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Effect of AM fungus on phosphorus nutrition of maize and pigeon pea in alfisols as influenced by different phosphorus amendments of North Carolina Rock Phosphate (NCRP)

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ABSTRACT

A greenhouse investigation was conducted to determine the effect of AM fungus on phosphorus nutrition of maize and pigeon pea in alfisols as influenced by different phosphorus amendments of North Carolina Rock Phosphate (NCRP). This experiment consisted of 24 treatments resulting from factorial combination of two plant species, two levels of AM fungal (*Glomus aggregatum*) inoculation and six levels of phosphorus amendments. The extent of mycorrhizal fungal colonization in roots of pigeon pea was higher than that of maize. Inoculation of soil with AM mycorrhizal fungus caused significant increase in total phosphorus uptake of pigeon pea as well as maize. The extent of increase in total phosphorus uptake due to mycorrhizal inoculation in pigeon pea was higher than maize. The phosphorus uptake efficiency of maize was lower than pigeon pea. Mycorrhizal colonization significantly reduced phosphorus utilization efficiency of both plant species. The results of this study suggest that application of NCRP bio-acidulated with *Bacillus* sp. and *Penicillium* sp. improves phosphorus nutrition of maize and pigeon pea and inoculation of soil with AM fungus increase that effect.

Keywords: AM fungi, NCRP, *Glomus aggregatum*, *Bacillus* sp., *Penicillium* sp., Phosphorus nutrition.

INTRODUCTION

In alfisols the available phosphorus status is low, which affects the crop productivity. These soils have high phosphorus retention capacity because, water soluble phosphorus added to these soils get converted into insoluble form of phosphorus by reacting with iron and aluminum. Goenadi *et al.* (2000) and Geel *et al.* (2016) reported the use of slow releasing phosphorus fertilizers, bone char and rock phosphate along with organic amendments to prevent P fixation problems by increasing available phosphorus status in P fixing soils. In this view, it may be economical to apply rock phosphate as a source of phosphorus along with organic amendments. The total phosphorus content of rock phosphate is high (> 13 % P) but its water-soluble phosphorus content is extremely low (Lehr, 1980). It is well known that rhizosphere microbiome plays a key role in plant phosphorus acquisition by solubilizing insoluble sources of phosphorus (Bais *et al.,* 2006). Several species of bacteria, fungi, cyanobacteria and yeasts are shown to solubilize different types of insoluble phosphates.

Goenadi *et al.* (2000) reported the use of *Aspergillus niger* to bio-activate Moroccan rock phosphate (MRP) and recorded significant increase in its citrate soluble phosphorus content. In this context, the bio-activation of rock phosphate with culture of phosphorus solubilizing microorganisms (PSMs) before application will not only increase phosphorus availability soon after application and may also sustain it, since these organisms have assured supply of energy substrates. Therefore, it is hypothesized that bio-activated rock phosphate may serve as a type of slow-release phosphorus fertilizer. However, the effect of application of bio-activated rock phosphate on phosphorus nutrition in plants appears to have not been demonstrated. It is well known that root morphological traits of plant species influence phosphorus acquisition efficiency (Erel *et al.,* 2017). Further, it is possible that the effectiveness of bio-activated rock phosphate may be enhanced due to inoculation of soil with arbuscular mycorrhizal (AM) fungi. AM fungi are a group of soil fungi which are having symbiotic association with plant roots and help to enhance the uptake of phosphorus. With this background, the objective of this investigation was to determine the effect of AM fungus on phosphorus nutrition of maize and pigeon pea in alfisols as influenced by different phosphorus amendments of North Carolina Rock Phosphate (NCRP).

MATERIALS AND METHODS

This greenhouse experiment was conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra (GKVK), Bangalore, to determine the effect of AM fungus on phosphorus nutrition of maize and pigeon pea in alfisols as influenced by different phosphorus amendments of NCRP.

Pure cultures of phosphate solubilizing bacterium, *Bacillus* sp. and phosphate solubilizing fungus, *Penicillium* sp. were obtained from the culture collection of biofertilizer production laboratory of the Department of Agricultural Microbiology, GKVK Campus. They were tested for phosphate solubilization efficiency using Sperber's medium. The zone of phosphorus solubilization on the medium was noticed with both the organisms, hence they were used to bio-activate rock phosphate in this study.

