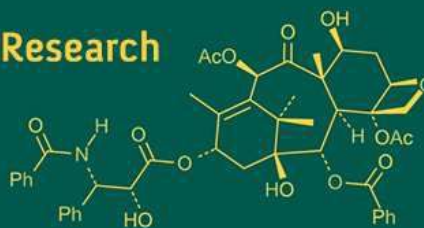


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Evaluation of nitrogenous bio-inoculants through nitrogen substitution for improving productivity and juice quality of sugarcane in calcareous soil of indo Gangetic plains of India

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Abstract

The experiment entitled "Evaluation of Nitrogenous Bio-inoculants for improving productivity and juice quality of sugarcane in Calcareous soil through Nitrogen Substitution by application of *Azotobacter* and *Acetobacter*" was conducted at Kalyanpur Farm, RPCAU, Pusa. The experiment was initiated in February 2024, the research aimed to assess the effects of nitrogen substitution using bio-inoculants (*Azotobacter* and *Acetobacter*) on sugarcane growth, nutrient uptake efficiency, and juice quality, with the ultimate goal of providing sustainable nitrogen management recommendations for sugarcane farmers. The study involved seven treatments with three replications, and the sugarcane genotype used was CoP-21438. The treatments varied in nitrogen application levels, including full and partial substitution of chemical nitrogen with bio-inoculants. Observations were recorded for plant growth parameters, yield attributes and juice quality at different stages of crop development. **Plant Growth:** The application of bio-inoculants showed a positive impact on plant growth. Treatment T₇, which included 75% nitrogen substitution along-with both *Azotobacter* and *Acetobacter*, resulted in the highest number of tillers (187.97 x 10³/ha) and plant height (287.53 cm), outperforming the control treatment T₁ (120.71 x 10³/ha tillers and 195.25 cm plant height). **Yield Attributes:** Cane girth, number of millable canes (NMC), and cane yield were also enhanced with bio-inoculant application. The highest cane girth (7.93 cm), NMC (130.17 x 10³/ha), and single cane weight (0.99 kg) were recorded in treatment T₇, resulting in a significantly higher cane yield of 128.87 t/ha compared to 77.13 t/ha in the control. **Juice Quality:** The use of bio-inoculants improved the quality of cane juice. The highest Brix (26.91%) and Pol (23.84%) were recorded in T₇, compared to the control treatment T₁ (Brix: 21.5%, Pol: 19.59%). However, there were no significant differences in juice purity among the treatments. In conclusion, the results of this study demonstrate the potential of nitrogenous bio-inoculants in enhancing both the productivity and quality of sugarcane in calcareous soils. The combination of *Azotobacter* and *Acetobacter*, when applied with reduced nitrogen levels, improved plant growth, yield, and juice quality, indicating that partial substitution of chemical nitrogen with bio-inoculants could be a viable strategy for sustainable sugarcane cultivation. These findings offer valuable insights for farmers aiming to adopt environmentally friendly practices while maintaining high sugarcane productivity.

Keywords: Sugarcane, bio-inoculants, *Azotobacter*, *Acetobacter*, juice quality

Introduction

Sugarcane (*Saccharum officinarum* L.) is one of the most important crops globally, playing a critical role in the production of sugar and biofuels. As a key source of raw materials for various industries, including food and bioenergy, sugarcane cultivation has expanded significantly over recent decades (Kumar *et al.* 2024a) [12]. In India, the second-largest producer of sugarcane in the world, the crop occupies about 4.6 million hectares of land, contributing nearly 370 million tonnes of sugarcane annually, the increasing demands on production systems, along with environmental concerns, have prompted researchers and farmers to explore sustainable agricultural practices, particularly in nutrient management (Singh *et al.*, 2024) [20]. The use of nitrogenous fertilizers has been a cornerstone of conventional agriculture, yet their overuse poses several risks to soil health and the environment (Meena *et al.*, 2024; Kumar *et al.*, 2025b) [20, 21, 16].

