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Microbially Improved Phosphorus Fertilizer for Rice Cultivation

J.P.H.U. Jayaneththi [1*], G. Seneviratne [2], H.M.S.P. Madawala [3] and M.G.T.S. Amarasekara [1]
[1] Faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama, Anuradhapura, Sri Lanka.
[2] National Institute of Fundamental Studies, Hanthana, Kandy, Sri Lanka.
[3] Faculty of Science, University of Peradeniya, Sri Lanka.
*Corresponding author email id: harshaniupulika@gmail.com

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Abstract **– Eppawala Rock Phosphate (ERP) has been identified as a virtuous alternative for Triple Super Phosphate (TSP). However, its application is limited due to low solubility. By enhancing the biosolubility, ERP may be used as a phosphorous (P) fertilizer for annual crops. Certain biofilms (BF1, BF2, BF3 and BF4) have been identified as potential ERP solubilizers, among them BF3 was most effective. Thus, this study was designed to test the potential of BF3-enriched ERP to replace the TSP in rice cultivation. A soil leaching tube experiment was conducted under laboratory condition with six treatments. Rice fertilizer mixture (CFE) recommended by the Department of Agriculture (DOA) was modified (CFM) by replacing TSP-P from BF3-enriched ERP-P with reduced dosages of nitrogen (N) and potassium (K). Different rates (65%, 85% and 100%) of BF3-enriched ERP in CFM were tested against CFE, and soil alone (control). The experiment was arranged in a Completely Randomized Design (CRD) with three replicates. In every two weeks, solubilized P was recovered by leaching for three months. Results reveled that, BF3 enriched 100% ERP recorded significantly ($p<0.05$) higher solubilized P in leachates. Further, it established significantly ($p<0.05$) highest cumulative solubilized P compared to TSP alone application. Overall, it can be conclude that BF3 enriched 100% ERP performed better than the DOA recommended TSP dosage. Therefore, BF3 enriched 100% ERP can be proposed as an alternative to TSP in rice cultivation. However, further studies are needed to evaluate the effectiveness of this BF3 enriched 100% ERP under field conditions.**

Keywords **– Biofilms, Biosolubilization, Department of Agriculture Fertilizer Recommendation, Eppawala Rock Phosphate, Triple Super Phosphate.**

I. Introduction

Rice (Oryza sativa L.) is one of the dominant food crops grown in Sri Lanka. It occupies approximately 34% (0.77 million ha) of total cultivated lands in Sri Lanka (RRDI Annual Report, 2011). On average, 560,000 ha of lands in Maha and 310,000 ha of lands in Yala are cultivated to reach average annual extent of rice about 870,000 ha of lands. Most farmers use synthetic chemical fertilizers as a nutrient supplement in rice cultivation. Urea, Triple Super Phosphate (TSP) and Muriate of Potash (MOP) are commonly applied synthetic fertilizers in rice cultivation. Amongst them TSP used as the main source of P in rice cultivation due to its high solubility since it is vital for short term fast-growing crops such as rice. However, TSP application leads to serious environmental issues such as accumulation of trace elements (Aluminum, Chromium, Nickel, Cadmium, Lead and Uranium etc.) in agricultural fields (Chandrajith *et al.*, 2011). Therefore, it is vital to find an alternative for replacing TSP from agricultural fields.

Eppawala Rock Phosphate (ERP) is considered as a cheap and environmentally-friendly alternative to TSP, despite few constraints. Due to low water solubility of ERP, its' direct application as a P fertilizer has been limited to few crops such as tea, rubber, coconut, export cash crops and other perennial crops. Due to the slow release of P, ERP is not recommended for short-term crops such as rice. Hence, ERP can apply in cooperatively with the biofilms to enhance its' solubility.

