International Journal of Plant & Soil Science

Volume 37, Issue 3, Page 261-275, 2025; Article no.IJPSS.133081
ISSN: 2320-7035

Assessment of Microbial and Enzymatic Activity of Plant-ratoon System in Sugarcane Rhizosphere in Indo-Gangetic Plains of India

S.K. Sinha [a++], Ajeet Kumar [b#*], Satish Chandra Narayan [c†], Sanjay Kumar [d++], Amrita Kumari [e‡] and Brajesh Kumar [f^]

[a] Department of Soil Science, Regional Research Station, Madhopur, Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur)-848125, Bihar, India.
[b] Department of Microbiology, College of Basic Sciences and Humanities (CBS&H), Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur)-848125, Bihar, India.
[c] Department of Plant Breeding, RRS, Madhopur Bihar-848125, India.
[d] Regional Research Station, Jhanjharpur, Madhubani, Bihar, India.
[e] Bihar Agricultural University, Sabour, Bhagalpur, 813210, India.
[f] Department of Soil Science, Post Graduate College of Agriculture, RPCAU, Pusa Samastipur, Bihar, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2025/v37i35364

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/133081

Original Research Article

Received: 18/01/2025
Accepted: 22/03/2025
Published: 26/03/2025

[++] Associate Professor -cum-Senior Scientist;
[#] Assistant Professor-cum-Scientist (Soil Science);
[†] Assistant Professor-cum- Scientist;
[‡] Assistant Professor;
[^] PG Research Scholar;
**Corresponding author: E-mail: ajeet.sri@rpcau.ac.in;*

Cite as: *Sinha, S.K., Ajeet Kumar, Satish Chandra Narayan, Sanjay Kumar, Amrita Kumari, and Brajesh Kumar. 2025. "Assessment of Microbial and Enzymatic Activity of Plant-Ratoon System in Sugarcane Rhizosphere in Indo-Gangetic Plains of India". International Journal of Plant & Soil Science 37 (3):261-75. https://doi.org/10.9734/ijpss/2025/v37i35364.*

ABSTRACT

A field experiment was carried out on sugarcane plant-ratoon system to develop nutrient management strategies for sustaining soil health, quality and sugarcane production at Research Farm, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar. Trials were conducted to test the efficiency of various fertilizers in sugarcane on solubility of applied inorganic fertilizer during spring season in calcareous soil. The pooled data revealed that number of millable cane (NMC), cane and sugar yield varied significantly due to integrated use of organic and inorganic fertilizer with bio-fertilizer in combination both in plant and ratoon crops. The significant increase in NMC, cane 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 (103.0×10^3 ha^{-1}), cane yield (85.8 t ha^{-1}) and sugar yield (11.21 t ha^{-1}) was recorded in treatment T9 i.e 75% NPK of RDF + *Acetobacter* + PSB + along-with Bio-compost @ 7.5 t ha^{-1}. Similar trend of result was recorded in ratoon crop also, 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 t ha^{-1}) and sugar yield (9.36 t ha^{-1}). The bio-compost improved overall performance of sugarcane. The uptake of nutrients by plant and ratoon followed the similar trend as cane yield. The treatment receiving RDF along with various biofertilizer significantly 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, P and K) 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.

Keywords: Sugarcane; ratoon; biofertilizers; bio-compost; PSB; enzymes.

