Original Scientific paper
10.7251/AGREN2403005P
UDC 633.18:631.8

COMPARISON BETWEEN SYNTHETIC CHEMICAL FERTILIZERS AND SOME BIOFERTILIZERS ON THE ROOT ENDOPHYTIC FUNGI AND RICE GROWTH

Nipuni Nayanathara PREMATHILAKA, Neelamanie YAPA*

Department of Biological Sciences, Faculty of Applied Sciences, Rajarata University of Sri Lanka, Mihintale, Sri Lanka
*Corresponding author: neelamanie@as.rjt.ac.lk

ABSTRACT

Rice (*Oryza sativa*) is the most consumed cereal in the world. However, the widespread use of synthetic chemical fertilizers in rice cultivation in Sri Lanka has compromised the sustainability of rice farming. A pot experiment was conducted to explore the impact of nitrogen-fixing bacteria; *Azospirillum* spp. and phosphorus-solubilizing bacteria *Pseudomonas* spp., *Bacillus* spp., and *Trichoderma* spp. based biofertilizers on the endophytic fungal population and rice growth. The fungal endophytes isolated from six treatments: T1: normal soil, T2: nitrogen-fixing *Azospirillum* spp., T3: phosphorus-solubilizing *Pseudomonas* spp., *Bacillus* spp.+rock phosphate, T4: *Trichoderma* spp., T5: *Azospirillum* spp.+*Pseudomonas* spp., + *Bacillus* spp.+*Trichoderma* spp.+ rock phosphate, T6: recommended dose of synthetic inorganic fertilizer. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Trichoderma* spp., and *Bipolaris* spp. were isolated as root endophytes. However, the statistical analysis using one-way ANOVA and the Tukey's pairwise comparison test indicated that there was no significant difference in the diversity and abundance of root endophytes among the treatments ($p>0.05$). Furthermore, considering various biometric parameters of rice, including shoot length, root length, number of tillers per plant, flag leaf length, dry biomass, 100-grain weight, number of grains per panicle, and harvest index showed significant differences ($p<0.05$) when comparing the control and the respective treatments. Moreover, there were significant variations in soil nitrogen and phosphorus concentrations, pH, and conductivity among the treatments. This suggested that the development and use of such biofertilizers could be served as a sustainable alternative, reducing the reliance on synthetic chemical fertilizers in rice cultivation in Sri Lanka.

Keywords: *Endophytes, Growth and yield parameters, Rice, Sustainability.*

INTRODUCTION

The global agricultural industry is facing an increasing challenge of feeding a growing population. In response to this challenge, various agricultural methods

have been employed to boost crop production and safeguard crops from pathogens and pests (Tilman *et al.*, 2002). These methods include the use of chemical or synthetic fertilizers, pesticides, and insecticides to enhance yields and protect against pest damage. However, the widespread use of these agro-chemicals has sparked significant public concern about the sustainability, safety, and reliability of our food supply (Daniel *et al.*, 2022).

Excessive use of synthetic fertilizers, which contain nitrogen (N), phosphorus (P), potassium (K), and sulfur (S), can weaken plant roots, increase disease susceptibility, acidify soil, and cause water eutrophication (Daniel *et al.*, 2022). The release of excess N through various pathways leads to environmental issues such as global warming, greenhouse gas emissions, nitrate pollution, soil degradation, and reduced soil microflora (Zhang *et al.*, 2023). Therefore, eco-friendly alternatives are essential for sustainable agriculture (Pathirana &Yapa, 2020). Biofertilizers have emerged as a sustainable alternative to synthetic fertilizers due to their ability to boost crop productivity while reducing environmental impacts (Sahoo *et al*., 2012). Biofertilizers are beneficial microorganisms, such as nitrogen-fixing, phosphate-solubilizing, potassium-solubilizing microorganisms, which enhance soil nutrient availability, induce disease resistance, tolerate abiotic stresses and increase plant growth and yield (Mahanty et al., 2017; Yapa *et al.*, 2022).

Rice, a staple food in Sri Lanka and a crucial global crop, requires significant water and nutrients like N, P and K for optimal growth (Sewwandi *et al.*, 2023). Research into endophytes, microorganisms living within plant tissues without causing harm, offers potential benefits for rice farming (Gouda *et al.*, 2016). Nearly all terrestrial plants are believed to harbor endophytic fungi, making them a promising focus for improving agricultural practices (Reis *et al*., 2022). Recent research has emphasized the rich biodiversity and ecological significance of endophytic fungi, which interact symbiotically with host plants and other microbiomes (Alam *et al.*, 2021). These fungi contribute to soil sustainability and environmental protection in an eco-friendly and cost-effective manner. In rice, endophytic microorganisms have been shown to promote growth, and improve disease resistance (Naik *et al.*, 2009). The present study focused on how biofertilizers containing beneficial bacteria and fungi affect the diversity of endophytic fungal populations and the growth of rice.

MATERIALS AND METHODS

Study Site. The experiment was conducted as a pot experiment under natural light conditions in a planthouse at the Faculty of Applied Sciences, Rajarata University of Sri Lanka, located in Mihintale, Anuradhapura district, Sri Lanka (GPS 8.3534, 80.5021). The study area receives an annual rainfall of 1000–1500 mm, with temperatures ranging between 28 and 32 °C throughout the year.

Sample collection, isolation of microorganisms and identification - Soil samples were collected from rice fields around Mihintale following the standard sampling techniques. Four samples were taken from each site at a depth of 0-15 cm and combined to create one composite sample. Any surface litter was removed before

sampling. In the laboratory, the soil was mixed thoroughly for uniformity. A subsample of soil was air-dried for determining chemical and physical characteristics, including measuring and recording the electrical conductivity and pH of the samples (Manzoor *et al*., 2017). 1g from the each collected soil samples was measured and suspended in 10 ml of sterile distilled water to make a soil suspension. Then, 10- fold serial dilutions were made from each sample up to 10^{-8}. Aliquots (1 mL) of dilutions 10^{-1}, 10^{-3}, 10^{-5}, 10^{-8} were spread plated onto four different selective media: Pikovskaya's (PKV) medium for phosphate-solubilizing bacteria (PSB), King's B (KB) medium for fluorescent pseudomonads, BTB-1 medium for *Azospirillum* spp., potato dextrose agar (PDA) medium for *Trichoderma* spp. All plates were incubated at 32 °C for optimal growth of target organism.

