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Optimizing Grain Yield by Enhancing Paddy Soil and Root Mycorrhizal Associations under Modern Bio-Organo Mineral Fertilizer

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ABSTRACT

Rice (*Oryza sativa* L.) is the most commonly consumed staple food crop for approximately half of the world's population, playing a crucial role in food security. In Sri Lanka, paddy cultivation heavily relies on chemical fertilizers (CF), which pose significant environmental and health risks. This study investigates the effectiveness of biofilm biofertilizers (BFBF) combined with bio-organo-mineral (BOM) fertilizers as a sustainable alternative to CF in enhancing rice productivity. Field experiments were conducted across four major rice-growing districts in Sri Lanka (Ampara, Anuradhapura, Polonnaruwa, and Puttalam) during the 2023-2024 *Maha* season. Treatments included BFBF + BOM, CF alone, and control (no amendments) using 100 m^2 triplicate plots in RCBD design at each location (n = 36). Key findings revealed that BFBF + BOM significantly increased root mycorrhizal colonization index (RMCI) and soil mycorrhizal spore count (SMSC) compared to CF alone. Moreover, BOM fertilizers enhanced topsoil total phosphorus content (TSTPC) while maintaining comparable yields with reduced input quantities. This study underscores the potential of BFBF and BOM fertilizers in promoting sustainable rice cultivation by improving nutrient cycling, enhancing microbial diversity, and reducing dependency on chemical inputs. These findings contribute to the development of environmentally friendly farming practices that ensure long-term agricultural sustainability.

KEYWORDS: Biofilm based bio-organo mineral fertilizers, Mycorrhizae, Paddy, Root colonization index, Spores

INTRODUCTION

Rice is a staple food for over half of the world's population, and its cultivation plays a crucial role in global food security (Gnanamanickam, 2009). In Sri Lanka, paddy is cultivated in two main seasons, *Maha* and *Yala*, which are dependent on the rainfall patterns.

In Sri Lanka, 98% of the rice-growing lands are cultivated with high yielding varieties (HYVs) which rely significantly on inorganic fertilizers (Silva *et al.*, 2020) and over 70% of the paddy farming areas are under chemical fertilizers (CF) (Weerahewa *et al.*, 2010). Sustainability of paddy cultivation is challenged by the excessive use of CF and CF depletes soil organic matter (SOM), decreases soil fertility and microbes, leads to environmental degradation and nutrient imbalances in the soil (Pahalvi *et al.*, 2021).

Organic farming has emerged as an environmentally friendly alternative that reduces reliance on chemical inputs and improves soil quality through the use of organic materials (Surekha *et al.*, 2010).

Biofilm biofertilizers (BFBF) have emerged as a successful tool for achieving this goal (Seneviratne and Jayasinghearachchi, 2005). The National Institute of Fundamental Studies (NIFS) in Sri Lanka has developed BFBF, a type of biofertilizer that contributes to significantly increased crop quality (Pathirana *et al.*, 2023) and yield by 20-30% while cutting down organic and CF use up to 50% (Premarathna *et al.*, 2021). BFBF has a potential in fully organic agriculture when incorporated with nutrient rich organic fertilizers or modern bio-organo-mineral fertilizers (BOM) (Navodya *et al.*, 2023). BOM fertilizers are a novel formulations trend of biofertilizers, which incorporate a mix of organic matter, minerals, and beneficial microorganisms in a biofilm matrix.

Mycorrhizae, represent a symbiotic association between beneficial soil fungi and plant roots, playing a crucial role in enhancing the absorption of phosphorus and other less mobile nutrients, by increasing the effective root surface area of plants (O'Keefe and Sylvia, 1991).

However, there is a lack of research on the growth of mycorrhizae in plants with the application of BOM and CF. This study seeks to bridge this gap in knowledge by examining the interactive influence of soil and root endophytic mycorrhizal association under BOM and CF application in rice cultivation.

METHODOLOGY

Location

Field experiments were conducted in 2023-2024 *Maha* season in four major paddy-growing districts of Sri Lanka (Ampara, Puttalam, Anuradhapura, and Polonnaruwa) which exhibit diverse climatic conditions, and elevations. The soil types also vary, including red-yellow podzolic with laterite, low humic gley, and reddish-brown earth (Ministry of Agriculture, 2014). Laboratory analyses were conducted in Microbial Biotechnology Unit, NIFS.

