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Development and Greenhouse Evaluation of PGPR-Based Biofertilizers Using Agro-Waste Carrier Materials

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Authors' contributions

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

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Abstract

Aims: This study aimed to develop eco-friendly biofertilizers derived from agro-waste materials and formulated with Plant Growth-Promoting Rhizobacteria (PGPR) as sustainable alternatives to chemical fertilizers.
Study Design: Experimental laboratory and greenhouse study.
Place and Duration of Study: The study was conducted in the Department of Microbiology, Federal University Oye Ekiti, Nigeria, between January and April 2025.
Methodology: Five rhizobacterial isolates- *Bacillus thuringiensis* (a), *Lysinibacillus sphaericus*, *Pseudomonas fluorescens*, *Bacillus thuringiensis* (b), and *Alcaligenes* sp., were characterized using

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morphological, biochemical, and molecular methods. The isolates were screened for plant growth-promoting traits, including indole-3-acetic acid (IAA) production, phosphate solubilization, siderophore production, ammonia production, and hydrolytic enzyme activities. Carrier-based formulations were prepared using sterilized sawdust (*Khaya* sp.) and rice bran (*Oryza sativa*). Microbial viability was monitored for eight weeks. Greenhouse trials using okra (*Abelmoschus esculentus*) evaluated plant growth compared with NPK fertilizer and an untreated control.

Results: All isolates exhibited multiple growth-promoting traits, while *L. sphaericus* uniquely showed protease activity. The formulations maintained high viability (1.29×10^7–8.91×10^7 CFU/g). Sawdust supported *L. sphaericus* and *B. thuringiensis* (b), whereas rice bran supported *P. fluorescens* and *B. thuringiensis* (a). Notably, *L. sphaericus* + sawdust and *P. fluorescens* + sawdust significantly enhanced plant height and biomass compared with NPK and control.

Conclusion: Agro-waste-based PGPR biofertilizers improved crop performance and offer a cost-effective, sustainable alternative to chemical fertilizers, promoting waste valorization and environmentally friendly agriculture.

Keywords: Lysinibacillus sphaericus; Pseudomonas fluorescens; Sustainable Agriculture; okra.

1. Introduction

Agricultural intensification has substantially increased crop productivity but has also contributed to soil chemical pollution and elevated greenhouse gas emissions, including carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4), which are key drivers of climate change (Menegat et al., 2022; Yan et al., 2023). The continued reliance on synthetic fertilizers raises concerns regarding environmental sustainability, soil health, and long-term agricultural resilience. In alignment with the United Nations Sustainable Development Goals (United Nations, 2015), sustainable nutrient management strategies are urgently required to balance productivity with environmental protection (FAO, 2018, Holt et al., 1994, Logan & De Vos, 2009).

Biofertilizers, particularly those based on plant growth-promoting rhizobacteria (PGPR), offer a promising alternative to chemical fertilizers. PGPR enhance plant growth through biological nitrogen fixation, phosphorus solubilization, phytohormone production, and stimulation of soil microbial activity (Saeed et al., 2021; Mus et al., 2016). Previous studies indicate that PGPR-based formulations can partially or fully substitute synthetic fertilizers without compromising yield, while potentially reducing input costs by 30–50% (Malusá and Vassilev, 2014; Bhardwaj et al., 2014, Rodríguez & Fraga, 1999, Varma & Jain, 2021). However, large-scale adoption remains limited due to formulation instability, unsuitable carrier materials, variable field performance, and cost-related constraints (Malusá et al., 2012).

Therefore, the present study aims to develop and evaluate a cost-effective, stable PGPR-based biofertilizer formulation using appropriate carrier materials. The research assesses its potential to enhance nutrient availability and plant growth performance while reducing dependence on synthetic fertilizers. By addressing both technical and operational limitations, this work contributes to advancing sustainable agricultural practices and supporting climate-resilient food production systems.

2. Materials and Methods

2.1 Rhizobacterial Isolates

Five rhizobacterial isolates were used in this study: IS 13-Bacillus thuringiensis (a), IS 10-Lysinibacillus sphaericus, IS 4A-Pseudomonas fluorescens, IS 9-Bacillus thuringiensis (b), and IS 14B-Alcaligenes sp. These isolates were screened for plant growth–promoting traits and enzyme production to confirm their potential prior to biofertilizer formulation and application.

2.2 Morphological and Biochemical Identification

Morphological characterization of the isolates was conducted using Gram staining as described by Smith and Brown (2021). Gram reaction and cell morphology were determined by microscopic examination.

