RESEARCH ARTICLE

Biofilm Enriched Rock Phosphate as a Potential Phosphorous Fertilizer To Replace Triple Super Phosphate In Rice Cultivation: A Preliminary Field Trial

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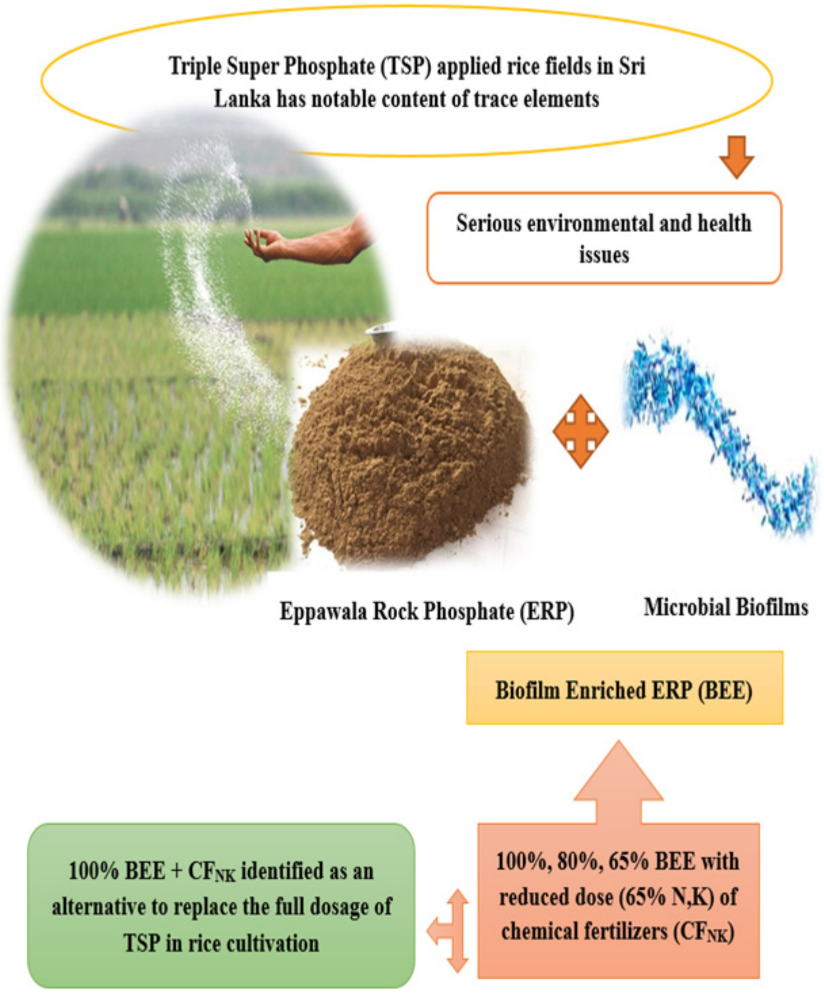

Highlights

- Application of Triple Super Phosphate (TSP) led to notable levels of trace elements in soils causing severe environmental and health issues
- Eppawala Rock Phosphate (ERP) is a cheap and environmental-friendly alternative to TSP despite low phosphorus solubility
- Microbial biofilms seem to be one of the most promising ways to enhance the solubility of ERP
- The study explored different proportions of biofilm-enriched ERP (BEE) to replace TSP in rice cultivation
- 100% BEE together with the CF_{NK} has showed promising results to replace TSP-P in rice cultivation

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Biofilm Enriched Rock Phosphate as a Potential Phosphorous Fertilizer To Replace Triple Super Phosphate In Rice Cultivation: A Preliminary Field Trial

