Enhancing Spinach growth and yield through the synergistic effects of Rhizobacter-Enriched compost

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Abstract: Plant Growth-Promoting Rhizobacteria (PGPR) and compost present promising solutions for bolstering agricultural sustainability by stimulating plant growth and yield while lessening dependence on chemical inputs. Our investigation delved into the combined effects of PGPR and compost on spinach cultivation, conducted at Amity University's Organic Farm. Employing a Randomized Block Design (RBD) with six treatments and three replications, we evaluated how these treatments influenced spinach growth parameters and photosynthetic pigment levels. Results showed that treatment T5 (AIOA Strain + Compost) outperformed others, significantly boosting plant height, leaf breadth, root length, and leaf count per plant. Furthermore, T5 exhibited elevated chlorophyll and carotenoid levels, indicating enhanced photosynthetic function and overall plant vigor. These outcomes underscore the potential synergy between PGPR and compost in fostering sustainable crop production. By diminishing reliance on chemical fertilizers and pesticides while enhancing soil health and nutrient cycling, integrating PGPR and compost-based biofertilizers offers an economical and environmentally friendly avenue for augmenting agricultural productivity and mitigating environmental hazards. Further exploration into optimization techniques and underlying mechanisms is crucial to fully harness the potential of these microbial and organic interventions across varied agroecological settings. Overall, this study contributes to advancing our knowledge of employing microbial and organic methods in sustainable agriculture, crucial for addressing global challenges in food security and environmental conservation.

Keywords- PGPR (Plant growth promoting rhizobacteria), rhizospheric soil, chlorophyll, strain, compost, biochar.

Introduction

Plant Growth-Promoting Rhizobacteria (PGPR) constitute a heterogeneous group of naturally occurring microorganisms that exert a pivotal influence on soil quality enhancement and plant yield improvement. Their capacity to augment nutrient availability within the rhizosphere, the zone of soil surrounding plant roots, has attracted considerable attention among both researchers and agricultural practitioners [1].

The application of PGPR in agricultural contexts holds substantial promise for ameliorating soil fertility, optimizing nutrient uptake efficiency, and augmenting crop yields. By leveraging the beneficial attributes of these microorganisms, farmers can curtail their dependence on chemical fertilizers and pesticides, thereby mitigating the environmental hazards linked with conventional agricultural practices [2]. Moreover, PGPR-based biofertilizers proffer a financially viable and eco-friendly avenue for fostering sustainable crop production systems [4,5]

Plant Growth-Promoting Rhizobacteria (PGPR) serve as indispensable facilitators of plant growth and vigor through both direct and indirect mechanisms. Directly, PGPR confer phytonutrients such as nitrogen fixation and solubilized minerals such as phosphorus (P), potassium (K), zinc (Zn), and iron (Fe), in addition to other essential mineral nutrients [6,7]. This direct nutritional provisioning significantly bolsters the overall health and developmental trajectory of plants by ensuring access to indispensable elements requisite for their growth processes.

Spinach cultivation necessitates meticulous attention to detail and adherence to specific environmental parameters. It is classified as a cool-season crop, thriving optimally within a temperature range of 10 to 21 °. This delineated temperature bracket is imperative for maximizing growth dynamics and ensuring the qualitative attributes of the harvested produce. Spinach garners acclaim for its nutritional potency, serving as a preeminent reservoir of vital nutrients such as calcium, iron, vitamin A, vitamin C, and folic acid [8,9]. Its exalted nutritional profile renders it a coveted choice among consumers who prioritize the integration of healthful dietary components.

Material and methods

Collection of Rhizospheric Soil Sample

The rhizospheric soil samples of Banana (Musa paradisiaca), member of Musaceae family was collected during the months of August-October 2023 from the four sites of agricultural farm of Banana situated at Amity University Uttar Pradesh, India (28.3239°N 77.1959°E). Agricultural practices at these sites strictly adhered to organic cultivation methodologies.

Each sampling occasion involved carefully removing three crop plants along with their surrounding soil from their respective locations. These plant-soil units were then transported to the laboratory in sealed zip lock bags under controlled conditions to preserve sample integrity. Upon reaching the laboratory, a detailed procedure was followed to systematically separate non-rhizospheric soil and large soil aggregates from the roots. The soil attached to the root systems of each plant was meticulously isolated and combined to create a composite rhizospheric soil sample representing each sampling site. These composite samples underwent standardized protocols for subsequent analysis.