Nitrogen is a vital macronutrient for plant growth and is required in large quantities, especially for sugarcane, which is a high-demand crop (Singh *et al.*, 2025) ^[26]. However, nitrogen management in sugarcane cultivation is challenging due to the crop's prolonged growing period and substantial nutrient requirements (Sinha *et al.*, 2024; Shraddha, *et al.*, 2026) ^[27, 31]. Traditionally, fertilizers, such as urea or ammonium nitrate, are applied to ensure optimal yields. Unfortunately, a significant portion of nitrogen applied to soils is lost through processes like volatilization, leaching (Kumar *et al.*, 2024b) ^[17], and denitrification. These losses not only efficiency of nitrogen use but also contribute to environmental issues such as groundwater contamination, eutrophication and the release of greenhouse gases. Moreover, in calcareous soils which are prevalent in many sugarcane-growing regions, nitrogen availability is often compromised due to high pH levels (Kumar, 2015; Singh *et al.*, 2022; Singh *et al.*, 2023) ^[9, 23, 22]. The alkaline nature of calcareous soils can inhibit nutrient uptake by plants, further reducing the effectiveness of nitrogen fertilizers (Kumar *et al.*, 2023a) ^[10, 24]. Thus, the development of strategic nitrogen use efficiency (NUE) in these soils is crucial for improving sugarcane productivity while minimizing environmental impacts through carbon sequestration (Kumar *et al.* 2024a) ^[12].

Bio-inoculants as a Sustainable Source in the recent years, there has been growing interest in the use of bio-inoculants-microorganisms that promote plant growth and improve soil fertility-as an alternative to chemical fertilizers (Kumar and Paswan., 2015) ^[9]. Bio-inoculants such as nitrogen-fixing bacteria can supplement for nitrogen management. These microorganisms, including *Azotobacter* and *Acetobacter*, have been shown to enhance plant growth by fixing atmospheric nitrogen and making it available to plants in a more sustainable manner (Kumar *et al.*, 2024d) ^[13]. In sugarcane, bio-inoculants have been reported to improve uptake, increase crop yield and enhance juice quality (Kumar *et al.*, 2026a) ^[30]. *Azotobacter* and *Acetobacter*: Key Nitrogen-Fixing Bio-inoculants *Azotobacter* and *Acetobacter* are endophytic free-living nitrogen-fixing bacteria that play an important role in sustainable agriculture by contributing to the nitrogen cycle in soil. *Azotobacter*, a well-studied bio-inoculant, is known for its ability to fix atmospheric nitrogen into a form that is available for plant uptake, promoting growth even in soils with low nitrogen content. Additionally, *Azotobacter* produces growth-promoting substances, which further enhance crop development. Similarly, *Acetobacter*, particularly *Gluconacetobacter diazotrophicus*, is another bacterium that has been widely studied for its symbiotic relationship with sugarcane (Kumar and Paswan., 2014) ^[8]. *Acetobacter* is capable of fixing atmospheric nitrogen and contributing significantly to the nitrogen the plant without requiring the energy inputs associated with chemical fertilizers. Research indicates that *Acetobacter* can thrive in the root zones of sugarcane plants, where it improved growth and yield. Furthermore, both *Azotobacter* and *Acetobacter* have been found to enhance the uptake of other essential nutrients, such as and potassium, thereby improving overall plant health.

Nitrogen Substitution for Sustainable Production

The substitution of chemical nitrogen fertilizers with nitrogenous bio-inoculants aligns with the principles of

sustainable agriculture, which emphasize reducing reliance on synthetic inputs while maintaining or enhancing crop productivity (Kumar., 2024) ^[1, 2]. By using bio-inoculants as partial replacements for chemical fertilizers, farmers can reduce the environmental footprint of agriculture, where nitrogen losses are prevalent. The integration of bio-inoculants into nutrient management strategies has been shown to enhance nitrogen use efficiency, promote soil health, and mitigate environmental impacts associated with excessive fertilizer use (Meena *et al.*, 2023) ^[21]. In calcareous soils, where nutrient availability is often limited by high pH levels, the role of bio-inoculants becomes even more critical. Studies have demonstrated that bio-inoculants can improve nutrient availability and uptake in such soils by altering the soil microbiome and enhancing soil structure. This, in turn, leads to better crop growth, higher yields, and improved quality of produce, such as sugarcane juice.

