Original Research Article

Assessment of soil microbial and enzymatic activity in the rhizospheric zone of sugarcane plant-ratoon system in Indo-Gangetic plains of India

Abstract

A field experiment was carried out to develop nutrient management strategies for sustaining soil health, quality and sugarcane production on sugarcane plant-ratoon system at Research Farm, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar. Trials were conducted to test the efficiency of various fertilizersin sugarcane on solubility of applied inorganic fertilizer during spring season in calcareous soil. The pooled data revealed that number of millable cane (NMC), cane yield, and sugar yield varied significantly due to integrated use of organic and inorganic fertilizer with bio-fertilizerin combination both in plant and ratoon crops. The significant increase in NMC, cane yield and sugar yield was recorded in the treatments receiving organic and inorganic fertilizer in combination with bio-fertilizer over control. The highest number of NMC ($103.0\times 10^3 ha^{-1}$), cane yield (85.8 t ha^{-1}) and sugar yield (11.21 t ha^{-1}) was recorded in treatment receiving 75% NPK of RDF + *Acetobacter* +PSB + along-with Bio-compost @7.5 t ha^{-1}. The residual effect of treatment receiving organic and inorganic fertilizer in combination with bio-fertilizer was also maximum pronounced on NMC (92.4×10^3 ha^{-1}), yield (79.6 tha^{-1}) and sugar yield (9.36 tha^{-1}) in ratoon crop under treatment T_9. The bio-compost improved overallperformance of sugarcane. The uptake of nutrients by plant and ratoon followed the similar trend as cane yield. The treatment receiving RDF along with various biofertilizersignificantly improved productivity of sugarcane over control. However, the efficiency of Bio-fertilizer was more pronounced under inorganic treated plots. The reduction in pH and increase in EC, organic carbon and available nutrients (N, PandK) in post-harvest soil was recorded in treatment receiving organics through bio-compost. The enzymatic activities was recorded for glycosidase, urease, acid phosphatase and dehydrogenase activities

Key words: Bio-compost, PSB, Nutrient uptake, microbial population, enzymes, Sugarcane.

Introduction

Sugarcane is widely cultivated throughout the Indo-Gangetic plains. More than 4.2 million hectares are under sugarcane cultivation in India alone, with an average cane yield of 60 tha^{-1}. Sugarcane (*Saccharum* species hybrids) is a very exhaustive and extracting crop that removes about 205 kg N, 55 kg P_2O_5, 275 kg K_2O, 30 kg S, 3.5 kg Fe, 1.2 kg Mn, 0.6 kg Zn and 0.2 kg Cu from the soil for a cane yield of 100 t ha^{-1},(Singh *et*

*al.*2007).Out of the total phosphorus (P) fertilizers applied to the crop, only 15-20% can be used and the rest is fixed in the soil as phosphates of Ca, Al or Fe depending on the soil reaction.A considerable amount of P is rapidly transformed into less available forms by forming a complex with Al or Fe in acid soils or Ca in calcareous soils (Lindsay et al., 1989) before plant roots had a chance to absorb it (Vikram, 2007).Consequently, due to the nature of this crop as extensive excavation of nutrient, the soils are becoming nutrient-deficient. In order to sustain productivity, the nutrients are applied each year at the recommended dose of fertilizer (RDF), which in the sub-tropical part of Bihar are 150 kg Nha^{-1} for the sugarcane main crop,85 kg of P_2O_5 and 60 kg of K_2O ha^{-1}while 170 kg N ha^{-1} as well as 50 kg of P_2O_5 and 60 kg of K_2O ha^{-1} for ratoon crop. The efficiency of sugarcane to utilize applied nitrogen ranges between 16% to 45%, as large quantities of applied N leached through the soil due to the percolating irrigation water(Yadav and Prasad 1992). Besides, the continuous use of chemical fertilizers causing deficiency in other micronutrients. In recent years, the yieldhavestagnated and factor productivity has declined with decrease in soil organic matter (SOM)content and deterioration in the physico-chemical and biological properties of the soil is the prime reasons for the declining yield (Garside 1997; Speir *et al.* 2004). Sugarcane farmers are switching over to alternative practicesto make sugarcane cultivation more sustainable and productive. Such farming practices, combined with the management of the farm and concurrently available renewable resources, results in the rejuvenation of the soils. The application of organic matterfrom such resources as animal manures, crop residues and green manuring has been shown to replenish organic carbon and improve soil structure and fertility (Parham *et al.* 2002; Saviozzi*et al.* 2002). Moreover, several kinds of microbial agents capable of fixing nitrogen or solubilising and mobilizing P and other nutrients are becoming an integral component..*Gluconacetobacterdiazotrophicus (GD)* (earlier known as *Acetobacter diazotrophicus*), a nitrogen-fixing bacteria associated with sugarcane as an endophyte, is present in high numbers (as high as 10^6 counts g^{-1} plant tissue) (Cavalcante and Dobereiner 1988). The exact role of such endophytic colonization, has not yet been elucidated, but the few inoculation experiments have been carried out which suggest that positive colonization contributes to plant in terms of improved plant height, nitrogenase activity, leaf nitrogen, biomass and yield. Field trials conducted have shown that inoculation by *GD* together with other diazotrophs can match yield equal to the application of 275 kg Nha^{-1} (Sevilla *et al.* 2001; Oliveira *et al.* 2002). In contrast, high levels of N fertilization negatively affect the population of endophytic bacteria in sugarcane. Apart from N fixation, other properties associated with GD are P-solubilization, production of plant growth hormone Indole acetic acid and the suppression of red rot disease (Suman *et al.* 2001), they reported that the native occurrence of *GD* in sugarcane varieties of sub-tropical India is very low,which may be enhanced through the inoculation of efficient isolates (Suman et al. 2005). Some sugarcane varieties have been found to

