World J Microbiol Biotechnol (2008) 24:1857–1865
DOI 10.1007/s11274-008-9684-8

ORIGINAL PAPER

Effect of rhizobia and rock biofertilizers with *Acidithiobacillus* on cowpea nodulation and nutrients uptake in a tableland soil

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Received: 22 October 2007 / Accepted: 28 January 2008 / Published online: 8 February 2008

Abstract Chemical fertilizers have been used in the cultivation of plants due to their high solubility and effect on crops yield. Biofertilizers with phosphate rock (PR) and potash rock (KR) plus sulfur inoculated with *Acidithiobacillus* may improve plant growth and contribute to addition of available P and K in soil. The effectiveness of biofertilizers from phosphate and potash rocks mixed with sulfur and *Acidithiobacillus* was studied in a Typic Fragiuldult soil of the Brazilian Northeast Tableland. Cowpea (cv. "IPA 206") was grown with and without rhizobia inoculation. Treatments were: (a) phosphate rock (1000 kg ha^{-1}); (b) Biofertilizers-B_P (250 and 500 kg ha^{-1}); (c) triple superphosphate-TSP (250 kg ha^{-1}); (d) potash rock (1000 kg ha^{-1}); (e) biofertilizer-B_K (250; 500 and 750 kg ha^{-1}); (f) potassium chloride-KCl (250 kg K_20 ha^{-1}); (g) control without P or K fertilization (P_0K_0). The soil was maintained under water submersion covered with black plastic (solarization process) for a period of 30 days. Biofertilizers (B_P and B_K) and soluble fertilizers increased plant growth and NPK uptake. Biofertilizers reduced soil pH, especially when applied in highest rates. Biofertilizers and TSP+KCl showed the best values of available P and K in soil. Rhizobial inoculation was effective on cowpea, but no nodules were formed by bacteria native from the soil, probably due to the effect of the solarization process. From obtained PK biofertilizers could be used as alternative for cowpea fertilization in Tableland soils.

Keywords *Rhizobium* · *Vigna unguiculata* · Apatite · Biotite · PK fertilization · Sulfur oxidation

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Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a legume widely cultivated in tropical countries due to its content on protein and amino acids and the high capacity for nitrogen fixation when inoculated with effective rhizobia bacteria. Strains of rhizobia previously screened for cowpea submitted to high temperature and acidic conditions showed great effectiveness on nitrogen fixation in Brazilian soils, and inoculation with these selected strains may be sufficient to supply the nitrogen requirements for good yields (Stamford et al. 1995; Martins et al. 1997; Rumjanek et al. 2005; Moreira et al. 2006).

The Typic Fragiudult soils of the Brazilian tableland (Embrapa 1999) represent an important portion of the arable soils in the world and the exploration of the agriculture in tropical regions depends on the maintenance of available phosphorus (P) and potassium (K) on the soil profile due to the low organic matter content and further to the role of these nutrients in the symbiotic process and in plant growth (Burity et al. 2000). Nitrogen fixation is directly affected by environmental factors such as acidity and temperature, although the establishment of the rhizobia is necessary for the maintenance of competitiveness and effectiveness (Stamford et al. 1995; Hungria et al. 2003; Moreira and Siqueira 2006; Shamseldin 2007).

Materials used for the manufacture of commercial fertilizers are mainly from sedimentary, igneous and biogenic origin. Conventional, chemically processed fertilizers are largely water-soluble and contain high available nutrient concentrations, and the direct application of natural rocks as fertilizers is limited due to their low solubility. Powdered rocks have been used in perennial crops together with soluble fertilizers to supply nutrients as slow release fertilizers, although the agronomic efficiency and economic use of rocks as fertilizers is not yet conclusive (van Straaten 2002).

Phosphorus and K in rocks generally do not occur in a form directly available to crops and must be modified physically, chemically or microbiologically to become an effective nutrient source for crops. Studies to isolate and screen microorganisms with the ability to promote higher solubilization of rocks have been carried out in tropical conditions, especially in interaction with microorganisms involved in processes of biological nitrogen fixation (Nahas 1999). Biofertilizers produced from natural rocks have a great advantage because they could be used in organic agriculture and may decrease the impact of soluble fertilizers on the environment (Stamford et al. 2003, 2006).

The effects of sulfur inoculated with *Acidithiobacillus* in soils and plant growth needs to be evaluated and compared with soluble fertilizers, because the acidity produced in the biological reaction could reduce soil pH, which may improve solubility of elements such as aluminum that may be harmful to plants (Stamford et al. 2006).

