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Evaluation of Nutrient Combinations Including Microbial Biofilm Treated Rock Phosphte for Maize Cultivation

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ABSTRACT

The Government of Sri Lanka has been spending billions of foreign exchange for more than a decade to provide fertilizer subsidy for synthetic fertilizers. Excessive use of chemical fertilizers adversely affects on soil fertility and ultimately crop productivity. Eppawala Rock Phosphate (ERP) is one of the low cost and alternative phosphorous fertilizer sources available in the country. At present, ERP is only used for perennial crops because of the low solubility of P. Since it is evident that low pH conditions increase solubility of ERP, microbes, specially fungi and their biofilm can be used to increase the solubility of ERP as they create acidic conditions. The objective of this study was to select the best nutrient combination including microbial biofilm treated ERP for maize cultivation. Five treatments were applied including a control; soil only (as the control); 100% Triple Super Phosphate, N, K (100% recommended dose by Department of Agriculture) mixed soil; 100% ERP, N, K and ERP solubilizing biofilm mixed soil; 50% ERP, N, K and ERP solubilizing biofilm mixed soil; 50% ERP, N, K, ERP solubilizing biofilm and maize biofilm (commercial biofilm product for maize produced by Lanka Bio Fertilizers Ltd.) mixed soil, 50% ERP, N, K and maize biofilm mixed soil. Maize was used as the test plant. Treatments were arranged according to Complete Randomized Design (CRD) with four replicates. Soil and plant parameters were measured and statistically analyzed. The maize biofilm treated plants applied with 50% ERP, N, K showed the significantly highest ($P<0.05$) dry mass production compared to other treatments including control. This preliminary study indicates that maize biofilm is more effective than ERP solubilizing biofilm in improving plant growth and perhaps even in increasing ERP solubilization. This study concludes that maize biofilm coupled with 50% ERP and 50% N and K is the most appropriate nutrient combination for maize. Further studies under field conditions are needed to understand effects and potentials of the biofilms

KEYWORDS: *Biofilm, Eppawala Rock Phosphate, Phosphorous, Zea Mays*

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1 Introduction

Phosphorus (P) is a naturally occurring element in the environment that can be found in all living organisms, soil and water. It is an essential component for physiological functions that are involved with energy transformations in the plant (Daniel *et al.,* 1996).Phosphorus is one of the 17 nutrients essential for plant growth. Adequate supply of phosphorous is vital for optimum growth and reproduction of plants. The total phosphorous concentration in agricultural crops generally varies from 0.1 to 0.5 percent (Anon, 1999).

Triple Super Phosphate (TSP) is the commonly used P fertilizer in Sri Lanka. However, it was reported that TSP contains hazardous elements such as arsenic and cadmium. Approximately 0.1 million tons of TSP is imported to Sri Lanka annually containing about 2100 kg of arsenic (Jayasumana et al., 2015). In addition government expense billions of foreign exchange to import TSP .Therefore, the use of synthetic fertilizer is not the best option to satisfy P requirement in crop production. Hence ,Eppawala RockPhosphate (ERP) is a better alternative to replace TSP.

Eppawala Rock Phosphate is directly applied for tea, rubber and coconut grown on acid soils (Walpola et al., 2003). However. It cannot be used for annual crops because of its low solubility. Organic acids produced by microorganisms as by-products of their metabolism are responsible for biological weathering of rocks and minerals (Neaman et al.,2005). Eppawala Rock Phosphate is also acid-soluble and activities that increase rhizosphere acidification possibly increase its solubility. Then the use of phosphate solubilizing microorganisms could bean interesting alternative solution for increasing solubility of ERP (Vassileva et al., 1997).

Biofilm (BF) formation is a prominent feature of microbial growth in nature. Certain microbes can attach to biotic or abiotic surfaces and differentiate to form complex, multi cellular communities called biofilms. The biofilms produced promising results in the P solubilization of rock phosphate (RP) (Jayasinghearachchi and Seneviratne, 2006).With the use of microorganisms, a biofilm has been developed at the National Institute of Fundamental Studies to increase the phosphate solubility of ERP.

Hence this study was designed to study the best nutrient combination including microbial biofilm treated rock phosphate, as an alternative for TSP in maize cultivation.