1. INTRODUCTION

“Sugarcane is widely cultivated throughout the Indo-Gangetic plains. More than 4.2 million hectares are under sugarcane cultivation in India, with an average cane yield of 60 tha^{-1}”(Kumar et al., 2023). “Sugarcane 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; Kumar et al., 2024). “Out of the total phosphatic 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” (Kumar et al*.,* 2013, Kumar et al*.,* 2014). “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, Kumar et al., 2014a). “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” (Sinha et al., 2024). “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” (Kumar et al., 2024). “Besides, the continuous use of chemical fertilizers causing deficiency in other micronutrients. In recent years, the yield have stagnated 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” (Kumar et al 2024a). “Sugarcane farmers are switching over to alternative practices to make sugarcane cultivation more sustainable and productive” (Kumar et al., 2023a). “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 matter from such resources such as animal manures, crop residues and green manuring has been shown to replenish organic carbon and improve soil structure and fertility” (Singh et al*.,* 2020;). “Moreover, several kinds of beneficial microflora capable of fixing nitrogen or solubilising and mobilizing P and other nutrients are becoming an integral component *Gluconacetobacter diazotrophicus* earlier known as *Acetobacter diazotrophicus*, a nitrogen-fixing bacteria associated with sugarcane as an endophyte, is present in high numbers ($1×10^6$ CFU)" (Rana et al., 2024; Kumar et al, 2024b, Kumar et al, 2024c). "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" (Kumar et al, 2024d). "Field trial data shown that inoculation by *GD* together with other diazotrophs can match yield equal to the application of 275 kg Nha^{-1}" (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" (Kumar et al*.* 2024), 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 (Kumar et al. 2023b). "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., *Azospirillum amazonense*, *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" (Kumar et al*.* 2024). "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" (Ajeet et al., 2023). 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 (Meena et al., 2023; Sinha et al. 2024a). "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 management approach has been observed highly beneficial. Phosphorus is the second most plant nutrient after nitrogen" (Kumar and Jha, 2021). "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" (Kumar et al., 2013, Ajeet et al., 2023). Thus, keeping in view the above all facts, a field experiment was conducted to study the integrated effect of manure, biofertilizer and inorganic fertilizer on soil properties, yield and quality in sugarcane plant-ratoon system under calcareous soil.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study was carried out in the Bihar, which 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 Burhi Gandak 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.

2.2 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 climate of Pusa belongs to subtropical climatic region of India. 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 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}.

2.3 Climate Requirement

Sugarcane 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 - 1500 mm is required during the vegetative growth followed by a dry period for ripening. Temperature: Growth of sugarcane requires a wide temperature 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 and sugar synthesis is about: 30°C; for sugar transport: 30-35°C; for Tillering: 33.3-34.4°C; for Root growth: 36°C and for shoot growth: 33°C. Relative humidity: sugarcane requires high humidity (80-85%) during grand growth period. Above 40% humidity coupled with warm weather favours vegetative growth of cane. A moderate humidity 45-65% coupled with limited water supply is required during the ripening. Sunshine hour: it requires at least 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: wind velocity exceeding 60 km/hr leading to lodging and cane breakage" (Kumar et al., 2018; Kumar et al., 2023).

2.4 Treatment Details

The research work was 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:

LIST 1. Treatment details

TreatmentNo.	:	Treatment details
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*
T_7	:	75% NPK + PSB
T_8	:	75% NPK + Bio-Compost (7.5 t/ha)
T_9	:	75% NPK + *Acetobacter* + PSB +Bio-Compost (7.5 t/ha)

Note: CFU of Acetobacter (10^9 cell/ml culture) and PSB (10^8 cell/ml culture) applied @ (5kg/ha); Spore count of Trichoderma viride (10^6 cell/ml culture) applied uniformly in all treatments except control plot. 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. The biocompost 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 fixer which 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 SRI, 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 culture powder was mixed with 50 Kg of dried cow dung powder and the mixture was broadcasted in furrow in moist condition

2.5 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, pol and purity %, from composite cane sample juice from each treatments as per standard procedures described (Kumar et al., 2023c). 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 samples were analyzed for N, P and K by the standard procedure.

2.6 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 by method described by Lindsay and Norvell, (1978). The quality of juice was determined by using procedure outlined by Spencer and Meade in 1964. Soil microbial colonies were determined using the methods of plate culture count.

2.7 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 (kg ha^{-1}) was worked out by multiplying the percentage of the nutrient in cane with the corresponding dry yields of the respective constituent.

