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INFLUENCE OF *Gluconacetobacter diazotrophicus* AND PSB WITH GRADED LEVELS OF MAJOR NUTRIENTS ON SUGARCANE GROWTH, YIELD AND QUALITY PARAMETERS

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Abstract: *A field experiment was conducted for three consecutive years (2012-13 to 2014-15) to study the effect of set treatment of Gluconacetobacter diazotrophicus and PSB as well as foliar spray of G. diazotrophicus on sugarcane yield, quality and saving of chemical N and P fertilizers. The set inoculation of Gluconacetobacter diazotrophicus + PSB coupled with 50% recommended N and 75% recommended P_2O_5 recorded the highest cane yield (174.69 t/ha) and the overall superiority in respect of all the growth, yield and quality parameters as a result of the highest count of Gluconacetobacter in cane at 6, 9 and 12 months (6.0, 8.0 and 8.11 x 10^4, respectively). This was statistically at par with foliar spray of G. diazotrophicus liquid culture at 60 days after planting @ 1 lit. in 500 lit water/ha + PSB @ 1.25 kg soil application in most of the parameters. The available N and P status of soil at harvest was significantly influenced by different fertilizer levels (N and P levels), inoculation treatments and their interactions as well. These results indicated the saving of 50% recommended N and 25% recommended P_2O_5 for suru sugarcane besides the improved yield, quality and sustenance of soil fertility.*

Keywords: *Sugarcane, Gluconacetobacter diazotrophicus, PSB, nitrogen, phosphorus.*

Introduction

Sugarcane (*Saccharum officinarum* L.) is an exhaustive crop that can uptake great amount of soil nutrients for its biomass production. In addition to micronutrients exportation, about 65 kg N, 90 kg P_2O_5 and 170 kg K_2O are taken up for a target yield of 50 t ha^{-1} (Kathiresan, 2008). The use efficiency of applied N fertilizers in sugarcane applied with recommended dose of N in the range of 250 to 400 kg ha^{-1} is only 20-30% and hence at every harvest of the crop, soil suffers a net loss of 50-100 kg N/ha. Similarly, out of the total phosphorus fertilizers applied to the crop, only 15-20% can be used, the rest is fixed in the soil as phosphates of Ca, Al or Fe depending on the soil reaction. A permanent manurial trial, conducted for 33 years at Anakpalle (Andhra Pradesh), revealed that sugarcane crop without addition of fertilizers yielded about 40 t ha^{-1} of cane annually. The soil nitrogen reserve under this crop, however, increased by 50% of the initial value which clearly indicated that the root associated diazotrophs contribute significant quantity of nitrogen for sustaining the production of sugarcane (Suman, 2003).

Inoculation of N fixing microbes to sugarcane have increased the cane yield by 5-15%, saved 25 kg fertilizer N ha^{-1} and also improved the juice quality parameter *viz*; sucrose and purity (Hari, 1995; Srinivasan and Naidu, 1987). *Gluconacetobacter diazotrophicus* is a nitrogen fixing bacterium highly specific to sugar-rich crops like sugarcane, sweet potato, pineapple, sugarbeet, etc. It was found to occur in the roots, stems, leaves (Cavalcante and Dobereiner, 1988; Li and MacRae, 1991; Reis *et al.*, 1994), rhizosphere soil and even in cane juice (Muthukumarasamy *et al.*, 1994) in appreciable number in the intercellular spaces of parenchyma and is considered as an obligate endophyte (Tejera *et al.*, 2003). It can excrete about half of its fixed nitrogen in a form that plants can use; excess nitrogen

fertilization decreases the population of *G. diazotrophicus* associated with sugarcane (Fuentez-Ramirez *et al*., 1999). It has also been reported that besides N fixation, all the strains of *G. diazotrophicus* produced indole acetic acid in a culture medium supplemented with tryptophan in the range of 0.14 to 2.42 µg ml^{-1} (Fuentez-Ramirez *et al*., 1993). Furthermore, it has been reported regarding its ability to solubilize insoluble inorganic phosphates from the soil and make available P for the inoculated crops. Hence, *Gluconacetobacter* inoculation to sugarcane significantly increased the cane girth, chlorophyll content, total nitrogen, cane length, number of millable canes resulting the cane yield increase by 42% over control (Chauhan *et al*., 2010). Thus, a field experiment was conducted during 2012-13 to 2014-15 to study the effect of *Gluconacetobacter diazotrophicus* and PSB with varying N and P levels on sugarcane yield, quality and possibility of saving of chemical fertilizer nitrogen and phosphorus.