Preparation of acidulated and bio-activated NCRP

NCRP (300g) was placed in four glass beakers. Distilled water (53ml) was added to one of these NCRP samples. The remaining 3 samples of NCRP received 53 ml of HCl solution, homogenized culture of *Bacillus* and homogenized culture of *Penicillium* respectively. The acidity level of these three solutions was similar. The volume of liquid added brought the moisture content of NCRP to 50% of its maximum water holding capacity. NCRP samples were incubated at 30 ^{o}C for 15 days in the incubator and during incubation period, 30 ml of distilled water was added to all samples to compensate evaporation loss during incubation. These NCRP samples were mixed uniformly at the rate of 200 mg of phosphorus per kg of soil before a day of planting.

Mycorrhizal inoculum production

The crude inoculum of AM fungus *Glomus aggregatum* was prepared by following the procedure proposed by Habte and Osorio (2001). The culture of this AM fungus was obtained from Soil Microbiology Laboratory of the Department of Agronomy and Soil Science, University of Hawaii, Honolulu, USA. This fungus is an obligate symbiont and was mass multiplied by using maize (*Zea mays* L.) as host. Soil samples in pots were mixed with this crude inoculum at the rate of 50 g per kg of soil.

Soil sample collection and processing

The soil used for this investigation was collected from an uncultivated field at GKVK campus, University of Agricultural Sciences, Bengaluru. Soil sample was pulverized, sieved through 4mm sieve and homogenized before placing in plastic pots at rate of 5 kg per pot. Soil moisture content was raised to 50 per cent of field capacity. Moistened soil was sterilized twice at an interval of 20 days in an autoclave at 121^{o}C and 15 lbs/sq. in. for 1 hour. Plastic pots containing sterilized soil were placed on benches in greenhouse to attain equilibration before imposition of treatments.

Nutrient application

Soil samples in pots (72) were amended with nutrient solution containing K, Mg, Cu, B, Mo, Zn, Mn and N in the form of KCl, $MgSO_4.7H_2O$, $CuSO_4.5H_2O$, H_3BO_3, $Na_2MoO_4.2H_2O$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$ and Ca $(NO_3)_2.4H_2O$ at the rate of 100, 50, 5, 10, 0.5, 10, 5 and 34.6 mg per kg soil, respectively. Soil samples were subjected to three cycles of wetting and drying to ensure uniform distribution of nutrients in the soil. After each wetting and drying cycle, soil samples in pots were thoroughly mixed to obtain a homogeneous mixture. Different formulations of NCRP were added at the rate of 200 mg P per kg soil and KH_2PO_4 was added at the rate of 50 mg P per kg soil.

Treatments

This experiment consisted of 24 treatments resulting from factorial combinations of 2 plant species, 2 levels of GA inoculation (GA- and GA+) and 6 types of phosphorus amendment (Unamended Control, Untreated NCRP, Acidulated NCRP, NCRP bio-activated with PSB, NCRP bio-activated with PSF and KH_2PO_4). Each treatment was replicated thrice, hence there were 72 experimental units in this factorial randomized complete block design experiment. Plants were harvested after 60 days of growth under greenhouse conditions.

Determination of per cent AM fungal colonization in roots

Fresh root samples (200 mg) were collected after harvesting, washed roots were used to determine the extent of AM colonization. Gridline intersect method outlined by Giovannetti and Mosse (1980) was followed to determine extent of AM colonization in roots of maize and pigeon pea after staining with acid fuchsin. Fresh root samples were immersed in 10% KOH solution placed in test tubes. These test tubes were placed in simmering water bath for 45 minutes to cause partial hydrolysis of root tissue. Alkali solution was decanted and 10% HCl was added to neutralize alkali and to cause acidification of roots. After decanting acid, acidified roots samples were stained with 0.2% acid fuchsin dissolved in lacto-glycerol. Staining solution was decanted and roots were de-stained with lacto-glycerol solution. The stained roots were placed on grid-line plates and observed using stereomicroscope at 40X magnification. Number of root intersections with AM fungal colonization were recorded and the per cent of mycorrhizal colonization was calculated by using formula.

$$\text{Mycorrhizal Colonization (\%)} = \frac{\text{Number of root intersections +ve for AM fungal colonization}}{\text{Total number of root intersections observed}} \times 100$$

Determination of phosphorus concentration in shoots and root samples

Phosphorus concentration in plant tissue samples was determined by following the phosphomolybdate blue colour method (Murphy and Riley, 1962). The phosphorus concentration was determined by comparing with a standard curve developed by using KH_2PO_4 as a source of phosphorus.

$$\text{PUE (mg of P absorbed / g root)} = \frac{\text{Total phosphorus uptake (mg / plant)}}{\text{Dry weight of root (g / plant)}}$$

Total phosphorus uptake

Total phosphorus uptake was computed as the summation of total phosphorus content of shoot and root.