Bio-inoculants in Improving Sugarcane Juice quality

In addition to their role in enhancing sugarcane growth and yield, bio-inoculants have been shown to positive the quality of sugarcane juice. Juice quality parameters such as Brix (a measure of sugar content), Pol (polarization or the amount of sucrose in the juice), and juice purity are critical for determining the commercial value of sugarcane. Research suggests that the application of nitrogenous bio-inoculants can lead to improvements in these parameters, likely due to the enhanced nutrient uptake and better overall plant health by bio-inoculants. In the present study, the application of *Azotobacter* and *Acetobacter* is expected to result in significant improvements in sugarcane juice quality. Preliminary data indicate that treatments in corporation of bio-inoculants yield higher Brix and Pol values compared to conventional nitrogen fertilizer applications (Sinha *et al.*, 2024) ^[27]. This enhancement in juice quality not only benefits farmers by increasing the commercial value of their produce but also aligns with consumer preferences for high-quality sugarcane juice products.

Significance of the Study

This research is particularly significant in the context of global efforts to promote sustainable agriculture and reduce the environmental impacts of intensive farming practices (Kumar *et al.*, 2014) ^[15]. The use of bio-inoculants as a substitute for chemical nitrogen fertilizers represents a promising approach to improving crop productivity while minimizing the negative effects of excessive fertilizer use. By focusing on sugarcane, a crop of major economic importance, this study has the potential to influence agricultural practices on a broad scale, particularly in regions where calcareous soils pose challenges to nutrient management (Kumar *et al.*, 2018) ^[11]. The findings from this research will provide valuable insights into the practical applications of bio-inoculants in sugarcane cultivation, offering farmers an environmentally friendly and economically viable alternative to chemical nitrogen fertilizers.

Materials and Methods

1. Experimental Site/Latitude/Longitude/Climate

The field experiment was conducted at Kalyanpur Farm, RPCAU, Pusa, the farm is situated at 52.0 m above mean sea level and 25°95' N latitude, 85°77' E longitude. The experimental site had a subtropical climate with distinct wet

and dry seasons. The experimental plot was well drained, medium upland and had uniform topography. The experimental site belongs to a region characterized by calcareous soils, which are high in calcium carbonate and present challenges for nutrient uptake due to their alkaline nature. The site was chosen for its typical representation of soil conditions found in major sugarcane-growing regions of Bihar, which require improved nutrient management strategies.

2. Plant Material

The sugarcane genotype used for the experiment was CoP-21438, which is well-adapted to local growing conditions and commonly cultivated in the region for its desirable agronomic traits, such as good juice quality and high yield potential.

3. Experimental Design

The experiment followed a Randomized Block Design (RBD) with seven treatments and three replications. The plot size for each treatment was 6 x 5.4 meters, with six rows of sugarcane per plot. Adequate spacing was maintained between plots to avoid interference between treatments. Treatments: The treatments were designed to test different combinations of nitrogen substitution using bio-inoculants (*Azotobacter* and *Acetobacter*) in addition to the recommended dose of fertilizers (RDF). The following seven treatments were applied: T₁: RDF 100% (150:85:60::N: P₂O₅:K₂O)-Control treatment with no bio-inoculants; T₂: RDF 100% + *Azotobacter* @ 5 kg/ha.; T₃: RDF 100% + *Acetobacter* @ 5 kg/ha; T₄: RDF 100% + *Azotobacter* @ 5 kg/ha + *Acetobacter* @ 5 kg/ha; T₅: RDF 75% (Nitrogen) + 100% (P₂O₅ and K₂O) + *Azotobacter* @ 5 kg/ha; T₆: RDF 75% (Nitrogen) + 100% (P₂O₅ and K₂O) + *Acetobacter* @ 5 kg/ha; T₇: RDF 75% (Nitrogen) + 100% (P₂O₅ and K₂O) + *Azotobacter* @ 5 kg/ha + *Acetobacter* @ 5 kg/ha.

