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Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields

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Abstract

Influence of application of phosphorus solubilizing bacteria (PSB), *Bacillus megatherium* var. *Phosphaticum*, at 10 kg ha^{-1} of lignite based culture with and without varying amounts of P fertilizer was studied on soil available P changes and sugarcane growth and yield. The PSB application increased the PSB population in the rhizosphere and the plant available P status in the soil. It also enhanced tillering, stalk population and stalk weight, and led to a cane yield increase of 12.6% over no application. When used in conjunction with P fertilizers, PSB reduced the required P dosage by 25%. In addition, it was found that 50% of the costly super phosphate could be replaced by rock phosphate (RP), a cheap source of P, when applied in conjunction with PSB. The PSB improved juice quality and sugar yields. The influence of PSB was greatest when RP constituted a part of the P fertilizers applied. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: *Bacillus megatherium*; 'Fixed' P; Ratoon crop; Residual effect; Rock phosphate; Soil available P; Sugarcane

1. Introduction

Phosphorus is a key nutrient required for higher and sustained productivity of sugar from sugarcane (*Saccharum* hybrid). Its influence on cane yield and juice quality has been well established and application of phosphorus has become an essential part of a sugarcane fertilizer programme (Blackburn, 1984). Phosphatic fertilizers are expensive and in developing countries like India, they are either imported or manufactured using imported raw material. Due to the increase in their cost in the recent past, there has been a trend towards the discontinuation of P fertilizer application or reduction in the amounts applied in India (Sundara and Natarajan, 1997). This is undesirable as it could lead to a fall in sugarcane productivity as has happened previously in some sugar factories of tropical India (Sundara, 1985).

Most soils contain substantial reserves of total P, most of it remains relatively inert, and only less than 10% of soil P enters the plant–animal cycle (Kucey et al., 1989). Consequently P deficiency is widespread and P fertilizers are almost universally required to maintain crop production. Although the P in these fertilizers is initially plant available, it rapidly reacts with the soil and becomes progressively less available for plant uptake. On being added to soils the soluble phosphates react with the constituents of the soil and form compounds which are less soluble, depending upon the soil. Thus in acid soils, the reaction products are aluminium and iron phosphates, in the predominantly calcareous soils, the reaction products are calcium phosphates. Different phosphatic fertilizers

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yield different reaction products. The formation of these reaction products depends upon the soil environment and the types of fertilizer material added. The reaction of phosphate solution with clay minerals leads to the formation of compounds with the general composition (H, Na, K, NH_4)·3(Fe, Al)PO_4·$N H_2O$, when the soil minerals were treated with phosphates of NH_4, K and Na (Haseman et al., 1950; Raychaudhury, 1976). The products obtained with calcium and magnesium phosphates were dicalcium and dimagnesium phosphates and members of variscite ($AlPO_4 \cdot 2H_2O$), barrandite (AlFe)($PO_4 \cdot 2H_2O$) and strengite ($FePO_4 \cdot 2H_2O$) isomorphous series. Das and Datta (1967 as quoted by Raychaudhury, 1976) reported the formation of breeshite ($CaHPO_4 \cdot 2H_2O$) and moletite ($CaHPO_4$) in black and brown soils when monoammonium phosphate and monocalcium phosphate were added to the soil. In acid soils, variscite ($AlPO_4 \cdot 2H_2O$) is formed with these fertilizers. NH_4-taranakite ($H_6NH_4Al_5PO_4)_8 \cdot 18H_2O$ is formed when soil is treated with monoammonium phosphate. Other reaction products identified were octocalcium phosphate, calcium aluminium phosphate, and colloidal ferric aluminium phosphate (Fe, Al, X)PO_4·$N H_2O$ with monocalcium phosphate. As a result most of the P applied (often as much as 90%) is rendered unavailable for crop uptake but is retained in insoluble form. In sugarcane, recovery of the applied P is around 10–15% (Humbert, 1968). Annual applications are often necessary to maintain adequate labile P. Thus, soils commonly have large reserves of 'fixed' P that could support long term crop requirements if it could be mobilized through appropriate soil management involving organic matter additions and/or use of P solubilizing microbes.