Phosphorus uptake efficiency

The phosphorus uptake efficiency of plants was calculated using the following formula

$$\text{PUEF (g biomass/ mg P)} = \frac{\text{Total bio-mass (g / plant)}}{\text{Total phosphorus uptake (mg / plant)}}$$

Statistical analysis

The data were subjected to two-way Analysis of Variance by factorial randomized complete block design and means were separated by the Duncan's multiple range test (DMRT).

RESULTS

Mycorrhizal colonization in roots

The extent of AM fungal colonization in roots of pigeon pea was higher than that of maize. The extent of AM colonization in roots of pigeon pea and maize grown in soil amended with NCRP formulations were significantly higher than those with KH_2PO_4 amendment. Further, the per cent AM colonization in roots of plants grown in soil without NCRP amendment was significantly lower than those grown in soil with NCRP application (**Fig. 1**).

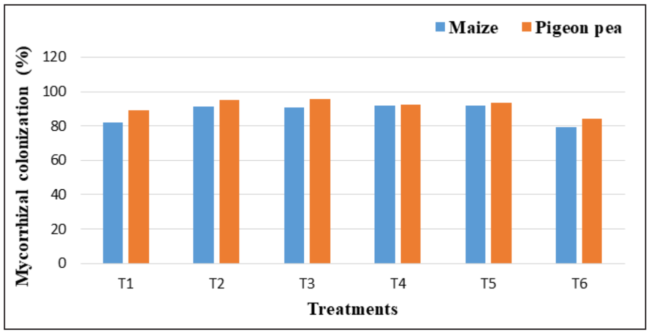

Fig. 1: Mycorrhizzal colonization of maize and pigeon pea as influenced by inoculation of soil with *Glomus aggregatum* and application of different sources of phosphorus. T_1- Unamended control; T_2- NCRP- Untreated; T_3- NCRP-Acidulated; T_4- NCRP-PSB (Bioactivated); T_5- NCRP-PSF (Bioactivated); T6- KH_2PO_4

Phosphorus concentration in shoot and root

In general, the concentration of phosphorus in shoots of pigeon pea was higher than those of maize (**Table 1**). Inoculation of soil with AM fungus caused significant increase in concentration of phosphorus in shoot and root of pigeon pea as well as maize (**Tables 1 and 2**). Although phosphorus concentration determined in this study was generally low, the concentration of phosphorus in plants grown in soil amended with KH_2PO_4 was higher than those of remaining plants.

Phosphorus content of shoot and root

Phosphorus content of shoot and root of maize was higher than those of pigeon pea (**Tables 3&4**). The colonization of roots by AM fungus markedly increased phosphorus content of shoot and root of maize by more than two times compared to non-mycorrhizal plants. The extent of increase of P content in pigeon pea was twenty times in case of shoot and six times in case of root. Higher phosphorus content of both shoot and root, was noticed in plants grown in soil amended with KH_2PO_4. It is interesting to note that phosphorus content of pigeon pea and maize grown in soil amended bio-activated NCRP and inoculated with AM fungus was significantly higher than their un-inoculated counterparts. However, in case of pigeon pea, AM colonization also resulted in increase of phosphorus content in plants grown in soil amended with acidulated as well as untreated NCRP. The inoculation of soil with AM fungus also resulted in enhancing phosphorus content of plants grown in soil amended with KH_2PO_4. The phosphorus content of shoot and root of maize was higher than that of pigeon pea irrespective of inoculation of soil with AM fungus and phosphorus amendment.

Table 1: Shoot phosphorus concentration of maize and pigeon pea as influenced by inoculation of soil with *Glomus aggregatum* and application of different sources of phosphorus.

	Phosphorus concentration in shoot (%)*			
	Maize		Pigeon pea	
Treatments	- GA	+ GA	- GA	+ GA
Unamended control	0.077e	0.010de	0.006f	0.039d
NCRP- Untreated	0.003e	0.009de	0.011f	0.086b
NCRP- Acidulated	0.002e	0.015cde	0.016ef	0.123a
NCRP-PSB (Bioactivated)	0.005e	0.032b	0.014ef	0.083b
NCRP-PSF (Bioactivated)	0.004e	0.022bc	0.016ef	0.070c
KH_2PO_4	0.025bcd	0.055a	0.021e	0.118a

*Means followed by the same letter within a plant species do not differ significantly at the 5 % level of significance.

