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RESEARCH ARTICLE

Assessing the Benefits of *Azotobacter* Bacterization in Sugarcane: A Field Appraisal

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Abstract Biofertilizers have long been assessed as powerful technology to obtain sustainable enhanced crop production. The present investigation revealed the positive effects of inoculation of *Azotobacter* biofertilizer on growth and yield parameters in sugarcane var. CoJ 83 under field conditions. Application of *Azotobacter* biofertilizer at both the nitrogen levels ($N_{75\%\ Rec}$ and $N_{100\%\ Rec}$ levels) resulted in significant increase in the cane yield over the respective controls. Maximum increase in cane yield was recorded by *Azotobacter* inoculation at recommended dose of nitrogen. Inoculation with *Azotobacter* at $N_{75\%\ Rec}$ level of N fertilizer resulted in cane yield that was observed to be statistically at par with $N_{100\%\ Rec}$ level. The application of this biofertilizer would not only be beneficial keeping in view the phenomenon of enhanced productivity using environmentally benign technology, but also would be useful to obtain better yield with improvement of the soil microbial ecology/soil food web.

Keywords *Azotobacter* · Cane yield · Diazotroph · PGP activity · Sugarcane

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Introduction

Agriculture is the predominant sector of Indian economy, which contributes approximately 26 percent to national income. It is also of greater importance since agricultural products (unprocessed/processed) provide the valuable foreign exchange. Indian agriculture is rapidly growing, however, on the cost of the fertility of the soils; particular reference to Punjab soils (an agriculture dominated northwestern Indian state); which, has been declining due to extensive input of chemical fertilizers to obtain enhanced yield. This hiked agrochemical input has aggravated to deterioration of soil health and soil foodweb leading to problems like altered microecological niches with depleted microbial diversity in terms of both species richness as well as population, which needs to be maintained by application of bioinoculants like *Azotobacter*. The high temperature and humidity prevailing during the majority of summer-rainy season in Punjab rapidly decomposes the organic matter content of soils (Kader et al. 2002). Moreover, high input of urea accelerates the decomposition of the organic matter, which result in stagnant or lower yields as well as lower fertilizer use efficiency from the same piece of land. Above all the higher doses of applied urea tend leach down to the fresh water aquifers and have many adverse impacts on the beneficial soil microflora and fauna particularly the soil diazotrophic count which is drastically altered in terms of number as well as diversity. To address these problems improvement of the overall management of crop through integrated nutrient management protocols involving the synergistic and combinatorial application of inorganic fertilizers along with organic amendments and biofertilizers is very useful. This would be a useful refuge to address the problems of decreased soil fertility and crop productivity. Nitrogen fixing biofertilizers, *Azotobacter* in particular, have a greater potential on application in non-leguminous crops.

The genus *Azotobacter* belongs to family Azotobacteriaceae including gram negative, free-living asymbiotic nitrogen fixing (up to 10–20 kg N ha^{-1}) diazotroph (Kader et al. 2002) that is readily isolated from the rhizospheric soils of a variety of non-leguminous crops viz., cereals like wheat, maize, rice, sorghum, cash crops like sugarcane, cotton and horticultural crops like tomato, brinjal, cabbage, potato etc. Apart from being a nitrogen fixer it also acts as a plant growth promoting (PGP) rhizobacteria by synthesis and secretion of PGP substances like Vitamin B complex (nicotinic acid, pantothenic acid, biotin), phytohormones (heteroauxins or IAA, gibberellins, kinetin), siderophores and fungistatic compounds that are instrumental in enhancing growth and development of the plant which results in enhanced yield on inoculation.

Among the various crops cultivated in India, sugarcane (*Saccharum officinarum*) is one of the prominent cash crop that is cultivated predominantly as an annual irrigated crop in both tropics and sub-tropics of India over an area of four million hectares and the production is estimated to be about 300 m t with a productivity of 70 t ha^{-1} annually (Sebastian et al. 2009). In the recent past, though the productivity of sugarcane crop has increased, the magnitude has been very small. In order to increase our national income, the sustainable production of such cash crops is imperative because of its importance in foreign exchange earnings. The sustainable production of sugarcane could be recovered by practicing the inoculation of biofertilizers particularly the *Azotobacter* bacterization. Production and aggressive marketing of indigenous and region specific biofertilizers would be a very useful trend to uplift the sugarcane production scenario. In the present study the multilocational field trial emphasizes the application of *Azotobacter* biofertilizer for improving the sugarcane crop yields by better nutrient supply as well as by the plant growth promoting properties of the microbial inoculant.