Biochemical identification was carried out using standard microbiological tests, including catalase (Hafezi and Khamar, 2024), indole (Barnwal and Saleh, 2025), citrate utilization (Barnwal and Saleh, 2025), urease (Yu et al., 2025), triple sugar iron (TSI) agar (Aryal, 2022), methyl red–Voges Proskauer (MR–VP) tests (Tu et al., 2025), and motility test using SIM medium (Cappuccino and Welsh, 2020). Test procedures and result interpretations followed established protocols.

2.3 Screening for Plant Growth-Promoting Traits

Phosphate solubilization was assessed on Pikovskaya's agar following incubation at 37 °C for five days. The phosphate solubilization index (PSI) was calculated and classified as low, medium, or high according to Oo et al. (2020) and Santos et al. (2025).

Indole-3-acetic acid (IAA) production was evaluated by culturing isolates in nutrient broth supplemented with 1% tryptophan for seven days at 37 °C. Following centrifugation, Salkowski reagent was added to the supernatant, and absorbance was measured at 530 nm. Results were used for relative comparison of IAA production (Oo et al., 2020).

Ammonia production was determined in peptone water using Nessler's reagent after 96 h incubation, while hydrogen cyanide (HCN) production was assessed on glycine-supplemented nutrient agar using picric acid-impregnated filter papers (Oo et al., 2020; Kashyap et al., 2021).

Siderophore production was evaluated by reacting culture supernatants with 2% ferric chloride solution, and color development was recorded as positive (Oo et al., 2020).

2.4 Enzyme Production Assays

Amylase activity was assessed on starch agar plates incubated at 37 °C for seven days, with iodine solution used to visualize hydrolysis zones (Dike et al., 2022). Protease activity was determined on skim milk agar plates after 24 h incubation, and clear zones around colonies were recorded as positive (Bibi et al., 2025).

2.5 Molecular Identification and Genomic Analysis

Genomic DNA was extracted using the ZymoBIOMICS™ DNA Miniprep Kit following the manufacturer's instructions. DNA quality was confirmed using spectrophotometry and agarose gel electrophoresis.

PCR amplification of the 16S rRNA gene was performed using primers 27F and 1492R. Amplified products were sequenced, and identification was carried out using BLASTn analysis against the NCBI GenBank database, with ≥98% sequence similarity considered confirmatory (Church et al., 2020).

Genome annotation was performed primarily for taxonomic confirmation of the isolates. Detailed functional genome mining for plant growth–promoting genes was beyond the scope of the present study and will be explored in future investigations.

2.6 Biofertilizer Formulation and Viability

Selected bacterial isolates were cultured in nutrient broth at 37 °C for 24 h under shaking conditions. Cells were harvested by centrifugation and adjusted to approximately 10^8 CFU mL^{-1}. Sterilized sawdust (Khaya sp.) and rice bran (Oryza sativa) were used as carrier materials and mixed with bacterial suspensions at a 1:5 (v/w) ratio (Akter et al., 2023).

Viability of the inoculated carriers was assessed after two and eight weeks of storage using serial dilution and plate counting, and results were expressed as $\log_{10}$ CFU g^{-1} (Aloo et al., 2022).

2.7 Greenhouse Experiment

A greenhouse experiment was conducted to evaluate the effect of biofertilizer application on okra (Abelmoschus esculentus L.), accession number NHAe 47–4, obtained from the National Horticultural Research Institute

(NIHORT), Ibadan, Nigeria. The greenhouse temperature was maintained under ambient conditions (25–30°C), consistent with standard greenhouse-based biofertilizer evaluations (Backer et al., 2018).

Surface-sterilized seeds were planted in 10-L plastic pots containing 8 kg of sterilized loamy soil. The soil was air-dried, sieved through a 2 mm mesh, and sterilized prior to use to minimize interference from native microbial populations, as recommended in PGPR inoculation studies (Basu et al., 2021). The experiment was arranged in a completely randomized design (CRD) with three replicates per treatment.

The treatments were as follows:

1. Uninoculated control
2. Sawdust-based biofertilizer inoculant
3. Rice bran-based biofertilizer inoculant
4. NPK fertilizer (15:15:15)

Biofertilizer formulations (5 g per pot) were applied two weeks after planting by thoroughly incorporating the carrier-based inoculant into the soil. NPK fertilizer (15:15:15) was applied tat a rate of 3 g per pot at the same time and uniformly mixed into the soil, serving as a conventional fertilizer control. Pots were watered regularly to maintain adequate soil moisture throughout the eight-week experimental period.

Plant growth parameters, including plant height, number of leaves, fresh weight, and dry weight, were measured at the end of the experiment (El-Sharkawy et al., 2024).