Means for main effects

	Phosphorus concentration in shoot (%)**	
Main effect	Maize	Pigeon pea
AM fungal inoculation		
- GA	0.008b	0.014b
+ GA	0.024a	0.090a
Treatments		
Unamended control	0.009c	0.023c
NCRP- Untreated	0.006c	0.049b
NCRP- Acidulated	0.008c	0.070a
NCRP-PSB (Bioactivated)	0.018b	0.049b
NCRP-PSF (Bioactivated)	0.013bc	0.042b
KH_2PO_4	0.040a	0.070a

**Means followed by the same letter within a main effect do not differ significantly at the 5% level of significance. -GA = Un-inoculated; +GA = Inoculated with *Glomus aggregatum*

Total phosphorus uptake

Trends noticed with that of total phosphorus uptake is similar to that of shoot and root phosphorus content (**Fig. 2**). Total phosphorus uptake of maize was higher than that of pigeon pea and inoculation of soil with AM fungus increased total phosphorus uptake by three times in case of maize and seventeen times in case of pigeon pea. The total phosphorus uptake of plants grown in soil amended with KH_2PO_4 was highest. Amendment of NCRP formulations did not significantly influence total phosphorus both under mycorrhizal as well as under non mycorrhizal condition. In general, inoculation of soil with AM fungus caused significant increase in total phosphorus uptake of maize and pigeon pea grown in soil amended with different NCRP.

Table 2: Root phosphorus concentration of maize and pigeon pea as influenced by inoculation of soil with *Glomus aggregatum* and application of different sources of phosphorus

	Phosphorus concentration in root (%)*			
	Maize		Pigeon pea	
Treatments	- GA	+ GA	- GA	+ GA
Unamended control	0.024c	0.028c	0.016d	0.036bc
NCRP- Untreated	0.029c	0.033bc	0.018d	0.048ab
NCRP- Acidulated	0.029c	0.044ab	0.015d	0.061a
NCRP-PSB (Bioactivated)	0.025c	0.053a	0.017d	0.037bc
NCRP-PSF (Bioactivated)	0.031c	0.028c	0.015d	0.035bc
KH_2PO_4	0.028c	0.049a	0.026cd	0.058a

*Means followed by the same letter within a plant species do not differ significantly at the 5 % level of significance.

Means for main effects

	Phosphorus concentration in root (%)**	
Main effect	Maize	Pigeon pea
AM fungal inoculation		
- GA	0.028b	0.018b
+ GA	0.039a	0.046a
Treatments		
Unamended control	0.026c	0.026c
NCRP- Untreated	0.031abc	0.033abc
NCRP- Acidulated	0.037ab	0.038ab
NCRP-PSB (Bioactivated)	0.039a	0.027bc
NCRP-PSF (Bioactivated)	0.029bc	0.025c
KH_2PO_4	0.039a	0.042a

**Means followed by the same letter within a main effect do not differ significantly at the 5% level of significance. -GA = Un-inoculated; +GA = Inoculated with *Glomus aggregatum*

Phosphorus uptake enhancement (PUEN) due to acidulation and bio-activation

The PUEN values presented in **table 5** were calculated considering phosphorus uptake of plants grown in soil amended with untreated NCRP as control. These values indicate the extent of increase in phosphorus uptake due to acidulation and bio-activation. These values in general suggest that acidulation and bio-activation does increase phosphorus availability to plants compared to applying untreated NCRP and phosphorus supply could be enhanced further by inoculation of soil with *Glomus aggregatum* (**Table 5**).

Phosphorus uptake enhancement (PUEN) due to inoculation of soil with *Glomus aggregatum*

The PUEN values presented in **table 6** were calculated considering phosphorus uptake by mycorrhizal plants by using phosphorus uptake of its non-mycorrhizal counterpart as control. These values suggest that PUEN due to AM colonization remain almost unaltered in case of pigeon pea, among six different treatments. However, in case of maize there was marked reduction in PUEN due to AM colonization noticed in plants grown in soil without phosphorus amendment as well as in plants grown in soil with KH_2PO_4 amendment.

