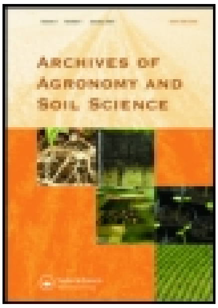

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Phosphorus-microbes interaction on growth, yield and phosphorus-use efficiency of irrigated cotton

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Phosphorus–microbes interaction on growth, yield and phosphorus-use efficiency of irrigated cotton

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The phosphorus-use efficiency of crops in high pH soil is low. A randomized complete block design in a 3 × 2 split-plot experiment was conducted on a high pH silt loam (Typic Ustochrepts) to evaluate whether P-solubilizing microbial (PSM) inocula were able to improve the P fertilization effects on irrigated cotton (*Gossypium hirsutum* L., cultivar CIM-482). Cotton was planted after seed treatment with PSM inoculation at 0, 22 and 44 kg P ha^{-1}. Results showed that soil microbial populations were significantly higher throughout the cotton-growing season in response to P fertilization and PSM inoculation. Both P fertilization and PSM inocula exerted a significant effect on cotton biomass and P uptake without an interaction. Economic analyses suggest that PSM inocula alone significantly increased P-use efficiency (8%), reduced cost and improved net income (by \$36 ha^{-1}) of irrigated cotton production. Moreover, the relationship between relative yield and P fertilization with PSM inocula showed that 95% of the maximum yield of cotton was produced at 22 kg P ha^{-1}, whereas in the absence of PSM inocula, 95% relative yield was obtained at 36 kg P ha^{-1}, a saving of ~39% applied P with PSM inoculation.

Keywords: PSM inocula; high pH soil; relative yield; P-use efficiency; economics

Introduction

Phosphorus is the second most important essential macronutrient vital for crop growth. Soils are usually supplemented with P in the form of chemical fertilizers. However, a large portion of P fertilizer is often immobilized rapidly after application through fixation and/or precipitation with highly reactive aluminum (Al^{3+}) and iron (Fe^{3+}) in acidic soils and calcium and magnesium in calcareous soils, and thus becomes unavailable to plants (Malik et al. 1992; Hao et al. 2002; Dorahy et al. 2004). Phosphorus is often considered as limiting nutrient in both low and high pH soils worldwide.

The phosphorus-use efficiency (PUE) of crops generally ranges from 10 to 25% (Adesemoye and Kloepper 2009). To improve PUE of crops, researchers have focused on using P-solubilizing microorganisms (PSM) in sustainable agricultural practices (Richardson 2001; Vessey 2003; Chen et al. 2006; Adesemoye and Kloepper

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2009). It is reported that PSM are involved in a range of processes and/or properties that influence the soil P cycle (Chen et al. 2006). PSM, in association with the plant rhizosphere, are capable of increasing P availability to plants either by solubilization of insoluble phosphates by production of acids or by mineralization of organic phosphates (Cunningham and Kuiack 1992; Rodriguez and Fraga 1999; Orhan et al. 2006). Insoluble phosphates are transformed into soluble forms by microbial–plant excretion of organic acids containing hydroxyl and carboxyl groups that chelate phosphate-bound reactive cations (Al, Fe and Ca) and improve P availability in the soil (Cunningham and Kuiack 1992; Nahas 1996; Adesemoye and Kloepper 2009). Moreover, PSM improve the agronomic efficiency of applied P fertilizers (Venadan and Subramanian 2000). Several studies have shown the beneficial effects of PSM inoculums on agronomic crops (Chabot et al. 1993; Singh and Kapoor 1999).

High fertilizer costs coupled with low PUE often limit cotton production in soil with higher contents of Ca and Mg carbonates (Malik et al. 1992). Moreover, limited information is available on the effects of PSM inocula to improve PUE of irrigated seed cotton on high pH soil under semi-arid climatic conditions (Illmer and Schinner 1992; Dorahy et al. 2004; Rochester 2007). The objective of this study was to evaluate the effectiveness of PSM inoculations on growth and yield, nutrient uptake and economics of irrigated seed cotton at various levels of P fertilization on a high pH soil under semi-arid climate.

