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Response of consortium of ENFB with levels of nitrogen on nutrient concentration on different stages, total uptake of n at harvest and n balance sheet of pre-seasonal sugarcane (Second Ratoon)

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Abstract

The experiment was conducted at PGI Farm, MPKV, Rahuri during 2015-16 to study the "Effect of consortium of nitrogen fixing bacteria on yield, quality and nutrient uptake of pre-seasonal sugarcane (Second Ratoon)". In this experiment 6 treatments and 4 replications used in randomized block design. There were four levels of nitrogen 0, 25, 50 and 100% and 100% P₂O₅, 100% K₂O along with and without foliar spray of consortium of endophytic nitrogen fixing bacteria. The result revealed that nutrient uptake and total nitrogen uptake of pre-seasonal sugarcane (Second Ratoon) were increased with foliar spray of consortium of endophytic bacteria along with 25% N of RDF. Among all the treatments, 100% N (RDF) recorded significantly highest yield and quality. The nitrogen balance sheet of experimental field shown negative balance towards nitrogen in soil.

Keywords: sugarcane, second ratoon, consortium, endophytes

Introduction

Sugarcane (*Saccharum officinarum L.*) is a member of the Andropogoneae tribe of the Poaceae (grass) family. It is a major crop in the tropical and semi-tropical area, where it is grown for the production of sugar and ethanol purpose. Sugarcane is capable of fixing atmospheric nitrogen in association with the bacterium *Gluconacetobacter diazotrophicus* (Yamada *et al.*, 1998) [11]. Unlike legumes and other nitrogen-fixing plants that form root nodules in the soil in association with bacteria *G. diazotrophicus* lives within the intercellular spaces of the sugarcane's stem (Dong *et al.*, 1994) [3]. It is reported that nitrogen at rate of about 250 kg ha⁻¹ along with some nitrogen fixers such as *Azotobacter* and *Azospirillum* has a significant effect on ratoon cane yield. Consortium of endophytic bacteria includes various bacteria like, *Acetobacter spp.*, *Agrobacterium spp.*, *Burkholderia spp.*, *Azospirillum spp.*, *Herbaspirillum spp.*, *Azoarcus spp.* etc. Endophytes are defined as microorganisms living inside the plant organs and tissues without causing disease symptoms, have become highly interesting models to study plant microbe interactions (More, 2012) [5]. Diazotrophic, non-legume plant growth promoting bacteria are able to support plant growth at low nitrogen conditions by a combination of nitrogen fixation, increasing the availability of soil nutrients, promoting root growth by hormonal signaling, and controlling disease symptoms (Spaepen *et al.*, 2007) [9]. An increase in biomass production and leaf N-content of both micropropagated and sugarcane developed from buds when endophytic Diazotrophic bacteria was inoculated together with arbuscular mycorrhizal fungi (Muthukumarasamy *et al.*, (1999) [6].

The application of RDF significantly increased cane and commercial cane sugar yield of pre-seasonal sugarcane and it was on par with 25% N with foliar application of consortium of endophytic bacteria at 3 L ha⁻¹ at 60 days after planting. It could be concluded that 25% N + foliar application of consortium of endophytic bacteria was found beneficial for nutrient uptake of pre-seasonal sugarcane of cultivar Phule-265 (Dhole, 2015) [2] and (Rajbinde, 2016) [8].

Material and Methods

The present investigation was carried out during February 2016 to January 2017 (pre-seasonal) to study the effects of application of consortium of endophytic nitrogen fixing bacterial culture on Microbial Population (*Gluconacetobacter diazotrophicus*) in sugarcane second ratoon grown on Inceptisol (Vertic Haplustept).

The site for the experiment was at Post Graduate Institute Research Farm of Department of Soil Science and Agricultural Chemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra (India). First pre-seasonal ratoon sugarcane was harvested in the month of February 2016 and second ratoon crop was continued. No new seedling setts for gap filling were used. It was laid out in randomized block design with four replications and six treatments.

