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Performance Evaluation of a Carrier-Based *Azotobacter vinelandii* Biofertilizer on Growth Parameters of Selected Cereal Crops

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Abstract
This study evaluated the performance of a carrier-based *Azotobacter vinelandii* biofertilizer on the growth parameters of selected cereal crops—maize (*Zea mays*), rice (*Oryza sativa*), millet (*Pennisetum glaucum*), and guinea corn (*Sorghum bicolor*)—under pot experimental conditions. Soil physicochemical properties were determined using standard analytical procedures. *Azotobacter vinelandii* was isolated from soil using Jensen's medium and identified based on morphological, biochemical, and molecular characteristics. Rice husk was used as the carrier material and inoculated with *A. vinelandii* at a concentration of 1×10^7 CFU g^{-1}, followed by incubation for 28 days. The experiment was conducted in triplicate, and growth parameters—including plant height, number of leaves, leaf area, chlorophyll content, and root length—were measured at 5, 10, and 15 days after germination. Data were analyzed using one-way analysis of variance (ANOVA) with SPSS version 20. Plants treated with the *Azotobacter* biofertilizer exhibited significantly higher plant height, leaf area, chlorophyll content, and root length compared to the uninoculated control ($p < 0.05$), while no significant difference was observed in leaf number at early growth stages. These findings demonstrate the effectiveness of carrier-based *A. vinelandii* biofertilizer in enhancing early growth performance of cereal crops and support its potential use as a sustainable alternative to chemical fertilizers.

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Keywords: Biofertilizer, *Azotobacter vinelandii*, Cereal Crops, Growth Parameters, Pot Experiment

Introduction

Environmental degradation remains a significant global challenge, with the indiscriminate use of chemical fertilizers contributing substantially to soil deterioration and ecological imbalance. Prolonged and excessive application of synthetic fertilizers disrupts soil physicochemical properties, reduces microbial diversity, and adversely affects long-term agricultural productivity (Indumathi, 2017) [6]. These concerns have intensified the search for sustainable and environmentally friendly alternatives to conventional fertilizers.

Biofertilizers are formulations containing living microorganisms that enhance plant growth by increasing the availability of essential nutrients through biological processes such as nitrogen fixation, phosphorus solubilization, and the synthesis of plant growth–promoting substances (Vessey, 2003; Singh & Verma, 2020) [18, 15]. When applied to soil, seeds, or plant surfaces, these microorganisms colonize the rhizosphere and improve nutrient uptake, thereby promoting sustainable crop production.

Carrier-based biofertilizers are among the most commonly used formulations and typically consist of effective microbial inoculants supported by organic carrier materials (Accinelli *et al.*, 2009) [1]. However, the development and large-scale application of biofertilizers in many developing countries are constrained by limited access to suitable carrier materials and inadequate production technologies (Khavazi *et al.*, 2007) [8]. Agricultural wastes, such as rice husk, offer a promising alternative as low-cost, readily available carrier materials.

Agricultural wastes are generated in large quantities from crop production and processing activities, and their improper disposal poses environmental and public health concerns (Obi *et al*., 2016) [11]. Effective agricultural waste management strategies emphasize the conversion of these residues into value-added products, such as biofertilizers, thereby reducing environmental pollution while enhancing soil fertility (Sud *et al*., 2008; Hai & Tuyet, 2010) [16, 5].

Among plant growth–promoting rhizobacteria (PGPR), *Azotobacter* species are widely recognized for their ability to fix atmospheric nitrogen, synthesize phytohormones, and enhance nutrient availability in non-leguminous crops. These free-living diazotrophs play a crucial role in improving crop growth and reducing dependency on chemical nitrogen fertilizers, particularly in cereal crop production systems (Singh *et al*., 2013) [14]. Their application is especially relevant in developing countries such as Nigeria, where the high cost and limited accessibility of synthetic fertilizers remain major constraints for smallholder farmers.

The present study aimed to formulate a carrier-based *Azotobacter vinelandii* biofertilizer using rice husk as a carrier material and to evaluate its effects on the growth parameters of selected cereal crops under pot experimental conditions.

Materials and Methods

Study Area

The study was conducted in the Department of Microbiology, Faculty of Sciences, Sokoto State University, Sokoto State, Nigeria.

Collection and Processing of Carrier Material

Rice husk was collected as agricultural waste from a rice processing facility and transported to the laboratory in sterile polyethylene bags. The material was air-dried and ground to a particle size of approximately 0.5–1.0 mm. The processed rice husk was sterilized by autoclaving at 121°C for 60 minutes in polypropylene bags. After sterilization, the carrier material was allowed to cool and stored in airtight zip-lock bags until further use.