2.8 Data Collection and Statistical Analysis

Plant growth parameters were recorded at regular intervals and at harvest. Data were analyzed using one-way analysis of variance (ANOVA) in SPSS version 27.0 and GraphPad prism. Treatment means were compared using Tukey's HSD test at P = .05 after verifying assumptions of normality and homogeneity of variance.

3. Results and Discussion

3.1 Morphological, Biochemical and Molecular Identification

The results of morphological identification, based on Gram staining and supported by biochemical characterization (Table 1), revealed that all five bacterial isolates were rod-shaped, with four being Gram-positive and one (IS4A) Gram-negative. The isolates appeared to belong to a variety of taxa, including Bacillus, Pseudomonas, Alcaligenes, and Lysinibacillus, according to the results of these preliminary investigations. The morphological and biochemical characteristics of the isolates were largely consistent with contemporary descriptions of these genera based on polyphasic taxonomic approaches integrating phenotypic and molecular features (Patel and Gupta, 2020).

Although molecular identification confirmed the taxonomic identity of the isolates, comprehensive functional genome analysis was not undertaken in this study. Future work will focus on genome mining to identify genes associated with IAA biosynthesis, phosphate solubilization, siderophore production, and other plant growth–promoting traits.

3.2 Screening of Rhizobacterial Isolates for Plant Growth-Promoting Traits and Enzyme Production

The screening of rhizobacterial isolates for plant growth-promoting (PGP) traits and enzyme production (Table 1) demonstrated that the isolates possess multiple complementary PGP and biocontrol attributes. This finding aligns with the current understanding that effective PGPR enhance plant growth through synergistic mechanisms rather than a single functional pathway (Basu et al., 2021). Most isolates produced indole-3-acetic acid (IAA) (Fig. 1), a key phytohormone known to stimulate root elongation, promote lateral root formation, and enhance nutrient uptake, thereby contributing to increased plant biomass (Ruzzi and Aroca, 2020).

Table 1. Morphological and Biochemical Characteristics of Bacterial Isolates

Isolates	Gram Reaction	Catalase Test	Citrate Test	Urease Test	Indole Test	Motility Test	Methyl Red Test	Voges Proskauer Test	Triple sugar Iron Test			Molecular identified Organisms
									L	G	S	
IS4A	-	-	-	+	+	-	+	-	+	-	+	*Pseudomonas fluorescens*
IS9	+	+	+	-	-	+	-	-	+	-	+	*Bacillus thuringiensis* (b)
IS10	+	+	+	+	-	+	-	-	+	-	+	*Lysinibacillus sphaericus*
IS13	+	+	+	-	-	+	-	-	+	+	+	*Bacillus thuringiensis* (a)
IS14B	+	+	+	-	-	+	-	-	+	-	+	*Alcaligenes faecalis*

Key: L: Lactose, G: Glucose, S: Sucrose, +: Positive, - : Negative

Table 2. Evaluation of plant growth-promoting and bio-control attributes in bacterial isolates

Isolates	Phosphate Solubilization	IAA	Ammonia Production	Siderophore Production	HCN	Protease	Amylase
IS4A	-	+	+	+	-	-	+
IS9	+	+	+	+	-	-	+
IS10	-	+	+	+	-	+	+
IS13	+	-	+	+	-	-	+
IS14B	-	+	+	+	-	-	+

Key: "+": indicates positive activity or presence of the trait; "-": indicates absence or undetectable levels of the trait.

Phosphate solubilization (Fig. 2), observed in selected isolates, underscores the strain-specific nature of this trait and its critical role in mobilizing insoluble phosphorus through organic acid secretion and enzymatic processes, ultimately improving phosphorus bioavailability in soil systems (Timofeeva et al., 2022). The consistent production of ammonia and siderophores further highlights the multifunctional capacity of the isolates. These metabolites contribute to nitrogen availability and enhanced iron acquisition under limiting conditions, while also supporting indirect suppression of phytopathogens through competitive interactions within the rhizosphere (Basu et al., 2021).

Although none of the isolates produced hydrogen cyanide (HCN), this is not uncommon, as cyanogenesis is limited to certain strains (Glick, 2012). Limited protease activity alongside widespread amylase production further indicates metabolic versatility and potential roles in pathogen inhibition and nutrient cycling. Collectively, these findings highlight the multifunctional potential of the isolates as biofertilizer and biocontrol agents.

