



Efficacy of combined beneficial micro-organisms in the vegetative growth of sugarcane (*Saccharum officinarum*) variety phil 2006-2289 with reduced recommended nitrogen

Angelie Kate Baliuag-Caluducan*, Nonito Pattugalan

Cagayan State University, Piat Campus, Baung, Piat, Cagayan, Philippines

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Abstract

This study investigates the use of beneficial microorganisms, specifically *Acetobacter* and *Azospirillum*, as a sustainable alternative to synthetic nitrogen fertilizers in sugarcane (*Saccharum officinarum*) production in Piat, Cagayan. Due to the environmental and economic challenges associated with excessive nitrogen fertilizer use, this research aimed to assess the impact of these microorganisms on sugarcane growth while reducing reliance on synthetic nitrogen. The study was conducted using a randomized complete block design (RCBD) with four treatments, including a control and three levels of nitrogen application (100%, 50%, and 25%) combined with beneficial microorganisms. Key growth parameters, including plant height, number of millable tillers, stalk diameter, and leaf color intensity, were measured. Results showed that the application of beneficial microorganisms significantly enhanced sugarcane growth, with reduced nitrogen inputs (up to 50%) still yielding comparable or even superior results in plant height and number of millable tillers compared to the control. These findings suggest that *Acetobacter* and *Azospirillum* can effectively improve sugarcane productivity while reducing the environmental impacts of excessive nitrogen fertilizer use. The study recommends the adoption of microbial inoculants as part of sustainable fertilizer management practices in sugarcane farming.

*Corresponding Author: Angelie Kate Baliuag-Caluducan ✉ katecaluducan.sra@gmail.com

Introduction

Sugarcane (*Saccharum officinarum*) is a major crop valued for its sugar content and potential for bioenergy. To achieve optimal yields, it requires substantial nitrogen input, commonly supplied through synthetic fertilizers. However, excessive fertilizer use has led to serious environmental concerns, including water pollution, greenhouse gas emissions, and soil degradation (Foley *et al.*, 2011; Tilman *et al.*, 2002). These issues highlight the urgent need for sustainable alternatives (Lal, 2012).

In Piat, Cagayan where the URC-SURE CARSUMCO mill district is situated, sugarcane productivity has declined due to high input costs and management challenges, as reported by the Sugarcane Regulatory Administration (SRA). Given the crop's heavy nutrient demands, reliance on chemical fertilizers has become both economically and environmentally unsustainable. One promising solution involves the use of beneficial microorganisms (BMOs), such as *Azospirillum*, *Rhizobium*, and *Acetobacter*, which fix atmospheric nitrogen and enhance plant nutrient uptake (Giller and Cadisch, 1995; Glick, 2012). These microbes improve soil fertility, support plant growth, and reduce the need for synthetic inputs (Ladha *et al.*, 2016; Calvo *et al.*, 2014). They also suppress pathogens, decompose organic matter, and detoxify soils (Compant *et al.*, 2009; Du Jardin, 2015; Raaijmakers *et al.*, 2008).

This study evaluated the effectiveness of BMOs on sugarcane production in Piat, Cagayan, where adaptation to local soil and climate conditions was a key consideration. While such microbial technologies have shown success in the Visayas region, their performance in Northern Luzon remains underexplored. The research aimed to support local farmers by promoting cost-effective, sustainable practices that boost yield and reduce environmental impact. Specifically, the study aimed to assess the impact of beneficial microorganism on the growth of sugarcane under Piat Cagayan condition in terms of plant height, number of millable tillers, stalk diameter and leaf color and to evaluate the

potential BMO to reduce the dependence on synthetic nitrogen fertilizers.

Materials and methods

Materials

The experiment utilized the following materials: sugarcane seed pieces (Phil 2006-2289) variety as planting material, inorganic fertilizers, and beneficial microorganisms (such as *Acetobacter* and *Azospirillum*), culture media, molasses, 20-liter container and sprayer. Other materials used include: sensitive weighing balance, meter stick, ruler, record book, pen, placards and string.

Soil sampling

Soil samples were collected from the experimental area in a random zigzag pattern. Approximately one kilogram of soil was gathered from each sampling site. The collected soil samples were mixed together and air dried for three days. One kilogram of the composite soil samples was submitted to DA-CVIAL for nutrient analysis. Result of the soil analysis served as basis for the rate of fertilizers applied per treatment. Additionally, bacterial plate count was also made to determine the population of microorganism before and after the conduct of the study.

Experimental design and procedures

The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replications. The treatments applied were as follows:

Treatment 1- Control (100% RR NPK)

Treatment 2- *Acetobacter* and *Azospirillum* + 100 % NPK based on soil analysis

Treatment 3- *Acetobacter* and *Azospirillum* + 25% nitrogen and 100%PK based on soil analysis

Treatment 4- *Acetobacter* and *Azospirillum* + 50 % nitrogen and 100%PK based on soil analysis based on soil analysis

Each plot measures 9 meters × 6 meters with a two-meter-wide alleyway maintained between treatments and replications.

