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Effect of gypsum and sulfur with *Acidithiobacillus* on soil salinity alleviation and on cowpea biomass and nutrient status as affected by PK rock biofertilizer

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ABSTRACT

The reclamation of saline soils requires the application of amendments such as gypsum and organic matter. The effects of gypsum and sulfur inoculated with *Acidithiobacillus* applied individually for the alleviation of saline soils is recognized, but the effects of mixed proportions of these products, especially on plant parameters, have not been studied. The aim of this study was to evaluate the effects of gypsum (G) and sulfur inoculated with *Acidithiobacillus* (S*) at various rates and mixing proportions, S*:G, on the alleviation of soil salinity. A control treatment that did not receive amendment was included, but the seeds did not germinate in this treatment. The effect of PK rock biofertilizer in interaction with the amendment treatments was also observed in cowpeas. The seeds were inoculated with effective *Bradyrhizobium* strains. At 45 days of growth, the cowpea shoot dry weight (DW) and the nutrient uptake in the shoots (total N, Na, P and K) were determined in the plants, and the soil was analyzed for pH, exchangeable Na, and available P and K. The results indicate that sulfur inoculated with *Acidithiobacillus* reduced soil pH, especially when applied without gypsum. The biofertilizer effectively increased the shoot biomass and nutrient uptake and increased the available P and K in the amended soil, and the best results were observed when the biofertilizer was used in interaction with S* and G in a mixing proportion equivalent to 50:50. In saline soil with high pH, PK rock biofertilizer seems to be an important factor for improving soil quality and may be an alternative to P and K fertilization.

1. Introduction

The increasing world population and demand for agricultural products are responsible for rapid changes in land patterns, including intensified use and new input techniques to maximize the productivity of field crops (Figueiredo et al., 1999; Rodrigues et al., 2013; Stamford et al., 2008; Silva et al., 2012), especially in arid and semiarid regions where animal production is limited by a widespread deficiency of protein.

The use of legume species, for example, cowpea, is of great importance because they may provide nitrogen to the system through the process of biological nitrogen fixation (BNF) and may supply protein without the addition of soluble nitrogen fertilizers (Sprent, 2009).

Soil salinity and sodicity are important factors in proposed solutions in semiarid regions because the high content of exchangeable sodium negatively affects nutrient availability and increases soil pH (Jalali et al., 2008; Qadir et al., 2005). Several studies have been performed in semiarid regions, and in general, they agree that the reduction of productive soils is due to salinity, sodicity and pH problems and that salinity and pH control are necessary to improve the productivity and quality of economic crops (Qadir et al., 2008).

Gypsum has been frequently used as a sodic soil amendment, particularly due to its low cost. However, it requires application in large amounts because its effect on the reduction of soil pH is not significant (Jalali et al., 2008). In contrast, sulfur inoculated with *Acidithiobacillus* produces sulfuric acid (El Tarabily et al., 2006) and contributes decisively to soil pH reduction and to the improvement of the poor physical conditions frequently found in alkaline soils (Qadir et al., 2008).

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Studies carried out in soils of the Brazilian semiarid region reported that sulfur inoculated with *Acidithiobacillus* can be a promising means for the reclamation of saline sodic soils because, in general, it reduced soil pH (Stamford et al., 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 amended soil; however, the effects on plant growth were not evaluated in that study.

Sulfur-oxidizing bacteria have a significant ecological impact in nature due to their role in promoting the availability of elemental sulfur that is oxidized to sulfate and also in contributing to the release of nutrients contained in the soil, especially phosphorus (El-Tarabily et al., 2006). Sulfur-oxidizing bacteria occur naturally in soils, but these species are relatively less abundant in agricultural soils. Therefore, to obtain a satisfactory effect on saline soil reclamation the sulfur-oxidizing bacteria should be introduced into the soil.

Recent studies have described the potential of rock biofertilizers produced from natural phosphate- and potassium-bearing rocks and mixed with sulfur inoculated with *Acidithiobacillus* oxidizing bacteria to increase nutrient availability due to the acidity produced by the sulfuric acid, which acts to release nutrients from the minerals contained in the rocks (Stamford et al., 2007, 2008; Berger et al., 2013). Because of this acidic reaction, PK rock biofertilizers are an important factor whose potential has not been fully studied, especially in arid and semiarid conditions with alkaline soils.