Soil Sample Collection and Physicochemical Analysis

Soil samples were collected from farmland located in the Kurfi area of Sokoto State using sterile polythene bags and transported to the laboratory for analysis. The soil was air-dried, homogenized, and sieved to remove debris. Physicochemical properties of the soil, including pH, electrical conductivity, moisture content, water-holding capacity, total organic carbon, total nitrogen, available phosphorus, potassium, calcium, magnesium, sodium, iron, zinc, manganese, and copper, were determined using standard analytical procedures.

Isolation of *Azotobacter vinelandii*

Azotobacter vinelandii was isolated from the soil following the method described by Maheswari and Kalaiyarasi (2015) [10]. Ten grams of air-dried soil sample was suspended in 90 mL of sterile distilled water and serially diluted up to 10^{-6}. An aliquot (0.1 mL) of the appropriate dilution was spread-plated onto Jensen's agar medium, with the pH adjusted to 7.0 using 1 N HCl or 1 N NaOH. The plates were incubated at 28°C for 5–7 days, after which distinct colonies characteristic of *Azotobacter* species were selected and purified by repeated streaking on Jensen's agar. Pure cultures were maintained on agar slants at 4°C for subsequent analyses.

Identification of Bacterial Isolate

The bacterial isolates were characterized based on colonial morphology, microscopic examination, and biochemical tests in accordance with standard procedures described in *Bergey's Manual of Systematic Bacteriology*. Gram staining was performed to determine cell morphology and Gram reaction. Biochemical tests conducted included catalase, oxidase, citrate utilization, urease, starch hydrolysis, nitrate reduction, and ammonia production, following established methods (Cheesbrough, 2006; Oyeleke & Manga, 2008; Tille, 2014) [4, 12, 17]. Carbohydrate utilization tests were carried out using glucose, maltose, mannitol, sucrose, and starch to confirm the identity of *Azotobacter vinelandii*. Molecular identification was performed as a confirmatory method, and the results were consistent with the morphological and biochemical characteristics, confirming the isolate as *Azotobacter vinelandii*.

Biofertilizer Formulation

The biofertilizer was formulated using sterilized rice husk as the carrier material. Each gram of dry carrier material was inoculated with *Azotobacter vinelandii* at a concentration of approximately 1×10^7 CFU g^{-1}, with the initial moisture content adjusted to 60–65%. The inoculated material was incubated at 35°C in 1,000 mL conical flasks for 28 days and mixed every 3–4 days to ensure uniform microbial distribution and aeration. Uninoculated sterilized rice husk served as the control formulation (Yang & Chen, 2003) [19]. A total of 500 g of carrier material was prepared per experimental setup.

Experimental Design and Pot Experiment

The experiment was conducted in a screen-house using a completely randomized design. Four cereal crops—rice (*Oryza sativa*), maize (*Zea mays*), millet (*Pennisetum glaucum*), and guinea corn (*Sorghum bicolor*)—were used as test plants. Healthy seeds were sown in plastic pots containing soil amended with the formulated biofertilizer, while control pots contained soil without biofertilizer application. Each treatment was set up in triplicate. The pots were watered regularly to maintain adequate moisture for germination and growth.

Growth parameters, including plant height, number of leaves, leaf area, chlorophyll content, and root length, were measured at 5, 10, and 15 days after germination.

Statistical Analysis

Data obtained from the experiment were analyzed using descriptive and inferential statistics. Results were expressed as mean ± standard deviation of triplicate determinations. Statistical significance between treatments was assessed using one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$, using SPSS version 20 for Windows.

Results and Discussion

Soil Physicochemical Properties

The physicochemical characteristics of the experimental soil are presented in Table 1. The soil was slightly acidic, with a pH of 5.4, and exhibited low electrical conductivity (0.356 dS m^{-1}), indicating minimal salinity. The moisture content and water-holding capacity were 28% and 58.2%, respectively, suggesting favorable conditions for microbial activity and plant growth.

Nutrient analysis revealed relatively low levels of total nitrogen (0.16 mg L^{-1}) and available phosphorus (6.56 mg L^{-1}), conditions under which the application of nitrogen-fixing biofertilizers is expected to have a pronounced effect. The presence of essential micronutrients such as iron, zinc, manganese, and copper further support the suitability of the soil for cereal crop cultivation. These baseline soil properties provided an appropriate medium for evaluating the growth-promoting potential of *Azotobacter vinelandii* biofertilizer.