Land preparation

A total land area of 777 square meters was prepared four weeks prior to the start of the study. Land preparation involved two rounds of plowing followed by two rounds of harrowing. The purpose of these operations was twofold: first, to promote the germination of weed seeds, and second, to incorporate dried weeds and crop stubbles into the soil, facilitating and hastening their decomposition. By allowing weed seeds to germinate and incorporating organic material into the soil, this preparation process aims to create a favorable environment for the sugarcane crop to be studied.

Preparation of planting materials

The cane points used in the experiment were approximately six (6) months old and selected from healthy sugarcane plants exhibiting vigorous vegetative growth, complete tillering, and free from pests and diseases. The stalks were cut into seed pieces, each containing three healthy buds. To prevent disease and insect infestation, the seed pieces were treated by submerging them in lukewarm water for two (2) hours prior to planting.

Furrowing

Furrow was made prior to planting at a distance of 1.3 meter apart using tractor. Furrowing was done a day before planting.

Planting

The sugarcane seed pieces were planted along the furrows, spaced 50 cm apart. The planting materials were then covered with a sufficient layer of damp soil to promote root development.

Watering

The newly planted canes were irrigated with an equal amount of water either early in the morning or late in the afternoon to promote optimal germination. Additional watering was performed as needed.

Reproduction of beneficial microorganism mixture

Mixed *Acetobacter* and *Azospirillum* was requested from Sugar Regulatory Administration-Luzon

Agricultural Research and Extension Center (SRA-LAREC) Laboratory and was multiplied under controlled conditions and prepared as a mixture. In one (1) gallon container with 3.5 liters of distilled water, dilute 60 grams molasses and 100mL nitrogen fixers (mixture of *Acetobacter* and *Azospirillum*) volume to one gallon mixed. The rubber band was covered and was placed in shaker. The container will need regular shaking for two to three days. Mix the three components in one gallon container to make up the BMO.

Application of beneficial microorganism

The inoculum containing *Acetobacter* and *Azospirillum* was applied directly into the furrows at thirty days after planting (30 DAP). A total of 20 liters of the bio-microbial organism (BMO) mixture was diluted in 100 liters of water and evenly applied to the furrow area. A second application of the same inoculum was conducted as a soil drench around the root zone at sixty days after planting (60 DAP), using 100 mL per plant hill, to promote early colonization and enhance microbial activity in the rhizosphere. Both applications were carried out early in the morning, ensuring that the soil had adequate ambient moisture to support microbial survival and activity.

Fertilizer management

The amount and time of application for the different inorganic fertilizer was based from the result of the soil analysis. For Treatments 3 and 4, the recommended nitrogen rate was reduced by 25% and 50% respectively, to evaluate the effect of lower nitrogen inputs when combined with beneficial microorganisms (BMO) inoculants.

Statistical analysis

All data were arranged and collated using Microsoft excel. Analysis of Variance (ANOVA) at 5% and 1% level of significance was used to determine the significance if there are significant differences on the different sources of variations. Least Significant Difference (LSD) was used as post hoc analysis to determine pairwise mean comparison on differences on the different treatments.

Results and discussion

Plant height

Fig. 1 illustrates the average plant height of sugarcane as influenced by varying levels of inorganic nitrogen and the application of combined beneficial microorganisms (BMOs). The tallest plants were recorded in Treatment 2, which received 100% of the recommended inorganic nitrogen rate supplemented with BMOs, with a mean height of 127.80 cm. This was closely followed by Treatment 3 (75% nitrogen + BMOs) and Treatment 4 (50% nitrogen + BMOs), with mean plant heights of 127.00 cm and 124.67 cm, respectively. In contrast, the shortest plants were observed in the control treatment (Treatment 1), which received neither inorganic nitrogen nor BMOs, with an average height of 109.93 cm.

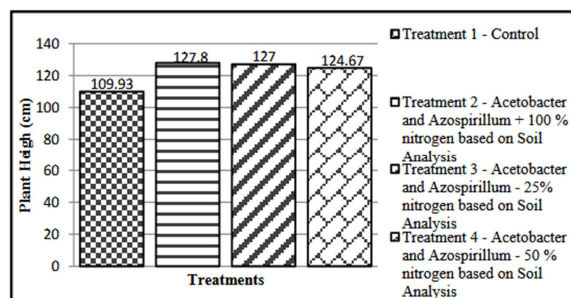


Fig. 1. Average plant height (cm) of sugarcane as affected by reduced levels of nitrogen application combined with a mixture of beneficial microorganisms at Piat, Cagayan, March 2025

Statistical analysis revealed no significant differences in plant height among Treatments 2, 3, and 4, indicating that reducing nitrogen by up to 50% did not significantly affect plant growth when combined with BMOs. However, all three treatments showed significantly greater plant height compared to the control. These results suggest that the application of beneficial microorganisms can effectively enhance sugarcane growth, even when the recommended nitrogen fertilizer rate is reduced.