We undertook this study based on the hypothesis that the gypsum and sulfur inoculated with *Acidithiobacillus*, applied at different rates and mixed proportions, would alleviate soil salinity. The effect of PK rock biofertilizers in combination with these amendment treatments on biomass yield and nutrient uptake of cowpeas was also tested in this study.

2. Materials and methods

2.1. Production of PK rock biofertilizer

The P and K rock biofertilizers were produced at the Horticultural Experimental Station of the Federal University Rural of Pernambuco (UFRPE) in furrows (each 10.0 m long, 1.0 m wide and 0.5 m deep). For each biofertilizer, 4000 kg of natural phosphate with total P of 240 g kg^{-1}, purchased from Irece (Bahia), and potash rock (biotite) with total K of 100 g kg^{-1}, purchased from Santa Luzia (Paraiba), Brazil, were mixed with 400 kg of elemental sulfur.

The sulfur was inoculated with Acidithiobacillus thiooxidans (Stamford et al., 2007), also used to produce the PK rock biofertilizer. The bacteria were grown in 2000 ml Erlenmeyer flasks containing 1000 ml of specific culture medium (El Tarabily et al., 2006) and autoclaved for 30 min at 120 °C. The materials (phosphate and potash rocks mixed with sulfur) were incubated for 60 days, and the humidity was maintained near field capacity.

Analysis of the P and K rock biofertilizer, extracted by (A) Mehlich 1 solution and (B) citric acid according to Embrapa-Empresa Brasileira de Pesquisa Agropecuária (2009) showed: (P biofertilizer): pH 3.8, available *P* (A) = 60 (g kg^{-1}) and (B) = 48 (g kg^{-1}); (K biofertilizer–BK): pH 3.3, available *K* (A) = 10 (g kg^{-1}) and (B) 0.5 (g kg^{-1}).

2.2. Soil classification, site and analyses

An alluvial soil classified as Sodic Salic Fluvic Neosol medium textured (Embrapa-Empresa Brasileira de Pesquisa Agropecuária, 2006) was used. The soil (0–30 cm layer) was collected from a small farm area in the County of Pesqueira in the semiarid region of Pernambuco state, Northeast Brazil, at 7° 59′ 0″ south latitude, 38° 19′ 16″ west longitude and altitude of 500 m. The soil was air dried, sieved (5 cm sieve), mixed, analyzed according to the Embrapa-Empresa Brasileira de Pesquisa Agropecuária (2009) methodology and kept in pots (10 kg capacity). The soil chemical analysis showed the following results: pH (H_2O) = 10.5; electrical conductivity (C.E.) = 22.7 dS m^{-1}; exchangeable cations Na^+ = 35.5 $cmol_c$ dm^{-3}; K^+ = 8.7 $cmol_c$ dm^{-3}; Ca^{2+} = 87.9 $cmol_c$ dm^{-3}; and Mg^{2+} = 19.6 $cmol_c$ dm^{-3}. The analyses were processed using the PerkinElmer 3110 Atomic Absorption Spectrophotometer.

2.3. Rhizobia inoculation, fertilization and experimental conditions

Cowpea seeds (cv. IPA 206) were surface-sterilized in 70% ethyl alcohol for 1 min, followed by immersion in $HgCl_2$ (1:500) for 0.5 min, and washed six times with sterile water. The seeds (4 per pot) were sown at a depth of 5 mm, and when emergence was completed (5 DAE), the seedlings were thinned to one per pot. All of the plants were inoculated with mixed *Bradyrhizobium* strains (NFB 516 + NFB 700), selected for salinity conditions in a previous experiment (Stamford et al., 2004), applying 2 ml pot^{-1} of rhizobia culture containing more than 10^8 CFU ml^{-1}.