Isolation and Identification of *Azotobacter vinelandii*

Azotobacter species were successfully isolated from the soil samples using Jensen's medium. The isolates formed milky to creamy, convex, and viscous colonies characteristic of *Azotobacter*. Microscopic examination revealed Gram-negative, small rod-shaped cells. Biochemical characterization showed positive reactions for catalase, oxidase, citrate utilization, nitrate reduction, starch hydrolysis, and carbohydrate utilization tests, including glucose, maltose, and mannitol (Table 2).

These morphological and biochemical characteristics are consistent with those reported for *Azotobacter vinelandii* in previous studies (Maheswari & Kalaiyarasi, 2015) [10], confirming the identity of the isolate used for biofertilizer formulation.

Effect of *Azotobacter vinelandii* Biofertilizer on Plant Height

Plant height measurements showed variable responses at early growth stages but consistent improvement following biofertilizer application at later stages. At 5 days after germination, control plants exhibited slightly greater heights in rice and maize; however, by 10 and 15 days after germination, plants treated with *Azotobacter vinelandii* biofertilizer showed significantly higher plant heights across all cereal crops ($p < 0.05$).

The observed increase in plant height may be attributed to biological nitrogen fixation and the production of phytohormones such as *indole-3-acetic acid* (IAA) by *Azotobacter* species, which enhance cell elongation and division. These findings agree with the reports of Kushare *et al*. (2009) [9], who demonstrated significant increases in cereal crop height following *Azotobacter* inoculation.

Effect on Number of Leaves

The number of leaves did not differ significantly between treatments at 5 and 10 days after germination ($p > 0.05$). However, by day 15, biofertilizer-treated plants exhibited higher leaf numbers across all crops compared to the control. Rice, maize, millet, and guinea corn treated with *Azotobacter* recorded mean leaf numbers of 3.67, 4.67, 5.00, and 5.00, respectively, compared with lower values in the control.

The delayed but positive effect on leaf number suggests that the influence of *Azotobacter* biofertilizer becomes more pronounced as bacterial populations establish in the rhizosphere and improve nutrient availability. Similar trends have been reported by Chaudhary and Sharma (2019) [3], who observed enhanced vegetative growth following microbial inoculation.

Effect on Leaf Area and Chlorophyll Content

Leaf area increased significantly in biofertilizer-treated plants throughout the experimental period. The enhanced leaf expansion observed in treated plants indicates improved photosynthetic surface area, which is essential for biomass accumulation. Chlorophyll content followed a similar trend, with no marked differences at early stages but significantly higher values in *Azotobacter*-treated plants at 10 and 15 days after germination.

The increase in chlorophyll content may be linked to improved nitrogen availability and enhanced uptake of micronutrients involved in chlorophyll synthesis. These results corroborate the findings of Jain *et al*. (2021) [7], who reported that microbial inoculation improves chlorophyll concentration and photosynthetic efficiency in cereal crops.

Effect on Root Length

Root length was consistently greater in plants treated with *Azotobacter vinelandii* biofertilizer compared to the control. At 15 days after germination, treated rice, maize, millet, and guinea corn recorded root lengths of 5.33 cm, 20.33 cm, 19.33 cm, and 17.83 cm, respectively, while control plants exhibited comparatively shorter roots.

Enhanced root development facilitates greater soil exploration and nutrient uptake, thereby supporting improved aboveground growth. The findings align with those of Saber *et al*. (2012) [13], who reported that biofertilizer application significantly enhances root architecture and nutrient absorption in cereal crops.