Distinct colonies of *Azospirillum* spp. were identified on BTB-1 plates based on morphology and color after 2 days and sub-cultured for further confirmation (Bashan *et al*., 2011). *Pseudomonas* spp. colonies with fluorescent pigment on KB plates were identified and sub-cultured for confirmation (Rainey *et al.,* 2014). Phosphate-solubilizing *Bacillus* spp. were identified by clear halos on PKV plates and confirmed through sub-culturing and biochemical identification methods (Mayadunna *et al.*, 2023). *Trichoderma* spp. colonies on PDA plates were sub-cultured with antibiotics for confirmation based on characteristic morphology (e.g., aerial mycelia, conidia).

Morphological characteristics of colonies such as shape, size, margin, elevation, surface, and texture were noted for each bacterial colony (Manzoor *et al*, 2017). Motility of bacterial cells were tested by the hanging drop method. Biochemical tests were conducted to identify the bacterial colonies at the generic level, following the procedures outlined in Bergey's Manual of Systematic Bacteriology. Gram stain, endospore stain (Hussey and Zayaitz, 2007), catalase test (Reiner, 2010), oxidase test (Shields and Cathcart, 2010), KOH test, starch hydrolysis test (Lal, 2012), casein hydrolysis test (Salisbury and Likos, 1972), and gelatin hydrolysis test (Cruz and Torres, 2012) were carried out for specific bacterial identification.

Trichoderma spp. colonies were identified morphologically, started white and became pale to dark green as they mature due to spore production. The colony surface was initially velvety but became rough or bumpy as spores develop, with an even and well-defined margin. (Siddiquee, 2017). Slide culture was done to observe microscopic characteristics of *Trichoderma* spp.

Preparation of inoculums

Pure cultures of *Bacillus subtilis*, *Pseudomonas* spp., *Azospirillum* spp., and *Trichoderma* spp. were used to prepare bacterial inoculants using sterile water, following the McFarland method. Bacterial cultures were standardized to a turbidity level of 0.5 McFarland, while fungal cultures were standardized to a 1 McFarland standard for optimal testing.

Pot trial for determination of the effect of the biofertilizer

A pot trial was conducted in the greenhouse at Rajarata University of Sri Lanka, to study the impact of biofertilizers on the growth and yield of the BG 358 rice variety, which can be harvested after 3.5 months of growth. Seeds were obtained from the regional agricultural center in Anuradhapura, Sri Lanka. Soil from rice fields in Anuradhapura was collected and sterilized for nursery preparation. The seeds underwent a two-step germination process and were then sown in nursery trays filled with sterilized soil. Environmental parameters were monitored throughout the study, with irrigation provided daily. After 14 days, seedlings were transplanted into individual pots filled with compost and soil from the rice fields in Anuradhapura, and submerged conditions were maintained throughout (Fig. 1).

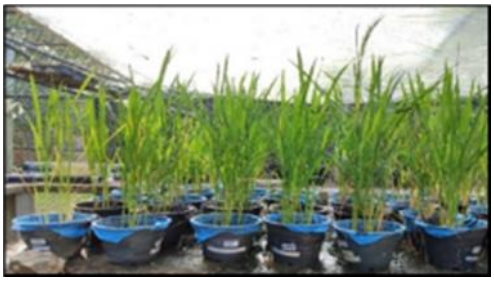

Figure 1- Ten (10) weeks old cultivated rice plants

The treatments for the pot trial were as follows:

T1: Normal soil

T2: Nitrogen-fixing bacteria (*Azospirillum* spp.)

T3: Phosphorus-solubilizing bacteria (*Pseudomonas* spp., *Bacillus* spp.) + Rock phosphate

T4: *Trichoderma* spp.

T5: Nitrogen-fixing bacteria (*Azospirillum* spp.) + Phosphorus-solubilizing bacteria (*Pseudomonas* spp., *Bacillus* spp.) + *Trichoderma* spp. + Rock phosphate

T6: Recommended rates of inorganic fertilizers

Evaluation of endophytic fungal population

Surface sterilization - Root samples were washed with running water to remove debris and soil particles. They were then sequentially immersed in 70% ethanol for 3 minutes, 5% sodium hypochlorite for 2 minutes, and 70% Clorox solution for 1 minute. Afterwards, the samples were rinsed three times with sterile distilled water. To validate surface sterilization, the last rinsing water was plated on Potato Dextrose Agar (PDA) medium containing tetracycline to prevent bacterial growth. The absence of fungal growth in the culture indicated successful surface sterilization (Schulz et al., 1993).

Isolation of endophytic fungi- Surface sterilized **r**oot samples were cut into 1 cm segments and surface sterilized. Four segments from each sample were randomly selected for isolation. The segments were dried and placed onto PDA plates with 50 µg mL^{-1} of Tetracycline to prevent bacterial contamination. Plates were sealed

and incubated at room temperature for 5-8 days. Developing hyphal tips from fungal colonies were transferred onto fresh PDA plates for obtaining pure cultures. Plates were observed daily for fungal growth, and all observed fungi were sub-cultured for purification.

Morphological and microscopic identification of endophytic fungi: Fungal endophytes were identified by observing colony morphological characteristics on PDA in Petri dishes. After 7 days of incubation, traits such as colony shape, size, elevation, surface, margin, color, pigmentation, and the presence of fruiting bodies and spore structures were recorded. Stereo and compound microscopes were used, following standard mycological protocols (Kichu *et al.*, 2020). Slides of each fungal species were prepared by slide culture method and examined under a compound light microscope (Alsharari *et al*., 2022). Identification of the isolates was done primarily based on available identification keys (Ainsworth, 1961).

Isolation rate (IR) was determined as the number of isolates obtained from plant segments divided by the total number of segments incubated. Colonization frequency percentage (CF%) of the endophytic fungi was calculated as, CF% = (Number of segments colonized by an endophyte / Total number of segments analyzed) * 100 (Hata and Futai, 1995).