derive up to 70% of their nitrogen requirement through biological nitrogen fixation (Boddey*et al.* 2001). Various kinds of bacteria such as *GD*, *Herbaspirillum* spp., *Azospirillumamazonense*, *Burkholderia* spp., capable of fixing nitrogen have been reported to colonize the epidermis of sugarcane stem and roots, of which Gluconacetobacter seems to contribute substantially to nitrogen nutrition of the plant (James *et al.* 1994).Sugarcane respond positively to organic sources to meet its nutrient requirements; however, the effect of organic sources together with GD on yield and the availability and balance of nutrients in the soil along with biological and physical status and overall sustainability of the system need to be ascertained. Furthermore, it has been reported regarding its availability to solubilise insoluble inorganic phosphates from the soil and make available P for the inoculated crops (Banger *et al.*1993). The indiscriminate use of chemical fertilizer, apart from their high cost often leads to nutritional imbalance which causes deterioration in soil health and decreases the yield. The present study designed to evaluate the effect of manures with bio-inoculants on the sugarcane and its subsequent ratoon in terms of the productivity of the sugarcane crop and subsequent ratoons as well as availability, uptake and balance of soil nutrients. Thus maintenance of fertility and productivity through combination of organics, inorganic and bio-fertilisers to harness maximum advantage.Manure has been considered as a value input to the soil. No single source of plant nutrients i.e. chemical fertilizers, manures or bio-fertilizers can meet the entire nutrient requirement of crop in intensive cultivation. It is a need for nutrient replenishment through organic waste, fertilizer and bio-fertilizer. For sustainability in sugarcane yield and sugar production, the integrated nutrient use has been observed highly beneficial (Banger et al. 1994; Chaudhary and Sinha (2001). Phosphorus is the second most plant nutrient after nitrogen (Donahue et al., 1990). Phosphate solubilizing bacteria (PSB), phosphate solubilizing fungi (PSF) and Actinomycetes has a greater potential for conversion of insoluble phosphate to soluble phosphate ions by many investigators (Chabot et al., 1993; Pal, 1998). Thus, keeping in view the above all facts, a field experiment was conducted to study the integrated effect of manure,biofertilizer and inorganic fertilizeron soil properties, yield and quality in sugarcane plant-ratoon system under calcareous soil.

Material and methods

Description of the study area

The study was carried out in the Bihar state of India. Bihar is situated in the eastern part of India in between latitudes 24°20'10"N and 27°31'15"N and longitudes 83°19'50"E and 88°17'40"E. It is an entirely land–locked state, in a subtropical region of the temperate zone. The experimental site situated on the bank of the river BurhiGandak at Pusa located in Samastipur, district of Bihar. The experimental research farm is situated at

$25^0$98'N latitude, $85^0$67'E longitude and at an altitude 52.0 m above mean sea level and annual rain fall is about 1000 mm.

Soil condition of experimental site

The field experiment was conducted for three consecutive years first year as main plant crop followed by two years in ratoon-crop at Research Farm of Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur) Bihar. The experiment was executed on medium upland having uniform in topography. The experimental site comes under Ustic moisture regime. The experimental soil belongs to Entisols soil order, Fluvents suborder and great group Typic Ustifluvent. The climate of Pusa belongs to subtropical climatic region of India. The experimental soil had sandy loam textural class as per whitney's textural triangle. Soil is calcareous in nature and the soil contains free calcium carbonate approximately 34%. Soil is moderately fertile in nature, with bulk density of 1.39 Mg m^{-3}. The analysis of initial experimental soil indicates slightly alkaline having pH (1:2.5) 8.25, EC 0.29 dsm^{-1}, $CaCO_3$ 31.63%, low in organic carbon 4.5g ha^{-1}, medium in available N 228.0 $kgha^{-1}$, medium in P_2O_5 22.2 kg ha^{-1}, and low in K_2O 112.1 kg ha^{-1}.

Climate Requirement

Sugarcane is a tropical plant. It grows successfully in regions where the climate is more or less tropical but it may also grow in sub tropics too as in north India. ***Rainfall:*** A total rainfall between 1100 and 1500 mm is required during the months of vegetative growth followed by a dry period for ripening. ***Temperature:*** Growth of sugarcane is closely related to temperature. It requires a wide temperature range from over 38°C. Optimum temperature required for germination is 27° to 33°C. Temperature below 27°C is injurious to the cane, reduce tillers and above 38°C adversely affect the sprouting. ***Ideal temperature: requires for*** Carbon assimilation: 30°C; Sugar synthesis: 30°C; Sugar transport: 30-35°C; Tillering: 33.3-34.4°C; Root growth: 36°C; Shoot growth: 33°C. ***Relative humidity:*** Growth of sugarcane requires high humidity (80-85%) during grand growth period. Above 40% humidity coupled with warm weather favours vegetative growth of cane. A moderate value of humidity 45-65% coupled with limited water supply is required during the ripening phase. ***Sunshine hour:***it requires at least Sunshine of 7-9 hrs./day. ***Frost:*** Severe cold weather inhibits bud sprouting in ratoons and arrest cane growth; at temperature 1°C to 2°C the cane leaves and meristem tissues are killed. ***Wind:*** High velocity winds exceeding 60 km/hr are harmful for canes leading to lodging and cane breakage.

