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Effect of consortium of endophytic n fixing bacteria on microbial population *Gluconacetobacter diazotrophicus* in pre-seasonal sugarcane second ratoon

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Abstract

Field experiment was conducted during the year 2016-17 at Post Graduate Institute Farm, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.S.) to study the effect of consortium of endophytic nitrogen fixing bacteria on microbial population and other yield parameters of pre-seasonal sugarcane (second ratoon). The experiment was laid out in randomized block design with four replications and six treatments. There were four levels of nitrogen 0, 25, 50, and 100% of recommended dose of nutrients (RDF) with 100% P₂O₅, 100% K₂O along with and without foliar spray of consortium of endophytic nitrogen fixing bacteria. 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.) The application of 250 kg N, 115 kg P₂O₅ and 115 kg K₂O ha⁻¹ (RDF) to second ratoon sugarcane significantly increased cane and CCS yield as well as microbial population, followed by 25% N with foliar application of consortium of endophytic bacteria (T₄). The microbial population (*Gluconacetobacter diazotrophicus*) of sugarcane (top and cane) at 180 DAHR was ranged from 9.75 x 10⁷ cfu ml⁻¹ (T₁) to 34.25 x 10⁷ cfu ml⁻¹ (T₅). was ranged from 9.75 x 10⁷ cfu ml⁻¹ (T₁) to 34.25 x 10⁷ cfu ml⁻¹ (T₅). The microbial population depicts the role of N fixation and consortia in sugarcane by use of consortium by its population at 180 and at harvesting. Use of consortium results in 75% saving in N fertilizer even in second ratoon.

Keywords: sugarcane, consortium, acetobacter, microbial population, second ratoon

Introduction

Sugarcane is botanically known as *Saccharum officinarum* (2n=80) belongs to family Poaceae. It is commercial crop grown under well and command areas of Maharashtra. India is the second largest in area under sugarcane (5.038 million ha) and production is 361 MT per year. The average productivity of sugarcane in India is 71.70 tonne ha⁻¹ with sugar recovery of 10.25 %. In Maharashtra 936 thousand hectares of land with average productivity is 77.4 tonne ha⁻¹. The country's requirement by 2025 AD has been projected at 625 MT cane, which means that there is need to raise the productivity of sugarcane and sustain the same.

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) [3]. 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) [6].

Acetobacter Diazotrophicus is nowadays known as *Gluconacetobacter Diazotrophicus*. It is an acid loving bacterium, require pH 4.0 to 4.5 for growth and nitrogen fixation. It showed positive growth at 25, 30 and 40°C temperature. It has potential to fix atmospheric nitrogen upto 300 kg ha⁻¹. Besides this, it is also able to solubilize insoluble phosphate in culture broth due to acid production (Bhowmik and Konde, 1997 and Mowade and Bhattacharyya, 2000) [1, 4]. N₂-fixing bacterial endophytes, such as *Burkholderia* spp., *Agrobacterium* spp., *Azoarcus* spp., *Herbaspirillum* spp., *Azospirillum* spp. and *Gluconacetobacter Diazotrophicus* been found within the tissues of some crops and grasses, and partially contribute to the nitrogen

requirement of the host plants. Therefore, looking to the importance of nitrogen fixing bacteria, their growth and activity for plant, the current investigation was carried out with a view to study of 'Effect of consortium of endophytic nitrogen fixing bacteria on Microbial Population (*Gluconacetobacter diazotrophicus*) in Pre-Seasonal Sugarcane (second ratoon).

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 sets for gap filling were used. It was laid out in randomized block design with four replications and six treatments. 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.

Biofertilizers

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.).

Treatment details

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:

- 100% P₂O₅ and 100% K₂O common to all treatments except T₁.
- RDF of pre-seasonal sugarcane (ratoon) (250:115:115N, P₂O₅, K₂O ha⁻¹) is applied for pre-seasonal sugarcane (second ratoon).
- DAHR-days after harvesting of ratoon.

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. Microbial population of *Gluconacetobacter diazotrophicus* was estimated using Serial dilution plate technique proposed by Chhonkar *et al.* (2007)^[2] and the data on microbial count was tabulated and analyzed statistically by the method described by Panse and Sukhatme (1985)^[5].

Result and discussions

The data on microbial population (*Gluconacetobacter diazotrophicus*) of sugarcane (top and cane) at 180 DAHR is presented in table 1 and Fig 2. The microbial population in sugarcane at 180 DAHR was ranged from 9.75 x10⁷cfu ml⁻¹ (T₁) to 34.25 x10⁷cfu ml⁻¹ (T₅).