Fungal diversity indices- Endophytic Fungal species diversity was reported as species richness (R), Shannon's diversity index (H′) (Feranchuk *et al*., 2018), and Simpson's index of dominance (D′) (Simpson, 1949). These metrics were calculated for each treatment using online tools (available at: http://www.alyoung.com/labs/biodiversity_calculator.html). Species richness indicated the number of different species present, while Shannon's diversity index measured overall diversity based on the abundance of each species. Simpson's index of dominance quantified the dominance of species within each treatment. These calculations provided insights into the diversity of endophytic fungi across the treatments.

Biometric data collection- Biometric data, including plant height, number of leaves per plant, flag leaf length, and tillers per plant, were recorded at four- time intervals: 3, 6, 9, and 12 weeks after transplantation. Yield related attributes per plant, such as number of productive tillers, panicle length, grains per panicle, hundred-grain weight, dry plant biomass, and harvesting index, were recorded at harvest. (Kalamulla *et al*., 2022).

Soil chemical analysis - Total Nitrogen content in each treatment were assessed using Kjeldahl's method (Bremner, 1960; Abrams *et al*. 2014), which involves digestion, distillation, and ammonia determination). Phosphorus content in soil was determined using the Vandate-molybdate method **(**Rajani, 2019)

Viable microbial cell count: The number of viable microorganisms in each treatment was assessed by a colony-forming unit (CFU) per 1 g of soil samples. This involved serial dilution of samples, plating onto agar plates, and incubation for colony growth. The CFU count per 1 g of soil was calculated based on the number of countable colonies on plates and the dilution factor. This count was performed three times during the study period.

Statistical analysis- The treatments were arranged in a randomized complete block design (RCBD) with five replicates. Data were statistically analyzed using One-way ANOVA and the Tukey's Pairwise Comparisons test.

RESULTS AND DISCUSSION

The effect of bacterial and fungal biofertilizers on endophytic fungal population

It was observed that there were 47 fungal isolates out of 96 plant segments. Although the number of fungal isolates in different treatments were not statistically different ($p<0.05$), the highest number of isolates were observed from treatments T2 (*Azospirillum* spp.) and T6 (inorganic fertilizer), while the lowest number was from T5 (*Azospirillum* spp. + *Bacillus* spp. + *Pseudomonas* spp. + *Trichoderma* spp.) (Fig. 2).

Table 1. The isolation rates of endophytes and their colonization frequencies under different treatments

Treatment	Isolation Rate	Colonization Frequency
T1	0.5625 ±0.239	56.25%
T2	0.625 ±0.144	62.5%
T3	0.4375 ±0.239	43.75%
T4	0.375 ±0.144	37.5%
T5	0.3125 ±0.125	31.25%
T6	0.625 ±0.144	62.5%

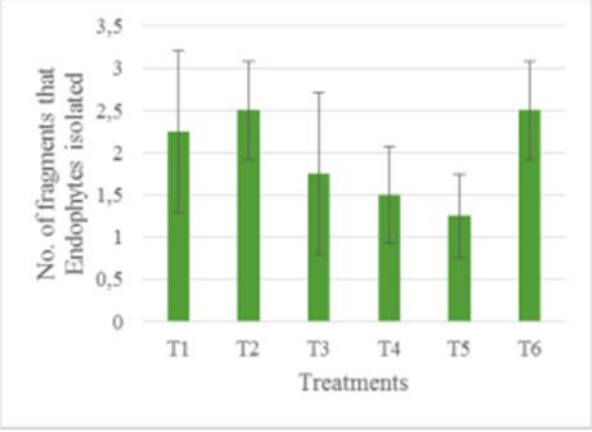

Figure 2. Average number of fragments of endophytes isolated from each treatment

The colonization frequency (CF%) of endophytic fungi was assessed for six treatments. The highest colonization frequencies were observed in treatments T2

(*Azospirillum* spp.) (62.5%), and T6 (inorganic fertilizer), while the lowest, was seen in treatment T5 (*Azospirillum* spp. + *Bacillus* spp. + *Pseudomonas* spp. + *Trichoderma* spp.) (31.25%). This indicated that T5 had lower rates of endophyte isolation and colonization compared to other treatments (Table1).

Diversity indices of the endophytic fungi

Table 2. Diversity indices of the endophytic fungi isolated from the six treatments

	Shannon's diversity index (H′)	**Shannon Equitability Index (EH)**	**Simpson's Diversity Index (D)**	**Dominance Index (1 – D)**	**Berger-Parker Dominance Index**	**Margalef Richness Index**
T1	1.523	0.9463	0.139	0.861	0.3333	1.8205
T2	1.557	0.967	0.133	0.867	0.3	1.7372
T3	1.351	0.975	0.143	0.857	0.285	1.5417
T4	1.011	0.92	0.267	0.733	0.5	1.1162
T5	0.673	0.97	0.4	0.6	0.6	0.6213
T6	1.029	0.937	0.311	0.689	0.5	0.8686

The diversity indices of endophytic fungi (EF) isolated from six treatments were analyzed using multiple indices: Shannon-Weiner, Simpson's, Simpson's diversity (1 - D), Berger-Parker Dominance, and Margalef Richness. The highest diversity, based on Simpson's index of diversity, was observed in T5. However, Shannon's index, which considers both richness and evenness, has been identified T5 as the most diverse. Instead, T2 had a higher Shannon's diversity index, indicating more species and a more even distribution (Table 2). Despite slight differences in fungal endophytic diversity among treatments, statistical analysis revealed no significant difference ($p>0.05$).