Materials and Methods

The experiment was conducted at four different locations viz., Regional Research stations at Gurdaspur and Faridkot, Sugarcane farm Ladhowal, and PAU campus during 2008–2009 using *Azotobacter chroococcum* culture. The experiment was conducted in a sandy loam soil of medium fertility. The area normally receives about 1,047 mm of rainfall (maximum during the 874 mm monsoon period, June to September) in 60 rainy days and the temperature conditions are moderate with monthly mean maximum temperature ranging from 33–40°C during May to June and minimum temperature from 5.0–11.0°C during December to January. The bright sunshine days in the months of May to October ranged from 4.3 to 9.9. The corresponding values in November to April were 3.8–11.8 respectively.

A total of four treatments comprised of 75 and 100% of the recommended nitrogen (112.5 and 150 kg ha^{-1} respectively) with and without *Azotobacter chroococcum* were tested in randomized block design (RBD) using five replications. The plot size was 5.4 m × 6.0 m (6 rows of 6.0 m spaced 0.9 m apart) for the plant crop. The treatments consisted of the most popular sugarcane variety of Punjab CoJ 83. The standard cultivation techniques for cultivation of sugarcane (as mentioned in package and practices, PAU) were followed at all the locations with a seed rate of 60,000 two budded setts ha^{-1}, single super phosphate 30 kg P_2O_5 ha^{-1} applied as a basal dose at the time of planting. Nitrogen was applied as urea in two equal splits as per the treatments at 45 and 90 days after planting.

Azotobacter culture for the experiment was procured from Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India. The bacterial culture was mass multiplied in Jensen's broth medium and 48 h old microbial growth was mixed with activated charcoal to form carrier based biofertilizer containing bacterial population above 10^8 colony forming units per gram of the inoculant. Ten kilograms of charcoal based biofertilizer $hectare^{-1}$ was applied in the furrow before plantation of the sugarcane clumps in the field. The biofertilizer was covered with soil by light earthing up followed by irrigation.

The cane height, cane girth and cane yield data was recorded at the harvesting stage and the cane yield data was converted to tonne per hectare. The chlorophyll content was determined using Anderson and Boardman (1964) method. The quality data was recorded for brix % juice, pol % juice and purity % juice from composite juice of 10 canes in each of four replications as per standard procedures described by Chen James (1985). Brix was measured by hydrometry. The clarified juice was analysed with Sucromat (digital automatic saccharimeter) for pol% and purity%. Commercial Cane Sugar per cent (CCS%) was calculated by using Winter's formula. Sugar yield (CCS/ha) was obtained by multiplying cane yield (t/ha) with CCS%. The *Azotobacter* establishment in the soil was assessed by analyzing the soil samples (0–15 cm) collected from each experimental plot by enumerating the viable cell count on Jensen's agar

Table 1 Effect of inoculation of *Azotobacter* on growth and yield attributing characters and yield in sugarcane variety CoJ 83