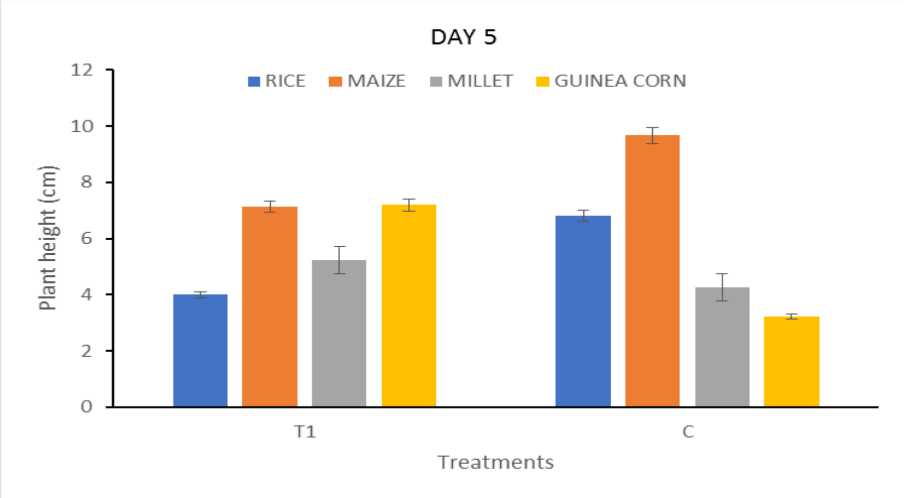

Fig 1: Plant height at day 5

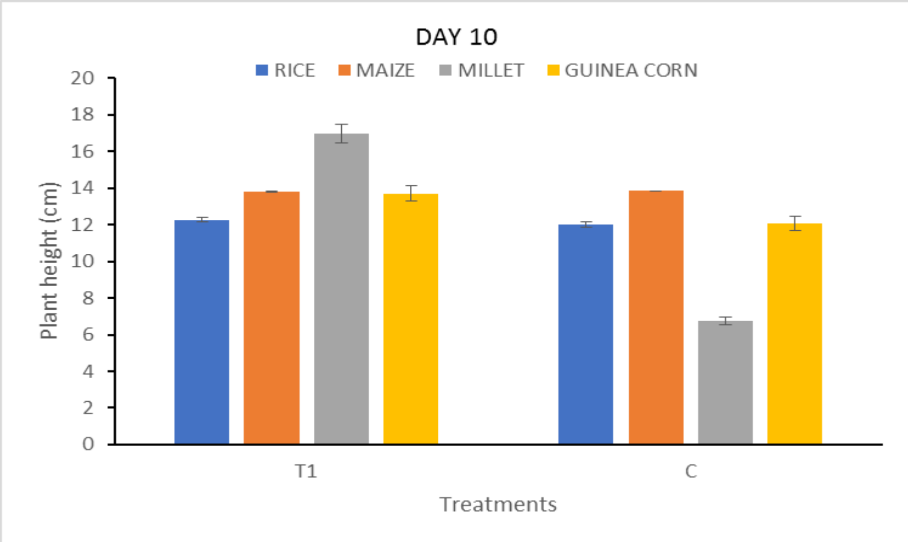

Fig 2: Plant height at day 10

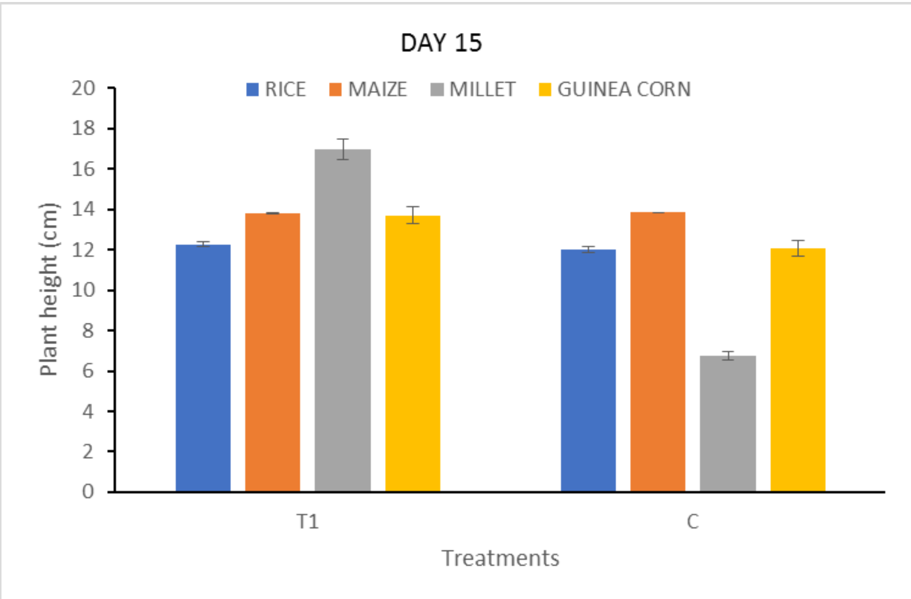

Fig 3: Plant height at day 15

Values are expressed as mean ± standard deviation of triplicate determination P value at ($p<0.05$) is considered as statistically significant