A greenhouse experiment was carried out to study the effects and effectiveness of biofertilizers produced from phosphate and potash rocks mixed with elemental sulfur inoculated with *Acidithiobacillus*, applied in a Brazilian acidic soil with a low level of available P and K. The biofertilizers were tested as an alternative to P and K soluble fertilizers. The effects and interactions of rhizobial inoculation with the PK biofertilizers were also studied in the soil maintained under water submersion and covered with black plastic during a period of 30 days.

Material and methods

Production of P and K biofertilizers

The P and K biofertilizers were produced at the Federal Agricultural University of Pernambuco (UFRPE) in the Horticultural Experimental Station, using two furrows (10-m long, 1-m wide and 0.5-m deep). For each biofertilizer 4,000 kg of phosphate rock (apatite) with 11% total P, purchased from Irecê (Bahia), Brazil was appplied, and 4,000 kg of potash rock (biotite) with 9% total K, purchased from Santa Luzia (Paraíba), Brazil, following the process described by Stamford et al. (2006).

The sulfur oxidative bacteria were grown in 2000-ml Erlenmeyer flasks containing 1000 ml of culture medium 9K (Garcia Junior 1991) and sterilized for 30 min at 120°C. The Erlenmeyer flasks were shaken (150 rev/min) for 5 days at 30°C. The materials (phosphate and potash rocks mixed with elemental sulfur) were incubated for 60 days, maintaining humidity near field holding capacity. To avoid excessive humidity due to rain and to increase the efficiency of the oxidative bacteria, the furrows were covered with black plastic.

Analysis of the natural rocks, P biofertilizer and K biofertilizer, using methodology (A) Mehlich 1 and (B) extraction with 2% citric acid, according to Embrapa (1997), presented the following results: (P-biofertilizer-B_P)-pH = 3.8, available P (A) = 60 (g kg^{-1}) and (B) = 48 (g kg^{-1}); (K biofertilizer-B_K)-pH = 3.3, available K (A) = 10 (g kg^{-1}) and (B) = 5 (g kg^{-1}); (phosphate rock)-pH = 7.2, available P (A) = 22 (g kg^{-1}) and (B) = 25 (g kg^{-1}); (potash rock)-pH = 8.8, available K (A) = 0.3 and (B) ND (not determined with 2% citric acid).

Soil and experimental conditions

The experiment was conducted in a greenhouse at temperature 30–35°C (minimum–maximum) and relative humidity 80–85% (minimum–maximum). Pots were filled with samples (0–30 cm) of a Typic Fragiudult soil with low available P and K, collected in the Sugar cane Experimental Station of the Federal Agricultural University of Pernambuco State in the District of Carpina, latitude 7° 51′ 4″ S, longitude 35° 14′ 27″ W, altitude 178 m, located in the Tropical Rainforest Zone of Pernambuco state, Northeast of Brazil. The soil was air dried and sieved (5 mm). Lime was not applied because the soil pH (5.8) and content of exchangeable cations Ca^{2+} and Mg^{2+} (28 $mmol_c$ dm^{-3}) were adequate for cowpea.

The main objective of the experiment was to evaluate the effects of P and K biofertilizers applied in different levels compared with conventional soluble fertilizers (triple superphosphate and potassium choride) and PK natural rocks on cowpea growth, total N, P and K uptake, and cowpea nodulation. The effects of the P and K biofertilizers on pH and available P and K in soil were also studied.

Analysis of chemical and physical attributes of the soil (Embrapa 1997) presented the following results: pH (water) 5.8; exchangeable cations ($mmol_c$ dm^{-3}): Al^{3+} 0.5, Ca^{2+} 20.0, Mg^{2+} 8.8; available P (Mehlich 1) 4.2 (mg dm^{-3}), available K (Mehlich 1) 10.0 (mg dm^{-3}); total N (g kg^{-1}) 0.8; organic C (g kg^{-1}) 8.7; particles density (g dm^{-3}) 2.60; global density (g dm^{-3}) 1.54; and sand, silt and clay content (g kg^{-1}) 91.0, 4.4 and 4.6, respectively.