Biofilms are assemblages of microorganisms that are attached to biotic or abiotic surfaces and embedded in a matrix of polymers (Khan and Joergensen, 2009). In vitro development of these biofilms as biofilmed biofertilizers (BFBFs) is one of the effective ways to enhance solubility in certain synthetic fertilizers such as ERP. Several studies conducted to test the potential of replacing TSP from biofilm-enriched ERP in rice cultivation. In 2017, Jayaneththi et al. developed a biofilm- enriched ERP using the most effective biofilm formulation (BF3) out of the four biofilms (BF1, BF2, BF3 and BF4) with a potential ERP solubilizing ability by the National Institute of Fundamental Studies (NIFS), Kandy. However, the BF3-enriched ERP did not show promising results in comparison to the DOA recommended TSP dosage for rice cultivation under controlled conditions. They suggested continuing the study by changing the ERP rates enriched with BF3 to replace the TSP in rice cultivation (Jayaneththi *et al.,* 2017). Therefore, this study is focused on testing the ability of replacing TSP by applying different rates of ERP enriched with BF3 in rice cultivation.

II. Methodology

A leaching tube experiment was conducted in the Soil and Water Science Laboratory at the Faculty of Agriculture, Rajarata University of Sri Lanka. Paddy soil (Reddish Brown Earth (RBE) and Low Humic Gley (LHG)soil catena) was collected from a farmer field at Puliyankulama in Anuradhapura, which belongs to the DL1b agro-ecological region with an average annual temperature of 27 ^{o}C and average annual rainfall of 1,368 mm.

The recommended chemical fertilizer (CFE) dosage for rice by the Department of Agriculture (DOA) was modified (CFM) by replacing TSP with ERP enriched with BF3 (BF3 was spread on ERP at a rate of 1.7 L/100 kg as recommended by the NIFS). Six treatments were used including DOA recommended fertilizer (CFE) where TSP is replaced with different rates of ERP enriched with BF3 and controls (soil alone). The tubes were arranged in a completely randomized design with three replicates for each treatment (Table 1).

Table 1. Six treatments used for leaching tube experiment.

Treatments	
T1	100% CFE
T2	65% CFM L1 + BF3
T3	65% CFM L2 + BF3
T4	65% CFM L3 + BF3
T5	100% CFE (only N, K)
T6	Control (soil alone)
CFM (L1) - Urea (65%) MOP (65%) BF3 enriched ERP (100%)	
CFM (L2) - Urea (65%) MOP (65%) BF3 enriched ERP (80%)	
CFM (L3) - Urea (65%) MOP (65%) BF3 enriched ERP (65%)	

Leaching Tube Experiment

Field soils and river sand were sieved (using a 2 mm sieve) separately before mixing them together at a weight ratio of 1:1. The soil: sand mixture was sieved again using the 2 mm sieve before the experiment. The leaching tubes (50 ml) were filled with the 100 g of soil: sand mixture and placed a glass wool pad (about ¼ - inch) on the surface of the soil to avoid dispersion (Fig. 1). The initial weight of each tube was measured and recorded. Measured amounts of fertilizers for the treatments were mixed thoroughly with 25 ml distilled water and added to each leaching tube. The rate of fertilizer applications (inorganic fertilizers and biofilm-enriched fertilizers) were aligned with the DOA and NIFS recommendations for rice. After adding the treatments, tubes were stoppered and incubated at 35 ^{o}C under laboratory condition. The weights of leaching tubes were measured once in 3 days and the initial weight was maintained by adding distilled water. The leachates were collected in 2- weekly intervals (Stanford and Smith, 1972) and the available P contents of the leachates were measured using the Olsen method (Olsen *et al.*, 1954). At the end of the study period (3 months), cumulative solubilized P was calculated. Soils in the leaching tubes were analyzed for biomass C (Anderson and Domsch, 1972), pH (Anderson and Ingram, 1993) and organic matter content (OM) (Baker, 1976) at the beginning and at the end of the study period.

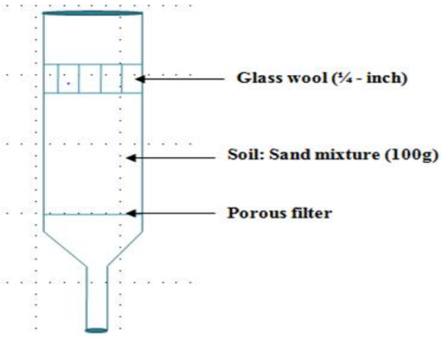

Fig. 1. Schematic diagram of a leaching tube (50 ml) used for the experiment.