Materials and Methods

A field experiment was conducted in a 3 x 5 factorial randomized block design (FRBD) with three replications on sugarcane variety CoM 0265 during *Suru* season (January planting) of 2012-13, 2013-14 and 2014-15 at Central Sugarcane Research Station, Padegaon, Tal. Phaltan, Dist. Satara (Maharashtra State, India) to study the effect of *Gluconacetobacter diazotrophicus* and PSB set treatment with varying N and P levels on sugarcane yield, quality and possibility of saving of chemical fertilizer nitrogen and phosphorus. The plot size employed was 6.0 x 6.0 m (5 Rows of 6 m). The biofertilizers *viz*., *G. diazotrophicus* and PSB were procured from Biological Nitrogen Fixation Scheme, College of Agriculture, Pune. For treatment of sugarcane bud sets, the two eye bud sets were dipped in the biofertilizer suspension prepared by mixing 10 kg *G. diazotrophicus* or 1 lit. liquid culture of *G. diazotrophicus* and 1.25 kg PSB in 100 lit. water per hectare as per treatments for 30 minutes prior to planting. The crop was applied with fertilizers as per the treatment details given as under.

Treatment details:

Factor A:

1. 100 % recommended NP (250:115 kg N and P_2O_5/ha)
2. 50 % RD-N + 75 % RD-P_2O_5 (i.e. 125:86 kg N and P_2O_5/ha)
3. 50 % RD-N + 50 % RD-P_2O_5 (i.e. 125:57 kg N and P_2O_5/ha)

Factor B: Biofertilizers treatments/methods

1. Uninoculated control.
2. Set treatment - *G. diazotrophicus* 10 kg + PSB 1.25 kg in 100 lit water/ha for 30 min before planting (Recommended check).
3. Set treatment - *G. diazotrophicus* liquid culture @ 1 lit. in 100 lit water/ha + PSB 1.25 kg soil application through 100 kg compost at planting.
4. Foliar spray of *G. diazotrophicus* liquid culture at 60 days after planting during morning hrs. @ 1 lit. in 500 lit water/ha + 1.25 kg PSB soil application through 100 kg compost at 60 days after planting in furrows.
5. Set treatment - *G. diazotrophicus* liquid culture @ 1 lit. in 100 lit water/ha + PSB 1.25 kg soil application through 100 kg compost at planting + foliar spray of liquid culture at 60 days after planting during morning hrs. @ 1 lit. in 500 lit water/ha.

Note:

Recommended K_2O (115 kg/ha), FYM/ Compost (20 t/ha) and pretreatment of sets with dimethoate + carbendazim were common to all the treatments.

The observation on germination at 30 days after planting was recorded. The data on cane height, internode length, cane girth, number of millable canes (NMC), cane yield, commercial cane sugar (CCS%) and sucrose content in seed cane was recorded at harvest. The population of *G. diazotrophicus* in cane juice was determined at 6, 9 and 12 months by using serial dilution and plating technique (Aneja, 2003). The soil samples were also analyzed for the available N and P after harvest as per the standard procedure described by Jackson

(1967). The data were subjected to statistical analysis by employing the standard methods of analysis of variance (Panse and Sukhatme, 1985) using MSTATC package.

Results and Discussion

The pooled results on sugarcane growth, yield and quality parameters are presented in Table 1 and 2. The fertilizer levels and inoculation treatments had a significant influence on all the growth and yield parameters, CCS % and *Acetobacter* population in canes and the soil N and P status at harvest.

Among the fertilizer levels, the F_2 level (50% N & 75 % P) recorded the highest germination (51.12 %), millable cane height (219.78 cm), total cane height (264.72 cm), number of internodes (20.70/cane), internode length (13.57 cm), cane girth (12.22 cm), number of millable canes i.e. NMC (92220/ha), cane yield (159.49 t/ha), CCS % (13.31 %), CCS yield (21.30 t/ha), count of *Gluconacetobacter* at 6, 9 and 12 months (4.97, 7.00 and 7.17 x 10^4 respectively) and available N (188.84 kg/ha) and P (16.44 kg/ha) status of soil at harvest. However, it was at par with F_1 i.e. 100 % N and P in respect of germination (50.36 %), number of internodes (20.43/cane), cane girth (12.03 cm), NMC (91740/ha) and CCS % (13.13 %).

