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Cowpea nodulation, biomass yield and nutrient uptake, as affected by biofertilizers and rhizobia, in a sodic soil amended with *Acidithiobacillus*

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ABSTRACT. Sodic soils require application of amendments as gypsum and organic matter. Many types of compost have been tested in sodic soils reclamation; however, these materials often do not provide satisfactory pH reduction. A recent study reported effective effects applying mixture of gypsum and sulfur inoculated with *Acidithiobacillus* in sodic soils with high pH and exchangeable sodium, though the effects on plant parameters were not evaluated. The present study was conducted to verify the effects of BPK rock biofertilizers on nodulation, biomass yield and nutrient uptake in cowpea compared with mineral fertilizer after sodic soil amendment. The BPK biofertilizers and PK mineral fertilizer were applied at different rates, and plants were inoculated with effective rhizobia strains. A control that did not receive PK fertilization was included. The results indicated that gypsum and sulfur with *Acidithiobacillus* reduced the soil's pH and the amount of soil exchangeable sodium. BPK rock biofertilizer increased cowpea nodulation, biomass yield and nutrient uptake. The native rhizobia in the soil exhibited effectiveness in cowpea growth; displaying similar results compared with the rhizobia inoculated plants. BPK biofertilizers may be used as alternative to mineral PK fertilizers in sodic soils after the application of gypsum and sulfur inoculated with *Acidithiobacillus*.

Keywords: *Vigna unguiculata*, soil amendment, sulfur-gypsum interaction, sulfur oxidation, sulfur oxidizing bacteria.

Nodulação, produção de biomassa e absorção de nutrientes em caupi por biofertilizantes e rizóbios em um solo sódico com *Acidithiobacillus*

RESUMO. Solos salinos sódicos necessitam aplicação de condicionadores como gesso e matéria orgânica. Vários compostos foram avaliados como condicionadores para solos sódicos; entretanto, frequentemente estes materiais não promovem satisfatória redução no pH. Pesquisa recente demonstrou efeitos efetivos da aplicação da mistura de gesso e enxofre inoculado com *Acidithiobacillus* em solos sódicos com alto pH e sódio solúvel, todavia, os efeitos na planta não foram avaliados. O trabalho procurou verificar os efeitos de biofertilizantes de rochas (BPK), na nodulação, produção de biomassa e absorção de nutrientes em caupi comparado com fertilizantes minerais após adição dos condicionadores. Biofertilizantes (BPK) e fertilizantes minerais PK foram aplicados em diferentes doses, e as plantas inoculadas com rizóbios efetivos. Tratamento controle sem fertilização com PK foi incluído. Os resultados indicaram efeito do gesso e enxofre com *Acidithiobacillus* na redução do pH e sódio trocável. O biofertilizante de rochas (BPK) aumentou a nodulação, a biomassa e a absorção de nutrientes. Os rizóbios nativos do solo favoreceram o crescimento do caupi, apresentando resultados similares quando comparado com plantas inoculadas com rizóbios efetivos. O biofertilizante de rochas (BPK) mostrou ser alternativa para fertilizantes minerais com P e K em solos sódicos, após adição de gesso e enxofre inoculado com *Acidithiobacillus*.

Palavras chave: *Vigna unguiculata*, condicionador do solo, interação enxofre-gesso, oxidação do enxofre, bactéria oxidante do enxofre.

Introduction

The growing world population and the demands for fertilizers have led to changes in agricultural cropping systems and have intensified the use of new techniques to produce maximum yields from field crops. As a result, agricultural lands need to be improved, particularly those of arid and semiarid soils, because of the effects of salinization. The use of legume species, for example, cowpea, is of great importance because these species may provide nitrogen to the system through N_2 fixation and may supply protein without the application of mineral nitrogen fertilizers (SPRENT, 2009), particularly under drought conditions (LABUSCHAGNE et al., 2008).

In semiarid regions, the soil salinity and sodicity are the most important factors that require consideration, particularly in view of the effects of the high sodium carbonate content on the uptake of nutrients because of the increase in the soil's pH, amount of $NaSO_4$ and amount of NaCl (JALALI et al., 2008; QADIR et al., 2005). Several studies conducted in semiarid regions have shown that reductions in plant yield are a function of soil salinity. These studies have also indicated that it is necessary to control for this specific problem to improve the productivity and quality of crops (QADIR et al., 2008).

