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RESEARCH ARTICLE

Coinoculaton of endophytic diazotroph with PGPR and AM fungi for enhancing sugarcane production

■ **E. Jamuna, S. Thiruvarassan and P. Sridhar**

SUMMARY

The field experiment was conducted at Sugarcane Research Station, Cuddalore during 2010 – 2012, to evaluate the response of sugarcane variety CoC 24 to the application of bioinoculants *viz*., *Gluconoacetobacter diazotrophicus,* AM fungi and Azophos (*Azospirillum* and phosphobacteria), under different levels of N, P_2O_5 and K_2O inorganic fertilizer in plant and ratoon crop. The results revealed that the application of mycorrhizae, G. *diazotrophicus,* Azospirillum and phosphobacteria significantly produced higher cane yield in plant crop. The application of *Gluconoacetobacter diazotrophicus* @10 kg + AM fungi + Azophos @10 kg + 75 % NPK recorded the maximum germination and tiller population and also maximum mean millable cane population of 1.32 lakhs /ha, cane yield (137.45 t/ha) and sugar yield (16.96 t/ha). Similar results were recorded with the ratoon crop. The population of *Azospirillum* and *Pseudomonas* are higher compared to that of *Glucanoacetobacter diazotrophicus* and phosphobacteria. The application of bioinoculants improves soil microbial biomass and their by enhancing soil organic matter content. The applied bacterial sources helps in nitrogen fixation and also in continuous mobilizing and solubilisation of nutrients and their persistence and colonization in soil is an added advantage and also enhances the soil fertility. The usage of these bioinoculants inturn reduces the inorganic fertilizer input and thereby reduces the cost of cultivation.

Key Words : Sugarcane, Growth, Yield attributes, Nitrogen fixing bacteria, Phosphorous solubilising, Phosphobacteria, Mobilizing, AM fungi, Yield parameters

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Sugarcane is a very demanding crop as for a cane yield of 100 t/ha it removes about 205 kg N, 55 kg P_2O_5, 275 kg K_2O and a large amount of micronutrients from soil (Yaduvanshi and Yadav, 1990). Since its fertilizer consumption is higher than that of other crops it has negative effect on soil health in the long term. In order to sustain productivity major nutrients are provided each year at the recommended application rates of 150 kg/ha of N and 60 kg each of P_2O_5 and K_2O for sugarcane. The efficiency of sugarcane to utilize N range between 16 and 45% as large quantities of applied N leach down through soil layer due to irrigation (Yadav and Prasad, 1992). Deteoration in the physico-chemical and biological properties of soil is considered to be the prime reason for declining sugarcane yield and productivity. The biofetilizer application increases crop growth through combination of BNF, growth promoting / hormonal substances, increased availability of soil nutrients and disease resistance. The importance of biofertilizer lies in the ability to supplement/ mobilize soil nutrients with minimal use of non renewable resources.

Johri (2006) reported that some of the sugarcane varieties have been found to derive upto 70% of their nitrogen requirement through biological nitrogen fixation. Since than various kinds of bacteria such as *Glucanoacetobacter diazotrophicus*, *Herbaspirillum* spp., *Azospirillum amazonense*, *Burkholderia* spp., capable of fixing nitrogen have been reported to colonize the epidermis of sugarcane stem and roots of which Glucanoacetobacter seems to contribute substantially to nitrogen nutrition of the plant (Dobereiner *et al.,* 1995). *G. diazotrophicus* a nitrogen fixing endophyte is found in high number in all part of sugarcane (10^2 – 10^6 per g fresh weight) and its better colonization in sugarcane is probably due to tis capability to grow in the presence of high sugar and low pH. Besides sugarcane it colonizes many other sugar and non sugar crops also. Production of plant growth hormones is the other beneficial trait associated with *G. diazotrophicus* (Sevilla *et al.,*1998). Field trials conducted in sugarcane with *Glucano acetobacter diazotrophicus* with other diazotrophs can match yield level equal to 275kg N/ha application (Sevilla *et al*., 2001; Muthukumarasway *et al*., 2002; Oliveria *et al*., 2002).

In India where sugarcane occupies over 4 million / ha, interest in exploitation of such microbes has increased due to an increase in prices of chemical fertilizers following withdrawal of subsidy on them. Concerning the above problems the current study was focused on the use of bioinoculants to enhance the sugarcane growth and also to assess the functional potentialities in relation to plant growth promoting activities like IAA, phosphate solubilisation and nitrogenase activity with the following objectives of standardizing the efficient combination of bioinoculants for maximizing sugarcane productivity and exploring the possibility of reduction in inorganic fertilizer input through bioinoculant application.