Treatment details

The research work was planned and conducted in RBD with nine treatments and three replications.Plot size was 9.24 m x 5.40 m. Test crop was sugarcane (cv. B.O.154). BC was applied one month before sugarcane crop planting. The treatments included: T_1:RDF for main plant: 150:85:60; RDF for Ratoon crop: 170:50:60; T_2:

100 % NPK + *Acetobacter*; T_3:100% NPK + PSB; T_4:100% NPK+Bio-Compost (@5 t/ha^{-1}); T_5:100% NPK+*Acetobacter* +PSB + Bio-Compost (5 t/ha); T_6:75% NPK+ *Acetobacter;* T7: 75% NPK + PSB ; T8: 75% NPK + Bio-Compost (7.5 t/ha); T9: 75% NPK + *Acetobacter* + PSB +Bio-Compost (7.5 t/ha).

Note: *Acetobacter* (*10^9* cell/ml culture) and PSB (*10^8* cell/ml culture) applied @ (5kg/ha); Trichoderma (*10^6* cell/ml culture) applied uniformly in all treatments except control plot

Input details:

Recommended dose of fertilizer (RDF): The RDF for main crop is N: P_2O_5: K_2O: : 150: 85: 60 and for ratoon crop it was 170:50:60, were applied through Urea, DAP and MOP. The T_1 contains only RDF while from treatment T_2 to T_5 contains 100% NPK along with different Biofertilizer and bio compost. The treatment T_6 to T_9 contains 25 % reduced dose of 100% NPK along with Biofertilizer. Half of N and whole K were applied through inorganic fertilizer at the time of planting of sugarcane and the rest half N was top dressed at the time of earthing up. *Bio-Compost*:The BC was brought from New Swadeshi Sugar Mill, Narkatiyaganj, Bihar. The BC used in this experiment was characterized as per the standard procedure and found that it contains 36 % C, 1.53 % N, 1.50 % P, and 3.10 % K as well as micronutrients contents as Zn 102.3 (mg kg^{-1}); Mn 19.64 (mg kg^{-1}), Cu 11.5 (mg kg^{-1}) and Fe 46 (mg kg^{-1}).*Acetobacter culture:*It works as endophytic nitrogen fixerwhich contains 10^6Cell/mL of culture. *PSB culture*: it contains 10^6Cell/mL of culture. *Freshly prepared* PSB cultures were taken from the Biofertilizer unit of Sugarcane Research Institute, Pusa. Five kilograms of compost based bio-fertilizer (PSB) hectare^{-1} was applied in the furrow before plantation of the sugarcane clumps in the field. The bio-fertilizer was covered with soil by light earthing up followed by irrigation.*Trichoderma culture:Trichoderma* culture was directly applied in soil. The 2.5 Kg of *Trichoderma* powder was mixed with 50 Kg of dried cow dung powder and the mixture was broadcasted in furrow.

Growth and yield parameters

The data related with cane height, cane girth and cane yield was recorded at the harvesting stage and cane yield was computed to tonne per hectare. The data of juice quality was recorded for brix, poland purity %, from composite cane sample juice from each treatments as per standard procedures described (Chen, 1985). Brix was measured by polarimeter. 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 t/ha) was obtained by multiplying cane yield (t/ha) with CCS%. The crop was harvested and plant sampleswere analyzed for N, P and K by the standard procedure.

Soil analysis

Soil samples were analyzed for pH and EC in 1:2 soil suspension ratios. The organic carbon was estimated (Walkley and Black, 1934). The available N was determined by using alkaline permanganate method (Subbiah and Ashija, 1956), available P was analyzed by method described (Olsen et al. 1954), and available K was determined by flame photo metrically as described (Jackson, 1973). The soil physical properties were analyzed by method described (Black, 1965).The available micronutrients cations were analysed method describe (Lindsay and Norvell, (1978). The quality of juice was determined using procedure outlined (Spencer and Meade, (1964). Soil microbial colonies were determined using the methods of plate culture count as described (Li, and Teng, 2008).

Plant analysis (N, P, K content and uptake)

The canes sampled for dry matter determination at harvest were utilized for chemical estimation. The dried samples were ground to fine powder (100 mesh sieves) and about ten g of representative sample from the powdered material was preserved in labeled brown paper bags for chemical estimation. The nitrogen, phosphorus and potassium content were determined by Microkjeldahl method, molybdovandate phosphoric acid method and flame photometric method, respectively. The uptake of nitrogen, phosphorus and potassium ($kgha^{-1}$) was worked out by multiplying the percentage of the nutrient in cane with the corresponding dry yields of the respective constituent.

Soil microbiological analysis

The populations of bacteria, fungi and Actinomycetes were quantified by dilution plate-count techniques on a range of culture media for microorganisms. Ten grams of rhizosphericsoil were aseptically weighed and transferred to flasks containing 95 mL sterile water, which were shaken for 10min at 200rpm on a rotary shaker. While the suspension was in motion, 1mL were withdrawn and added to 9mL sterile water in a screw-cap flask and shaken for 1 min; 1mL of this suspension were transferred to 9mL sterile water, and the process was repeated to a final dilution of 10^{-6}, 10^{-4}, 10^{-2} for bacteria, fungi and Actinomycetes respectively. The dilutions from $10^{-2}$to 10^{-6}were spread onpetriplatescontaining Thornton's Medium (1922), Rose-bengal Agar (Martin, 1950)and Kenknight and Munaier's medium, for bacteria, fungi and Actinomycetes, and incubated at 28±2°C for 4, 3 and 5 d, respectively. After the incubation, colonies were counted. The microbial populations were determined for five replicates.