In the pots with each amendment treatment, after the incubation period (45 days) but prior to sowing, P and K biofertilizers were mixed and applied at the rate recommended for cowpeas (IPA-Instituto Agronômico de Pernambuco, 2008). During the experimental period, the soil moisture was maintained near field holding capacity by daily application of water with the following chemical attributes: E.C. = 0.2 dS m^{-1}; cations ($cmol_c$ dm^{-3}) Na^+ = 2.4, K^+ = 0.3, Ca^{+2} = 1.1, Mg^{+2} = 1.0.

The cowpea plants were harvested 45 days after planting (DAP), and the following were determined: shoot dry weight (DW), total N, total Na, total P and total K in the shoots, according to the Embrapa-Empresa Brasileira de Pesquisa Agropecuária (2009) methodology. The soil samples were analyzed for pH, total N and available P, K and exchangeable Na, following Embrapa-Empresa Brasileira de Pesquisa Agropecuária (2009).

2.4. Experimental design, plant and determinations, statistical analysis

A greenhouse experiment, set up in a factorial $(4 \times 5) + 1$, was conducted in a randomized block design with four replicates. The following amendment treatments were used: 4 rates of amendments (0.8; 1.6; 2.4; 3.2 t ha^{-1}) and 5 mixing percentage proportions S*:G (100:0; 75:25; 50:50; 25:75 and 0:100). A control treatment without PK fertilization or gypsum and sulfur was added. After the incubation period, the inoculated cowpeas were seeded and fertilized with the PK rock biofertilizer. No plant analysis was performed in the control treatment due to lack of germination.

The analysis of variance used SAS Learning Edition 4.1 (SAS Institute, 2011), and means were compared by the Tukey test ($p \leq 0.05$). For soil pH, exchangeable Na and electrical conductivity, the comparisons include the control treatment and were conducted separately for overall rate effects and for proportion effects. The remaining variables were compared according to the significant effects from the analysis of variance.

3. Results

3.1. Effects of amendments on soil attributes before PK biofertilizer application

The effects of the amendment treatments on the soil before cowpea growth (Table 1) showed significant differences ($p \leq 0.05$).

Table 1
Soil pH and exchangeable cations in a saline sodic soil of the Brazilian semiarid region amended with sulfur inoculated with *Acidithiobacillus* (S*) and gypsum (G) at different rates and mixed percentage proportions (S*:G) before PK rock biofertilizer application (-PK).

Rates proportion (S*:G)	Soil pH (H_2O)	Exchangeable Na (cmol dm^{-3})	Electrical conductivity (dS m^{-1})
Control 0:0	9.9A	23.0A	8.5A
Rate 0.8 t ha^{-1}	8.6B	15.3B	4.1B
1: 0	8.4b	15.1a	4.0b
0.75: 0.25	8.5ab	15.4a	3.2b
0.50:0.50	8.6a	15.5a	2.9b
0.25:0.75	8.7a	15.6a	5.6a
0.00:1.00	8.8a	15.1a	4.7b
Rate 1.6 t ha^{-1}	8.3C	13.4B	3.7B
1: 0	7.8c	16.0a	4.0a
0.75: 0.25	8.0bc	14.2a	4.5a
0.50:0.50	8.1bc	14.0a	2.8b
0.25:0.75	8.3ab	10.2b	4.0a
0.00:1.00	8.6a	10.6b	3.1b
Rate 2.4 t ha^{-1}	8.0C	9.9C	3.3C
1: 0	7.1c	12.6a	4.7a
0.75: 0.25	7.3c	9.4b	5.0a
0.50:0.50	8.1a	9.6b	2.0b
0.25:0.75	8.3a	9.0b	2,1b
0.00:1.00	8.4a	8.9b	2.6b
Rate 3.2 t ha^{-1}	7.4D	10.4C	3.8C
1: 0	6.6c	10.9a	4.4a
0.75: 0.25	7.1b	10.5a	4,2a
0.50:0.50	7.3b	10.4a	2.4b
0.25:0.75	7.8b	10.2a	2.2b
0.00:1.00	8.4a	10,2a	2.6b
(%) C.V.	10.9	12.9	13.7

Means followed by different letters are significantly different by the Tukey test ($p = 0.05$). Uppercase letters compare overall rate effects, and lowercase letters compare proportions (S*:G) regardless of the applied rate. In the control treatment, PK biofertilizer was not applied, and the seeds did not germinate. C.V. (Coefficient of variation).