The nitrogen, phosphorus and potassium were applied to crop through urea (46% N), single superphosphate (16% P₂O₅) and muriate of potash (60% K₂O) as per RDF for pre-seasonal sugarcane. There were different levels of nitrogen 0, 25, 50 and 100% of recommended dose of fertilizer with 100% P₂O₅ and 100% K₂O along with and without foliar sprays of consortium of endophytic nitrogen fixing bacteria at 60 days after ratooning.

An *Acetobacter diazotrophicus* culture and consortium of endophytic nitrogen fixing bacteria (consortium includes *Acetobacter spp.*, *Azospirillum spp.*, *Azoarcus spp.*, *Agrobacterium spp.*, *Burkholderia spp.*, *Herbaspirillum spp.*) for the fixation of nitrogen in plant biologically was obtained from Department of Microbiology, Vasantdada Sugar Institute, Manjari, Pune (M.S.). *Acetobacter diazotrophicus* culture foliar application was given @ 2.5 kg ha⁻¹ at 60 DAHR and liquid consortium of endophytic bacteria was applied as foliar application @ 3 L.ha⁻¹ according to treatments at 60 DAHR.

The different levels of nitrogen, *Acetobacter diazotrophicus* culture and consortium of endophytic nitrogen fixing bacteria were applied in combination as per following treatment through soil and foliar sprays.

- T1 - Absolute control
 T2 - RDF +100% N (250 kg N ha⁻¹)
 T3 - 50% N + *Acetobacter diazotrophicus* @ 2.5 kg.ha⁻¹ (foliar spray at 60 DAHR)
 T4 - 25% N + Consortium of endophytic bacteria @ 3 L.ha⁻¹ (foliar spray at 60 DAHR)
 T5 - Consortium of endophytic bacteria @ 3 L.ha⁻¹ (foliar spray at 60 DAHR)
 T6 - Without consortium of endophytic bacteria

Note

1. 100% P₂O₅ and 100% K₂O common to all treatments except T₁.

2. RDF of pre-seasonal sugarcane (ratoon) (250:115:115N, P₂O₅, K₂O ha⁻¹) is applied for pre-seasonal sugarcane (second ratoon).
 3. DAHR-days after harvesting of ratoon.

Intercultural operations like weeding, spraying of insecticide, fertilizer application and schedule of irrigation for ratoon sugarcane crop was carefully followed. The cop variety used was Co- 265. The data obtained was carefully analyzed and appropriately interpreted as per the methods described in "Statistical Methods for Agricultural Workers" by Panse and Sukhatme, (1985)^[7].

Results and Discussions

Nutrient Concentration

The data on nutrient concentration of top and cane at 6 month stage are presented in Table 1. The nitrogen content in sugarcane top was ranged from 0.92% (T₁) to 1.25% (T₂). The treatment 100% N RDF showed higher value and it was at par with 25% N + foliar application of consortium of endophytic bacteria (T₄). Similarly, percent N in cane was ranged from 0.61% (T₁) to 0.81% (T₂). The treatment T₂ showed the highest N content over other treatments except 25% N + foliar application of consortium of endophytic bacteria (T₄) which was at par with T₂ treatment.

Phosphorus content in top at 6 month was ranged from 0.21% (T₁) to 0.27% (T₄). Phosphorus content in top in T₄ treatment i.e. 25% N + foliar application of consortium of endophytic bacteria was significantly increased P content (0.27%) and at par with T₂ and T₃ i.e. RDF (0.26% and 0.25% respectively). Concentration of P in cane ranged from 0.18% (T₁) to 0.22% (T₄). The T₄ treatment showed significantly higher P content over other treatments except T₂ i.e. RDF 100% N (0.21%).

Potassium content in top at 6 month was ranged from % (T₁) 1.18% to 1.56% (T₂). Treatment T₂ i.e. RDF 100% N showed the highest K concentration in top over other treatments except 25% N + foliar application of consortium of endophytic bacteria (T₄) which is at par with T₂. Concentration of K in cane was ranged from 0.59% (T₁) to 0.73% (T₂). Treatment T₂ i.e. 100% N showed significantly higher K content (0.73%) over other treatment except T₅ i.e. foliar application of consortium of endophytic bacteria (0.72%) and T₄ i.e. 25% N + Foliar application of consortium of endophytic Bacteria (0.71%).