2.8 Soil Microbiological Analysis

The populations of bacteria, fungi and actinomycetes were quantified by serial dilution plate-count techniques on a range of culture media for microorganisms, to a final dilution of 10^{-6}, 10^{-4}, 10^{-2} respectively. The dilutions were spread on petriplates containing 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 5d, respectively. After the incubation, colonies were counted.

2.9 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 $g^{-1}h^{-1}$.

2.10 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.

3. RESULTS AND DISCUSSION

3.1 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 treatment T_9 receiving 75 % NPK of RDF + *Acetobacter* + PSB along with Bio-compost @7.5 t/ha produced highest NMC (103.0 x10^3/ha) and yield (85.8 t/ha) of plant crop as compare to control. 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 also. "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 findings of many scientists such as" (Nagaraju et al. 2000; Virdia and Patel 2010). Yadav et al. (2018) reported that addition of 10 t ha^{-1} FYM/compost along with 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.

3.2 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 t ha^{-1}) recorded in treatment T_9, which was at par with T_5 and that of lowest was observed in control. "A field study to evaluate the response of sugarcane varieties to application of *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 un inoculated control", Similar findings were recorded Kumar et al., 2024; Sinha et al., 2024. Kumar et al., 2025. The use of *Azotobactor*, *Azospirillum* and Phosphorus fixing bacteria (*Bacillus mangatherium*) alone or in combined use significantly increased the sugar yield.

3.3 Nutrient Uptake

Data regarding the nutrient uptake is presented in Table 2 revealed that, nutrient uptake by plant and ratoon was found 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; Kumar et al., 2025a. "The use of phosphate solubilising bacteria as inoculants simultaneously increase P uptake by the plant and crop yield" (Kumar et al.2014). "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 rhizospheric zone especially for mineralization of crop residues and other dissected root parts" (Kumar et al.2014).

3.4 Soil Properties

Addition of organic manure with bio-fertilizer in 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 (0.39 dSm^{-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 Meena et al. (2024). There was significant effect of treatments receiving biocompost on 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}) and K_2O (114.8 to 136.6 kg ha^{-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" (Kumar et al., 2024). "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 biocompost treated 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 biocompost in improvement of physical and chemical condition of soil may be attributed to improvement in organic matter status in organic manure treated soil resulted in buildup of soil fertility for sustainable sugarcane production" (Sinha et al. 2024; Kumar et al., 2023). The Table 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.

3.5 Microbial Populations

The microbial population *viz*. bacteria, fungi, actinomycetes and *acetobacter* significantly increased with addition of organic manure and bio-fertilizer over control. The highest population of bacteria (42.8 x 10^6), fungi (29.3 x 10^4), actinomycetes (28.7 x 10^2) and *acetobacter* (34.8 x 10^6) were recorded 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 et al., (2014) also reported that "the population of all the groups of microbes 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 biofertilizer treated 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".

3.6 Soil Enzyme Activity

Data regarding soil enzyme activity revealed that, the enzyme activity is influenced by the soil characteristics related to nutrient availability and soil microbial activity processes which modified the potential soil enzyme mediated 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-compost having 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" (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_9 which 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" (Meena et al. 2024).