4. Soil Preparation and Crop Establishment

The experimental field was ploughed and prepared according to standard agronomic practices. Soil testing was conducted before the experiment to determine the baseline nutrient status, particularly nitrogen, phosphorus, potassium and pH levels. Based on the soil test results, the required quantities of chemical fertilizers were calculated and applied as per treatment specifications. Planting: Sugarcane setts were planted manually at a row spacing of 90 cm with a depth of about 8-10 cm. The setts were treated with appropriate fungicides to prevent any initial infections.

5. Bio-inoculant Application

The bio-inoculants *Azotobacter* and *Acetobacter* were applied in their respective treatments at a rate of 5 kg/ha. The inoculants were mixed with organic carriers and applied to the soil near the root zones of the plants during planting. The inoculants were used from a bio-fertilizer production unit, of sugarcane research institute, Pusa and their quality

was tested before use to ensure viability and effectiveness. The bio-inoculants were applied both as a seed treatment and through soil application. In the seed treatment method, sugarcane setts were soaked in slurry of bio-inoculants before planting.

6. Fertilizer Application

Chemical fertilizers were applied in the form of urea, single super phosphate (SSP) for phosphorus and muriate of potash (MOP) for potassium. These were applied in split doses, with nitrogen applied at planting and in two top dressings at 45 and 90 days after planting. Phosphorus and potassium were applied as basal doses at the time of planting.

7. Irrigation and Crop Management

The crop was irrigated through a surface irrigation system at intervals depending on weather conditions. Weeds were controlled through manual weeding. Pest and disease management practices were followed using integrated pest management (IPM) strategies, ensuring minimal pesticide use to avoid interference with microbial bio-inoculants.

8. Observations and Data Collection

Observations were made at critical growth stages and data were collected on plant growth parameters, yield attributes and juice quality as follows: A. Plant Growth Parameters, Number of tillers per hectare at 120 DAP, Plant height (cm) at 180 DAP, Cane girth (cm) at the maturity stage. B. Yield Attributes and Cane Yield, Number of millable canes (NMC) at maturity, Single cane weight (kg) at harvest, Total cane yield (t/ha) at harvest. C. Juice Quality Parameters, like Brix (%): A measure of total soluble solids, indicating sugar content; Pol (%): A measure of sucrose content in the juice; Juice purity (%): Calculated as the ratio of Pol to Brix.

9. Statistical Analysis

The collected data were analyzed statistically using analysis of variance (ANOVA) to determine the significance of treatment effects on plant growth, yield attributes, and juice quality. The least significant difference test was applied at a 5% probability level to compare treatment means. The standard error of the mean (SEM) was calculated to assess the variability within treatments. All data were presented in tabular form to provide clarity on the effects of bio-inoculants on sugarcane productivity and juice quality.

10. Soil and Environmental Monitoring

Soil samples were taken from each plot before planting and after harvest to assess changes in nutrient levels and microbial activity. Key soil parameters measured included pH, organic carbon content, nitrogen, phosphorus, potassium, and microbial populations (*Azotobacter* and *Acetobacter* counts). These measurements were important for understanding the influence of bio-inoculants on soil health and nutrient dynamics.

Results and Discussions

Table 1: Plant growth parameters, Yield attributes and cane yield of sugarcane crop under the influence of application of Nitrogenous Bio-inoculants in sugarcane cultivation in Calcareous soil.

Treatments	Tillers (x 10 ³ /ha)	Plant Height (cm)	cane girth (cm)	NMC (x 10 ³ ha ⁻¹)	Single cane weight (kg)	Cane Yield (t ha ⁻¹)
T ₁	120.71	195.25	6.54	98.89	0.78	77.13
T ₂	161.97	258.76	6.86	118.32	0.81	95.84
T ₃	164.12	265.93	7.60	121.12	0.89	107.80
T ₄	165.32	269.45	7.73	134.51	0.94	126.44
T ₅	162.21	265.18	7.81	131.14	0.94	123.27
T ₆	171.38	273.45	7.89	129.98	0.95	123.48
T ₇	187.97	287.53	7.93	130.17	0.99	128.87
CD (0.05)	24.35	31.63	1.08	17.54	0.12	32.84

Table 2: Cane juice quality and sugar yield of sugarcane crop under the influence of application of Nitrogenous Bio-inoculants in sugarcane cultivation in Calcareous soil.

