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Potential of Microbial Solubilization of Rock Phosphate for Use in Sustainable Agriculture: Does Biochar Application Enhance Microbial Solubilization?

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Authors' contributions

This work was carried out in collaboration between both authors. Author NY designed the study, performed the statistical analysis, wrote the protocol. Author SJ wrote the first draft of the manuscript. Authors SJ and NY managed the analyses of the study. Authors SJ and NY managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to investigate the strategies for microbial solubilization of Eppawala rock phosphate in Sri Lanka as an alternative to chemical acidulation by using biochar and microbial inoculants. A pot experiment was carried out in the plant house at Faculty of Applied Sciences, Rajarata University of Sri Lanka, Mihintale, Sri Lanka. The treatments were different combinations of field soil, biochar, mycorrhizae and *Pseudomonas fluorescens* with rock phosphate (RP). The experimental treatments were arranged in a completely randomized block design (CRBD) with eight replicates and the treatment means were compared by the Tukey's test (p<0.05). The soil available phosphorus (P) and leaf P were estimated after 120 days of growing maize in pots. Total bacterial and fungal counts of the soil and mycorrhizal colonization were also estimated. Results showed that the highest available soil phosphorus was observed in biochar, mycorrhizae and *P. fluorescens* addition with 3% RP and highest leaf phosphorus was observed in biochar and

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mycorrhizae with 3% RP. The addition of biochar to the soil containing RP caused a significant increase (p<0.05) of solubilized P in soil. These results suggested that biochar can be used to enhance microbial RP solubilization and mycorrhizae inoculants to increase P uptake. Significantly high bacterial count and fungal count were observed in biochar, *P. fluorescens* and RP added and mycorrhizae, *P. fluorescens* and RP treatments respectively. Greater efficiency of P solubilizing bacteria has shown with the addition of biochar and through co-inoculation with mycorrhizae. Findings of this research increase the prospects of using biochar and P solubilizing microorganisms (PSM) including *P. fluorescens* and mycorrhizae for RP solubilization.

Keywords: Rock phosphate; microbial solubilization; biochar; Pseudomonas fluorescens; mycorrhizae.

1. INTRODUCTION

Scientists have identified phosphorus (P) scarcity in sustainable agricultural systems [1]. Phosphorus is one of seventeen nutrients essential for plant growth and survival. It is the second important plant nutrient after nitrogen, which highly affects the growth of plants by influencing the cell division and development, energy transport (ATP and ADP), photosynthesis, respiration, signal transduction and macromolecular biosynthesis. Phosphorus is frequently deficient for crop production and is required by crops in relatively large amounts. The total P concentration in agricultural crops generally varies from 0.1 to 0.5 percent [2].

The agricultural sector is the foundation in Sri Lankan economy and more than 70% of the population living in rural areas depends on farming as their major source of income. With the demand of synthetic fertilizers has been increased within the agricultural sector in Sri Lanka, resulted several negative effects to the human health and the environment. Farmers use various types of phosphorus fertilizers such as triple super phosphate (TSP), single super phosphate (SSP) and ammonium phosphate (AP). However, environmental concerns and human negative health impacts have led to the search for sustainable way of P nutrition of crops. Moreover, plants can use only a small amount of this P since 75–90% of added P is precipitated by metal–cation complexes, and rapidly becomes fixed in soils [3]. Rock phosphate (RP) is sparingly soluble phosphate and P can be used in organic agriculture and it contains the mineral apatite [Ca_5 $(PO_4)_3OH$, F,Cl]. TSP and AP are more soluble than RP [4].