Table 1. 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	69.0	59.1	53.8	53.2	-	-	6.28	5.29	-	-
T_2	75.0	73.5	62.6	60.2	16.36	13.15	7.40	6.60	17.83	24.76
T_3	78.0	76.6	66.9	65.4	24.34	22.93	7.80	7.00	24.20	32.33
T_4	93.0	88.1	80.5	73.8	49.63	38.72	10.18	8.62	62.10	62.94
T_5	96.0	89.5	81.7	77.5	51.86	45.67	10.69	9.28	70.22	75.43
T_6	89.8	88.8	77.9	74.7	44.80	40.41	9.58	9.16	52.55	73.16
T_7	71.0	68.2	58.2	57.8	8.18	8.64	6.52	6.23	3.82	17.77
T_8	95.4	89.3	82.4	78.5	53.15	47.55	10.32	9.31	64.33	75.99
T_9	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	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	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	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	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	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	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	133.5	17.96	145.5	120.9	11.38	133.7	45.50	562.4	195.8	39.32	516.3	183.4
T_8	192.6	18.71	206.1	178.7	16.97	179.7	54.22	669.8	232.68	49.69	598.27	224.3
T_9	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	8.29	0.28	4.4	1.38	10.25	226.4	23.4	114.8
T_2	8.17	0.28	4.6	1.37	10.36	252.7	26.7	119.5
T_3	8.16	0.29	4.7	1.36	10.37	250.9	29.8	123.3
T_4	8.09	0.32	6.5	1.34	12.10	253.2	34.3	129.3
T_5	7.76	0.34	6.6	1.33	12.07	256.6	36.5	132.5
T_6	8.11	0.33	6.2	1.34	11.57	246.8	34.9	126.7
T_7	8.10	0.34	6.3	1.35	11.42	235.3	35.2	124.4
T_8	7.85	0.38	6.7	1.33	11.83	243.8	29.9	129.4
T_9	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
T_8	8.26	0.74	0.83	2.80
T_9	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	23.2	-	13.3	-	11.8	-	17.7	-
T_2	26.9	15.95	14.7	10.53	13.7	16.10	26.2	48.02
T_3	27.8	19.83	20.2	51.88	14.9	26.27	24.8	40.11
T_4	32.5	40.09	20.3	52.63	20.4	72.88	29.9	68.93
T_5	37.7	62.50	26.8	101.50	22.6	91.53	31.2	76.27
T_6	34.9	50.43	20.6	54.89	20.3	72.03	28.9	63.27
T_7	33.9	46.12	19.5	46.62	19.5	65.24	28.0	58.19
T_8	36.3	56.37	26.4	98.49	23.1	95.76	28.2	59.32
T_9	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	-

Table 6. 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	218	15	319	0.20
T_2	345	26	428	0.96
T_3	389	32	457	1.08
T_4	540	37	850	1.20
T_5	576	39	993	1.93
T_6	365	30	443	0.98
T_7	397	35	469	1.18
T_8	403	36	561	1.21
T_9	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

"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. 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" (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".

4. CONCLUSION

In conclusion, the study demonstrated that the integrated application of organic and inorganic fertilizers, along with bio-fertilizers such as *Acetobacter* and PSB, significantly improved sugarcane productivity and soil health. The treatment combining 75% NPK of RDF with *Acetobacter*, PSB, and bio-compost (T_9) recorded the highest cane yield (85.8 t/ha), sugar yield (11.21 t/ha), and nutrient uptake while enhancing soil fertility and microbial activity. Improved soil enzymatic activity, increased organic carbon, and better nutrient availability in post-harvest soil were observed, indicating enhanced soil health. The results confirm that integrated nutrient management is a sustainable strategy for improving sugarcane yield and soil quality in the Indo-Gangetic plains. Thus from present experiment it is concluded that integrated use of biocompost along with various bio-fertilizer improved fertility status of soil with improvement in enzymatic activities and population of microbes.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENTS

This work was financially supported by New Swadeshi Sugar Mills, Narkatiyaganj (Distillery Division), West Champaran, Bihar in collaboration with *Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur)-848125, Bihar, India* through adhoc Project entitled "Evaluation of bio-fertilizer with bio-compost and bio-methanted distillery effluent in sugarcane crop" and experiment were executed at Agricultural research farm of RPCAU, Pusa, Bihar.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Ajeet Kumar, Sunita Kumari Meena, S.K. Sinha and A. K. Singh (2023) Mechanism of Microbial Dissolution of Insoluble Phosphorus. *Agriblossom*. 3 (7): 19-23. ⟨hal-04935392⟩.

