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BIOFILM TREATED EPPAWALA ROCK PHOSPHATE AND FELDSPAR AS SUBSTITUTES FOR TRIPLE SUPER PHOSPHATE AND MURIATE OF POTASH IN RICE (*Oryza sativa* L.) CULTIVATION

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ABSTRACT

The excessive and prolonged use of synthetic fertilizers such as Triple Super Phosphate (TSP) and Muriate of Potash (MOP), apart from Urea has led to serious environmental concerns, including soil and water pollution and the accumulation of heavy metals in agroecosystems. These issues have posed long-term risks to both ecosystem health and human well-being. This study evaluated biofilm-enriched Eppawala Rock Phosphate (BFE) and biofilm-enriched Feldspar (BFF) as bio-mineral fertilizers with biofilm biofertilizer (BFBF), proposed as a sustainable alternative to TSP and MOP. The experiment was conducted in a farmer managed paddy field using a Randomized Complete Block Design (RCBD) with three replicates, comparing BFE + BFF + BFBF practice with the current BFBF practice. Key parameters assessed included soil physicochemical properties, plant growth and yield, microbial activity, and heavy metal accumulation. Results indicated a significant ($p<0.05$) increase in exchangeable soil K in plots treated with the new BFBF practice, due to enhanced potassium availability with the BFF application. No statistically significant differences ($p>0.05$) were observed between the two practices for soil moisture, organic carbon, microbial biomass carbon, total phosphorus, total nitrogen, shoot and root dry weights, endophytic diazotroph populations, and crop yield. Furthermore, plant and soil cadmium (Cd) and iron (Fe) concentrations remained within safe limits and did not differ significantly between the two practices, indicating no adverse effects from the application of BFE and BFF. These findings suggest that biofilm-enriched P and K mineral fertilizers can effectively replace synthetic TSP and MOP without compromising rice growth, yield, or soil and plant health. The use of BFE and BFF within a BFBF system offers a promising approach for enhancing nutrient sustainability, reducing environmental impact, and supporting the transition toward organic and sustainable rice cultivation.

Keywords: Biofilm, Eppawala Rock Phosphate, Feldspar, Rice

INTRODUCTION

Extensive dependence on chemical fertilizers in agricultural systems has been a key driver of environmental deterioration, progressive soil health decline, and the accumulation of toxic compounds in agroecosystems (Pahalvi *et al*., 2021). In particular, the excessive application of nitrogen, phosphorus and potassium based fertilizers, such as Urea, Triple Super Phosphate (TSP) and Muriate of Potash (MOP) has raised critical environmental concerns. These include eutrophication of water bodies, soil nutrient imbalances, and diminished fertilizer use efficiency, all of which pose risks not only to ecosystems but also to human health (Alori *et al*., 2017). In response to the growing demand for sustainable nutrient management strategies, biofertilizer technologies have emerged as an ecologically viable alternative (Chaudhary *et al*., 2020). Among these, biofilm biofertilizers (BFBF) have gained increasing attention due to their unique mode of action. Biofilms consist of beneficial microorganisms embedded in a self-produced extracellular polymeric substance matrix, forming structured microbial consortia (Singh *et al*., 2020). This biofilm architecture confers multiple advantages over conventional free-living microbes. Biofilms improve the survival and persistence of microbes in the rhizosphere, support beneficial microbial interactions (Wu *et al*., 2019), aid in nutrient cycling, strengthen plant defenses against pathogens, and enhance resilience to abiotic stresses (Ajijah *et al*., 2023). Additionally, they assist in the solubilization and mobilization of key nutrients (Rekadwad and Khobragade, 2017). While biofilms naturally occur in the soil, their limited abundance reduces their beneficial effects in the soil system like improved nutrient cycling, climate resilience etc. Biofilms developed in industrial setting to be used as biofertilizers are termed BFBFs, which are vital for boosting agricultural productivity through sustainable and eco-friendly methods (Buddhika *et al*., 2016).