Improvement in P availability to crops through microbial means was first demonstrated in Russia. Several groups of micro-organisms (fungi, bacteria, actinomycetes) are known to help dissolve fixed P (Sundara Rao and Sinha, 1963). During the 1950s, farmers in the USSR and several eastern European countries inoculated a large proportion of their agricultural soils with a fertilizer consisting of kaolin impregnated with spores of the bacterium *Megatherium viphosphateum*. This bacterium was later named *Bacillus megatherium* var. *Phosphaticum* and the fertilizer was called phosphobacterin (Cooper, 1959; Menkina, 1963). Crop yield increases resulting from the addition of *B. megatherium* to Soviet soils were reported to range from 0 to 70%. However, experiments in the United States did not show similar increases (Smith et al., 1961). Experiments in India indicated positive responses to phosphobacterin application (Sundara Rao and Sinha, 1963; Sundara Rao et al., 1963; Bajpai and Sundara Rao, 1971; Gaur, 1990; Marwaha, 1995).

Phosphobacterins in general have been found effective in solubilizing inorganic P in the soils. The solubilization effect is generally due to the production of organic acids such as citric, glutamic, succinic, lactic, oxalic, glyoxalic, maleic, fumeric, tartaric and α-ketobutyric acids as has been observed in the liquid medium (Bardia and Gaur, 1972; Mishustin and Naumova, 1956; Pareek and Gaur, 1973). The action of organic acids has been attributed to their chelation property. Because P solubilizing micro-organisms render more phosphates into solution than is required for their growth and metabolism, the surplus gets absorbed by plants (Alexander, 1977).

In sugarcane, there have been only a few studies on the influence of P solubilizers. Yadav and Singh (1990) observed increase in germination per cent, tiller number, cane yield and P uptake by inoculating P solubilizer (*B. megatherium*) with different doses of phosphatic fertilizers in Bihar, India under alluvial soils. Kathiresan et al. (1995) and Kumaraswamy et al. (1992) noted an increase in cane yield by the application of phosphorus solubilizing bacteria (PSB) along with FYM. Sundara and Natarajan (1997) also suggested the possibilities of saving phosphatic fertilizer (single super phosphate, 16% P_2O_5). These isolated studies were of preliminary nature and were limited to a single season only (plant crop). Besides, there were no reports available about the usage of rock phosphate (RP) along with P solubilizers so that economy in P rate could be achieved. Considering the importance of P nutrition in sugarcane and the need for economizing P fertilizer use, the reported experiments were carried out.

2. Materials and methods

Field experiments were conducted at the Sugarcane Breeding Institute, Coimbatore (11°N, 77°E) during 1995–1998 on a vertisol of medium fertility (initial available N, P and K of 86, 5.3 and 189 mg kg^{-1}, respectively) to study the effect of PSB (*B. megatherium* var. *phosphaticum*) application with or without P fertilizers on the changes in the soil available P level and sugarcane and sugar yields. The treatments comprised application of 100, 75 and 50% recommended rate of P (34.9 kg ha^{-1}) either entirely through single super phosphate (SSP, 6.99% P) or half through SSP + half through rock phosphate (RP, 7.86% P) along with or without PSB application at 10 kg ha^{-1} of lignite based culture having a bacterial load of 10^8 cells g^{-1}, and two controls, viz. no PSB or P fertilizer, and PSB application alone (10 kg culture ha^{-1}). The resulting treatment combinations (14) are presented in Table 1. Treatments were applied to both plant and ratoon crops. To study the residual effects, one half of the ratoon crop under each treatment in each replication was grown without re-imposing the treatments.

The experiment was laid out in a randomized block design with three replications. The plot size was 7.2 m × 6.1 m (8 rows of 6.1 m spaced 0.9 m apart) for the plant crop and 3.6 m × 6.1 m for the ratoon. The trial was first planted in March 1995 and harvested 12 months later in March 1996, and its ratoon was grown during 1996–1997. Two more crops (one plant + one ratoon) were grown separately in the adjoining field during 1996–1998. Crops were subjected to the standard package of practices which included a seed rate of 60,000 two-bud setts ha^{-1}, applications of N and K dosages of 280 and 100 kg ha^{-1}, respectively, applied in two equal splits of 45 and 90 days of planting, and in ratoon, in three splits, at ratooning, 30 and 60 days later. Phosphatic fertilizers were applied as a basal dressing before planting. Phosphobacterial culture (*B. megatherium* var *phosphaticum* mixed in lignite powder at 10^8 cfu g^{-1}) was applied a month after planting or ratooning by mixing the culture with fine soil of the experimental fields.