Isolation of PGPB

Rhizobacteria were obtained from the soil sample through the serial dilution method in Luria-Bertani (LB) medium, consisting of 10 g/L peptone, 5 g/L yeast extract, and 10 g/L NaCl, with 1.5% agar added. In this procedure, 0.1 mL of suitable dilutions of the soil sample was spread onto LB agar plates and then placed in an incubator at 28°C for 24 hours.

Experimental design

Field experiment were performed at the experimental Organic Farm of Amity University Noida for research on Enhancing Spinach Growth and Yield Through the Synergistic Effects of Rhizobacter-Enriched Compost in Gautam Buddha Nagar. Latitude: 28.54 o N and 77.39 o E longitude and at an altitude of 200m above the sea level.

Experimental details:

The experiment was laid out in Randomized block design (RBD) with 6 treatments and each treatment was replicated 3 times. The various treatments were randomly throughout the plots unbiased.

Experimental Design

T1	Control
T2	Compost (3kg)
T3	(AIOA Strain+biochar) (100ml +2kg)
T4	AIOA Strain (200ml)
T5	(AIOA Strain+Compost) (100ml+3kg)
T6	Biochar (2kg)

Layout detail of experiment

Design	Randomized block design(RBD)
Number of treatments	6
Number of replications	3
Plot size	32sqm(8m*4m)
Row to row spacing	25cm
Plant to plant spacing	10cm
Total number of plots	24
Date of sowing	20th November
Method of sowing	Broadcasting
Seed rate	15kg/ha

Determination of photosynthetic pigments: Total Chlorophyll

To determine chlorophyll content a half gram of fresh leaf was crushed in 10 ml of 80% methanol, then the resulting extract was centrifuged at 10,000 rpm for 10 minutes. Absorbance readings were taken using a spectrophotometer and chlorophyll a and b levels were computed based on the method outlined by [10].

Analysis of morphological growth parameters of Spinach plants

After bacterial inoculation, the spinach plants were harvested, and various growth parameters were assessed. This included measuring plant height, number of leaves, width of leaf, root length. To determine the fresh weight, the roots and shoots of spinach plants were excised using a sterile blade and weighed separately on an electronic balance.

Statistical analyses

All data of Spinach growth parameters obtained after inoculation of bacteria in the field were analysed by one-way ANOVA followed by Tukey's test with bacterial inoculation method considered as independent variable. All the statistical analyses were calculated at significance level $p = 0.05$ through SPSS software. The experiments were performed in triplicates, the mean and standard deviation were calculated using Microsoft Excel 2016.

Results

Isolation and collection of the bacteria from rhizospheric soil of Banana Plant

The soil sample obtained from Amity University's organic farm was employed for bacteria isolation. Serial dilution technique was used to dilute the soil sample. 0.1 mL of the diluted soil was spread onto LB Agar plates comprising 10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, and 1.5% agar, and then placed in a 28°C incubator for 24 hours. Morphologically distinct colonies were chosen and streaked on additional LB Agar plates. After further incubation, the streaked bacterial isolates were sub-cultured and utilized for subsequent investigations.

