB. Keeping in view all above discussion and based on the findings of Arif et al. (2018), the research was conducted to evaluate the effects of PSB coated DAP with 25-50% of recommended P for improving PUE and yield of maize.

2. Materials and Methods

2.1. Experimental site

Lab study was conducted in the laboratory of Department of Soil and Environmental Sciences while field study was carried out at the research area of MNS University of Agriculture Multan, Pakistan (30°08'51"N 71°26'36"E, 122.19 m) during 2017-2018.

2.2. Experimental soil characteristics

The soil samples (0-15 cm depth) from the experimental site were collected and analyzed (Table 1). The soil used was of loam texture. The soil of experimental site was of loam texture with deficient nutritional and organic matter status and alkaline in nature (Table 1). The soil from the same experimental field was used during pot study.

Table 1: Experimental field's physico-chemical soil characteristics

Parameter	Unit	Value
Textural class	-	Loam
Organic matter	%	0.49
ECe	dS m^{-1}	2.11
pH	-	8.00
CEC	Cm_c kg^{-1}	5.29
Total N	%	0.039
Available P	mg kg^{-1} soil	6.5
Extractable K	mg kg^{-1} soil	110

2.3. Lab study

The treatment plan of lab study included control (soil without DAP, T_1), 100% of recommended P_2O_5 from commercial DAP (T_2) and 75% of recommended P_2O_5 from PSB inoculants (PSBI) and three incubation periods (30, 45 and 60 days of incubation). The lab study was laid down using two factorial CRD with four replications. Fine ground soil (100 g cup^{-1}) taken from experimental field was mixed with FFC commercial DAP (1.00 g cup^{-1}) and polymer coated DAP (0.75 g cup^{-1}). The DAP mixed soil was filled in disposable cups and placed in an incubator (Syano Incubator) for incubation up to 60 days. Water required for soil saturated paste was measured and 75% of the calculated water (equivalent to field capacity) was added to each cup and weighed. Later on, the moisture in the soil of each cup was maintained on weight basis.

2.4. PSB isolation and inoculation on DAP grains

University of Agriculture, Faisalabad, Pakistan provided a pre-isolated strain (P-10) of PGPR containing ACC-deaminase and phosphatase activity. The strain was multiplied using general purpose medium (GPM) in the conical flasks (250 mL) and used for coating over diammonium phosphate (DAP) using methods discussed by Arif et al. (2018) for coating of PSB on single superphosphate (SSP).

2.5. Field study

Like lab study, a field study was also conducted to evaluate the effects of PSB coated DAP on nutrient use efficiency, growth and yield of maize. Field study was laid out according to randomized complete block design (RCBD) with four replicate. The detail of treatment plan was: T_1 = Control (without P_2O_5 application; DAP_0), T_2 = 100% of recommended P_2O_5 from DAP (DAP_{100}), T_3 = 75% of recommended P_2O_5 from PSB coated DAP (PSB-DAP_{75}) and T_4 = 50% of recommended P_2O_5 from PSB coated DAP (PSB-DAP_{50}). In all treatments, N and K_2O were applied @ 150 and 90 kg ha^{-1} whereas the recommended rate for P_2O_5 was 120 kg ha^{-1}.

2.5.1. Land preparation, treatment application, sowing and data collection

The seedbed was prepared after ploughing and planking. For bed preparation, seedbed planter was used. On each side of bed (2.5 feet wide), seeds of maize was sown manually while maintaining plant-to-plant distance 2 feet. The source of N and K used were Urea and SOP, respectively. All the amount of P and K fertilizers was added before sowing and N was added in three splits i.e. before sowing, at first irrigation and at fourth irrigation. The field was irrigated at Field capacity. For weed control, pre-emergence "Pendimethalin" was used while post-emergent weeds were eradicated manually. For control of sucking and chewing insects, Syngenta company products were used after pest scouting.

The data related to growth attributes was taken before harvesting of crop while yield attributes was taken after harvest of crop. Similarly, data related different physiological parameters such as photosynthetic rate, transpiration, chlorophyll contents was collected using Portable Chlorophyll meter SPAD-502 and IRGA (Infra-Red Gas Analyzer) as discussed in Ahmed et al. (2020).

