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Soil enzymatic activity and yield of sugarcane short crop as influenced by fertilizers and microbial inoculants

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Abstract
A field experiment was conducted at Agricultural Research Station, Perumallapalle, Tirupati, Acharya N. G. Ranga Agricultural University, Andhra Pradesh, India during 2021-22 to study the effect of fertilizers and microbial inoculants on soil enzymatic activity and yield of sugarcane short crop. The experiment was laid out in randomized block design with ten treatments and replicated thrice. The treatments consist of 75%, 100% and 125% RDF in combination of solid and liquid microbial inoculants (*Gluconacetobacter*, PSB and KSB). Data on soil urease, dehydrogenase, acid and alkaline phosphatase enzyme activity and yield were recorded. Among the ten treatments 100% RDF along with soil application of liquid *Gluconacetobacter* @ 10 kg ha^{-1}, PSB @ 1.25 kg ha^{-1} and KSB @ 1.25 kg ha^{-1} resulted significantly the highest urease (256, 290 and 265 µg urea hydrolyzed g^{-1} soil h^{-1} at tillering, grand growth and harvest stages, respectively), dehydrogenase (69.05, 77.87 and 73.80 µg TPF g^{-1} soil h^{-1} at tillering, grand growth and harvest stages, respectively), acid phosphatase (44.82, 52.84 and 47.05 µg *p*-nitrophenol g^{-1} soil h^{-1} at tillering, grand growth and harvest stages, respectively) and alkaline phosphatase activity (76.25, 91.34 and 83.26 µg *p*-nitrophenol g^{-1} soil h^{-1} at tillering, grand growth and harvest stages, respectively). Significantly the highest cane yield (97 t ha^{-1}) was recorded with 100% RDF along with sett treatment with liquid *Gluconacetobacter* @ 10 kg ha^{-1}, PSB @ 1.25 kg ha^{-1} and KSB @ 1.25 kg ha^{-1}.

Keywords: Enzymatic activity, inorganic fertilizers, microbial inoculants, sugarcane and yield

Introduction
Sugarcane is the most important commercial crop of India and plays a vital role in the agricultural as well as industrial economy. Sugarcane is a multipurpose crop that provides sugar, fiber, bio-fuel and manure apart from many by-products. It constitutes the major raw material for sugar production and for making gur and khandasari. Sugarcane has unique character of ratooning as several succeeding crops are raised from a single planting which is an integral component of the sugarcane production system.

Sugarcane (*Saccharum officinarum*) is a nutrient exhaustive crop that can uptake great amount of soil nutrients for its biomass production. In addition to micronutrient 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) [9]. A permanent manurial trial, conducted for 33 years at RARS, Anakapalle (Andhra Pradesh), revealed that sugarcane crop without addition of fertilizers yielded about 40 t ha^{-1} of cane annually. The soil available nitrogen, increased by 50% from the initial value which was clearly indicated that the root-associated diazotrophs contributed significant quantity of nitrogen for sustaining the production of sugarcane (Suman 2003) [14]. Inoculation of N-fixing microbes to sugarcane has increased the cane yield by 5-15% and also improved the juice quality parameter, *viz*., sucrose and purity (Hari 1995) [7].

Gluconacetobacter diazotrophicus is a nitrogen-fixing bacterium highly specific to sugar-rich crops. It can excrete about half of its fixed nitrogen in plant available form. It also produced Indole acetic acid in a culture medium supplemented with tryptophan in the range of 0.14 to 2.42 g ml^{-1} (Fuentez *et al*., 1993) [6]. Furthermore, it has the ability to solubilize inorganic phosphates from the soil and make available for the crops. Hence, *Gluconacetobacter* inoculation to sugarcane significantly increased the cane length, dry

matter production and number of stalks, resulting higher cane yield. PSB application helps in solubilizing the fixed form of phosphates into available form by production of organic acids. The K releasing bacteria (KSB) is more effective in releasing potassium from inorganic and insoluble fractions of soil K through solubilization. The soil microbial biomass is fundamental to maintaining soil functions because it represents the main source of soil enzymes that regulate transformation processes of elements in soils. Soil enzyme activities have been proposed as appropriate indicators because of their intimate relationship to soil biology, and rapid response to change in soil management. Phosphomonoesterase is a generic name for a group of enzymes which catalyse the hydrolysis of esters of phosphoric acid in releasing phosphate and is of paramount importance as a soil quality indicator (Trasar-Cepeda *et al*., 2008) [17]. Dehydrogenase exist as an integral part of intact cells, involved in oxidative phosphorylation, and reflect the total oxidative potential of the soil microbial community (Dick 1997) [4]. Keeping this background in view, a field experiment was conducted to study the effect of soil application and sett treatment of solid and liquid *G. diazotrophicus,* PSB and KSB along with fertilizers on soil enzymatic activity and yield of sugarcane short crop.