A huge phosphate rock deposit estimated to be over 40 million metric tons was discovered in Eppawala (North Central Province) in Sri Lanka [5]. Large differences exist between solubility of the sparingly soluble Eppawala rock phosphate (ERP) from the readily soluble TSP, SSP and AP [6]. The primary sources of P fertilizers are RPs that is chemically solubilized with inorganic acids. This process includes more negative effects to people as well as the environment [7]. The use of phosphate solubilizing microorganisms (PSM) is more natural and environmentally friendly method of modifying rock phosphate to use in sustainable agriculture. The PSM which secrete organic acids such as citric acid, gluconic acid, lactic acid and oxalic acid have the ability to solubilize the insoluble P into soluble form [8]. However, it was demonstrated that during the solubilization process, PSM released fluoride from the rock phosphate [9]. It causes a strong decrease in P solubilization. As a soil amendment, biochar is very effective to remove fluoride while it is released from RP and it could increase the overall efficiency of RP solubilization [10].

2. OBJECTIVE

Therefore, this study was aimed to investigate the strategies for ERP solubilization using microbial inoculants such as arbuscular mycorrhizal fungi (AMF) and *Pseudomonas* spp. with the combination of biochar as soil amendment, which facilitate microbial growth, activity and interactions.

3. METHODOLOGY

A pot experiment was carried out in the plant house at Faculty of Applied Sciences, Rajarata University of Sri Lanka in Mihintale, Sri Lanka during July to December 2015. The day temperature in the plant house during the trial was 31-35°C and night temperature was 30-32°C with the 68% relative humidity. Annual precipitation in the area is between 1000-1500 mm and it receives inter monsoon and the North-East monsoon rains mostly.

Soil was gathered from the top soil layer in the field of Mihintale, which has not been cultivated in for a long period and RP was obtained from Lanka Phosphate Limited, Eppawala in Anuradhapura district, North Central Province, Sri Lanka. The soil type was Alfisols. The biochar was produced from wood chips and air- dried maize kernels and stalks (3:2 ratio) by using two -barrel method at a 450–550°C pyrolysis temperature. Two metal barrels of small and large ones were taken for this procedure. For the production of biochar, organic biomass was filled in to the small barrel and the lid was closed. The small barrel was placed inverted position inside the large barrel. The dried organic matter in the inner barrel was heated to about 450–550°C by burning with firewood in the space between the inner and outer barrels. After the biochar production it was ground by passing through a sieve. The AMF inoculant was prepared by the trap cultured maize plant roots. For trap culturing root samples were collected from organically grown maize field in Eppawala and soil collected from the land in Mihintale, which was left fallow for ten years. After trap cultured, the root samples were stained and checked the AMF colonization potential [11] before the soil application.

Pseudomonas fluorescens, isolated from forest soil in Mihintale and a soil sample from Eppawala rock phosphate deposit. As the first step, one gram of soil was mixed in 10 mL distilled water and prepared 10^{-1}–10^{-5} dilution series. The spread plates were prepared from the dilution series using King's B (KB) medium and incubated in temperature of 30°C. King's B medium is recommended for distinguishing fluorescent pseudomonads from non-fluorescent pseudomonads. The *Pseudomonas fluorescens* colonies were transferred to the flasks of 25 mL nutrient broth (NB). They were placed on the shaker for 72 hours, shaking at 100 rpm and 30°C, until reach the density approximately of 10^8-10^9 CFU/ mL in NB [12].

Treatments were as follows, T_1: field soil (FS) (control), T_2: sterilized field soil (SFS) and 3% RP (w/w), T_3: field soil (FS) and 3% RP (w/w), T_4: field soil (FS), 20% biochar and 3% RP (w/w), T_5: field soil (FS), AMF and 3% RP (w/w), T_6: field soil (FS), *P. fluorescens* and 3% RP (w/w), T_7: field soil (FS), 20% biochar, AMF and 3% RP (w/w), T_8: field soil (FS), 20% biochar, *P. fluorescens* and 3% RP (w/w), T_9: field soil (FS), AMF, and 3% RP (w/w), T_{10}: field soil (FS), 20% biochar, AMF, *P. fluorescens* and 3% RP (w/w). Twenty per cent of biochar (w/w) was added in pots of respective treatments and uniformly ground biochar was mixed with the soil. The total weight of the filled pot was three kilograms.