The concentration of different nutrients in the top and cane at 6 month stage indicated that the foliar application of consortium of endophytic bacteria with 25% N of RDF was favourable for maintaining higher values of N and P.

Table 1: Nutrient concentration of top and cane as influenced by levels of nitrogen with consortium of endophytic bacteria at 6 month of pre-seasonal sugarcane (second ratoon).

Tr. No.	Treatment	Nutrient concentration (%)					
		N		P		K	
		Top	Cane	Top	Cane	Top	Cane
T ₁	Absolute control	0.92	0.61	0.21	0.18	1.18	0.59
T ₂	RDF (100% N)	1.25	0.81	0.26	0.21	1.56	0.73
T ₃	50% N + <i>Acetobacter diazotrophicus</i> (Foliar spray)	1.21	0.70	0.25	0.20	1.46	0.68
T ₄	25% N + Consortium of endo. bacteria (foliar application)	1.24	0.80	0.27	0.22	1.53	0.71
T ₅	Foliar application of consortium of endo. bacteria	1.21	0.71	0.24	0.20	1.49	0.72
T ₆	Without consortium of endophytic bacteria	1.17	0.63	0.22	0.19	1.24	0.62
S.E. ±		0.006	0.009	0.005	0.004	0.011	0.007
C.D. at 5%		0.017	0.026	0.016	0.013	0.034	0.021

The data on nutrient concentration of top and cane at 9 month stage are presented in Table 2. The nitrogen content in sugarcane top was ranged from 0.93% (T₁) to 1.26% (T₂). The

treatment 100% N RDF showed higher value and it was at par with 25% N + foliar application of consortium of endophytic bacteria (T₄). Similarly, percent N in cane was ranged from

0.64% (T₁) to 0.83% (T₂). The treatment T₂ showed the highest N content over other treatments except 25% N + foliar application of consortium of endophytic bacteria (T₄) which was at par with T₂ treatment *i.e.* RDF (100% N).

Phosphorus content in top at 9 month was ranged from 0.23% (T₁) to 0.31% (T₄). Phosphorus content in top in T₄ treatment *i.e.* 25% N + foliar application of consortium of endophytic bacteria was significantly increased P content (0.31%) which was at par with T₂ *i.e.* RDF 100% N (0.29%). Concentration of P in cane ranged from 0.18% (T₁) to 0.25% (T₄). The T₄ treatment showed significantly higher P content over other treatments except T₂ *i.e.* RDF 100% N (0.25%).

Potassium content in top at 9 month was ranged from % (T₁)

1.19% to 1.57% (T₂). Treatment T₂ *i.e.* RDF 100% N showed the highest K concentration in top over other treatments except 25% N + foliar application of consortium of endophytic bacteria (T₄) which is at par with T₂. Concentration of K in cane was ranged from 0.61% (T₁) to 0.75% (T₂). Treatment T₂ *i.e.* 100% N showed significantly higher K content (0.75%) over other treatment except T₅ *i.e.* foliar application of consortium of endophytic bacteria (0.74%).

The concentration of different nutrient in the top and cane at 9 month stage indicated that the foliar application of consortium of endophytic bacteria with 25 % N of RDF was favourable for maintaining higher values of N, P and K.

Table 2: Nutrient concentration of top and cane as influenced by levels of nitrogen with consortium of endophytic bacteria at 9 month of pre-seasonal sugarcane (second ratoon).

Tr. No.	Treatment	Nutrient concentration (%)					
		N		P		K	
		Top	Cane	Top	Cane	Top	Cane
T ₁	Absolute control	0.93	0.64	0.23	0.20	1.19	0.61
T ₂	RDF (100% N)	1.26	0.83	0.29	0.24	1.58	0.75
T ₃	50% N + <i>Acetobacter diazotrophicus</i> (Foliar spray)	1.23	0.72	0.28	0.23	1.48	0.71
T ₄	25% N + consortium of endo. bacteria (Foliar spray)	1.25	0.81	0.31	0.25	1.57	0.73
T ₅	Foliar application of consortium of endo. bacteria	1.22	0.73	0.26	0.22	1.51	0.74
T ₆	Without consortium of endophytic bacteria	1.19	0.65	0.23	0.21	1.27	0.63
S.E. ±		0.006	0.007	0.006	0.003	0.006	0.004
C.D. at 5%		0.020	0.022	0.018	0.009	0.019	0.013