Materials and Methods

A field experiment was conducted during 2021-22 at Agricultural Research Station, Perumallapalle, Tirupati, Acharya N. G. Ranga Agricultural University, geographically situated at 13° 36' 761" N latitude and 79° 20' 704" E longitude with an altitude of 182.9 m above the mean sea level, which falls under Southern agroclimatic zone of Andhra Pradesh. The experiment soil was sandy loam in texture, neutral in reaction (7.36), non saline (0.232 dS m^{-1}), low in organic carbon (0.49%) and available nitrogen (212 kg ha^{-1}) whereas medium in available phosphorus (40.12 kg ha^{-1}) and high in available potassium (282 kg ha^{-1}). The experiment consist of ten treatments *viz*., T_1: 100% RDF, T_2 : 125% RDF, T_3: 100% RDF + soil application of solid *Gluconacetobacter* + PSB + KSB, T_4 : 100% RDF + sett treatment with solid *Gluconacetobacter* + PSB + KSB, T_5 : 75% RDF + soil application of solid *Gluconacetobacter* + PSB + KSB, T_6 : 75% RDF + sett treatment with solid *Gluconacetobacter* + PSB + KSB, T_7 : 100% RDF + soil application of liquid *Gluconacetobacter* + PSB + KSB, T_8 : 100% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB, T_9 : 75% RDF + soil application of liquid *Gluconacetobacter* + PSB + KSB and T_{10} : 75% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB. The experiment was laid out in randomized block design with three replications. The swarnamukhi variety of sugarcane was sown. The recommended dose of inorganic fertilizers 224:112:112 kg $N:P_2O_5$ and K_2O ha^{-1}, respectively were applied as per the treatments. Solid *Gluconacetobacter*, PSB and KSB were applied @ 10 kg ha^{-1} each as soil application. The recommended dose of solid biofertilizers for sett treatment was 10 kg - 1.25 kg - 1.25 kg ha^{-1} of *Gluconacetobacter*, PSB and KSB, respectively. Recommended dose of liquid *Gluconacetobacter*, PSB and KSB for soil application was 1 L, 1.25 L and 1.25 L ha^{-1}, respectively. The same quantity of liquid *Gluconacetobacter*, PSB and KSB was used for sett treatment. All the other recommended practices were adopted as per the crop requirement.

Initial soil sample was collected from 0-15 cm depth before planting of the crop. Five soil samples were collected randomly and mixed thoroughly, dried under shade, passed through 2 mm sieve and labelled. The soil samples (0-15 cm) were also collected from each treatment plot at tillering, grand growth stage and after harvest and processed for analysis. The processed soil samples were analyzed for soil enzymatic activities by using standard procedures. Soil urease activity was determined by the method given by Evazi and Tabatabai (1977) [5]. The urease activity is expressed as µg urea hydrolyzed g^{-1} soil h^{-1}. The dehydrogenase activity in the soil samples was determined as described by Casida *et al*. (1964) [3]. The amount of dehydrogenase activity of the sample was expressed as µg TPF g^{-1} soil h^1. The procedure of Tabatabai and Bremner (1969) [16] and Evazi and Tabatabai (1977) [5] were adopted for the assay of acid and alkaline phosphatase activities, respectively. The enzyme activities were calculated from the liberated p-nitrophenol and expressed as µg of *p*-nitrophenol released g^{-1} soil h^{-1}. The data on cane yield per plot has been recorded from each treatment and expressed in terms of t ha^{-1}. The data was statistically analyzed by following the analysis of variance for randomized block design as outlined by Panse and Sukhatme (1985) [13]. Statistical significance was tested with 'F' test at 5 percent and 1 per cent level of probability. Further, multiple comparison tests have been done using Duncan's multiple range test (DMRT) to identify the homogenous groups of treatments using SPSS-20.