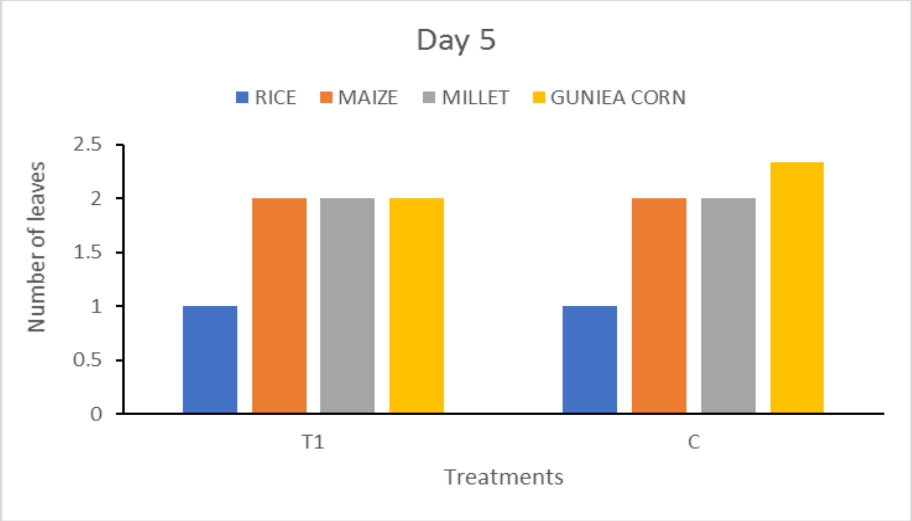

Fig 4: leaf number at day 5

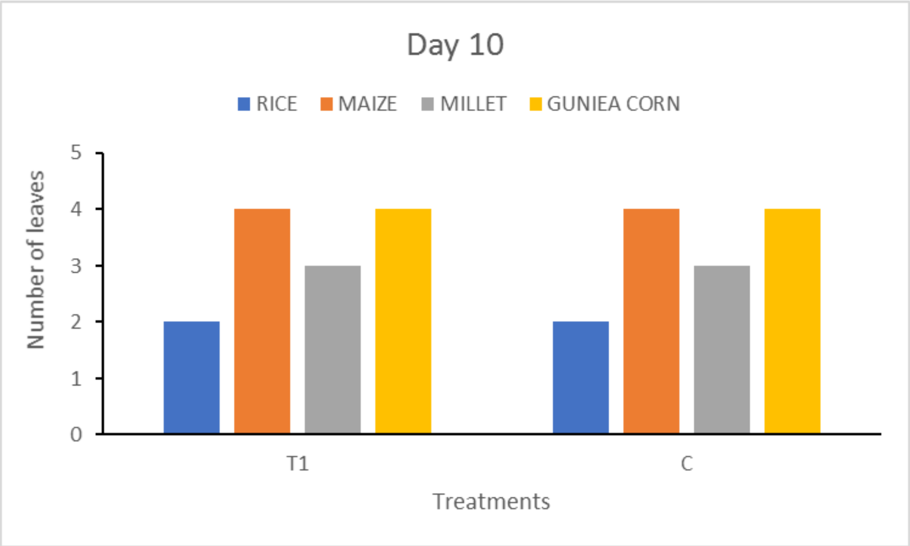

Fig 5: leaf number at day 10

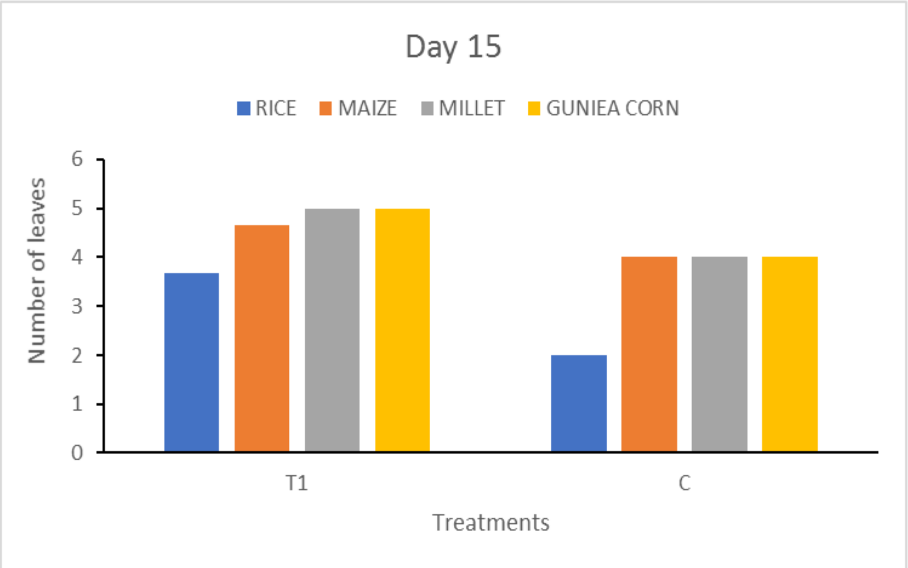

Fig 6: leaf number at day 15

Values are expressed as mean ± standard deviation of triplicate determination P value at ($p<0.05$) is considered as statistically significant