Rice (*Oryza sativa* L.) is the staple food crop in Sri Lanka, cultivated over approximately 1.3 million hectares annually across both Yala and Maha seasons, accounting for nearly 29% of the total agricultural land (DOA, 2024). In recent years, BFBFs have been introduced in major rice-producing districts such as Ampara, Polonnaruwa, Kurunegala, and Monaragala (Premarathna *et al*., 2021; Rathnathilaka *et al*., 2023). However, there is limited scientific evidence supporting the effectiveness of BFBFs in fully replacing imported fertilizers with locally sourced materials. Locally available mineral resources, including Eppawala Rock Phosphate (ERP: 28% to 42% of P2O5) and Feldspar (6% to 10% of K) are abundant and inexpensive but their agricultural use is constrained by poor solubility and low nutrient bioavailability. Recent advancements in biofilm-mediated mineral solubilization have demonstrated promising outcomes in converting such low-solubility minerals into bioavailable nutrient forms (Henagamage, 2024). Therefore, the application of biofilm-treated ERP (BFE) and biofilm-treated feldspar (BFF) may offer a sustainable alternative to conventional fertilizers. However, the use of these materials for improving crop growth, nutrient uptake, and soil fertility in paddy fields is still not well studied in Sri Lanka. This study aims to evaluate the performance of BFE and BFF as substitutes for TSP and MOP respectively, in rice cultivation across diverse agroecological zones of Sri Lanka.

METHODOLOGY

Experimental location and experimental design

The field experiments were conducted in five farmer managed fields located in Ampara (DL1b), Polonnaruwa (DL1c), and Monaragala (IM1c). According to cultivation history, the selected fields ensured that the paddy crop exhibited responsiveness to soil-applied P and K. Laboratory experiments were conducted at the National Institute of Fundamental Studies (NIFS), Hantana Road, Kandy. The farmer fields were arranged according to Randomized Complete Block Design. Each practice was replicated three times, and rice was cultivated using the broadcasting method. The rice variety BG-350 with a growth duration of 3.5 months was used. All agronomic practices, including watering, weed control, and pest and disease management, were implemented following the guidelines prescribed by the Department of Agriculture (DOA).

Fertilizer practices

Two practices, current BFBF and new BFBF were tested in farmer managed fields to evaluate their effectiveness (Table 1). The efficacy of BFBF was not tested here because it has already been proven that its effect is real and significant even under large-scale paddy cultivations (Ekanayake *et al*., 2024). The BFBF was applied to the paddy fields of 0.01 ha by mixing 500 mL of BFBF with 4 L of fine sand at 2 weeks and 6 weeks after broadcasting.

Table 1: Different fertilizer practices used in in the experiment

Practice	Description
T1- BFBF	[2.5 L of BFBF with 225 kg chemical fertilizer (CF)/ha (Urea 150, TSP 40 and MOP 35 kg/ha)] *225 kg/ha is ca. 65% of CF recommendation of DOA
T2-New BFBF	(BFF - 128 kg/ha and BFE - 92 kg/ha to replace P & K, Urea - 150 kg/ha + BFBF 2.5 L/ha)

Sample collection

At the 50% flowering stage, three rice plants were randomly selected from each plot, and they were carefully uprooted with the root zone soil by gently digging around the plant.

Plant analysis

The soil was removed carefully from the plant roots. Then, the plants were washed carefully without damaging the root system. Roots and shoots were separated and oven-dried at 65 ºC until it reaches a constant weight and then root dry weight (RDW) and shoot dry weight (SDW) were recorded using a top-loading balance. At harvest, five crop cuts (1 m × 1 m) were taken from each experimental plot. The grain yield from these cuts was recorded and used to calculate the total grain yield per plot.

Soil analysis

Soil pH, soil moisture (SM), total nitrogen (STN), total phosphorous (STP), potassium (SP), and soil organic carbon (SOC) were analyzed before and after fertilizer application. In fresh soil analysis, soil pH was measured in the supernatant using a pH meter (Anderson and Ingram, 1993). Also, SM content was measured using the oven dry method (105 ^{o}C) (Anderson and Ingram, 1993). The rest of the soil samples were air-dried. Subsamples were taken from the air-dried soil samples and were crushed well. Then a sub sample was passed through the 2 mm sieve. Sieved soil was ground using mortar and pestle. It was passed through 0.5 mm sieve. The SOC was measured by using the Colorimetric method described by Baker (1976). The STN and STP were determined using the Kjeldahl method (Bremner, 1996) and colorimetric method as described by Anderson and Ingram (1993), respectively. The SP was analyzed by using the atomic absorption spectrophotometer.

Microbial analysis

Surfaces of the plant leaf parts were sterilized using 70% ethanol, and they were crushed for enumerating Endophytic diazotrophs (ED), which were counted by culturing at 10^{-3} and 10^{-5} dilutions in combined carbon medium. The colony counts were taken at 48 hours after inoculation.

