

## Effect of Associative Nitrogen Fixing and Phosphate Solubilising Bioagents on Growth, Yield and Quality of Sugarcane

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**ABSTRACT.** Field investigations were carried out at Regional Research Station, Visveswaraya Canal Farm, Mandya of Karnataka, India to study the effect of inoculation of nitrogen fixing and phosphate solubilising microorganisms (PSM) on growth, yield and quality of sugarcane. Soil inoculation of nitrogen fixers viz., *Azotobacter* in plant crop and *Azospirillum* in ratoon crop ensure increased cane yields of 10–15% as a result of perceptible improvement in various growth and yield parameters. Enhanced nutrients use efficiency as evident from their higher availability and uptake, especially of nitrogen, were suggestive of economy in fertilizer nitrogen up to 20% or 50 kg ha<sup>-1</sup>. The leaching loss of nitrate nitrogen (NO<sub>3</sub>-N) and consequent pollution of ground water was greatly reduced due to inoculation by *Azotobacter* and *Azospirillum*. *Azospirillum* is more rewarding in compacted soils of ratoon crop while, *Azotobacter* in light textured soils.

Soil inoculation of *Agrobacterium radiobacter* and *Bacillus megaterium* in plant crop and *Aspergillus awamori* and *Bacillus megaterium* in ratoon crop exhibit 8–10% higher cane yields besides economy in fertilizer phosphorus up to 25%. Further, enhanced availability and uptake of phosphorus indicated better P use efficiency and P solubilising capacity of PSM. The effect due to PSM is more pronounced with soluble sources of phosphorus viz., single super phosphate in conjunction with pressmud. The bacterial cultures viz., *Agrobacterium radiobacter* and *Bacillus megaterium* were efficient solubilisers of phosphorus in plant crop whilst, the fungus *Aspergillus awamori* in ratoon crop. Inoculation of *Azotobacter* and *Azospirillum* among nitrogen fixers and *Agrobacterium radiobacter* among PSM exhibited jaggery (Gur) of higher quality and grade.

### INTRODUCTION

Fertilizer management with an integrated and ecofriendly approach seems to be the desirable route to achieve the objective of high yield, cane quality with sustainable productivity of both cane and sugar. Nitrogen (N) nutrition assumes primacy in sugarcane production as it is instrumental in improving yield and quality of sugarcane. Reduced N use efficiency (NUE) due to leaching and volatilisation losses, increasing cost of N fertilizers have added to reduced cost : benefit ratios. The leaching loss of nitrate N (NO<sub>3</sub>-N) is said to be the potential threat to ground water pollution and World Health Organisation (WHO) standard for drinking water with nitrate content is 100 mg l<sup>-1</sup> (Biswas *et al.*, 1992). Thus, economic and environmental considerations have prompted the need to stimulate research on alternate but cheaper, renewable and ecofriendly supplementer of

N. The advent of acetylene reduction assay for measuring nitrogenase enzyme activity has led to the establishment of biological N fixation (BNF) in sugarcane rhizosphere (Dobereiner *et al.*, 1972). *Azotobacter* a free-living soil bacteria and *Azospirillum* an associative, microaerophilic symbiotic soil bacterium offer a great potential as biological N fixers in sugarcane crop. *Acetobacter diazotrophicus* an obligate endophytic bacterium is capable of fixing 200–250 kg N ha<sup>-1</sup> per season meeting substantial part of N demand of sugarcane crop (Lima *et al.*, 1987; Dobereiner, 1990).

Phosphorus (P) is the second most important but deficient nutrient in sugarcane production. As a consequence of high cost of water soluble chemically processed phosphatic fertilizers and reduced P use efficiency due to fixation as insoluble phosphates, imbalance in NPK ratios are anticipated which eventually leads to reduced productivity of sugarcane. Mobilisation of insoluble P in soils through microbially mediated system assumes practical importance in increasing P use efficiency and associated growth related parameters in sugarcane. The soil isolates such as *Agrobacterium radiobacter*, *Bacillus megaterium var. phosphaticum*, *Aspergillus awamori* *etc.*, have been reported to be efficient phosphate solubilising microorganisms (PSM) even with low grade phosphates such as rock phosphate, slag, bonemeal *etc.* (Gaur, 1990; Marwaha, 1995). The reports on the capabilities of such organisms suffer from major drawback as they are mostly of *in vitro* experimentation and showed solubilisation under highly artificial conditions of culture media (Chhonkar, 1994).