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Abstract: Rock Phosphate (RP), derived from natural apatite mined from Eppawala (ERP), is identified as a possible substitute for Triple Super Phosphate (TSP). However, its application is limited due to low solubility. If the bio-solubility is enhanced, ERP can be used as a phosphorous (P) fertilizer for annual crops. The present study focused on using an effective biofilm formulation identified after screening several known phosphate solubilizing biofilms, and introducing a biofilm-enriched ERP (BEE) as an alternative for TSP in rice cultivation. Two field trials were conducted in two consecutive seasons, dry season in 2018 (*Yala*) and the wet in 2018/2019 (*Maha*). The existing chemical fertilizer dosage (CF) recommended for rice by the Department of Agriculture (DOA) was modified by fully replacing TSP-P with BEE, along with 65% nitrogen (N) and potassium (K) fertilizers (CF_{NK}). Three different rates of BEE, 65, 80 and 100% (equivalent to TSP-P recommended by the DOA) were tested (CF_{NK} + 65 BEE, CF_{NK} + 80 BEE and CF_{NK} + 100 BEE) against CF. Results revealed that the rate of 100% BEE performed better than that of other rates of BEE (65% and 80%), while the same recorded significantly higher grain yields in both seasons compared to all other treatments. Moreover, plant growth parameters *viz*. shoot and root dry masses, height, and panicle length were also recorded higher values with CF_{NK} + 100BEE application than the control (CF). Therefore, the BEE can be suggested as an alternative to TSP for rice cultivation, with an added advantage of using a reduced dosage of N and K fertilizers. However, further studies are needed to test the BEE under different soil and climatic conditions in the country.

***Keywords*;** Biofilms; Biofertlizers; Eppawala Rock Phosphate; Rice cultivation; Triple Super Phosphate.

INTRODUCTION

Rice (*Oryza sativa L.*) is one of the dominant cereals grown in Sri Lanka. Furthermore, rice is the staple food of the majority of Sri Lankans. Rice cultivation currently occupies approximately 708,000 ha of land (Department of Senses and Statistics, 2021). On average, 460,000 ha are being cultivated annually in the *Maha s*eason (a wet season from September to March) and 310,000 ha in the *Yala* season (dry season from May to August). Most farmers use chemical fertilizers (CFs) in rice cultivation. Urea, Triple Super Phosphate (TSP) and Muriate of Potash (MOP) are the commonly applied CFs in rice cultivation (Ministry of Agriculture, 2005). Amongst them, TSP is used as the main source of phosphorus (P) in rice cultivation due to its high solubility for short-term fast-growing crops such as rice. However, the extensive use of chemical fertilizers in agriculture has led to serious environmental and health issues due to heavy metal pollution (Al, Cr, Ni, Cd, Pb and As) (Chandrajith *et al*., 2011). In the recent history, the chronic kidney disease has become a major health concern in Sri Lanka with a high prevalence in agricultural areas, thus some attributing to heavy metal pollution due to the extensive usage of agrochemicals (Rajapakshe *et al.,* 2016). Therefore, finding of alternatives to replace CFs is crucial.

Eppawala Rock Phosphate (ERP) is considered as a cheap and environmental-friendly alternative to TSP despite few inherent constraints such as low phosphorus solubility, which has limited its direct application as a potential P fertilizer in place of TSP for perennial crops. Over the years, studies have been carried out to enhance the P solubility in rock phosphates, but with limited success (Biswas *et al.,* 2022). The P solubilizing microbes have also been tested (Panhwar *et al.,* 2011; Illmer & Schinner, 1992) with limited results.

Biofilms are assemblages of microorganisms attached to biotic or abiotic surfaces and embedded in a self-created matrix of polymers (Khan *et al.,* 2010). Incorporation of biofilmed biofertilizers (BFBFs) is one of the most promising ways to enhance the solubility of natural fertilizers such as ERP. Some laboratory trials conducted to test the potential of replacing TSP from biofilm-enriched ERP (BEE) in rice have yielded some encouraging results (Jayaneththi *et al.,* 2017). A previous study tested four biofilm formulations (BF1 – BF4) and observed some promising results with BF3. Accordingly, the biofilm formulation, BF3, has been identified as the most effective phosphate solubilizer out of the four biofilms, and it was used in the present study to explore the suitable application proportion of BEE to replace TSP in rice cultivation.

MATERIALS AND METHODS

A field experiment was carried out in a farmer-managed

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field at Puliyankulama in Anuradhapura (North Central Province of Sri Lanka), which belongs to the DL_{1b} agroecological region. Fields were prepared according to the recommendations given by the DOA. Rice variety BG 352 (3.5 months old) was used. Crop was established by broadcasting the water-soaked pre-germinated seeds at the recommended rate. The experiment was arranged in a Randomized Complete Block Design (RCBD), with three replicates for each treatment. The plot size was 3 m × 6 m.

The soil at the experimental site was clayey in texture and classified as poorly drained Reddish Brown Earth (Panabokke, 1996) that generally consisted of low organic matter (1.74%) with a soil reaction close to neutral (pH= 7.24). However, soils seem to have a moderate level of available N (75.14 ± 0.14 mg kg^{-1}) and close to critical or deficient level of available P content (10.11 ± 1.24 mg kg^{-1}). Potassium is considered sufficient (112.6 ± 1.62 mg kg-1) for growing rice (Portch & Hunter, 2002).