2.6. Fertilizer uptakes and use efficiency

Following procedure given by Wolf (1982), Jackson (1962) and Chapman and Pratt (1961), N, P and K concentration in shoot and corn grains were determined. The collected plant samples and grains were dried in Air flow oven at 65˚C till constant weight, ground and stored in a plastic bag till wet digestion. The wet digestion was done using mixture of hydrogen peroxide and sulphuric acid (Ryan et al., 2001). From digested samples, N was measured using Kjeldhal and distillation method whereas P was measured by following spectrophotometric vanadium phospho-molybdate method (Olsen et al., 1954). K content from digested samples was measured using flame photometer (Chapman and Pratt, 1961). N, P, K uptakes were calculated as product of N, P and K concentration and dry weight $plant^{-1}$, respectively while different forms of nutrient use efficiency were also calculated using following formulae used by Ahmed et al. (2020):

$$\mathrm{AUE}\ (\mathrm{kg\ kg^{-1}}) = \frac{\mathrm{GY_T - GY_C}}{\mathrm{NA}}$$

$$\mathrm{AR}(\%) = \frac{\mathrm{NU_T - NU_C}}{\mathrm{NA}} \times 100$$

$$\mathrm{PUE}\ (\mathrm{kg\ kg^{-1}}) = \frac{\mathrm{GY_T - GY_C}}{\mathrm{NU_T - NU_C}}$$

$$\mathrm{IR}\ (\%) = \frac{\mathrm{NU_T - NU_C}}{\mathrm{NU_T}} \times 100$$

$$\mathrm{UTE}(\%) = \frac{\mathrm{GY_C}}{\mathrm{GY_T}} \times 100$$

$$\mathrm{FSF}(\%) = \frac{\mathrm{GY_T - GY_C}}{GY_\mathrm{T}} \times 100$$

Whereas AUE = agronomic use efficiency, GYT = Grain yield in treated plot, GYc = Grain yield in control plot, NUT = nutrient uptake in treated plot, NUc = nutrient uptake in control plot, NA = nutrient applied, AR = Apparent recovery, PUE = Physiological use efficiency, IR = Internal remobilization, UTE = Utilization efficiency and FSF = Fertilizer stress factor. Physiological use efficiency, IR = Internal remobilization, UTE = Utilization efficiency and FSF = Fertilizer stress factor.

Statistical analysis

All the data was analysed using completely randomized design (CRD) for two-way analysis of variance (ANOVA) in lab study while randomized complete block design (RCBD) for one-way ANOVA in field study. The difference in mean values were compared using LSD test at 5% probability level (Steel et al., 1997). The data was analysed statistically using Statistix 8.1©.

3. Result and Discussion:

3.1. Variations in available P under controlled conditions

The data related to available P from PSB-DAP (75%) and commercial DAP (100%) at 30, 45 and 60 days of incubation is shown in Table 2. Results of Table 2 showed a gradual increase in available P with the passage of time. At 60 days of incubation, Olsen P was found at higher from PSB-DAP and commercial DAP. Results show that the Olsen P from 1.00 g commercial DAP recommend P from PBS-DAP and 0.75 g PBS-DAP was statistically at par (Table 2). This might be happened due to the physiological role of PBS strain coated over DAP. As the soil used was alkaline and deficient in organic matter, the P released from the commercial DAP was immediately fixed in the soil where PSB strain was not used. Arif *et al.* (2018) also used the same strain of PSB but coated over single super phosphate (SSP) and reported significant improvements in P uptake and use efficiency in cotton.

3.2. Plant biomass and chlorophyll contents

The findings of field study clearly depict that plants fertilized with PSB-DAP had higher plant dry mass than those plants fertilized with commercial DAP (Fig. 1). Data related to plant dry biomass also reveal that the effect of 100% P from commercial DAP and 50% P from PSB-DAP. However, chlorophyll contents