Results and Discussion

The data pertaining to soil enzymatic activity at different growth stages presented in Table 1 and 2.

Urease activity

Urease activity has significantly increased from initial to grand growth stage and later decreased at harvest (Table 1). Significantly the highest urease activity (256, 290 and 265 µg urea hydrolyzed g^{-1} soil h^{-1} at tillering, grand growth and harvest stages, respectively) in sugarcane was observed with 100% RDF + soil application of liquid *Gluconacetobacter* + PSB + KSB (T_7). The next best treatment was 100% RDF + soil application of solid *Gluconacetobacter* + PSB + KSB (T_3) (249, 283 and 262 µg urea hydrolyzed g^{-1} soil h^{-1}, at tillering, grand growth and harvest stages, respectively) which was on par with 100% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB (T_8) (247, 280 and256 µg urea hydrolyzed g^{-1} soil h^{-1}, at tillering, grand growth and harvest stages, respectively). Urease activity of soil was higher when combined application of recommended dose of NPK fertilizers and microbial inoculants were applied as compared to 100% recommended dose of inorganic NPK alone. These results are in line with the findings of Buragohain *et al*. (2017) [2] which showed increased urease activity on addition of biofertilizers with chemical fertilizers. Higher N status in soil stimulated heterotrophic microbial activity and resulted in higher activity of hydrolytic enzymes in turn, urease activity was enhanced Vajantha *et al*. (2010) [18].

Table 1: Soil urease and dehydrogenase enzymes activity at different stages of sugarcane short crop as influenced by application of microbial inoculants and fertilizers

Treatments	Urease activity (µg urea hydrolyzed g^{-1}soil h^{-1})			Dehydrogenase activity (µg TPF g^{-1} soil h^{-1})		
	Tillering stage	Grand growth stage	After harvest	Tillering stage	Grand growth stage	After harvest
T_1	179^{g}	208^{h}	186^{g}	49.82^{g}	62.32^{f}	54.15^{f}
T_2	196^{f}	221^{g}	201^{f}	52.23^{f}	64.54^{e}	56.54^{e}
T_3	249^{b}	283^{b}	262^{a}	66.21^{b}	75.20^{b}	72.67^{a}
T_4	244^{c}	274^{c}	252^{b}	63.45^{c}	72.23^{c}	69.34^{b}
T_5	239^{cd}	239^{f}	215^{e}	56.21^{e}	69.00^{d}	62.13^{d}
T_6	218^{d}	259^{d}	233^{c}	60.16^{d}	69.50^{d}	63.76^{d}
T_7	256^{a}	290^{a}	265^{a}	69.05^{a}	77.87^{a}	73.80^{a}
T_8	247^{b}	280^{b}	256^{b}	65.34^{b}	74.80^{b}	69.86^{b}
T_9	210^{e}	251^{e}	221^{d}	62.80^{c}	71.70^{cd}	66.60^{c}
T_{10}	242^{c}	270^{cd}	238^{c}	63.04^{c}	72.03^{c}	68.90^{b}
F-Value	79.08**	59.41**	118.05**	14.01**	14.06**	29.37**
P-Value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

**Significant at P = 0.01 level

Note : Same set of alphabets indicates no significant difference or at par with each other (DMRT)

Dehydrogenase

Dehydrogenase activity has significantly increased from initial to grand growth stage and later decreased at harvest (Table 1). Significantly the highest dehydrogenase activity (69.05, 77.87 and 73.80 µg TPF g^{-1} soil h^{1}, at tillering, grand growth and harvest stages, respectively) in sugarcane was observed with 100% RDF + soil application of liquid *Gluconacetobacter* + PSB + KSB (T_7). The treatments 100% RDF + soil application of solid *Gluconacetobacter* + PSB + KSB (T_3) (66.21,75.20 and 72.67 µg TPF g^{-1} soil h^{-1}, at tillering, grand growth and harvest stages, respectively) and 100% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB (T_8) (65.34, 74.80 and 69.86 µg TPF g^{-1} soil h^{-1}, at tillering, grand growth and harvest stages, respectively) were on par with each other. Soil enzymatic activities are the direct expression of the soil community to metabolic requirements and available nutrients. Dehydrogenase activity is directly correlated with the applied microbial load and also the moisture content. Higher dehydrogenase activity with integrated application of fertilizers and microbial inoculants might be due to increased microbial load which facilitated the organic matter degradation process in soil and there by dehydrogenase activity. These results are in line with the findings of Antony *et al*. (2019) [1] and Buragohain *et al.* (2017) [2].