The experiment was carried out in pots (8-L) containing 10 kg of soil grown with cowpea, each plus the respective fertilizer mixed with the soil, and with distilled water applied up to 2 cm of the surface. The process of submersion was conducted for 30 days and each pot was covered with black plastic during this period. After the incubation period, four seeds of cowpea (cv. IPA 206) were sown, adjusting the final population to two seedlings in each pot, seven days after the sowing. The harvest was realized 40 days after emergency (DAE) and the following parameters were determined: nodule numbers and shoot dry weights (65°C for 72 h); total N, total P and total K in shoots; total N by the Kjeldhal semi-micro method using an auto-analyser (Tecator 1030), total P and K in shoots following Malavolta et al. (1989). Soil pH and available P and K in the soil were determined using the Embrapa (1997) methodology.

Cowpea cultivar and rhizobial growth and inoculation

The seeds of cowpea (cv. "IPA 206") were surface sterilized (Vincent 1970) and inoculated with the strains of *Bradyrhizobium* spp. NFB 516 and NFB 700 isolated from Brazilian soils and selected in previous experiments for efficient nitrogen fixation on cowpea under high temperature and acidic soil conditions (Stamford et al. 1995). In the preparation of the inoculants, the strains were purified (Vincent 1970) using yeast mannitol extract agar (YMA medium) with bromothymol blue as indicator. In a following step they were transferred in duplicate to 125 ml Erlenmeyer flasks containing 50 ml of liquid yeast mannitol (YM medium) and incubated in a rotary shaker (150 rev/min) at a controlled temperature of 28–30°C for 5 days. After this time period the inoculum of the two strains (NFB 516 and NFB 700) containing 10^8 c.f.u. ml^{-1} were mixed in rate 1:1 and inoculated with liquid culture (2 ml pot^{-1}).

Experimental design and statistical analysis

The experiment was set up in a factorial arrangement, conducted in a randomized block design with 4 replicates. Treatments were: (a) phosphate rock (1000 kg ha^{-1}); (b) Biofertilizers-B_P (250 and 500 kg ha^{-1}); (c) triple superphosphate-TSP (250 kg ha^{-1}); (d) potash rock (1000 kg ha^{-1}); (e) biofertilizer-B_K (250; 500 and 750 kg ha^{-1}); (f) potassium chloride-KCl (250 kg K_20 ha^{-1}); (g) control without P or K (P_0K_0). Fertilizers were applied following the maximum recommendation for cowpea (Stamford et al. 1990). Rhizobia treatments were: inoculation with rhizobia (strains NFB 516 and NFB 700) selected for acid soils (Stamford et al. 1995) and uninoculated (control).

The statistical analysis was achieved by analysis of variance that included main effects of fertilization and rhizobia treatments and as well as their interactions, using the software SAS (SAS Institute 1999). Differences among treatment means were determined by Tukey test ($P \leq 0.05$). In soil determinations means represent 8 replicates because rhizobia inoculation did not affect the soil parameters. Number of nodules was not normally distributed because of the high frequency of zero values in the uninoculated treatment.

Results

Nodules and shoot dry matter

In the pot experiment the results of nodules and shoot dry matter are presented in Table 1. Nodulation promoted by rhizobia native from the soil was not observed, due to the water submersion for one month under black plastic. This fact could be due to the high temperature at the greenhouse. It was however sufficient to cause disturbance in nodulation and nodules biomass production in the cowpea plants. The strains applied by inoculation were selected for high temperature and acidic conditions and nodulated satisfactorily, especially when the biofertilizers with P and K plus sulfur inoculated with *Acidithiobacillus* were used.

The treatments with P showed significant effects ($P \leq 0.05$) in both nodules and shoot dry biomass. Comparing the data for the biofertilizer levels, in this study, concerning the patterns of the three sources used for cowpea fertilization, significant differences in dry matter yield occurred (Table 1). The comparative performance of the biofertilizers indicates that it is necessary to test different rates of P and K biofertilizers application in field conditions.

Cowpea showed a higher dry shoot biomass when it received the PK-biofertilizers plus sulfur and *Acidithiobacillus* or chemical fertilizers, which are significantly different when compared with the other PK treatments, with the exception of levels B_{250}, which did not differ from either P_0K_0 or P $rock_{1000}$ + K $rock_{1000}$. Biofertilizer plus sulfur with *Acidithiobacillus* achieved greater cowpea shoot dry matter yield than phosphate rock and the control treatment without PK fertilizer (P_0K_0), although it was not different from any of the remaining fertilizers.

