Sustainable utilization of Rock Phosphate for improved phosphorus nutrition of rice (*Oryza sativa* L.), black pepper (*Piper nigrum* L.) and cinnamon (*Cinnamomum verum* J. Presl) by using microbial inocula

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Abstract

Realizing the deteriorative effects of chemical phosphorus (P) fertilizer, scientists seek for better alternatives to improve plant P nutrition while sustaining soil health. Being a low solubilized P fertilizer, Eppawala Rock Phosphate (ERP) is recommended only for perennials. For annual crops, Triple superphosphate (TSP) is imported at a high cost. Hence this study mainly focused on improving the solubility of ERP. As ERP is a non-renewable resource, exploring a way of sustainable and eco-friendly utilization is vital while improving plant P nutrition. In this study, the approach of ERP solubilization by P solubilizing microorganisms (PSM) was done in two ways: (i) Application of P solubilizing microorganisms directly as biofertilizers by introducing into the soil and (ii) Using PSM for *in-vitro* ERP solubilization to produce a liquid P fertilizer (LPF).

A native *Aspergillus* sp., a P solubilizing bacterium and an Arbuscular mycorrhizae (AM), *Glomus mosseae* were employed. Initially, molecular identification was done to confirm the identity of PSM. P solubilizing bacteria (PSB) was identified as *Paraburkholderia tropica* (Synonym *Burkholderia tropica*) whereas fungus (PSF) was identified as *Aspergillus japonicus*.

For direct application as a biofertilizer, mass cultivation was done. Five different agricultural waste materials: rice straw, rice husk, coir dust, saw dust and sugarcane bagasse were employed as base material. Those were supplemented either with broken maize seeds or rice bran. The base materials were tested alone and in combinations either with broken maize seeds or rice bran at the ratios of 1:1, 2:1, 3:1 and 5:1. Among the tested materials the best mass culturing medium for *A. japonicus* and *P. tropica* were found to be rice straw supplemented with broken maize seeds at a ratio of 2:1 and rice straw supplemented with rice bran at a ratio of 1:1, respectively.

The factors affecting *in-vitro* ERP solubilization (time, aeration, C and N concentration) were optimized for *A. japonicus*, *P. tropica* and for the combination of *A. japonicus* and *P. tropica*. It was observed the P solubilization of *A. japonicus* (170.36±3.81 mg P/ L) was superior in the medium which contained 0.5 g of ammonium sulphate & 10 g of glucose, aerated at 100 rpm oscillation for 72 hours.

Rice (*Oryza sativa* L.) pot experiment with P biofertilizer contained sixteen treatments with combinations of PSM and different P fertilizer levels. Growth and yield parameters, soil available P, shoot P uptake and microbial counts were obtained at three growth stages of rice, i.e. vegetative, reproductive and ripening stage. The data were statistically analyzed using one way ANOVA, two way ANOVA and multiple mean comparison using PROC GLM in SAS 9.0 version. The tested parameters were significantly different ($p \leq 0.05$) among the treatments. In general, the treatments with PSM improved the available soil P, plant P uptake and the grain yield. The maximum grain yield per plant was obtained in *A. japonicus* with 50% TSP and remaining 50% P_2O_5 replaced by ERP (4.78±0.38 g) and the next highest was in combined inocula with 50% TSP and remaining 50% P_2O_5 given by ERP (4.26±0.17 g). Both were on par with the DOA recommended treatment and selected for the field experiment.

Rice pot experiment with LPF contained six different treatments and the data were obtained at three growth stages. Treatment with LPF (split application) showed the maximum treatment effect in the parameters tested and reported the highest significant grain yield per plant (3.82±0.59 g). The next highest was obtained in treatment having combination of 50% TSP and the remaining 50% P_2O_5 by LPF (2.93±0.38 g). These two treatments were on par with each other and selected for the field trial.

Rice field experiment was carried out in RRDI, Batalagoda. It contained ten treatments repeated in three blocks. Similar parameters were tested as in pots. A positive effect was observed in treatments with PSM and LPF. Treatments with inocula recorded the highest

significant soil P content even at the harvesting stage revealing the effectiveness of applied PSM. *A. japonicus* was the most persistent in soil and retained in the range of 10^4 cfu/g soil throughout the experiment. The maximum grain yield per hill was recorded in treatment with LPF (22.69±1.32 g) followed by the treatment having *A. japonicus* along with 50% TSP and remaining 50% P_2O_5 given by ERP (21.27±1.32 g), both were on par with the recommendation. Overall results exhibited the possible reduction of TSP by 50% in the presence of the promising P biofertilizer, *A. japonicus*.

Pepper (*Piper nigrum* L.) and cinnamon (*Cinnamomum verum* J. Presl.) pot experiments were conducted separately in the University of Colombo. Each pot experiment contained twenty four treatments with combinations of PSM and P fertilizer levels; twelve were with inocula and the other twelve were counterparts with no inocula. Growth parameters, soil available P, shoot P uptake and microbial counts were obtained at three destructive sampling stages; 10 week time interval after inoculation. Generally, a significant treatment effect was not observed in the growth parameters except for root dry weight. At each sampling session, treatments with the inocula recorded significantly high soil P (except for mycorrhizae treatments) compared to their counterparts. In both experiments, P solubilization was significantly high in treatments having fungus and bacteria with recommended ERP and in the treatment having same inocula with 50% of recommended ERP. During the later stages, P solubilization was significantly high in treatments having fungus either with recommended ERP or with 50% of recommended ERP. Improvement of shoot P uptake was also observed in these treatments as well. Similar to rice experiment *A. japonicus* was more persistent in soil than *P. tropica*. Hence, *A. japonicus* with 50% of recommended ERP has a potential to be used as a promising P biofertilizer for perennial crops.

Phosphate biofertilizer has a potential to solubilized fix P in soil, hence the residual P in selected fertilized and non-fertilized black pepper and cinnamon cultivations were investigated prior to extending the treatments to field. It was found that root zone soil of fertilized cultivations contained large amount of total P compared to non-fertilized cultivation indicating the possible accumulation of P due regular fertilization over a long period of time. Therefore, development of strategies to utilize residual P pool for improved plant P nutrition can lead to a reduction in the cost of production and sustainable utilization of ERP reservoir.