Acid phosphatase

Acid phosphatase activity has significantly increased initially from tillering to grand growth stage and decreased at harvest by the combined application of microbial inoculants and fertilizers (Table 1). Significantly the highest acid phosphatase (44.82, 52.84 and 47.05 µg *p*-nitrophenol g^{-1} soil h^{1}, at tillering, grand growth and harvest stages, respectively) in sugarcane was observed with 100% RDF + soil application of liquid *Gluconacetobacter* + PSB + KSB (T_7). The treatments 100% RDF + soil application of solid *Gluconacetobacter* + PSB + KSB (T_3) (41.98, 50.85 and 46.89 µg *p*-nitrophenol g^{-1} soil h^{-1}, at tillering, grand growth and harvest stages, respectively) and 100% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB (T_8) (41.56, 50.42 and 43.21 µg *p*-nitrophenol g^{-1} soil h^{-1}, at tillering, grand growth and harvest stages, respectively) were on par with each other. Higher acid phosphatase activity was recorded with combined application of microbial inoculants and fertilizers might be ascribed to the increased population of microorganisms due to availability of substrate which in turn release this enzyme of extracellular origin. These findings are in agreement with the studies of Nath *et al*. (2012).

Alkaline phosphatase

Alkaline phosphatase activity has significantly increased initially from tillering to grand growth stage and decreased at harvest by the combined application of microbial inoculants and fertilizers (Table 1). Significantly the highest alkaline phosphatase (73.80, 88.96 and 78.66 µg *p*-nitrophenol g^{-1} soil h^{1}, at tillering, grand growth and harvest stages, respectively) in sugarcane was observed with 100% RDF + soil application of liquid *Gluconacetobacter* + PSB + KSB (T_7). The treatments 100% RDF + soil application of solid *Gluconacetobacter* + PSB + KSB (T_3) (74.28, 89.26 and 82.34 µg *p*-nitrophenol g^{-1} soil h^{-1}, at tillering, grand growth and harvest stages, respectively) and 100% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB (T_8) (73.80, 88.96 and 78.66 µg *p*-nitrophenol g^{-1} soil h^{-1}, at tillering, grand growth and harvest stages, respectively) were on par with each other. Higher alkaline phosphatase activity observed with integrated application of inorganic fertilizers and microbial inoculants might be due to conjunctive use of biofertilizers and fertilizers which augmented activities of soil microflora resulting greater release of soil enzymes. (Buragohain *et al*., 2017) [2]. These results are in confirmity with the findings of Kaur *et al*. (2017) [10] and Vajantha *et al*. (2012) [21].

Table 2: Soil acid and alkaline phosphatase enzymes activity at different stages of sugarcane short crop as influenced by application of microbial inoculants and fertilizers.

Treatments	Acid phosphatase activity (µg *p*-nitrophenol g^{-1} soil h^{-1})			Alkaline phosphatase activity (µg *p*-nitrophenol g^{-1} soil h^{-1})		
	Tillering stage	Grand growth stage	After harvest	Tillering stage	Grand growth stage	After harvest
T_1: 100% RDF	28.34^{f}	36.56^{h}	31.82^{f}	57.34^{g}	72.08^{g}	60.18^{h}
T_2: 125% RDF	30.54^{e}	39.21^{g}	35.23^{e}	62.78^{f}	75.23^{f}	63.45^{g}
T_3: 100% RDF + soil application of solid *Gluconacetobacter* + PSB + KSB	41.98^{b}	50.85^{b}	46.89^{a}	74.28^{b}	89.26^{b}	82.34^{a}
T_4: 100% RDF + sett treatment with solid *Gluconacetobacter* + PSB + KSB	40.34^{c}	48.98^{c}	42.65^{b}	72.12^{c}	87.16^{c}	78.20^{b}
T_5: 75% RDF + soil application of solid *Gluconacetobacter* + PSB + KSB	34.23^{de}	45.34^{e}	40.21^{c}	69.65^{d}	84.12^{d}	74.87^{cd}
T_6:75% RDF + sett treatment with solid *Gluconacetobacter* + PSB + KSB	36.98^{d}	43.98^{ef}	38.15^{d}	66.34^{ef}	80.32^{e}	69.67^{ef}
T_7: 100% RDF + soil application of liquid *Gluconacetobacter* + PSB + KSB	44.82^{a}	52.84^{a}	47.05^{a}	76.25^{a}	91.34^{a}	83.26^{a}
T_8: 100% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB	41.56^{b}	50.42^{b}	43.21^{b}	73.80^{b}	88.96^{b}	78.66^{b}
T_9: 75% RDF + soil application of liquid *Gluconacetobacter* + PSB + KSB	37.23^{cd}	46.67^{d}	38.97^{d}	67.65^{e}	80.87^{e}	71.56^{e}
T_{10}: 75% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB	39.65^{c}	48.67^{c}	42.12^{b}	72.45^{c}	86.80^{c}	76.34^{c}
F-Value	27.88**	58.42**	20.31**	170.68**	62.56**	61.62**
P-Value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