These findings are supported by several studies on plant growth-promoting microorganisms (PGPMs). According to Bhardwaj *et al.* (2014), PGPMs enhance plant development by improving nutrient availability, producing phytohormones such as auxins and

cytokinins, and facilitating nitrogen fixation. Glick (2012) also emphasized that beneficial microbes like *Azospirillum* and *Rhizobium* can stimulate shoot elongation through hormone modulation and improved nutrient acquisition. Similarly, Calvo *et al.* (2014) and Ladha *et al.* (2016) noted that microbial inoculants improve root health and soil structure, enabling more efficient water and nutrient uptake, thereby supporting above-ground biomass, including plant height.

Number of millable tiller per hill

The average number of millable tillers per sugarcane hill was significantly influenced by the application of reduced nitrogen levels, supplemented with combined beneficial microorganisms (Fig. 2).

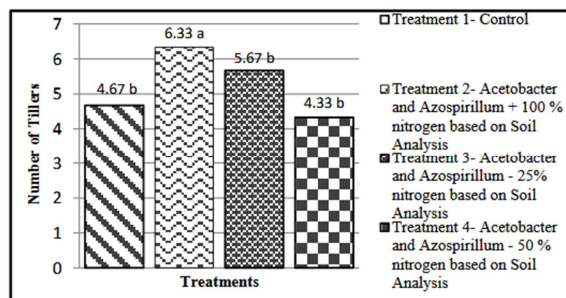


Fig. 2. Average number of millable tiller per hill of sugarcane as affected by reduced levels of nitrogen application combined with a mixture of beneficial microorganisms at Piat, Cagayan, March 2025.

The average number of millable tillers per hill was significantly influenced by the application of reduced nitrogen levels, supplemented with fixed amount of *Acetobacter* and *Azospirillum*. T2 recorded the highest number of millable tillers per hill with a mean of 6.33. Subsequent mean values were recorded for T3 (5.67) and T4 (5), respectively. Plants from the control plots, exhibited the lowest number of millable tillers, with a mean of 4.67.

Analysis of variance on number of millable tillers per hill revealed significant differences among the various treatments tested. Comparison among treatment means showed that Treatment 2 (100% nitrogen + beneficial microorganisms) outperformed all other treatments. While no significant difference was observed between

the plants treated with 25% and 50% nitrogen, these treatments showed significant differences when compared to Treatment 1 (the control group).

These findings suggest that the application of reduced nitrogen levels and mixture of beneficial microorganisms, enhances the production of millable tillers in sugarcane, which means that supplementation of BMOs potentially reduces the dependency on high nitrogen inputs while improving the growth of sugarcane. This is consistent with the findings of Gontia-Mishra *et al.* (2015) and Xu *et al.* (2018), that the application of beneficial microorganisms, such as nitrogen-fixing bacteria or mycorrhizal fungi, can enhance nutrient availability and improve plant growth even under limited nutrient conditions. Moreover, the synergistic effect of microorganisms and reduced nitrogen application is well-documented, as microorganisms can enhance nitrogen utilization efficiency and reduce nitrogen loss through volatilization or leaching (Pettit *et al.*, 2017).

Diameter of stalk (mm)

Results revealed highly significant differences among treatments, indicating that varying nitrogen levels combined with microbial inoculants significantly affected stalk development. Among the treatments, Treatment 2 which received the full recommended nitrogen rate along with BMO produced the thickest stalks, with an average diameter of 3.67 cm. This suggests that the synergistic effect of *Acetobacter* and *Azospirillum*, when combined with adequate nitrogen supply, enhances nutrient uptake and hormonal stimulation, resulting in more vigorous cane growth and thicker stalks.

Treatments 1 and 3 recorded a lower but similar average diameter of 3.20 cm, while Treatment 4 which had the most reduced nitrogen level (50%) resulted in the thinnest stalks, with an average of 3.07 cm. This downward trend indicates that although BMO supplementation aids plant development, substantial nitrogen reduction beyond a threshold may limit optimal stalk growth due to insufficient nutrient supply for cellular expansion and structural integrity.

These findings are consistent with those of Kumar *et al.* (2019), who reported that the combined application of nitrogen fertilizer and *Azospirillum* significantly increased sugarcane stalk girth due to enhanced nitrogen fixation and plant hormone production. Similarly, Yadav and Verma (2017) observed that the use of *Acetobacter diazotrophicus* in conjunction with reduced nitrogen levels led to improved stalk thickness and overall biomass in sugarcane, supporting the potential of integrating microbial inoculants into fertilization strategies.