One hundred grams of crushed *Gliricidia* leaves and *Panicum* grass leaves were added to each pot to maintain adequate nitrogen (N) and potassium (K) in the soil. The treatments were arranged in a Completely Randomized Block Design (CRBD) with eight replicates. Maize (*Zea mays* L.) was grown as a test plant and pots were irrigated daily with 200 mL water. Three maize seeds were planted in each pot and thinned to one after the second week. Inoculation was done over the seeds after planting with *P. fluorescens* at a population of 1×10^8 cell/g soil. Above ground plant material was harvested after 120 days and dry matter yield per single plant and amount of plant phosphorus and available soil phosphorus were determined by using atomic absorption spectrophotometer (Technicon-UTBP) following the method described by Murphy and Riley [12].

Soil microbial analyses were also carried out following the standard procedures and using selective culture media. Bacterial, fungal and *Pseudomonas fluorescens* colony counts were estimated by using tryptone soy agar, rose bengal agar and King's B medium respectively. Fine lateral roots of maize plants were collected and stained according to the procedure published by McGonigle et al., [13]. After stained the roots were observed under high power (x40) of the light microscope and count the AM fungal colonization potential in each treatment. Results were statistically analyzed and Tukey's Studentized Range (HSD) test was used to compare the mean values at a significance level of $p< 0.05$.

4. RESULTS AND DISCUSSION

The results indicated that RP applied treatments contained significantly higher amount of available soil P than the control (Fig.1). T_4 and T_{10} treatments were shown significantly higher amount of soil P. This was indicated that biochar alone (T_4) and biochar with AMF and *Pseudomonas* added treatments were shown the increase P solubilization with comparing other treatments (Fig. 1). Significantly higher ($p<0.05$) leaf P was observed in biochar and AMF with 3% RP (T_7). Eppawela rock phosphate

had 38% of P_2O_5 and it was not in solubilize form as $H_2PO_4^-$ or HPO_4^{2-}; the form of P, which can be absorbed by the plants. It was revealed that available soil P concentration, which was assumed that the RP solubilization was achieved by AMF and *Pseudomonas* sp. with biochar.

Rodriguez and Fraga [14] suggested that the inoculated rhizobacteria could release P ions from insoluble P sources and the released P was taken up by the external arbuscular mycorrhizal mycelium because the rhizobacteria cannot transfer P to roots. *Pseudomonas fluorescens*, which was inoculated in the present study, is one of the rhizobacteria that influences solubilization of insoluble phosphorus. According to Ahonen et al., [15] the inoculation of P solubilizing microorganisms is also a promising technique because it can increase phosphorus availability in soils fertilized with rock phosphates.

It appeared that there was a significant difference among treatments (p=0.02) in final bacterial count over the control. The highest final bacterial count (Fig. 3) was observed in 20% biochar and *P. fluorescens* with 3% RP (T_8).

T_4, T_7, T_8, and T_{10} were soils amended with biochar in this experiment. The application of biochar to the soil might be formed the favourable niche for the growth of bacteria. Pores within a biochar particle are large enough to accommodate soil microorganisms, to the exclusion of their larger predators [16]. It is possible to increase the water retention capacity by adding biochar, thus increasing the suitability of amended soils as microbial habitat [17].

Sterilized field soil with 3% RP (T_2) was used to check whether RP solubilization could occur without microbial activities. Soil was sterilized by autoclaving. However, in T_2, after establishment of maize, the tested soil consisted of comparatively low bacterial population (Fig. 3). This may be due to the effective establishment of environmental bacteria after the application of sterilized soil via air and water. The substrate in soil available for microbial establishment, after sterilization, tends to be utilized by the more competitive microorganisms.