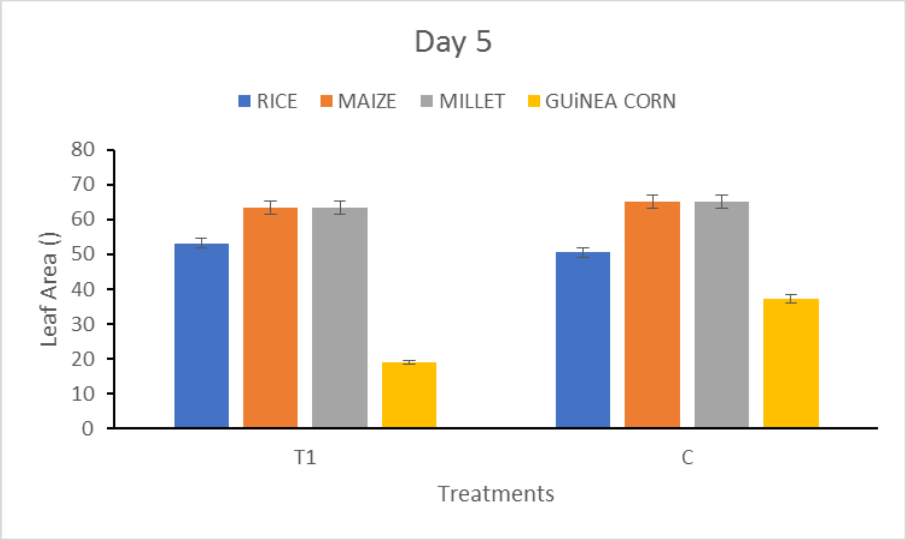

Fig 7: leaf area at day 5

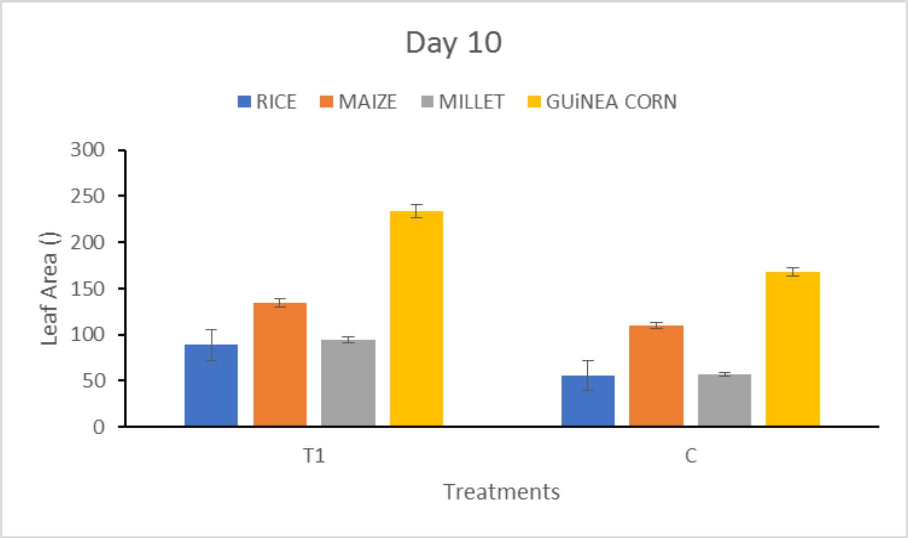

Fig 8: leaf area at day 10

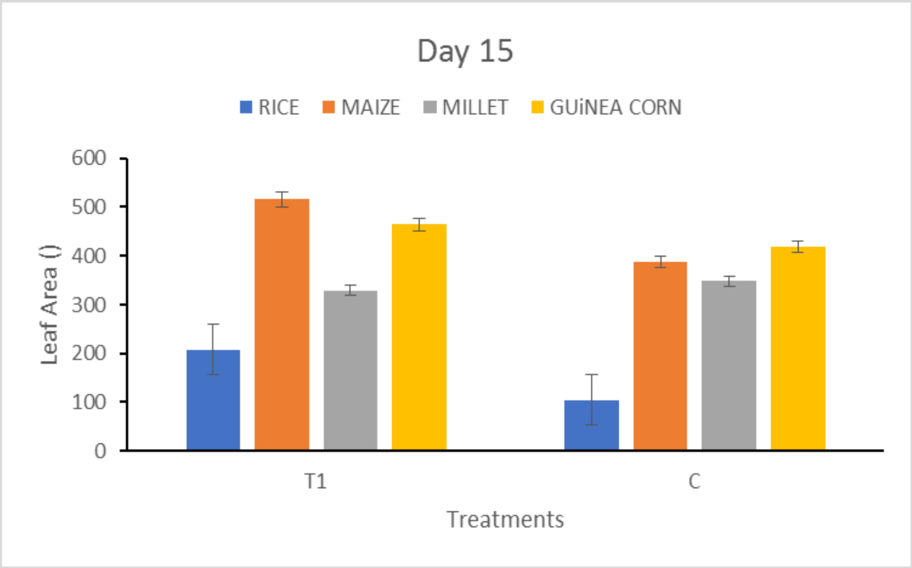

Fig 9: Leaf Area Day 15

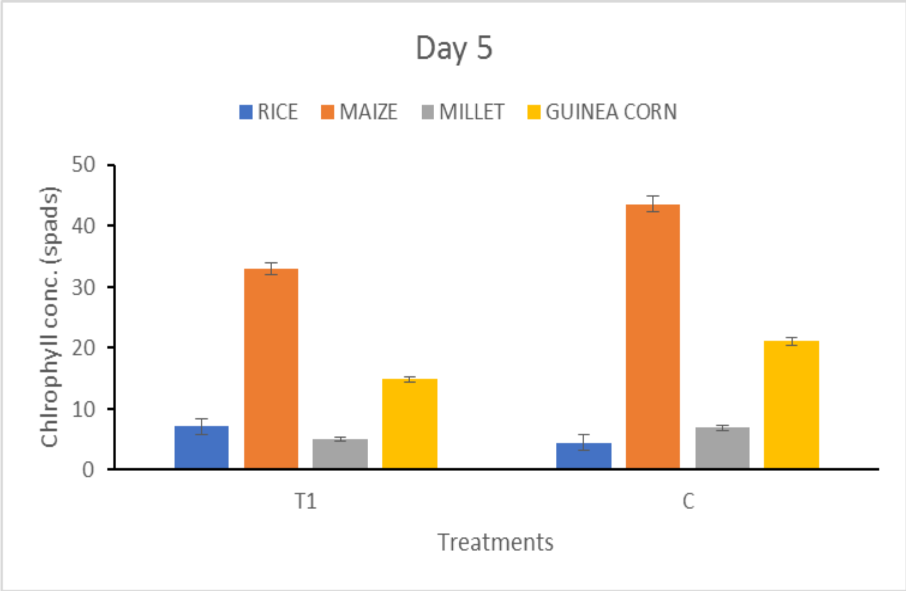

Fig 10: chlorophyll content at day 5

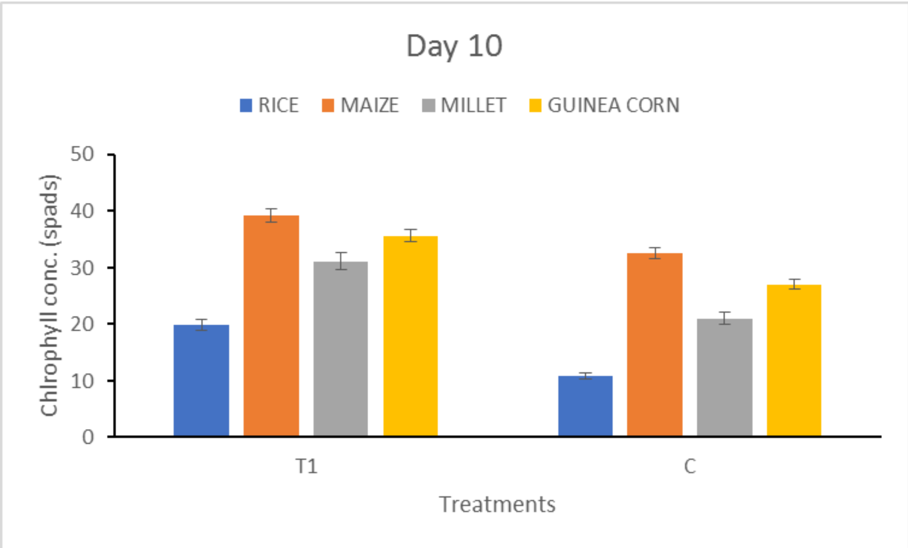

Fig 11: Chlorophyll Content at Day 10

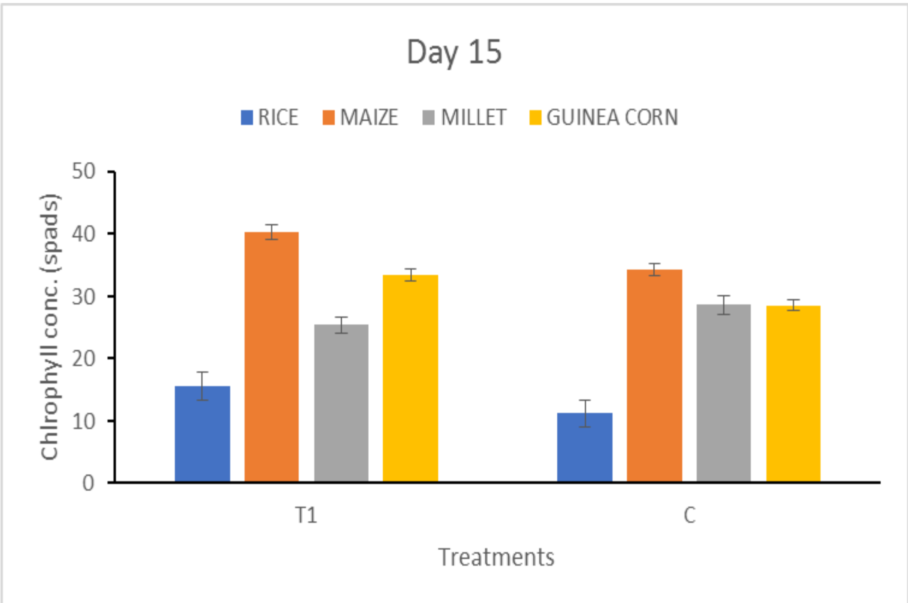

Fig 12: chlorophyll content at day 15

Values are expressed as mean ± standard deviation of triplicate determination P value at ($p<0.05$) is considered as statistically significant

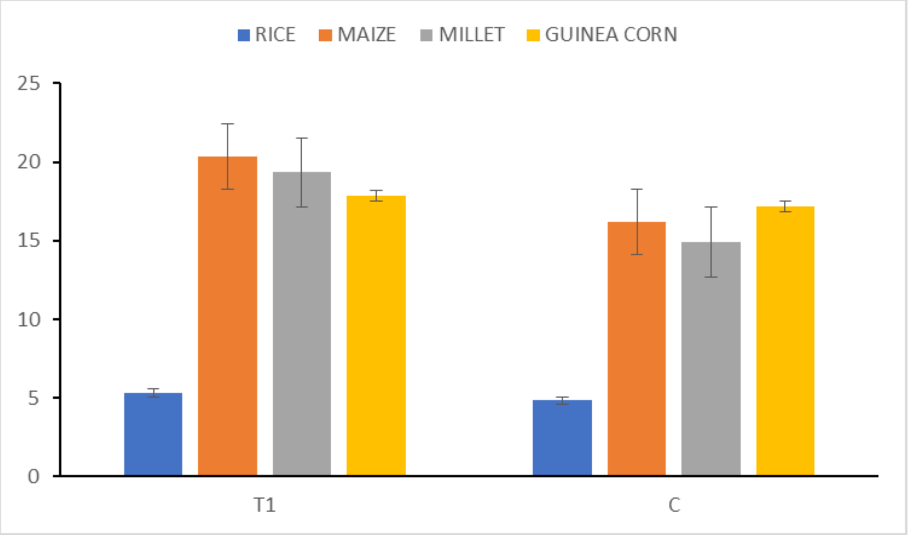

Fig 13: Root length at day 15

Values are expressed as mean ± standard deviation of triplicate determination; Values with different superscripts (lower case) in the same column are significantly different at $p<0.05$; Values with different superscripts (Upper case) in the same row are significantly different at $p<0.05$