Materials and methods

Site characteristics

The field experiment was conducted at the Central Cotton Research Institute (29°22′N latitude and 72°45′E longitude), Multan, Pakistan in 2005 and 2006. The soil is a high $CaCO_3$ content Sultanpur silt loam (coarse, silty, mixed, hyperthermic, Typic Ustochrepds) widely distributed in the farm and extensively used for growing irrigated cotton. Basic soil characteristics in 0–30 cm soil depth were: pH, 8.1; electrical conductivity, 2.2 dS m^{-1}; organic matter, 4.9 g kg^{-1}; total N, 0.4 g kg^{-1}; ammonium acetate extractable K, 126 mg kg^{-1}; sodium bicarbonate extractable P, 6 mg kg^{-1}; and total P, 0.92 g kg^{-1}. The climate of the region is characterized by two distinct seasons, a very hot and humid summer from May to September and a winter from November to March. Mean annual rainfall ranged from 140 to 180 mm. About two-thirds of the rainfall is received during the monsoon period (mid-June to September).

PSM population culture and seed treatment

Soil samples before sowing of cotton crop were collected from the experimental field at 0–15 cm depth and analyzed for PSM isolation and culture in the Soil Bacteriology laboratory of the Ayub Agricultural Research Institute, Faislabad, Pakistan. Peat was used as a carrier for PSM inocula. A sample of 250 g finely ground peat was mixed with 50 g cane sugar and 200 mL distilled water to make a homogeneous paste. Cultured PSM inocula (*Bacillus* sp.) were thoroughly mixed with the paste (at 50% moisture level, w/w) with total viable counts (TVC; *Bacillus* sp.) of 6×10^7 cells g^{-1}. Delinted cotton seed was uniformly coated with the paste at a rate of 70 g (3×10^7 TVC) kg^{-1} seed for 1 h before planting.

Experimental treatments and cultural practices

A randomized complete block design in 3 × 2 split-plot arrangement with four replications (plot size 12 × 13 m) for each treatment combination was laid-out in the field. Experimental treatments were consisted of three rates of P fertilization: 0, 22 and 44 kg ha^{-1} as the main plot and two seed treatments with PSM (untreated vs. treated) as subplots.

Cotton was planted in mid-May at a spacing of 75 cm between rows and 30 cm between plants. Basal fertilization of 150 kg N ha^{-1} as urea and 41 kg K ha^{-1} as sulfate of potash was applied to all the plots. Phosphorus was applied in the form of triple superphosphate (TSP) with 20% soluble P. A full dose of P, K and one-third N was applied at the time of planting. The remaining N was applied in two equal splits, at flowering and boll formation stages, respectively. Cultural practices were performed as required. Cotton was furrow irrigated 12 times during the cropping season with a total volume of 2800 m^3. Seed cotton was harvested manually from each plot. P concentration in seed, lint, bur, leaf, and stalk was determined according to Ryan et al. (2001).

PUE of cotton was calculated using the following equation:

$$\text{PUE}(\%) = \left[\begin{pmatrix}\text{P uptake by}\\ \text{fertilized plants}\end{pmatrix} - \begin{pmatrix}\text{P uptake by}\\ \text{control plants}\end{pmatrix} * 100\right] \Big/ (\text{P applied}).$$

Soil sampling and analysis

Composite soil samples were collected from 0–15 and 0–30 cm depths prior to the establishment of the experiment in 2005, at maximum vegetative growth (75 days after planting; DAP), and maturity (150 DAP) of the cotton. A portion of the field-moist soil (0–30 cm soil depth) was sieved through a 2-mm mesh, air-dried at room temperature for 15 days before analysis for selected soil properties. Soil pH was measured by a glass electrode pH meter. Electrical conductivity of the saturated extract was measured by the conductivity meter. Soil K was extracted by 1 M neutral ammonium acetate and determined in the extract by flame photometry. Sodium bicarbonate extracted P was determined by the ascorbic acid method (Murphy and Riley 1962). Soil total P was determined by wet digestion (Olsen and Sommers 1982). The Walkley and Black wet-oxidation method was used to measure total soil organic carbon (Jackson 1973).

Another portion of the 2-mm sieved field-moist soil collected from 0–15 cm depth was taken for measuring PSM populations. For assessing PSM population, the processed soils were serially diluted, spread plated in Petri dishes containing Pikovaska's agar (Pikovskaia 1948), and incubated at $30 \pm 1°$C for 7 days. The tricalcium phosphate clearing zone forming colonies (Figure 1) were counted as PSM cells (total PSM counts g^{-1} of oven-dried equivalent soil weight).