According to the results there was a significant difference among treatments (p<0.05) in fungal count over the control. The highest fungal count (Fig. 4) was observed in microbial inoculum addition with 3% RP (T_9). In T_1, T_3 and T_6 the final fungal count was relatively high. The lowest final fungal count was observed in 20% biochar and microbial inoculum addition with 3% RP (T_{10}). T_4, T_7, T_8, and T_{10} were shown relatively low final fungal counts when compared to the other treatments with biochar application.

T_1: FS (control), T_2: SFS + 3% RP, T_3: FS + 3% RP, T_4: FS + 20% biochar + 3% RP, T_5: FS + AMF + 3% RP, T_6: FS + *P. fluorescens* + 3%

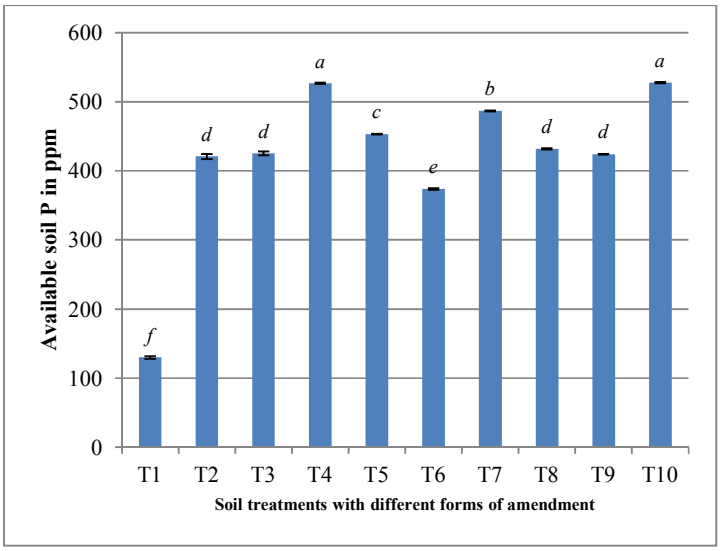

Fig. 1. Changes of available soil phosphorus with different treatments
Means denoted with different letters are significantly different at p<0.05

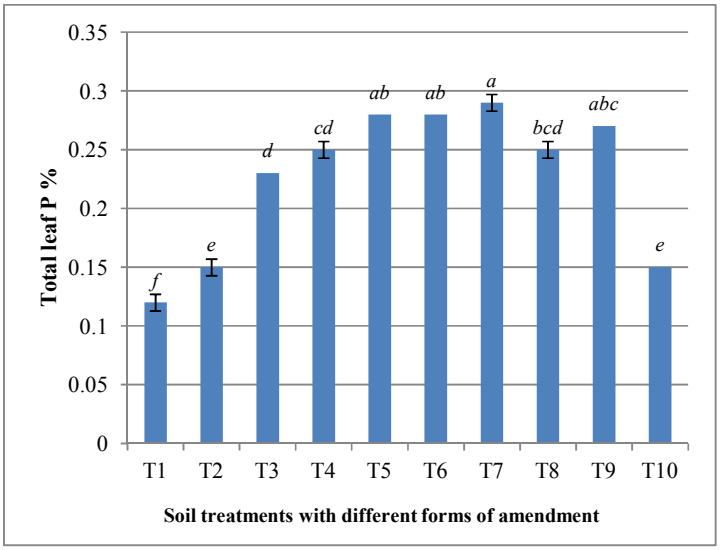

Fig. 2. Changes of leaf phosphorus with different treatments
Means denoted with different letters are significantly different at p<0.05

