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Biofertilizers and performance of *Paenibacillus* in the absorption of macronutrients by cowpea bean and soil fertility

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Key words:
PRPG
rock powder
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potassium

ABSTRACT

The study was conducted in a greenhouse at the Agricultural Research Corporation of Rio Grande do Norte (EMPARN), in order to evaluate the effect of biofertilizers and potassium phosphate rocks, triple superphosphate (TSP) and potassium chloride (KCl), crushed rocks on the chemical properties of the soil and performance of the bacteria *Paenibacillus polymyxa* in the absorption of macronutrients by the cowpea bean crop. An Ultisol (10 kg per pot) was used with the addition of biofertilizers at levels 40, 70, 100 and 200% of recommendation for TSP and KCl, inoculated or not with bacteria. There was an effect of fertilization on the absorption of macronutrients, being the best results for P and K with TSP + KCL, and N, Ca and Mg for the biofertilizers. In the chemical properties of the soil, the pH was lower in biofertilizers of higher levels, but it was better especially with its addition to P (BPK200). For Ca it was better with TSP + KCl, and Mg with pure rock. The *P. polymyxa* did not influence in absorption of elements by the plant. The biofertilizers and rocks could satisfy the nutrient needs of the plants by making them potential for sustainable agriculture.

Palavras-chave:
RPCP
pó de rocha
fósforo
potássio

Biofertilizantes e performance do *Paenibacillus* na absorção de macronutrientes pelo feijão caupi e fertilidade do solo

RESUMO

O trabalho foi conduzido em casa de vegetação, na Empresa de Pesquisa Agropecuária do Rio Grande do Norte (EMPARN) para avaliar o efeito de biofertilizantes de rochas potássica e fosfatadas, superfosfato triplo (SFT) e cloreto de potássio (KCl), rochas moídas nas propriedades químicas do solo e no desempenho da bactéria *Paenibacillus polymyxa* na absorção de macronutrientes na cultura do feijão caupi. Utilizaram-se 10 kg de um Argissolo Amarelo Distrófico por vaso com adição dos biofertilizantes nos níveis 40, 70, 100 e 200% da recomendação para SFT e KCl, inoculados ou não com a bactéria. Houve efeito da fertilização na absorção dos macronutrientes sendo os melhores resultados para P e K com SFT+KCL, e N, Ca e Mg para os biofertilizantes. Nas propriedades químicas do solo o pH foi mais reduzido nos biofertilizantes de maiores níveis porém melhorando com sua adição especialmente para P (BPK200). Para Ca foi melhor com o SFT+KCl, e para Mg rocha pura. O *P. polymyxa* não influenciou na absorção dos elementos na planta. Os biofertilizantes e as rochas supriram a necessidade de nutrientes das plantas revelando-se como potencial para uma agricultura sustentável.

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Introduction

The natural fertility of most of Brazilian soils reduces crop production, making it necessary to use fertilizers to correct the nutrient deficiencies and increase crop yields. Such practice in agriculture made the country highly dependent on fertilizers either by the need for external supply of raw material, or by high quantity used and high cost, creating problems in production and economy of the country (Cola & Simão, 2012).

In Brazil, sales of fertilizers to the end customer closed the first half of 2012 with 11.727 million tons, showing an increase of 5.6% compared to the same period of 2011. For the same period, there was a national production of 4.489 million tons and 7.833 million tons of imports, compared to 208,000 t of exports of fertilizers and NPK formulations, and only 5.612 million tons of stocks of intermediate products (ANDA, 2012). The deficit conditions of the chemical fertilizers seriously commit the competitiveness of agribusiness, to the extent that Brazil has this strong external dependence on agrominerals inputs.

Among the most promising alternatives for fertilizer, stone powder is noted, which provides the use of crushed rocks rich in micronutrients and macronutrients as restorative and renewing sources of the soil, can help reduce highly soluble chemical products such as fertilizers in the NPK form (Beneduzi et al., 2013).