Table 3: Shoot phosphorus content of maize and pigeon pea as influenced by inoculation of soil with *Glomus aggregatum* and application of different sources of phosphorus

	Phosphorus content in shoot (mg/plant)*			
	Maize		Pigeon pea	
Treatments	- GA	+ GA	- GA	+ GA
Unamended control	0.050d	0.135d	0.016d	0.178d
NCRP- Untreated	0.081d	0.363cd	0.033d	0.788c
NCRP- Acidulated	0.052d	0.574cd	0.064d	1.460b
NCRP-PSB (Bioactivated)	0.130d	1.108c	0.052d	0.810c
NCRP-PSF (Bioactivated)	0.121d	0.926c	0.056d	0.796c
KH_2PO_4	2.798b	6.467a	0.183d	4.219a

*Means followed by the same letter within a plant species do not differ significantly at the 5 % level of significance.

Means for main effects

	Phosphorus content in shoot (mg/plant)**	
Main effect	Maize	Pigeon pea
AM fungal inoculation		
- GA	0.539b	0.067b
+ GA	1.595a	1.376a
Treatments		
Unamended control	0.093b	0.097d
NCRP- Untreated	0.222b	0.416c
NCRP- Acidulated	0.313b	0.762b
NCRP-PSB (Bioactivated)	0.619b	0.432c
NCRP-PSF (Bioactivated)	0.523b	0.426c
KH_2PO_4	4.633a	2.201a

** Means followed by the same letter within a main effect do not differ significantly at the 5 % level of significance. -GA = Un-inoculated; +GA = Inoculated with *Glomus aggregatum*

Phosphorus uptake efficiency (PUE)

Data on PUE of maize and pigeon pea as influenced by inoculation of soil with *Glomus aggregatum* and application of different forms of phosphorus are presented in **table 7**. The PUE of maize was lower than that of pigeon pea. Mycorrhizal colonization of roots significantly enhanced PUE of both plant species. The magnitude of this increase was nearly seven times in case of pigeon pea, from 0.599 to 4.403 mg of P per gram dry weight of root. It is interesting to note that inoculation of soil with *Glomus aggregatum* significantly increased PUE of pigeon pea in all six treatments. However, in case of maize, such increase was noticed in plants grown in soil amended with NCRP bio-activated with PSMs and KH_2PO_4. In the remaining three treatments, un-amended control, NCRP-untreated and NCRP-acidulated, mycorrhizal fungal inoculation of soil increased PUE of maize marginally, but the extent of increase was not statistically significant.

Table 4: Root phosphorus content of maize and pigeon pea as influenced by inoculation of soil with *Glomus aggregatum* and application of different sources of phosphorus

	Phosphorus content in root (mg/plant)*			
	Maize		Pigeon pea	
Treatments	- GA	+ GA	- GA	+ GA
Unamended control	0.076f	0.089f	0.010e	0.026de
NCRP- Untreated	0.148ef	0.454cd	0.015e	0.053bcde
NCRP- Acidulated	0.203ef	0.525c	0.011e	0.094b
NCRP-PSB (Bioactivated)	0.160ef	0.485c	0.012e	0.060bcd
NCRP-PSF (Bioactivated)	0.153ef	0.294de	0.012e	0.077bc
KH_2PO_4	0.765b	1.089a	0.042cde	0.293a

*Means followed by the same letter within a plant species do not differ significantly at the 5% level of significance.

Means for main effects

	Phosphorus content in root (mg/plant)**	
Main effect	Maize	Pigeon pea
AM fungal inoculation		
- GA	0.250b	0.016b
+ GA	0.489a	0.100a
Treatments		
Unamended control	0.082d	0.018c
NCRP- Untreated	0.301bc	0.034bc
NCRP- Acidulated	0.364b	0.052b
NCRP-PSB (Bioactivated)	0.322bc	0.036bc
NCRP-PSF (Bioactivated)	0.224c	0.045bc
KH_2PO_4	0.925a	0.168a

**Means followed by the same letter within a main effect do not differ significantly at the 5 % level of significance. -GA = Un-inoculated; +GA = Inoculated with *Glomus aggregatum*