Soil enzyme activities

The β-glucosidase activity was estimated by using p-nitrophenyl-β-D-glucoside (PNG) as a substrate and incubating 1 g of soil with 0.25 ml toluene, 4 ml modified universal buffer (pH 6), and 1 ml PNG solution (25 mM) for 1 h at 37°C (Eivazi and Tabatabai 1988). After incubation, 1 ml of $CaCl_2$ solution and 4 ml Tris

buffer (pH 12) were added, and absorbance was taken at 400 nm using a spectrophotometer. The activity of β-glucosidase was expressed as μg PNG g^{-1} dwt h^{-1} at 37°C. The urease activity was determined by using urea as a substrate as described by Yao et al. (2006). Five grams of moist soil was incubated with 1 ml methylbenzene, 10 ml of 10% urea 20 ml citrate buffer (pH 6.7) for 24 h at 37°C. One milliliter of filtered soil solution, 1 ml of sodium phenolate, and 3 ml of sodium hypochlorite were added and diluted to 50 ml, and absorbance was determined at 578 nm using a spectrophotometer. The activity of urease was expressed as NH_3-N g^{-1} h^{-1} at 37°C. Acid phosphatase activity was analyzed using p-nitrophenyl phosphate (p-NPP) as substrate as described by Schneider et al. (2000). Five grams of moist soil was mixed with 20 ml acetate buffer (pH 5.2) and 100 mM p-NPP and incubated at 30°C for 30 min. After incubation, 1 ml of $CaCl_2$ and 4 ml of 0.2 M NaOH were added after incubation in order to terminate the reaction. The absorbance was determined using the spectrophotometer at 405 nm. The activity of AP was expressed as μg p-NPP g^{-1} h^{-1} at 30°C. Dehydrogenase activity was measured using triphenyl tetrazolium chloride (TTC) as a substrate (Thalmann 1968), where the TTC solution (0.3–0.4 g/100 ml) was mixed with 5 g of moist soil and incubated for 24 h at 30°C. After incubation, 40 ml of acetone was added, and absorbance was determined at 546 nm using a spectrophotometer. The activity of dehydrogenase was expressed as μg TTC g^{-1} h^{-1}.

Statistical analysis

Analyses of variance (AVOVA) and standard deviations were performed separately at individual sampling dates, using measurements within each plot. All statistical analyses were performed using SPSS version 11.5. The data obtained were analyzed statistically after harvest of second ratoon crops. Data pertaining to different parameters is presentedin tables.

Results and discussion

Effect on NMC, yield and sugar yield

Integrated nutrient application had significant impact on number of millable cane, yield and sugar yield of plant and ratoon of sugarcane (Table 1). The significant increase in cane yield was recorded in the treatments receiving organic manure in combination with bio-fertilizer over control. The treatment T_9 receiving 75 % NPK of RDF + *Acetobacter* +PSBalong with Bio-compost @7.5t/ha produced highest NMC (103.0 $x10^3$/ha) and yield (85.8 t/ha) of plant crop. Similarly, residual effect of treatment T_9 was more pronounced on NMC (92.4 x 10^3/ha) and yield (79.6 t/ha) of ratoon crop. The result indicated that application of NPK through both from organic and inorganic sources along with bio-fertilizer were found beneficial for obtaining higher yield of plant and ratoon crop. However, difference in yield was significantly at par with treatment T_5 and T_8 receiving bio-compost @ 5t ha^{-1} and 7.5 t ha^{-1}respectively. The results are in agreements with findingsof many scientists

(Nagaraju et al. 2000; Virdia and Patel 2010). Yadav et al. (2018) reported that addition of 10 t ha^{-1}FYM/compost alongwith inorganic fertilizers on the basis of soil test + bio fertilizers (Azotobactor + PSB) @ 12.5 kg ha^{-1} each had a positive effect on sugarcane growth and yield in both plant and ratoon crops.

Sugar Yield

The effect of bio-fertilizer and bio-compost along with inorganic fertilizer slightly improved sugar yield in plant and ratoon crop. The highest sugar yield (11.21 tha^{-1}) in treatment T_9, which was at par with $T_5$and T_8receiving biocompostand lowest was observed in control. A field study to evaluate the response of sugarcane varieties to application of nitrogen fixing diazotrophs viz., *Azotobactor*, *Azospirillum* and *Gluconacetobacter* under different levels of fertilizer nitrogen, reported significant improvement in yield and sugar content of bio-fertilizer inoculated sugarcane plants compared to uninoculated control,Hari and Srinivasan, (2005).Thakur and Singh (1996) reported that the use of different bio-fertilizers like *Azotobactor*, *Azospirillum* and Phosphorus fixing bacteria (*Bacillus mangatherium*) alone or in combined use of these micro-organisms significantly increased the sugar yield.