2. Methodology:

The study was carried out at the National Institute of Fundamental Studies (NIFS), Kandy. Duration of the study was six months. Soil samples were collected from the maize growing field in Kahagollarawa area. After removing uppermost vegetative cover, the soils were collected. Eppawala Rock Phosphate samples were taken from the products of Lanka Phosphate Ltd. containing 28% of P2O5 content. Maize variety Pacific was used as the test crop. Evaluation of nutrient combinations including microbial biofilm treated rock phosphate for maize cultivation

Biofilm was developed in a biofilm formation medium as described in protocols in the Microbial Biotechnology Unit (MBU) of the National Institute of Fundamental Studies (NIFS). Biofilm was centrifuged (SANYO Harrier 18/80) at 4000 rpm for 20 minutes (25 oC) and pH of the supernatant was measured. ERP was treated with biofilm at the pH 3.3. A commercially available product in the market namely Maize Bio Film was also included to treatments.

Five treatments [ie: (i)Department of Agriculture (DOA)fertilizer recommendation for maize, (ii) 100%Bio film treated ERP+100% N, K, of DOA (iii) 50%BF treated ERP+50% N, K (iv)50%BF treated ERP+Maize BF+50% N, K (v) 50%ERP+Maize BF+50% N,K] with a control were tested at the greenhouse of National Institute of Fundamental Studies (NIFS) as a pot experiment. Soil collected from Kahagollarawa area, was air dried, sieved and composited prior to pot filling and each pots were filled with equal weight of soil. Treatments were added as split application at 12 days and one month after germination. Experiment was arranged according to Complete Randomized Design (CRD) with four replicates.

Soil samples drawn from each treatment were analyzed for soil pH, soil moisture content, soil available and total phosphorous before and 4 weeks after sowing. Analysis for soil pH, soil moisture content, available and total phosphorous was performed by standard protocols explained in Tropical Soil Biology and Fertility (Anderson and Ingram ,1993).

Plant height, number of leaves per plant and stem diameter were measured at 12 days, 4weeks and 8 weeks after germination. Chlorophyll content of the leaves was also measured using a chlorophyll meter (Chlorophyll meter SPAD-502). Dry matter weight of plant samples was taken after drying at 600C.

The data were subjected to Analysis of Variance (ANOVA) in Minitab 16 version. Means were compared using Tukey's method at $p < 0.05$.

3. **Results and Discussion:**

3.1 Soil parameters

Soil samples drawn from each treatments after one month of the application of fertilizer combinations were analyzed for pH, moisture content, available phosphorous and total phosphorous (Table 01).

Table 01: Variability of soil parameters one month after treatments application

Treatment	pH	Moisture (%)	Available P (%)	TotalP (%)
100%(TSP,Urea,MOP)	5.5 ±0.03 cd	9.81 ±2.90 **a**	0.000627 ±0.16 x10^{-4} **a**	0.01199± 16x10^{-4}**a**
100%(ERP,Urea,MOP) +ERP BF	5.5 ±0.03 **d**	10.77 ±1.47 **a**	0.000361 ±0.09 x10^{-4}**b**	0.00411± 1.5x10^{-4}**b**
50%(ERP,Urea,MOP)+ ERP BF	5.6 ±0.03 **c**	11.64 ±1.30 **a**	0.000363 ±0.22 x10^{-4}**b**	0.00009± 1.2x10^{-4}**c**
50%(ERP,Urea,MOP)+ ERP BF+M.BF	5.6 ±0.02 **c**	12.26 ±1.06 **a**	0.00037 ±0.30 x10^{-4}**b**	0.0035± 0.63$10^{-4}$**bc**
50%(ERP,Urea,MOP)+ M. BF	5.9 ±0.04 **b**	10.64 ±1.15 **a**	0.00027 ±0.03 x10^{-4}**c**	0.0048± 2.5x10^{-4}**b**
CONTROL	6.6 ±0.07 **a**	10.44 ±2.60 **a**	0.000241 ±0.2 x10^{-4}**c**	0.0021±1.7x10^{-4}**c**
INITIAL SOIL	6.7 ±0.01	9.65 ±0.05	0.000305 ±0.04 x10^{-4}	0.001± 0.13 x10^{-4}