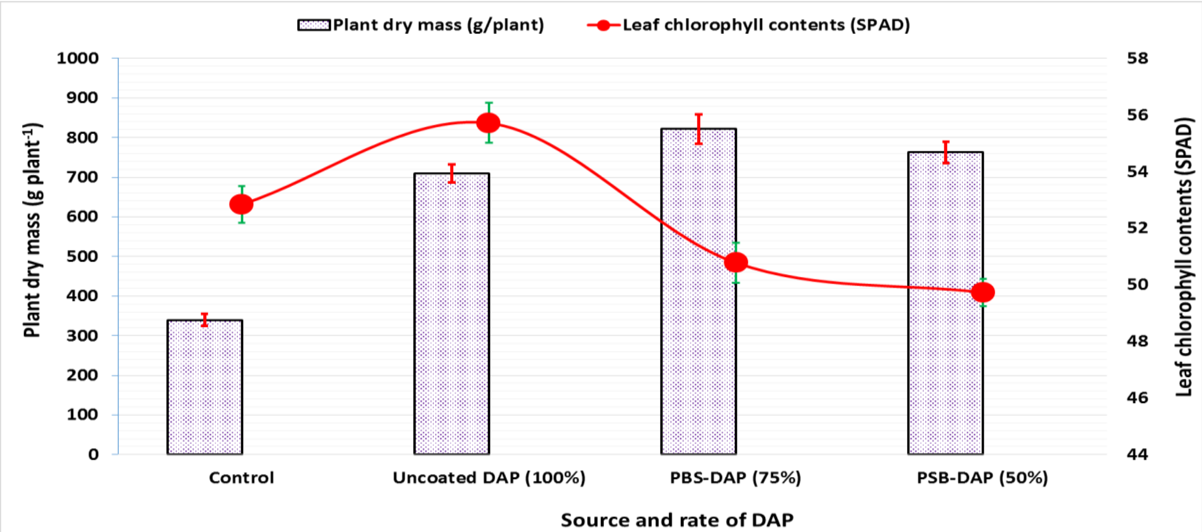

Fig. 1. Comparative effect of P from uncoated DAP and PSB-DAP on plant dry mass and chlorophyll contents of maize. Note: PSB-DAP = PSB coated DAP

Table 3. Variations in growth variables of maize due to polymer coated DAP and uncoated DAP under field conditions

Treatment	Cob weight (g cob^{-1})	1000-grains weight (g)	Grain yield		Water Use Efficiency	
			g plant^{-1}	tons acre^{-1}	Plant WUE	Photosynthetic WUE
Control	136 c	235 c	108 c	1.886 c	18.6 c	0.37 c
DAP_{100}	284 b	423 b	248 b	4.312 b	41.3 b	0.45 b
$PSB\text{-}DAP_{75}$	329 a	490 ab	273 a	4.748 a	45.6 a	0.71 a
$PSB\text{-}DAP_{50}$	305 ab	503 a	250 b	4.356 b	46.3 a	0.77 a
LSD value	*17.740*	*92.462*	*21.073*	*0.30217*	*12.614*	*0.2952*

Values sharing same letter(s) in each column do not differ by each other at p = 0.05 according to LSD test. Plant WUE (g/mmol m^{-2} s^{-1}), Yield per acre was calculated using 17242 plants per acre.

declined in plants where P source was PSB-DAP. This decline in chlorophyll content might be due to lower supply of N from PSB-DAP_{75} and PSB-DAP_{50}. But contrary to chlorophyll contents, improvement in plant dry mass either due to PSB-DAP_{75} or PSB-DAP_{50} might be occurred due to more supply of P to plant roots as it was observed in lab study that PSB showed positive effect on Olsen P.

3.3. Grain yield attributes and water use efficiency

We observed that the plants having supply of P from PSB-DAP_{75} and PSB-DAP_{50} improved cob weight, 1000-grains weight and yield per plant compared to yield of plants treated with 100% P from uncoated DAP (Table 3). Significant improvement in cob weight and 1000-corn grains was also recorded due to the dynamic role of PSB. The PSB not only reduced the DAP rate of application but also resulted in 10% more grain yield. This might be happened due to physiological role of P in grain and root development. Like yield per plant, similar improvements were also found in water use efficiency (WUE). We also noticed a gradual increase in both types of WUE; Plant water use efficiency and photosynthetic water use efficiency due to P supply from PSB-DAP_{75} and PSB-DAP_{50} as compared 100% P supply from uncoated DAP (T_2). Arif *et al.* (2018), Yaseen *et al.* (2018) and Billah *et al.* (2015) also reported improvement in yield attributes cotton and wheat due to microbial activity. Improvements in grain weight and water use efficiency occurred might be due to sufficient P supply which is essential for maturity, photosynthetic WUE and formation and quality of seed (Memon *et al.*, 2011).

3.4. Plant NPK contents

In calcareous soils, nutrient uptake is of greater concern. Nutrient losses in these soils are higher i.e. 60-90, 90, 60 and 60% losses of N, P, K and other nutrients, respectively. We used the sum of shoot and grain N contents (mg NPK plant-1) as an approximate measure of total N uptake by

Table 4. Comparative effects of PBS-DAP and uncoated DAP on plant N, P and K concentration and uptake in maize under alkaline/ calcareous conditions

Treatment	NPK concentration ($mg\ g^{-1}$)			NPK contents ($mg\ plant^{-1}$)*		
	N	P	K	N	P	K
Control (T_1)	7.1 b	3.65 c	6.4 c	32 c	0.39 c	33 c
DAP_{100} (T_2)	10.2 a	4.21 b	8.0 b	90 a	1.04 b	45 c
PSB-DAP_{75} (T_3)	7.7 b	4.52 a	10.4 a	60 b	1.23 a	91 a
PSB-DAP_{50} (T_4)	8.7 b	4.36 b	9.8 a	50 b	1.09 b	68 b
LSD values	*2.2044*	*0.1533*	*1.1731*	*25.103*	*0.1725*	*16.367*