Soil samples (0–20 cm) were collected from 10 spots at random from the experimental field before planting and a composite sample was used for initial soil analysis. Soil samples (0–20 cm, five spots in each plot and mixed) were collected from all the experimental plots (42 in the plant crop, 84 in the ratoon) at 90, 180 and 300 days after planting/ratooning (to represent formative, grand growth and ripening phases, respectively, Sundara, 1998) were analysed for available P (Olsens's P) (Jackson, 1973). During the

Table 1
The effect of PSB and P fertilizer application on PSB population and available P status in the soil and sheath P

Treatment	PSB population ($\times 10^4$/g)			Soil available P (mg/kg)			Sheath P (% on sugar free dry weight basis)		
	TP[a]	GGP[b]	RP[c]	TP	GGP	RP	TP	GGP	RP
T_1—control	20.41	22.32	20.61	4.4	4.8	4.0	0.149	0.132	0.110
T_2—PSB	26.23	26.36	21.41	5.7	5.6	4.8	0.153	0.142	0.119
T_3—100% P as SSP	22.31	23.32	20.46	6.8	7.1	5.3	0.162	0.142	0.121
T_4—75% P as SSP	19.21	20.46	18.36	5.9	5.7	4.9	0.158	0.139	0.120
T_5—50% P as SSP	19.01	20.21	17.90	5.1	4.9	4.1	0.153	0.133	0.114
T_6—T_3 + PSB	28.10	29.36	24.62	7.5	7.7	6.2	0.164	0.146	0.124
T_7—T_4 + PSB	30.46	29.02	24.51	7.2	7.0	5.9	0.161	0.146	0.122
T_8—T_5 + PSB	27.60	28.03	23.10	6.5	6.8	4.6	0.158	0.132	0.122
T_9—100% P (50% as SSP + 50% as RP)	23.30	25.16	21.32	7.2	7.3	5.6	0.148	0.132	0.113
T_{10}—75% P (50% as SSP + 50% RP)	20.01	20.10	19.26	6.1	5.9	5.1	0.144	0.131	0.115
T_{11}—50% P (50% as SSP + 50% as RP)	19.62	20.75	18.10	5.6	5.2	4.6	0.144	0.132	0.115
T_{12}—T_9 + PSB	33.16	36.41	28.21	8.2	8.3	6.8	0.159	0.149	0.123
T_{13}—T_{10} + PSB	33.41	33.41	30.41	8.3	8.2	7.0	0.155	0.148	0.119
T_{14}—T_{11} + PSB	30.53	30.62	27.36	7.9	7.5	6.5	0.154	0.144	0.118
LSD ($P = 0.05$)	2.30	3.02	3.6	0.2	0.3	0.3	0.006	0.006	0.005

[a] Tillering phase.
[b] Grand growth phase.
[c] Ripening phase.

same crop stages, PSB population in the soil (Gaur, 1990) and sheath P status of the crop (Clements, 1980) were determined. For assessing the PSB population, the moisture content of the soil samples was estimated by drying the samples in hot air oven at 103 °C and the loss in weight was calculated. The soil samples were serially diluted and spread plated in Petri plates containing Pikovskaya's agar (Pikovskaya, 1948) and incubated at 30 °C for 7 days. The tricalcium phosphate clearing zone forming bacterial colonies were counted as phosphate solubilizing bacterial count. The population of phosphate solubilizing bacteria was then expressed per gram on dry weight basis.

Germination on 35 days of planting, shoot population on 90 days and stalk number, stalk weight and plot yields at harvest were recorded. Juice samples were extracted from 10 canes cut at random in each plot. Brix and sucrose were determined following the standard procedures (Meade and Chen, 1977). From the values of brix and sucrose, commercial cane sugar per cent (CCS %) was calculated as CCS $\% = 1.022S–0.292B$, where S and B are sucrose and brix per cent, respectively, in the juice (Mathur, 1961). Cane yield data recorded from the net plots were converted to yield per hectare. From cane yield and CCS % data, sugar yield was calculated as is usually done (in sugarcane experiments in India). The data from the various years/crops were pooled (since there were no yearly or crop differences with respect to treatment effects), tested through analysis of variance and means were separated using LSD at $P \leq 0.05\%$. The data on the residual effects were analysed separately.