Bastida, F., Moreno, J.L., Hernandez, T., and García, C. (2007) The long-term effects of the management of a forest soil on its carbon content, microbial biomass and activity under a semi-arid climate. *Appl Soil Ecol* 37(1-2):53–62. https://doi.org/10.1016/j.apsoil.2007.03.010.

Bhalerao, V.P., Jadhav, M.B, and Bhoi, P.G. (2006) Effect of spent wash, press mud and compost on soil properties, yield and quality of seasonal sugarcane. *Indian Sugar* 6 (9): 57-65.

Black, C.A.(1965). *Methods of soil Analysis,* Part 1, physical properties. *American Soc. Agronomy.* Inc. Madison, Wisconsin, USA, 1-768.

Boddey, R.M., Polidoro, J.C, Resende, A.S., Alves, B.J.R, and Urquiaga, S. (2001). Use of the ^{15}N natural abundance technique for the quantification of the contribution of N_2 fixation to sugarcane and other grasses. *Aust. J. Plant Physiol*. 28: 889–895.

Bonanomi, G., D'Ascoli, R, Antignani, V., Capodilupo, M., Cozzolino, L., Marzaioli, R., Puopolo, G., Rutigliano, F.A., Scelza, R., Scotti, R., Rao, M.A., Zoina, A. (2011) Assessing soil quality under intensive cultivation and tree orchards in Southern Italy. *Appl Soil Ecol* 47(3):184-194.

Casida, L.E., Jr, Klein, D.A., Santoro, T. (1964). Soil dehydrogenase activity. *Soil Sci* 98: 371-376.

de Medeiros, E.V., Notaro, K.A., de Barros, J.A., Moraes, W.S., Silva, A.O, Moreira, K.A. (2015) Absolute and specific enzymatic activities of sandy entisol from tropical dry forest, monoculture and intercropping areas. *Soil Tillage Res* 145:208-215.

Eivazi, F., and Tabatabai, M.A. (1988). Glucosidases and galactosidases in soils. *Soil Biol Biochem* 20:601-606.

Jackson, M.L. (1973). *Soil* Chemical analysis, Ed. Prentices Hall of India Pvt. Ltd. New Delhi.

Kandeler, E., Kampichler, C., Horak, O. (1996). Influence of heavy metals on the functional diversity of soil microbial communities. *Biol Fertil Soils* 23:299-306.

Kong, C.H., Wang, P., Zhao, H., Xu, X.H., Zhu, Y.D. (2008). Impact of allelochemical exuded from allelopathic rice on soil microbial community. *Soil Biol Biochem* 40(7):1862-1869.

Kotroczo, Z., Veres, Z., Fekete, I., Krakomperger, Z., Toth, J.A., Lajtha, K., Tothmeresz, B. (2014). Soil enzyme activity in response to long-term organic matter manipulation. *Soil Biol Biochem* 70:237-243.

Kumar Ajeet and C. K. Jha (2021) Sugarcane: Crop of the Future*. Agriculture & Food: E-Newsletter*. 3 (6): 61-63. ⟨hal-04934993⟩.

Kumar Ajeet, Meena Sunita Kumari, Sinha S.K., Minnatullah, Singh A.K. and Singh Sanjay Kumar (2023b) Isolation and biochemical characterization of endophytic bacterium *Gluconacetobacter diazotrophocus* from native sugarcane cultivar of middle gangetic plains of India. DOI : 10.21203/rs.3.rs-3193451/v1

Kumar Ajeet, S.K. Sinha and A.K. Singh (2024b) Microbiome Management in Sugarcane Rhizosphere: Unraveling the Sweet Microbial Symphony. *New Era Agriculture Magazine..,* 2 (10): 34-37. March, 2024; https://hal.science/hal-04788422.