**Significant at P = 0.01 level

Note : Same set of alphabets indicates no significant difference or at par with each other (DMRT)

Seed cane yield

Cane yield of sugarcane short crop was significantly differed with microbial inoculants and fertilizers application (Table 2). Significantly the highest seed cane yield (97 t ha^{-1}) was recorded with the application of 100% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB (T_8) followed by 100% RDF + sett treatment with solid *Gluconacetobacter* + PSB + KSB (T_4) (92 t ha^{-1})**.** The control (100% RDF) (T_1) produced significantly the lowest cane yield (69 t ha^{-1}). The highest cane yield with 100% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB might be due to direct utilization of sugars present in setts by microbes as a food source which inturn leads to more microbial multiplication and leads to production of growth promoting substances. It helps in more growth with high photosynthesis and most of substrates move from source to sink *i.e.*, cane leads to more cane yield Vajantha *et al*. (2019) [19]. Sufficient quantity of nutrients supplied to plant through chemical fertilizers provides readily available nutrients and application of biofertilizers may hasten the constant nutrient supply by nitrogen fixation in the rhizosphere, solubilization of mineral nutrients, enhanced rooting and plant establishment, better uptake of low mobile ions such as P, improved nutrient cycling, improved plant tolerance to stress (biotic and abiotic) and amelioration of physical and biological environment. (Surendran and Vani, 2013) [15]. Similar results were reported by Indi *et al*. (2014) [8], Murumkar *et al*. (2017) [11] and Vajantha *et al*. (2014) [20].

Table 3: Seedcane yield of sugarcane short crop as influenced by application of microbial inoculants and fertilizers

Treatments	Seedcane yield (t ha^{-1})
T_1: 100% RDF	69^{h}
T_2: 125% RDF	77^{f}
T_3: 100% RDF + soil application of solid *Gluconacetobacter* + PSB + KSB	85^{d}
T_4: 100% RDF + sett treatment with solid *Gluconacetobacter* + PSB + KSB	92^{b}
T_5: 75% RDF + soil application of solid *Gluconacetobacter* + PSB + KSB	74^{g}
T_6:75% RDF + sett treatment with solid *Gluconacetobacter* + PSB + KSB	82^{e}
T_7: 100% RDF + soil application of liquid *Gluconacetobacter* + PSB + KSB	88^{c}
T_8: 100% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB	97^{a}
T_9: 75% RDF + soil application of liquid *Gluconacetobacter* + PSB + KSB	81^{e}
T_{10}: 75% RDF + sett treatment with liquid *Gluconacetobacter* + PSB + KSB	84^{d}
F-Value	80.08**
P-Value	<0.01

**Significant at P = 0.01 level

Note : Same set of alphabets indicates no significant difference or at par with each other (DMRT)

Conclusion

It can be concluded that that combined application of 100% RDF + soil application of liquid *Gluconacetobacter* @ 1 l ha^{-1} + PSB @ 1.25 l ha^{-1} + KSB @ 1.25 l ha^{-1} is the most efficient nutrient management practice to obtain better growth, soil enzymatic activity and higher yields of sugarcane short crop.

Hence, it is the best practice to sustain higher productivity and to achieve economic profitability in Southern Agroclimatic Zone of Andhra Pradesh.

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