The values with same letters are not significantly different at 0.05 probability level. According to table 01, all the treated soil showed higher acidic level compared with the control. The production of organic acids by microbes results in decrease in soil pH. This leads to develop an acidic environment around the rhizosphere. Microbial acid production is also important for suppressing plant pathogens (Bandara et al, 2006). The highest moisture percentage was recorded in the soil treated with ERP solubilizing biofilm, maize biofilm coupled with 50% ERP,MOP and Urea. The lowest moisture percentage was observed in the soil treated with DOA recommendation. However, no significant variation of soil moisture was observed among treatments. The highest available P concentration was recorded in the treatment fertilized with DOA recommendation and the lowest value was depicted in the control. However no significant difference of available P was observed among other treatments. The highest total P concentration was recorded in the treatment fertilized with DOA recommendation .

3.2 Plant parameters

Plant growth is a process of biomass accumulation and is a consequence of the interaction of the photosynthesis, long-distance transport, respiration, water relations and mineral nutrition processes (Lambers et al.,1998). The crucial yield components of maize are mainly the number of plants per area unit and their mass. Revilla et al. (1999) and Vrzalet al. (1999) found the parameters such as plant mass, height,

number of leaves per plant, number of leaves under the ear, length of flowering, grain mass and portion of grain on total yield are very significant with respect to total production of maize.

In this preliminary study plants parameters were measured in order to determine the effects of different treatments on growth of tested maize plants.

3.2.1 Plant height

Figure 01 shows plant height of different treatments one month after germination. Treatments having same letters do not show significant difference. There is a significantly higher plant height in treatment 05 when compared to the control. However no significant difference was observed among other treatments.

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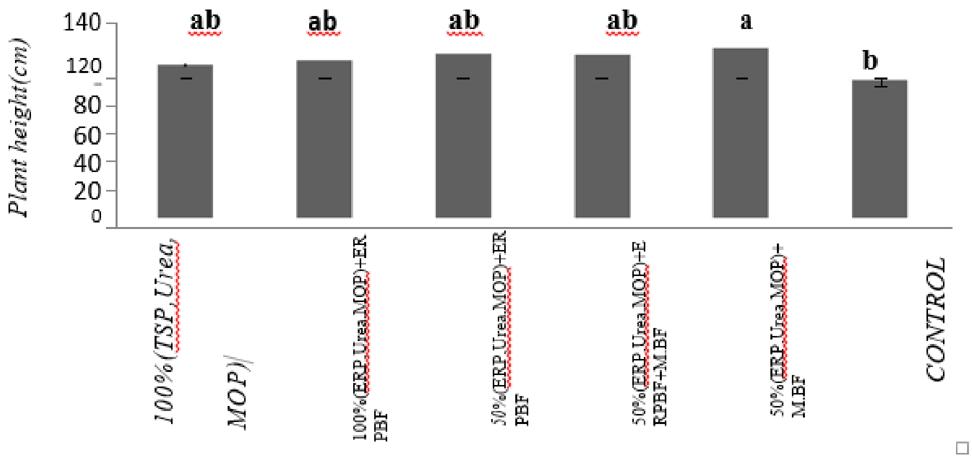

Figure 1:Variability in plant height two months after germination

3.2.2 Stem diameter

Figure 2 illustrates the variability in stem diameter with different treatments two months after germination.