The use of crushed rocks in agriculture also plays the role of fertilizers restorative of nutrients for crops, among them the cowpea bean (*Vigna unguiculata* (L.) Walp.), one of the most widely consumed legume in the world and responsible for employment generation and income in rural and therefore a very important economic partner. The cowpea bean is a major source of vegetable protein sources of low cost, especially for low-income populations (Vieira et al., 2010; Lima Filho et al., 2013). In 2011 in Brazil were collected around 1.6 million hectares, with production of 822 thousand tons, an average of 525 kg ha^{-1}, where most production is concentrated in the Northeast, with 84% of the acreage and 68% of national production. The culture of cowpea bean holds each year 1.2 million direct jobs (EMBRAPA, 2013).

One of the most promising alternative mechanisms for increasing crop yields is the use of biotechnology, such as association with microorganisms that exert important functions for nutrition and survival of the host by different mechanisms, such as increased absorption of nutrients in solution soil, as found by (Silva et al., 2006). Another important type of biotechnology for agriculture and that has gained the attention of researchers is the isolation and use of soil microbes with greater ability to solubilize rock phosphate, particularly for the possibility of interaction with N_2-fixing microorganisms (Sridevi & Mallaiah, 2009).

A new model of agriculture is necessary in current agricultural scenario that reconciles physical processes, chemicals and biological that are basic functioning of soils that sustain agricultural production (Vieira, et al. 2010; Silva et al. 2011; Cola & Simão, 2012). The use of biological processes for improvement of agricultural productivity is likely to be one of the most important tools for agriculture in the current world, mainly due to emerging demand for reducing dependence on chemical fertilizers and the need for sustainable agriculture development (Dastager et al., 2011; Pereira et al., 2013).

The objective of this study was to evaluate the effect of fertilization with biofertilizer, chemical fertilizer and crushed rocks on plant and soil chemical properties, as well as the influence of inoculation with the bacterium *P. polymyxa* in the absorption of nutrients in the cultivation of cowpea bean.

Material and Methods

The experiment was conducted in a greenhouse at the Experimental Station Dr. Rommel Mosque Faria, Agricultural Research Company in Rio Grande do Norte (EMPARN), located in the municipality of Parnamirim, State of Rio Grande do Norte, Brazil, geographic coordinates 5° 55' 45 "S and 35° 11' 21" W.

The cowpea bean (*Vigna unguiculata* [L.] Walp.) was used as test plant, the experiment being conducted following a randomized complete block design, in factorial 7 x 2, corresponding to 7 fertilization treatments and 2 inoculations with *P. polymyxa*, with four replications. The fertilization treatments were: FPK100 (fertilization fertilizer superphosphate + potassium chloride dose corresponding to 100% of the recommended amount according to the chemical analysis of the soil) for cowpea bean; RPK100 (fertilization with rock phosphate + potash dose corresponding to 100% of the recommended amount for superphosphate and potassium chloride, respectively); BPK200 (fertilization with biofertilizers with phosphate + potassium dose corresponding to 200% of the recommended amounts for superphosphate and potassium chloride, respectively); BPK100 (fertilization with biofertilizers with phosphate + potash dose corresponding to 100% of the recommended amounts for superphosphate and potassium chloride, respectively); BPK70 (fertilization with biofertilizers with phosphate + potassium dose corresponding to 70% of the recommended amounts for superphosphate and potassium chloride respectively); BPK40 (fertilization with biofertilizers with phosphate + potassium dose corresponding to 40% of the recommended amounts for superphosphate and potassium chloride, respectively), T (absolute control without application of chemical fertilizers, biofertilizers and crushed rocks). The two inoculations were with *P. polymyxa* and without *P. polymyxa*.

