Enhancement of phosphorus solubility in rock phosphate through the addition of biochar, *Pseudomonas fluorescens* and arbuscular mycorrhizae

S.G.R. Jayawardhana, and P.N. Yapa*
Faculty of Applied Sciences, Rajarata University of Sri Lanka, Mihintale,
*pnyapa40@yahoo.co.uk

Introduction

Phosphorus (P) is one of the major plant nutrients which highly affects the growth and development of plants. The total P concentration in agricultural crops generally varies from 0.1 to 0.5 percent. Since the world population increases, the application of chemical fertilizers in crop cultivation has increased dramatically. Farmers use various types of phosphorus fertilizers such as triple super phosphate (TSP), single super phosphate (SSP), ammonium phosphate (AP) and rock phosphate (RP). RP 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 (Withers *et al.*, 2005).

A phosphate rock deposit estimated to be over 40 million metric tons was discovered in Eppawala (North Central Province) in Sri Lanka (Jayawardana, 1976). Considering the adverse consequences in imported TSP used in agriculture sparingly soluble RP might be utilized in Sri Lanka if appropriate solubility is achieved. It is a challenge to find different strategies to process RP cost effectively to make more soluble fertilizer for short term crops and maximize the crop yield. Many materials and compounds available in soil or added into soil play a great role in P availability. Among them biochar, arbuscular mycorrhizal fungi (AMF) and P solubilizing bacterial strains (PSM) are prominent. Biochar is a type of soil amendment made from pyrolysis of biomass feedstock. It has several chemical and physical properties which facilitates microbial activities as well as mineral retention and exchange (Lehmann *et al.*, 2006).AMF, in their association with rootlets, secrete acid phosphates which dissolves insoluble P, facilitating plant P nutrition. (Duponnois and Plenchette, 2003).Some mechanisms in of PSM strain, *Pseudomonas*enhance P availability from rock phosphate (Zaida *et al.*, 2003). Synergistic interactions between AMF and P solubilizing microorganisms (PSM) help plant growth. PSM solubilize and release H_2PO_4 from unavailable forms of P and AMF help in the uptake of H_2PO_4 ions from soil (Bagyaraj *et al.*, 2015).

Therefore, this study was aimed to investigate the strategies for RP solubilization using microbial inocula such as arbuscular mycorrhizal fungi and *Pseudomonasfluorescens*in combination of biochar as soil amendment which facilitates microbial growth, activity and interactions.

Methodology

A pot experiment was carried out in the plant house at Faculty of Applied Sciences, Rajarata University of Sri Lanka in Mihintale (soil type is Reddish Brown Earth), during July to December 2015. Soil was gathered from 0-15 cm depth from an agricultural field in Mihintale, which has been kept fallowing for three years. High grade Eppawala rock phosphate (HERP) was obtained from Lanka Phosphate Limited, Anuradhapura. The biochar was produced from wood chips and air dried maize kernels and stalks. It was produced at a 450 – 550 ^{0}C pyrolysis temperature using two barrel method. AMF inoculum

was prepared by the trap cultured maize plant roots. After trap cultured, the root samples were stained and checked the potential AMF colonization (McGonigle *et al.*, 1990) before the soil application. *P. fluorescens* were isolated from forest soil in Mihintale and soil sample from Eppawala rock phosphate deposit.First the spread plates were prepared from the soil dilution series using King's B (KB) medium and incubated at 30^0C temperature. King's B medium is recommended for distinguishing fluorescent pseudomonads from non fluorescent pseudomonads.

After 24 hours the colonies were observed under ultraviolet illuminator (356 nm). Green fluorescence colonies were identified as *Pseudomonas fluorescens* and further identified by biochemical tests such as Gram test, oxidase test and catalase test. They were Gram negative, oxidase positive and catalase positive. The identified colonies were sub cultured on KB medium. The pure *P. fluorescens* colonies were transferred to 25 mL nutrient broth (NB). They were placed on the shaker for 72 hours to a density approximately of 10^8- 10^9 CFU/ mL in NB with shaking at 100 rpm and 30 ^{0}C (Hussain *et al.*, 2009). After 5 mL of broth culture was added to soil in each pot according to particular treatment.