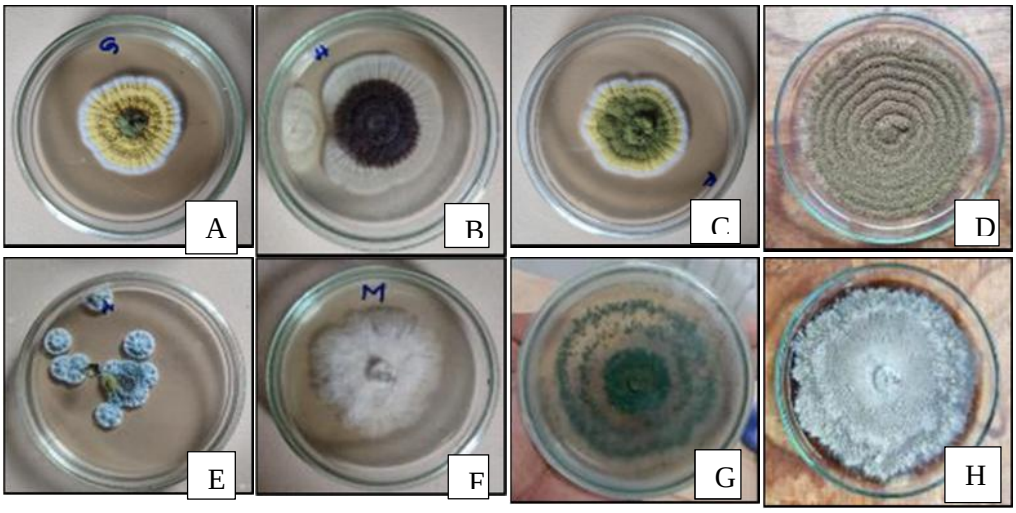

Figure 3. Colony morphology of isolated endophytic fungi (A) Aspergillus flavus (B) Aspergillus niger (C) Aspergillus spp. 3 (D) Aspergillus spp. 4 (E)Penicillium spp. (F) Fusarium spp. (G) Trichoderma spp. (H) Bipolaris spp.

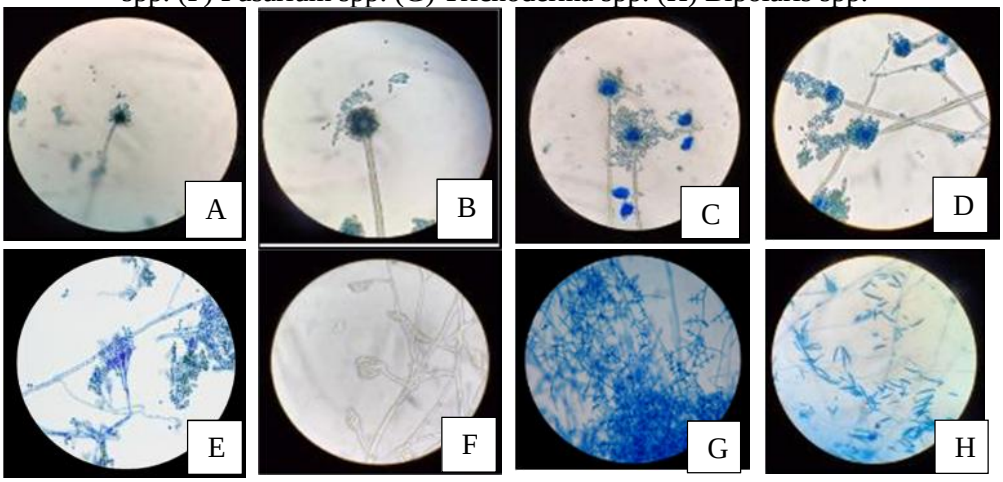

Figure 4. Microscopic view of isolated endophytic fungi (A) Aspergillus flavus, (B) Aspergillus niger, (C) Aspergillus spp. 3 (D) Aspergillus spp. 4 (E) Penicillium spp. (F) Fusarium spp. (G) Trichoderma spp. (H) Bipolaris spp.

The fungal endophytes isolated from the 6 treatments are primarily common genera found in soil. They are *Aspergillus flavus*, *Aspergillus niger* and other two *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Trichoderma* spp. and *Bipolaris* spp. (Fig. 3 and Fig. 4).

Effect of bacterial and fungal biofertilizers on the growth of rice

Growth parameters

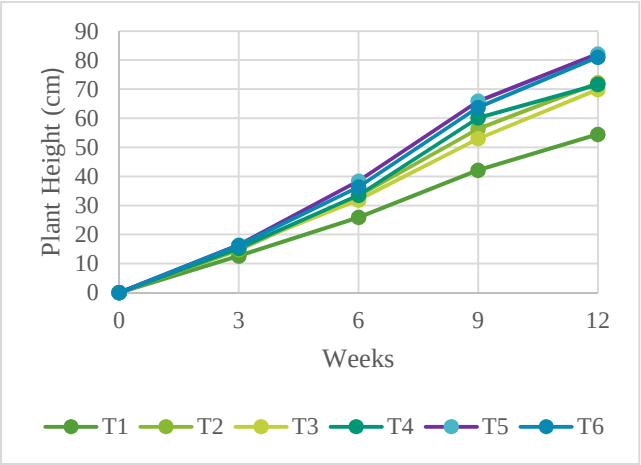

Figure 5. Change of shoot length with the time

Shoot length (cm)- Initially, the plants grew steadily from 0 cm to 70 cm over the first 9 weeks. In the following 3 weeks, the growth rate slowed, reaching 80 cm. This indicated that the overall growth of rice approximately 80 cm in 12 weeks (Fig. 5). Statistical analysis confirmed the significant difference among treatments ($p<0.05$), implying that the growth differences are substantial and not due to chance. Among the treatments, T5, which combined biofertilizer, showed the highest growth rate, indicating the most effective growth-promoting effect (Table 3). This enhanced productivity can be attributed to the synergistic effects of the combined biofertilizers, which improve nutrient availability and uptake by rice plants.