Treatment	Gurdaspur	Faridkot	Ladhowal	PAU	Pooled data over locations
Cane length or plant height (cm pl^{-1})					
$N_{75\%\ Rec}$	256.0	188.8	194.0	233.0	218.0
$N_{100\%\ Rec}$	242.0	189.8	192.1	252.0	219.0
$N_{75\%\ Rec}$ + Azoto	255.0	187.2	195.8	244.0	220.5
$N_{100\%\ Rec}$ + Azoto	290.0	190.0	196.6	259.0	233.9
CD @ 5%	NS	NS	NS	8.5	7.3
CV	3.23	4.27	4.71	4.85	4.27
SE(±)	15.1	0.7	3.0	2.83	5.41
Number of tillers (000/ha)					
$N_{75\%\ Rec}$	221.79	130.9	99.4	145.0	149.3
$N_{100\%\ Rec}$	183.16	134.1	129.9	151.7	149.7
$N_{75\%\ Rec}$ + Azoto	218.54	137.7	107.4	153.3	154.2
$N_{100\%\ Rec}$ + Azoto	207.81	139.0	136.1	158.3	160.3
CD @ 5%	12.60	NS	9.88	8.12	10.2
CV	3.22	4.31	5.12	4.78	4.36
SE(±)	4.21	0.32	3.29	2.70	2.63
Number of millable canes (000/ha)					
$N_{75\%\ Rec}$	91.82	93.8	84.6	113.3	95.9
$N_{100\%\ Rec}$	92.65	98.7	94.0	119.7	101.3
$N_{75\%\ Rec}$ + Azoto	103.45	97.9	88.1	118.5	102.0
$N_{100\%\ Rec}$ + Azoto	126.93	99.7	96.0	123.5	111.5
CD @ 5%	5.30	NS	7.7	NS	6.5
CV	3.11	4.67	4.78	4.97	4.38
SE(±)	1.76	0.28	2.56	0.30	1.23
POL (%) Juice					
$N_{75\%\ Rec}$	18.82	17.74	19.64	19.94	19.0
$N_{100\%\ Rec}$	19.15	17.82	20.06	21.02	19.5
$N_{75\%\ Rec}$ + Azoto	18.96	18.10	19.80	20.19	19.3
$N_{100\%\ Rec}$ + Azoto	19.20	18.10	20.00	21.07	19.6
CD @ 5%	NS	NS	NS	NS	NS
CV	3.4	4.56	3.98	4.62	4.14
SE(±)	0.4	0.5	0.6	0.7	0.55
CCS %					
$N_{75\%\ Rec}$	13.18	12.40	13.66	13.87	13.3
$N_{100\%\ Rec}$	13.44	12.54	14.04	14.68	13.7
$N_{75\%\ Rec}$ + Azoto	13.35	12.68	13.76	14.12	13.5
$N_{100\%\ Rec}$ + Azoto	13.47	12.58	13.90	14.73	13.7
CD @ 5%	NS	NS	NS	NS	NS
CV	3.43	4.21	4.09	5.02	4.19
SE(±)	0.3	0.4	0.5	0.6	0.45
Yield (t/ha)					
$N_{75\%\ Rec}$	59.1	61.74	71.44	71.3	65.87
$N_{100\%\ Rec}$	60.8	66.15	77.00	81.1	71.60
$N_{75\%\ Rec}$ + Azoto	60.0	64.50	74.00	77.3	68.70
$N_{100\%\ Rec}$ + Azoto	69.1	68.30	79.56	85.3	75.67
CD @ 5%	4.66	3.6	4.57	4.5	2.15
CV	2.99	4.07	3.88	5.9	4.21
SE(±)	1.55	1.2	1.52	1.51	0.71

medium (cfu g^{-1} dry soil wt.) using dilution spread plating technique of the samples at initial, at tillering and at harvesting stages. Soil samples were also used for the estimation of soil organic carbon content (Walkley and Black 1934) and soil available nitrogen (Subbiah and Ashija 1956). Data regarding various plant growth and yield parameters as well as yield were subjected to analysis of variance (ANOVA) using CPCS1 software at CD @ 5%. The meterological data was recorded from Department of Meterology, PAU, Ludhiana for the experimental year.

Results

Biofertilizers undoubtedly have long been assessed to enhance growth and yield of inoculated plants. In the present field trial, *Azotobacter* inoculation in sugarcane at both the nitrogen levels positively altered the morphological characters as well as yield parameters (Table 1). The inoculation with *Azotobacter* did not improve the cane juice quality as measured in terms of Pol % juice and CCS % of the sugarcane juice (Table 1). Similar trend was observed for the CCS (t/ha) and per cent survival of sugarcane tillers (Table 2). The chlorophyll content (mg g^{-1} of fresh leaf tissue) of sugarcane sampled twice exhibited similar trend of increase in total chlorophyll content by *Azotobacter* inoculation at both the nitrogen levels with maximum increase observed in *Azotobacter* + $N_{100\%\ Rec}$ treatment (Fig. 1).

In individual multilocational trials, an appreciable numerical increase in cane yield at all the locations was observed by inoculation with *Azotobacter* over respective controls. However, in the pooled data over locations, *Azotobacter* culture inoculation at both the nitrogen levels ($N_{75\%\ Rec}$ and $N_{100\%\ Rec}$ levels) resulted in significant increase in cane yield over the respective controls (Table 1). This evidently clears the benefits of application of *Azotobacter* in sugarcane.