Statistical analysis

Data were analyzed statistically using SAS (SAS Institute 2008). Simple effects of P fertilization and PSM inoculations over time and their interaction were evaluated using two-way analysis of variance and *F*-protected least significant difference test ($p \leq 0.05$) unless otherwise mentioned. Relative yield of cotton was regressed on

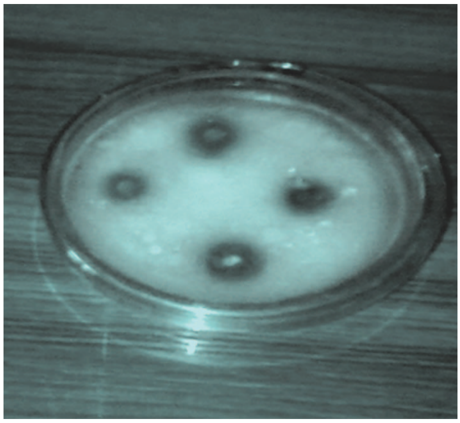

Figure 1. Solubilization of tricalcium phosphate and halozone formation by phosphate-solubilizing microbial (PSM) populations.

P levels with and without PSM inocula using the boundary line technique (Webb 1972) to calculate routine fertilization rates of P and PSM inocula to achieve at least 95% of irrigated cotton yields.

Economic analysis

Economic analysis was done by calculating the total cost of production; market value and total income from seed cotton produced in control and in P use with or without PSM inocula. Net income was calculated by deducting the total income of control and PSM inocula at 0 P-level from the respective total income obtained at applied P-levels of 22 and 44 kg ha^{-1}. The difference between mean values of control and PSM inocula across P-levels was taken as net income obtained from PSM inocula.

Results and discussion

Soil PSM population dynamics

Soil analysis showed that PSM populations increased over time with P fertilization and PSM inoculations (Table 1). P fertilization significantly increased PSM populations irrespective of season. PSM populations were significantly increased by 15–27% at maximum vegetative growth and 41–69% at maturity of cotton by P fertilization as compared with the control. The P fertilized plots treated with PSM inocula maintained a level of PSM populations than the control plots. In other words, PSM inocula were effective at increasing PSM populations, irrespective of P fertilization.

Averaged across P fertilization rates, the initial PSM populations of 11×10^5 TVC g^{-1} of soil increased at maximum vegetative growth (75 DAP) and then decreased considerably at maturity (150 DAP) of cotton. Even in the control, PSM populations increased over time from their initial values. Although PSM populations decreased at cotton maturity, the populations were higher compared with those at the pre-plant stage. Averaged across years, PSM populations increased at maximum

Table 1. The effect of phosphorus (P) fertilization and phosphate-solubilizing microbial (PSM) inocula on PSM populations at maximum vegetative growth and maturity of irrigated cotton in high pH soil under a semi-arid climate (data combined over 2005 and 2006).

		PSM population (Total PSM counts ($\times 10^5$ g^{-1} soil))		
P level ($kg\ ha^{-1}$)	PSM inocula	Initial (0 DAP)	Max vegetative growth (75 DAP)	Maturity (150 DAP)
Mean of all P levels	Control	11	181B[1]x[2]	29By
	PSM	11	214Ax	50Ay
			P × PSM interaction	
0	Control	11	157	23
	PSM	11	188	35
	Mean		173b[3]x	29by
22	Control	11	185	30
	PSM	11	213	52
	Mean		199ax	41ay
44	Control	11	200	34
	PSM	11	240	63
	Mean		20ax	49ay

Notes: [1]Means separated by upper case letter (A vs. B) in each column are not significantly different at $p \leq 0.05$ between PSM treatments. [2]Means separated by lower case letter (x and y) in each row are not significantly different at $p \leq 0.05$ between cotton growth stages. [3]Means separated by lower case letter (a vs. b) in each column are not significantly different among $p \leq 0.05$ at P levels.

vegetative growth (by 18%) and maturity (by 72%) of cotton over controls. PSM populations, in general, were higher at maximum vegetative growth than at cotton maturity.