MATERIAL AND METHODS

The experiment was conducted for a period of 2 year (2010 - 2012) at Sugarcane Research Station, Cuddalore with ten treatments with three replications in Randominised Block Design in plant and ratoon crop. The sugarcane variety taken for the study was CoC24. The mean maximum and minimum temperature of the location was 31.7°C and 24.1°C, respectively. The mean annual rainfall was 1200 mm. The soil of the experimental field was sandy clay loam, with low available N (186.84 kg ha^{-1}), medium in available 'P' (16.5 kg ha^{-1}) and medium in available potash (265 kg ha^{-1}). The pH of the soil is 7.2. The bioinoculants *viz., G. diazotrophicus,* AM fungi and Azophos (*Azospirillum* and phospho bacteria) was used along with inorganic fertilizer.

The microbial inoculants were applied as soil application @ 10 kg/ ha of *G. diazotrophicus* and Azophos, 25 kg of VAM / ha in two equal splits at 30th and 60th day after planting. The data collected on germination count, tiller population, millable cane population, cane yield commercial cane sugar per cent, sugar yield were pooled and analysed. The yield were recorded along with the quality parameters. The soil samples were collected at 45th and 105th day after planting of plant crop and ratoon crop. The population of bioinoculants was enumerated by pour plate technique (James 1958)

RESULTS AND DISCUSSION

The plant and the ratoon crop was raised during 2010-2012 with sugarcane variety CoC24. The microbial bioinoculants were applied as per the treatment schedule. The inorganic fertilizers were also applied as per schedule. Regarding germination and tiller counts, the application of *Gluconoacetobacter diazotrophicus* @10 kg + AM fungi @ 25 kg/ ha + Azophos @10 kg + 75 % NPK recorded higher germination of 87.36 % and maximum tiller population of 1,94,185 / ha (Table 1). Application of *G. diazotrophicus* @10 kg/ ha+ AM fungi @ 25 kg/ ha + Azophos @ 10 kg/ ha + 75 % of the recommended NPK also recorded significantly maximum mean millable cane population and cane yield with 1.32 lakhs /ha and 137.45 t/ha, respectively, followed by the application of *G. diazotrophicus* @10 kg + AM fungi @ 25 kg/ ha + Azophos @10 kg + 100 % NPK with 1.29 lakhs millable cane/ha. The CCS% and sugar yield also recorded maximum with 10.7 % and 16.96 t/ha, respectively with the application of *G. diazotrophicus* @10 kg/ ha+AM fungi @ 25 kg/ ha + Azophos @ 10 kg/ ha + 75 % of the recommended NPK (Table 2). As that of plant crop similar trend was observed with ratoon crop with maximum millable cane population of 1.22 lakhs/ha in plot treated with *G. diazotrophicus* @10 kg/ ha+ AM fungi @ 25 kg/ ha + Azophos @ 10 kg/ ha + 75 % of the recommended NPK (Table 2).

The treatment with *G. diazotrophicus* + AM fungi + Azophos + 75 % NPK recorded the maximum population of G. *diazotrophicus* (39.33 x10^4 cfu/ml and 44.00 x10^4 cfu/ml), *Azospirillum* (35.33 x10^4 cfu/ml and x 50.66 x 10^4 cfu/ml), phosphobacteria (32.00 x10^4 cfu/ ml and 41.00 x10^4 cfu/ml)and *Pseudomonas* (46.66 x10^6 cfu/ml and x 52.00 x 10^6 cfu/ml) at 45th and 10^{5th} DAP, respectively and similar trend was observed with ratoob crop also (Table 4 and 5).

The increase in yield and enhanced quality parameters was due to the combined effect of the

Table 1: Effect of combined application of bio inoculants with NPK fertilizers on yield germination and tiller population in plant and ratoon crop

Sr. No.	Treatments	Plant crop		Ratoon crop	
		Germination percentage	Tiller population ('000/ha)	Sprouting percentage	Tiller population ('000/ha)
1.	*G. diazotrophicus* + 75 % NPK	82.93	178.72	85.93	180.72
2.	AM fungi + 75 % NPK	77.86	175.89	85.86	177.89
3.	Azophos + 75 % NPK	84.09	179.21	86.09	183.21
4.	*G. diazotrophicus* + AM fungi + 75 % NPK	86.06	187.57	87.34	193.38
5.	*G. diazotrophicus* + Azophos + 75 % NPK	86.71	188.29	89.51	190.29
6.	AM fungi + Azophos + 75 % NPK	83.46	191.38	88.76	189.57
7.	*G. diazotrophicus* + AM fungi + Azophos + 75 % NPK	87.36	194.11	94.25	196.11
8.	*G. diazotrophicus* + AM fungi + Azophos + 100% NPK	86.58	194.18	90.18	191.18
9.	Recommended NPK (100%) alone	83.20	194.16	84.20	185.16
10.	75% of recommended NPK alone	83.46	167.67	83.46	154.67
	Mean	84.17	185.12	87.56	184.22
	S.Ed	4.13	0.22	4.13	0.32
	CD (0.05)	8.67	0.46	8.67	0.58