Among the inoculation treatments, the set treatment with *G. diazotrophicus* 10 kg + PSB 1.25 kg in 100 lit water/ha for 30 min before planting (I_2) recorded the highest germination (51.68 %), millable cane height (219.92 cm), total cane height (264.76 cm), number of internodes (20.88/cane), internode length (13.54 cm), cane girth (12.19 cm), NMC (92290/ha), cane yield (157.62 t/ha), CCS % (13.29%), CCS yield (21.00 t/ha), count of *Gluconacetobacter* in cane at 6, 9 and 12 months (4.93, 6.83 and 7.07 x 10^4 respectively) and available N (188.11 kg/ha) and P (16.36 kg/ha) status of soil at harvest. However, it was at par with I_5 (Set treatment - *G. diazotrophicus* broth culture @ 1 lit. in 100 lit water/ha + PSB 1.25 kg soil application through compost at planting + foliar spray of broth culture at 60 days after planting @ 1 lit. in 500 lit water/ha) in respect of all the parameters except count of *Gluconacetobacter* in cane at 6 months (4.28 x 10^4). It was also at par with I_3 (Set treatment with *G. diazotrophicus* broth culture @ 1 lit. in 100 lit water/ha + PSB 1.25 kg soil application through compost at planting) in respect of the parameters *viz.*, germination (49.57 %), number of internodes (20.35/cane), internode length (13.15 cm), cane girth (12.07 cm), NMC (91130/ha), cane yield (151.95 t/ha), CCS % (13.08 %) and count of *Gluconacetobacter* in cane at maturity (6.55 x 10^4). The treatment I_4 i.e. foliar spray of *G. diazotrophicus* broth culture at 60 days after planting @ 1 lit. in 500 lit water/ha + PSB 1.25 kg soil application through compost at planting was also on par with T_2 in respect of cane girth (11.97 cm) and NMC (90430/ha) and was next superior for the cane yield (148.23 t/ha). The factorial interactions were significant only for the total cane height, number of internodes, CCS %, CCS yield, count of *Gluconacetobacter* in cane at 6 and 9 months and available N and P status of soil at harvest. However, the fertilizer level, F_2 (50 % N & 75 % P) coupled with the inoculation treatment, I_2 (the set treatment with *G. diazotrophicus* 10 kg + PSB 1.25 kg in 100 lit water/ha for 30 min before planting) recorded the highest cane yield (174.69 t/ha) and the overall superiority in respect of all the growth, yield and quality parameters as a result of the highest count of *Gluconacetobacter* in cane at 6, 9 and 12 months (6.0, 8.0 and 8.11 x 10^4 respectively). It was closely followed by F_2I_5 (164.32 t/ha) and F_2I_3 (163.86 t/ha) for cane yield.

Gluconacetobacter inoculation to sugarcane has been reported to significantly increase the plant height, cane length and NMC over control resulting in the increased cane yield (Chauhan *et al.*, 2010). Moreover, the nitrogen fixing bacterial inoculations to sugarcane have also been able to improve the cane girth (Hari, 1995; Srinivasan and Naidu, 1987). Over 40% of atmospheric nitrogen fixed was contributed by *Acetobacter diazotrophicus* besides increased cane yield by 10 to 25% (Bhor *et al.*, 2006). The improvement in sugarcane yield to the tune of 7 to 10 t $acre^{-1}$ and in sugar recovery by 0.5 to 1.0% with 50% reduction in the recommended dose of

chemical nitrogen by use of the nitrogen fixing *Acetobacter diazotrophicus* has been reported by Muthukumarasamy *et al.* (2002). It has been reported that the number and activity of endophytic bacteria is reduced when the sugarcane crop is grown under high or optimal nitrogen input levels (Fuentez-Ramirez *et al.*, 1999; Muthukumarasamy, 2002; Suman, 2003). The available N and P status of soil at harvest was significantly influenced by different fertilizer levels (N and P levels), inoculation treatments and their interactions as well. The increase in soil nitrogen reserve under sugarcane crop by 50 % of the initial value due to the nitrogen fixation by root associated diazotrophs has been reported by Suman (2003).