Field Experiments and Treatments

The treatments of the study were (a) BFBF + NPK BOM fertilizer practice (BFBF is a fungal-bacterial biofilm) (Seneviratne, 2008) which is now a patented [Sri Lanka patent no. 15958 (2013)] commercial product, and hence exact composition cannot be revealed due to Intellectual Property Rights reasons, [2.5 L/ ha of BFBF with 500 kg BOM/ ha (N 275, P50, K 175 kg/ ha)]), (b) CF alone [340 kg CF/ ha [Urea 225, TSP 55, MOP 60 kg/ ha)] and (c) control (no amendments). Consecutive treatment plots were taken as a block design in each site with three replicate fields. Bw367, Bg310, Bg403, and Bg360 varieties were allocated to the respective sites of Anuradhapura, Puttalam, Ampara, and Polonnaruwa.

Collection and Preparation of Soil and Plant Samples

Soil samples from a depth of 0-30 cm were collected from the experimental rice fields. At the 50% flowering stage, three randomly selected rice hills were carefully uprooted with rhizosphere ensuring minimal damage to the root system. Subsequently, these samples were transported to NIFS – Hantana for additional laboratory experiments.

Soil Analysis

TSTPC: Top Soil Total Phosphorous Content, LSTPC: Low Soil Total Phosphorous Content, TSBD: Top Soil Bulk Density, LSBD: Low Soil Bulk Density, TSPPS: Top Soil Phosphorous Pool Size, and LSPPS: Low Soil Phosphorous Pool Size were determined.

Soil available phosphorous content (mg/ kg) was measured using a portable soil detector (Model: RS-TRREC-N01-1) at the field. The colorimetric method was used to determine the total phosphorous (%) (Anderson and Ingram., 1993). Bulk density (g/ cm^3) was measured (Blake and Hartge, 1982) and the soil phosphorous pool size (kg/ha) was obtained by integrating soil total phosphorous content and bulk density.

Yield Analysis

At crop maturity, 5 m × 5 m crop cuts were utilized non-randomly within each plot. The dry grain weights from these crop cuts were measured and the grain yield was calculated in kg/ ha.

Isolation of AMF Spores and Enumeration of Soil Mycorrhizal Spore Count (SMSC)

Spores were separated by wet sieving and decanting technique (Gerdemann and Nicolson, 1963), followed by sucrose centrifugation (1750 rpm). Spores were then washed onto a 45 µm sieve and observed under a light microscope (×400). Spores were enumerated using a hemocytometer (Avin, 2020) and soil mycorrhizal spore count (SMSC) for each treatment was calculated. Spores were identified based on the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) and mycorrhiza identification manual (Figure 1).

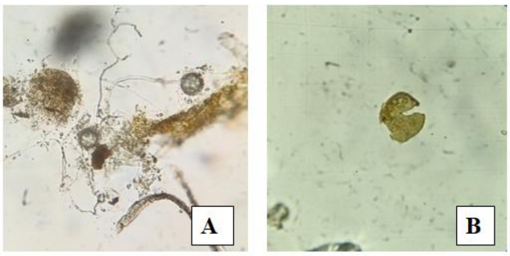

Figure 1. Microscopic views of (A) Mycorrhizal spores, (B) Mycorrhizal spore carps (×400)

Determination of Root Mycorrhizal Colonization Index (RMCI) by Staining

The root samples were washed carefully and cut into 1 cm segments. Then boiled in 10% KOH for 30 min at a temperature of 90 ºC to remove polyphenol compounds. KOH was poured off, and the roots were washed with tap water for 3 times. If the roots turned to dark in color, they were bleached with 30% alkaline H_2O_2 for 10-20 min and acidified with 1% for 3-4 min. Finally, roots were stained with cotton-blue in lacto phenol stain. Stained root specimens were placed on a drop of 70% glycerin on a slide and observed under the microscope. The slide intersection method was used to quantify root colonization (Giovannetti and Mosse, 1980) (Figure 2). The root colonization index (RT%) was quantified using the following equation (1).