However, beneficial influence of artificial inoculation of N fixers and PSM better known as biofertilizers or biological software in sugarcane has been reported under diverse agroclimatic conditions with positive gain in crop yields and economy in fertilizer N and P. Therefore, their differential behaviour towards varying agroclimatic conditions, varieties *etc.*, prompted a detailed location specific field study on the efficacy of some selected bioagents in sugarcane in the Cauvery basin in southern Karnataka. The objectives of the investigations were to study besides, growth, yield and quality parameters of sugarcane, availability and uptake of major nutrients, economy in fertilizer N and P and quality and grading of jaggery (Gur) as influenced by N fixers and PSM. The possible reduction in the leaching loss of NO<sub>3</sub>-N due to N fixers and the utilization of natural and cheaper sources of P due to PSM were programmed as added objectives.

## MATERIAL AND METHODS

Field investigations were carried out at Regional Research Station, Visveswaraya Canal Farm, Mandya, Karnataka, India to study the effect of inoculation of N fixers and PSM independently on growth, yield and quality of sugarcane plant and ratoon during 1995–98. The soils of the study area is classified as Ustalfs with a sandy clay loam texture. They are neutral in reaction with low organic carbon and available N, medium in available P and low in available K. The four field experiments include two each on N fixers and PSM on both plant and ratoon sugarcane. Four N fixers *viz.*, *Azotobacter chroococcum*, *Azospirillum brasilense*, *Acetobacter diazotrophicus* (local and Brazilian isolates) superimposed with three levels of N *viz.*, 150, 200 and 250 kg ha<sup>-1</sup> were tried in split plot design. *Azotobacter chroococcum* and *Azospirillum brasilense* as carrier based inoculants were applied to soil at 2.5 kg ha<sup>-1</sup> in two equal splits at 30 and 60 days after planting in

plant crop and 15 and 45 days after stubble shaving in ratoon experiment. The liquid cultures of *Acetobacter diazotrophicus* (local and Brazilian isolates) were diluted with sugarcane juice and inoculated through set treatment in plant and drenching the stubbles in ratoon experiments. The levels of N as per the treatments were imposed in four splits in plant and three splits in ratoon experiments as per the recommendations of the region. The varieties used were *cv. Co 7804* and *cv. Co 419* in plant and ratoon experiments respectively. Simultaneously, the treatments were simulated in pot culture study for collection and analysis of leachate for  $\text{NO}_3\text{-N}$ .

The studies on PSM include three species of PSM *viz.*, *Agrobacterium radiobacter*, *Bacillus megaterium* and *Aspergillus awamori* superimposed with two levels of P *i.e.*, 75 and 100% recommended P and three sources of P *i.e.*, single superphosphate (SSP), SSP+mussorie rock phosphate (MRP) to supplement 50% P each, SSP+pressmud (a byproduct of sugar industry) to supplement 50% P each were tried in split-split-plot design in plant and ratoon sugarcane. The PSM were inoculated through soil as carrier based inoculants at  $10 \text{ kg ha}^{-1}$  at 30 days after planting of sugarcane and 15 days after of stubble shaving in ratoon experiments. The levels and sources of P as per treatments were imposed in full at planting and 30 days after stubble shaving in ratoon experiments.

The growth, yield and quality parameters of sugarcane were recorded at appropriate stages of growth adopting standard procedures commonly in all the experiments. The nutrient indices, uptake and availability of major nutrients were estimated adopting standard procedures (Jackson, 1973; Subbaiah and Asija, 1956). The jaggery samples prepared in the laboratory at harvest were analysed and graded according to net remnant (NR) values (Khanna and Chakravarthi, 1954). The leachate samples collected periodically from field and pot culture studies were analysed for  $\text{NO}_3\text{-N}$  by Devarda alloy method (Sankaran, 1966). The statistical analysis was done using Fisher's method of analysis of variance technique.

## RESULTS AND DISCUSSION

### Effect of N levels and N fixers on growth, yield and quality of Sugarcane- plant and ratoon

#### Growth and yield parameters

The N levels and the N fixers had no perceptible influence on initial growth parameters such as germination, tillering capacity, shoot population both in plant and ratoon crop experiments. The leaf area index (LAI) and leaf area duration (LAD) in general exhibited marked improvement due to inoculation of *Azotobacter* and *Azospirillum* at 6<sup>th</sup> month of growth and harvest stages in both plant and ratoon experiments. The higher LAI and LAD are attributable for increase in number and size of leaves, extended source capacity and stay green character (Arabidopsis) as a result of production of plant growth regulators (PGR) and biologically active metabolites by the bioagents (Marwaha, 1995). The values of LAI and LAD were higher in plant crop than in ratoon and declined sharply towards harvest. The crop growth rate (CGR) responded positively to both N fertilization and N fixers *viz.*, *Azotobacter* and *Azospirillum* in plant crop arguably due to higher LAI and