Because of its low cost, gypsum ($CaSO_4.2H_2O$) has been frequently used for sodic soil reclamation and to achieve sulfur fertilizer (JAGGARD; ZHAO, 2011). However, the use of gypsum for sodic soil reclamation requires its application in large amounts, and the resulting reduction in the soil pH is not substantial (STAMFORD et al., 2002, 2003 and 2004). The inoculation of the sulfur-oxidizing bacteria *Acidithiobacillus* with elemental sulfur generates sulfuric acid, and, different from gypsum application, the H^+ released by the acid may contribute decisively to reduce the pH in sodic soils (STAMFORD et al., 2007).

Sulfur-oxidizing bacteria have a significant ecological impact. These bacteria promote the availability of elemental sulfur in the soil through its oxidation to sulfate, also contributing to the solubilization of the otherwise-unavailable soil phosphorus (EL-TARABILY et al., 2006). Although sulfur-oxidizing bacteria occur naturally in soils, these species are relatively less abundant in agricultural soils. Therefore, to obtain satisfactory effects for sodic soil reclamation, sulfur-oxidizing bacteria should be introduced into the soil.

Studies that have been conducted in the soils of semiarid Northeast Brazil have reported that the inoculation of sulfur with *Acidithiobacillus* may be a promising means for the reclamation of saline sodic soils because of the resulting strong reduction in pH of the soil (STAMFORD et al., 2002, 2003 and 2004). Indeed, Stamford et al. (2007) used a mixture of sulfur inoculated with *Acidithiobacillus* and gypsum in equal proportions and observed excellent results in the attributes of the soil; however, the accompanying effects on plant growth were not evaluated. Furthermore, the plant parameter effects of PK rock biofertilizers applied to amended sodic soils have not been reported in the literature.

Cowpea (*Vigna unguiculata* L. Walp) is one of the most important grain crops in the semi-arid region of Northeast Brazil. It is produced mostly on small, family farms under low-input agricultural systems, with no fertilizer application. Thus, biological nitrogen fixation (BNF) must have an important role in the plant nutritional status (CLARK et al., 2005). The overall aim of this study was to evaluate the effects of PK rock biofertilizers on several cowpea parameters (nodulation, shoot biomass and nutrient uptake) in a sodic soil after gypsum and sulfur inoculated with *Acidithiobacillus* (in equal proportions, 50:50) at a rate of 2.4 ton ha^{-1}. The effects of the PK rock biofertilizers compared with those of the mineral fertilizers (simple superphosphate and sulfate chloride) and rhizobial inoculation were also investigated.

Material and methods

Experimental conditions and soil analyses

A greenhouse experiment was conducted in jars containing sodic soil (10 kg) collected in the Brazilian semiarid region, District of Serra Talhada, Pernambuco State. The climate of the study site is characterized by a high potential evapotranspiration throughout the year (1500 to 2000 mm $year^{-1}$) and low, concentrated and erratic rainfall (mean annual rainfall approximately 700 mm) that is typical of the Brazilian Northeast Semiarid region. The soil has been classified as Neosoil Fluvic Sodic with a medium texture (EMBRAPA, 2006). Soil samples were collected at a depth of 0-20 cm and were air dried, sieved (using a 2 mm sieve) and mixed. The chemical analyses were conducted using the Embrapa (2009) methodology and produced the following results: pH (H_2O) = 10.5; electrical conductivity (E.C.) = 22.7 dS m^{-1} and exchangeable cations ($mmol_c\,dm^{-3}$) $Na^+ = 355$, $K^+ = 87$, $Ca^{2+} = 879$ and $Mg^{2+} = 196$.

Soil reclamation

The sodic soil was initially amended with a mixture of gypsum (G) and sulfur inoculated with *Acidithiobacillus thiooxidans* (S*). This application used 2.4 ton ha^{-1} of the mixture in 50:50 (w:w) percentage proportions (G:S*), as based on the methodology that was described by Stamford et al. (2007). To obtain the best results from the bacterial oxidation, the soil was incubated for 45 days after the application of amendments and was irrigated daily using slightly saline water (0.2 dS m^{-1}) that was obtained from the Serra Talhada Research Station. After the incubation period, water (2 liter pot^{-1}) was added to promote a leaching layer. Subsequently, soil samples were collected and analyzed for the pH, electrical conductivity (E.C.), Ca^{2+}, Mg^{2+} Na^+ and K^+ using inductively coupled plasma-atomic emission spectroscopy ICP-AES (Perkin Elmer).