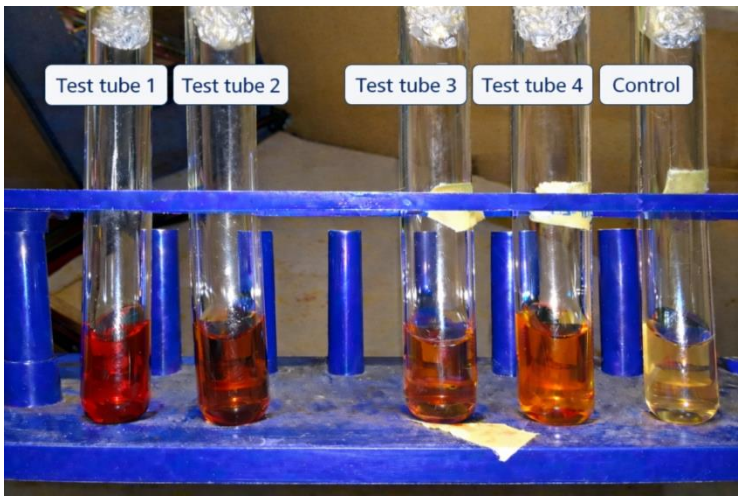

Fig. 1. Qualitative detection of indole-3-acetic acid (IAA) production by rhizobacterial isolates using Salkowski's reagent. The color change from pink to red indicates IAA production, with varying intensities reflecting the relative amounts of IAA synthesized by each isolate. Test tubes 1 and 2 show the strongest IAA production, while the lighter colors in subsequent tubes indicate lower production levels

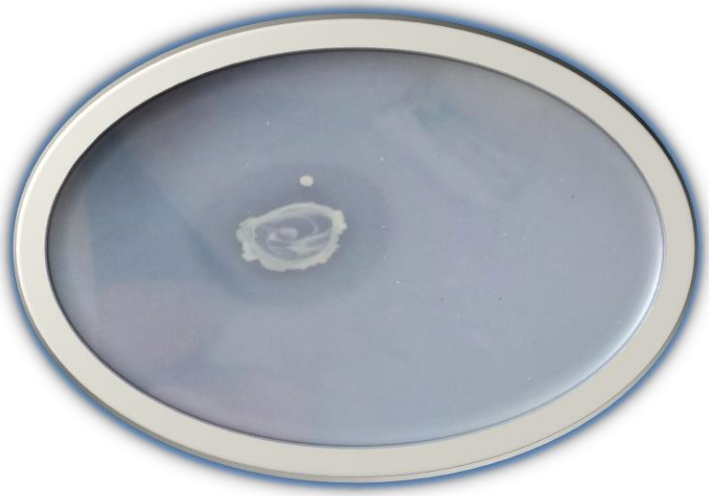

Fig. 2. Phosphate solubilization by the bacterial isolate on Pikovskaya's agar, evidenced by a clear halo zone surrounding the spot inoculum

3.3 Biofertilizer Viability

The viability of each bacterial isolate was assessed using two carrier materials, sawdust and rice bran. Comparison of the bacterial populations in these formulations (Table 3) provides insight into the suitability of these agro-based materials as carrier substrates for bioinoculant production. All formulations maintained substantial bacterial populations, with counts ranging from 1.29×10^7 to 8.91×10^7 CFU g^{-1}.

Sawdust-based formulations supported higher populations of Bacillus thuringiensis (b), Lysinibacillus sphaericus, and Alcaligenes sp., whereas Pseudomonas fluorescens and B. thuringiensis (a) exhibited greater survival in rice bran–based formulations. These differences underscore the influence of carrier composition on microbial persistence. The findings are consistent with bioformulation studies indicating that organic carrier materials significantly affect microbial survival, shelf life, and functional performance (Basu et al., 2021).

Table 3. Comparison of Viable Counts (CFU/g) of Bacterial Isolates in Sawdust and Rice Bran Formulations

Isolates	Sawdust (CFU/g)	Rice Bran (CFU/g)	Higher Viability
P. fluorescens	1.30×10^7	1.91×10^7	Rice Bran
B. thuringiensis (b)	1.43×10^7	1.33×10^7	Sawdust
L. sphaericus	8.91×10^7	8.50×10^7	Sawdust
B thuringiensis (a)	1.33×10^7	1.85×10^7	Rice Bran
Alcaligenes sp.	1.35×10^7	1.29×10^7	Sawdust