In the pooled data over locations 4.29% ($N_{75\%\ Rec}$ level) and 5.68% ($N_{100\%\ Rec}$ level) increase in cane yield was observed over respective controls by *Azotobacter* inoculation while inoculation of *Azotobacter* resulted in maximum of 13.65% (at $N_{75\%\ Rec}$ level) and 8.41% ($N_{100\%Rec}$ level) increase in sugarcane yield over control at Gurdaspur and PAU locations respectively (Fig. 2). *Azotobacter* inoculation at recommended dose of nitrogen resulted in maximum increase in cane yield. However, *Azotobacter* inoculation + $N_{75\%\ Rec}$ level of N fertilizer resulted in cane yield that was observed to be statistically at par with $N_{100\%\ Rec}$ level. It shows that *Azotobacter* inoculation can be useful in saving 25% of the applied nitrogen fertilizer without impeding the cane yield, which may be attributed to the diazotrophic behavior of this soil microbe.

The inoculated culture was observed to get established with maximum viable cell count at $N_{100\%\ Rec}$ + *Azotobacter* treatment in all the experiments (Table 3). Thus the culture has been observed to be competent enough to get established in the rhizosphere of the growing plants in the presence of high (recommended level) of N fertilizer as well as without the application of organic fertilizer however it exhibited seasonal and temporal alterations in number. In addition to increased yield and better net returns, soil health was improved by establishment of the inoculated culture and soil fertility was also improved, may be meager by increased soil available nitrogen (Table 4).

Table 2 Effect of inoculation of *Azotobacter* on CCS and survival percent in sugarcane variety CoJ 83

Treatment	Gurdaspur	Faridkot	Ladhowal	PAU
CCS (t/ha)				
$N_{75\%\ Rec}$	7.79	7.66	9.76	9.89
$N_{100\%\ Rec}$	8.17	8.30	10.81	11.90
$N_{75\%\ Rec}$ + Azoto	8.01	8.18	10.18	10.91
$N_{100\%\ Rec}$ + Azoto	9.31	8.59	11.06	12.56
CD @ 5%	0.16	0.13	NS	0.24
CV	3.20	3.91	7.81	4.82
SE(±)	0.05	0.04	0.06	0.08
Survival %				
$N_{75\%\ Rec}$	41.40	71.66	85.11	78.14
$N_{100\%\ Rec}$	50.58	73.60	72.36	78.91
$N_{75\%\ Rec}$ + Azoto	47.34	71.10	82.0	77.30
$N_{100\%\ Rec}$ + Azoto	61.08	71.73	70.54	78.02
CD @ 5%	NS	NS	NS	NS
CV	1.88	2.70	1.21	1.36
SE(±)	2.76	1.80	2.63	0.90

Discussion

As far as the growth parameters are concerned there are reports which support the positive effect of inoculation of diazotrophic bacteria, particular instance is the report by Nagaraju et al. (2000) which advocated that height and weight of millable canes were influenced favorably both due to N-fertilization and application of press mud cakes (PMC) with *Azotobacter*. Hari and Srinivasan (2005) have also observed better results regarding both the morphological and yield parameters in sugarcane in combination treatment i.e. biofertilizer + chemical fertilizer treatment than using either treatments alone. Similarly, Shankaraiah and Kalyanamurthy (2005) have recorded positive influence of biofertilizer application on the yield parameters viz., height, weight and diameter of millable cane due to increasing levels of fertility and addition of PMC in