The data on nutrient concentration of top and cane at harvest are presented in Table 3. The nitrogen content in sugarcane top was ranged from 0.91% (T₁) to 1.24% (T₂). The treatment 100% N RDF showed higher value and it was at par with 25% N + foliar application of consortium of endophytic bacteria (T₄). Similarly, percent N in cane was ranged from 0.59% (T₁) to 0.78% (T₂). The treatment T₂ showed the highest N content over other treatments except 25% N + foliar application of consortium of endophytic bacteria (T₄) which was at par with T₂ treatment.

Phosphorus content in top at harvest was ranged from 0.22% (T₁) to 0.29% (T₄). Phosphorus content in top in T₄ treatment *i.e.* 25% N + foliar application of consortium of endophytic

bacteria was significantly increased P content (0.28%) and at par with T₂ *i.e.* RDF 100% N (0.28%). Concentration of P in cane ranged from 0.19% (T₁) to 0.24% (T₄). The T₄ treatment showed significantly higher P content over other treatments except T₂ *i.e.* RDF 100% N (0.22%). Concentration of different nutrient in the top and cane at harvest stage indicated that the foliar application of consortium of endophytic bacteria as well as foliar application with *Acetobacter diazotrophicus* was favourable for maintaining higher values of N, P and K upto harvest.

The increase in leaf N content was also reported by (Muthukumarasamy, 1999) [6] in sugarcane plant inoculated with endophytic bacteria.

Table 3: Nutrient concentration of top and cane as influenced by levels of nitrogen with consortium of endophytic bacteria at harvest of pre-seasonal sugarcane (second ratoon).

Tr. No	Treatment	Nutrient concentration (%)					
		N		P		K	
		Top	Cane	Top	Cane	Top	Cane
T ₁	Absolute control	0.91	0.59	0.22	0.19	1.21	0.63
T ₂	RDF (100% N)	1.24	0.78	0.28	0.22	1.60	0.78
T ₃	50% N + <i>Acetobacter diazotrophicus</i> (Foliar spray)	1.22	0.69	0.26	0.20	1.50	0.73
T ₄	25% N + Foliar application of consortium of endo. bacteria	1.23	0.77	0.29	0.24	1.59	0.75
T ₅	Foliar application of consortium of endo. bacteria	1.21	0.72	0.25	0.21	1.53	0.76
T ₆	Without consortium of endophytic bacteria	0.94	0.63	0.23	0.20	1.30	0.65
S.E. ±		0.004	0.007	0.006	0.008	0.005	0.006
C.D. at 5%		0.013	0.022	0.019	0.025	0.014	0.018

Total uptake of nutrients

Data on total uptake of nitrogen, phosphorus and potassium are presented in Table 4 and Fig 1. The recommended dose of fertilizer with 100% N without foliar sprays of consortium of endophytic bacteria gave significantly higher nitrogen uptake in RDF 100% N (373.52 kg ha⁻¹) over all other treatments except T₄ *i.e.* 25% N + foliar application of consortium of endophytic bacteria (316.36 kg ha⁻¹). The treatment T₂ gave significantly higher phosphorus uptake (76.52 kg ha⁻¹) over all other treatments except T₄ *i.e.* 25% N + foliar application

of consortium of endophytic bacteria (76.04 kg ha⁻¹). The total uptake of potassium was significantly highest in RDF treatment (105.86 kg ha⁻¹).

Total nitrogen uptake by sugarcane plant was ranged from 134.21 kg ha⁻¹ (T₁) to 373.53 kg ha⁻¹ (T₂). Although, the application of RDF (T₂) was significantly showed higher nitrogen uptake. The treatment T₄ *i.e.* 25% N + foliar application of consortium of endophytic bacteria (316.36 kg ha⁻¹) shown comparable effect of total nitrogen uptake. This indicates that at lower dose of nitrogen the efficiency of

nitrogen fixation by endophytes might have enhanced and resulted into increase in nitrogen uptake. The data indicated that the 25% N with foliar application of consortium of endophytic bacteria also maintained the nitrogen uptake pool in sugarcane plant in spite of reduction of 75% N dose (187.5 kg N ha⁻¹). Thus, signifying the role of consortium of endophytic bacteria in N fixation. The use of foliar sprays of consortium of endophytic bacteria showed significantly higher N uptake over uninoculated treatment. Similar result were obtained by Indira and Bagyaraj (1997)^[4] and Boddey *et al.* (1995)^[1] in sugarcane.