Production of the P and K biofertilizers

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

The sulfur-oxidizing bacteria were grown in 2,000 mL Erlenmeyer flasks that contained 1,000 mL of culture medium 9K sterilized for 30 min. at 120°C. The Erlenmeyer flasks were shaken (150 revolutions $min.^{-1}$) for 5 days at 30°C. The materials (phosphate and potash rocks that were mixed with the elemental sulfur) were incubated for 60 days. The humidity was maintained at a level that was near the field holding capacity. The furrows were covered with black plastic to avoid excessive humidity due to rain and to increase the efficiency of the oxidative bacteria. An analysis of the natural-rock P and K biofertilizer using extractions with (A) Mehlich 1 and (B) 2 % citric acid, according to Embrapa (2009), yielded the following results: (P-biofertilizer)-pH = 3.8 and available P (A) = 60 (g kg^{-1}) and (B) = 48 (g kg^{-1}) and (K biofertilizer-BK)-pH = 3.3 and available K (A) = 10 (g kg^{-1}) and (B) = 5 (g kg^{-1}).

Experimental design

Following the soil amendment procedure of adding gypsum and elemental sulfur that were inoculated with *Acidithiobacillus*, an experiment was conducted using a factorial arrangement (6 + 1) x 5 and performed according to a randomized block design with 4 replicates. The phosphorus and potassium (P+K) were applied using two sources: (a) P + K mineral fertilizer (simple superphosphate + potassium sulfate) and (b) rock biofertilizer produced by phosphate rock and potassium rock plus sulfur inoculated with *Acidithiobacillus* (BP + BK). The two sources were applied at three rates: P_1K_1 (the recommended rate for cowpea), $P_{1.5}$ $K_{1.5}$ (1.5 times the recommended rate) and P_2K_2 (2.0 times the recommended rate). A control group (P_0K_0) that was not treated with P and K fertilization was also included in the study.

The quantities of the fertilizers that were used in the experiment followed the recommendations for irrigated cowpea grown in the semiarid region of Pernambuco State (IPA, 2008) and were estimated as follows: (a) soluble P fertilizer (simple superphosphate = 300, 450 and 600 kg ha^{-1}) + soluble K fertilizer (potassium sulfate = 80, 120 and 160 kg ha^{-1}) and (b) biofertilizer from phosphate rock (BP= 250, 500 and 750 kg ha^{-1}) + biofertilizer from potash rock (BK= 80, 120 and 160 kg ha^{-1}).

The five treatments to supply nitrogen to the cowpea plants were: three rhizobia (NFB 700, Semia 6156 and BR 3267) applied individually, the mineral fertilization (N_{100}) with ammonium sulfate (500 kg ha^{-1}), and the N control treatment (N_0) with no N source applied in which the plants may receive N furnished by N_2 fixation from the native rhizobia and mineral N from the soil (low total N). The effective strains recommended for cowpea in Northeast Brazil (NFB 700, Semia 6156 and BR 3267) were applied individually. Strain NFB 700 was selected for cowpea grown in saline sodic soils, strain Semia 6156 was purchased from the Brazilian Inoculant Center (Mircen, Brazil), and strain BR 3267 was purchased from CNPAB (*Centro Nacional de Pesquisa em Agrobiologi*a - EMBRAPA, Rio de Janeiro State, Brazil). Both of the strains that were purchased are recommended to produce commercial inoculants for cowpea.

Seed inoculation

Prior to sowing, the seeds of cowpea (cv. IPA 206) were inoculated with strains NFB 700, Semia 6156 and BR 3267, applied individually. In the treatments with the mineral N application and the control with no N applied (N_0) the plants were not inoculated with rhizobia. The inoculants were produced at the Soil Microbiology Laboratory (University Federal Rural of Pernambuco) and were grown in a yeast mannitol medium that was prepared at a minimal concentration of 10^8 (UFC mL^{-1}), in accordance with Stamford et al. (1995).