Fig.1. Bacterial culture inoculated in Luria-bertani medium

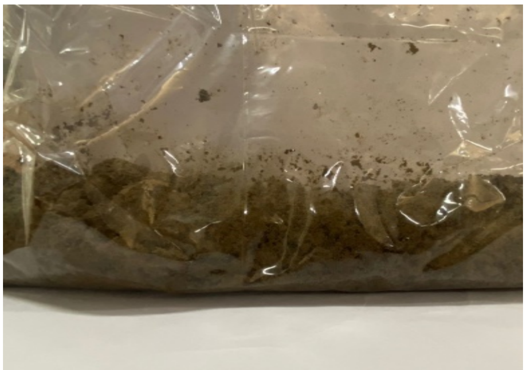

Fig.2. Rhizospheric soil collected in a plastic bag

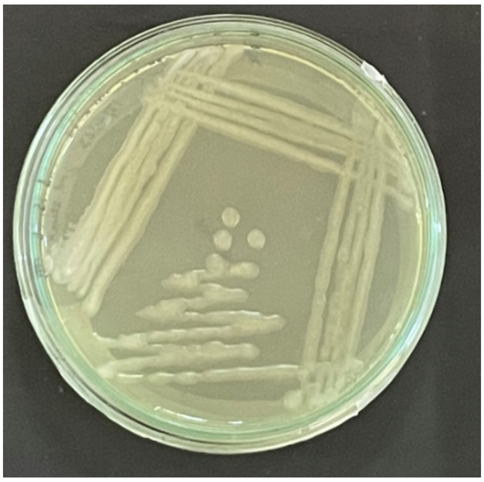

Fig.3. Bacterial culture streaked on LB agar Plate

Experimental details

The experiment was planned using a Randomized Block Design (RBD), featuring six distinct treatments, each treatment replicated thrice. These treatments comprised a control group (T1), compost application (T2), biochar integrated with bacterial AIOA strain (T3), sole application of bacterial AIOA strain (T4), a combination of bacterial AIOA strain and compost (T5), and exclusive biochar application (T6). Each treatment was randomly allocated across the experimental plots to ensure impartial distribution.The experimental plots comprised 24 units, each spanning 32 square meters (8m x 4m). Within these plots, rows were spaced 25cm apart, with a 10cm distance between individual plants, optimizing space utilization while upholding experimental integrity. Spinach seeds were uniformly sown across all plots on November 20th, employing a broadcasting technique for even seed dispersion.To attain optimal plant density and uniformity, a seed rate of 15kg/ha was applied across the experimental plots.

Fig.4. Plot layout for spinach treatments

Determination of chlorophyll content

In chlorophyll estimation, it was observed that in treatment T5 (AIOA strain + compost), the chlorophyll a content was recorded as 19.74382mg/L, while the chlorophyll b content was measured at 33.46097mg/L , resulting in a total chlorophyll content of 53.20479mg/L. In comparison, treatment T1 (control) exhibited lower values. However, in treatment T4 (AIOA Strain), the chlorophyll a content was found to be 21.402629mg/L, the chlorophyll b content was 30.275854mg/L, and the total chlorophyll content was 51.678483 mg/L.

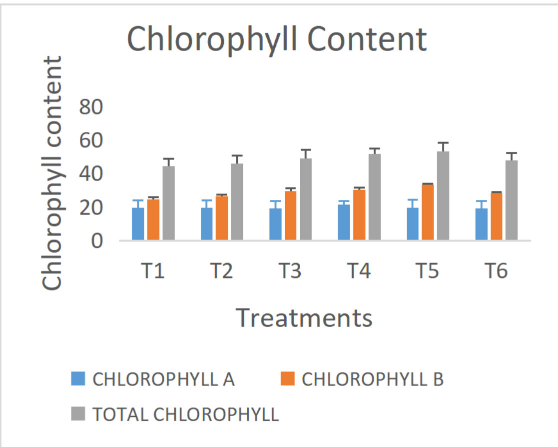

Fig.5. Graphical analysis of spinach Chlorophyll content treatments

Growth Indicators

Plant Height

Based on the growth estimation, treatment T5 (AIOA Strain + Compost) demonstrated the most significant effect on plant height , with a recorded plant height of 36.3 cm, surpassing all other treatments. Conversely, the control treatment (T1) exhibited comparatively shorter plants, measuring 22.4 cm in height. Notably, in treatment T3 (AIOA Strain + Biochar), the plant height reached 27.667 cm, showing an increase compared to treatment T4 (AIOA Strain), where the plant height was slightly lower at 26.867 cm. These observations were made at 90 days after sowing (DAS), highlighting the efficacy of the (AIOA Strain + Compost) combination in promoting superior plant growth.

Width of Leaf

Based on the growth estimation, treatment T5 (AIOA Strain + Compost) demonstrated the tallest plants, with a recorded plant height of 7.633 cm, surpassing all other treatments. Conversely, the control treatment (T1) exhibited comparatively shorter plants, measuring 4.567 cm in height. Notably, in treatment T4 (AIOA Strain), the plant height reached 6.500cm, showing an increase compared to treatment T3 & T6 (AIOA Strain), where the plant height was slightly lower at 6.167 cm. These observations were made at 90 days after sowing (DAS), highlighting the efficacy of the AIOA Strain+Compost combination in promoting superior plant growth.