Total phosphorus uptake by sugarcane was ranged from 48.96 kg ha⁻¹ (T₁) to 76.52 kg ha⁻¹ (T₄). The 25% N with foliar application of consortium of endophytic bacteria (T₄) showed significantly higher phosphorus uptake over all other treatments except RDF (T₂). The results indicated that the 25% N with foliar application of consortium of endophytic bacteria maintained the phosphorus uptake pool in sugarcane plant. Thus, role of consortium of endophytic bacteria in P uptake of sugarcane was also significant as compared to RDF. Similar observation was also recorded by Dhole (2015)^[2] and Rajbinde (2016)^[8].

Total potassium uptake by sugarcane was ranged from 225.64 kg ha⁻¹ (T₁) to 379.03 kg ha⁻¹ (T₂). The treatment T₂ (RDF 100% N) showed significantly higher K uptake (379.03 kg ha⁻¹) over rest of the treatment. Results indicated that the

application of 25% N with foliar application of endophytic bacteria showed significantly higher uptake of N and P, which saved 75 % N dose (187.5 kg ha⁻¹) as compared to RDF.

An improvement in sugarcane growth and nutrient uptake by fertilizing with different levels of urea (0, 75 and 150 kg N ha⁻¹) and inoculating with *Acetobacter diazotrophicus* was also observed by Suman *et al.* (2005)^[10].

Nitrogen balance study

The data in respect of nitrogen balance study are presented Table 5. All the treatments showed negative balance towards nitrogen in soil. Maximum negative balance in soil was observed in T₄ treatment, 25% N with foliar application of consortium of endophytic bacteria (-262.75 kg ha⁻¹) followed by T₅ treatment, foliar application of consortium of endophytic N fixing bacteria (-223.06 kg ha⁻¹). The study indicated that there is a mining of soil available nitrogen from soil where endophytic bacteria was used as foliar sprays in absence of N fertilizer or with lower dose of N fertilizers.

Soil nitrogen balance was computed by making the summation of initial soil available nitrogen and nitrogen added through chemical fertilizers. This summation was subtracted from the summation of total uptake of nitrogen by sugarcane and soil available nitrogen at harvest of second ratoon sugarcane.

Table 4: Total uptake of nutrients at harvest of pre-seasonal sugarcane (second ratoon) as influenced by levels of nitrogen with consortium of endophytic bacteria.

Tr. No.	Treatment	Total uptake of nutrients (kg ha ⁻¹)								
		N			P			K		
		Cane	Top	Total	Cane	Top	Total	Cane	Top	Total
T ₁	Absolute control	103.13	31.08	134.21	19.70	29.26	48.96	64.72	160.92	225.64
T ₂	RDF (100% N)	308.38	65.15	373.53	29.08	47.44	76.52	105.86	273.17	379.03
T ₃	50% N + <i>Acetobacter diazotrophicus</i> (Foliar application)	216.21	52.32	268.53	25.08	41.69	66.77	93.29	245.51	338.80
T ₄	25% N + foliar application of consortium of endophytic bacteria	256.40	59.96	316.36	29.49	46.55	76.04	94.36	258.99	353.35
T ₅	Foliar application of consortium of endophytic bacteria	136.64	46.88	183.52	23.68	37.42	61.10	87.80	226.73	314.53
T ₆	Without consortium of endophytic bacteria	117.27	35.81	153.08	22.16	32.88	55.04	72.36	188.12	260.48
	S.E. ±	14.66	2.37	14.81	1.06	1.12	1.74	1.30	2.26	2.59
	C.D. at 5%	44.17	7.15	44.65	3.20	3.38	5.26	3.92	6.82	7.82