Plants inoculated with rhizobial strains only differed significantly in shoot dry biomass from uninoculated plants when receiving PR + PK. However, dry biomass was generally higher for inoculated plants for all PK treatments. There was a significant difference between rhizobia-inoculated and -uninoculated plants, and a close relation

Table 1 Effects of rhizobia inoculation and PK fertilization on nodules and shoot dry biomass of cowpea (cv. IPA 206) in a Brazilian tableland soil with low available P and K

PK treatments (kg ha^{-1})		KCl_{250}	BK_{250}	BK_{500}	BK_{750}	K_0
Nodules dry biomass (mg $plant^{-1}$)						
No P applied (P_0)	−rhizobia	–	–	–	–	–
	+rhizobia	140cA	270cA	180bA	260cA	–
Biofertilizer P_{250}	−rhizobia	–	–	–	–	
	+rhizobia	390bB	880aA	690aA	720aA	
Biofertilizer P_{500}	−rhizobia	–	–	–	–	
	+rhizobia	830aA	720aA	600aBC	460bC	
Triple perphosphate$_{250}$	−rhizobia	–	–	–	–	
	+rhizobia	440bA	480bA	200bB	–	
P $rock_{1000}$ + K $rock_{1000}$	−rhizobia	–				
	+rhizobia	–				
Shoot dry biomass (g $plant^{-1}$)						
No P applied (P_0)	−rhizobia	5.7bB	6.0bA	6.4aA	4.9abC	2.8
	+rhizobia	5.4bAB	6.1bA	5.9abAB	3.2bC	2.7
Biofertilizer P_{250}	−rhizobia	5.6bA	5.9bA	4.4bB	3.5bB	
	+rhizobia	6.7aA	6.4abA	6.5aA	5.8aB	
Biofertilizer P_{500}	−rhizobia	6.5aA	6.5abA	5.6abB	4.7abB	
	+rhizobia	6.9aA	7.5aA	5.2bB	5.4aB	
Triple superphosphate$_{250}$	−rhizobia	4.8cA	4.9cA	4.7bA	4.1bA	
	+rhizobia	5.9abA	5.8bA	4.4bB	4.4bB	
P $rock_{1000}$ + K $rock_{1000}$	−rhizobia	4.2c				
	+rhizobia	4.5				

%C.V. Nodules dry matter = 19.54; Shoot dry matter = 8.74

L.S.D. = Nodules biomass = rows 218.4, columns 197.7; Shoot biomass = rows 0.512, rows 0.387

Biofertilizer P_{250} and P_{500} correspond to application of P biofertilizer at levels 250 and 500 kg ha^{-1}; BK_{250}, K_{500}, and K_{750} application of K biofertilizer at levels 250, 500, and 750 kg ha^{-1}; P $rock_{1000}$ + K $rock_{1000}$; application of apatite and biotite in level of 1000 kg ha^{-1}, respectively. Triple Superphosphate$_{250}$ is the recommended P fertilization for cowpea in Brazilian tableland soil (Cavalcanti et al. 1998). Different letters on the top of each bar indicate significant difference at $P \leq 0.05$ (Tukey test); upper case letters compare data in rows and lower letters in columns

between rhizobial inoculation and PK fertilization was observed.

Total NPK content in shoot dry biomass

PK fertilizer application had significant response in the total N uptake in shoot dry matter (Table 2), without significant overall difference between phosphorus sources, especially for inoculated plants. Uninoculated plants receiving PR+KR or B_{250}, although not significantly different from the remaining treatments receiving P and K were not different from those not receiving P and K.

Differences in total N accumulation in shoots indicate a positive effect of P application (Table 2), with B_{500} and B_{750} achieving significantly higher results. Total N concentration in shoot dry matter (data not shown) was not significantly different between the P treatments ($P \leq 0.05$), although higher N concentration occurred in P_0K_0, probably due to growth dilution.

Plants inoculated with rhizobia showed no differences in total N in shoot among the PK fertilizers applied. Higher quantities of total nitrogen accumulation in shoots were obtained on plants inoculated with rhizobial strains, especially when combined with any kind of P and K fertilizer.

Total P accumulation in dry matter shoots of cowpea (Table 3) showed significant response when they received different sources of phosphorus, especially when biofertilizers plus sulfur inoculated with *Acidithiobacillus* were applied and with triple superphosphate. Phosphate rock and phosphate rock plus sulfur uninoculated with *Acidithiobacillus* showed results greater than the control treatment (P_0K_0).