Statistical Analysis

The statistical analysis was performed using one-way ANOVA and means were separated using Tukey's HSD test. Microsoft Excel was used to prepare the graphs.

III. RESULTS AND DISCUSSION

Initial Soil Analysis

Paddy soil (RBE and LHG soil catena) was collected from a farmer field, Puliyankulama. This soil belongs to DL1b agro ecological region with an average annual temperature of 270C and average annual rainfall of 1368 mm. Collected soils were initially analysed for certain chemical, physical and biological properties such as pH, available N and P, exchangeable K, organic matter and biomass C (Table 2).

Table 2. Nutrient contents of the 0-15cm soil layer of RBE-LHG catena measured at prior to the experiment.

Soil Property	**Average ± SD**
pH(1:5soil:H_2O)	7.4 ± 0.01
Available P(mg/kg)	14.31 ± 1.75
Available N (mg/kg)	77.32 ± 0.34
Exchangeable K (mg/kg)	102.6 ±1.5
Organic matter (%)	1.23 ± 0.35

Soil Property	Average ± SD
Microbial biomass C (mg/kg)	2.68 ± 1.25

The reaction of the initially collected soil ranged around neutral condition (7.2). It was ranged within the optimum pH range for rice cultivation in dry zone. According to Bandara et al., 2005, optimum pH range for low country dry zone paddy cultivation is 6.2-7.2.

Soil organic matter is significantly influence to maintain the soil physical, chemical and biological properties for an optimum plant growth. The initial value OM of the soil was 1.14%. In 2005, Bandara et al., revealed that 3-5 % is most optimum OM range low country dry zone paddy cultivation. Therefore, OM content of soil (1.14%) used for this experiment was very low at initial level.

Nutrient availability and productivity of agro ecosystems mainly depend on the size and activity of the microbial biomass (Friedel *et al.*, 1996). Therefore microbial biomass may be used as early and sensitive indicators of soil quality for comparisons of soils under different managements. This soil initially established a lowest microbial biomass (2.68 mg/g) since the field was a continuous cultivation filed for two, three seasons with the application of chemical fertilizers.

The available N content of soil was 77 mg/ kg indicating optimum for rice cultivation (Bandara *et al.*, 2005). Available P concentration was 14.3 mg/kg and sufficient for rice but it was not ranged around the optimum range for rice (24-24 mg/kg) (Bandara et al., 2005). Further, P concentrations were less 10 mg/kg are known as P deficient soils (Kumaragamage and Indraratne, 2011). Potassium is also one of another critical nutrient which significantly influenced on growing of rice plant by enhancing the number of spikelets per panicle, filled grain percentage, recovering from toxicity and improving the rigidity of pseudo stems etc. Initial soil collected for this experiment showed good condition of exchangeable K for paddy cultivation (102.62 mg/kg). But it was lower below the optimum exchangeable K (117-156 mg/kg) for low land paddy cultivation in RBE-LHG catena.

Solubilized P in Leachates

In first two (14 days) and four weeks (28 days) of leachates analysis; 100% BF3 enriched ERP (T2) solubilized statistically similar P for 100% DOA recommended CF (N and K only) treatment (T5). Followed by other weeks, T2 showed a significantly higher solubilized P over other treatments. Overall, at the end of the leachates analysis the significantly ($p<0.05$) highest solubilized P was recorded in 100% BF3 enriched ERP applied treatment than the other treatments (Figure 2).