Heavy metal analysis

Soil and plant samples were analyzed for heavy metal concentrations following microwave-assisted acid digestion. Soil samples were digested using a microwave digestion system (MARS 6, MarsXpress model) with concentrated nitric acid (HNO_3) as the digesting agent. First, a 0.25 g portion of each ground sample was weighed into digestion tubes, followed by the addition of 4 mL of concentrated HNO_3. The samples were then digested at 180 °C for 20 minutes. After digestion, the mixture was diluted to a final volume of 10 mL using ultra-pure water. The digested samples were first filtered through Whatman No. 42 filter paper and subsequently through syringe filters to ensure clarity. For plant samples, collected tissues were first rinsed with tap water and then with deionized water to remove surface dust and contaminants. The cleaned samples were air-dried, ground to a fine powder, and stored in sealed polyethylene bags inside a desiccator until analysis. A 0.25 g subsample was weighed into digestion tubes and treated with 3 mL of trace metal–grade HNO_3. Digestion was carried out in the microwave system at 180 °C for 15 minutes. After cooling, the digests were diluted to 25 mL with Milli-Q water and filtered through cellulose acetate filters (0.45 µm pore size). A reagent blank was prepared alongside each batch to account for any contamination introduced during sample handling. The elemental concentrations were measured using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Ultra-pure water was pre-filtered using a 0.4 µm microbial filter for use in sample preparation and dilution. Calibration standards were prepared from a multi-element stock solution. Since arsenic (As^{2+}) was not present in the stock solution, a separate As^{2+} standard solution was prepared. Concentrations of heavy metals and micronutrients were then determined using the ICP-OES.

Data analysis

The normality of data for each variable across the practices was first assessed using a normality test. Based on the results, one-way ANOVA was applied. Mean separation was performed using Tukey's HSD test. All statistical analyses were conducted using Minitab version 17.

RESULTS AND DISCUSSION

The initial soil data were averaged as follows. Since there were no significant differences between the experimental locations, the data were pooled (Table 2).

Table 2: Initial soil parameters before application of fertilizers

pH	SM (%)	STN (%)	STP (%)	SOC (%)	SP (mg/kg)
6.48 ± 0.11	55.78 ± 1.57	0.04 ± 0.01	0.06 ± 0.01	1.03 ± 0.12	14.59 ± 2.31

Values represent mean± standard error.

The table summarizes the effects of the current and the new BFBF practices on various parameters, including soil physicochemical properties, microbial activity, plant biomass, heavy metal accumulation in soil and plant tissues and final grain yield (Table 3). These measurements were taken to assess the comparative effectiveness of both BFBF practices in enhancing soil fertility, promoting plant health, and ensuring food safety while maintaining crop productivity.

Table 3: Soil, plant and microbial parameters of the two practices

Parameters	Current BFBF practice	New BFBF practice
pH	6.17 [b] ±0.17	6.52 [a] ±0.06
SM (%)	57.57 [a] ±3.44	47.10 [b] ±4.17
SOC (%)	1.26 [a] ±0.11	1.37 [a] ±0.14
MBC (mg/kg)	1.26 [a] ±0.11	1.42 [a] ±0.14
STP (%)	0.12 [a] ±0.01	0.12 [a] ±0.01
STN (%)	0.49 [a] ±0.09	0.53 [a] ±0.12
SP (mg/kg)	21.38 [b] ±3.17	31.57 [a] ±5.70
SDW (g)	4.73 [a] ±0.72	3.46 [a] ±0.80
RDW (g)	5.37 [a] ±1.56	3.55 [a] ±0.76
ED (x10^3 CFU/mL)	63.70 [a] ±1.76	67.15 [a] ±2.12
Plant Cd (ppb)	0.43 [a] ±0.21	0.22 [a] ±0.05
Plant Fe (ppm)	11.92 [a] ±6.90	16.30 [a] ±11.48
Soil Cd (ppb)	0.39 [a] ±0.08	0.50 [a] ±0.11
Soil Fe (ppm)	1.19 [a] ±1.03	4.34 [a] ±3.88
Yield (kg/ha)	6587 [a] ±596	6382 [a] ±665

Values represent mean± standard error. Means followed by the same superscripts in a same row are not significantly different at 0.05 probability level according to Tukey's HSD test.

Soil parameters

The results revealed that soil pH increased within the favorable range in the new BFBF practice over the present BFBF practice adopted by the farmers. Moreover, the soil moisture decreased in the new BFBF practice over the present practice. Interestingly, soil potassium increased in the new BFBF practice over the current BFBF practice possibly due to the action of BFF. In BFF, microbial biofilms have enhanced the enzymatic and metabolic activity of potassium solubilizing bacteria that leads to decomposed K-feldspar and released K to the soil (Geisseler and Scow, 2014; Ahmad *et al*., 2016). As such, it has been observed that the K requirement of the rice plant can be fulfilled with the application of eco-friendly bio-mineral fertilizers like BFF while replacing chemical fertilizers like MOP. The application of MOP to the soil adds a high amount of chloride (Cl^-) in arid and semi-arid climates (Tariq *et al*., 2011). Excess Cl^- application reduces soil microbial activity (Pereira *et al*., 2019). Therefore, considering the results of the present study, the new BFBF practice can be considered as a more eco-friendly method in supplying K requirement to rice cultivation.