Treatments were comprised of T_1: soil only, T_2: sterilized soil and 3% RP, T_3: soil and 3% RP, T_4: soil, 20% (v/v) biochar and 3% RP, T_5: soil, mycorrhizae and 3% RP, T_6: soil, *P. fluorescens* and 3% RP, T_7: soil, 20% (v/v) biochar, mycorrhizae and 3% RP, T_8: soil, 20% (v/v) biochar, *P. fluorescens* and 3% RP, T_9: soil, mycorrhizae, *P. fluorescens* and 3% RP, and T_{10}: soil, 20% (v/v) biochar, mycorrhizae, *P. fluorescens* and 3% RP. The treatments were arranged in a Completely Randomized Block Design (CRBD) with eight replicates. Maize (*Zea mays* L.) was grown as a test plant. Growth parameters were recorded within four weeks intervals. Plant height and length of leaves were measured by using steel tape. Number of leaves were recorded including shaded leaves. Relative growth rate was calculated.

After 120 days of seeding, available soil phosphorus and total leaf phosphorus were measured with using atomic absorption spectrophotometer (Technicon-UTBP). All the yield parameters were taken after uprooting the plants. Dry weight of kernels was measured after dried in oven within 60^0C temperature overnight. Then 100 seed weight was determined. Results were statistically analyzed using Tukey's Studentized Range (HSD) test and the mean values were compared at a significance level of $p< 0.05$.

Results and Discussion

Results indicated that there was a significant difference among treatments ($p<0.05$) in all measured parameters over the control. The highest available soil phosphorus was observed in biochar AMF and *Pseudomonas fluorescens* addition with 3% RP (T_{10}) and highest leaf phosphorus was observed in biochar and AMF with 3% RP (T_7). In the treatments of T_5, T_6, T_7, T_8 and T_9 the available soil P was decreased while the total leaf P was relatively increased (Table 1).

Sterilized soil with 3% RP (T_2) was shown the higher relative growth rate, leaf numbers, and dry weights of shoots and roots (Table 2). Higher growth and yield parameters of T_2 may be due to soil heating (during sterilization) might have increased the solubility of certain mineral nutrients. Growth rate and yield were shown lower in biochar added treatments (T_4, T_7, T_8 and T_{10}) and it might be due to biochar adsorbed nutrients and they were temporarily unavailable to plant (Lehmann *et al.*, 2011).

Table 1. Available soil phosphorus and total leaf phosphorus changes with different treatments

Treatment	Available soil Phosphorus (ppm)	Total leaf Phosphorus (%)	Soil pH	Soil electrical conductiv (mS/cm)
T_1	130±2.001 ^{f}	0.12±0.007 ^{f}	7.27±0.01 ^{bc}	82±1.0 ^{g}
T_2	420.8±3.698 ^{d}	0.15±0.007 ^{e}	7.32±0.02 ^{b}	130±1.0 ^{ab}
T_3	425.4±2.701 ^{d}	0.23±0.000 ^{d}	7.19±0.01 ^{c}	109.5±1.5 ^{e}
T_4	526.85±1.047 ^{a}	0.25±0.007 ^{cd}	7.6±0.01 ^{a}	128±1.0 ^{b}
T_5	453.3±0.403 ^{c}	0.28±0.000 ^{ab}	7.07±0.035 ^{d}	110±2.0 ^{e}
T_6	373.85±1.047 ^{e}	0.28±0.000 ^{ab}	6.98±0.005 ^{d}	97±1.0 ^{f}
T_7	486.7±0.700 ^{b}	0.29±0.007 ^{a}	7.62±0.01 ^{a}	115±1.0 ^{d}
T_8	432±1.103 ^{d}	0.25±0.007 ^{bcd}	7.59±0.035 ^{a}	110.8±1.0 ^{e}
T_9	424.25±0.453 ^{d}	0.27±0.000 ^{abc}	7.19±0.005 ^{c}	121.5±0.5 ^{c}
T_{10}	527.75±0.849 ^{a}	0.15±0.000 ^{e}	7.61±0.01 ^{a}	132±2.5 ^{a}

Means denoted with different letters are significantly different at $p< 0.05$.
T_1: Soil only (control), T_2: Sterilized soil + 3% RP, T_3: Soil + 3% RP, T_4: Soil + 20% biochar + 3% RP, T_5: Soil + AMF + 3% RP, T_6: Soil + *Pseudomonas fluorescens*+ 3% RP, T_7: Soil + 20% biochar + AMF + 3% RP, T_8: Soil + 20% biochar + *Pseudomonas fluorescens*+ 3% RP, T_9: Soil + AMF + *Pseudomonas fluorescens*+ 3% RP, T_{10}: Soil + 20% biochar + AMF + *Pseudomonas fluorescens*+ 3% RP.