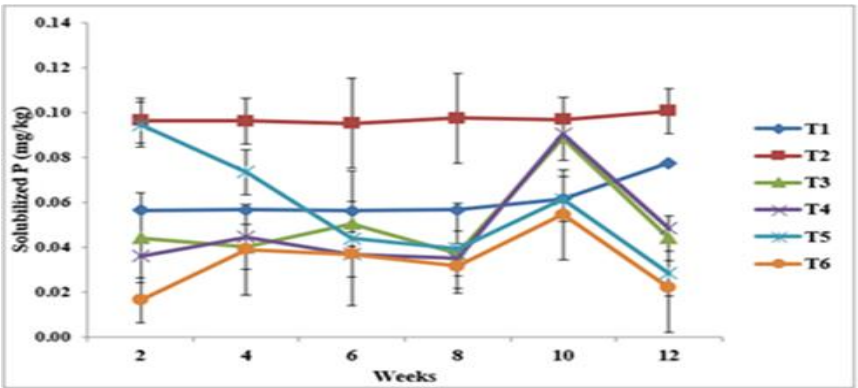

Fig. 2. Solubilized P of leachates in 2 weekly intervals.

Eppawala rock phosphate is a locally available natural resource containing about 28% to 40% of P2O5 depending on the mining technique and the nature of apatite crystals in the matrix (Dahanayake and Subasinghe, 1989). According to Pitawala et al., 2002 solubility of row ERP is very low. The reported approximate citric acid solubility varies from 4 to 6%. Although it is an important phosphorous fertilizer, its usage is not recommended for annual crops like rice due less solubility in normal soil conditions. Phosphorous solubilizing biofilms are capable of enhancing the solubility of ERP while secreting many organic acids (He *et al.*, 2002). These biofilms are used as biofertilizer since 1950s (Kudashev, 1956; Krasilinikov, 1957). Release of P from insoluble and fixed forms is an import aspect regarding P availability in soils. It is confirmed with this study that 100% BF3 enriched ERP showed a significantly ($p<0.05$) highest performance in P solubilization process compared to TSP applied treatments. Triple Super Phosphate is very soluble and after application it might have been quickly fixed in high P fixing soils such as RBE. Generally, available P concentrations are low RBE soil and it is varied from 1 to 87 mg/kg (Kendaragama *et al.*, 2009). The high P fixation ability is the main reason which leads to deficient level of P. Combine application of microbial biofilms with ERP into P fixing soils such as soils collected from RBE-LHG catena for this experiment, would be the reason for enhancing the P solubilization process compared to highly soluble TSP fertilizer. Microbial biomass of biofilms assimilates soluble P and prevents it from adsorption or fixation (Khan and Joergensen, 2009). Microbial community influences soil fertility through soil processes as decomposition, mineralization, and storage/release of nutrients. Microorganisms enhance the P availability to plants by mineralizing organic P in soil and by solubilizing precipitated phosphates (Chen *et al.*, 2006; Kang *et al.*, 2002; Pradhan and Sukla, 2006). These bacteria in the presence of labile carbon serve as a sink for P by rapidly immobilizing it even in low P soils (Bunemann *et al.*, 2005).

Cumulative Solubilized P

Cumulative values of solubilized P in every two weeks were calculated at the end of the three months of leaching tube experiment (Figure 3). Similarly as two weeks analysis, in cumulative solubilized P also significantly ($p<0.05$) highest was recorded in 100% BF3 enriched ERP. Biofilms would be able to enhance the solubility of ERP while assimilating more solubilized P in microbial cells by preventing the fixation of P in soils (Khan and Joergensen, 2009).