Root length

Based on the growth estimation, treatment T5 (AIOA Strain + Compost) demonstrated the tallest plants, with a recorded plant height of 11.367 cm, surpassing all other treatments. Conversely, the control treatment (T1) exhibited comparatively shorter plants, measuring 6.067cm in height. Notably, in treatment T6 (Biochar), the plant height reached 7.800cm, showing an increase compared to treatment T4 (AIOA Strain), where the plant height was slightly lower at 7.467 cm. These observations were made at 90 days after sowing (DAS), highlighting the efficacy of the AIOA Strain+Compost combination in promoting superior plant growth.

Leaves per plant

Based on the growth estimation, treatment T5 (AIOA Strain + Compost) demonstrated the tallest plants, with a recorded plant height of 34.300 cm, surpassing all other treatments. Conversely, the control treatment (T1) exhibited comparatively shorter plants, measuring 22.767cm in height. Notably, in treatment T3 (AIOA Strain+Biochar), the plant height reached 27.667, showing an increase compared to treatment T4 (AIOA Strain), where the plant height was slightly lower at 26.867 cm. These observations were made at 90 days after sowing (DAS), highlighting the efficacy of the AIOA Strain+Compost combination in promoting superior plant growth.

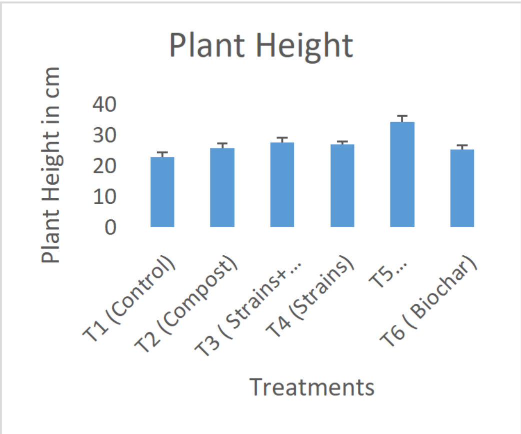

Fig.6. Graphical analysis of spinach plant height according to treatments

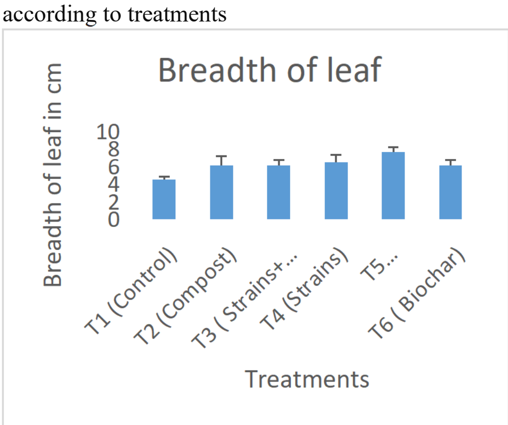

Fig.7. Graphical analysis of spinach plant's breadth of leaf

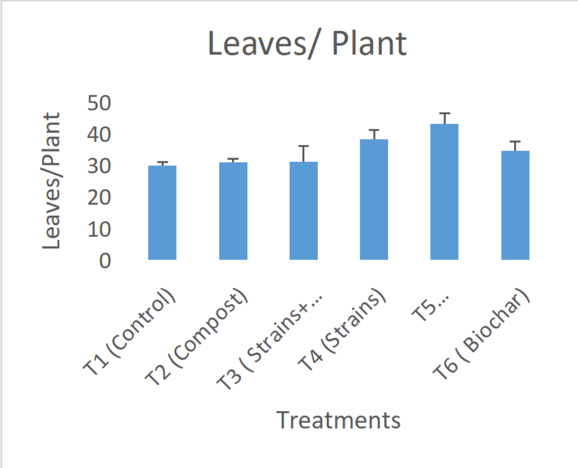

Fig.8. Graphical analysis of spinach Leaves/plant for different treatments

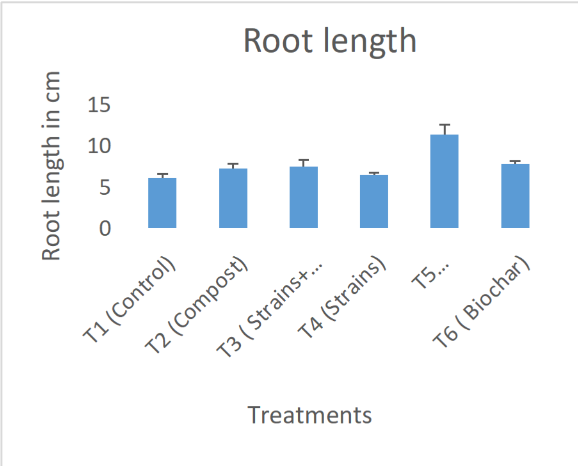

Fig.9. Graphical analysis of spinach root length for different treatments

Discussion

The findings of this study underscore the significant potential of harnessing Plant Growth-Promoting Rhizobacteria (PGPR) and compost to enhance spinach growth and yield. Through a series of experiments and analyses, we have demonstrated the positive impact of these microbial and organic amendments on various aspects of spinach cultivation.

Effect of Treatments on Chlorophyll

Our results indicate that treatment T5 (AIOA Strain + Compost) exhibited the highest chlorophyll content, with chlorophyll a, chlorophyll b, and total chlorophyll levels surpassing those of other treatments. This suggests that the synergistic interaction between PGPR and compost may stimulate chlorophyll synthesis, thereby enhancing photosynthetic efficiency and overall plant vigor.

These findings are consistent with previous studies illustrating the role of PGPR in promoting chlorophyll production and plant growth [10]

Impact of Treatments on Growth Parameters

Treatment T5 (AIOA Strain + Compost) consistently outperformed other treatments in terms of various growth parameters, including plant height, breadth of leaf, root length, and number of leaves per plant. This indicates that the combined application of PGPR and compost significantly enhanced spinach growth and development compared to individual treatments or the control. The observed increase in growth parameters highlights the potential of PGPR and compost as effective biofertilizers for sustainable crop production.