Plant parameters and statistical analysis

To estimate the dry biomass of the nodules and shoots, the plants were harvested approximately 45 days after the planting date. The nodules and shoots were separated, and their dry biomass values were determined. The total N in shoot dry matter was analyzed using the semimicro-Kjeldhal method following Malavolta et al. (1997) using a Kjeltec autoanalyzer (Model 1030). The total P, K, Ca and Mg were determined using the nitroperchloric digestion method in accordance with Malavolta et al. (1997). The soil was analyzed following Embrapa (2009) methodology.

The statistical analyses were performed using an analysis of variance that included the main effects of the fertilization, the rhizobia treatments and the interaction between these factors. SAS software was used to implement the analyses (SAS INSTITUTE, 1999). The differences among the treatment means were analyzed using the Tukey test ($p \leq 0.05$). All of the variables were normally distributed; a normal distribution was appropriate for the nodule biomass because zero values did not occur in the data from the uninoculated treatment.

Results and discussion

Soil attributes after the amendment application

The analysis of the sodic soil after the amendment with elemental sulfur inoculated with *Acidithiobacillus* (S*) and gypsum (G) (50:50) applied at a rate of 2.4 ton ha^{-1} and prior to planting showed a pH of 8.0 and exchangeable cation values (mmol$_c$ dm^{-3}) of $Na^+ = 99$, $Ca^{+2} = 16$ and $Mg^{+2} = 2.3$. The data show a reduction in the soil's pH and Na^+, Ca^{+2} and Mg^{+2} exchangeable cations after the amendment.

The significant effect of the sulfur inoculated with *Acidithiobacillus* and gypsum as amendment agrees with the results that were obtained by Stamford et al. (2007). The pH reduction that was produced by the BP and BK biofertilizers produced from rocks that were inoculated with *Acidithiobacillus* was described by Stamford et al. (2006) for sugar cane grown in an acidic soil from the Brazilian Coastal Tableland Zone. These authors concluded that biofertilizers may be used as sources of P and K and that it is important to note the strong effect of these fertilizers in reducing the soil pH.

The effect of the amendment procedure on the reduction of exchangeable sodium was significant (the Na^+ values decreased from 230 to 99 mmol$_c$ dm^{-3}), probably due to the production of sulfuric acid by the oxidizing *Acidithiobacillus*. This effect was also significant when the BPK rock biofertilizers were applied. The results indicated sodium values that were less than those used to classify sodic soil. The increase in the exchangeable Ca^{+2} and Mg^{+2} that was produced by the application of the BPK rock biofertilizers resulted from the high content of calcium in the rock phosphate (12 mg g^{-1}) and magnesium in the biotite rock (9 mg g^{-1}), the materials that were used to produce the BP and BK biofertilizers.

Nodules and plant biomass

The values of the nodules dry biomass of the cowpea plants grown in the saline soil of the Brazilian semiarid region that was amended with a 50:50 mixture of gypsum and sulfur inoculated with *Acidithiobacillus*, fertilized with N mineral source and BPK biofertilizers and inoculated with different recommended *Bradyrhizobium* strains and a control treatment with no N source (native rhizobia) are shown in Table 1.

The PK rock biofertilizer (BPK) administration and rhizobial inoculation increased the nodulation. The treatments that received the BPK biofertilizers produced a greater amount of nodule dry biomass than did the controls ($p < 0.05$). The best results were obtained when the BPK biofertilizer was applied at the highest rate, particularly when the plants were inoculated with the Semia 6156 rhizobia strain. The rhizobia that were native to the soil were efficient at producing nodulation in the treatments that received the BPK fertilization. The amount of nodule dry biomass was drastically reduced when the mineral N fertilizer (500 kg ha^{-1}) in the form of ammonium sulfate was applied, independent of BPK fertilization, whereas the amount of nodule biomass was not affected in the control group, probably because the soil had satisfactory levels of available P and K.

Table 1. Nodules and shoot dry biomass of cowpea as affected by PK mineral fertilizers, BPK rock biofertilizer and inoculation with rhizobia strains (NFB 700, Semia 6156 and BR 3267) compared with N mineral and control with no N applied (native rhizobia), in a sodic soil of the Brazilian semiarid region after amendment with gypsum and sulphur inoculated with *Acidithiobacillus*.