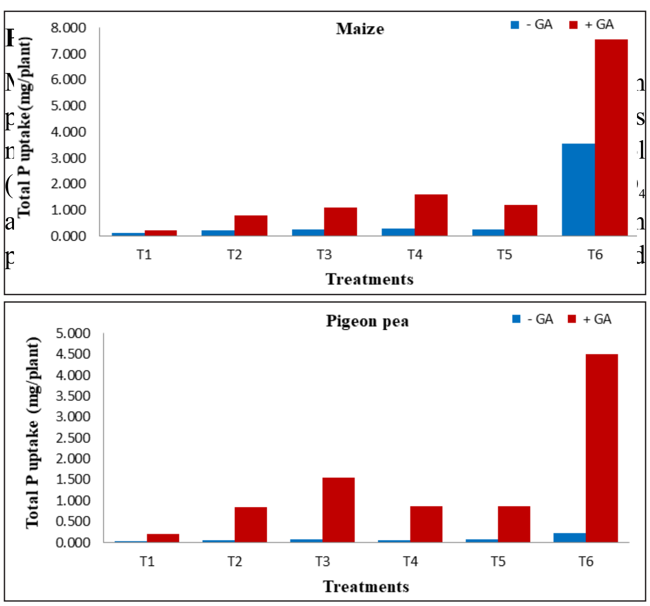

Fig. 2. Total P uptake by maize and pigeon pea as influenced by inoculation of soil with *G. aggregatum* and application of different sources of phosphorus. T_1- Control; T_2- NCRP-Untreated; T_3- NCRP- Acidulated; T_4- NCRP-PSB (Bioactivated); T_5- NCRP-PSF (Bioactivated); T_6- KH_2PO_4.

Table 5: Phosphorus uptake enhancement by maize and pigeon pea due to acidulation and bio-activation of rock phosphates

	PUEN (%)			
	Maize		Pigeon pea	
Treatments	- GA	+ GA	- GA	+ GA
NCRP- Acidulated	10.19	25.75	36.00	45.85
NCRP-PSB (Bioactivated)	21.03	48.74	25.00	3.60
NCRP-PSF (Bioactivated)	16.42	33.11	29.41	3.60

PUEN- Phosphorus uptake enhancement.

The phosphorus uptake enhancement due to acidulation and bio-activation of rock phosphate

$$NCRP = \frac{\text{Total phosphorus content in treated NCRP} - \text{Total phosphorus content in un-treated NCRP}}{\text{Total phosphorus content in treated NCRP}} \times 100$$

Table 6: Phosphorus uptake enhancement (PUEN) by maize and pigeon pea due to inoculation of soil with *Glomus aggregatum*

	PUEN (%)	
Treatments	Maize	Pigeon pea
Unamended control	43.75	86.34b
NCRP- Untreated	71.26	94.01
NCRP- Acidulated	76.57	95.11
NCRP-PSB (Bioactivated)	81.59	91.83
NCRP-PSF (Bioactivated)	76.70	92.15
KH_2PO_4	51.80	95.01

PUEN- Phosphorus uptake enhancement.

The phosphorus uptake enhancement due to inoculation of soil with *Glomus aggregatum* was calculated using the following formula.

$$PUEN\,(\%) = \frac{\text{Total phosphorus content of inoculated plant} - \text{Total phosphorus content of un-inoculated plant}}{\text{Total phosphorus content of inoculated plant}} \times 100$$

treatments PUEF of pigeon pea was markedly lower than that of maize. In general, PUEF of maize and pigeon pea colonized with *Glomus aggregatum* and grown in soil amended with KH_2PO_4 was lowest. The PUEF values of maize and pigeon pea grown in soil amended with NCRP in different formulations and uninoculated with AM fungus were not significantly different (**Table 8**).

DISCUSSION

The effect of inoculation of soil with AM fungus on phosphorus nutrition of plant species grown in soil amended with different types of rock phosphate has been the subject of several studies earlier. In general, the results of these investigation indicate enhanced phosphorus uptake in plants colonized with AM fungi. Effect of application of bio-acidulated NCRP and inoculation of soil with *Glomus aggregatum* on phosphorus nutrition of maize and pigeon pea

Data on the extent of mycorrhizal colonization in roots of maize and pigeon pea observed in this study was in good agreement with earlier studies. Mycorrhizal colonization in root of pigeon pea was significantly higher than that of maize.