Significantly higher PSM populations at maximum vegetative growth of cotton suggested that the young and actively growing cotton plants may have provided a favorable environment to support higher PSM populations. By contrast, a significant decrease in the number of PSM populations at maturity of cotton was related to the decreased live root activity. A higher proportion of PSM populations in the rhizosphere are metabolically more active than PSM populations in other environments (Zoysa et al. 1999; Vazquez et al. 2000; Ponmurugan and Gopi 2006). As a result, PSM populations were affected synergistically by activities and/or excretion of the living roots of young cotton plants. Young plant roots selectively stimulate the growth of different microbial species including PSM in the rhizosphere via root exudation of sugars, amino acids, organic acids, hormones and vitamins (Kourtev et al. 2003; Bais et al. 2004). Consequently, PSM populations responded more to root exudation of young and growing cotton plants than to that of matured plants. A significant increase in PSM populations by P fertilization was due to the positive effects of P on plant root exudation of metabolically active compounds in the soil.

Cotton biomass distribution, yield, and phosphorus uptake

PSM inocula increased cotton fruit biomass with a simultaneous decrease in stalk production. Moreover, PSM inocula have significantly increased seed cotton yield by 3% over the control. Similarly, P fertilization significantly increased cotton fruit biomass (by 3–5%) and yield (by 9–16%), but decreased stalk and leaf biomass production (Table 2). Egamberdiyeva et al. (2004) reported that phosphate-solubilizing bacteria combined with phosphate had a significant effect on dry matter accumulation of cotton plant. P fertilization and PSM inocula did not exert any significant interactions on cotton biomass and yields, however, the PSM inocula improved P fertilization effects on cotton fruit biomass and yields.

Among the cotton biomass components, the highest P concentration was found in seed followed by leaves, stalks and burs, respectively. PSM inocula did not increase the cotton P content significantly except in leaves (by 9%) compared with control. By contrast, P fertilization alone significantly increased the P concentration by 7–11% in seeds, 8–12% in leaves, 18–24% in stalks and 33% in burs (Table 3). However, lint P concentration was not affected significantly by P fertilization or PSM inocula.

The highest P uptake was observed in cotton seeds (by 37–65%) followed by leaves (by 20–28%) and stalks (by 9–15%), respectively (Table 4). Averaged across P fertilization rates, PSM inocula significantly increased P uptake in cotton seeds (by 17%) and stalks and leaves (by 6–7%), including total P uptake by 17% compared with control. Moreover, PSM inocula increased PUE by 8%. P fertilization significantly increased the P uptake in cotton seeds (by 37–65%), leaves (by 20–28%) and stalks (by 9–15%). Total P uptake by cotton was increased by 29–50% in response to P fertilization. PUE of cotton was significantly and nonlinearly increased (by $> 20\%$) in response to P fertilization. Moreover, the PSM inocula synergistically improved the P uptake and PUE of cotton, irrespective of P treatments.

Significantly higher cotton yield by P fertilization with or without PSM inocula may be due to increasing diversion of the vegetative portion (leaves + stalk) towards the reproductive portion (fruit/seed) of cotton. PSM inocula could have synergistically influenced the effects of P fertilization to facilitate greater diversion of

Table 2. The effect of phosphorus fertilization (P) and phosphate-solubilizing microbial (PSM) inocula on irrigated cotton biomass production and yields under a semi-arid climate (data combined over 2005 and 2006).

P level (kg ha^{-1})	PSM inocula	Biomass production (%)			Yield ($Mg\ ha^{-1}$)
		Leaf	Stalk	Fruit	
Mean of all P levels	Control	17.9A[1]	30.3A	51.8B	1.89B
	PSM	16.9A	28.3B	54.7A	1.95A
		P × PSM interaction			
0	Control	18.6ns	32.8	48.6	1.75
	PSM	18.2	29.6	52.2	1.79
	Mean	18.4a[2]	31.2a	50.4c	1.77c
22	Control	18.2	29.5	52.3	1.89
	PSM	17.0	27.7	55.1	1.97
	Mean	17.6a	28.6b	53.7b	1.93b
44	Control	16.8	28.6	54.6	2.02
	PSM	15.4	27.6	56.9	2.08
	Mean	16.1b	28.1b	55.8a	2.05a

Notes: [1]Means separated by upper case letters in each column are not significantly different at $p \leq 0.05$ between PSM treatments. ns indicates a non-significant interaction of P levels × PSM inocula. [2]Means separated by lower case letters in each column are not significantly different at $p \leq 0.05$ among P levels.

Table 3. The effect of phosphorus fertilization (P) and phosphate-solubilizing microbial (PSM) inocula on phosphorus concentration of irrigated cotton biomass under semi-arid climate (data combined over 2005 and 2006).