Kumar Ajeet, S.K. Sinha and A.K. Singh (2024c) Nurturing Growth: The Role of Microbiome in Phosphorus Solubilization for Sustainable Agriculture. *New Era Agriculture Magazine.* 2 (11): 91-93, April, 2024, https://hal.science/hal-04788436

Kumar Ajeet, S.K. Sinha and A.K. Singh (2024d) Harnessing the Power of Sulphur Solubilizing Bacteria for enhancing Agricultural Production Potential*. New Era Agriculture Magazine.* 2 (12): 31-34. https://hal.science/hal-04788449.

Kumar Ajeet, Sinha S.K., Singh Sanjay Kumar, Rana Lalita, Singh A.K., Kumari Sunita, Kumar Amrendra, Singh Harendra and Paswan Sudhir (2025) Influence of Intercropping and Planting Techniques on Sugarcane Yield and Nutrient Absorption in the North West Alluvial Plains of Bihar. *AATCC Review*, 13 (1): 144-153. https://hal.science/hal-04962582.

Kumar Ajeet, Sunita Kumari Meena, S.K. Sinha and A.K. Singh (2023a) Fostering Sugarcane Cultivation in the Indo-Gangetic Plains: Unveiling the Potential of Bio-Fertilizers and Sustainable Farming Practices. *Agriblossom*. 3 (11): 1-5. ⟨hal-04935427⟩.

Kumar, Ajeet (2016) Soil Health and Soil Quality: - The Fundamental Keys to a Sustainable and Productive Agriculture; *Pg. No. 48-54;* souvenir *of International Symposium on Management of Rice based agricultural system under stress prone environment;* organized by Rajendra Agricultural University, Bihar during 17-19, March, 2016. https://hal.science/hal-04972899.

Kumar, Ajeet., Chattopadhyay, S. and Meena, S.K. (2023) Soil Fertility Assessment of Sugarcane Growing Villages in Samastipur District of Bihar. *Environment and Ecology.* 41 (2): 759-764, April-June 2023. https://hal.science/hal-04676939.

Kumar, Ajeet., Choudhary A., and Kumar Mukul (2018) Impact of climate change and their mitigation for better sugarcane production- A Review; *International Journal of Agricultural Sciences.* 14 (2): 431-441. https://hal.science/hal-04676732.

Kumar, Ajeet., Choudhary, C.S., Paswan, D., Kumar, B. and Arun, A. (2014a) Sustainable Way for Enhancing Phosphorus Efficiency in Agricultural Soils through Phosphate Solubilizing Microbes – A *Review. An Asian Journal of Soil Science* (An International Refereed Research Journal). 9 (2): 300-310 https://hal.science/hal-04676707.

Kumar, Ajeet., Jha, Shankar and Maurya, B.R. (2013) In vitro Solubilization of Tricalcium Phosphate by Phosphate Solubilizing Fungi Isolated from Crop Rhizosphere, *RAU Journal of Research*. 23 (1&2): 14-20 (2013). https://hal.science/hal-04972636.

Kumar, Ajeet., Maurya, B.R. and Jha, Shankar (2014) *In Vitro* Solubilization of Rock Phosphate by Fungus Isolated From Agricultural Soil. *RAU Journal of Research.* 24 (1&2) 39-48. https://hal.science/hal-04972626

Kumar, Ajeet., Meena, S. K., Sinha, S.K., Singh, A.K., Minnatullah, and Singh, S. K. (2024) Isolation and biochemical characterization of endophytic bacterium *Gluconacetobacter diazotrophocus* from native sugarcane cultivar of middle gangetic plains of India. *Indian Journal of ecology*, 51(1): 104-112. DOI: https://doi.org/10.55362/IJE/2024/4202.

Kumar, Ajeet., Meena, S.K., Minnatullah, Sinha, S.K. and Singh A.K. (2023) Chapter No.:28: Sustainable Sugarcane production in the Era of Climate Change: Mitigation and Adaptation Efforts, Pg. 195-204. In Souvenir cum Edited Book: Innovations in Agriculture for Sustainable Food Systems and Farmers' Income – A Climate Resilient perspective. 31st annual conference of AERA during 7-9 December 2023. ⟨hal-04881517⟩.