Table 7: Phosphorus uptake efficiency (PUE) of maize and pigeon pea as influenced by inoculation of soil with *Glomus aggregatum* and application of different sources of phosphorus

	PUE (mg P/g dry weight of root)*			
	Maize		Pigeon pea	
Treatments	**- GA**	**+ GA**	**- GA**	**+ GA**
Unamended control	0.272e	0.463cde	0.285g	2.012ef
NCRP- Untreated	0.279e	0.402cde	0.440g	4.741bc
NCRP- Acidulated	0.245e	0.615cde	0.739fg	6.348a
NCRP-PSB (Bioactivated)	0.301de	1.284b	0.668fg	4.060cd
NCRP-PSF (Bioactivated)	0.266e	0.804bcd	0.557fg	3.031de
KH_2PO_4	0.869bc	2.265a	0.899fg	6.209ab

*Means followed by the same letter within a plant species do not differ significantly at the 5% level of significance

Means for main effects

	PUE (mg P/g dry weight of root)**	
Main effect	**Maize**	**Pigeon pea**
AM fungal inoculation		
- GA	0.372b	0.599b
+ GA	0.972a	4.403a
Treatments		
Unamended control	0.368c	1.148c
NCRP- Untreated	0.340c	2.592ab
NCRP- Acidulated	0.430c	3.544a
NCRP-PSB (Bioactivated)	0.793b	2.364b
NCRP-PSF (Bioactivated)	0.535bc	1.794bc
KH_2PO_4	1.567a	3.554a

**Means followed by the same letter within a main effect do not differ significantly at the 5 % level of significance. -GA = Un-inoculated; +GA = Inoculated with *Glomus aggregatum*

Plant species have been observed to differ in their susceptibility to colonization by AM fungi (Rengel, 2002).

In a greenhouse study involving four species of *Leucaena* and four species of *Sesbania*, Manjunath and Habte (1991) observed reduction in AM colonization in roots of Sesbania only at soil solution phosphorus levels higher than 0.08 mg/l. This clearly indicates that application of bio-acidulated NCRP at the rate of 200 mg of phosphorus per kg soil will not increase soil solution phosphorus concentration to a level that would interfere with AM colonization of root.

Further, studies of Buwalda *et al.* (1983) and Asimi *et al.* (1980) suggested that phosphorus concentration in roots also regulate the extent of root colonization by AM fungi and high root phosphorus concentration reduces AM colonization. Application of NCRP formulations did not increase root phosphorus concentration to a level that would limit the development of AM fungi in root system of maize and pigeon pea. On the contrary, the concentration of phosphorus in roots of maize and pigeon pea noticed in this study was markedly lower than the levels noticed in earlier studies and were at that levels which would encourage optimal development of AM fungi their root system (**Table 1 and 2**).

Table 8: Phosphorus utilization efficiency (PUEF) of maize and pigeon pea as influenced by inoculation of soil with *Glomus aggregatum* and application of different sources of phosphorus

	PUEF (g biomass/mg of P)*			
	Maize		Pigeon pea	
Treatments	**- GA**	**+ GA**	**- GA**	**+ GA**
Unamended control	9.740bc	8.125cd	14.377a	2.775ef
NCRP- Untreated	13.308ab	7.556cde	9.710b	1.366f
NCRP- Acidulated	15.408a	5.314cdef	7.120cd	0.932f
NCRP-PSB (Bioactivated)	12.975ab	3.353ef	7.858bc	1.387f
NCRP-PSF (Bioactivated)	15.632aa	5.153cdef	7.466bcd	1.733f
KH_2PO_4	4.315def	1.950f	5.138de	0.961f

*Means followed by the same letter within a plant species do not differ significantly at the 5 % level of significance

Means for main effects

	PUEF (g biomass/mg of P)**	
Main effect	**Maize**	**Pigeon pea**
AM fungal inoculation		
- GA	11.896a	8.595a
+ GA	5.422b	1.523b
Treatments		
Unamended control	8.932a	8.579a
NCRP- Untreated	10.432a	5.538b
NCRP- Acidulated	10.361a	4.026bc
NCRP-PSB (Bioactivated)	8.164a	4.623bc
NCRP-PSF (Bioactivated)	10.392a	4.599bc
KH_2PO_4	3.182b	3.050c

**Means followed by the same letter within a main effect do not differ significantly at the 5% level of significance. -GA = Un-inoculated; +GA = Inoculated with *Glomus aggregatum*

Significant increase in phosphorus concentration and phosphorus content of plant tissue and total phosphorus uptake of maize and pigeon pea grown in soil amended with bio-acidulated NCRP and inoculation with AM fungi, observed in the current study is consistent with earlier investigations (Piccini and Azcon, 1987; Linderman 1992; Kucey *et al.,* 1989; Gyaneshwar *et al.,* 2002). The culture medium of PSMs was known to be acidic and is known to contain several organic acids such as, citric acid, oxalic acid, butyric acid, malonic acid, lactic acid, succinic acid, tartaric acid and gluconic acid (Bhattacharyya and Jain, 2000). These acids would solubilize phosphorus from NCRP soon after addition of homogenized media. Further, application of NCRP bio-acidulated with homogenized medium would last long because it contains live propagules which would use energy substrates present in bio-activated NCRP and soil as well as those supplied by plants.