Cost : Benefit analysis

The economics of effect of *G. diazotrophicus* and PSB at varying N and P levels on sugarcane yield and quality is depicted in Table 3A and 3B. Among the inoculation treatments, set treatment with lignite based culture of *G. diazotrophicus* 10 kg + PSB 1.25kg in 100 lit water/ha for 30 min (I_2) recorded the highest B:C ratio (2.93). Among fertilizer levels, the F_2 level (50% N & 75 % P) recorded the highest B:C ratio (2.97). The interaction of fertilizer levels and inoculation treatments showed that F_2I_2 i.e. 50 % N & 75 % P coupled with the set treatment of lignite based culture of *G. diazotrophicus* 10 kg + PSB 1.25 kg in 100 lit water/ha for 30 min recorded the highest B:C ratio (3.26). This was followed by F_2I_3 (3.06), F_2I_5 (3.04) and F_2I_4 (2.97). It indicated that by employing the treatment of sugarcane bud sets before planting or spray of liquid culture at 60 days after planting one can save 50 % recommended N and 25 % recommended P_2O_5 fertilizer requirement of the crop.

Conclusion

The overall results indicated that for *suru* sugarcane, the bud sets can be treated in the solution of 10 kg *Gluconacetobacter diazotrophicus* + 1.25 kg PSB in 100 lit. water/ha for 30 min before planting or if set treatment is not done before planting, *Gluconacetobacter diazotrophicus* liquid culture can be sprayed @ 1 lit. in 500 lit water/ha at 60 days after planting during morning hrs. and 1.25 kg PSB application in soil by mixing with 100 kg compost in furrows coupled with 50% of recommended N and 75% of recommended P_2O_5 for improved sugarcane yield, quality and sustenance of soil fertility. The results also indicated the saving of 50% N and 25% P_2O_5 for *suru* sugarcane.

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Table 1: Effect of *Gluconacetobacter diazotrophicus* and PSB at varying N and P levels on growth parameters of sugarcane (pooled suru 2012-13, 2013-14 & ratoon 2012-13)

Fertilizers	Inoculation treatments	% Germination at 30 DAP	Millable cane height (cm)	Total cane height (cm)	No. of internodes/ cane	Internode length (cm)	Cane girth (cm)	NMC (1000/ha)
F1	I1	50.31	211.39	256.33	20.22	12.79	11.81	90.31
(100% N & P)	I2	51.09	218.94	261.00	20.62	13.48	12.16	92.50
	I3	49.63	214.00	259.39	20.43	13.10	12.05	92.01
	I4	50.15	212.67	257.83	20.36	13.04	12.03	91.51
	I5	50.60	215.72	259.44	20.54	13.29	12.09	92.35
F2	I1	47.29	200.83	243.83	18.87	12.23	11.46	86.42
(50% N, 75 % P)	I2	54.78	230.39	280.06	21.92	14.29	12.53	94.20
	I3	51.58	221.67	266.67	20.89	13.77	12.43	93.36
	I4	49.50	220.17	263.17	20.71	13.57	12.19	93.06
	I5	52.44	225.84	269.89	21.13	13.99	12.48	94.07
F3	I1	45.48	198.00	235.11	18.57	12.06	11.01	84.01
(50% N & P)	I2	49.17	210.44	253.22	20.11	12.85	11.88	90.16
	I3	47.50	205.27	247.88	19.72	12.58	11.72	88.03
	I4	47.75	203.00	245.44	19.41	12.54	11.69	86.73
	I5	47.33	207.32	251.71	20.04	12.64	11.78	88.52
Fertilizers	S.E.±	0.64	1.55	1.61	0.15	0.11	0.07	0.51
	C.D. at 5 %	1.80	4.35	4.53	0.43	0.32	0.19	1.44
Ino. Treatments	S.E.±	0.82	2.00	2.08	0.20	0.15	0.09	0.66
	C.D. at 5 %	2.32	5.62	5.85	0.55	0.41	0.24	1.86
Interactions	S.E.±	1.43	3.46	3.60	0.34	0.25	0.15	1.14
	C.D. at 5 %	NS	NS	10.13	0.96	NS	NS	NS
	C.V. %	8.63	4.87	4.21	5.06	5.77	3.73	3.79