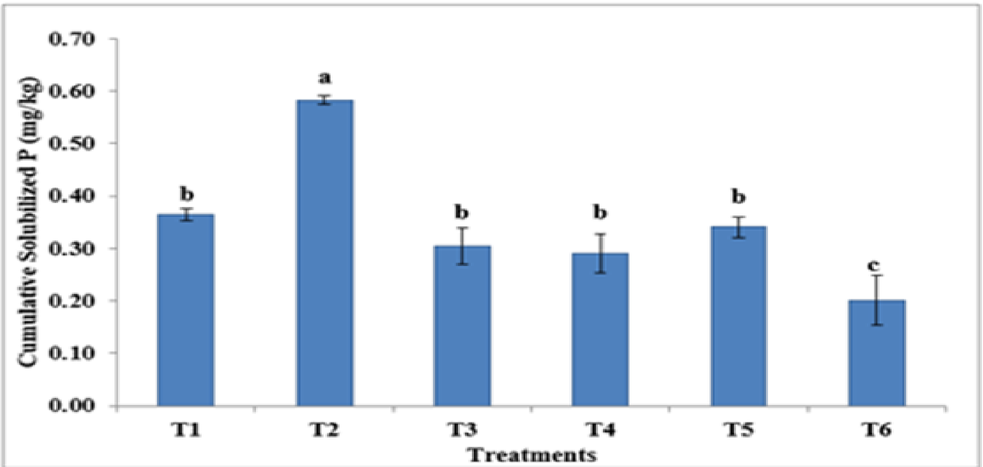

Fig. 3. Cumulative solubilized P of the leachates at end of the three months of leaching tube experiment. Different letters indicate statistically significant differences at 5% probability level according to the Tukey's mean comparison test.

Biomass C, pH and Organic Matter

In three months, higher microbial biomass C was resulted in soils with biofilm incorporated treatments (T2, T3 and T4) compared to inorganic fertilizer alone applications and control (Fig. 4 a). Introduced microbes and subsequent emergence of cyanobacterial and fungal communities with the application of biofilms could be the source for soil microbial biomass enhancement of the biofilms applied treatments compared to chemical fertilizers applied treatments. Generally, the microbial biomass releases a huge quantity of organic carbon which is produced from a variety of decomposition process thus it might be the source of highest organic matter contents that are released with the application of 100% BF3 enriched ERP (Fig. 4 c).

Microbial biofilms apply various approaches to make phosphorus accessible for plants to absorb including lowering soil pH, chelation, and mineralization. Among them principal mechanism for solubilization of soil P is lowering of soil pH by microbial production of organic acids or the release of protons. In present study, soil reactions of all treatments changed is soil reactions of all treatments changed into slightly acidic from the initial neutral pH (pH = 7.4) during the solubilization process (Fig. 4 b).