The best result in soil pH (pH 6.9) was observed when sulfur was applied at a rate of 3.2 t ha^{-1}, in a mixing proportion (S*:G) equivalent to 100:0. The effects on exchangeable Na^+ revealed a great reduction when higher levels of gypsum (G) and sulfur inoculated with *Acidithiobacillus* bacteria (S*) were applied. When S* and G were applied in the mixing proportions (S*: G) equivalent to 25:75 and 0:100, the best values of soil exchangeable Na^+ (8.9 and 9.0 $cmol_c$ dm^{-3}) were obtained. Some results of the exchangeable Na^+ after soil amendment were found to be below 15 ($cmol_c$ dm^{-3}), and Na^+ in these concentrations do not promote injurious problems in tolerant plants.

The soil electrical conductivity (Table 1) showed that salinity below 4.0 (mS m^{-1}) was obtained with G and S* applied at higher rates, especially when the amendments were used in the same percentage proportion (50:50).

3.2. Effects of amendment on cowpea parameters after PK biofertilizer application

The results of the gypsum (G) and sulfur inoculated with *Acidithiobacillus* (S*) treatments on cowpea shoot dry weight (DW) after the application of PK biofertilizer are presented in Table 2. It was observed that the effect on cowpea growth was dependent upon the amendment treatment. In the control treatment (soil without G and S* and without biofertilizer application), the seeds did not germinate, and no results were obtained on the plant parameters. The best response of the amendment treatments on cowpea growth was obtained when the higher rates (2.4 t ha^{-1} and 3.2 t ha^{-1}) were applied and when S* and G were applied in the mixing proportion (S*:G) equivalent to 50:50. In general, the best results were observed when S*:G was applied in the percentage proportions equivalent to 25:75 and 50:50.

The effects of the amendments on total N and total Na^+ in the cowpea shoots are shown in Table 3 and on total P and total K in Table 4. An interaction effect was observed, and the best results for total N, total P and total K accumulation in shoot dry biomass were obtained when the S* and G were applied in a percentage proportion equivalent to 50:50. In reference to the total P and K uptake, a greater concentration of both nutrients was observed when S* and G were applied at higher rates.

Table 2
Cowpea dry weight (DW) in a sodic soil amended with a mixture or gypsum (G) and sulfur inoculated with *Acidithiobacillus* (S*), applied at different rates and percentage proportions (S*:G) after PK biofertilizer application.

Amendment	Rate of amendment (t ha^{-1})				
	0,8	1,6	2,4	3,2	Mean
(S*:G)	Cowpea dry weight (g $plant^{-1}$)				
100: 0	0.92bA	0.88bA	0.82dA	0,88cA	0.87D
75: 25	1.60aA	1.13bB	1.29cC	1.11bC	1.33C
50: 50	1.65aB	1.87aC	2.54aA	2.55aA	2.15A
25: 75	1.52aC	1.99aA	1.63bB	1.94aA	1.77B
0: 100	0.98bB	0.89bB	1.32cA	0.82cB	1.00D
(%) CV	8.2				

G corresponds to gypsum, and S* is sulfur inoculated with *Acidithiobacillus*. Means followed by different letters are significantly different at $p = 0.05$ by Turkey test. Capital letters compare the different rates and small letters the mixed proportions (S*:G). C.V. (Coefficient of variation).

3.3. Effects on soil attributes after cowpea harvest

The data obtained from the soil analyses (pH, exchangeable Na) of the samples collected after the cowpea harvest and fertilized with PK rock biofertilizer are presented in Tables 5 and 6. The soil pH was affected by the amendment treatments, and a reduction in the soil pH was observed when the sulfur inoculated with *Acidithiobacillus* was applied, especially at the highest rates. The effect of the sulfur inoculated with *Acidithiobacillus* was evident, and gypsum had no sensible effect on the soil pH. The lowest value of soil pH (6.9) was obtained when the amendment was used at

Table 3
Total N and total Na in shoot biomass of cowpea with PK rock biofertilizer application, grown in a sodic soil after amendment with gypsum (G) and sulfur inoculated with *Acidithiobacillus* (S*) applied at different rates and mixed proportions (S*:G).