Values are from the 10^{-4} dilution

3.4 Greenhouse Experiment

3.4.1 Effect of Different Treatments on the Plant Height of Abelmoschus esculentus

Plant height increased steadily from the second to the eighth week after planting under all treatments (Fig. 3). The plants that served as control consistently recorded the lowest mean heights throughout the study, while bio-fertilizers with organic substrates generally produced superior vegetative performance. By the eighth week, plants treated with Lysinibacillus sphaericus + sawdust achieved the highest heights, with mean plant height (33.84 ± 13.87 cm). This was closely followed by Pseudomonas fluorescens + sawdust (29.78 ± 10.87 cm) and rice bran only (26.25 ± 9.30 cm), whereas plants receiving only sawdust treatment were shorter. The enhanced plant height observed in biofertilizer-treated plants may be attributed to multiple, complementary mechanisms. Pseudomonas fluorescens and Lysinibacillus sphaericus have been reported to promote plant growth through nitrogen fixation, phosphate solubilization, and the production of phytohormones such as indole-3-acetic acid (IAA) (Naureen et al., 2017; Pantoja-Guerra et al., 2023). In this study, Sawdust-based carriers have also been shown to sustain higher bacterial populations and improve inoculant efficacy in several formulations (Aloo et al., 2022). Thus, combining a PGP-competent strain such as L. sphaericus with sawdust likely produced a synergistic effect: the bacterium supplies growth-promoting metabolites and nutrient-mobilizing activities, while the sawdust carrier preserves viable cells during storage and facilitates their gradual release into the rhizosphere.

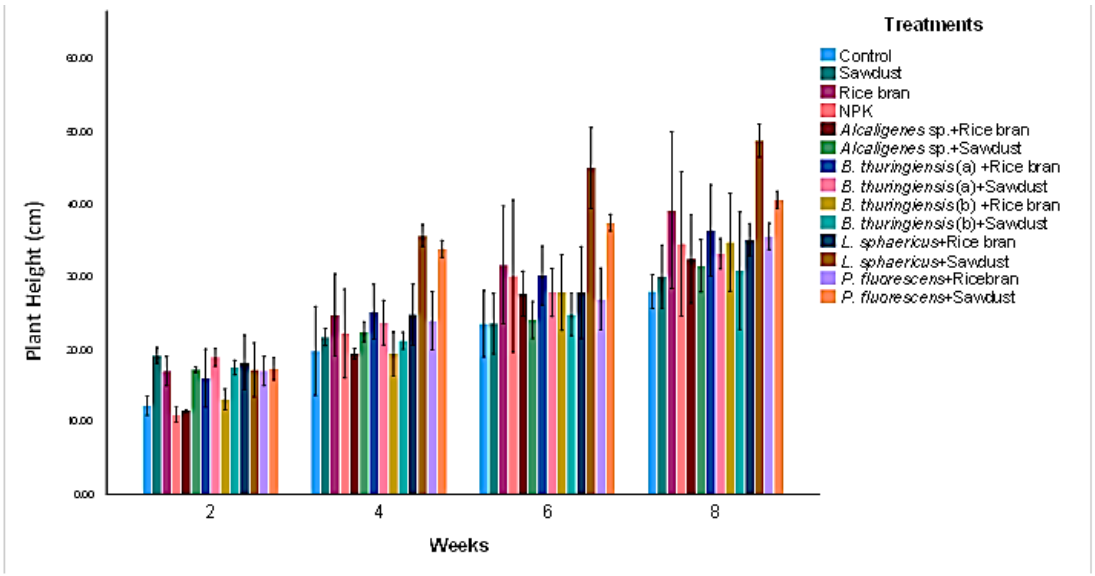

Fig. 3. Effect of Different Treatments on Plant Height. Mean plant height of plants under different treatment groups. Error bars represent standard deviation (SD)