		Biomass P concentration (%)				
P level (kg ha^{-1})	PSM inocula	Leaf	Stalk	Bur	Seed	Lint
Mean of all P levels	Control	0.23B[1]	0.18A	0.09A	0.28A	0.02A
	PSM	0.25A	0.20A	0.09A	0.30A	0.02A
		P × PSM interaction				
0	Control	0.23ns	0.17	0.09	0.27	0.01
	PSM	0.25	0.18	0.09	0.29	0.01
	Mean	0.24b[2]	0.17b	0.09b	0.28b	0.01b
22	Control	0.26	0.19	0.11	0.29	0.02
	PSM	0.27	0.2	0.12	0.31	0.02
	Mean	0.26a	0.20a	0.12a	0.30a	0.02a
44	Control	0.27	0.2	0.12	0.31	0.02
	PSM	0.28	0.21	0.12	0.32	0.02
	Mean	0.27a	0.21a	0.12a	0.31a	0.02a

Notes: [1]Means separated by upper case letters in each column are not significantly different at $p \leq 0.05$ between PSM treatments. ns indicates a non-significant interaction of P levels × PSM inocula. [2]Means separated by lower case letter in each column are not significantly different at $p \leq 0.05$ among P levels.

Table 4. The effect of phosphorus fertilization (P) and phosphate-solubilizing microbial (PSM) inocula on phosphorus uptake and phosphorus-use efficiency (PUE) of irrigated cotton under semi-arid climate (data combined over 2005 and 2006).

		P uptake (kg ha^{-1})				
P level (kg ha^{-1})	PSM inocula	Leaf	Stalk	Fruit	Total	PUE (%)
Mean of all P levels	Control	2.8B[1]	3.6A	14.6A	20.9B	0
	PSM	3.0A	3.8A	17.1A	23.9A	8.1
		P × PSM interaction				
0	Control	2.4ns	3.3	10.9	16.6	0
	PSM	2.7	3.5	12.8	19.0	0
	Mean	2.5b[2]	3.4b	11.8c	17.8c	0b
22	Control	2.8	3.6	14.7	21.2	20.9
	PSM	3.1	3.8	17.7	24.6	25.5
	Mean	3.0a	3.7a	16.2b	22.9b	23.2a
44	Control	3.1	3.9	18.1	25.1	19.3
	PSM	3.2	4.0	20.9	28.2	26.4
	Mean	3.2a	3.9a	19.5c	26.7a	22.8a

Notes: [1]Means separated by upper case letter in each column are not significantly different at $p \leq 0.05$ between PSM treatments. ns indicates non-significant interaction of P levels × PSM inocula. [2]Means separated by lower case letters in each column are non-significant at $p \leq 0.05$ among P levels.

vegetative growth to reproductive growth. These results are in conformity to the findings of Malik et al. (1990) who reported higher yields of cotton than vegetative biomass with P fertilization.

A significant increase in biomass P concentration and uptake by cotton was closely related with P fertilization. Higher plant growth and P concentration with PSM (e.g. *Pseudomonas fortinii*) inocula was reported by Bartholdy et al. (2001). It is reported that *Bacillus* spp. inoculation transform insoluble P into soluble forms by secreting acidic compounds and subsequently result in higher P uptake and crop yields (Cakmakci et al. 2006; Canbolat et al. 2006; Orhan et al. 2006). Moreover, a significantly higher P content of cotton seeds was due to increased translocation of assimilates into the seed by P fertilization. Rochester (2007) reported that P uptake by cotton ranged between 18 and 43 kg ha^{-1} in response to P fertilization. In our studies, P uptake was 17–28 kg ha^{-1} by cotton, which is well within the range as reported by Rochester (2007). Since seed is a biological entity for survival and lint production of cotton, it is expected to maintain a higher concentration of nutrients including P than other components of the plant. Dorahy et al. (2004) reported that P uptake by cotton at cut-out point was 21 ± 1.2 kg ha^{-1} and of this, two-thirds (15 ± 1.9 kg ha^{-1}) was accumulated in seed.

The significant increase in PUE of cotton by 8% with PSM inocula and up to 26% with P fertilization was more than the already reported PUE of 20% by P fertilization. The improved PUE could be attributed to PSM application to cotton. Dorahy et al. (2004) have also reported similar values of cotton PUE at the cut-out point in the range of 24–67%.