Table 3. Growth-attributing characters of the rice plant according to different fertilizer application

Treatment	Number of tillers per plant	Number of leaves per plant	Flag leaf length (cm)	Root length of rice plants	Shoot dry weight per plant (g)	Root dry weight per plant (g)
T1	2.25±0.95 ^{d}	16.25±1.70^{c}	14.7±1.82^{d}	8±0.71^{d}	6.51±0.93^{d}	2.14±0.91^{c}
T2	4±0.81^{cd}	21±2.44^{b}	22.32±0.90^{c}	9.72±0.60^{c}	8.92±0.98^{cd}	2.11±0.67^{c}
T3	4.25±0.57^{cd}	23.75±1.25^{ab}	22.4±0.95^{c}	12.9±0.49^{b}	11.57±0.81^{bc}	2.95±0.54^{bc}
T4	5±0.81^{bc}	22.75±1.25^{b}	26±1.47^{b}	14.05±0.82^{ab}	14.02±1.62^{b}	3.98±0.68^{b}
T5	7±1.82^{ab}	26.75±1.5^{a}	30.87±1.31^{a}	15.22±0.85^{a}	17.52±1.08^{a}	5.87±0.45^{a}
T6	7.75±1.70 ^{a}	26.75±0.95^{a}	31.62±1.25^{a}	9.12±0.85^{cd}	19.27±1.92^{a}	4.42±0.63^{ab}

The means under each parameter followed by the same letter are not significantly different ($p \leq 0.05$) according to Tukey's pairwise comparisons. Values are means

± standard errors, calculated from four replicates. Different letters (a, b, c, d) indicate significant differences among fertilizer treatments.

Yield parameters

Yield attributing factors were determined such as number of grains per panicle, number of productive tillers, weight of 100 grains and the harvest index (HI). Analysis of these yield parameters revealed significant difference ($p<0.05$) among treatments compared to the control (Table 4).

Harvest index is a measure of the economic yield of a crop, calculated as the ratio of grain yield to total biomass yield. In simple terms, it shows how much of the plant's total biomass is harvested as grains. The highest HI showed by T6 followed by T5, T4 and T3. The control (T1) has the lowest harvest index. Statistical analysis confirmed that a significant difference between these treatments ($p<0.05$), indicating that the observed variations in average panicle lengths are not due to a chance.

Table 4. Yield-attributing characters of the rice plant according to the different fertilizer application

Treatment	Dry weight of 100 seeds	No. of productive tillers	Panicle length	No. of grains per panicle	Harvesting Index
T1	1.28 ± 0.10^{c}	1.75 ± 0.5^{c}	18.05 ± 1.17^{c}	83.5 ± 17.31^{d}	0.255 ± 0.037^{d}
T2	1.33 ± 0.16^{bc}	2.75 ± 0.95^{bc}	19.42 ± 1.10^{c}	99.25 ± 14.26^{cd}	0.287 ± 0.038^{cd}
T3	1.49 ± 0.12^{abc}	3 ± 0.81^{bc}	21.67 ± 1.15^{b}	126.25 ± 21.32^{bc}	0.337 ± 0.013^{bc}
T4	1.59 ± 0.15^{ab}	4 ± 0.81^{b}	23.45 ± 1.14^{b}	146.75 ± 14.88^{ab}	0.390 ± 0.038^{b}
T5	1.73 ± 0.08^{a}	6 ± 0.81^{a}	25.97 ± 0.55^{a}	165 ± 7.70^{a}	0.474 ± 0.043^{a}
T6	1.75 ± 0.05^{a}	6.25 ± 0.95^{a}	26.85 ± 0.41^{a}	168.5 ± 4.79^{a}	0.495 ± 0.038^{a}

The means under each parameter followed by the same letter are not significantly different ($p \leq 0.05$) according to Tukey's pairwise comparisons. Values are means ± standard errors, calculated from four replicates. Different letters (a, b, c, d) indicate significant differences among fertilizer treatments.

Table 5. Basic chemical parameters and total microbial counts of the collected soil samples from each treatment

Treatment	Soil pH (after 12 weeks)	Soil Conductivity (µs)
T1	6.542 ± 0.156^{ab}	84.923 ± 21.417^{d}
T2	6.967 ± 0.179^{a}	134.093 ± 26.860^{cd}
T3	5.717 ± 0.331^{c}	147.5 ± 24.925^{bcd}
T4	6.343 ± 0.248^{b}	193.60 ± 42.362^{bc}
T5	6.112 ± 0.333^{bc}	224.4 ± 46.595^{ab}
T6	6.255 ± 0.188^{bc}	274.70 ± 40.977^{a}

The soil pH values range from about 5 to 7, with the lowest pH in T3 and the highest in T2, indicating slightly acidic conditions influenced by different treatments (Table 5). Soil conductivity, ranging from 75 to 300 µs and indicating

moderately saline conditions, is highest in T6 (inorganic fertilizer) due to soluble salts, followed by T5 and T4, while T2 and T3 have the lowest conductivity, likely due to a smaller impact from their respective treatments.

The treatment with the addition of combined biofertilizer and rock phosphate (T5), was shown the highest number of total culturable bacteria after 9 weeks. This indicated that T5 promotes a thriving bacterial community in the soil, potentially due to improved nutrient availability, the presence of beneficial compounds for certain bacteria, or the other factors. In contrast, T1, representing normal soil, exhibits the lowest number of total culturable bacteria after 9 weeks. The other treatments such as T2, T3, T4, and T6 were indicated the higher bacterial counts than T1 but lower than T5, placing them between these extremes (Fig. 5, Fig. 6).