Amendment	Rate of amendment (t ha^{-1})				
	0.8	1.6	2.4	3.2	Mean
(S*:G)	Total N uptake in cowpea shoot dry biomass (mg plant^{-1})				
100: 0	154bA	152bA	150cA	154cA	152D
75: 25	192aA	188bA	198cA	188bA	191B
50: 50	214aA	227aA	248aA	241aA	232A
25: 75	208aB	215aA	226bB	242aB	223A
0: 100	169bB	158bB	196bA	167bB	172C
(%) CV	14.5				
	Total Na uptake in cowpea shoot dry biomass (mg plant^{-1})				
100: 0	24.9bA	24.8bA	20.8cB	26.0bA	24.1C
75: 25	27.5bB	25.0bA	22.6cB	26.6bB	25.4B
50: 50	37.1aA	20.8bB	37.3aA	31.7aAB	31.7A
25: 75	29.6bA	32.6aA	29.8bA	30.4aA	30.6A
0: 100	16.7cB	24.4bA	26.4bA	18.4cB	21.4C
(%) CV	15.8				

G corresponds to gypsum, and S* is sulfur inoculated with *Acidithiobacillus*. Means followed by different letters are significantly different at $p = 0.05$ by Turkey test. Capital letters compare the different rates and small letters the mixed proportions (S*:G). C.V. (Coefficient of variation).

Table 4
Total P and total K in shoot biomass of cowpea grown with PK rock biofertilizer applied in a sodic soil after amendment with gypsum (G) and sulfur inoculated with *Acidithiobacillus* (S*) at different rates and mixed proportions (S*:G).

Amendment	Rate of amendment (t ha^{-1})				
	0.8	1.6	2.,4	3.2	Mean
(S*:G)	Total P uptake in cowpea shoot dry biomass (mg plant^{-1}				
100: 0	6.6bB	6.5bB	5.0cB	11.1aA	7.3C
75: 25	11.2aA	12.0aA	9.5bB	9.3aB	10.5B
50: 50	12.0aB	13.8aB	18.9aA	11.3aB	14.0A
25: 75	11.4aB	14.7aA	11.8bAB	14.3aA	13.0A
0: 100	7.1bA	7.0bA	9.4bA	8.7bA	8.1C
% (CV)		13.4			
	Total K uptake in cowpea shoot dry biomass (mg plant^{-1})				
100: 0	30.1bA	32.4bA	16.8dB	24.6cAB	26.0
75: 25	58.6aA	38.7bB	37.7cB	41.9bB	44.2
50: 50	48.9aC	24.8cD	84.6aA	52.2bB	52.7
25: 75	40.0abC	58.1aB	54.0bB	72.6aA	56.2
0: 100	37.1bAB	29.2bB	43.0cA	28.3cB	34.4
% (CV)		12.6			

G corresponds to gypsum, and S* is sulfur inoculated with *Acidithiobacillus*. Means followed by different letters are significantly different at $p = 0.05$ by Turkey test. Capital letters compare the different rates and small letters the mixed proportions (S*: G). C.V. (Coefficient of variation).

the highest rate (3.2 kg ha^{-1}) and in a mixing proportion (S*:G) equivalent to 100:0.

After application of the PK biofertilizer, the available P and K in the soil increased when the amendment treatments were applied at the higher rates (Table 6), especially when sulfur (S*) and gypsum (G) were applied at the higher rates and in the percentage proportion 50:50. The positive effect of the PK biofertilizer on the available P and K is evident from these results, probably because in the treatments with higher amounts of sulfur inoculated with *Acidithiobacillus,* the sulfuric acid produced by the oxidizing bacteria increases the release of the nutrients contained in the rocks and promotes an increase in available P and K in the soil, which is derived from the P and K rocks used to produce the PK biofertilizer.

The effect of the amendment on the reduction of exchangeable N^{+} was significant, probably due to the effect of the sulfuric acid produced by the sulfur-oxidizing bacteria *Acidithiobacillus*. This effect was significant when the PK rock biofertilizers were applied.