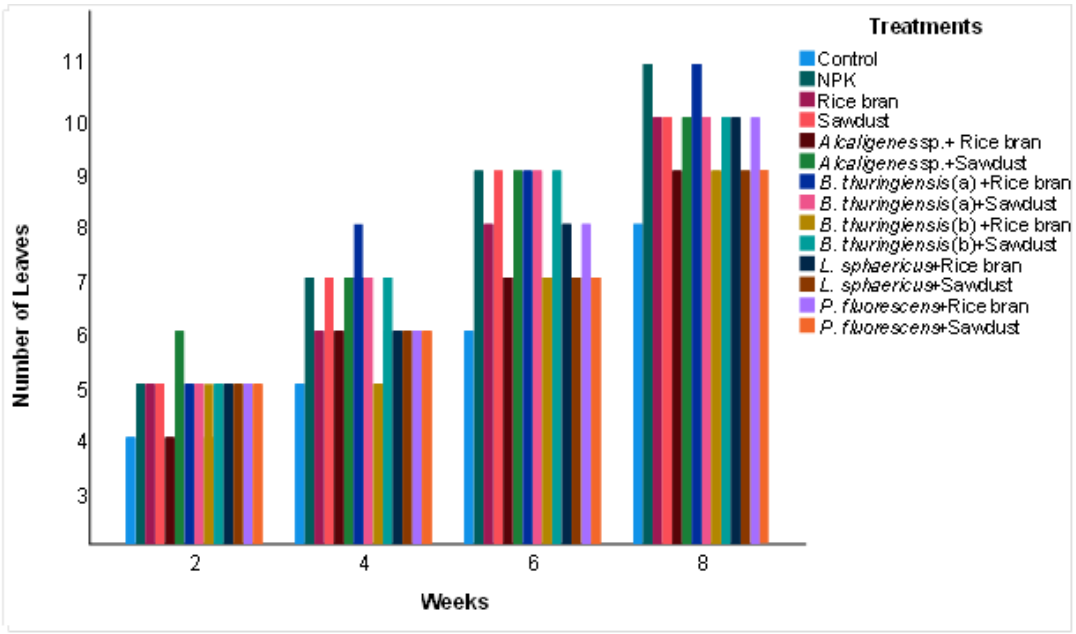

Fig. 4. Effect of Different Treatments on Leaf Number. Mean leaf number of plants under different treatment groups

3.4.2 Effect of Different Treatments on the Leaf Number of Abelmoschus esculentus

The number of leaves in Abelmoschus esculentus (okra) plants increased progressively across the growth period in all treatments, with variations in magnitude among treatments (Fig. 4). At week 8 of the experiment, the treatment combining Bacillus thuringiensis (strain a) with rice bran achieved the highest mean number of leaves (7.58 ± 2.50), closely followed by the conventional inorganic fertilizer (NPK) treatment (7.50 ± 2.46). Among the remaining treatments, those receiving rice bran only, sawdust only, Alcaligenes sp. + rice bran, Bacillus thuringiensis (a) + sawdust, Bacillus thuringiensis

(b) + sawdust, Lysinibacillus sphaericus + rice bran and Pseudomonas fluorescens + rice bran maintained intermediate leaf numbers. The remaining treatments, including the untreated control, showed comparatively lower leaf numbers, with the control producing the fewest leaves. The superior performance observed in the B. thuringiensis + rice bran treatment on plant leaf number is likely promoted through two complementary pathways: (i) rice bran served as a nutrient rich organic substrate, providing macro and micronutrients, enhancing microbial activity, and improving soil fertility; and (ii) the inoculated bacterium contributed plant growth promoting traits such as phytohormone (e.g., indole 3 acetic acid) production, nutrient solubilisation (for phosphorus and potassium), and improved root nutrient uptake. Recent studies have shown that PGPR can significantly enhance plant growth and nutrient availability, particularly when integrated with organic amendments that improve microbial establishment and soil nutrient dynamics (Mustapha et al., 2025; Sharma et al., 2024).

3.4.3 Effect of Different Treatments on the Stem Girth of Abelmoschus esculentus

The result from Fig. 5 demonstrates a clear pattern that the stem girth of Abelmoschus esculentus increased steadily from week 2 to week 8 across all treatments, with the lowest values observed in the untreated control and the greatest thickening seen in the plants treated with combinations of bacterial inoculants plus organic carriers. Treatments such as Bacillus thuringiensis + sawdust and B. thuringiensis (b) + rice bran yielded greater stem-girth values than either NPK or any single treatment. The observed progressive increase in stem girth of Abelmoschus esculentus from week two to week eight across all treatments aligns with previous findings showing that okra amended with organic compost develops significantly thicker stems compared to control plants (Tandoh et al., 2024). Bandopadhyay (2020) also demonstrated that Bacillus thuringiensis enhanced the vegetative performance of okra under field conditions, though stem girth was not quantified. The current findings extend that work by showing that B. thuringiensis combined with sawdust or rice bran not only increased biomass but also significantly improved stem thickening. This implies that the organic carriers provided a microenvironment that enhanced microbial colonization and nutrient mineralization, translating into improved vascular growth and cambial activity.