3. Results

3.1. PSB and P fertilizer effects on PSB population and available P status

PSB application without any P fertilizer increased PSB population and soil available P status at different stages of the crop (Table 1). However, when PSB was used in conjunction with P fertilizer, a much greater effect was observed. All the treatments involving P fertilizer + PSB improved the PSB population and the available P levels in the soil significantly over the P fertilizer treatments without PSB. The PSB populations in treatments involving PSB applications were maintained upto grand growth phase (GGP) and then declined towards ripening phase. The PSB population and the available P levels were higher in treatments where P was given partly through RP and PSB was applied (T_{12}, T_{13} and T_{14}) than in treatments where P was given entirely through SSP and PSB was applied (T_6, T_7 and T_8).

The PSB populations were not significantly influenced by the changes in the P fertilizer rates whether applied with PSB or otherwise. However, the available P levels declined significantly when P rates were reduced to 75 and 50% without PSB addition. But when PSB was supplied with P fertilizers there were no significant changes in the available P levels owing to reduction in the P rate to 75%; but further reduction to 50% caused significant decline even with PSB.

Sheath P content at different growth stages increased by the applications of PSB, P fertilizers and P fertilizers along with PSB in the order mentioned. The sheath P levels were higher in treatments where RP and PSB formed a part of the treatment.

3.2. PSB and P fertilizer application effects on yield components and yield

Data on the effects of PSB and P fertilizer treatments on stalk population, stalk weight, cane yield, CCS % and sugar yield are presented in Table 2. The PSB application increased all the parameters significantly over control. The recommended P application through SSP increased the stalk population, stalk weight and cane yield over control and only PSB application. Addition of PSB to the recommended P rate did not further influence the yield and its components. When the P rate was reduced to 75 or 50% without the addition of PSB, significant reductions in the stalk populations, stalk weight and cane yield occurred. But when the P rate was reduced to 75% with the addition of PSB, no such reductions were noticed. However, further reduction of the rate to 50% with the addition of PSB reduced the yield.

Substitution of SSP with RP by 50% at different P rates caused significant reduction in cane yield as compared to the corresponding P rates supplied entirely through SSP. But no such reductions were noticed when RP was used along with PSB. Thus treatments in which P was supplied at recommended rate through SSP, SSP + PSB, 75% P through

Table 2
The effect of PSB and P fertilizer treatments on stalk population, stalk weight, cane yield, CCS % and sugar yield

Treatment	Stalk population ('000/ha)	Stalk weight (kg)	Cane yield (t ha^{-1})	CCS %	Sugar yield (t ha^{-1})
T_1—control	102.3	1.38	101.4	12.46	12.63
T_2—PSB	106.6	1.47	112.1	12.63	14.16
T_3—100% P as SSP	113.3	1.51	125.5	13.02	16.34
T_4—75% P as SSP	112.7	1.48	113.3	12.54	14.21
T_5—50% P as SSP	104.1	1.46	103.6	12.51	12.96
T_6—T_3 + PSB	120.4	1.46	126.2	12.76	16.10
T_7—T_4 + PSB	118.7	1.47	126.3	12.87	16.25
T_8—T5 + PSB	110.3	1.44	114.6	12.56	14.39
T_9—100% P (50% as SSP + 50% as RP)	105.4	1.46	106.4	12.56	13.36
T_{10}—75% P (50% as SSP + 50% as RP)	102.1	1.49	101.2	12.47	12.62
T_{11}—50% P (50% as SSP + 50% as RP)	106.1	1.42	101.3	12.46	12.62
T_{12}—T_9 + PSB	118.4	1.46	127.1	13.20	16.77
T_{13}—T_{10} + PSB	120.3	1.46	125.3	13.30	16.66
T_{14}—T_{11} + PSB	112.6	1.44	115.4	12.81	14.78
LSD ($P = 0.05$)	5.9	0.05	7.8	0.28	1.01

SSP + PSB, 100% P (through SSP + RP) + PSB or 75% P (through SSP + RP) + PSB were at par in cane yield components and cane yield.

The CCS % was better in treatments where P was applied entirely through SSP or P fertilizers + PSB at 100 or 75% of the dosage. The RP + PSB applied plots in general registered a higher level of CCS %. Sugar yield increased significantly by the application of the entire P through SSP or by 100 or 75% through P fertilizer + PSB. The sugar yield trend was similar to that of the cane yield.