Table 5
Soil pH and exchangeable Na in a sodic soil amended with sulfur inoculated with *Acidithiobacillus* (S*) and gypsum (G) applied at different rates and mixed proportions after cowpea growth with PK rock biofertilizer.

S*: G	Rate of amendment applied (t ha^{-1})				
	0.8	1.6	2.4	3.2	Mean
	Soil pH (water 1.0:2.5)				
100: 0	5.4c	5.0c	5.0b	5.0b	5.0D
75: 25	5.2b	5.5b	5.5b	5.1b	5.3C
50: 50	5.3b	5.7b	5.9ab	4.9c	5.4C
25: 75	5.6b	6.3a	6.4a	5.8b	6.0B
0: 100	5.9a	6.9a	6.6a	7.1a	6.6A
Control (0:0)	9.9				
(%) CV	3.9				
	Exchangeable Na in soil (mmol$_c$ dm^{-3})				
100: 0	79a	75a	68a	50a	68A
75: 25	68c	61b	46b	41b	54B
50: 50	64c	55c	38c	29c	46C
25: 75	63c	47d	34c	28c	43C
0: 100	66c	58c	46b	31b	45C
Mean	68A	59B	46C	38D	
Control (0:0)	230				
(%) CV	12.9				

G corresponds to gypsum, and S* is sulfur inoculated with *Acidithiobacillus*. Means followed by different letters are significantly different at $p = 0.05$ by Turkey test. Capital letters compare the different rates and small letters the mixed proportions (S*:G). C.V. (Coefficient of variation).

Table 6
Available P and K in a sodic soil amended with sulfur inoculated with *Acidithiobacillus* (S*) and gypsum (G) applied at different rates and mixed proportions (S*:G) after cowpea growth with PK rock biofertilizer.

Amendment	Rate of amendment (t ha^{-1})				
	0.8	1.6	2.4	3.2	Mean
(S*:G)	Available P in soil (mg dm^{-3})				
100: 0	71aB	73aB	79aA	82aA	76A
75: 25	79aA	77aA	70aB	76bA	76A
50: 50	72bC	76aB	76aB	85aA	77A
25: 75	65aB	65bB	72aC	82aA	71A
0: 100	52bC	72aB	72aB	82aA	69A
(%) CV	15.3				
	Available K in soil (mg dm^{-3})				
100: 0	214aA	234aA	221aA	221aA	222A
75: 25	234aA	208aA	227aA	214aA	221A
50: 50	195abA	221aA	199bB	208aA	205A
25: 75	182bA	182bA	195bB	201aA	190A
0: 100	155cB	152bA	149bB	158bA	153B
(%) CV	11.0				

G corresponds to gypsum, and S* is sulfur inoculated with *Acidithiobacillus*. Means followed by different letters are significantly different at $p = 0.05$ by Turkey test. Capital letters compare the different rates and small letters the mixed proportions (S*:G). C.V (Coefficient of variation).

4. Discussion

4.1. Effects of amendments on soil attributes before PK biofertilizer application

The soil pH was affected by the amendment application, and the reduction was more evident when sulfur inoculated with *Acidithiobacillus* was applied, especially at the highest rates. Stamford et al. (2007) observed similar results in experiments to compare the application of gypsum and the application of sulfur inoculated with *Acidithiobacillus* at different rates in Brazilian sodic soils from the semi-arid region. The authors showed the evident effect of the sulfur inoculated with *Acidithiobacillus* on the decrease of soil pH, and gypsum produced little effect on this soil characteristic, as described by Qadir et al. (2008).

The soil chemical results after application of the PK biofertilizer and after the cowpea harvest showed that the soil pH was drastically reduced by the PK biofertilizer application, and a pH lower than 5.0 was observed, probably due to the effect of the biofertilizer pH (3.0–3.5) promoted by the oxidizing bacteria *Acidithiobacillus thiooxidans,* which reacts in soil to produce sulfuric acid. The acidifying effect of biofertilizer produced from phosphate rock plus sulfur inoculated with *Acidithiobacillus* was reported by Stamford et al. (2004, 2008) in studies carried out in the acid soils of the low coastal plateau of Northeast Brazil (Pernambuco state).