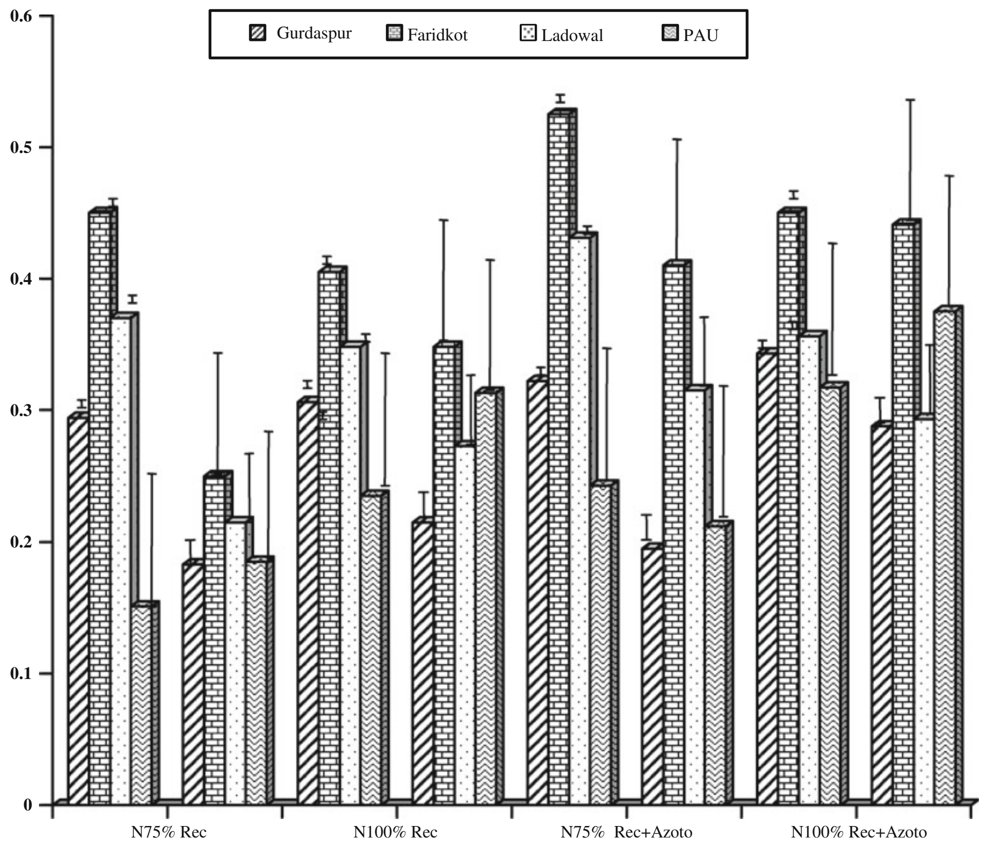

Fig. 1 Effect of inoculation of *Azotobacter* on chlorophyll content (mg g^{-1} fresh leaf tissue)

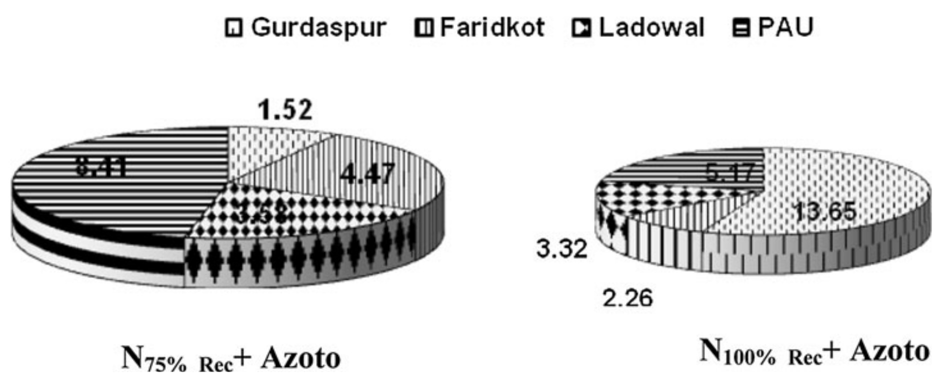

Fig. 2 Effect of inoculation of *Azotobacter* on per cent increase in cane yield of sugarcane in multilocational trials for the year 2008–2009

Table 3 *Azotobacter* count (cfu $g^{-1} \times 10^4$) in soil samples collected from sugarcane field at different plant growth stages

Treatment	Initial count	$N_{75\%\ Rec}$		$N_{100\%\ Rec}$		$N_{75\%\ Rec}$ + Azoto		$N_{100\%\ Rec}$ + Azoto	
		Tillering	Harvesting	Tillering	Harvesting	Tillering	Harvesting	Tillering	Harvesting
Gurdaspur	36.0	31.0	39.6	29.6	40.3	32.3	45.5	41.0	43.5
Faridkot	23.0	25.0	29.4	30.0	39.0	35.5	43.3	46.5	48.6
Ladhowal	17.0	33.0	40.0	36.0	43.0	31.0	52.5	40.3	59.5
PAU	25.0	37.5	40.0	36.5	61.0	38.5	52.0	34.0	65.0

general. Similarly, in a pot experiment on two Okra cultivars, Shaheen et al. (2007) have reported enhanced plant growth, pod yield and quality by application of two bacterial biofertilizers *Azospirillium* and/or *Azotobacter*.