Kumar, Ajeet., Meena, S.K., Sinha, S.K. and Singh, A.K. (2023c) Chapter No.:18: Strategies for Enhancing Sugarcane Yields by Harnessing the Power of Ripening and the Use of Ripeners in Sugarcane cultivation, Pg. 185-196. In Souvenir cum Edited Book: Sustainable sugarcane production and utilization: Issues and Initiatives. ISBN: 978-81-966957-2-9. Annual group meet-2023, All India coordinated Research Project on sugarcane, October 26-27, 2023. https://hal.science/hal-04881488.;

Kumar, Ajeet., Singh, S. K., Meena, S. K., Sinha, S. K., Rana, L., Singh, A. K., Kumar, A., Singh, H., & Paswan, S. (2025a). A Comprehensive Review on Impact of Intensive Use of Nitrogenous Fertilizer on Nitrate Contamination in Groundwater under Sugarcane Based Cropping System in Indo-Gangetic Plains of India. International Journal of Environment and Climate Change, 15(2), 286–300. DOI: https://doi.org/10.9734/ijecc/2025/v15i24727.

Kumar, Ajeet., Singh, S. K., Meena, S.K., Sinha, S.K. and Rana L (2024) Groundwater contamination with nitrate and human health risk assessment of North East alluvial plains of Bihar. *International Journal of Environment and Climate Change*, 14(3), 17–31. https://doi.org/10.9734/ijecc/2024/v14i34016.

Kumar, B., Sinha, S. K., Kumar, Ajeet., and Kumari, A. (2024a). Exploring the Impact of Organic-Inorganic Coupling on Nutrient Use Efficiency and Cane Yield in Calcareous Soils of the Indo-Gangetic Plains of India. *Journal of Advances in Biology & Biotechnology*, 27(6), 644-656. https://doi.org/10.9734/jabb/2024/v27i6924

Li, X., and Sarah, P. (2003). Enzyme activities along a climatic transect in the Judean Desert. *Catena* 53:349-363.

Lindsay, W.L., and Norvell, W.A. (1978). Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* 42, 421-428.

Maharajan, M., Sanaullah, M., Razavi, B.S., Kuzyakov, Y. (2017). Effect of land use and management practices on microbial biomass and enzyme activities in subtropical top- and sub-soils. *Appl Soil Ecol* 113:22-28.

Martin J P. (1950). Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69:215-32. Univ. California Citrus Experiment Station, Riverside, CA.

Meena S.K., Kumar Ajeet, Rana Lalita, Suman S.N. and Singh A.K. (2023) Chapter No.:25: Strategies for Effective fertilization and nutrient management in sugarcane: Integrated nutrient management, Pg. 225-234. In Souvenir cum Edited Book: Sustainable sugarcane production and utilization: Issues and Initiatives. ISBN: 978-81-966957-2-9. Annual group meet-2023, AICRP on sugarcane, October 26-27, 2023. https://hal.science/hal-04881496.

Moghimian, N., Hosseini, S.M., Kooch, Y., Darki, B.Z. (2017) Impacts of changes in land use/cover on soil microbial and enzyme activity. *Catena* 157:407-414.

Oliveira, A.L.M., Urquiaga, S., Dobereiner, J., Baldani, J.I. (2002) The effect of inoculating endophytic N_2-fixing bacteria on micropropagated sugarcane plants. *Plant Soil* 242:205-215.

Olsen, S.R, Coles, C.V., Watanabe, P.S., and Dean, L.N. (1954). Estimation of available Phosphorus in soil by Extraction with sodium bicarbonate, *USDA Circular*, 939.

Prasad, B., and Sinha, M.K. (1984) Structural characteristics of humic and fulvic acids isolated from soil and poultry litter. *Journal of Indian Society of Soil Science* 32: 165-167.