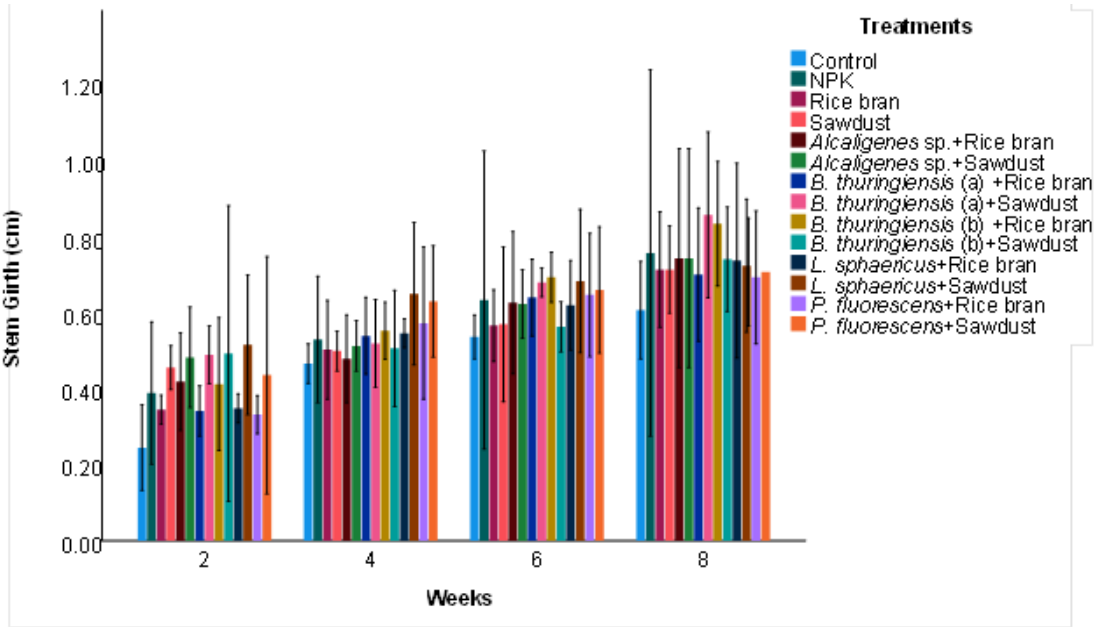

Fig. 5. Effect of Different Treatments on Stem Girth. Mean stem girth of plants under different treatment groups. Error bars represent standard deviation (SD)

3.4.4 Effect of Different Treatments on the Fresh and Dry Weight of Abelmoschus Esculentus

The results presented (Figs. 6 and 7) show clear differences among the treatments in terms of both fresh and dry weight of Abelmoschus esculentus (okra). A distinct trend is evident across the two parameters: treatments that combined microbial inoculants with organic carriers, particularly sawdust, produced markedly higher fresh and dry biomass compared to the control, or rice bran alone.

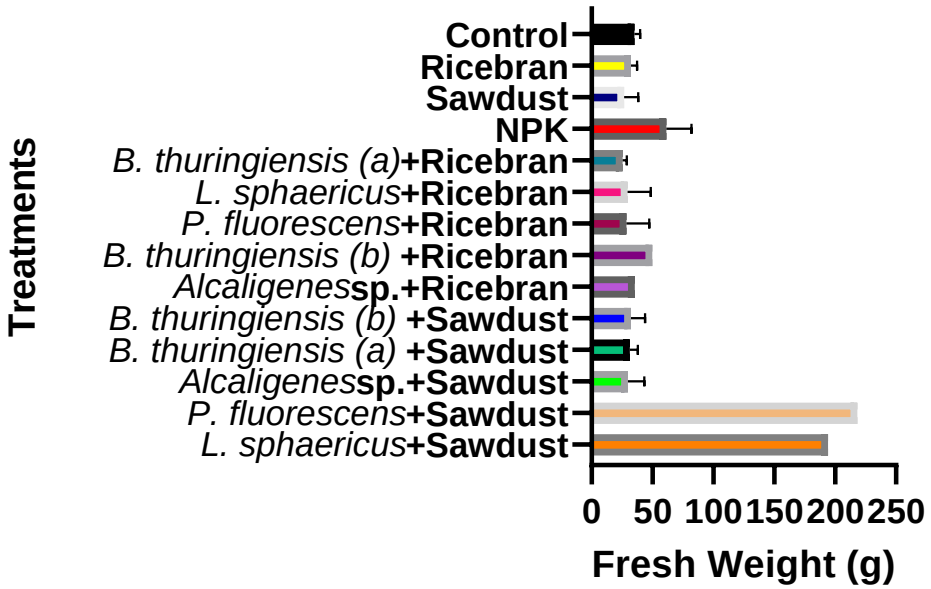

Fig. 6. Effect of Different Treatments on Fresh Weight. Mean fresh weight of plants under different treatment groups. Error bars represent standard deviation (SD)