Treatments	Juice Quality (%)		
	Brix	Pol	Purity
T ₁	21.5	19.59	89.76
T ₂	22.4	21.23	89.37
T ₃	22.5	21.58	88.74
T ₄	26.98	24.82	89.44
T ₅	25.88	21.53	89.9
T ₆	25.89	21.61	89.22
T ₇	26.91	23.84	89.78
CD (0.05)	NS	NS	NS

Results and Discussions:

Plant growth, Yield attributes and cane yield parameters

Tillers (x 10³/ha): The number of tillers per hectare was significantly higher in treatments involving bio-inoculants compared to the control. The maximum tiller count (187.97 x 10³/ha) was recorded in treatment T₇ (75% RDF + *Azotobacter* + *Acetobacter*), which was significantly higher than the control (120.71 x 10³/ha). **Plant Height (cm):** Similarly, plant height was positively influenced by the application of bio-inoculants. Treatment T₇ (75% RDF + *Azotobacter* + *Acetobacter*) had the tallest plants at 287.53 cm, compared to 195.25 cm in the control. **Cane Girth (cm):** The application of bio-inoculants also led to an increase in cane girth, with treatment T₇ showing the highest girth (7.93 cm), while the control treatment had the lowest (6.54 cm). **NMC (Number of Millable Canes x 10³/ha):** There was a substantial increase in the number of millable canes in bio-inoculated treatments. The maximum NMC was recorded in treatment T₄ (134.51 x 10³/ha) and T₇ (130.17 x 10³/ha), both significantly higher than the control (98.89 x 10³/ha). **Single Cane Weight (kg):** The weight of individual canes also increased with bio-inoculant application, with the highest weight (0.99 kg) observed in T₇. **Cane Yield (t/ha):** The highest cane yield (128.87 t/ha) was recorded in T₇, a substantial increase over the control (77.13 t/ha). All bio-inoculant treatments significantly outperformed the control in terms of yield. Similar kind of results has been reported by Sinha *et al.*, 2024; Kumar and Jha., 2021; Kumar *et al.*, 2024 [27, 3, 12].

The results clearly demonstrate that the application of nitrogenous bio-inoculants, either singly or in combination, significantly enhanced sugarcane growth, yield attributes, and cane yield. The treatments involving both *Azotobacter* and *Acetobacter* (T₇) consistently performed better across all parameters, showing the synergistic effect of these bio-inoculants. The increase in tiller count, plant height, and

cane girth can be attributed to the improved nitrogen availability and enhanced nutrient uptake facilitated by bio-inoculants, particularly in calcareous soils (Kumar *et al.*, 2022) [4, 5] where nutrient availability is often limited due to high pH. The ability of *Azotobacter* and *Acetobacter* to fix atmospheric nitrogen and promote root development likely contributed to better crop growth (Kumar and Maurya, 2006) [34]. Furthermore, the increase in single cane weight and overall cane yield highlights the potential of bio-inoculants to partially substitute chemical nitrogen fertilizers without compromising productivity. This is especially relevant for sustainable agriculture, as reducing chemical fertilizer inputs can mitigate environmental risks while maintaining high yields (Singh *et al.*, 2026) [28, 29].

Juice Quality and Sugar Yield

Brix (%): Brix values, which indicate the total soluble solids in the juice, were significantly higher in treatments involving bio-inoculants. Treatment T₄ (100% RDF + *Azotobacter* + *Acetobacter*) had the highest Brix (26.98%), followed closely by T₇ (75% RDF + *Azotobacter* + *Acetobacter*) with 26.91%, compared to the control (21.5%).

Pol (%): Pol, a measure of sucrose content, also increased significantly in bio-inoculated treatments. The highest Pol (24.82%) was recorded in T₄, with T₇ showing similar results (23.84%). The control had a significantly lower Pol value (19.59%).