Treatments

There were five treatments, including the 100% CF dosage recommended by the DOA for rice (Urea: 225 kg ha^{-1}, TSP: 55 kg ha^{-1} and MOP: 60 kg h^{-1}) (CF), and CF modified by replacing TSP-P (55 kg ha^{-1}) fully with BEE (92 kg ha^{-1}) with 65% (reduced) doses of N and K (CF_{NK} + BEE). The different rates of BEE were tested with CF_{NK} as 100%, 80% and 65% (CF_{NK} + 100% BEE, CF_{NK} + 80% BEE and CF_{NK} + 65% BEE). The DOA recommended fertilizer dosage (CF) without TSP (CF_{NK}) and a control without any fertilizer (CTR) were also tested (Table 1).

To develop BEE, the BF3 P-solubilizing biofilm (Jayaneththi *et al.,* 2021) was sprayed on ERP at the rate of 1.7 L per 100 kg as recommended by the National Institute of Fundamental Studies, (NIFS), Kandy, Sri Lanka.

Table 1: Different treatments (T1 – T6) used in the field experiment.

Treatment Number	Treatments
T1	CF
T2	CF_{NK+} 100% BEE
T3	CF_{NK+} 80% BEE
T4	CF_{NK+} 65% BEE
T5	CF_{NK}
T6	CTR

CF = Chemical Fertilizer; BEE = Biofilm-enriched ERP; CTR = Control (without any fertilizer)

All chemical and biofertilizer-enriched fertilizers were broadcasted as they were in solid forms. All management practices such as irrigation, weeding, pest and disease management were carried out according to the recommendations given by the DOA. At the end of the growing season, five random plants (excluding plants along edges of plots) were destructively harvested to measure root and shoot dry masses, panicle length and plant height. Numbers of filled grains and unfilled grains per panicle were also counted in each plant. Plants were also harvested (excluding the edge plants) from each subplot (1 m × 1 m) and grain yield was recorded after air drying.

RESULTS AND DISCUSSION

Plant growth parameters

Plants exposed to the treatment CF_{NK}+100% BEE (T2) showed higher values for all measured plant growth parameters i.e. root and shoot dry mass, panicle length and plant height, compared to other treatments (Figure 1 and 2). In both seasons, root and shoot dry masses of CF_{NK} +100 % BEE (T2) noted significantly *($p<0.05$)* higher values, while the lowest was recorded in CTR (Figure 1 A and B). However, the average plant growth (in terms of plant height and dry biomass) was lower during the dry season than that of the wet season, possibly due to climatic conditions prevailed in the season. In consistent with plant biomass, plant height and panicle lengths were also recorded significantly higher values (at $p < 0.05$) in CF_{NK} + 100% BEE (T2) (Figure 2 A, B). In favour, previous studies also showed a positive role of microbial biofilms in rice cultivation, where rice rhizosphere can serve as a potential repository for microbes involved in solubilizing P from various sources. Bacterial and fungal biofilms are known to produce an array of different organic acids that could effectively dissolve different forms of P in soil (Beheshti *et al.,* 2021).

Yield parameters

Filled and un-filled grain weights were taken as yield parameters and shown in Figure 2. In both seasons, CF_{NK} +100% BEE recorded the highest filled grain weights and the lowest un-filled grain weights (Figure 3).

The application of phosphate solubilizing biofilm-enriched natural fertilizer as an alternative to chemical P fertilizers is an environmental-friendly approach for improving crop yield in various soils (Etesami, 2020). These selective biofilms may increase the solubility of unavailable (precipitated) P through various known mechanisms, including lowering the soil pH, chelation, and mineralization (Etesami, 2020). Hence, this would be a promising way to manage relatively unavailable P in soils and improve the fertilizer use efficiency of P fertilizers in paddy fields (Wu *et al.,* 2016). In the present study, the biofilm used in enriching ERP (Jayaneththi *et al.,* 2021) is consisted of nitrogen-fixing and P-solubilizing bacteria and root-associated fungi, and produced eventually high yields in both *Yala* (1,984 kg ha^{-1}) and *Maha* (6,181 kg ha^{-1}) seasons (Table 2) when it is provided at a rate of 100% (CF_{NK}+ 100% BEE). The control plots (without any fertilizer) recorded significantly *($p<0.05$)* lower yields in both seasons (1,119 kg ha^{-1} in *Yala* and 3,312 kg ha^{-1} in *Maha*). However, the overall grain yield of the *Yala* season was lower than the average rice yield perhaps due to the low rainfall received during the season.