It was used an Argissolo Amarelo Distrófico sandy/medium texture (EMBRAPA, 1999) or Ultisol in the FAO soil classification, collected in Agricultural Research Corporation of Rio Grande do Norte (EMPARN). Soil samples were collected in the topsoil (0-0.20 cm), with the following chemical characteristics: pH in water = 5.8; P = 2 mg dm^{-3}; K = 0.08; Na = 0, 03; Ca = 0.71; Mg = 0.39; Al = 0.0 and H = 1.73 cmol$_c$ dm^{-3}; soil density = 1.36 kg dm^{-3}; sand = 933; silt = 27; clay = 40 g kg^{-1}.

The strains used in this study were obtained from different collections and are listed in Table 1.

Table 1. Genres of bacteria used in bioassay with their origins

Types of bacterias	Access code	Origin
Paenibacillus polymyxa	421	Laboratory of Bacterial Physiology, Instituto Oswaldo Cruz - Fiocruz, Dr. Leon Rabinovitch.
Bradyrhizobium japonicum.	3267	Fungal culture collection of the Instituto Oswaldo Cruz - Fiocruz, Dr. Maria Inez de Moura Sarquiz

The strain of the genus *P. polymyxa* was grown using TSB (Tryptic soya broth) to 33º C/150 rpm and stored on TSB agar (Silva et al., 2006) and strain of the genus *B.japonicum* in YMA (mannitol, yeast extract and agar) with congo red as indicator (Vincent, 1970) at 28 ºC and 150 rpm.

The various cultivation of cowpea bean (*Vigna unguiculata* [L.] Walp.) was BRS Potiguar of EMPARN, whose seeds were previously sterilized in 70% alcohol for 1.5 min, and soon after, in hypochlorite solution 1% sodium per 2.5 min. The seeds were then washed successively in distilled water.

For the assay, 10 kg of soil per pot was used and six seeds were sown at a depth of 3.5 cm where the inoculation was made by placing 2 mL of the liquid culture of *B.japonicum* per seed as a source of organic nitrogen and 1 mL of culture *P. polymyxa* per seed. After eight days of planting was done thinned two plants per pot. The soil was maintained with a water content of 80% of field capacity.

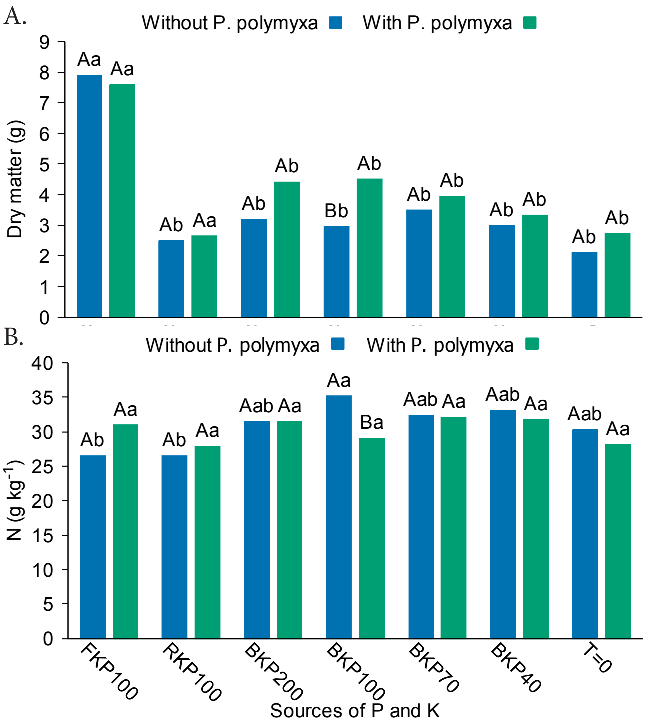
Plants were harvested 45 days after planting, where the following parameters were evaluated: dry matter (ADM); contents of macronutrients (N, P, K, Ca, Mg) in ADM and contents of the elements P, K, Ca, Mg in soil according to EMBRAPA (1999).