Table2. Plant growth and yield parameters changes with different treatments

Treatment	Relative growth rate	Number of leaves	Length of leaves (cm)	Shoot dry weight (g)	Root dry weight (g)	Kernel dry weight (g)	Dry weight of 100 seeds (g)
T_1	0.23±0.003 ^{e}	12.5±0.25^{b}	72.58±2.6^{b}	59.91±4.312 ^{b}	7.54±0.821 ^{c}	23.46±2.2 ^{d}	19.74±0.28 ^{f}
T_2	0.29±0.003 ^{a}	15±0.38^{a}	89.94±1.9^{a}	103.7±7.885 ^{a}	22.66±2.549 ^{a}	47.86^{a}±2.9 ^{a}	28.17±0.73 ^{a}
T_3	0.27±0.005 ^{bcd}	14±0.65 ^{ab}	76.13±2.7^{b}	60.78±5.865 ^{b}	10.67±1.444 ^{bc}	25.14±4.2 ^{cd}	24.08±0.2 ^{d}
T_4	0.26±0.002 ^{d}	13±0.38^{ab}	76.89±1.9^{b}	60.32±4.483 ^{b}	9.36±1.167 ^{bc}	30.72±2.3 ^{bcd}	23.16±0.24 ^{e}
T_5	0.28±0.004^{abc}	14±0.36^{ab}	73.43±2.2^{b}	64.48±7.173^{b}	11.47±1.06^{bc}	32.99±5.7^{abcd}	23.99±0.05^{de}
T_6	0.29±0.002^{ab}	14.75±0.378^{a}	79.19±2.184^{ab}	78.57±5.866^{ab}	10.27±1.397^{bc}	44.08±2.5^{ab}	26.98±0.6^{b}
T_7	0.27±0.002^{cd}	13.25±0.648^{ab}	75.51±2.569^{b}	66.81±5.565^{b}	12.10±2.029^{bc}	39.60±2.8^{abc}	23.01±0.2^{e}
T_8	0.27±0.003^{abc}	13.75±0.327^{ab}	73.3±2.197^{b}	55.29±4.986^{b}	10.37±2.470^{bc}	34.73±1.9^{abcd}	23.32±0.1^{de}
T_9	0.29±0.003^{ab}	14.5±0.5^{a}	79.31±2.924^{ab}	77.93±5.482^{ab}	15.72±1.330^{ab}	42.81±4.3^{ab}	25.4±0.4^{c}
T_{10}	0.27±0.003^{bcd}	14±0.5^{ab}	77.25±1.873^{b}	61.52±3.546^{b}	13.08±1.274^{bc}	32.54±3.0^{abcd}	26.13±1.0^{b}

Means denoted with different letters are significantly different at $p< 0.05$.
T_1: Soil only (control), T_2: Sterilized soil + 3% RP, T_3: Soil + 3% RP, T_4: Soil + 20% biochar + 3% RP, T_5: Soil + AMF + 3% RP, T_6: Soil + *Pseudomonas fluorescens*+ 3% RP, T_7: Soil + 20% biochar + AMF + 3% RP, T_8: Soil + 20% biochar + *Pseudomonas fluorescens*+ 3% RP, T_9: Soil + AMF + *Pseudomonas fluorescens*+ 3% RP, T_{10}: Soil + 20% biochar + AMF + *Pseudomonas fluorescens*+ 3% RP.

Conclusions

The results concluded that biochar, AMF and P. fluorescens are significantly increasing the P solubility in HERP in short term applications. Also they increased the soil P availability and improved P nutrition, growth and yield of maize plants. Short term influence of high amount of (20% v/v) biochar in plant growth and yield is already less than the long term influence (reported earlier). Soil heating is increase in concentration of certain soluble mineral nutrients availability.

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