Values sharing same letter(s) in each column do not differ by each other at p = 0.05 according to LSD test. *NPK uptake

Table 5. Comparison among different forms of PUE in maize grown on calcareous soil

Treatment	PAUE ($g\ g^{-1}$)	PRE (%)	PPUE ($g\ g^{-1}$)	IPR (%)	PUTE (%)	PSF (%)
Control (T_1)	-	-	-	-	-	-
DAP_{100} (T_2)	37 c	17 c	215 a	62 c	44 b	56 a
PSB-DAP_{75} (T_3)	59 b	30 b	197 c	68 a	40 b	40 b
PSB-DAP_{50} (T_4)	76 a	37 a	204 b	64 b	43 a	43 b
LSD values	*12.614*	*4.132*	*5.234*	*1.042*	*2.2044*	*10.422*

Values sharing same letter(s) in each column do not differ by each other at p = 0.05 according to LSD test. P agronomic use efficiency (PAUE), P recovery efficiency (PRE), P physiological use efficiency (PPUE), Internal P remobilization (IPR), P utilization efficiency (PUTE) and P stress factor (PSF) plant. We noted the greatest N uptake in plants subjected to treatment T4 while the least N uptake in plants of control treatment without application of fertilizers (Table 4). Table 3 also suggests that the P uptake by plant varies with P supply source to plants. Our calculations explore that the P uptake is dependent on P source. The P released from peaks in plants subjected to treatment T3 (Table 4). In contrast, P coming out from DAP resulted in a decrease in P uptake by plant. In nutshell, we suggest the usage of PSB based fertilizers in calcareous soil for effective P uptake by plants. The P uptake by plants significantly affected plant biomass. The plants subjected to treatment T_2 showed the greatest N uptake while the plants of control treatment had the least N and K uptake (Table 4). The plants with greater P uptakes also expressed greater K uptake and vice versa (Table 4). Yaseen et al. (2018) also reported similar findings.

3.5. Phosphorus use efficiency

Maize plants subjected to different DAP fertilizers responded differently for PUE. Plants subjected to treatment T_4 (PSB-DAP_{50}) had the highest PAUE (204 g grain/g P applied), and PRE (37%) and PUTE (43%), followed by T_3 (PSB-DAP_{75}) (Table 5). Alike, Table 5 also reveal that the plants subjected to PSB-DAP_{75} showed the highest IPR (68%). We suggest that this higher PRE is due to higher P uptake by the plants supplied with PSB-DAP (Table 4). The decline in PPUE in treatment T_3 (PSB-DAP_{75}) and treatment T_4 (PSB-DAP_{50}) i.e. 197 g grain/g and 204 g grain/g P applied, respectively, were observed compared to treatment T_2 i.e. 215 g grain/g P applied (Table 5). This decline in PPUE suggests effective conversion of absorbed P into grains or economical portion of maize. The plants subjected to different forms of DAP behave contrarily for P utilization efficiency (PUTE) and P stress factor (PSF). The treatment PSB-DAP_{75} had the lowest PUTE (40%) compared to uncoated DAP (44%) whereas PSF in plants treated with PSB-DAP_{75} was 40% compared to 56% PSF in plants subjected to uncoated DAP, indicating that PSB kept P in available form and enhanced P uptake (Table 4). These improvements in P use efficiency occurred might be due to physiological role of P and effective P uptake in plants. Arif *et al.* (2018), Yaseen *et al.* (2018) and Billah *et al.* (2015) also reported similar findings in PUE in cotton and wheat respectively due to PSB. Improvements in yield attributes occurred might be due to sufficient P supply which is essential for maturity, photosynthetic WUE and formation and quality of seed (Memon *et al.*, 2011).

4. Conclusion

PSB coated DAP (Phosphatic bio fertilizer) caused a significant increase in plant biomass, chlorophyll contents, water and P use efficiency and ultimately corn grain yield even after application of 25 to 50% P from recommended P for maize. This study suggests that P biofertilizer performed better under reduced application of 50% of the recommended P. It also suggests that PSB coated DAP has a great potential to cut down pressure on chemical fertilizers and P exhausting resources. Based on the findings of these studies, researchers and breeders can develop such P responsive cultivars, having potential to utilize major portion of unavailable P.

5. Conflict of Interest

There is no conflict of interest among the authors related to this work.

6. Acknowledgement:

The authors are thankful to the Soil Microbiology Lab, ISES, UAF for provision of PSB strains and Soil Fertility Lab, ARRI, Faisalabad for providing facility of soil and plant analysis.

7. Author's Contribution:

All the authors contributed in their domains related to lab and field study.

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