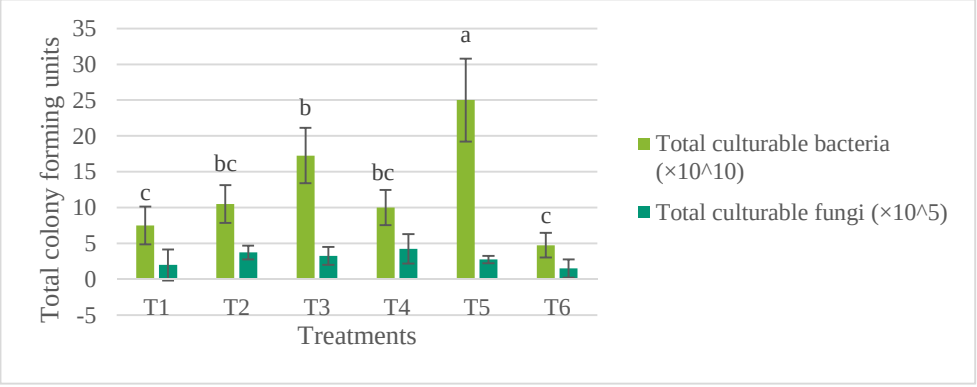

Figure 6. Microbial counts of the collected soil samples from each treatment

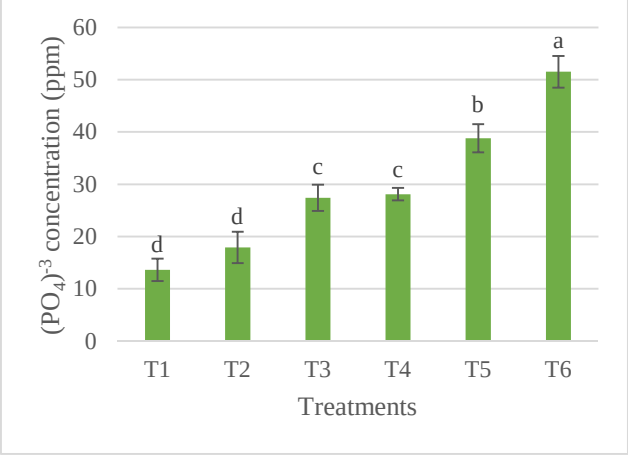

Figure 7. $(PO_4)^{-3}$ concentration of soil in different treatments

Nutrient uptake- It was shown that the highest soil (PO4)-3 concentration, in T6 indicated a high level of readily available phosphate in the soil. T1 and T2, representing normal soil and nitrogen-fixing Azospirillum spp. respectively, have the lowest soil $(PO_4)^{-3}$ concentrations, suggesting limited natural phosphate availability and minimal impact of nitrogen fixing bacteria on phosphate mobilization. However, the nitrogen fixation needs more energy to breakdown triple bond of N_2 hence, absorb more soluble phosphates from the soil.

Table 6. Mean Nitrogen percentage in soil, shoot and root samples of each treatment

Treatment	Total N% in soil	Total N% in root	Total N% in shoot
T1	0.0910 ± 0.0180^{a}	0.6304 ± 0.0511^{e}	1.4955 ± 0.0576^{d}
T2	0.1085 ± 0.0134^{a}	0.7285 ± 0.0302^{d}	1.5656 ± 0.0462^{d}
T3	0.1120 ± 0.0114^{a}	0.8511 ± 0.0239^{c}	1.3694 ± 0.0699^{d}
T4	0.1733 ± 0.1040^{a}	1.0017 ± 0.0542^{b}	1.8703 ± 0.1557^{c}
T5	0.1716 ± 0.0176^{a}	1.3099 ± 0.0478^{a}	2.6619 ± 0.0605^{b}
T6	0.3292 ± 0.0435^{a}	1.2644 ± 0.0239^{a}	3.0121 ± 0.0943^{a}

All the treatments were shown relatively low soil N%, suggesting potential nitrogen deficiency. T5 (combined treatment with bacteria and rock phosphate) has the highest root N%, followed by T6 (inorganic fertilizer). T1 (normal soil) has the lowest root N%. T5 (combined treatment) has the highest shoot N%, significantly higher than all the other treatments. T3 (phosphorus-solubilizing bacteria and rock phosphate) has the lowest shoot N% (Table 6).

In the present study microorganisms capable of fixing nitrogen and solubilizing phosphates were selected for their potential benefits. *Azospirillum* spp. was identified as a nitrogen-fixing microorganism, while *Bacillus* sp., *Pseudomonas* sp., and *Trichoderma* sp. were recognized as phosphate and potassium solubilizing microorganisms. The impact of bacterial and fungal biofertilizers on endophytic fungal populations is complex, influenced by the specific properties of the biofertilizers, plant species, and environmental conditions. The observed effects on endophyte populations can be attributed to beneficial microorganisms competing for resources, triggering systemic resistance in plants, and producing antibiotics and enzymes that target pathogenic endophytes (Qin *et al.,* 2011). *Trichoderma* species can parasitize other fungi, reducing pathogenic endophytes. Additionally, beneficial microbes can create favorable environments for certain endophytes, enhancing nutrient uptake, stress tolerance, and plant growth (Naik *et al*., 2009).

The discovery of many potential plant pathogenic genera as endophytes supports the theory that endophytes can act as latent pathogens (Schulz *et al.*, 1993). However, they can also live commensally or mutualistically within their hosts at different life stages, deriving nutrients and protection while enhancing host resistance through antibiotically active metabolites (Redman *et al.*, 2002).

Normal Soil serves as a valuable baseline for comparison, highlighting the need for nutrient amendments in deficient soils. Understanding natural soil nutrient

dynamics is crucial for developing sustainable management practices. The electrical conductivity of soil indicates the presence or absence of salts, with higher values suggesting higher ion concentrations and vice versa (Mayadunna *et al.*, 2023). In this study, the electrical conductivities of soils from various treatments differed significantly, with no observable correlation with other factors.

Phosphorus-solubilizing bacteria might release organic acids as they solubilize phosphorus, leading to a decrease in soil pH (Alori *et al*., 2017). Inorganic fertilizer often contains acidic compounds like ammonium sulfate, potentially the reason for its lower pH. The T5 might have a cumulative effect from phosphorus-solubilizing bacteria and other components influencing pH.

In T5, the synergy of bacteria, rock phosphate, and *Trichoderma* spp. enhances nutrient uptake efficiency and promotes plant growth more effectively than using individual components. This approach could reduce reliance on inorganic fertilizers, decreasing associated environmental and cost drawbacks (Sabry, 2015). Also, *Azospirillum* (Suhag, 2016) and *Trichoderma* spp. may also contribute to beneficial soil microbial communities and improve soil structure over time. However, the long-term effectiveness and sustainability compared to inorganic fertilizers need further investigation. Optimizing the specific bacterial combination and application strategies is crucial for maximizing benefits.