Further, the increased phosphorus uptake observed in this study could also be attributed to synergistic interaction between AM fungi and PSMs by which the phosphorus released by the phosphate solubilizers is rapidly taken up by mycorrhizal mycelium making it available for plant growth

(Toro *et al.,* 1997). These fungi are known to absorb phosphorus at lower soil solution phosphorus levels at which plants will not be able to absorb. Although AM fungi do not solubilize phosphorus, rapid absorption of phosphorus by their hyphae from soil solution may induce further dissolution from insoluble sources in soil.

Phosphorus uptake efficiency

Phosphorus uptake efficiency of maize and pigeon pea grown in soil inoculated with *Glomus aggregatum* were higher than their non-mycorrhizal counterparts. Higher phosphorus uptake efficiency in plants effectively colonized with AM fungi grown in fumigated soil was observed (Manjunath and Habte, 1991). It is well known that AM fungal hyphae ramifying in soil will greatly increase surface area of root for absorption of phosphorus beyond the nutrient depletion. AM hyphae could also explore smaller soil pore for nutrients which cannot be accessed by fine roots and root hairs. Physiological and biochemical changes in roots caused due to the AM fungal colonization may also influence phosphorus uptake efficiency by reducing K_m value of roots for phosphorus (Zhu *et al.,* 2001; Jamal *et al.,* 2002; Ponce *et al.,* 2004).

It is interesting to note that PUE of pigeon pea was higher than that of maize both under mycorrhizal as well as under nonmycorrhizal condition. Ishikawa *et al.* (2002) demonstrated the ability of pigeon pea root exudates to solubilize iron phosphates.

Phosphorus utilization efficiency

An indirect relationship between phosphorus utilization efficiency and quantity of phosphorus supplied has been observed earlier (Koide, 1991). Mycorrhizal colonization has also been observed to cause reduction in phosphorus utilization efficiency (Manjunath and Habte, 1989; Bolan *et al.,* 1994). Lower nutrient use efficiency in plants grown at higher level of nutrient availability is mainly because of accumulation of nutrients. Although PUEF of mycorrhizal plants and plants grown at higher levels of nutrient amendment was low, phosphorus accumulated in plant tissue will be available for biomass production at later stages of plant growth. It was interesting to note that PUEF of maize was higher than pigeon pea. The plant species which are less dependent on AM fungi are known to be most efficient in utilizing phosphorus for biomass production (Verbruggen *et al.,* 2012). Maize with its higher root length, root surface area and root density than pigeon pea was known to be less dependent on AM fungi for uptake of phosphorus.

Data on phosphorus uptake enhancement due to acidulation, bio-acidulation and inoculation of soil with *Glomus aggregatum* (**Tables 5&6**) suggest that the effect of AM colonization is more pronounced than acidulation and bio-acidulation. Rock phosphate application although increases the total phosphorus content, it will not affect soil solution phosphorus level markedly (Tinker, 1980). The AM mycorrhizal hyphae network developed in soil is known to absorb phosphorus at lower soil solution phosphorus levels, at which plant roots will not be able to absorb it (Marschner and Dell, 1994).

The results of these investigations clearly illustrate that NCRP bio-acidulated with homogenized media of PSMs has the potential to enhance phosphorus uptake markedly and this effect could be maximized by effective colonization of roots by AM fungi.

CONCLUSION

The present investigation revealed that bio-acidulated NCRP with homogenized media of PSMs has the potential to enhance phosphorus uptake of pigeon pea as well as maize markedly and inoculation of soil with AM mycorrhizal fungus caused significant increase in phosphorus nutrition in maize and pigeon pea. The results of this study conclude that application of NCRP bio-acidulated with *Bacillus* sp. and *Penicillium* sp. improves phosphorus nutrition of maize and pigeon pea and inoculation of soil with AM fungus increase that effect. Therefore, with this investigation, P fixation problems in alfisols can be reduced and improve the plant performance in alfisols with phosphorus nutrition.

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