LAD during grand growth period. Higher dry matter production due to inoculation of *Azotobacter* followed by *Azospirillum* in plant crop and *Azospirillum* in ratoon crop at 6<sup>th</sup> month are attributable for improvement in LAI, LAD and CGR. The height and weight of millable cane expressed significant improvement due to addition of N fixers viz., *Azotobacter* followed by *Azospirillum* in plant crop. Whilst addition of N up to 200 kg ha<sup>-1</sup> and *Azospirillum* alone expressed higher height and weight of millable cane in ratoon cane (Table 1).

### Cane and sugar yield

Inoculation of *Azotobacter* registered the highest cane yield followed by *Azospirillum* at the recommended N level of 250 kg ha<sup>-1</sup> in plant crop. In ratoon crop, *Azospirillum* excelled with the highest cane yield of 126 Mg ha<sup>-1</sup> at the same level of N. Improvement in yield following the inoculation of N fixers is ascribed for increase in height and weight of millable cane. The increase in cane yield was to a tune of 21.22 Mg ha<sup>-1</sup> and 9.33 Mg ha<sup>-1</sup> due to *Azotobacter* and *Azospirillum* respectively, over no inoculation in plant crop. Whilst, it was to a tune of 5.44 Mg ha<sup>-1</sup> and 3.67 Mg ha<sup>-1</sup> due to *Azospirillum* and *Azotobacter* respectively over no inoculation in ratoon crop (Table 1 and 2). This establishes the superiority of *Azotobacter* in plant crop and *Azospirillum* in ratoon crop. *Azotobacter* is reported to perform better in semi dry loamy and sandy soils which are less compacted with better aeration as observed under plant crop soil conditions (Srinivasan and Naidu, 1987). *Azospirillum* being microaerophilic and known to maintain high nitrogenase activity in low oxygen tension environment substantiates its effectiveness under compacted soils of ratoon crop. *Azospirillum* expressed significantly the highest millable cane population in ratoon crop which is attributable for higher survival rate due to the production of PGR. It is evident from the data that the yield levels either on par or higher than that obtainable at recommended N level could be maintained even at reduced N level of 200 kg ha<sup>-1</sup> upon inoculation with *Azotobacter* and *Azospirillum* in plant crop and *Azospirillum* in ratoon crop. This suggests the possibility of saving fertilizer N to an extent of 20% or 50 kg ha<sup>-1</sup> indicating better N use efficiency. The juice quality parameters were not influenced due to imposed treatments. This is anticipated since N fertilization at the recommended level (250 kg ha<sup>-1</sup>) had no deleterious effect on juice quality. The sugar yield improved significantly due to the inoculation of *Azotobacter* with N fertilization which is essentially due to improved cane yield rather than juice quality parameters (Table 2).

### Tissue indices and uptake of major nutrients

The index leaf N (3–6 leaf blades) is of paramount importance since it reflects the physiological status of plants (Alexander, 1973; Clements, 1980). Application of *Azotobacter* expressed significant increase in N index at both the stages of growth in plant crop. The N index declined from an average value of 2.39% at 6<sup>th</sup> month to 1.05% at harvest exhibiting negative correlation with age. Lakshmikantham *et al.* (1970) observed maximum leaf N at formative and grand growth period and progressive fall with advancement in age. *Azotobacter* registered higher uptake of N of 377 kg ha<sup>-1</sup> at harvest stage (Table 3). This is attributable for higher dry matter production coupled with higher