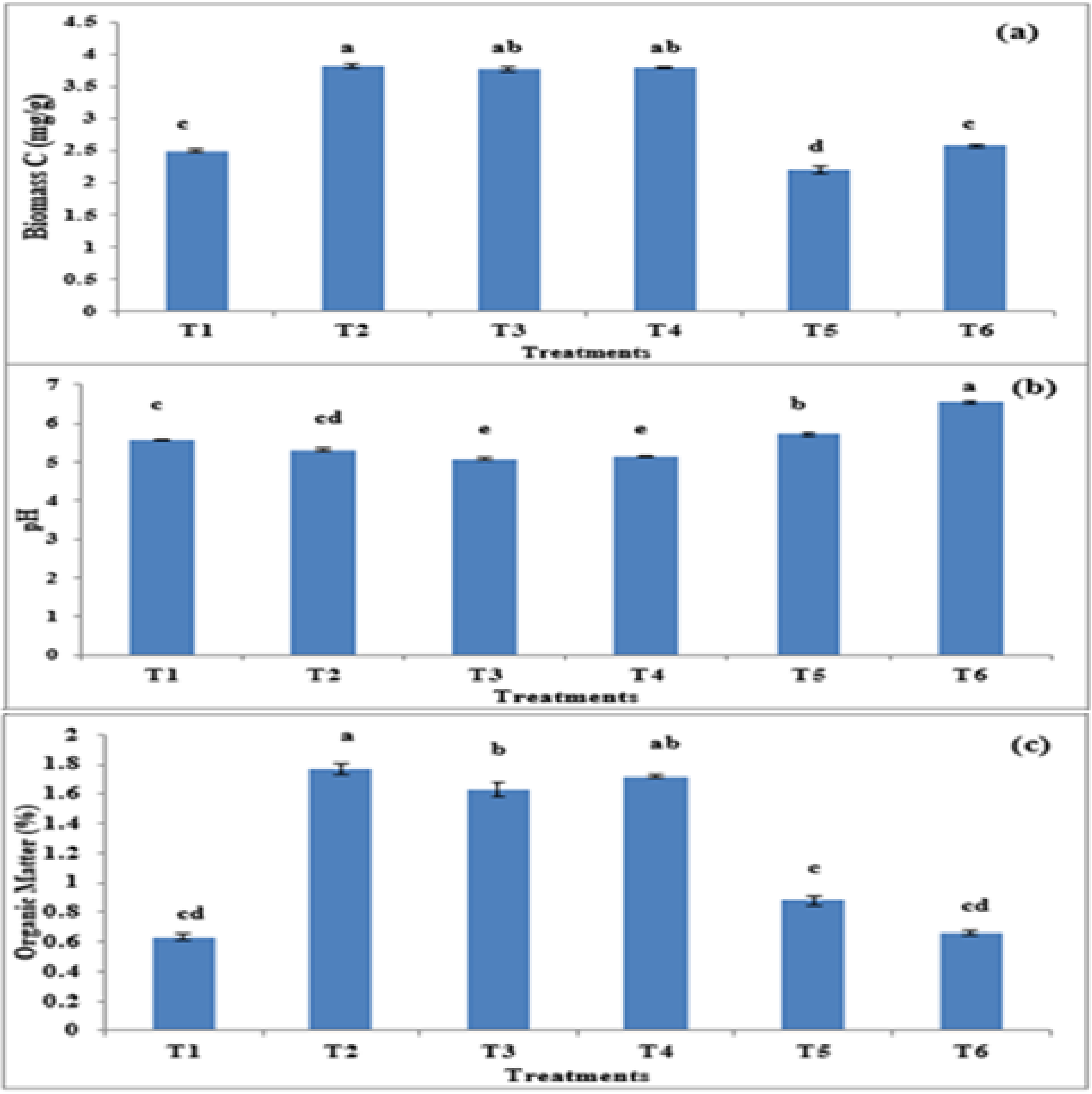

Fig. 4. (a) Biomass C (b) pH and (c) Organic matter content of soils remaining in leaching tubes at the end of the three months. Different letters indicate statistically significant differences at 5% probability level according to the Tukey's mean comparison test.

Available N, P and Exchangeable K

Biofilm treated ERP resulted significant improvement in uptake of N, P and K (Fig. 5). At the end of the three months, available N, P and K contents of soils were fallen rather below compared to initial levels highlights the highest capacity of biofilm incorporated treatments on nutrient uptake. In this regards, microbial biofilms play a significant role by secreting plant growth promoting (PGP) substances which leads to enhance the root growth, thus increasing the nutrient uptake, compared to synthetic fertilizers. Applications of biofilms to the soil under suitable conditions secrete metabolites, acids and enzymes, which make deficient nutrients readily available to the plant (Meena *et al.*, 2015).