Nutrient uptake

The nutrient uptake by plant and ratoon (Table 2) significantly increased due to application of organic manure and bio-fertilizer along with inorganic fertilizer over control. The highest uptake was recorded in treatment T_9 and lowest was recorded in control. The data further revealed that among major nutrients relatively higher K uptake was recorded which was followed by N and P. The higher yield coupled with management of nutrients through organic and inorganic sources in T_9 resulting more nutrients uptake Bhalerao, et al. (2006).The use of phosphate solubilising bacteria as inoculants simultaneously increase P uptake by the plant and crop yield (Banger *et al.*1993). The principal mechanism for mineral phosphate solubilisation is the production of organic acid and acid phosphatases play a major role in the mineralization of organic phosphorus in soil. Ratoon cultivation requires more nitrogen in comparison to main crop because the activity of bacteria in ratoon crop is more in rhizospheric zone especially for mineralization of crop residues and other dissected root parts. Chemical fertilizers should be applied only after 3 weeks of stubble shaving.

Soil Properties

Addition of organic manure with bio-fertilizerin combination with inorganic fertilizer significantly improved the soil fertility in terms of organic carbon in particular and availability of macro and micro nutrients (N, P, K, Zn, Cu, Mn and Fe) in general with reduction in bulk density of post-harvest soil (Table 3). The application of organics in combination with inorganic fertilizer and bio-fertilizer significantly decreased pH and lowest being in T_9(7.69) and highest in control (8.29). In contrast, significant increase in EC was recorded in

bio-compost treated plot with maximum increase in T_9 ($0.39dSm^{-1}$). The reduction in pH might be due to production of organic acids due to decomposition of biocompost followed by increase in salt content of soil due to mineralization, which increase EC of soil. The soil pH reduced while EC increased due to application of biocompost as reported by Bhalerao, et al. (2006). There was significant effect of treatments receiving biocomposton organic carbon and available N, P_2O_5, K_2O and micro nutrient of soil after harvest of crop over control. The highest (7.3 g ha^{-1}) organic carbon was observed in T_9 over control. The treatments varied significantly for available nutrients with N (226.4 to 265.4 kg ha^{-1}), P_2O_5 (23.4 to 37.9 kg ha^{-1})andK_2O (114.8 to 136.6 $kgha^{-1}$).The increase in soil nitrogen reserve under sugarcane crop by 50% of the initial value due to the nitrogen fixation by root associated diazotrophs helping sustained production of sugarcane (Suman 2003).The buildup of soil available nutrient could be attributed to greater multiplication of microbes due to addition of organic manure, which helps in mineralization as well as solubilization of native nutrients. The data also indicated that cations especially Ca^{2+}+Mg^{2+} content of soils significantly increased in treatments of bio-compost.This might be resulted due to solubilization of nutrients by complexation of nutrients by humic and fulvic acid present in biocompost as reported by Prasad and Sinha (1984).The result also indicated that application of only inorganic fertilizer (T_1) was not effective for maintenance of soil health in sugarcane plant as reflected from initial value. Soil available nutrients and organic carbon sustained in all the organic manure and bio-fertilizer treated plots. The bulk density of post-harvest soil varied significantly (1.32 to 1.38 g/cm^3) with addition of organic manure and bio-fertilizer(Table 3). The reduction in bulk density resulted in increased pore space of soil with increasing level of organic manure. The reduction in bulk density may be attributed to the buildup of organic carbon content of soil in Biocomposttreated plots. The maximum reduction (1.32 g/cm^3) in bulk density was recorded in treatment T_9 as compared to control. Beneficial effect of Biocompostin improvement of physical and chemical condition of soil may be attributed to improvement in organic matter status in organic manure treated soil resulted in buildupof soil fertility for sustainable sugarcane production (Sinha et al. 2014; Jha et al. 2015). The table No. 4, reflects the Effect of biofertilizer with bio-compost on soil micro nutrients at harvest in sugarcane plant-ratoon system. The Fe, Zn, Cu, And Mn contents varies from 6.5 - 8.50; 0.66 - 0.79; 0.76 - 0.89 and 2.10 - 2.89 mg/kg, respectively,

Microbial Populations

The microbial population viz. bacteria, fungi, Actinomycetes,and Acetobacter significantly increased with addition of organic manure and bio-fertilizerover control. The highest population of bacteria (42.8×10^6), fungi (29.3×10^4), Actinomycetes (28.7×10^2) and Acetobacter(34.8×10^6) were observed in treatment $T_9$and lowest microbial count observed in control (Table 5). These results explained the improvement in microbial

population of soil due to application of organics. Kumar *etal*., (2015) reported that in both plant and ratoon crops enumeration of *Azotobactor*, PSB, Fungi, Bacteria, Actinomycetes in rhizosphere indicated that the population of all the groups was higher when bio-fertilizers were applied in combination with inorganic fertilizers. Microorganism utilized organic carbon as a source of energy for nourishment which resulted in proliferation of soil microorganism. The increased activity of microflora in organic manure and biofertilizertreated soil may be due to high organic matter build up with application of organic manure. The shift in microbial population signifies the maintenance of soil fertility and productivity due to faster rate of decomposition and speedy mineralization of organic materials.