**Table 1. Growth and yield parameters as influenced by N levels and N fixers.**

Character	LAI		LAD (days)		Dry matter (t ha <sup>-1</sup> )		CGR (g.m <sup>-2</sup> .d <sup>-1</sup> )	Height of millable cane (m)		Weight of millable cane (kg)	
	at 6 <sup>th</sup> month		10 <sup>th</sup> month-harvst		at 6 <sup>th</sup> month			Plant	Ratoon	Plant	Ratoon
Plant/Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Plant	Ratoon	Plant	Ratoon
<b>N levels</b>											
150 kg ha <sup>-1</sup>	7.09	3.62	205	142	25.85	18.30	19.79	2.50	2.01	1.47	1.23
200 kg ha <sup>-1</sup>	7.47	4.00	207	160	27.83	18.63	25.63	2.72	2.14	1.62	1.32
250 kg ha <sup>-1</sup>	7.31	3.88	205	153	27.14	17.34	28.35	2.83	2.19	1.69	1.34
<b>N fixers</b>											
<i>Azotobacter chroococcum</i>	7.99	4.03	219	148	30.92	18.17	36.17	2.89	2.13	1.72	1.31
<i>Azospirillum brasilense</i>	7.74	3.98	216	166	28.35	20.19	25.10	2.70	2.16	1.61	1.33
<i>Acetobacter diazotrophicus</i> (Local)	6.89	3.86	192	139	23.94	18.00	20.41	2.64	2.11	1.57	1.30
<i>Acetobacter diazotrophicus</i> (Brazil)	6.79	3.94	204	158	26.26	18.17	19.61	2.60	2.10	1.55	1.29
Control	7.07	3.36	198	149	23.90	15.94	21.66	2.55	2.07	1.52	1.27
<b>LSD (P=0.05)</b>											
N levels	NS	NS	NS	NS	NS	NS	8.283	0.089	0.135	0.053	0.083
N fixers	0.785	0.481	19.648	19.303	3.159	2.697	6.801	0.075	0.069	0.045	0.042
<b>LSD - Least significant difference</b>		<b>NS - Non significant</b>									

Table 2. Cane yield and sugar yield as influenced by N levels and N fixers.

Character Plant/ratoon N levels/N fixers	Cane yield (Mg ha <sup>-1</sup> )								Sugar Yield (Mg ha <sup>-1</sup> )			
	Plant				Ratoon				Plant			
	150 kg ha <sup>-1</sup>	200 kg ha <sup>-1</sup>	250 kg ha <sup>-1</sup>	Mean	150 kg ha <sup>-1</sup>	200 kg ha <sup>-1</sup>	250 kg ha <sup>-1</sup>	Mean	150 kg ha <sup>-1</sup>	200 kg ha <sup>-1</sup>	250 kg ha <sup>-1</sup>	Mean
<i>Azotobacter Chroococcum</i>	166.67	181.67	189.00	179.11	114.67	119.00	112.67	118.78	23.92	25.79	26.77	25.49
<i>Azospirillum brasilense</i>	154.67	170.33	176.67	167.22	113.00	122.33	126.33	120.55	20.99	24.27	24.43	23.23
<i>Acetobacter diazotrophicus</i> (Local)	151.33	167.00	172.00	163.44	115.67	118.00	119.67	117.78	21.10	23.64	24.42	23.05
<i>Acetobacter diazotrophicus</i> (Brazil)	150.33	165.33	168.67	161.44	111.33	117.67	112.33	117.11	21.38	23.47	23.65	22.83
Control	142.33	160.33	171.00	157.89	106.33	120.33	118.67	115.11	19.91	22.94	24.06	22.30
Mean	153.07	168.93	175.47		112.20	119.47	121.93		21.46	24.94	24.67	
LSD (P=0.05)												
N levels		5.44				7.424				0.683		
N fixers		4.69				3.791				1.026		
N levels × N fixers		NS				NS				NS		
N fixers × N levels		NS				NS				NS		

LSD - Least significant difference    NS - Non significant

**Table 3. N index, N uptake, soil available N, soil *Azotobacter* count,  $\text{NO}_3\text{-N}$  in leachate and cost : benefit ratio as influenced by N levels and N fixers.**

Character Plant/Ratoon	N index (%) at 6 <sup>th</sup> month		N uptake (kg ha <sup>-1</sup> ) at harvest		Soil av. N (kg ha <sup>-1</sup> ) at 8 <sup>th</sup> month	$\text{NO}_3\text{-N}$ in leachate (mg l <sup>-1</sup> )		Soil <i>Azotobacter</i> count (Xx'000 g <sup>-1</sup> )	Cost:benefit ratio	
	Plant	Ratoon	Plant	Ratoon	Plant	Field sampling	Pot sampling	Plant	Plant	Ratoon
<b>N levels</b>										
150 kg ha <sup>-1</sup>	2.28	1.79	195	198	258	46.25	56.25	6.00	2.30	2.38
200 kg ha <sup>-1</sup>	2.52	1.92	225	178	323	60.62	59.38	7.17	2.50	2.51
250 kg ha <sup>-1</sup>	2.38	2.06	284	192	342	73.75	61.25	10.83	2.57	2.53
<b>N fixers</b>										
<i>Azotobacter chroococcum</i>	2.60	2.25	377	207	359	30.62	50.62	12.78	2.63	2.45
<i>Azospirillum brasilense</i>	2.26	2.03	210	206	311	56.87	50.00	-	2.45	2.50
<i>Acetobacter diazotrophicus</i> (Local)	2.35	1.92	214	186	303	65.62	66.88	-	2.40	2.45
<i>Acetobacter diazotrophicus</i> (Brazil)	2.48	1.71	190	190	312	85.62	56.87	-	2.37	2.44
Control	2.29	1.70	183	158	253	62.50	70.00	2.41	2.38	2.49
<b>LSD (P=0.05)</b>										
N levels	NS	0.152	17.725	NS	22.263	2.237	1.437	-	-	-
N fixers	0.182	0.225	25.832	36.434	43.410	4.970	6.06	-	-	-