$$RT\ (\%) = \left(\frac{TLM}{RL}\right) \times 100\% \quad \ldots\ldots\ldots\ldots(1)$$

Where,

RL = Root length

TLM = Total lengths of AMF structures

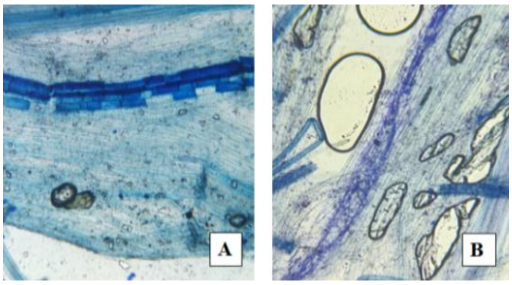

Figure 2. Microscopic views of mycorrhizal colonization of roots

(A) Mycorrhizal arbuscules, (B) Mycorrhizal hyphae (×400)

Data Analysis

The statistical data analysis was performed using Tukey's (HSD) test in Minitab 21 version. The probability level considered for statistical significance was 0.05.

RESULTS AND DISCUSSION

Effect of Fertilizer Treatments on Microbial Parameters

Different fertilizer applications that are used in rice cultivation have significantly affected the mycorrhizal colonization index in roots and mycorrhizal spore count. Both RMCI and SMSC were significantly higher in the BOM fertilizer practice compared with the 100 % CF practice (Figure 3; $p = 0.004$ and $p = 0.000$, respectively).

This indicates that the BOM fertilizer was more effective in promoting root mycorrhizal colonization in plant roots compared to the CF. The increased RMCI content with the use of BFBF in paddy cultivation has also been observed previously (Seneviratne *et al.*, 2009). Also, these results suggest that BOM fertilizer significantly enhances the soil mycorrhizal spore population. However, the increased SMSC with the use of BFBF in paddy cultivation has not been observed before.

Effect of Fertilizer Treatments on Yield and Soil Parameters

TSTPC was significantly higher in the BOMF practice than in the farmers' 100% CF practice (Table 1; $p= 0.046$). The increased topsoil total phosphorous content with the use of BFBF has been observed previously (Premarathna *et al.*, 2021). However, low soil phosphorous content (LSTPC), topsoil bulk density (TSBD), low soil bulk density (LSBD), topsoil phosphorous pool size (TSPPS), low soil phosphorus pool size (LSPPS), and yield were not significantly impacted by the fertilizer treatments (Table 1; $p > 0.05$).

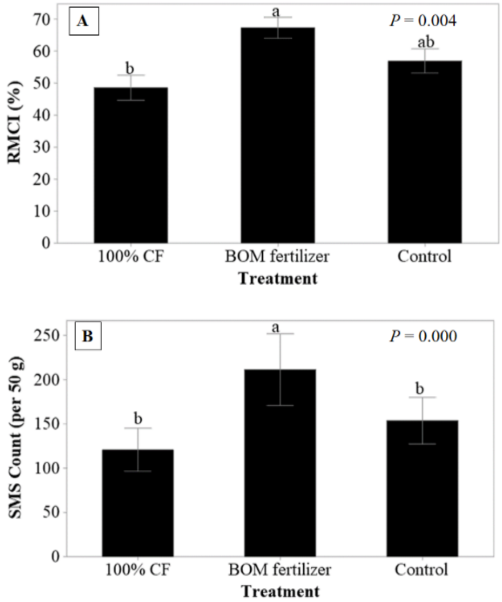

Figure 3. Effect of BOM fertilizer and 100% CF treatments on (A) Root mycorrhizal colonization index (RMCI) and (B) soil mycorrhizal spore count (SMSC)

Bars are standard errors of the mean. Different letters indicate significant differences according to Tukey's HSD test.