In general, there was significant response of rhizobial inoculation in total phosphorus in shoot dry matter of cowpea. The best results of total phosphorus accumulation

Table 2 Effects of P and K fertilization and rhizobia inoculation on total N accumulation on shoot dry biomass of cowpea (cv. IPA 206) grown in a Brazilian tableland soil with low available P and K

PK treatments		KCl_{250}	BK_{250}	BK_{500}	BK_{750}	K $rock_{1000}$	K_0
Total N in shoot dry biomass (mg plant^{-1})							
No P (P_0)	−rhizobia	40.7cdB	67.8bA	69.3cA	69.6bA	64.0A	51.4B
	+rhizobia	152.6aA	100.9abB	142.5aA	92.0abC	70.0C	
Biofertilizer P_{250}	−rhizobia	98.9bA	51.2bcB	41.5cB	33.8cC		
	+rhizobia	151.0aA	170.0aA	148.7aA	118.3aB		
Biofertilizer P_{500}	−rhizobia	66.5cB	54.0bcB	45.7cB	54.8cB		
	+rhizobia	156.8aA	174.8aA	101.4bB	90.7abB		
TSP_{250}	−rhizobia	59.8cB	77.0bA	81.5bcA	75.8abA		
	+rhizobia	75.6bcC	150.0aAB	98.9bB	91.4abB		
	−rhizobia	64.0c					
	+rhizobia	75.0c					

%C. V. 14.54

L.S.D. Total N = rows l3.29, columns 10.06

Biofertilizer P_{250} and P_{500} correspond to application of P biofertilizer at levels 250 and 500 kg ha^{-1}; BK_{250}, K_{500}, and K_{750} application of K biofertilizer at levels 250, 500, and 750 kg ha^{-1}; P $rock_{1000}$ + K $rock_{1000}$; application of apatite and biotite in level of 1000 kg ha^{-1}, respectively. Triple $Superphosphate_{250}$ is the recommended P fertilization for cowpea in Brazilian tableland soil (Cavalcanti et al. 1998). Different letters on the top of each bar indicate significant difference at $P \leq 0.05$ (Tukey test); upper case letters compare data in rows and lower letters in columns

in shoots were obtained in plants inoculated with rhizobial strains, especially when it was applied together with biofertilizers and chemical PK-fertilizer.

In reference to the effects of fertilization on total K accumulation in shoot dry matter, it was observed that the effects of K-biofertilizers were not clear, and greater quantities were obtained when KCl was applied together with TSP and BP in level 500 kg ha^{-1}. Probably the effects of acid production by application of biofertilizers plus sulfur inoculated with *Acidithiobacillus* reduce the soil pH (Table 4) which increases exchangeable Al in soil that may promote injurious effects in plant growth and reduction on nutrient absorption. Similar results were obtained by Stamford et al. (2006) when applying PK biofertilizers in a Brazilian tableland soil grown with sugarcane.

Soil pH and available P and K

The soil pH decreased significantly when biofertilizers plus sulfur inoculated with *Acidithiobacillus* were applied, especially in the biofertilizers produced with higher levels of sulfur (Table 4). Decrease in soil pH due to the application of biofertilizers in higher levels was observed, but injurious effects on plant growth were not detected (Table 1). Stamford et al. (2007) using sulfur inoculated with *Acidithiobacillus* and gypsum in different rates and mixed proportions for amendment of a sodic soil from the Brazilian semiarid region observed the effect on soil pH reduction.

The highest effects of fertilization treatments on available phosphorus in soil were observed with biofertilizers application (Table 5). Indeed it is possible that the effect of the lower pH is a consequence of the application of biofertilizers with sulfur inoculated with oxidative bacteria. This suggests that soil acidification may improve solubility of P and K from natural rocks, and this data demonstrates the influence of sulfuric acid produced metabolically by *Acidithiobacillus* oxidative bacteria.

The effect of biofertilizers on increased available P in soil was significant and the highest amounts were obtained when applied biofertilizers P_{250} and P_{500}, which were 26% greater than the values of triple superphosphate and eight times greater than the control without P application (P_0K_0).

Highest values of available K in soil were detected in treatments receiving PK-biofertilizers, especially when applied biofertilizer K_{500} and K_{750}, and chemical fertilizer (KCl_{250}) as shown in Table 5. It seems that the availability of P and K from phosphate and potash rocks is a continuous process, because sulfuric acid is continuously produced and may increase phosphate rock solubilization as indicated by the low values of soil pH (Table 4). PK-Biofertilizer with higher amount of sulfur inoculated with *Acidithiobacillus* (B_{750}), had great potential to improve cowpea yields and showed higher amount of available P and K in the soil after plant harvest (Table 5).