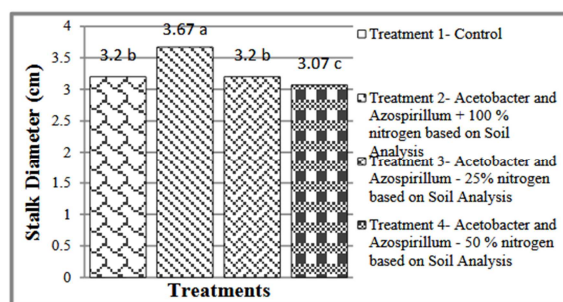


Fig. 3. Average diameter of sugarcane stalk as affected by reduced levels of nitrogen application combined with a mixture of beneficial microorganisms at Piat, Cagayan, March 2025.

Leaf color

The effect of reduced nitrogen application combined with a mixture of beneficial microorganisms on the leaf color of sugarcane is shown in Fig. 4. Treatment 2 exhibited the darkest leaf color, followed by Treatments 3, 4 and 1. The observed darker leaf color in T2 is due to the function of the beneficial microorganisms in enhancing the nitrogen uptake which results to the improvement in the nutrient utilization efficiency. This is consistent with previous research finding where in the supplementation of microorganisms, particularly nitrogen-fixing bacteria and mycorrhizal fungi, can promote better nutrient absorption in plants, even under conditions of limited nitrogen (Gontia-Mishra *et al.*, 2015; Muthukumar *et al.*, 2018). These microorganisms have been known to form symbiotic relationships with plants, helping to increase the bioavailability of essential nutrients such as nitrogen, which in turn can enhance plant growth and physiological characteristics like leaf color (Marschner *et al.*, 2012).

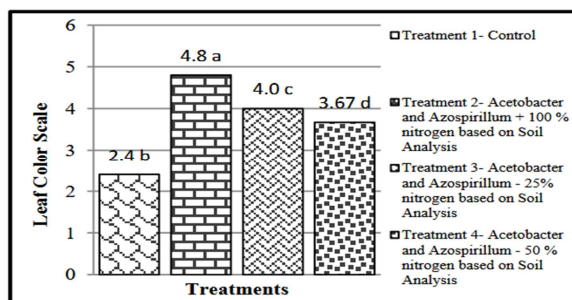


Fig. 4. Average diameter of sugarcane stalk as affected by reduced levels of nitrogen application combined with a mixture of beneficial microorganisms at Piat, Cagayan, March 2025.

In addition, the darker leaf color observed in treatments supplemented with microorganisms, even at reduced nitrogen levels, is attributed to the increased chlorophyll content and enhanced photosynthetic efficiency, which are often associated with improved nutrient status (Mancuso *et al.*, 2013). The ability of microorganisms to mitigate nitrogendeficiency and stimulate plant growth has been well- documented in crops such as sugarcane (Pettit *et al.*, 2017), where microbial inoculants have been shown to improve leaf chlorophyll levels and overall plant health.

The fact that Treatment 2, had the highest nitrogen level, recorded the darkest leaves, aligns with studies that suggest nitrogen is a key factor in chlorophyll synthesis and plant vigor (Rana *et al.*, 2019). However, the effectiveness of beneficial microorganisms in maintaining leaf color and plant health, even when nitrogen is reduced, demonstrates the potential of using microorganisms as a sustainable practice to optimize fertilizer use while maintaining or improving crop performance (Joshi *et al.*, 2020).

The ability to enhance plant physiological traits, such as leaf color, without excessive nitrogen input, can contribute to more sustainable agricultural practices by reducing reliance on synthetic fertilizers, which are often associated with negative environmental impacts, including soil degradation and water pollution (Bashan *et al.*, 2014; Smith *et al.*, 2019).

Conclusion

The integration of *Acetobacter* and *Azospirillum* with reduced nitrogen fertilization significantly enhanced sugarcane vegetative growth in Piat, Cagayan. Plants treated with these beneficial microorganisms showed increased height, more millable tillers, and darker leaves—indicators of improved nitrogen use efficiency. While maximum growth was achieved with full nitrogen levels, reducing nitrogen by up to 50% alongside microbial inoculants had minimal negative effects, suggesting a viable strategy for lowering fertilizer inputs.

To support more sustainable and cost-effective sugarcane cultivation, farmers are encouraged to adopt *Acetobacter* and *Azospirillum* as part of their nutrient management practices. This approach can reduce reliance on synthetic nitrogen fertilizers by up to 50% without compromising yield, leading to lower production costs and reduced environmental impact.

Continued research is advised to assess the long-term effects on soil health, yield stability, and farm sustainability.

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