PK fertilization (Sources/ rates)	N mineral fertilization 500 kg ha^{-1}	Control-N (native rhizobia)	Rhizobia inoculation		
			BR 3267	NFB 700	SEMIA 6156
	Nodules dry biomass (mg plant^{-1})				
BPK Biofertilizer $_{1,0}$	4^{Ab} ± 1.1	152^{Ba} ± 45.8	145^{Ba} ± 43.8	188^{Ba} ± 56.7	176^{Ba} ± 53.1
BPK Biofertilizer $_{1,5}$	9^{Ac} ± 2.7	229^{Ab} ± 69.1	221^{Bb} ± 66.6	225^{Bb} ± 67.9	329^{Aa} ± 99.3
BPK Biofertilizer $_{2,0}$	5^{Ab} ± 1.5	303^{Aa} ± 91.3	331^{Aa} ± 99.9	311^{Aa} ± 93.9	354^{Aa} ± 96.8
PK Fertilizer $_{1,0}$	5^{Ac} ± 2.0	162^{Bb} ± 54.9	188^{Bb} ± 45.8	213^{Bb} ± 66.6	305^{Aa} ± 77.8
PK Fertilizer $_{1,5}$	7^{Ac} ± 2.7	182^{Bab} ± 5.2	152^{Bb} ± 50.0	221^{Ba} ± 85.7	258^{Aa} ± 76.7
PK Fertilizer $_{2,0}$	9^{Ac} ± 2.9	150^{Bb} ± 47.9	166^{Bb} ± 41.9	284^{Aa} ± 62.4	261^{Aa} ± 58.4
Control (P_0K_0)	10^{Ac} ± 6.9	159^{Bab} ± 8.0	139^{Bb} ± 41.8	207^{Ba} ± 62.4	194^{Ba} ± 58.5
	Shoot dry biomass (g plant^{-1})				
BPK Biofertilizer $_{1,0}$	7.99^{Aa} ± 1.13	6.45^{Aab} ± 0.91	6.75^{Aab} ± 0.96	5.68^{Ab} ± 0.81	5.56^{Ab} ± 0.79
BPK Biofertilizer $_{1,5}$	7.91^{Aa} ± 1.12	6.51^{Aab} ± 0.92	6.45^{Aab} ± 0.92	5.47^{Ab} ± 0.78	5.85^{Ab} ± 0.83
BPK Biofertilizer $_{2,0}$	6.79^{Aa} ± 0.96	5.51^{Bb} ± 0.78	5.61^{ABb} ± 0.80	6.27^{Aa} ± 0.89	5.91^{Aab} ± 0.84
PK Fertilizer $_{1,0}$	7.07^{Aa} ± 1.00	5.72^{ABab} ±0.81	5.59^{ABab} ± 0.79	6.52^{Aa} ± 0.93	6.38^{Aa} ± 0.90
PK Fertilizer $_{1,5}$	6.27^{Aa} ± 0.89	6.71^{Aa} ± 0.95	5.60^{ABab} ± 0.80	5.37^{Ab} ± 0.76	5.95^{Aab} ± 0.84
PK Fertilizer $_{2,0}$	6.42^{Aa} ± 0.91	6.10^{Aa} ± 0.87	6.51^{Aa} ± 0.92	5.47^{Ab} ± 0.78	5.63^{Aab} ± 0.80
Control (P_0K_0)	5.34^{Ba} ± 0.76	5.33^{Ba} ± 0.90	5.06^{Ba} ± 0.72	5.90^{Aa} ± 0.84	5.73^{Aa} ± 0.81

Within topics, values followed by different letters are significantly at ($p \leq 0.05$) using the Tukey test. Upper case letters compare data in columns. (PK sources) and lower case compare data in rows (mineral N and rhizobia strains). C.V. (%) Dry nodules biomass = 30.18; dry shoot biomass = 14.19.

The amount of nodule dry biomass was drastically reduced when the mineral N fertilizer (500 kg ha^{-1}) in the form of ammonium sulfate was applied, independent of the BPK fertilization. In the control group, the amount of nodule biomass was not affected, probably because the soil had satisfactory levels of available P and K.