Phosphorus-Solubilizing bacteria (T3) and Nitrogen fixing bacteria (T2) might addressed specific nutrient deficiencies (phosphorus or nitrogen) while potentially having less impact on other soil properties compared to inorganic fertilizers. The inoculation of beneficial microorganisms into the rhizosphere of rice plants enhances nutrient availability, which, when absorbed by the plants, positively influences their growth and yield parameters. The application of combined biofertilizers to rice plants resulted in increased yield components, including the number of effective tillers per plant, panicle length, and the number of grains per panicle. The biofertilizers enhanced the allocation of additional nitrogen (N) and phosphorus (P) to panicles, which increased productivity and grain yield. Interestingly, plants treated with combined biofertilizer produced higher yield same as inorganic fertilizer. This finding supports the promotion of ecological agriculture as an alternative to the exclusive use of chemical fertilizers.

The combined use of *Trichoderma* with various other plant growth promoting rhizobacteria has shown significant synergistic effects in enhancing plant growth, improving nutrient uptake, and increasing crop yields. A field experiment at Jabalpur, India in 2017-18 showed that the combined application of *Trichoderma viride*, *Pseudomonas fluorescence*, and *Azotobacter chroococcum* significantly improved the growth and yield of chilli cv. Arka Lohit. The treatment resulted in the tallest plants and the highest yield, with the best results recorded in the combined treatment of *Trichoderma* and PGPR (Singh and Sharma, 2019). According the another study the inoculation of *Azospirillum* sp. not only promoted rice growth but also influenced association of bacteria with rice in both the base and shoot of the plant (Bao *et al.*, 2013). Hossain *et al.*, (2015) revealed that the

Azospirillum inoculation significantly increased all plant growth parameters, and seed germination as well.

While inorganic fertilizer provides a quick and effective solution for nutrient deficiencies, its drawbacks necessitate exploring alternative approaches. The application of bacteria, rock phosphate, and *Trichoderma* spp. (T5) were shown more promising approach of sustainable nutrient use in rice farming. However, further research is needed to fully understand and optimize its long-term benefits and ensure its wider applicability. Ultimately, a combination of strategies considering specific soil conditions, plant needs, and long-term sustainability goals are likely to be the most effective approach for managing soil fertility and plant growth.

CONCLUSIONS

This study revealed that the colonization and diversity of endophytic fungi were not significantly affected by the use of biofertilizers or synthetic inorganic fertilizers. But there can be long term effects of applying these fertilizers. The application of biofertilizers in rice fields significantly increased shoot and root growth in rice plants under submerged conditions, leading to improved plant productivity, and yield. Therefore, biofertilizers have the potential to optimize rice yield sustainably. Since there was no significant difference of yield increment of rice between biofertilizer and inorganic fertilization, biofertilizers can be used as an alternative to synthetic inorganic fertilizer in rice farming.

REFERENCES

Abrams, D., Metcalf, D., & Hojjatie, M. (2014). Determination of Kjeldahl nitrogen in fertilizers by AOAC Official Method SM 978.02: effect of copper sulfate as a catalyst. *Journal of AOAC International*, *97*(3), pp.764-767.

Ainsworth, G. C. (1961). Mycology Manual. *British Medical Journal*, *2*(5264), p.1409.

Alam, B., Lǐ, J., Gě, Q., Khan, M. A., Gōng, J., Mehmood, S., Yuán, Y. & Gǒng, W. (2021). Endophytic fungi: from symbiosis to secondary metabolite communications or vice versa? *Frontiers in Plant Science*, *12*, p.791033.

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology*, *8*, p.971.

AlSharari, S. S., Galal, F. H., & Seufi, A. M. (2022). Composition and diversity of the culturable endophytic community of six stress-tolerant dessert plants grown in stressful soil in a hot dry desert region. *Journal of Fungi*, *8*(3), p,241.

Bao, Z., Sasaki, K., Okubo, T., Ikeda, S., Anda, M., Hanzawa, E., Kakizaki, K., Sato, T., Mitsui, H. & Minamisawa, K. (2013). Impact of *Azospirillum* sp. B510 inoculation on rice-associated bacterial communities in a paddy field. *Microbes and Environments*, *28*(4), pp.487-490.

Bashan, Y., Trejo, A., & de-Bashan, L. E. (2011). Development of two culture media for mass cultivation of *Azospirillum* spp. and for production of inoculants to enhance plant growth. *Biology and Fertility of Soils*, *47*(8), pp.963-969.

Bremner, J. M. (1960). Determination of nitrogen in soil by the Kjeldahl method. *The Journal of Agricultural Science*, *55*(1), pp.11-33.

Dela Cruz, T. E. E., & Torres, J. M. O. (2012). Gelatin hydrolysis test protocol. *American Society for Microbiology*. pp.20161-10.

Daniel, A. I., Fadaka, A. O., Gokul, A., Bakare, O. O., Aina, O., Fisher, S., Burt, A.F., Mavumengwana, V., Keyster, M. & Klein, A. (2022). Biofertilizer: the future of food security and food safety. *Microorganisms*, *10*(6), p.1220.

Feranchuk, S., Belkova, N., Potapova, U., Kuzmin, D., & Belikov, S. (2018). Evaluating the use of diversity indices to distinguish between microbial communities with different traits. *Research in Microbiology*, *169*(4-5), pp.254-261.

Gouda, S., Das, G., Sen, S. K., Shin, H. S., & Patra, J. K. (2016). Endophytes: a treasure house of bioactive compounds of medicinal importance. *Frontiers in Microbiology*, *7*, p.1538.

Hata, K., & Futai, K. (1995). Endophytic fungi associated with healthy pine needles and needles infested by the pine needle gall midge, Thecodiplosis japonensis. *Canadian Journal of Botany*, *73*(3), pp.384-390.

Hossain, M. M., Iffat, J., Salina, A., Rahman, M. N., & Rahman, S. M. B. (2015). Effects of Azospirillum isolates isolated from paddy fields on the growth of rice plants. *Research in Biotechnology*, *6*(2), pp.15-22.

Hussey, M. A., & Zayaitz, A. (2007). Endospore stain protocol. *American Society for Microbiology*, *8*, pp.1-11.