Soil enzyme activity

Soil enzyme activity is influenced by the soil characteristics related to nutrient availability and soil microbial activity processes which modified the potential soil enzymemediated substrate catalysis as reported by Kandeler et al. (1996). In this study, the activity of all the enzymes was higher under $T_{9,}$ the soils were applied with bio-composthaving high carbon content and added greater SOM. This suggests that the enzyme activities are governed by the availability of carbon sources and SOM decomposition. The presence of *Trichoderma* in all the treatments helps in rapid decomposition of soil organic matter.The intensive management practices under sugarcane cultivation constantly disturb the soil and regular removal of organic layer restricted the supply of substrate for microbes present in rhizosphere, thereby reduces the enzyme activities. Kotroczo et al. (2014) reported that under different treatments of detritus input and removal, the enzyme activities were more influenced by root activity rather than aboveground organic matter availability. In this case, the higher activity of rhizosphere in sugarcane cultivation increased the enzyme activities. Previous studies reported a reduction in soil enzyme activities following the conversion of forests into cultivated lands observed by several workers (Vinhal-Freitas et al. 2017; Silva et al. 2019). Urease regulates the transformation of soil nitrogen and is involved in the hydrolysis of urea into ammonia and CO_2 (Kong et al. 2008). The urease activity is influenced by various soil properties including pH, soil nutrient supply, soil nitrogen, and N fertilizers (Moghimian et al. 2017). In this study, the highest urease activity (44 (NH_3-N g^{-1} h^{-1})) was evaluated in T_9which is at par with T_5. Our findings were similar to previous findings indicating greater urease activity under higher level of bio-compost than lower level of biocompost, indicating that the availability of fresh SOM for microbial decomposition enhances the microbial activity in soil and increases the enzyme activity (de Medeiros et al. 2015). Contrastingly, in cultivated fields, high urease activity was found despite low values of soil carbon and soil nitrogen. This can be explained by the regular supply of urea fertilizer in the field. Also, a strong positive correlation of urease activity with soil organic matter supported its increased activity (Zeng et al. 2009).

Dehydrogenase activity in soil serves as an indicator of the microbiological redox system and microbial oxidative activities in soil (Casida Jr et al. 1964). It indicates the respiratory activity of the soil and can be used as a measure of microbial activity in semiarid climates (Bastida et al. 2007). The reduced content of labile carbon and soil carbon are suggested to decrease the activity. Bonanomi et al. (2011) reported a reduction by 84% in dehydrogenase activity in a low-input management regime as compared with the high-input management regime. de Medeiros et al. (2015) reported the dehydrogenase activity in soils under different intercropping areas found the lowest activity in Cajanus cajan, Vignia unguiculata monoculture. The study reported that soil disaggregation and weeding along with low vegetation cover attributed to reduced enzyme activity. Further, in dry climate conditions the abiotic stress to microbial activity due to high temperature and low soil moisture influence the organic matter oxidation by dehydrogenase (Li and Sarah 2003). In addition, β-glucosidase activity in soil is linked to the release of carbohydrates in soil, which provides a major substrate for soil microorganisms. The positive impact of the soil carbon with β-glucosidase activity indicated that soil organic matter content is the major factor in its activity (Vinhal-Freitas et al. 2017). Corroborating with our results, Silva et al. (2019) evaluated β-glucosidase activity under tropical native forest, protected area, reported reduced activity under the cultivated field; and suggested a closed linking of β-glucosidase with soil organic carbon and soil organic matter content. de Medeiros et al. (2015) demonstrated similar β-glucosidase activity among tropical dry forest and intercropping soils with less aggressive management practices. Similarly, the acid phosphatase activity was also higher under T_9(1100 µg p-NPP g^{-1} h^{-1})as compared to other treatments, which is at par with T_5. The activity of acid phosphatase activity is also influenced by soil pH, nutrients, soil carbon, soil nitrogen, soil phosphorus, soil organic matter quality and quantity, microbial community structure, soil moisture, and soil temperature as mentioned by many scientist (Hendriksen et al. 2016; Maharajan et al. 2017). Raiesi and Beheshti (2015) indicated that soil pH is the main regulator of acid phosphatase activity, and narrow pH ranges attributed to no significant changes after natural forest conversions.

Conclusion:

The results suggested that the application of nitrogen fixer like *Acetobacte*r, organic matter decomposer like *Trichoderma* and PSB used in cultivation of sugarcane have significantly reduced the application of 25% recommended dose of NPK. Hence, integrated use of bio-compost and inorganic fertilizer along with PSB and Acetobacter improved the soil health, which ultimately enhances productivity of sugarcane and sugar recovery with improvement in microbial community structure and enzymatic activity in the rhizospheric zone. Thus it is concluded that integrated use of bio-compost along with various bio-fertilizer improved fertility status of soil with improvement in enzymatic activities and population of microbes.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Allowed to publish

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Table1. Effect of biofertilizer with bio-compost on NMC, yield and sugar yield in sugarcane plant- ratoon system (*pooled data of two years for Ratoon crop)