In addition, soil total P content did not significantly differ between the two practices. Likewise, comparable amounts of P have been supplied by the BFE when compared to the use of synthetic P fertilizers like TSP. The low solubility of ERP and its high cost of conversion to a chemical P fertilizer have been addressed by the application of microbial biofilms on ERP, when developing BFE. If P applied in a synthetic form, most of the P is trapped with Mg, Ca, Fe and Al in both acidic and alkaline soils causing the soil P pool unavailable for plants (Hinsinger, 2001). Moreover, TSP contains large amounts of potentially toxic trace elements like Cd, As and Pb (Premarathne *et al*., 2011). Therefore, considering the results of the present study, the new BFBF practice can be considered as more eco-friendly method in supplying P requirement to rice cultivation. Soil total nitrogen (STN), organic carbon (SOC) and microbial biomass carbon (MBC) were not significantly different between the two practices. Nitrogen cycling involves multiple biological and chemical processes, and the substitution of chemical P and K fertilizers with bio-mineral fertilizers makes availability of nutrients other than nitrogen ecofriendly (Robertson and Groffman, 2015). Similarly, MBC remained unchanged, possibly reflecting stable organic substrate availability and the resilience of soil microbial communities to moderate changes in fertilizer inputs within the study period (Li *et al*., 2018).

Plant growth and yield

No significant differences ($p>0.05$) in plant growth were observed between the two practices. There was no significant difference in the crop yield. The current practice yielded 6587 kg/ha, while the new practice yielded 6382 kg/ha, suggesting comparable crop productivity. These results align with a previous study, replacing TSP completely with biofilm-enriched ERP (at 100 % replacement rate) combined with reduced N and K fertilization resulted in grain yields statistically similar to those attained with the DOA recommended chemical fertilizer dosage (Jayaneththi *et al*., 2023). Moreover, another pot and soil leaching experiment reported that biofilm-enriched ERP showed a similar grain yield to DOA recommended chemical fertilizer dosage (Jayaneththi *et al*., 2018).

Microbial parameters

Both practices supported similar levels of endophytic colonization, with no significant difference (~ 64–67 x 10^3 CFU/mL). This range is well within the typical densities reported for rice endophytes. The previous studies have found endophytic populations in rice roots and shoots ranging from 10^4 to 10^5 CFU/g (fresh weight), and occasionally up to 10^6 CFU/g depending on plant part and inoculation strategy (Piromyou *et al*., 2015; Setiawati *et al*., 2021).

Heavy metal and micronutrient

No statistically significant differences (P>0.05) were found between the new and current practices in terms of plant Cd accumulation (0.22 and 0.43 ppb, respectively) and plant Fe uptake (16.30 and 11.92 ppm, respectively). Similarly, soil Cd (0.50 and 0.39 ppb, respectively) and soil Fe levels (4.34 and 1.19 ppm, respectively) showed no significant (p>0.05) variation between the two practices. The application of BFBF is considered important for improving soil quality by regulating heavy metal levels. This is supported by Henagamage *et al*. (2022), who reported that *Trichoderma harzianum*–*Bacillus subtilis* biofilm effectively reduced heavy metal concentrations in soil while enhancing overall soil health.

CONCLUSIONS

This study demonstrated that when BFE and BFF are applied as parts of a biofilm-based biofertilizer (BFBF) system, they act as effective substitutes for conventional TSP and MOP, respectively, in rice cultivation. The new BFBF practice significantly enhanced exchangeable soil potassium levels while maintaining comparable results to the current BFBF practice in terms of soil fertility parameters, plant growth, microbial activity, and crop yield. Importantly, the application of BFE and BFF did not result in increased accumulation of heavy metals, confirming their safety for agricultural use. These findings highlight the potential of BFE and BFF as sustainable, locally sourced bio-mineral fertilizers that can support environmentally friendly rice production while reducing dependence on synthetic inputs.

Recommendation:

Future research should explore the long-term impacts of this BFBF system on soil health and crop productivity across diverse agroecological regions.

Conflict of interest

The authors declare no conflict of interest

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