Several other studies also reported that P solubilizing biofilms play a leading role in adjusting nutrient cycling (e.g., C, N, P, and K) and energy flows and regulating the nutrient sinks in the soil (Lu *et al.,* 2016). Therefore, P solubilizing biofilms have been reported to play an

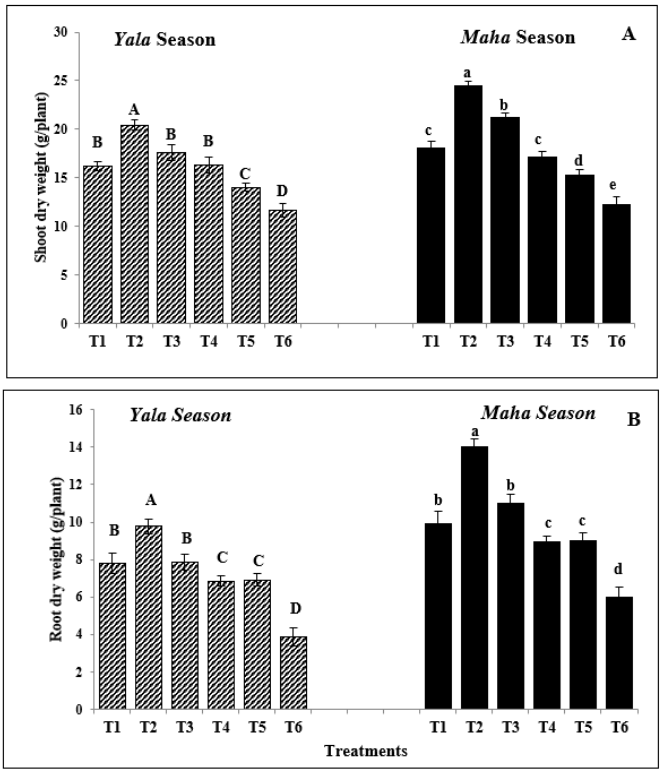

Figure 1: Mean (A) shoot dry and (B) root dry mass of rice plants at harvest under different treatments (T1 – T6). Different letters indicate statistically significant differences at 5% probability level resulted from Tukey's mean comparison test for *Yala* and *Maha* seasons separately. Treatments: T1-CF; T2- CF_{NK} + 100% BEE; T3-CF_{NK} + 80% BEE; T4- CF_{NK} + 65% BEE; T5-CF_{NK} and T6-CTR]

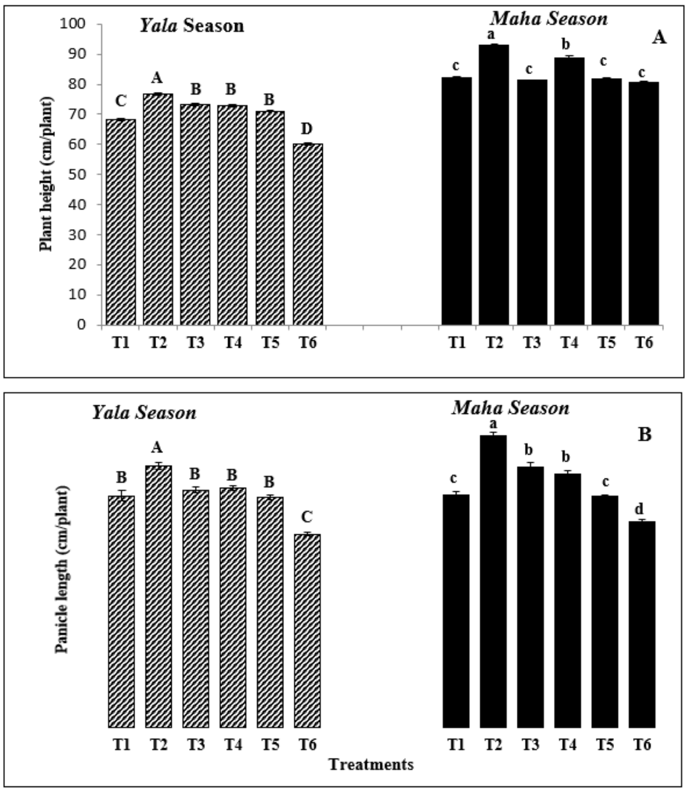

Figure 2: Mean (A) plant height and (B) panicle length of rice plants at harvest under different treatments (T1-T6). Different letters indicate statistically significant differences at 5% probability level resulted from Tukey's mean comparison test for *Yala* and *Maha* seasons separately. Treatments: T1-CF; T2- CF_{NK} + 100% BEE; T3-CF_{NK} + 80% BEE; T4- CF_{NK} + 65% BEE; T5-CF_{NK} and T6-CTR]