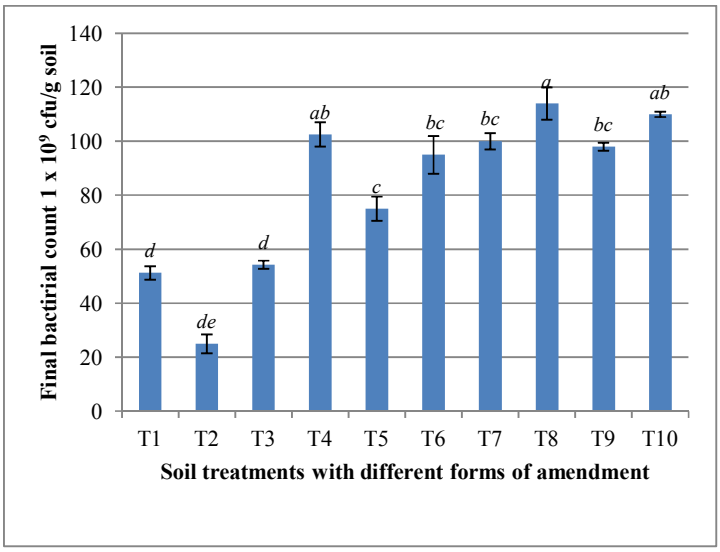

Fig.3. Changes of final bacterial count with different treatments.
Means denoted with different letters are significantly different at p< 0.05

RP, T_7: FS + 20% biochar + AMF + 3% RP, T_8: FS + 20% biochar + *P. fluorescens* + 3% RP, T_9: FS + AMF + *P. fluorescens* + 3% RP, T_{10}: FS + 20% biochar + AMF + *P. fluorescens* + 3% RP.

It was revealed that there was a significant difference among treatments (p<0.05) in the *Pseudomonas fluorescens* count and percentage AMF colonization over the control. The highest *P. fluorescens* count (Fig. 5) as well as highest percentage AMF colonization (Fig.6) were observed in microbial inoculums addition with 3% RP (T_9).

The *P. fluorescens* count was somewhat close to the application of *P. fluorescens* inoculums in the pot experiment. T_6, T_8, T_9 and T_{10} initially consisted of the added *P. fluorescens* inoculums and it was shown a relatively high number of final *P. fluorescens* count. The PSM can solubilize and mineralize P from inorganic and organic pools of total soil P and may be used as

inoculants, in order to increase plant P availability [18]. The PSM which excrete organic acids such as citric acid, gluconic acid, lactic acid, oxalic acid etc. have the ability to solubilize the insoluble P into the soluble form in soil applications [19].

T_7 and T_{10} consisted of AMF inoculums and 20% added biochar. So that may have facilitated the biochar soil amendment in increasing the colonization of AMF abundantly in these treatments. Maize has high arbuscular mycorrhizal colonization potential and hence increases soil phosphorus availability [20]. One of the positive effects of biochar is to increase mycorrhizal association when applied to soil [21].

With both biochar addition and mycorrhizal abundance, opportunities for exploiting a potential synergism that could positively affect soil quality are brought about. According to Warnock et al., [21], biochar can also increase the ability of AMF to assist their host in resisting infection by plant pathogens. According to Ortas et al., [22] mycorrhizal fungi on the host plant

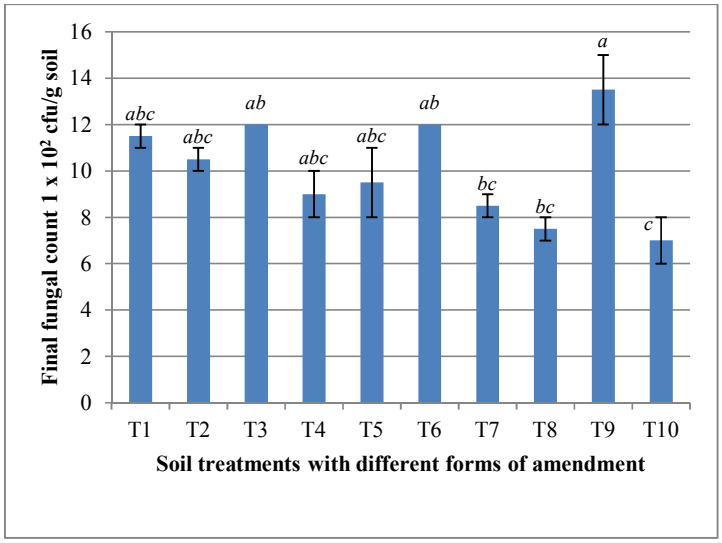

Fig.4. Changes of final fungal count with different treatments
Means denoted with different letters are significantly different at p< 0.05