The chlorophyll content is also positively influenced by inoculation may be partly because of the enhanced nutritional availability due to increased number of lateral rootlets and partly due to higher supply of fixed nitrogen to the growing tissue and organs supplied by diazotrophic inoculants. Hari and Srinivasan (2005) advocated that inoculation of *Azospirillum* and *Azotobacter* with 100% urea application resulted in significant increase in total chlorophyll compared to the control treatment in sugarcane leaves sampled at different regular time intervals (viz., 20, 40 and 60th days).

The enhancement criteria for the growth and yield attributing characters have been translated in increased yield of sugarcane in the present field trial. Chandrasekar et al. (2005) revealed that application of nitrogen fixing bacteria at all levels and in combination with chemical nitrogen resulted in increase in growth, yield and biochemical components w.r.t. the control (without biofertilizers and chemical nitrogen). Likewise, Hari and Srinivasan (2005) in a field study to evaluate the response of sugarcane varieties to application of nitrogen fixing diazotrophs viz., *Azotobacter*, *Azospirillum and Gluconacetobacter* under different levels of fertilizer nitrogen, reported significant improvement in the yield and sugar content of biofertilizer inoculated sugarcane plants compared to uninoculated control.

The highest yield of the sugarcane has been reported in treatment *Azotobacter* + $N_{100\%\ REC}$ which signifies the other additional plant growth promoting benefits apart from the mere diazotrophic action of the *Azotobacter* inoculation in sugarcane. Similar results have been quoted by application of biofertilizers (*Azotobacter* and *Azospirillum*) alongwith 100% urea treatment highest yields of millet (*Echinochloa frumentacea* (Roxb.) were obtained compared to control (Chandrasekar et al. 2005).

The *Azotobacter* inoculation resulted in multiplication and establishment of the culture in the inoculated field soil. Being PGP and plant probiotic bacteria *Azotobacter* count increase is a good signature of the improved soil microbial status and hence better soil health. Kaur et al. (2008) have also reported establishment of larger populations of *Azotobacter chrococcum* Mac 27 *lac* z^+ in rhizospheric soil of wheat and clover plants in organic fertilizer treatments in comparison to chemical fertilizer alone treatments. The inoculation in the present field trial resulted in a meager but increase in the available nitrogen due to diazotrophic properties which signifies the maintenance if not

Table 4 Effect of inoculation of *Azotobacter* on physicochemical characteristics of the soil samples from different locations on harvesting

Treatment	Gurdaspur				Faridkot			
	pH	EC ds/m	Available soil N (kg ha^{-1})	Soil OC (%)	pH	EC ds/m	Available soil N (kg ha^{-1})	Soil OC (%)
$N_{75\%\ Rec}$	7.50	0.163	340.5	0.570	8.15	0.445	322.6	0.465
$N_{100\%\ Rec}$	7.47	0.172	349.4	0.585	8.25	0.492	376.3	0.435
$N_{75\%\ Rec}$ + Azoto	7.55	0.194	367.4	0.578	8.15	0.441	349.4	0.480
$N_{100\%\ Rec}$ + Azoto	7.60	0.127	376.3	0.593	8.35	0.613	430.1	0.390
	Ladhowal				PAU			
$N_{75\%\ Rec}$	7.95	0.427	358.4	0.450	7.50	0.120	162.0	0.340
$N_{100\%\ Rec}$	7.85	0.200	403.2	0.563	7.77	0.132	171.0	0.360
$N_{75\%\ Rec}$ + Azoto	7.75	0.215	403.2	0.578	7.81	0.144	165.0	0.340
$N_{100\%\ Rec}$ + Azoto	7.75	0.258	412.6	0.563	7.84	0.152	174.0	0.360

Initial data on physicochemical characteristics of soil

Location	pH	EC	Available soil N (kg ha^{-1})	Soil OC (%)
Gurdaspur	7.51	0.153	330.5	0.56
Faridkot	8.20	0.45	315.7	0.45
Ladowal	7.78	0.25	338.4	0.51
PAU	7.61	0.14	158.4	0.35

improvement of the soil fertility. Hari and Srinivasan (2005) however, have reported that biofertilizer inoculation did not influence the soil available nitrogen status.

Conclusions

Overall the inoculation of *Azotobacter* carrier based inoculants in sugarcane increased the growth and yield attributing characters and thus the cane yield in the present investigation making the biofertilizer technology to be substantially useful for sustainable enhanced productivity and yield of sugarcane.

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