Table 1. Yield and soil parameters under different fertilizer practices in rice cultivation

Parameter	BOM	CF	Control	P value
Yield (kg/ ha)	5874[a] ± 450	5394[a] ± 533	5034[a] ± 557	0.524
TSTPC (%)	3.408[a] ± 0.66	1.631[b] ± 0.30	2.030[ab] ± 0.45	0.046
LSTPC (%)	1.282[a] ± 0.41	0.434[a] ± 0.15	0.945[a] ± 0.23	0.124
TSBD (g/ cm^3)	1.014[a] ± 0.09	0.888[a] ± 0.09	1.020[a] ± 0.09	0.538
LSBD (g/ cm^3)	1.178[a] ± 0.06	1.126[a] ± 0.08	1.166[a] ± 0.07	0.867
TSPPS (kg/ ha)	17469[a] ±4666	10129[a] ±3305	16160[a] ± 5169	0.469
LSPPS (kg/ ha)	7541[a] ±2902	3411[a] ±1452	5274[a] ± 1801	0.404

Mean ± SE in each column. Within rows, values with different letters differ significantly ($p < 0.05$, Tukey's HSD test). Yield, TSTPC: Top Soil Total Phosphorous Content, LSTPC: Low Soil Total Phosphorous Content, TSBD: Top Soil Bulk Density, LSBD: Low Soil Bulk Density, TSPPS: Top Soil Phosphorous Pool Size, LSPPS: Low Soil Phosphorous Pool Size. BOM (n=36), CF (n=36)

Two main positively correlated relationships were observed. First, SMSC was directly related to the RMCI ($r = 0.501$, $p = 0.02$). A similar relationship was previously observed by Sivakumar, (2013) in sugarcane fields as well as Kalamulla *et al.* (2022) in paddy fields.

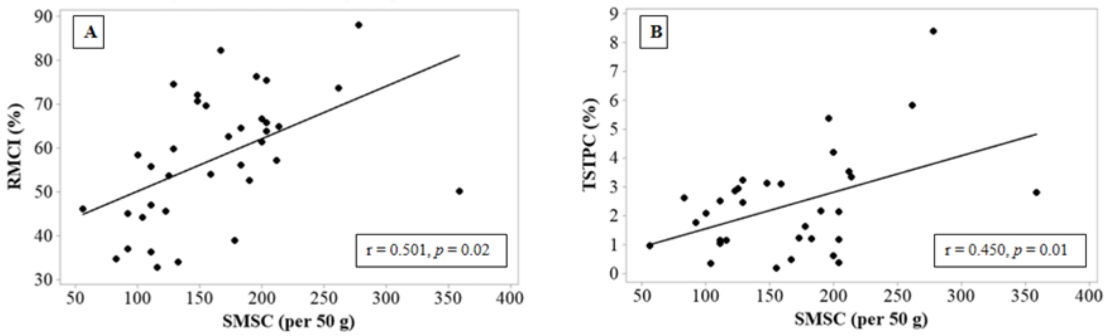

Figure 4. Matrix plot of (A) root mycorrhizal colonization index (RMCI) vs soil mycorrhizal spore count (SMSC) and (B) topsoil total phosphorous content (TSTPC) vs soil mycorrhizal spore count (SMSC)

Also, there was a positive correlation between SMSC and TSTPC (r = 0.450, p = 0.01). However, previous reports showed a negative relationship between SMSC and TSTPC (Khakpour and Khara, 2012).

Therefore, BOM fertilizer can be the reason for the observed positive relationship. According to the department recommendation, 10 tons of organic fertilizers or 340 kg of unsustainable chemical fertilizers should be added to a 1 ha (Weerasinghe, 2023). However, only 500 kg of eco-friendly, sustainable BOM fertilizer amount was needed to get a comparable yield. This can be attributed to the positive effect of modern BOM fertilizer to increase the microbial abundance such as mycorrhizal abundance. Mycorrhizae produce P solubilizing enzymes and increase the P uptake in plants (O'Keefe and Sylvia, 1991). P is an energy molecule and a vital constituent of major biomolecules such as DNA, RNA, ATPs, phosphate esters, and phospholipids. (Marschner, 2012). Also, P is very important in microbial dynamics (Kutu *et al.*, 2019) and it helps to increase plant growth and yield (Zhang *et al.*, 2019).

CONCLUSIONS

It is clear from this study that BOM practice helps to increase soil mycorrhizal abundance and their association with plant roots. Moreover, BOM fertilizers enhanced topsoil total phosphorus content (TSTPC) while maintaining comparable yields with reduced input quantities. This study highlights the potential of BFBF and BOM fertilizers in promoting sustainable rice cultivation by improving nutrient cycling, enhancing microbial diversity, and reducing dependency on chemical inputs.

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