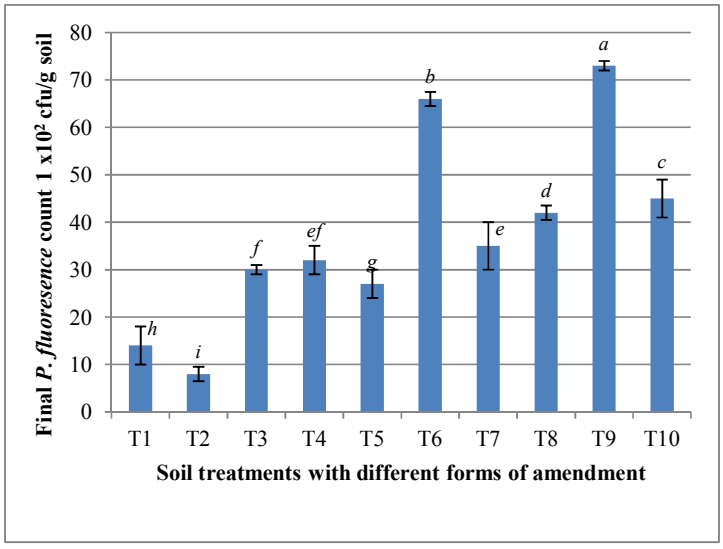

Fig.5. Changes of *Pseudomonas fluorescens* count with different treatments
Means denoted with different letters are significantly different at p< 0.05

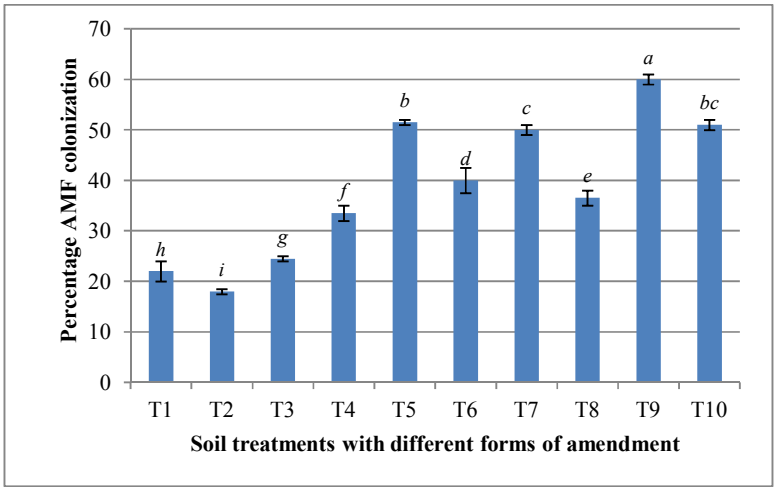

Fig. 6. Changes of percentage arbuscular mycorrhizal fungi (AMF) colonization with different treatments

Means denoted with different letters are significantly different at p< 0.05

increases phosphorus fertilization rate, which is mainly due to the capacity of the mycorrhizal fungi to absorb phosphate from soil and transfer it to the host roots.

It was observed that T_9 possessed a high *Pseudomonas fluorescens* count and also higher percentage of maize root AMF colonization. Beyond the phosphate solubilization, many P solubilizing microorganisms increase the mycorrhizal root colonization by production of specific metabolites such as vitamins, amino acids and hormones [23].

5. CONCLUSION

The results concluded that biochar with AMF and *P. fluorescens* have the potential of ERP solubilization in short term applications. Also biochar and microbial inoculums increased the soil P availability and increased P nutrition, growth and yield of maize plants. The potential of dual inoculation with AMF and *Pseudomonas fluorescens* with the biochar needs to be further evaluated under different crop and agro-climatic conditions, particularly in the field.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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