The effect of mineral N on cowpea nodulation in a sodic soil of the Brazilian semiarid region (Pesqueira, Pernambuco State) has been reported by Stamford et al. (2002), and the effect of mineral N on cowpea nodulation in a Brazilian tableland acidic soil has been reported by Stamford et al. (2008). The effectiveness of strain Semia 6156 has also been reported by Freitas et al. (2003) for pigeon pea plants grown in a saline soil of the Brazilian semiarid region. In reference to the effectiveness of the native rhizobia in the soil, Silva et al. (2008) have shown that the native soil rhizobia in Paraiba State, Brazil, were more effective than the effective strains that are recommended for cowpea by various Brazilian laboratories.

Stamford et al. (2002 and 2003) observed effective cowpea nodulation in a study conducted in the sodic soil of the Brazilian semiarid region of Pernambuco State. This previous study evaluates the effect of the application of gypsum in comparison to sulfur inoculated with *Acidithiobacillus*. In the present study, the cowpea plants that were inoculated with the recommended strains exhibited more effective nodulation than the uninoculated plants. Similar results were reported by Stamford et al. (2005) in experiments using leucena and mimosa tree legumes.

The results for the shoot dry biomass of the cowpea plants grown in saline sodic soil amended with the gypsum and sulfur inoculated with *Acidithiobacillus*, fertilized with an N mineral source and BPK biofertilizers and inoculated with different recommended *Bradyrhizobium* strains and a control treatment with no N source (native rhizobia) are also shown in Table 1.

In a previous assay (data not presented) cowpea seeds do not germinate in unamended soil, likely because of the high sodium content of the exchangeable soil. In general, we observed a significant effect of the P and K fertilization, which was evident from the comparisons of the biofertilizers (BPK) and the soluble mineral fertilizers with the control treatment (P_0K_0). The findings that were obtained using strains NFB 700 and Semia 6156 did not differ from these results.

The highest shoot dry biomass for the cowpea plants was observed when the soluble mineral fertilizers and biofertilizers were applied at 1.5 times the recommended rate for P and K. The positive effect of mineral fertilization and BPK biofertilizer is evident from these results. However, a reduction in the shoot dry biomass was displayed when the BPK biofertilizers were applied at the highest rate (P and K at 2 times the recommended rate). In accordance with Malavolta et al. (1997), this result was likely a consequence of the reduction in the soil pH. Similar results have been reported by Stamford et al. (2006) after tests using PK rock biofertilizers in sugar cane and by Lima et al. (2007) in an experiment with lettuce. The reduction in the shoot dry matter that was observed when the highest rate of BPK rock biofertilizer was applied may have resulted from the low pH of the P and K biofertilizers (3.0 and 3.5, respectively).

Nutrient uptake

The total N content of the dry biomass of the cowpea shoots is shown in Table 2. In general, the best results for the total N were obtained when the plants were fertilized with mineral nitrogen and the BPK rock biofertilizers were applied at the highest rate. The total N accumulation in the shoot dry biomass did not present significant differences among the treatments. However, it is very important to note that the plants inoculated with strain NFB 700 and without P and K fertilization presented similar total N values.

The total N accumulation in the shoot dry biomass did not present significant differences. However, it is very important to note that the plants that were inoculated with strain NFB 700 and without P and K fertilization presented similar total N values. This observation indicates that this strain was effective in soils that had sufficient levels of P and K, as suggested by Stamford et al. (1995). These authors emphasized that this strain is known to possess effective nitrate reductase and nitrogenase activities and may be effective in the presence of mineral nitrogen.

The total levels of P and K accumulation in the shoot dry biomass of the cowpea plants are presented in Table 3. The values of total P and K in the shoot dry biomass showed no significant differences across the BPK fertilization treatments (sources and rates) and the N sources that were used (mineral nitrogen and inoculation with different rhizobia strains). This finding may result from the high content of available P (48.2 mg dm^{-3}) and K (0.32 $mmol_c$ dm^{-3}) in the soil, which can supply sufficient amounts of P and K to cowpea plants. No significant response was observed for the total amount of calcium in the cowpea shoot dry biomass (data not shown). This result may be explained by the fact that all of the treatment groups received Ca, which was supplied by the application of the gypsum for saline soil reclamation and by the apatite of the BP biofertilizer, and a high amount of Mg, which was released from the biotite rock.

Table 2. Total N accumulation in the shoot dry biomass of cowpea as affected by PK mineral fertilizers, PK rock biofertilizer and inoculation with rhizobia strains (NFB 700, Semia 6156 and BR 3267) compared with N mineral and native rhizobia, in a sodic soil of the Brazilian semiarid region after amendment with gypsum and sulphur inoculated with *Acidithiobacillus*.