Kalamulla, R., Sandaruwan, D., Karunarathna, S. C., Stephenson, S. L., Tibpromma, S., Elgorban, A. M., Al-Rejaie, S., Yapa, P.N. & Suwannarach, N. (2022). Assessment of community dynamics of arbuscular mycorrhizal fungi in the rice (*Oryza sativa* L.) Rhizosphere and potential application as biofertilizer. *Sustainability*, *14*(24), p.16537.

Kichu, A., Ajungla, T., Nyenthang, G., & Yeptho, L. (2020). Colonial and Morphological characteristics of soil fungi from Jhum Land. *Indian Journal of Agricultural Research*, *54*(1), pp.1-9.

Lal, A., & Cheeptham, N. (2012). Starch agar protocol. *American Society for Microbiology*, *1*, pp.1-9.

Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, P., Das, B., Ghosh, A., & Tribedi, P. (2017). Biofertilizers: a potential approach for sustainable agriculture development. *Environmental Science and Pollution Research*, *24*, pp.3315-3335.

Manzoor, M., Abbasi, M. K., & Sultan, T. (2017). Isolation of phosphate solubilizing bacteria from maize rhizosphere and their potential for rock phosphate solubilization–mineralization and plant growth promotion. *Geomicrobiology Journal*, *34*(1), pp.81-95.

Mayadunna, N., Karunarathna, S. C., Asad, S., Stephenson, S. L., Elgorban, A. M., Al-Rejaie, S., Kumla, J., Yapa, N. & Suwannarach, N. (2023). Isolation of phosphate-solubilizing microorganisms and the formulation of biofertilizer for sustainable processing of phosphate rock. *Life*, *13*(3), p.782.

Naik, B. S., Shashikala, J., & Krishnamurthy, Y. L. (2009). Study on the diversity of endophytic communities from rice (Oryza sativa L.) and their antagonistic activities in vitro. *Microbiological Research*, *164*(3), pp.290-296.

Pathirana, B. K. W. & Yapa, P.N. (2020). Evaluation of different carrier substances for the development of an effective pelleted biofertilizer for rice (*Oryza Sativa* L.) Using Co-inoculated bacteria and arbuscular mycorrhizal fungi. *Asian Journal of Biotechnology and Bioresource Technology*, 6 (1), pp. 1-10.

Qin, S., Xing, K., Jiang, J. H., Xu, L. H., & Li, W. J. (2011). Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. *Applied Microbiology and Biotechnology*, *89*, pp.457-473.

Rainey, P. B., Desprat, N., Driscoll, W. W., & Zhang, X. X. (2014). Microbes are not bound by sociobiology: response to Kümmerli and Ross-Gillespie (2013). *Evolution*, *68*(11), pp.3344-3355.

Rajani, A. (2019) 'Forms of Phosphorus'. Available at: https://www.researchgate.net/publication/335209942_Forms_of_phosphorus (Accessed: 21 June 2024).

Redman, R. S., Sheehan, K. B., Stout, R. G., Rodriguez, R. J., & Henson, J. M. (2002). Thermotolerance generated by plant/fungal symbiosis. *Science*, *298*(5598), pp.1581-1581.

Reiner, K. (2010). Catalase test protocol. *American Society for Microbiology*, *1*(1), pp.1-9.

Reis, J. B. A. D., Lorenzi, A. S., & do Vale, H. M. M. (2022). Methods used for the study of endophytic fungi: A review on methodologies and challenges, and associated tips. *Archives of Microbiology*, *204*(11), p.675.

Sabry, A. K. (2015). Synthetic fertilizers; role and hazards. *Fertilizer Technology*, *1*, pp.110-133.

Sahoo, R.K., Bhardwaj, D. and Tuteja, N. (2012) 'Biofertilizers: a sustainable eco-friendly agricultural approach to crop improvement', in Tuteja, N. (ed.) *Plant acclimation to environmental stress*. New York, NY: Springer New York, pp. 403-432.

Salisbury, W. A., & Likos, J. J. (1972). Hydrolysis of casein: a differential aid for the indentification of Serratia marcescens. *Journal of Clinical Pathology*, *25*(12), pp.1083-1085.

Schulz, B., Wanke, U., Draeger, S., & Aust, H. J. (1993). Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. *Mycological Research*, *97*(12), pp.1447-1450.

Sewwandi, M. C., Somarathna, W. G. R. S., Mubarak, A. N. M., Nashath, M. N. F., & Kumara, A. D. N. T. (2023). Evaluating the growth and yield performances of selected rice (*Oryza sativa* L.) varieties grown in organic fertilizer. *Journal of Agricultural Sciences,17(1),* pp.54-67

Shields, P., & Cathcart, L. (2010). Oxidase test protocol. *American Society for Microbiology*, pp.1-9.

Siddiquee, S., & Siddiquee, S. (2017). Morphology-based characterization of Trichoderma species. *Practical Handbook of the Biology and Molecular Diversity of Trichoderma Species from Tropical Regions*, pp.41-73.

Simpson, E. H. (1949). Measurement of diversity. *Nature*, *163*(4148), pp.688-688.

Singh, P., & Sharma, D. P. (2019). Response of *Trichoderma viride* and plant growth promoting Rhizobacteria (PGPR) on growth and yield of chilli cv. Arka Lohit. *Journal of Pharmacognosy and Phytochemistry*, *8*(4), pp.1495-1497.

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature*, *418*(6898), pp.671-677.

Yapa, N., Lakmali, D., Zoysa, D., Silva, K. S., Manawadu, C., Herath, B. M., Madhushan, A., Perera, G., Ratnayakae, O., Kapilan, R. Rathnayake, A., Sirisena, A., I. Asad, S., Karunarathna, S. C., & Bamunuarachchige, C. (2022). Biofertilizers: An Emerging Trend in Agricultural Sustainability. *Chiang Mai Journal of Science*, 49(3 Special Issue II), pp.608–640.

Zhang, Z., Masuda, Y., Xu, Z., Shiratori, Y., Ohba, H., & Senoo, K. (2023). Active nitrogen fixation by iron-reducing bacteria in rice paddy soil and its further enhancement by iron application. *Applied Sciences*, *13*(14), p.8156.