Treatments	NMC (000/ha)		Yield (t/ha)		Cane yield Response over control (%)		Sugar yield (t/ ha)		Sugar Yield Response over control (%)	
	Plant	Ratoon*	Plant	Ratoon*	Plant	Ratoon*	Plant	Ratoon*	Plant	Ratoon*
T_1: RDF main plant: 150:85:60; RDF for Ratoon crop: 170:50:60	69.0	59.1	53.8	53.2	-	-	6.28	5.29	-	-
T_2: 100 % NPK + *Acetobacter*	75.0	73.5	62.6	60.2	16.36	13.15	7.40	6.60	17.83	24.76
T_3: 100% NPK + PSB	78.0	76.6	66.9	65.4	24.34	22.93	7.80	7.00	24.20	32.33
T_4: 100% NPK + Bio-Compost (5t/ha)	93.0	88.1	80.5	73.8	49.63	38.72	10.18	8.62	62.10	62.94
T_5: 100% NPK+ *Acetobacter* + PSB + Bio-Compost (5 t/ha)	96.0	89.5	81.7	77.5	51.86	45.67	10.69	9.28	70.22	75.43
T_6: 75% NPK + *Acetobacter*	89.8	88.8	77.9	74.7	44.80	40.41	9.58	9.16	52.55	73.16
T_7: 75% NPK + PSB	71.0	68.2	58.2	57.8	8.18	8.64	6.52	6.23	3.82	17.77
T_8: 75% NPK + Bio-Compost (7.5 t/ha);	95.4	89.3	82.4	78.5	53.15	47.55	10.32	9.31	64.33	75.99
T_9: 75% NPK + *Acetobacter* + PSB +Bio-Compost (7.5 t/ha).	103.0	92.4	85.8	79.6	59.48	49.62	11.21	9.36	78.50	76.93
CD (P=0.05)	**8.01**	11.12	**5.89**	**6.20**	**-**	**-**	**0.90**	**0.90**	**-**	**-**
SEm±	**2.57**	3.98	**2.53**	**3.79**	**-**	**-**	**0.29**	**0.28**	**-**	**-**

Table 2. Effect of biofertilizer with bio-compost on uptake of nutrients in sugarcane plant-ratoon system (*pooled data of two years for Ratoon crop)

Treatments	Uptake of macro nutrient (kg/ha)						Uptake of micro (g/ha)					
	Plant			Ratoon*			Plant			Ratoon		
	N	P	K	N	P	K	Zn	Fe	Mn	Zn	Fe	Mn
T_1: RDF main plant: 150:85:60; RDF for Ratoon crop: 170:50:60	121.5	11.34	129.6	107.0	8.99	114.2	42.04	548.3	192.7	37.38	490.7	183.6
T_2: 100 % NPK + *Acetobacter*	146.9	13.38	152.6	141.4	12.40	144.5	49.48	561.4	253.8	42.83	610.6	215.4
T_3: 100% NPK + PSB	155.1	14.50	165.1	149.6	13.52	157.2	50.94	564.4	228.6	44.84	625.8	217.3
T_4: 100% NPK + Bio-Compost (5t/ha)	187.6	17.59	183.3	177.1	16.79	180.2	48.30	652.4	227.6	48.45	637.3	221.6
T_5: 100% NPK+ *Acetobacter* + PSB + Bio-Compost (5 t/ha)	191.8	19.43	213.14	182.1	17.69	186.8	51.10	673.82	235.4	50.82	643.5	227.4
T_6: 75% NPK + *Acetobacter*	172.4	12.76	199.3	162.3	17.06	184.1	47.30	605.6	211.8	43.28	570.5	215.6
T_7: 75% NPK + PSB	133.5	17.96	145.5	120.9	11.38	133.7	45.50	562.4	195.8	39.32	516.3	183.4
T8: 75% NPK + Bio-Compost (7.5 t/ha);	192.6	18.71	206.1	178.7	16.97	179.7	54.22	669.8	232.68	49.69	598.27	224.3
T9: 75% NPK + *Acetobacter* + PSB +Bio-Compost (7.5 t/ha).	196.9	20.89	221.92	195.40	19.93	198.5	56.13	679.61	239.96	53.24	657.40	237.5
CD (P=0.05)	**13.38**	**1.64**	**17.69**	**14.04**	**2.41**	**12.25**	**3.17**	**6.36**	**5.05**	**2.98**	**14.38**	**10.6**
SEm±	**4.18**	**0.46**	**4. 86**	**4.62**	**0.71**	**3.79**	**1.30**	**2.41**	**2.81**	**1.05**	**3.93**	**3.14**

Table 3. Effect of biofertilizer with bio-compost on soil properties (0-30 cm depth) after harvest in sugarcane plant- ratoon system

Treatment	pH	EC (dS/m)	Organic Carbon (g/kg)	Bulk density (g/cm^3)	$Ca^{2} + Mg^{+}$ (m/L)	Available Nutrients (kg/ha)		
						N	P_2O_5	K_2O
T_1: RDF main plant: 150:85:60; RDF for Ratoon crop: 170:50:60	8.29	0.28	4.4	1.38	10.25	226.4	23.4	114.8
T_2: 100 % NPK + *Acetobacter*	8.17	0.28	4.6	1.37	10.36	252.7	26.7	119.5
T_3: 100% NPK + PSB	8.16	0.29	4.7	1.36	10.37	250.9	29.8	123.3
T_4: 100% NPK + Bio-Compost (5t/ha)	8.09	0.32	6.5	1.34	12.10	253.2	34.3	129.3
T_5: 100% NPK+ *Acetobacter* + PSB + Bio-Compost (5 t/ha)	7.76	0.34	6.6	1.33	12.07	256.6	36.5	132.5
T_6: 75% NPK + *Acetobacter*	8.11	0.33	6.2	1.34	11.57	246.8	34.9	126.7
T_7: 75% NPK + PSB	8.10	0.34	6.3	1.35	11.42	235.3	35.2	124.4
T8: 75% NPK + Bio-Compost (7.5 t/ha);	7.85	0.38	6.7	1.33	11.83	243.8	29.9	129.4
T9: 75% NPK + *Acetobacter* + PSB +Bio-Compost (7.5 t/ha).	7.69	0.39	7.3	1.32	12.85	265.4	37.9	136.6
CD (P=0.05)	**0.03**	**0.05**	**0.60**	**0.01**	**0.75**	**09.39**	**1.99**	**4.32**
SEm±	**0.01**	**0.12**	**0.20**	**0.002**	**0.24**	**3.22**	**0.64**	**2.08**

Table-4. Effect of biofertilizer with bio-compost on soil micro nutrients at harvest in sugarcane plant-ratoon system.