Overall Performance of *Azotobacter* Biofertilizer

Overall, the application of carrier-based *Azotobacter vinelandii* biofertilizer significantly improved key growth parameters of the tested cereal crops under pot experimental conditions. The positive responses observed in plant height, leaf area, chlorophyll content, and root length highlight the potential of *Azotobacter* biofertilizer as a sustainable alternative to chemical fertilizers, particularly in nutrient-deficient soils.

Conclusion

The findings of this study demonstrate that the application of a carrier-based *Azotobacter vinelandii* biofertilizer significantly enhanced the early growth performance of selected cereal crops, including rice, maize, millet, and guinea corn, under pot experimental conditions. Compared with the uninoculated control, biofertilizer-treated plants exhibited improved plant height, leaf area, chlorophyll content, and root length, while leaf number showed a positive response at later growth stages.

The observed growth enhancement can be attributed to the ability of *Azotobacter vinelandii* to fix atmospheric nitrogen, produce plant growth–promoting substances, and improve nutrient availability in the rhizosphere. The use of rice husk as a carrier material further highlights the potential of agricultural waste valorization in sustainable biofertilizer production.

Overall, this study supports the use of carrier-based *Azotobacter* biofertilizers as an eco-friendly, cost-effective alternative to chemical fertilizers, particularly in nutrient-deficient soils. Integrating biofertilizer technology into conventional nutrient management strategies may contribute to improved crop productivity, reduced environmental degradation, and enhanced soil health. Further field-based studies are recommended to validate these findings under diverse agroecological conditions.

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