Economic analysis of irrigated cotton production

The economics of irrigated seed cotton production varied significantly by P fertilization and PSM inocula treatments (Table 5). The highest total income from cotton production was \$1020 at 44 kg P ha^{-1} followed by \$964 at 22 kg P ha^{-1}, compared with control (\$887). In other words, the net income was \$77 and \$132 when P was applied at 22 and 44 kg ha^{-1}, respectively. Likewise, PSM inocula increased net income by \$37 ha^{-1} over the control. Irrespective of P fertilization rates, PSM inocula improved net income.

Table 5. Economic analysis of irrigated cotton production in response to phosphorus (P) fertilization and phosphate-solubilizing microbial (PSM) inocula under a semi-arid climate (data combined over 2005 and 2006).

		Economics (\$ ha^{-1})			
P level (kg ha^{-1})	PSM inocula	Total cost	Market vaue	Total income	Net income[1]
Mean of all P-levels	Control	362.9A[2]	1301.7B	938.7B	–
	PSM	367.8A	1343.1A	975.3A	36.3
			P × PSM interaction		
0	Control	334.7ns	1209.6	875.0	–
	PSM	338.2	1237.5	899.5	24.5
		336.5c[3]	1223.6c	887.3c	
22	Control	362.9	1302.0	939.0	64.0
	PSM	369.1	1358.6	989.5	90.0
		366.0b	1330.3b	964.3b	77
44	Control	391.2	1393.4	1002.2	127.2
	PSM	396.1	1433.1	1037.0	137.5
		393.7a	1413.3a	1019.6a	132.4

Notes: [1]Income growth by P fertilization and PSM inocula. [2]Means separated by upper case letter in each column are not significantly different at $p \leq 0.05$ between PSM treatments. ns indicates a non-significant interaction of P levels × PSM inocula. [3]Means separated by lower case letter in each column are not significantly different at $p \leq 0.05$ among P levels.

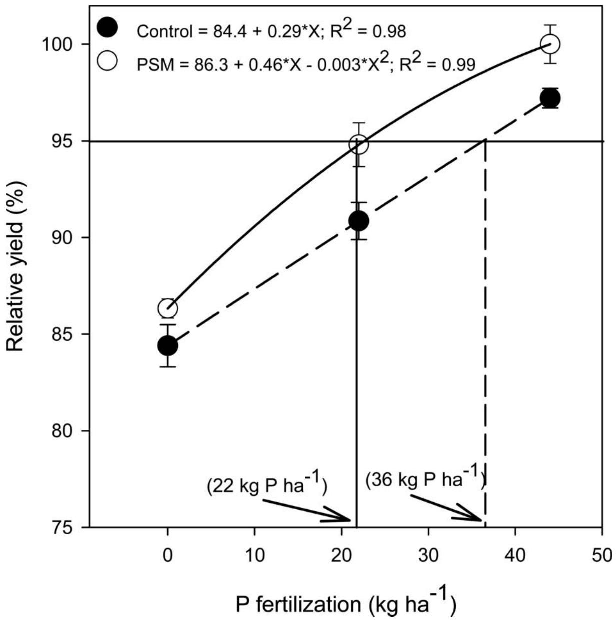

Figure 2. Effect of phosphorus (P) fertilization and phosphate-solubilizing microbial inocula on relative yield of irrigated cotton in a semi-arid climate.

When the relative yield of cotton was plotted over P fertilization levels, PSM inocula showed a nonlinear response and the control treatment showed a linear response (Figure 2). An extrapolation of the relationship showed that P fertilization at 22 kg ha^{-1} with PSM inocula is sufficient for near maximum production (~95%) of seed cotton. In contrast, the relationship between P fertilization and relative yield showed that 95% of the cotton yield was produced when P applied at 36 kg ha^{-1} without any PSM inocula.

Integration of half dose of P fertilizer (22 kg ha^{-1}) with PSM inocula produces crop yield economically comparable with the normal rates of P fertilization (at 44 kg ha^{-1}) and through reduced amount of inputs, and improved PUE, the production cost is minimized and net return is maximized. A higher rate of P fertilization without PSM inocula is required to produce near maximum yields of cotton (~95%) due to less availability of applied P to cotton from greater P fixation by Ca and Mg carbonates in high pH soil.

Conclusion

Cotton seed treatment with PSM inocula had a significant impact on PUE to increase cotton yields. The PSM inocula were found effective to complement the reduced rates of phosphorus fertilization for growing seed cotton on high pH soil. Moreover, PSM inocula with reduced rates of phosphorus fertilization (20 to 25 kg ha^{-1}) have maximized cotton yields and improved farm economics.

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