Treatment	**Soil Micro Nutrients (mg/kg)**			
	Fe	**Zn**	**Cu**	**Mn**
T_1	6.50	0.66	0.76	2.10
T_2	6.80	0.68	0.77	2.21
T_3	7.21	0.71	0.78	2.31
T_4	8.40	0.75	0.85	2.60
T_5	8.11	0.73	0.87	2.70
T_6	8.10	0.73	0.86	2.50
T_7	7.70	0.72	0.84	2.51
T8	8.26	0.74	0.83	2.80
T9	8.50	0.79	0.89	2.89
CD (P=0.05)	**0.06**	**0.05**	**0.02**	**0.17**
SEm±	**0.03**	**0.02**	**0.01**	**0.05**

T_1: RDF main plant: 150:85:60; RDF for Ratoon crop: 170:50:60; T_2: 100 % NPK + *Acetobacter;* T_3: 100% NPK + PSB; T_4: 100% NPK + Bio-Compost (5t/ha); T_5: 100% NPK+ *Acetobacter* + PSB + Bio-Compost (5 t/ha); T_6: 75% NPK + *Acetobacter;* T_7: 75% NPK + PSB; T8: 75% NPK + Bio-Compost (7.5 t/ha);T9: 75% NPK + *Acetobacter* + PSB +Bio-Compost (7.5 t/ha).

Table-5. Effect of biofertilizer with bio-compost on microbial population of soils after harvest in sugarcane plant-ratoon system.

Treatments	Total microbial counts							
	Bacteria (cfu×10^6 g^{-1})	Population increase over control (%)	Fungi (cfu×10^4 g^{-1})	Population increase over control (%)	Actinomycetes (cfu× 10^2 g^{-1})	Population increase over control (%)	*Acetobacter*(cfu×10^6 ml^{-1})	Population increase over control (%)
T_1: RDF main plant: 150:85:60; RDF for Ratoon crop: 170:50:60	23.2	-	13.3	-	11.8	-	17.7	-
T_2: 100 % NPK + *Acetobacter*	26.9	15.95	14.7	10.53	13.7	16.10	26.2	48.02
T_3: 100% NPK + PSB	27.8	19.83	20.2	51.88	14.9	26.27	24.8	40.11
T_4: 100% NPK + Bio-Compost (5t/ha)	32.5	40.09	20.3	52.63	20.4	72.88	29.9	68.93
T_5: 100% NPK+ *Acetobacter* + PSB + Bio-Compost (5 t/ha)	37.7	62.50	26.8	101.50	22.6	91.53	31.2	76.27
T_6: 75% NPK + *Acetobacter*	34.9	50.43	20.6	54.89	20.3	72.03	28.9	63.27
T_7: 75% NPK + PSB	33.9	46.12	19.5	46.62	19.5	65.24	28.0	58.19
T_8: 75% NPK + Bio-Compost (7.5 t/ha);	36.3	56.37	26.4	98.49	23.1	95.76	28.2	59.32
T_9: 75% NPK + *Acetobacter* + PSB +Bio-Compost (7.5 t/ha).	42.8	84.74	29.3	120.30	28.7	143.22	34.8	96.61
CD (P=0.05)	5.92	-	3.04	-	6.32	-	4.33	-
SEm±	1.94	-	1.33	-	2.33	-	1.67	-

Table6. Effect of biofertilizer with bio-compost on soil enzyme activities of β-glucosidase, Urease, Acid phosphatase activity and Dehydrogenase activity, after harvest in sugarcane plant-ratoon system

Treatments	Soil enzyme activities			
	β-glucosidase (μg PNG g^{-1} dwt h^{-1})	Urease (NH_3-N g^{-1} h^{-1})	Acid phosphatase activity (μg p-NPP g^{-1} h^{-1})	Dehydrogenase activity (μg TTC g^{-1} h^{-1})
T_1: RDF main plant: 150:85:60; RDF for Ratoon crop: 170:50:60	218	15	319	0.20
T_2: 100 % NPK + *Acetobacter*	345	26	428	0.96
T_3: 100% NPK + PSB	389	32	457	1.08
T_4: 100% NPK + Bio-Compost (5t/ha)	540	37	850	1.20
T_5: 100% NPK+ *Acetobacter* + PSB + Bio-Compost (5 t/ha)	576	39	993	1.93
T_6: 75% NPK + *Acetobacter*	365	30	443	0.98
T_7: 75% NPK + PSB	397	35	469	1.18
T_8: 75% NPK + Bio-Compost (7.5 t/ha);	403	36	561	1.21
T_9: 75% NPK + *Acetobacter* + PSB +Bio-Compost (7.5 t/ha).	760	44	1100	1.98
CD (P=0.05)	**123.18**	6.30	**174.19**	**0.06**
SEm±	**43.69**	2.16	**53.72**	**0.18**