Raiesi, F., and Beheshti, A. (2015) Microbiological indicators of soil quality and degradation following conversion of native forests to continuous croplands. *Ecol Indic* 50:173-185.

Rana, Lalita, Navnit Kumar, Jitendra Rajput, Sumit Sow, Shivani Ranjan, Sarita Kumari, Jyostnarani Pradhan, Anil Kumar, S. N. Singh, Ajeet Kumar, CK. Jha, Meenu Kumari, Devendra Singh, and Ritwik Sahoo (2024). Unlocking Potential: The Role of Zinc Fortification Combating Hidden Hunger and Enhancing Nutritional Security. *Journal of Experimental Agriculture International*; 46 (10):625-42. https://doi.org/10.9734/jeai/2024/v46i102986.

Schneider, K., Turrion, M.B., and Gallardo, J.F. (2000). Modified method for measuring acid phosphatase activities in forest soils with high organic matter content. *Commun Soil Sci Plant Anal* 31(19-20):3077-3088.

Silva, E.O., de Medeiros, E.V., Duda, G.P., Junior, M.A.L., Brossard, M, de Oliveira, J.B., dos Santos, U.J., and Hammecker, C. (2019) Seasonal effect of land use type on soil absolute and specific enzyme activities in a Brazilian semi-arid region. *Catena* 172:397-407.

Singh S.K., Kumar Ajeet, Singh A.K. (2020) Technical Bulletin on Use of chemical and organic fertilizers, Pg. 1-32. Vide Technical Bulletin No. SS-1/2020. Tirhut College of Agriculture, Dholi, RPCAU, Pusa (Samastipur)-Bihar, India, pp. 1-32. https://hal.science/hal-04960937.

Sinha, S.K., Ajeet Kumar, Brajesh Kumar, Amrita Kumari and A.K. Singh (2024a) Chapter 1 Title: Influence of Organic-Inorganic Interaction on Nutrient Use Efficiency and Sugarcane Yield in Calcareous Soils of the Indo-Gangetic Plains, India; In Book: Current Research Progress in Agricultural Sciences. Volume.3. Page 1-22. https://doi.org/10.9734/bpi/crpas/v3/1426.

Sinha, S.K., Kumar, Ajeet., Kumari, A and Singh. A.K. (2024) The Integrated Effect of Organic Manure, Biofertilizer and Inorganic Fertilizer on Soil Properties, Yield and Quality in Sugarcane Plant-ratoon System under Calcareous Soil of Indo Gangetic Plains of India. Journal of Scientific Research and Reports (JSRR). 30(5): 193-206. https://doi.org/10.9734/jsrr/2024/v30i51934

Spencer, E.F., and Meade, G.P. (1964) Cane sugar hand book, 7th Ed. John Willey and sons, Inc., New York.

Subbiah, B.V., and Ashija, G.L. (1956) A rapid procedure for the estimation of available nitrogen in soils. *Current Science* 25: 259-266.

Thalmann, A. (1968) On the methodology for determining dehydrogenase activity in soil using Triphenyl tetrazolium chloride (TTC). *Landwirtsch Forsch* 21: 249-58.

Thornton, H.G. (1922) On the Development of a Standardized Agar Media for Counting Soil Bacteria with a Special Regards to the Repression of Spreading Colonies. Annals of Applied Biology, 9, 241-274.

http://dx.doi.org/10.1111/j.1744-7348.1922.tb05958.x.

Walkley, A., and Black, C.A. (1934) An examination of the digestion method for determining soil organic matter and proposed modifications of the chromic acid titration method. *Soil Science* 37: 29-38.

Yao, X.h., Min, H., Lu, Z.h., Yuan, H.P. (2006) Influence of acetamiprid on soil enzymatic activities and respiration. *Eur J Soil Biol* 42(2):120-6.

Peer-review history:
The peer review history for this paper can be accessed here:
https://pr.sdiarticle5.com/review-history/133081