LSD - Least significant difference

NS - Non significant

tissue N content. Although, similar trend was observed in ratoon crop, *Azospirillum* was equally effective with higher indices and uptake of N more conspicuously at lower N level of 200 kg ha<sup>-1</sup>. The values however, were lower in ratoon than in plant crop as ratoons are less efficient users of N (Hunsigi, 1982). Although the indices of P and K revealed no definite trend, their uptake increased significantly due to *Azotobacter* and *Azospirillum* by virtue of higher dry matter production. The mean K indices and uptake declined towards harvest exhibiting negative correlation with advancement in age.

#### Available soil nutrients and microbial load

The soil available N in general showed significant improvement due to inoculation of N fixers and the highest availability of 359 kg ha<sup>-1</sup> was recorded under *Azotobacter* at 8<sup>th</sup> month in plant crop (Table 3). The soil available N at harvest however showed marginally higher values due to bioagents and the sharp fall towards harvest is probably due to removal and exhaustion of N. The soil available P at harvest declined due to N fertilization and improved marginally due to inoculation of *Azotobacter* and *Azospirillum*. The soil available K however, did not differ due to treatments. The status of soil available nutrients in ratoon were not influenced significantly due to treatments.

The population of *Azotobacter* in the soil at 8<sup>th</sup> month increased significantly following its inoculation. The effect was more pronounced when *Azotobacter* was inoculated in higher doses of N (Table 3).

#### Quality and grading of jaggery

Inoculation of *Azospirillum* produce jaggery with higher sucrose and lower reducing sugars (RS) and NR values of above 65 and thus classified under "A<sub>1</sub>" grade with "Excellent" quality in both plant and ratoon crop experiments. This is attributable for higher uptake of N and P for their proper balance of 1 : 1.5 in juice (Ranadive, 1985). Unlike in plant crop, sucrose in jaggery increased with N fertilization with parallel reduction in RS content in ratoon crop which is assignable for reduced N use efficiency leading to lower N uptake besides earliness in maturity of ratoon crop (data not shown).

#### Economics of use of N fixers

Inoculation of *Azotobacter*, in plant crop and *Azospirillum* in ratoon crop in conjunction with recommended N level of 250 kg ha<sup>-1</sup> expressed higher net returns and cost benefit (C : B) ratios as a result of significant improvement in cane yield and economy in fertilizer N. The C : B ratios were 1 : 2.63 in plant crop and 1 : 2.50 in ratoon crop due to the inoculation of respective N fixers as against 1 : 2.38 and 1 : 2.49 respectively under no inoculation (Table 3) (data not shown).



### Leaching loss of NO<sub>3</sub>-N and ground water pollution

The NO<sub>3</sub>-N in the leachate increased with N fertilization while inoculation of *Azotobacter* and *Azospirillum* resulted in conspicuous reduction in the content of NO<sub>3</sub>-N in the leachate. Singh *et al.* (1987) reported that the loss of N increased with the increasing rates of application which brings about ground water pollution. Higher N absorption and better N use efficiency are the plausible reasons for lower levels of NO<sub>3</sub>-N in the leachates collected from the plots/pots treated with bioagents. Also, these bioagents are known to produce gummy substances called "Mucolites" which bind the soil particles for better structure leading to reduced leaching of nitrates. Further, the levels of NO<sub>3</sub>-N in the leachate from the treated plots/pots with the bioagents were much below the formidable limits of 100 mg l<sup>-1</sup> or 1.6 m.eq.l<sup>-1</sup> of drinking water as specified by WHO (Table.3).