Table 2 : Effect of combined application of bio inoculants with NPK fertilizers on yield attributes, juice quality, cane and sugar yield in plant crop

Sr. No.	Treatments	Millable cane population (' 000/ha.)	Cane yield (' 000/ha.)	CCS%	Sugar yield (t/ha)
1.	*G. diazotrophicus* + 75 % NPK	120.0	126.12	9.92	12.51
2.	AM fungi + 75 % NPK	117.2	123.56	9.72	12.01
3.	Azophos + 75 % NPK	118.7	125.79	9.80	12.33
4.	*G.diazotrophicus*+ AM fungi + 75 % NPK	124.8	131.25	10.34	13.57
5.	*G.diazotrophicus* + Azophos + 75 % NPK	123.5	130.55	10.22	13.34
6.	AM fungi + Azophos + 75 % NPK	121.2	129.84	9.95	12.92
7.	*G.diazotrophicus*+ AM fungi + Azophos + 75 % NPK	132.4	137.45	10.70	14.96
8.	*G.diazotrophicus*+ AM fungi + Azophos + 100% NPK	125.2	133.62	10.50	14.03
9.	Recommended NPK (100%) alone	120.8	128.19	10.50	12.88
10.	75% of recommended NPK alone	95.70	107.15	10.05	10.52
	Mean	119.95	127.352	10.17	12.907
	SEd	4.21	3.55	0.99	0.68
	CD	8.47	7.14	NS	1.38

Table 3: Effect of combined application of bio inoculants with NPK fertilizers on yield attributes, juice quality, cane and sugar yield in ratoon crop

Sr. No.	Treatments	Millable cane population (' 000/ha.)	Cane yield (' 000/ha.)	CCS%	Sugar yield
1.	*G. diazotrophicus* + 75 % NPK	116.20	118.42	10.37	12.05
2.	AM fungi + 75 % NPK	114.25	115.86	10.17	11.55
3.	Azophos + 75 % NPK	115.7	118.09	10.25	11.87
4.	*G.diazotrophicus*+ AM fungi + 75 % NPK	117.8	123.55	10.79	13.11
5.	*G.diazotrophicus* + Azophos + 75 % NPK	117.0	122.85	10.67	12.88
6.	AM fungi + Azophos + 75 % NPK	115.8	122.14	10.4	12.46
7.	*G.diazotrophicus*+ AM fungi + Azophos + 75 % NPK	122.4	129.75	11.15	14.5
8.	*G.diazotrophicus*+ AM fungi + Azophos + 100% NPK	118.2	125.92	10.95	13.57
9.	Recommended NPK (100%) alone	116.5	120.49	10.95	12.42
10.	75% of recommended NPK alone	89.70	99.45	10.5	10.06
	Mean	114.36	119.65	10.62	12.45
	SEd	6.37	5.13	0.86	0.21
	CD	13.38	11.07	NS	0.58

Table 4 : Enumeration of microbial population at 45th and 105th DAP (Plant crop)

Treatments	*Azospirillum* (x 10^5cfu/ml)		*G. diazotrophicus* (x 10^4cfu/ml)		Phosphobacteria (x 10^4cfu/ml)		*Pseudomonas* (x 10^6cfu/ml)	
	45th DAP	105th DAP	45th DAP	105th DAP	45th DAP	105th DAP	45th DAP	105th DAP
G. diazotrophicus + 75 % NPK	35.66	54.00	28.02	49.00	10.66	19.66	35.66	40.33
AM fungi + 75 % NPK	26.66	35.66	19.66	26.00	9.50	20.75	39.00	44.15
Azophos + 75 % NPK	36.00	50.33	15.33	22.50	20.00	32.00	37.66	41.70
G.diazotrophicus+ AM fungi + 75 % NPK	36.77	49.00	22.33	40.33	10.33	19.00	41.66	45.50
G.diazotrophicus + Azophos + 75 % NPK	41.00	50.50	23.16	39.33	18.50	28.66	37.66	45.00
AM fungi + Azophos + 75 % NPK	39.66	47.33	16.67	27.66	15.66	25.66	40.33	40.33
G.diazotrophicus+ AM fungi + Azophos + 75 % NPK	43.33	68.33	26.66	45.66	20.33	30.00	46.66	52.00
G.diazotrophicus+ AM fungi + Azophos + 100% NPK	39.66	59.00	25.12	44.66	19.66	28.66	42.66	49.00
Recommended NPK (100%) alone	24.66	36.33	10.66	15.66	6.66	15.33	20.66	23.66
75% of recommended NPK alone	25.00	30.66	12.50	19.50	4.13	14.00	20.00	28.66
	34.84	48.11	19.21	35.53	10.06	21.77	36.20	41.03