The effectiveness of biofertilizers, produced through mixing powdered rocks and elemental sulfur inoculated with *Acidithiobacillus*, were compared with P and K soluble fertilizer (triple superphosphate and potassium

Table 3 Effects of PK fertilization and rhizobia inoculation on nodules and shoot dry biomass of cowpea grown in a Brazilian tableland soil with low available P and K

PK treatments (kg ha^{-1})		KCl_{250}	BK_{250}	BK_{500}	BK_{750}	K_0
Total P in shoot dry biomass (mg $plant^{-1}$)						
No P applied (P_0)	−rhizobia	4.1cA	5.5cA	6.1bA	4.1cA	2.2
	+rhizobia	9.2abA	6.5bcA	6.7bA	4.8bcA	1.9
Biofertilizer P_{250}	−rhizobia	8.6bA	6.7bcA	6.8bA	5.8bA	
	+rhizobia	8.5bA	8.7cA	11.2aA	10.4aA	
Biofertilizer P_{500}	−rhizobia	7.7bA	9.8bA	10.9aA	8.7abA	
	+rhizobia	10.5abA	11.2bA	11.8aA	10.2aA	
Triple perphosphate$_{250}$	−rhizobia	10.9abAB	12.7aA	5.7bdB	7.7bAB	
	+rhizobia	12.8aA	15.1aA	10.1aA	11.4aA	
P $rock_{1000}$ + K $rock_{1000}$	−rhizobia	4.1				
	+rhizobia	4.7				
Total K in Shoot dry biomass (mg $plant^{-1}$)						
No P applied (P_0)	−rhizobia	230cA	157cAB	186cAB	124dB	105
	+rhizobia	355bcA	269bcA	315bA	344bA	99
Biofertilizer P_{250}	−rhizobia	381bcA	217bcBC	264bcB	160cdC	
	+rhizobia	516bA	248bcB	376bB	307bBC	
Biofertilizer P_{500}	−rhizobia	453bA	241bcB	271bcB	310bB	
	+rhizobia	603abA	348bB	360bB	335bB	
Triple superphosphate$_{250}$	−rhizobia	660aA	340bB	255cB	250cB	
	+rhizobia	740aA	473aC	551aBC	590aB	
P $rock_{1000}$ + K $rock_{1000}$	−rhizobia	123				
	+rhizobia	163				

%C.V. Total P in shoot dry matter = 11.36. Total K in shoot dry matter = 15.37

L.S.D. Total P biomass = rows 5.33, columns 3.10; Total K biomass = rows 42.170, columns 55.71

Biofertilizer P_{250} and P_{500} correspond to application of P biofertilizer at levels 250 and 500 kg ha^{-1}; BK_{250}, K_{500}, and K_{750} application of K biofertilizer at levels 250, 500, and 750 kg ha^{-1}; P $rock_{1000}$ + K $rock_{1000}$; application of apatite and biotite in level of 1000 kg ha^{-1}, respectively. Triple $Superphosphate_{250}$ is the recommended P fertilization for cowpea in Brazilian tableland soil (Cavalcanti et al. 1998). Different letters on the top of each bar indicate significant difference at $P \leq 0.05$ (Tukey test); upper case letters compare data in rows and lower letters in columns

chloride) and the improved availability of P and K in soil, suggesting that they could be important in P and K fertilization, especially in soils with neutral pH due to it acidification effect. In contrast it is necessary to take care when using the biofertilizers in acidic soils.

Discussion

This study has provided some information on the response of cowpea legumes after applying biofertilizers produced from phosphate and potash rocks plus elemental sulfur inoculated with *Acidithiobacillus* and its use as alternative to soluble P and K fertilizers.

According to Garcia Junior (1992) the production of sulfuric acid by *Acidithiobacillus* should be responsive to additional levels, since there are significant differences in biofertilizer pH and available P and K due to sulfur addition, and the differences among biofertilizers indicate a continuous effect proportional to the sulfur addition.