Purity (%): Purity, the ratio of Pol to Brix, was relatively consistent across treatments, with no significant differences observed between the control and bio-inoculated treatments. All treatments showed purity levels around 89%. The work is in conformity with the work of Kumar *et al.*, 2023 [10, 24], Kumar *et al.*, 2024 [2]; Kumar *et al.*, 2025a [16]. Kumar and Singh (2022) [23]; Singh *et al.*, (2026) [28, 29], highlighted the impact of climate change on soil microorganisms, emphasizing its role in maintaining soil health and plant-microbe interactions (Kumar *et al.*, 2026) [30]. This perspective aligns with our study, as soil microbial activity significantly influences nutrient availability, which in turn affects juice quality and sugar yield. The observed variations in sugar concentration and juice purity could be attributed to changes in soil microbial dynamics, as suggested by previous research

The application of nitrogenous bio-inoculants significantly improved cane juice quality, particularly in terms of Brix and Pol values. The higher Brix percentages observed in treatments T₄ and T₇ suggest that bio-inoculants not only enhance crop yield but also improve the concentration of

sugars in the juice, which is a critical factor for sugarcane producers. The increase in Pol values further supports the positive impact of bio-inoculants on juice quality. The improved sucrose content in bio-inoculated treatments can be attributed to better nutrient uptake and the enhanced physiological functioning of the plants due to the nitrogen-fixing capabilities of *Azotobacter* and *Acetobacter*. While the purity percentage did not show significant differences between treatments, the overall improvement in Brix and Pol values highlights the potential of bio-inoculants to enhance the commercial quality of sugarcane juice. These findings suggest that integrating bio-inoculants into nutrient management practices could benefit both sugarcane farmers and sugar processors by increasing the overall sugar yield per hectare.

Conclusion

The present study on the "Evaluation of Nitrogenous Bio-inoculants for Improving Productivity and Juice Quality of Sugarcane in Calcareous Soil through Nitrogen Substitution by Application of *Azotobacter* and *Acetobacter*" has yielded promising results that underline the potential of bio-inoculants in sustainable sugarcane production. The results from both tables clearly indicate that nitrogenous bio-inoculants, especially when applied in combination, significantly enhance both sugarcane productivity and juice quality. These findings are particularly relevant for sustainable agriculture, as they demonstrate that bio-inoculants can partially replace chemical nitrogen fertilizers without compromising yield or quality, thereby reducing the environmental footprint of sugarcane cultivation. The findings demonstrate that the integration of *Azotobacter* and *Acetobacter* into nutrient management strategies significantly enhances sugarcane growth, yield, and juice quality when compared to conventional nitrogen fertilization alone. The treatments involving a combination of both bio-inoculants, particularly at 25% nitrogen substitution (T₇), produced the highest number of tillers, plant height, cane girth, and cane yield, outperforming the control treatment with 100% RDF (T₁). Furthermore, the application of bio-inoculants resulted in a notable improvement in cane juice quality, with significant increases in Brix and Pol values, which are critical indicators of sugar content and commercial quality. These results suggest that bio-inoculants not only facilitate efficient nitrogen fixation but also promote overall plant health and nutrient uptake, even in challenging calcareous soils where nutrient availability is often restricted. The ability of *Azotobacter* and *Acetobacter* to enhance plant growth and productivity while reducing the reliance on chemical nitrogen fertilizers provides a sustainable and eco-friendly alternative for sugarcane cultivation. Thus it is finally concluded that, the partial substitution of chemical nitrogen fertilizers with *Azotobacter* and *Acetobacter* offers a viable pathway toward achieving sustainable intensification in sugarcane cultivation, especially in nutrient-limited calcareous soils, thus contributing to more sustainable agricultural systems.

Recommendations based on the results

The adoption of bio-inoculants as part of integrated nutrient management practices offers several advantages: *Improved Productivity*: Enhanced plant growth parameters and higher cane yield can contribute to increased profitability for sugarcane farmers. *Enhanced Juice Quality*: Improved Brix

and Pol values indicate better sugar recovery, benefiting sugar mills and the overall supply chain. *Environmental Sustainability*: The reduction in chemical nitrogen fertilizer usage helps mitigate environmental risks such as soil degradation, water contamination and greenhouse gas emissions. Overall, this study provides evidence that nitrogenous bio-inoculants can play a key role in promoting sustainable sugarcane production while maintaining or even improving yield and quality. Future research should focus on long-term field trials and the economic feasibility of large-scale bio-inoculant application to validate these findings further and promote widespread adoption among sugarcane farmers.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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