### Effect of levels and sources of P and PSM on growth, yield and quality of sugarcane-plant and ratoon

#### Growth and yield parameters

The growth parameters such as germination, tillering, shoot population, LAI and LAD were not influenced significantly due to treatments. The production of dry matter at harvest increased due to inoculation of *Agrobacterium radiobacter* and *Bacillus megaterium* in conjunction with SSP+PM and SSP alone. Marked improvement in crop growth rate (CGR) was observed due to inoculation of *Agrobacterium radiobacter* and *Bacillus megaterium* and the highest CGR of 32.54 g m<sup>-2</sup> d<sup>-1</sup> was observed with *Agrobacterium radiobacter*. The positive influence due to bioagents is attributable for production of plant growth regulators (PGR) besides increased availability and uptake of P. In ratoon crop, the shoot population increased due to P applied through SSP and SSP+PM and inoculation of *Aspergillus awamori*. The LAI and production of dry matter at harvest were influenced favourably due to the inoculation of PSM in conjunction with SSP+PM. Conjunctive use of SSP+PM and *Aspergillus awamori* registered the highest CGR of 22.75 g m<sup>-2</sup> d<sup>-1</sup>. The height and weight of millable cane improved markedly due to P applied through SSP + PM and SSP alone in plant and ratoon experiments. Among the PSM, *Agrobacterium radiobacter* and *Bacillus megaterium* in plant and *Aspergillus awamori* in ratoon crop expressed higher values of yield components (Table 4).

#### Cane yield and sugar yield

The relevant data are presented in Table 5. The perceptible improvement in various growth and yield parameters resulted in significant increase in cane yield due to SSP+PM and SSP alone in plant and ratoon experiments. Among the PSM, *Agrobacterium radiobacter* and *Bacillus megaterium* in plant crop and *Aspergillus awamori* and *Bacillus megaterium* in ratoon crop were significantly superior. Their inoculation ensured 10–11 Mg ha<sup>-1</sup> and 7–8 Mg ha<sup>-1</sup> of increased cane yield in plant and ratoon crop respectively. The data is further suggestive of economy in fertilizer P up to 26% due to inoculation of above PSM. Phosphorus applied through SSP is water-soluble, suitable for neutral soils due to less P fixation and meets the peak P demand in the early growth stages. Pressmud besides

**Table 4. Growth and yield parameters as influenced by levels and sources of P and PSM.**

Character	Dry matter (Mg ha <sup>-1</sup> ) at harvest		CGR (g.m <sup>-2</sup> .d <sup>-1</sup> )		Height of millable cane (m)		Weight of millable cane (kg)	
	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon
<b>P levels</b>								
75% Rec. P	42.95	37.38	29.87	19.66	2.75	2.27	1.61	1.33
100% Rec. P	43.75	37.76	29.86	19.70	2.80	2.30	1.64	1.34
<b>P sources</b>								
SSP	43.44	37.70	29.47	19.44	2.78	2.29	1.63	1.34
SSP + MRP	42.71	35.88	29.78	18.59	2.73	2.18	1.61	1.27
SSP + PM	43.90	39.13	30.34	21.00	2.81	2.38	1.65	1.39
<b>PSM</b>								
<i>Agrobacterium radiobacter</i>	44.79	37.22	32.54	19.80	2.86	2.26	1.68	1.32
<i>Bacillus megaterium</i>	44.48	38.25	30.21	20.12	2.84	2.32	1.67	1.36
<i>Aspergillus awamori</i>	42.07	38.50	28.02	20.40	2.69	2.34	1.58	1.36
Control	42.06	36.32	28.69	18.40	2.69	2.21	1.58	1.29
<b>LSD (P=0.05)</b>								
P levels	NS	NS	NS	NS	NS	NS	NS	NS
P sources	0.819	1.220	NS	1.664	0.05	0.074	0.030	0.043
PSM	1.102	1.451	3.384	1.807	0.07	0.088	0.042	0.052

LSD - Least significant difference    NS - Non significant

rich in P, adds organic matter, leads to better N nutrition and promotes cation exchange capacity (CEC) and are attributable for higher response to the conjunctive use of SSP+PM (Yaduvanshi and Yadav, 1990). Production of PGR and higher P solubilising efficiency are the probable reasons for higher response due to *Agrobacterium radiobacter* and *Bacillus megaterium*. The differential performance of *Aspergillus awamori* in ratoon crop is possibly due to better sporulation under fairly high organic matter content of ratoon soils. Inoculation of *Agrobacterium radiobacter* and *Bacillus megaterium* in plant crop and *Aspergillus awamori* and *Bacillus megaterium* in ratoon crop in conjunction with SSP+PM exhibited significant improvement in sugar yield. The juice quality parameters were not influenced significantly due to treatments.