PK fertilization (Sources and rates)	N mineral fertilization 500 kg ha^{-1}	Control - N (Native rhizobia)	Rhizobia inoculation		
			BR 3267	NFB 700	SEMIA 6156
	Total N accumulation in shoot (mg $plant^{-1}$)				
BPK Biofertilizer $_{1,0}$	$194^{Ab} \pm 31.1$	$234^{Aa} \pm 31.1$	$182^{Bb} \pm 30.0$	$199^{Bb} \pm 30.0$	$228^{Aa} \pm 35.1$
BPK Biofertilizer $_{1,5}$	$209^{Ab} \pm 32.7$	$248^{Aa} \pm 33.6$	$248^{Aa} \pm 30.6$	$236^{ABa} \pm 31.5$	$242^{Aa} \pm 37.5$
BPK Biofertilizer $_{2,0}$	$187^{Ab} \pm 28.8$	$231^{Aa} \pm 30.6$	$237^{Aa} \pm 31.6$	$250^{Aa} \pm 33.9$	$236^{Aa} \pm 36.5$
PK Fertilizer $_{1,0}$	$180^{Ab} \pm 29.6$	$252^{Aa} \pm 34.3$	$195^{Bb} \pm 31.3$	$232^{ABab} \pm 30.8$	$221^{Aab} \pm 36.7$
PK Fertilizer $_{1,5}$	$204^{Ab} \pm 31.8$	$267^{Aa} \pm 36.9$	$245^{Aab} \pm 36.0$	$242^{ABab} \pm 32.5$	$204^{ABb} \pm 35.8$
PK Fertilizer $_{2,0}$	$211^{Ab} \pm 32.1$	$243^{Aa} \pm 32.7$	$233^{Aa} \pm 32.9$	$253^{Aa} \pm 32.7$	$238^{Aa} \pm 35.6$
Control (P_0K_0)	$191^{Ab} \pm 29.6$	$197^{Bb} \pm 30.6$	$191^{Bb} \pm 33.6$	$209^{Bb} \pm 30.7$	$191^{Bb} \pm 30.6$

Within topics, values followed by different letters are significantly at ($p \leq 0.05$) using the Tukey test. Upper case letters compare data in columns (PK sources) and lower case compare data in rows (mineral N and rhizobia strains). C.V. (%) = 15.09.

Table 3. Total P and total K accumulation in the shoot dry biomass as affected by PK mineral fertilizers, BPK rock biofertilizer and inoculation with rhizobia strains (NFB 700, Semia 6156 and BR 3267) compared with N mineral and native rhizobia, in a sodic soil of the Brazilian semiarid region after amendment with gypsum and sulphur inoculated with *Acidithiobacillus*.