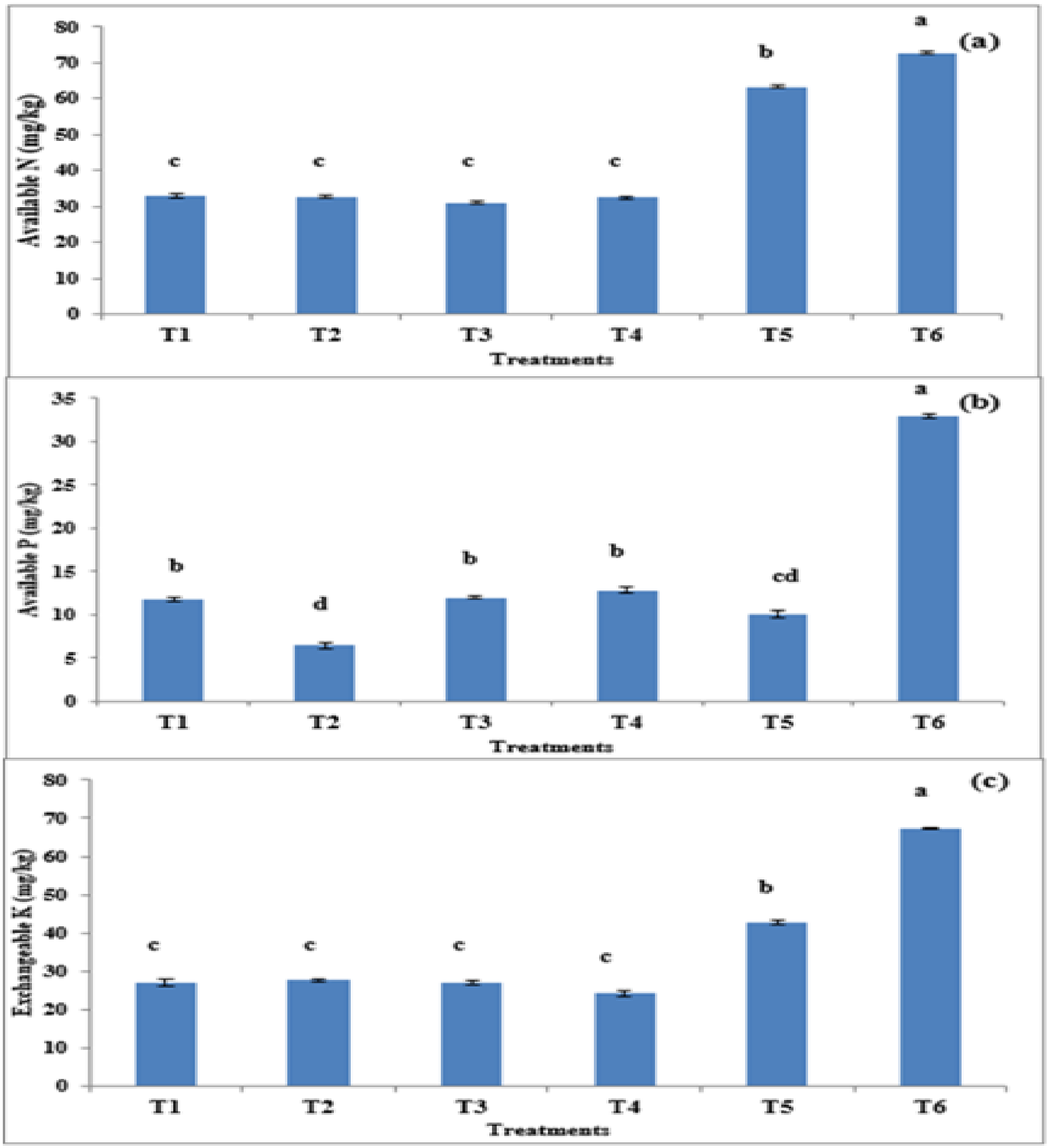

Fig. 5. (a) Available N (b) Available P and (c) Exchangeable K content of soils remaining in leaching tubes at the end of the three months. Different letters indicate statistically significant differences at 5% probability level according to the Tukey's mean comparison test.

IV. Conclusion

The results of the present studies conducted under laboratory (leaching tube experiment) and field conditions showed that the whole 100% of TSP-P should be replaced by BF3 enriched ERP-P in order to achieve the effect of DOA recommended TSP dosage in the chemical fertilizers alone application. In achieving this, only 65% of urea and MOP each was sufficient with the BF3 enriched 100% ERP. Therefore, the BF3 enriched 100% ERP can be suggested as an alternative to TSP, together with a reduction of 35% of urea and MOP each in rice cultivation. Further studies are however needed to test this under field with different soil and climatic conditions in the country.

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AUTHOR'S PROFILE

First Author
Mrs. J.P.H.U. Jayaneththi, Lecturer (Prob.)/ Faculty of Agriculture/ Rajarata University of Sri Lanka.

Second Author
Senior Prof. G. Seneviratne, Institute of Fundamental Studies/ Kandy, Sri Lanka.

Third Author
Prof. H.M.S.P. Madawala, Faculty of Science/ University of Peradeniya, Sri Lanka.

Fourth Author
Dr. M.G.T.S. Amarasekara, Senor Lecturer / Faculty of Agriculture/ Rajarata University of Sri Lanka.