Additionally, treatment T3 (AIOA Strain + Biochar) showed promising results, particularly in terms of plant height and root length, suggesting that the combination of PGPR with biochar may also contribute to improved plant growth. Biochar, known for its ability to improve soil structure and nutrient retention, may have synergistic effects when combined with PGPR, enhancing their efficacy in promoting plant growth [8]

Implications for Sustainable Agriculture

The findings of this study have important implications for sustainable agriculture practices. By utilizing PGPR and compost-based biofertilizers, farmers can reduce their dependence on chemical fertilizers and pesticides, thus mitigating environmental risks associated with conventional agricultural practices. Moreover, the use of microbial and organic amendments can improve soil health, nutrient cycling, and overall ecosystem resilience, contributing to long-term agricultural sustainability.

Conclusion

This study demonstrates the potential of harnessing Plant Growth-Promoting Rhizobacteria (PGPR) and compost to enhance spinach growth and yield in agricultural settings. Through a comprehensive experimental approach, we have elucidated the positive effects of these microbial and organic amendments on various growth parameters and photosynthetic pigment content of spinach plants.

Our results reveal that the combined application of PGPR and compost, particularly in treatment T5 (AIOA Strain + Compost), significantly promotes spinach growth compared to individual treatments or the control. The observed increase in plant height, breadth of leaf, root length, and number of leaves per plant highlights the efficacy of the synergistic interaction between PGPR and compost in enhancing plant vigor and development.

Furthermore, the determination of chlorophyll and carotenoid content indicates that treatment T5 exhibits higher levels of these photosynthetic pigments, suggesting improved photosynthetic efficiency and overall plant health. This finding underscores the importance of PGPR and compost in enhancing nutrient uptake and assimilation, thereby contributing to increased crop productivity.

The outcomes of this research have significant implications for sustainable agriculture, as the use of PGPR and compost-based biofertilizers offers a cost-effective and eco-friendly approach to enhance soil fertility, reduce chemical inputs, and mitigate environmental risks associated with conventional farming practices.

Moving forward, further investigation into the underlying mechanisms driving the observed effects, as well as optimization of application strategies for different crop species and agroecological conditions, will be valuable for maximizing the potential of PGPR and compost in sustainable crop production systems.

Overall, this study contributes to the growing body of knowledge on the utilization of microbial and organic amendments for improving agricultural sustainability and underscores the importance of integrating these approaches into modern farming practices to meet the challenges of food security and environmental stewardship.

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