Table 2: Effect of *G. diazotrophicus* and PSB at varying N and P levels on sugarcane yield, quality, *Acetobacter* population and soil N and P status (pooled suru 2012-13, 2013-14 & ratoon 2012-13)

Fertilizers	Inoculation treatments	Cane yield (t/ha)	CCS %	CCS yield (t/ha)	Acetobacter count (x 10^4)			Avail. Soil N (kg/ha)	Avail. Soil P (kg/ha)
					6 months	9 months	12 months		
F1	I1	146.76	12.97	19.03	2.22	4.50	5.11	182.00	15.54
(100% N & P)	I2	153.40	13.22	20.29	4.00	6.22	6.33	188.67	16.57
	I3	150.40	13.15	19.77	3.44	5.89	5.94	184.56	15.94
	I4	150.86	13.09	19.47	2.94	5.39	5.83	183.56	15.67
	I5	152.19	13.21	20.10	3.22	5.67	6.06	187.67	16.12
F2	I1	135.49	12.44	16.86	2.94	5.22	5.39	171.89	13.75
(50% N, 75 % P)	I2	174.69	13.70	23.96	6.00	8.00	8.11	195.44	17.29
	I3	163.86	13.48	22.10	5.28	7.33	7.44	191.89	17.02
	I4	160.07	13.37	21.30	5.11	6.89	7.22	191.33	17.00
	I5	164.32	13.56	22.28	5.50	7.56	7.67	193.67	17.16
F3	I1	123.49	12.29	15.09	3.39	5.22	5.44	172.33	14.05
(50% N & P)	I2	144.78	12.96	18.76	4.78	6.28	6.78	180.22	15.21
	I3	141.60	12.61	17.87	4.39	6.11	6.28	175.44	14.66
	I4	140.76	12.57	17.19	3.56	5.72	5.78	174.22	14.42
	I5	144.51	12.66	18.33	4.11	6.17	6.22	178.56	14.97
Fertilizers	S.E.±	1.96	0.07	0.28	0.11	0.10	0.15	1.06	0.10
	C.D. at 5 %	5.50	0.19	0.78	0.30	0.29	0.41	2.97	0.28
Ino. Treats.	S.E.±	2.53	0.09	0.36	0.14	0.13	0.19	1.36	0.13
	C.D. at 5 %	7.10	0.24	1.01	0.39	0.37	0.53	3.83	0.37
Interactions	S.E.±	4.37	0.15	0.62	0.24	0.23	0.33	2.36	0.23
	C.D. at 5 %	NS	0.42	1.75	0.67	0.64	NS	6.64	0.64
	C.V. %	8.79	3.45	9.56	17.68	11.15	15.43	3.86	4.33

Table 3 A: Economics of effect of *Gluconacetobacter diazotrophicus* and PSB at varying N and P levels on growth parameters of sugarcane

Fertilizers	Inoculation treatments	Cane yield (t/ha)	Gross returns (Rs/ha)	Cost of cultivation (Rs/ha)	Net returns (Rs/ha)	C:B ratio
F_1	I_1	146.76	293520	109286	184234	2.69
(100% N & P)	I_2	153.40	306800	109736	197064	2.80
	I_3	150.40	300800	109636	191164	2.74
	I_4	150.86	301720	110396	191324	2.73
	I_5	152.19	304380	110696	193684	2.75
F_2	I_1	135.49	270980	106731	164249	2.54
(50% N, 75 % P)	I_2	174.69	349380	107181	242199	3.26
	I_3	163.86	327720	107081	220639	3.06
	I_4	160.07	320140	107841	212299	2.97
	I_5	164.32	328640	108141	220499	3.04
F_3	I_1	123.49	246980	105681	141299	2.34
(50% N & P)	I_2	144.78	289560	106131	183429	2.73
	I_3	141.60	283200	106031	177169	2.67
	I_4	140.76	281520	106791	174729	2.64
	I_5	144.51	289020	107091	181929	2.70

Table 3 B: Two-way Table (Cost:Benefit Analysis)

Fertilizers	Inoculation treatments					Mean
	I_1	I_2	I_3	I_4	I_5	C:B ratio
F_1	2.69	2.80	2.74	2.73	2.75	2.74
F_2	2.54	3.26	3.06	2.97	3.04	2.97
F_3	2.34	2.73	2.67	2.64	2.70	2.61
Mean	2.52	2.93	2.83	2.78	2.83	