#### Tissue indices and uptake of major nutrients

Higher index tissue content *i.e.*, 3-6 leaf blades and uptake of N was observed due to P applied though SSP+PM and SSP alone and inoculation of *Agrobacterium radiobacter* and *Bacillus megaterium* in both plant and ratoon experiments. Similarly, higher P indices and uptake were recorded at the recommended level of P and SSP+PM and SSP alone in plant crop. Among the PSM, *Bacillus megaterium* and *Agrobacterium radiobacter* registered higher P indices and uptake of P (Table 5). Eventually, the conjunctive use of

Table 5. Cane yield, sugar yield, P index, uptake of P and cost : benefit ratio as influenced by levels and sources of P and PSM.

Character	Cane yield (Mg ha <sup>-1</sup> )		Sugar yield (Mg ha <sup>-1</sup> )		P index (%) at 6 <sup>th</sup> month		P uptake (kg ha <sup>-1</sup> ) at harvest		Cost : benefit ratio		
	Plant/Ratoon.	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon
<b>P levels</b>											
75% Rec. P		179.72	133.05	24.62	17.75	0.031	0.069	22.21	24.15	2.73	2.85
100% Rec. P		183.05	134.39	25.72	17.62	0.060	0.088	26.93	25.89	2.76	2.84
<b>P sources</b>											
SSP		181.75	134.17	24.75	17.67	0.049	0.075	24.10	24.95	2.73	2.83
SSP + MRP		178.71	127.70	24.89	16.66	0.029	0.060	22.02	17.70	2.70	2.72
SSP + PM		183.71	139.25	25.86	18.73	0.058	0.100	27.59	32.40	2.79	2.98
<b>PSM</b>											
<i>Agrobacterium radiobacter</i>		187.38	132.44	25.94	17.64	0.044	0.079	25.22	26.19	2.80	2.78
<i>Bacillus megaterium</i>		186.11	136.11	25.99	17.78	0.054	0.086	30.06	28.53	2.78	2.80
<i>Aspergillus awamori</i>		176.05	137.00	24.10	18.15	0.042	0.076	22.51	24.75	2.63	2.86
Control		176.00	129.28	24.64	17.18	0.042	0.073	20.49	21.15	2.64	2.75
<b>LSD (P=0.05)</b>											
P levels		NS	NS	NS	NS	-	-	4.349	NS	-	-
P sources		3.405	4.343	0.82	1.02	-	-	4.119	4.018	-	-
PSM		4.613	5.165	1.012	0.895	-	-	2.347	3.694	-	-

LSD - Least significant difference

NS - Non significant

above sources of P and PSM registered higher values of P indices and uptake. The positive influence due to PSM is apparently due to better solubilisation of fixed P in soil. Yadav and Singh (1990) reported increased P uptake following inoculation of *Bacillus megaterium*. The data on ratoon experiments revealed similar trend as observed in plant crop experiment. The K indices and uptake too have improved due to P addition through SSP+PM and inoculation of *Agrobacterium radiobacter* and *Bacillus megaterium* in plant crop while, uptake of K alone exhibited significant increase due to SSP+PM and *Agrobacterium radiobacter* in ratoon experiment (data not shown).

### Quality and grading of jaggery

Higher sucrose and lower RS and ash in jaggery were observed with SSP at recommended level of P and due to inoculation of *Agrobacterium radiobacter* in plant crop. As a result, recommended level of P fertilization through SSP in conjunction with *Agrobacterium radiobacter* produced jaggery of "A<sub>1</sub>" grade with "excellent" quality (data not shown). The beneficial effect of P on composition and clarity of juice are the attributable reasons for better quality of jaggery (Ranadive, 1985).

### Economics of use of phosphate solubilising microorganisms

In general, the inoculation of *Agrobacterium radiobacter* and *Bacillus megaterium* in plant crop and *Aspergillus awamori* in ratoon crop expressed higher net returns and cost benefit ratios. Finally the inoculation of *Agrobacterium radiobacter* in plant crop and *Aspergillus awamori* in ratoon crop in conjunction with SSP+PM registered higher monetary benefits obviously due to higher cane yields (Table 5).