PK fertilization (Sources and rates)	N mineral fertilization 500 kg ha^{-1}	Control-N (Native rhizobia)	Rhizobia inoculation		
			BR 3267	NFB 700	SEMIA 6156
	Total P in shoot dry biomass of cowpea (mg $plant^{-1}$)				
BPK Biofertilizer $_{1,0}$	$32.3^{ABa} \pm 5.49$	$41.1^{Aa} \pm 6.99$	$30.3^{Aa} \pm 5.15$	$31.0^{Aa} \pm 5.27$	$34.4^{Aa} \pm 5,85$
BPK Biofertilizer $_{1,5}$	$37.2^{Aa} \pm 6.33$	$44.0^{Aa} \pm 7.48$	$31.5^{Aa} \pm 5.36$	$33.5^{Aa} \pm 5.70$	$34.4^{Aa} \pm 5,85$
BPK Biofertilizer $_{2,0}$	$36.3^{Aa} \pm 6.17$	$48.2^{Aa} \pm 8.20$	$37.5^{Aa} \pm 6.38$	$37.0^{Aa} \pm 6.29$	$36.4^{Aa} \pm 6,19$
PK Fertilizer $_{1,0}$	$36.8^{Aa} \pm 6.26$	$39.5^{Aa} \pm 6.72$	$32.0^{Aa} \pm 5.44$	$38.3^{Aa} \pm 6.51$	$34.0^{Aa} \pm 5,78$
PK Fertilizer $_{1,5}$	$36.6^{Aa} \pm 6.23$	$39.8^{Aa} \pm 6.77$	$34.7^{Aa} \pm 5.90$	$37.6^{Aa} \pm 6.40$	$36.7^{Aa} \pm 6,24$
PK Fertilizer $_{2,0}$	$35.4^{Aa} \pm 6.02$	$41.0^{Aa} \pm 6.97$	$35.5^{Aa} \pm 6.04$	$35.5^{Aa} \pm 6.04$	$35.9^{Aa} \pm 6,11$
Control (P_0K_0)	$29.8^{Ba} \pm 5.07$	$31.5^{Ba} \pm 5.36$	$32.7^{Aa} \pm 5.56$	$32.5^{Aa} \pm 5.53$	$35.6^{Aa} \pm 6,06$
	Total K in shoot dry biomass of cowpea (mg $plant^{-1}$)				
BPK Biofertilizer $_{1,0}$	$173^{Ab} \pm 24.91$	$247^{Aa} \pm 35.57$	$181^{Ab} \pm 26.0$	$176^{Ab} \pm 25.3$	$207^{Aab} \pm 29.8$
BPK Biofertilizer $_{1,5}$	$176^{Ab} \pm 25.35$	$245^{Aa} \pm 35.28$	$211^{Aab} \pm 30.3$	$181^{Ab} \pm 26.0$	$243^{Aa} \pm 34.9$
BPK Biofertilizer $_{2,0}$	$180^{Ab} \pm 25.92$	$228^{Aa} \pm 32.83$	$204^{Aa} \pm 29.3$	$177^{Ab} \pm 25.4$	$216^{Aa} \pm 31.1$
PK Fertilizer $_{1,0}$	$193^{Ab} \pm 27.79$	$230^{Aa} \pm 33.12$	$171^{Ab} \pm 24.6$	$170^{Ab} \pm 24.4$	$191^{Ab} \pm 27.5$
PK Fertilizer $_{1,5}$	$211^{Aa} \pm 30.38$	$212^{Aa} \pm 30.53$	$173^{Aa} \pm 24.9$	$204^{Aa} \pm 29.3$	$197^{Aa} \pm 28.3$
PK Fertilizer $_{2,0}$	$195^{Aa} \pm 28.08$	$200^{Aa} \pm 28.80$	$181^{Aa} \pm 26.0$	$179^{Aa} \pm 25.7$	$193^{Aa} \pm 27.7$
Control (P_0K_0)	$175^{Aa} \pm 25.20$	$180^{Ba} \pm 25.92$	$171^{Aa} \pm 24.6$	$187^{Aa} \pm 26.9$	$185^{Aa} \pm 26.6$

Within topics, values followed by different letters are significantly at ($p \leq 0.05$) using the Tukey test. Upper case letters compare data in columns (PK sources) and lower case compare data in rows (mineral N and rhizobia strains). C.V. (%) = Available p = 17.01; Available K = 14.40.

The present study has provided new information on the response to biofertilizers that is produced by phosphate rock and elemental sulfur inoculated with *Acidithiobacillus*. The results of the present study demonstrate the effects of additional sulfur on phosphorus solubilization and agree with the results of previous experiments that were conducted by our research team (LIMA et al., 2007) and with the findings indicating that *Acidithiobacillus* should increase nutrient availability. The effects on the shoot biomass and the total N, P, K, Ca and Mg contents of the plants were compared with the effects after the application of commercial fertilizer. The results of this comparison are conclusive and indicate the effectiveness of biofertilizers, particularly when applied at the highest rate (2 times the recommendation) for use by low-income farmers, individuals who frequently cannot afford the higher-solubility phosphorus fertilizers (ERNANI et al., 2001).

Conclusion

The conclusions from this work are that the BPK biofertilizer produced from natural phosphate and biotite rocks plus sulfur inoculated with *Acidithiobacillus* have potential as alternative to soluble commercial fertilizers and when applied to the soil provided acidity due to the acid production by the oxidizing bacteria. Therefore, after the amendment with gypsum and sulfur inoculated with *Acidithiobacillus* the BPK biofertilizer is effective at reducing the pH of sodic soils. Moreover, the native rhizobia from the used soil were effective in the process of nitrogen fixation compared with the strains recommended for cowpea.

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