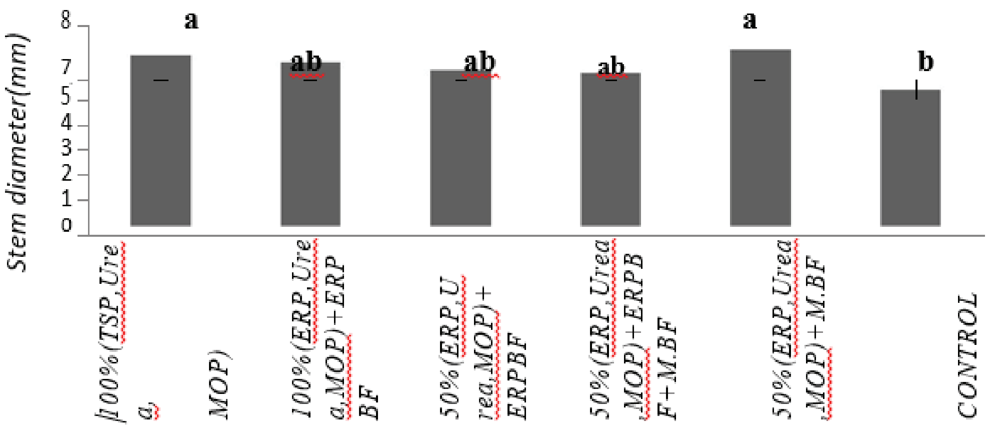

Figure 2:Variability in stem diameter two months after seeding

The corn plant requires N and P soon after germination to initiate the growth of stems, leaves and ear structures (Jones, 1985). The highest value of stem diameter was resulted from the treatments, maize biofilm coupled with 50% ERP,Urea, MOP and the treatment consists of 100% TSP, Urea, MOP.The control treatment showed the lowest value. There was no any significant different was observed among the treatments of ERP solubilizing biofilm coupled with 100%ERP, Urea, MOP and ERP solubilizing biofilm coupled with 50% ERP, Urea, MOP,and 50% ERP, Urea, MOP coupled with two types of biofilm.

3.2.3 Leaf Chlorophyll Content

Leaf chlorophyll is a factor directly related to the photosynthesis of plants (Gendouz and Maamari, 2011). The highest chlorophyll content was recorded in the treatment consists of 50% ERP, Urea, MOP coupled with Maize biofilm while the lowest value was recorded in the control ($p<0.05$).

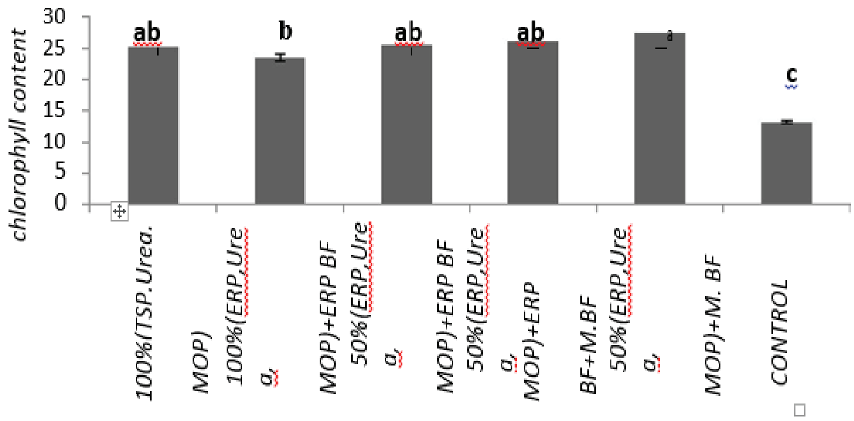

Figure 3:Variability in leaf chlorophyll content two months after germination

The treatments, 100%TSP, Urea, MOP, 50% ERP,Urea, MOP coupled with ERP solubilizing biofilm and 50%ERP.Urea,MOP coupled with both ERP solubilizing biofilm and maize biofilm were not significantly different with each other.