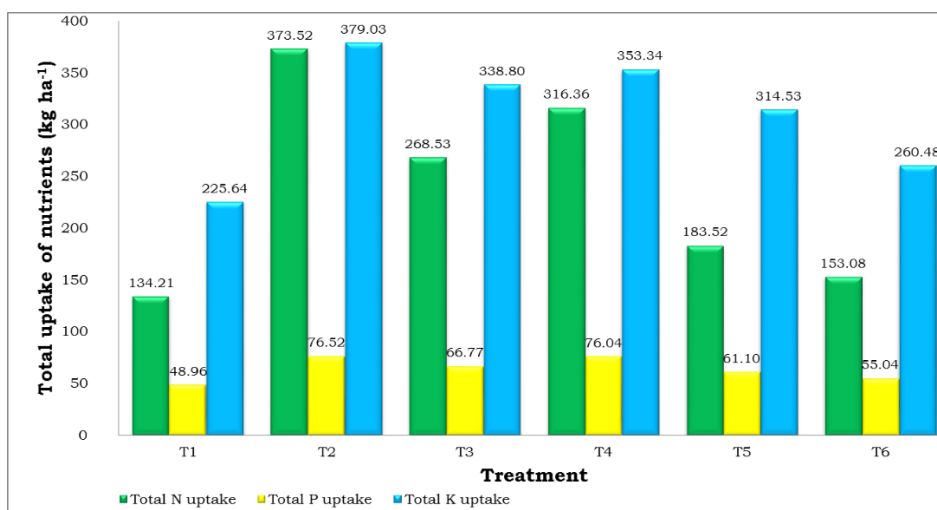


Fig 1: Total uptake of nutrients as influenced by levels of nitrogen with consortium of endophytic bacteria at harvest of pre-seasonal sugarcane (second ratoon).

All the treatments observed negative balance in respect of nitrogen. The negative balance of N was ranged from -157.22 kg ha⁻¹ (T₃) to -262.75 kg ha⁻¹ (T₄). The highest negative balance was noticed in T₄, 25% N with foliar application of

endophytic N fixing bacteria (-262.75) followed by T₅, foliar application of consortium of endophytic bacteria (-223.06). It showed that there is a possible mining of available nitrogen from soil where endophytic bacteria as foliar spray was used.

Table 5: Soil nitrogen balance as influenced by levels of nitrogen with consortium of endophytic bacteria at harvest of pre-seasonal sugarcane (second ratoon) (in kg ha⁻¹).

T. no.	Treatment	Initial avail. N (A)	N added (B)	Total N uptake (C)	Avail. N at Harvest (D)	N balance (A+B)-(C+D)
T ₁	Absolute control	136.7	0	134.21	153.66	-175.27
T ₂	RDF (100% N)	153.55	250	373.52	178.75	-158.68
T ₃	50% N + <i>Acetobacter diazotrophicus</i> (foliar application)	157.1	125	268.53	163.07	-157.22
T ₄	25% N + Conso. of endo. bacteria (foliar application)	148.79	62.5	316.36	150.53	-262.75
T ₅	Foliar application of conso. of endophytic bacteria	147.1	0	183.52	156.8	-223.06
T ₆	Without conso. of endophytic bacteria	139.67	0	153.08	144.26	-187.62

Conclusion

The recommended dose of fertilizer treatment (250 kg N, 115 kg P₂O₅ and 115 kg K₂O ha⁻¹) gave significantly higher nutrient uptake and total nitrogen uptake. These results were at par with treatment 25% N with foliar application of consortium of endophytic bacteria (T₄). Application of 25% of recommended dose of N (62.50 kg N ha⁻¹) + 100% P₂O₅ (115 kg P₂O₅ ha⁻¹) + 100% K₂O (115 kg K₂O ha⁻¹) and foliar application of consortium of endophytic bacteria @ 3 L.ha⁻¹ in 500 L of water at 60 days after harvesting of pre-seasonal sugarcane (second ratoon) positively beneficial for microbial population with ecofriendly use of nitrogenous fertilizer.

Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB, List no. 64. Int J Syst Bacteriol. 1998; 48:327-328.

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