The effects of PK treatments on shoot biomass and on accumulation of total N, P, and K in shoots, compared with soluble phosphate and potash fertilizers and without PK application, are conclusive and strongly indicate a possible role for biofertilizer use, especially for low-income farmers, who frequently are not able to afford with the use of more soluble PK fertilizers. Klepker and Anghinoni (1995) and Ernani et al. (2001) compared the effect of soluble phosphate and phosphate rock, and observed that phosphate rock was not competitive compared to soluble fertilizer (triple superphosphate). However, Stamford et al. (2005, 2006) showed similar effect of P biofertilizer produced from apatite rock when compared with the soluble fertilizer (triple superphosphate) in a tableland acidic soil with low available P and K grown with mimosa (*Mimosa caesalpiniifolia*) and sugarcane.

Table 4 Effect of PK fertilization in pH and exchangeable Al in a Brazilian tableland soil with low available P and K

PK treatments (kg ha^{-1})	KCl_{250}	BK_{250}	BK_{500}	BK_{750}	K_0
Soil pH					
No P applied-P_0	6.0aA	5.6aB	5.1aB	4.6aC	6.1
Biofertilizer P_{250}	5.1bA	5.0bA	4.5bB	4.3abB	
Biofertilizer P_{500}	4.9bA	4.5bAB	4.1bB	4.0bB	
Triple superphosphate$_{250}$	5.3bA	4.9bA	4.8abA	4.5aB	
P- rock$_{1000}$ + K rock$_{1000}$	5.4				
Exchangeable Al^{+3} ($cmol_c$ dm^{-3})					
No P applied (P_0)	0.00bB	0.00bC	0.07abB	0.14aC	0.00
Biofertilizer P_{250}	0.07cAB	0.10bcBC	0.20abA	0.29aB	
Biofertilizer P_{500}	0.13cAB	0.25bcA	0.38abA	0.64aA	
Triple Superphosphate$_{250}$	0.17aA	0.25aA	0.30aA	0.32 aAB	
P- rock$_{1000}$ + K rock$_{1000}$	0.00				

%C.V. pH = 6.49; Available Al^{+3}= 29.05

L.S.D. pH = rows 0.339, columns 0.256; exchangeable Al^{+3} = rows 0.182, columns 0.256

Biofertilizer P_{250} and P_{500} correspond to application of P biofertilizer at levels 250 and 500 kg ha^{-1}; BK_{250}, K_{500}, and K_{750} application of K biofertilizer at levels 250, 500, and 750 kg ha^{-1}; P rock$_{1000}$ + K rock$_{1000}$; application of apatite and biotite in level of 1000 kg ha^{-1}, respectively. Triple Superphosphate$_{250}$ is the recommended P fertilization for cowpea in Brazilian tableland soil (Cavalcanti et al. 1998). Different letters on the top of each bar indicate significant difference at $P \leq 0.05$ (Tukey test); upper case letters compare data in rows and lower letters in columns

Table 5 Effect of PK treatments in available P and K in a Brazilian tableland soil with low available P and K

PK treatments (kg ha^{-1})	KCl_{250}	BK_{250}	BK_{500}	BK_{750}	K_0
Available P in soil (mg dm^{-3})					
No P applied (P_0)	8.4Db	8.8cB	15.5bA	15.5cA	6.1
Biofertilizer P_{250}	49.2bcC	62.9aA	50.0aBC	56.4aAB	
Biofertilizer P_{500}	57.8aA	48.2bB	48.3aB	62.8aA	
Triple Superphosphate$_{250}$	38.0cB	44.4 bAB	49.0aA	42.1bAB	
P-rock$_{1000}$ + K rock$_{1000}$	24.5				
Available K in soil (mg dm^{-3})					
No P applied (P_0)	51.3dA	12.7aB	14.7bB	16.3bB	4.2
Biofertilizer P_{250}	65.7cA	17.0aB	19.3bB	17.3bB	
Biofertilizer P_{500}	95.7aA	18.3aB	18.7bB	18.3bB	
Triple Superphosphate$_{250}$	88.3bB	20.0aC	108aA	94.3aA	
P rock$_{1000}$ + K rock$_{1000}$	4.0				

%C.V. Available P = 11.55, K = 12.13

L.S.D. Available P = rows 5.11, columns 3.87; Available K = rows 11.25, columns 5.63