3.2.4 Shoot Dry Weight

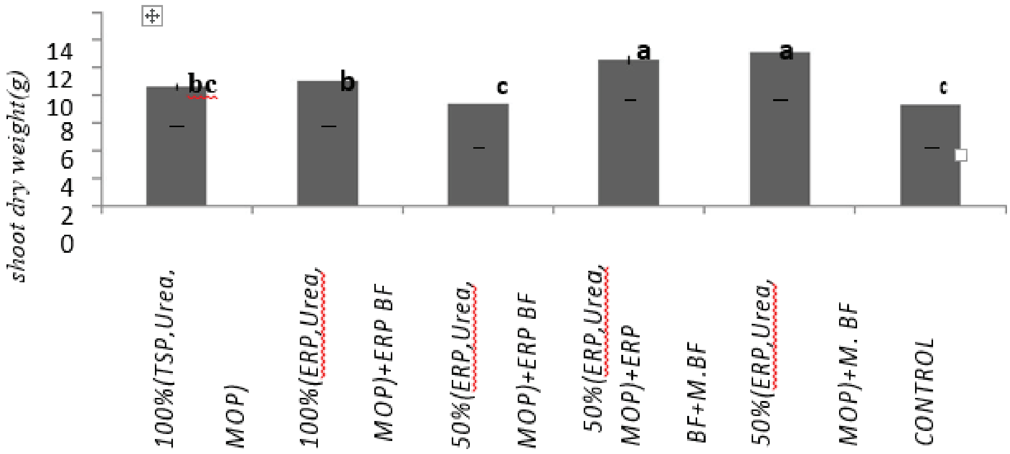

Figure 4:Variability in shoot dry weight two months after germination

Mustafa et al., (2014) reported that, the selection could be made for high yielding maize genotypes on the basis of the characters, such as fresh root length, root density, dry root weight, leaf temperature and dry shoot weight. Significantly higher shoot dry weight were recorded in 50% ERP, MOP, Urea coupled with maize biofllm and 50% ERP, MOP, Urea coupled with both biofilms ($p<0.05$).Out of these two treatments the 50% ERP, MOP, Urea coupled with Maize biofllm showed the highest value. It is very clear that these two treatments are superior than the treatment having DOA recommendation. Except these two treatments others did not show significant difference.

3.2.5 Root Dry Weight

Higher root dry weight was reported by two treatments (ie: treatment consists of 50% ERP, MOP, Urea coupled with Maize biofllm and the treatment consists of 50% ERP, MOP, Urea coupled with both biofilms , Maize biofllm and ERP solubilizing biofilm.)Evaluation of nutrient combinations including microbial biofilm treated rock phosphate for maize cultivation

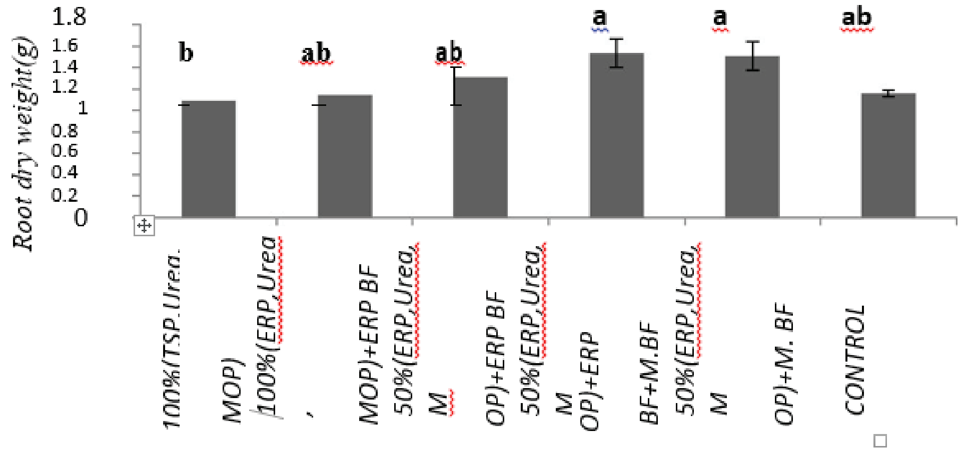

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Figure 5:Variability in root dry weight two months after germination

The plants,that treated with maize biofilm coupled with 50% ERP, Urea, MOP, showed significantly higher values for stem height, leaf chlorophyll content, shoot dry weight and root dry weight. The preliminary study, indicate that the maize biofilm coupled with 50% ERP, Urea, MOP has contributed considerably on plant growth of maize compared with other treatments. Reyes et al.(2002) also reported in a soil-plant microcosm, inoculation of maize with PenicilliumrugulosumIR-94MF resulted in increases of dry matter yields ranging from 3.6 to 28.6% when PRs were used as a P source. Under greenhouse and field conditions, Hameeda et al. (2008) showed that two phosphate-solubilizing bacteria, SerratiamarcescensEB 67 and Pseudomonas sp. CDB 35, increased the biomass of maize fertilized with phosphate rocks.

4. **Conclutions**

This preliminary study concluded that the maize biofilm coupled with 50% ERP,Urea,MOP is the most appropriate nutrient combination for maize. Maize biofilm is more effective than ERP solubilizing biofilm in improving plant growth, and perhaps even in increasing ERP solubilization.

The preliminary pot experiment suggest that the use of maize biofildm with ERP can replace the TSP. However, leaf sample analysis for P and also a filed experiments are needed for concrete conclusions.

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