3.3. Residual effects

Data regarding the residual effect studies are presented in Table 3. PSB application along with SSP

Table 3
Residual effect of plant crop treatment on the ratoon crop[a]

Treatment to plant crop	PSP population ($\times 10^4$/g)	Available P (mg/kg)	Stalk population ('000/ha)	Cane yield (t ha^{-1})	CCS %	Sugar yield (t ha^{-1})
T_1—control	18.03	4.3	70.26	64.4	10.86	6.99
T_2—PSB	18.21	4.2	70.35	63.3	10.93	6.92
T_3—100% P as SSP	18.31	5.0	80.12	78.3	11.01	8.62
T_4—75% P as SSP	18.25	4.8	78.36	76.1	10.81	8.23
T_5—50% P as SSP	18.28	4.6	72.20	71.4	10.80	7.71
T_6—T_3 + PSB	18.63	5.0	80.26	79.1	11.06	8.75
T_7—T_4 + PSB	18.56	4.9	80.16	76.1	10.94	8.33
T_8—T_5 + PSB	18.52	4.8	78.30	70.6	10.81	7.63
T_9—100% P (50% as SSP + 50% as RP)	17.94	4.8	76.41	72.3	11.01	7.96
T_{10}—75% P (50% as SSP + 50% RP)	17.96	4.6	76.23	68.1	10.92	7.44
T_{11}—50% P (50% as SSP + 50% as RP)	17.86	4.6	74.16	65.6	10.42	6.84
T_{12}—T_9 + PSB	22.58	6.0	87.56	84.9	11.68	9.92
T_{13}—T_{10} + PSB	21.26	5.8	82.15	78.1	11.41	8.91
T_{14}—T_{11} + PSB	20.14	5.3	80.41	78.1	11.10	8.67
LSD $P = (0.010)$	2.01	0.3	5.53	4.03	0.29	0.62

[a] PSB and available P are at 90 days of the ratoon crop.

alone to the plant crop had no residual effect in ratoon with respect to PSB population, available P status, cane growth, yield or quality. However, PSB application along with SSP + RP showed residual effects leading to a higher rhizosphere PSB population, available P in the soil, stalk population and cane and sugar yields.

4. Discussion

The results of this study indicated highly beneficial effects of PSB application to sugarcane. This is evident by comparing the data of the treatments where PSB was applied with those where it was not. For example, the mean cane and sugar yields of the treatments involving PSB (T_2, T_6, T_7, T_8, T_{12}, T_{13} and T_{14}) were 121 and 15.58 t ha^{-1}, respectively, as opposed to 107.5 and 13.53 t ha^{-1} in treatments without PSB (T_1, T_3, T_4, T_5, T_9, T_{10} and T_{11}). Thus the mean increases in cane and sugar yield by PSB application were 12.56 and 15.15%, respectively. This was due to increased PSB activity in the rhizosphere following PSB application and consequently by enhanced P solubilization as evidenced by the higher levels of plant available P in the soil at appropriate crop growth stages. That the crop was able to utilize more soil P was evident by the increased levels of tissue (sheath) P observed at various stages of crop growth. P uptake is highly correlated with cane yield (Sundara, 1994). Kucey et al. (1989) cite that several studies in various crops have indicated increased levels of available P in the soil following inoculation with PSB organisms.

Increase in plant available P and its enhanced uptake by the crops following PSB additions has led to an increase in tillering and thus stalk number and stalk growth ultimately leading to higher cane yields. Improvement in juice sucrose and purity were significant by PSB additions thus leading to higher CCS % and sugar yields. Increased availability of phosphorus facilitates better nitrogen utilization (Humbert, 1968) and thus better crop growth, besides improving juice purity (Clements, 1980).

An important result of much practical value is that PSB application enabled the rate of P fertilizer to be reduced by 25%. This is apparent from the similar cane and sugar yields in those treatments where the recommended rate of P was applied and in treatments where 25% of the rate was reduced but PSB was supplied. Additionally, RP could be applied to substitute partially (50%) the costly super phosphate when used in conjunction with PSB. The RP treatments also had a residual effect and showed improvement in juice quality. This suggests that increased use of RP with PSB may be beneficial for sustaining long term productivity of sugarcane.

5. Conclusion

Application of PSB has beneficial effects on sugarcane growth, yield and quality. A 25% reduction in the amount of P applied is possible when P fertilizer is used in combination with PSB. RP could be used to partially supplement the P requirement if applied with PSB.

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