The reduction in exchangeable Na^+ in the soil complex in the presence of gypsum supports the findings of Holanda et al. (2000), who described significant reduction in exchangeable Na^+ after the application of gypsum.

The use of sulfur inoculated with oxidizing bacteria is a very important subject of research regarding the effect of the application of this product on the availability of nutrients contained in the minerals, especially in the production of P and K biofertilizer from rocks. El Tarabily et al. (2006) reported the effect of different oxidizing bacteria on the availability of nutrients in calcareous soils. These results are in accordance with Stamford et al. (2007), using different rates and mixed percentage proportions of sulfur inoculated with *Acidithiobacillus* and gypsum to affect the soil attributes.

The results suggested that more studies are necessary to evaluate the effect of such amendments on the alleviation of saline soils, especially due to the effect on soil pH, because reduction of pH to approximately 6.0–6.5 may improve plant growth, especially in tropical crops.

4.2. Effects of amendment on cowpea plants after PK biofertilizer application

In the specific literature, there are no studies that explain the effect of rates and mixed proportions of gypsum and sulfur in relation to plant growth and nutrient uptake. The effect on shoot dry weight (DW) was probably due to the increased availability of P and K contained in the PK biofertilizer and originating from the P and K minerals contained in the natural phosphate and potash rock (biotite) submitted to inoculation with *Acidithiobacillus*. Similar results were observed by Stamford et al. (2008) when applying P and K rock biofertilizers on cowpeas grown in soil of the coastal tableland of Pernambuco State. In this study, the authors described greater increase in available K in the soil when K rock biofertilizer plus elemental sulfur with *Acidithiobacillus* were applied in organic matter (earthworm compound) with a neutral reaction (pH 7.9) that neutralized the soil acidity.

The results obtained in the present study may be explained by the fact that other native bacteria in the soil besides *Acidithiobacillus* improve P solubility and can release nutrients for plant growth, as reported by El Tarabily et al. (2006) in African soils with high pH. Stamford et al. (2004) found a significant effect of PK rock biofertilizer on P and K uptake by cowpea plants, and the best results were obtained when the higher rates of PK rock biofertilizers plus elemental sulfur inoculated with *Acidithiobacillus* were applied.

Stamford et al. (2008) and Berger et al. (2013) described significant results due to the application of PK rock biofertilizers plus earthworm compound on cowpeas inoculated with effective rhizobia, grown in a coastal soil from the Brazilian rain forest region, characterized by very low soil pH and low content of available P and K. The authors described that the observed positive effects are found due to the release of P and K from the rocks by the action of *Acidithiobacillus* that produces sulfuric acid, and the acidity of the biofertilizer was controlled by the addition of the earthworm compound with high pH (7.9).

Stamford et al. (2011) obtained similar response from applying PK rock biofertilizers plus earthworm compound to grapes (*Vitis vinifera)* in soil of the semiarid region with pH of 6.5. The authors concluded that the results represent the effect of the *Acidithiobacillus* bacteria, which promote solubilization of the minerals contained in the P and K rocks used to produce the PK biofertilizer, and the incorporation of earthworm compound is very important to control the acidity.

5. Conclusions

The application of gypsum (G) and sulfur with *Acidithiobacillus* (S^*) are not effective in the reclamation of sodic soils. Sulfur inoculated with *Acidithiobacillus* (S^*) is more efficient in alleviation of the soil pH and improves nutrient availability in the soil after application of PK rock biofertilizers.

The combined effects of gypsum (G) and sulfur with *Acidithiobacillus* (S^*) were observed, and the best results on cowpea growth and nutrient uptake were obtained when a mixture of S^* and G in the percentage proportion 50:50 was applied, especially at a rate of 2.4 t ha^{-1}.

The use of sulfur inoculated with oxidizing bacteria is very important to release the nutrients contained in the minerals and for the production of PK biofertilizer from phosphate and potash rocks.

The results suggested that further studies are necessary to explain the effect of these amendments in different soils, especially the effect on soil pH, because reduction of pH to approximately 6.0–6.5 in soils with high pH may improve plant growth, especially in tropical crops.

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