Table 5 : Enumeration of microbial population at 45th and 105th DAR (Ratoon crop)

Treatments	*Azospirillum* (x 10^5cfu/ml)		*G. diazotrophicus* (x 10^4cfu/ml)		Phosphobacteria (x 10^4cfu/ml)		*Pseudomonas* (x 10^6cfu/ml)	
	45th DAP	105th DAP	45th DAP	105th DAP	45th DAP	105th DAP	45th DAP	105th DAP
G. diazotrophicus + 75 % NPK	37.16	57	28.77	50	12.66	22.66	37.66	44.33
AM fungi + 75 % NPK	28.16	38.66	20.41	27	11.5	23.75	41	48.15
Azophos + 75 % NPK	37.5	53.33	16.08	23.5	22	35	39.66	45.7
G.diazotrophicus+ AM fungi + 75 % NPK	38.27	52	23.08	41.33	12.33	22	43.66	49.5
G.diazotrophicus + Azophos + 75 % NPK	42.5	53.5	23.91	40.33	20.5	31.66	39.66	49
AM fungi + Azophos + 75 % NPK	41.16	50.33	17.42	28.66	17.66	28.66	42.33	44.33
G.diazotrophicus+ AM fungi + Azophos + 75 % NPK	44.83	71.33	27.41	46.66	22.33	33	48.66	56
G.diazotrophicus+ AM fungi + Azophos + 100% NPK	41.16	62	25.87	45.66	21.66	31.66	44.66	53
Recommended NPK (100%) alone	26.16	39.33	11.41	16.66	8.66	18.33	22.66	27.66
75% of recommended NPK alone	26.5	33.66	13.25	20.5	6.13	17	22	32.66
	36.34	51.11	20.76	34.03	15.54	26.37	38.20	45.03

bioinoculants along with the inorganic fertilizers. The biofertilizers application enhanced the yield and quality parameters and also essential to maintain soil microflora population and protect soil fertility from deterioration. Significant changes in various plant growth parameters have been shown by the inoculation of various nitrogen fixing and plant growth promoting bacteria (Nayak *et al.,* 1986; Murty and Ladha, 1988 and Gunarto *et al.,* 1999). Sevilla *et al* (1998), have shown the benefits to sugarcane growth by using *Nif* – mutants of *Acetobacter*. In addition to nitrogen fixation the beneficial effects has been attributed to the production of plant growth hormones also (Glick, 1994).

Application of phosphorous from different sources *ie.* from inorganic and as bioinoculants (AM fungi and phosphobacteria) was found to be effective in sugarcane. Continuous availability of the valuable nutrients and their persistence and colonization in soil makes the soil more fertile and healthy. The mobilization of P from soil to the plants is mediated by hairy root systems of the mycorrhizal fungi through plant roots. It commonly infect plant roots, including those of sugarcane forming beneficial symbiotic relationships (Kelly *et al.,* 1997). The improvement in plant growth was attributed to an enhanced access of mycorrhizal root to soil phosphorous located beyond the rhizosphere (Sanders and Tinker. 1973) and infection by mycorrhizal fungi is significantly rduced at high soil phosphorous levels (Amijee *et al.,* 1989). Mycorrhiza was found to be compatible with nitrogen fixers *viz.*, *Rhizobium* (Hayman, 1986), *Acetobacter* and phosphate solubilising bacteria (Singh and Kapoor, 1999). It also holds good for sugarcane.

Conclusion:

The inoculation of bioinoculants is beneficial for sugarcane growth for increasing the plant vigour at lower nitrogen levels, consequently the amount of fertilizer could be reduced. AM fungi and phosphobacteria are very much essential to convert the unavailable form of the phosphorous source to available source and providing to the plants. The usage of these bioinoculants inturn reduces the inorganic fertilizer input and thereby reduces the cost of cultivation. With this references these bioinoculants can be recommended for their use in nutrient management and enhanced sugarcane productivity.

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