## CONCLUSIONS

Soil application of associative N fixers (Rhizocoenoses) viz., *Azotobacter* in plant crop and *Azospirillum* in ratoon crop ensure improvement in cane yield to a tune of 10–15%, economy in fertilizer N up to 20% and higher net returns and cost benefit ratios. Enhanced nutrients use efficiency especially of N as a result of higher availability and uptake and consequent reduction in leaching loss of N and ground water pollution have further demonstrated their practical utility in agriculture. *Azotobacter* performs better under light textured soil conditions of plant crop whilst, *Azospirillum* under compacted and less aerated soil conditions of ratoon crop. Among the PSM, the bacterial cultures viz., *Agrobacterium radiobacter* and *Bacillus megaterium* in plant crop and the fungus *Aspergillus awamori* in ratoon crop are efficient P solubilisers. Improvement in cane yields, economy in fertilizer P up to 25% are suggestive of cost effectiveness and higher net returns due to their inoculation. The superiority of PM signifies its practical utility as an alternate but cheaper supplementer of P in the presence of PSM.

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## REFERENCES

- Alexander, A.G. (1973). Sugarcane Physiology. A comprehensive study of Saccharum source – sink system. Elsevier Scientific Pub. Co., Amsterdam, Netherlands. pp. 442-487.
- Biswas, B.C., Naresh Prasad and Tewatia, R.K. (1992). Fertilizer use and environmental quality in India. Fert. News. 37(11): 15-17.
- Chhonkar, P.K. (1994). Crop response to phosphatic biofertilizers. Fert. News. 39(4): 39-41.
- Clements, H.F. (1980). Sugarcane crop logging and crop control. Principles and practices. USA University Press, Hawaii. Pp. 348.
- Dobereiner, J., Day, J. and Dart, P.J. (1972). Nitrogenase activity in the rhizosphere of sugarcane and some other tropical grasses. Plant and Soil. 47: 191.
- Dobereiner, J., (1990). Bacterium may triple sugarcane yields. Taiwan Sugar. Pp. 6.
- Gaur, A.C. (1990). Phosphate solubilizing microorganisms as biofertilizers. Omega Scientific Publishers, New Delhi.
- Hunsigi, G. (1982). Ratooning of sugarcane in India. Prabhat Printers, Mysore. pp. 10-24.
- Jackson, M.L. (1973). Soil chemical analysis. Asian Publishing House, New Delhi. Pp. 448.
- Khanna, K.L. and Chakravathi, A.S. (1954). Studies in gur quality in Bihar. Proc. 2<sup>nd</sup> Bien. Conf. Sug. Res. Dev. Workers. 11(4): 74-96.
- Laxmikantham, M., Narasimha Rao, G., Hanumantha Rao, P., Ramana Rao, P.V. and Narasimha Rao, P.V. (1970). Studies in crop logging of sugarcane. APAU, Hyderabad. pp. 5-24.
- Lima, E., Boddey, R.M. and Dobereiner, J. (1987). Quantification of biological nitrogen fixation associated with sugarcane using a <sup>15</sup>N aided nitrogen balance. Soil Boil. Biochem. 19: 165-170.
- Marwaha, B.C. (1995). Biofertilizer a supplementary source of plant nutrient. Fert. News. 40(7): 39-50.
- Ranadive, S.J. (1985). Review of work done on gur at Sugarcane Research Station, Padegaon. Proc. National Seminar-cum-group discussion on jaggery manufacture and storage. Pp. 100.
- Sankaran, A. (1996). Laboratory manual for agricultural chemistry. Asia Publishing House. pp. 96-97.
- Singh, I.P., Singh, B. and Bal, H.S. (1987). Indiscriminate fertilizer use vis-a-vis ground water pollution in Central Punjab. Ind. J. Agric. E.con. 42: 402-409.
- Srinivasan, T.R. and Naidu, K.M. (1987). Response of sugarcane varieties to biofertilizers under different soil conditions. Sugarcane (UK). 3: 5-11.
- Subbaiah, B.V. and Asija, S.L. (1956). A rapid procedure for estimation of available nitrogen in soils. Curr. Sci. 25: 259-260.
- Yadav, D.V. and Singh, T. (1990). Effect of *Bacillus megaterium* on solubilization of phosphatic fertilizers influencing yield P up-take by sugarcane. Bharatiya Sugar. 15(3): 15-23.

Yaduvanshi, N.P.S. and Yadav, D.V. (1990). Effects of sulphitation pressmud and nitrogen fertilizers on biomass, nitrogen economy and plant composition in sugarcane and on soil properties. *J. Agric. Sci. (Camb.)*, 114: 259-263.