Biofertilizer P_{250} and P_{500} correspond to application of P biofertilizer at levels 250 and 500 kg ha^{-1}; BK_{250}, K_{500}, and K_{750} application of K biofertilizer at levels 250, 500, and 750 kg ha^{-1}; P rock$_{1000}$ + K rock$_{1000}$; application of apatite and biotite in level of 1000 kg ha^{-1}, respectively. Triple Superphosphate$_{250}$ is the recommended P fertilization for cowpea in Brazilian tableland soil (Cavalcanti et al. 1998). Different letters on the top of each bar indicate significant difference at $P \leq 0.05$ (Tukey test); upper case letters compare data in rows and lower letters in columns

The results for addition of sulfur to phosphate rock without *Acidithiobacillus* inoculation differ from those of Lombardi (1981) that stated that sulfur addition to phosphate rock with or without *Acidithiobacillus* inoculation were similarly effective. Results reported by Stamford et al. (2005), showed that the effect of phosphate rock pelleting the sulfur inoculated with *Acidithiobacillus* promote bigger increases in total N and P in plant shoots than application of triple superphosphate and phosphate rock plus sulfur without *Acidithiobacillus*, but with smaller advantages compared to our results. Probably it is due to phosphate rock pelleting the sulfur inoculated with

Acidithiobacillus, in their experiment, which may reduce sulfuric acid production due to the limited oxygen and water for oxidizing the sulfur.

The effect of the PK treatments on soil pH was observed, especially, when the biofertilizers with higher levels of sulfur inoculated with *Acidithiobacillus* were applied, but plant growth and cowpea biomass yield were not affected by the reduction in soil pH, probably because this legume is tolerant to low pH as described by Stamford et al. (2003). This lack of negative response to low soil pH is most probably due to a combination of increased P and K availability, at least partly a consequence of low soil pH as related by He et al. (1996), and plant adaptation to acid soil conditions, since this species is largely characterized as presenting good growth in acid soils.

The efficiency of rhizobial inoculation in the pot experiments being not consistent with other authors may be due to the presence of poorly competitive rhizobial native population on the experimental site, as pointed out by Campos et al. (2001). The possibility of selecting rhizobial strains of cowpea according to their capacity of recovery from different kinds of stresses has been investigated (Serraj et al. 2001; Figueiredo et al. 2007). However, the non-specificity of the introduced strains and the occurrence of ineffective indigenous strains in soils limit the introductions of selected strains, thus limiting the potential contribution of N_2 fixation as reported to cowpea by Figueiredo et al. (1999) and Santos et al. (2005) in peanut. In the other hand, the efficiency of inoculated rhizobia strains could be influenced by the high acidity found in the soil, especially when biofertilizers are applied with high sulfur with *Acidithiobacillus*.

It is important to observe that the process of water submersion following the soil exposure to high temperature was not used in this work, yet as reference to the participation of indigenous rhizobia, which in further studies showed to be more competitive for nodule formation and in a general way are ineffective to tropical legumes (Stamford et al. 1995).

The water submersion process covering the soil with black plastic had a drastic effect and reduced totally the indigenous rhizobia from soil (producing no nodule biomass) and may contribute to the effectiveness of rhizobia introduced by inoculation. In a general way the effects of rhizobial inoculation with specific strains selected for cowpea in high temperature and acidic conditions were consistent in the pot trial, but these results need to be tested in field experiments.

The positive impact of the PK biofertilizer produced from apatite and biotite plus sulfur inoculated with *Acidithiobacillus* on the growth of cowpea points up great promise for improving the input of these products as an alternative for partial or total substitution of soluble PK fertilizers with low cost of production. The PK biofertilizer produced from apatite and biotite inoculated with *Acidithiobacillus* applied in high levels may be unfavorable to rhizobia inoculation and could reduce N_2 fixation.

The soil acidity influenced N_2 fixation, especially when biofertilizers with a high amount of sulfur with *Acidithiobacillus* were used, although the *Bradyrhizobium* strains introduced by inoculation showed efficient association with cowpea plants, corroborating the importance of inoculation.

Conclusion

Phosphate and potash biofertilizers produced from natural apatite and biotite plus sulfur inoculated with *Acidithiobacillus* has great potential to be used as alternative to phosphate and potassium chemical fertilizers in cowpea, especially in soils with low available P and K, suggesting also a good potential for other short term cultures. The biofertilizers may be applied taking into account the acidity effect in soils due to the acid production and especially in acidic and low buffered soils.

Acknowledgments Authors are indebted to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio e Tecnologia do Estado de Pernambuco (FACEPE) and the Program in Agronomy (Soil Science Postraduate Courses) of University Federal Rural of Pernambuco (UFRPE), for financial support and fellowships.

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