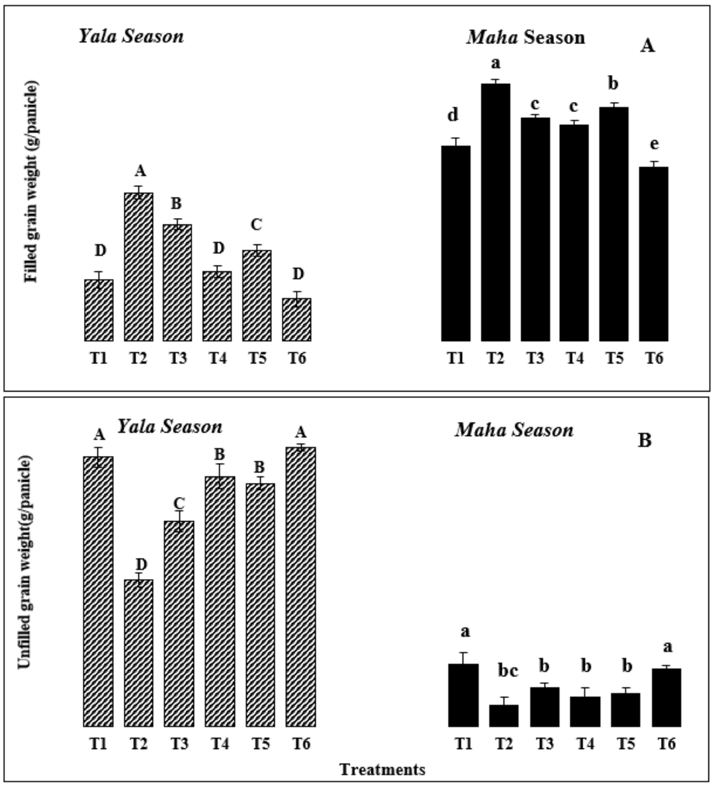

Figure 3: Mean (A) filled grain weight and (B) un-filled grain weight of rice plants at harvest under different treatments (T1-T6). Different letters indicate statistically significant difference at 5% probability level resulted from Tukey's mean comparison test for *Yala* and *Maha* seasons separately. Treatments: T1-CF; T2- CF_{NK} + 100% BEE; T3-CF_{NK} + 80% BEE; T4- CF_{NK} + 65% BEE; T5-CF_{NK} and T6-CTR]

Table 2: Grain yield (kg per ha) at the harvest of *Yala* (2018) and *Maha* (2018/2019) seasons.

Treatment	Yields	
	Yala season (2018) (kg ha^{-1})	*Maha* season (2018/2019) (kg ha^{-1})
CF	$1,710 \pm 11^{b}$	$5,632 \pm 6^{b}$
CF_{NK}+100%BEE	$1,984 \pm 23^{a}$	$6,181 \pm 9^{a}$
CF_{NPK+}80% BEE	$1,981 \pm 16^{a}$	$5,740 \pm 12^{b}$
CF_{NPK+}65% BEE	$1,705 \pm 2^{b}$	$5,190 \pm 8^{c}$
CF_{NK}	$1,837 \pm 13^{a}$	$5,283 \pm 4^{c}$
CTR	$1,119 \pm 17^{c}$	$3,312 \pm 6^{d}$

essential role in the P cycle, and if combined with chemical fertilizer it can be a potential source of P in rice cultivation (Etesami, 2020).

Different letters are statistically significant at 5 % probability level, resulting from Tukey's mean comparison test.

CONCLUSION

The results revealed the potential of using enriched ERP instead of TSP without risking any yield reductions in rice. The 100% dosage of biofilm enriched ERP (BEE) along with N and K fertilizer achieved almost a yield similar to that of DOA-recommended NPK dosage, thus indicating that 100% BEE along with the CF_{NK} is sufficient replacing the full dosage of TSP-P in rice cultivation. However, long-term experimental results are required to make more reliable recommendations.

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