The data on microbial population (*Gluconacetobacter diazotrophicus*) of sugarcane (top and cane) at harvest is presented in table 2 and Fig 2. The microbial population in sugarcane at 180 DAHR was ranged from 9.75 x10⁷cfu ml⁻¹ (T₁) to 34.25 x10⁷cfu ml⁻¹ (T₅). At harvesting stage of sugarcane second ratoon the microbial population ranged from from 27.25 x10⁷cfu ml⁻¹ (T₁) to 67.25 x10⁷cfu ml⁻¹ (T₅). The Microbial population of *Gluconacetobacter diazotrophicus* insugarcane plant of second ratoon showed significantly increased in all inoculated treatments, however the treatment T₅ i.e. foliar application of consortium of endophytic bacteria showed maximum population of *Gluconacetobacter diazotrophicus*.

Table 1: Effect of consortium of endophytic N fixing bacteria on microbial population (*Gluconacetobacter diazotrophicus*) in pre-seasonal sugarcane (second ratoon) at 180 DAHR.

Sr. No.	Tr. No.	Treatment	Microbial Count (x10 ⁷ cfuml ⁻¹)
			At 180 DAHR
1	T ₁	Absolute control	9.75
2	T ₂	RDF (100% N)	15.75
3	T ₃	50% N + <i>Acetobacter diazotrophicus</i> (Foliar application)	20.25
4	T ₄	25% N + foliar application of consortium of endophytic bacteria	29.75
5	T ₅	Foliar application of consortium of endophytic bacteria	34.25
6	T ₆	Without consortium of endophytic bacteria	16.25
S.E. ±			1.68
C.D. at 5%			5.04

Table 2: Effect of consortium of endophytic N fixing bacteria on microbial population (*Gluconacetobacter diazotrophicus*) in pre-seasonal sugarcane (second ratoon) at harvest.

Sr. No.	Tr. No.	Treatment	Microbial Count (x10 ⁷ cfuml ⁻¹)
			At Harvest
1	T ₁	Absolute control	27.25
2	T ₂	RDF (100% N)	48.5
3	T ₃	50% N + <i>Acetobacter diazotrophicus</i> (Foliar application)	59.25

4	T ₄	25% N + foliar application of consortium of endophytic bacteria	63.25
5	T ₅	Foliar application of consortium of endophytic bacteria	67.25
6	T ₆	Without consortium of endophytic bacteria	51.25
S.E. ±			2.39
C.D. at 5%			7.19

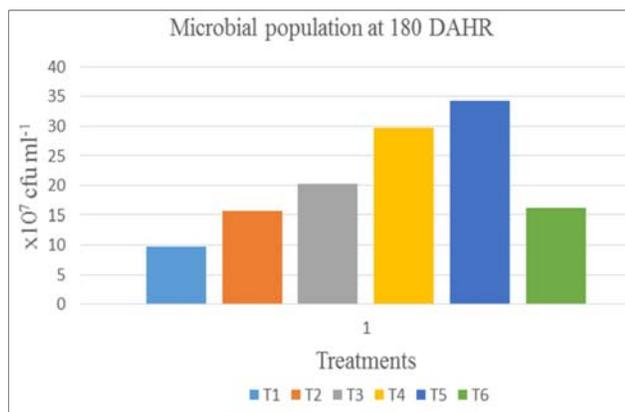


Fig 1: Effect of consortium of endophytic N fixing bacteria on microbial population (*Gluconacetobacter diazotrophicus*) in pre-seasonalsugarcane (second ratoon) at 180 DAHR.

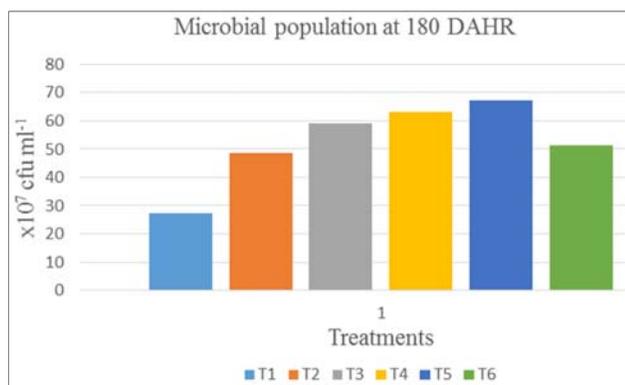


Fig 2: Effect of consortium of endophytic N fixing bacteria on microbial population (*Gluconacetobacter diazotrophicus*) in pre-seasonalsugarcane (second ratoon) at harvest

Conclusions

The recommended dose of fertilizer treatment (250 kg N, 115 kg P₂O₅ and 115 kg K₂O ha⁻¹) gave significantly higher number of tillers, cane and commercial cane sugar yield, yield attributing characters viz., no. of millable canes, total cane height, millable cane height, length and girth of internodes, total N, P and K uptake and net monetary returns.

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 P₂O₅ha⁻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.

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