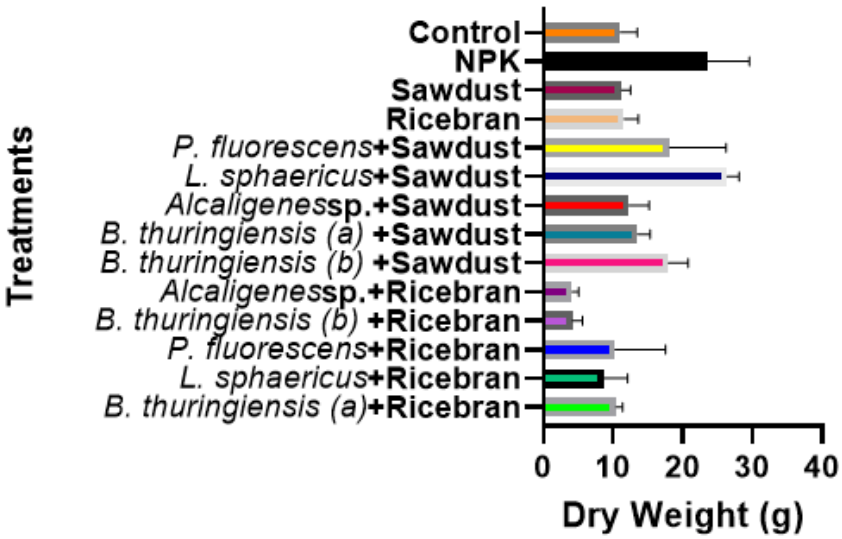

Fig. 7. Effect of Different Treatments on Dry Weight. Mean dry weight of plants under different treatment groups. Error bars represent standard deviation (SD)

For fresh weight, the highest values were recorded in Pseudomonas fluorescens + sawdust and Lysinibacillus sphaericus + sawdust treatments, both exceeding 200 g per plant, whereas the control

and NPK treatments recorded the lowest values (below 80 g per plant). Treatments containing rice bran as the carrier generally produced moderate increases (100–150 g per plant), indicating that sawdust supported more vigorous vegetative growth than rice bran.

A similar trend was observed for dry weight, where L. sphaericus + sawdust and P. fluorescens + sawdust again outperformed other treatments, recording dry biomass values around 35–40 g per plant, while control remained under 20 g per plant. In contrast, rice bran combinations and non-inoculated substrate treatments (sawdust or rice bran alone) exhibited intermediate results. The consistency of this pattern between fresh and dry weights indicates that the biomass enhancement observed in sawdust plus inoculum treatments was not solely due to increased tissue water content, but reflected genuine accumulation of plant structural material. The parallel increase in fresh and dry weight across treatments demonstrates that the improved growth responses resulted from true physiological gains rather than mere tissue water accumulation.

The dry matter accumulation indicates enhanced carbon fixation, protein synthesis, and cell wall formation traits typically associated with improved nutrient assimilation and photosynthetic efficiency under microbial inoculation (Samantaray et al., 2024). The L. sphaericus + sawdust treatment exhibited both high fresh and high dry weight, indicating an efficient conversion of assimilates into structural biomass, while moderate treatments such as B. thuringiensis (b) + rice bran produced lower dry weights, implying lower biomass density or less efficient nutrient utilization (Perveen et al. 2023).

4. Conclusion

The present study demonstrated that agro-waste–based carrier formulations incorporating plant growth-promoting rhizobacteria (PGPR) significantly enhanced the growth performance of okra under greenhouse conditions. The evaluated isolates exhibited multiple verified plant growth–promoting traits, including indole-3-acetic acid production, phosphate solubilization, siderophore production, ammonia production, and hydrolytic enzyme activity, indicating their multifunctional capacity to improve plant development through complementary mechanisms.

Carrier material played a critical role in determining microbial viability and subsequent plant growth responses. Sawdust-based formulations consistently supported higher bacterial populations and produced superior growth outcomes compared with rice bran–based formulations. In particular, the combination of Lysinibacillus sphaericus or Pseudomonas fluorescens with sawdust resulted in significantly greater plant height and biomass accumulation than both the uninoculated control and NPK fertilizer treatment. These findings suggest that appropriate carrier selection enhances inoculant stability, microbial persistence, and effective rhizosphere colonization.

Importantly, this study highlights the dual advantage of utilizing agro-waste materials for biofertilizer development: promoting sustainable waste valorization while reducing dependence on synthetic chemical fertilizers. The integration of locally available organic carriers with efficient PGPR strains provides a cost-effective and environmentally responsible approach to improving soil fertility and crop productivity.

Although the experiment was conducted under controlled greenhouse conditions, the promising results warrant further validation under field conditions to assess long-term performance, environmental variability, and scalability.

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 writing or editing of this manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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