The results were statistically analyzed using analysis of variance and comparison of means was performed using the Tukey test at 0.05 probability by using the software Assistat7.6.

RESULTS AND DISCUSSION

Figures 1A and 1B show the results obtained in relation to the production of shoot dry matter of cowpea bean and macronutrient content of nitrogen (N) contained within a function of P and K fertilization and inoculation with the bacteria of the genus *P. polymyxa*. With respect to dry matter (Figure 1A), it can be seen that treatment with chemical fertilizer (FKP100) showed significantly higher values ($p < 0.05$) compared to other treatments. This trend occurred in soils inoculated and not inoculated with *P. polymyxa*.

There was no significant difference in dry matter values between the control treatment and treatments with application of biofertilizers and crushed rock, although checked gains up to 114% in dry biomass (BKP100 = 4.50 g pot^{-1}) in relation to treatment control (T = 2.10 g pot^{-1}), when inoculated bacteria *P. polymyxa*, and increases up to 52% in dry biomass (BKP200 = 3.20 g pot^{-1}) when the absence of *P. polymyxa*. These increments in dry weight may be associated with direct mechanisms for the production of growth hormones (gibberellins, auxins), increased availability of iron and/or indirect effects as biocontrol, antibiotic production and competition for nutrients, as reported by Lal & Tabacchioni (2009). Moura et al. (2007) working with fertilization of melon found no difference ($p < 0.05$) among treatments, but there was a better result for the biofertilizer at the highest level (BP200) and the mixture of rocks (RP + RK) in double the recommended dose for chemical fertilizer plus sulfur. The same was reported by Stamford et al., (2004) working with cowpea bean inoculated with *Rhizobium*. Only treatment with *P. polymyxa* BKP100 inoculation caused a significant increase of shoot dry matter of cowpea bean (52%) compared to uninoculated soil. Rice plants inoculated with *Pseudomonas* and *Serratia* strains promoted plant growth and uptake of phosphate (Nico et al., 2012). It was also reported with bean by Jain et al. (2010).

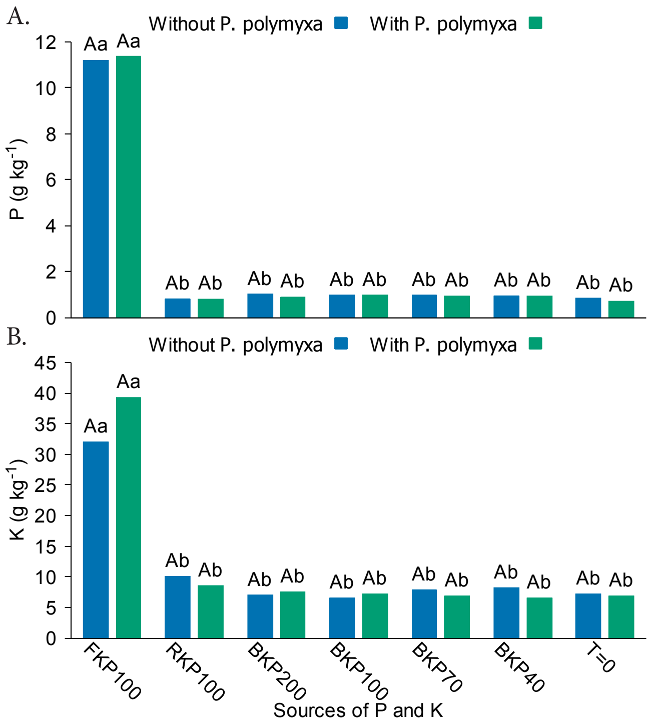


Capital letters compare inoculation treatments within the same fertilization treatment. Lowercase letters compare fertilization treatments within the same inoculation treatment. Means with the same letter do not differ statistically from each other at 0.05 probability by Tukey test

Figure 1. (A) Dry matter production of shoots of cowpea bean in relation to fertilization; (B) N content in shoots of cowpea bean in the studied treatments

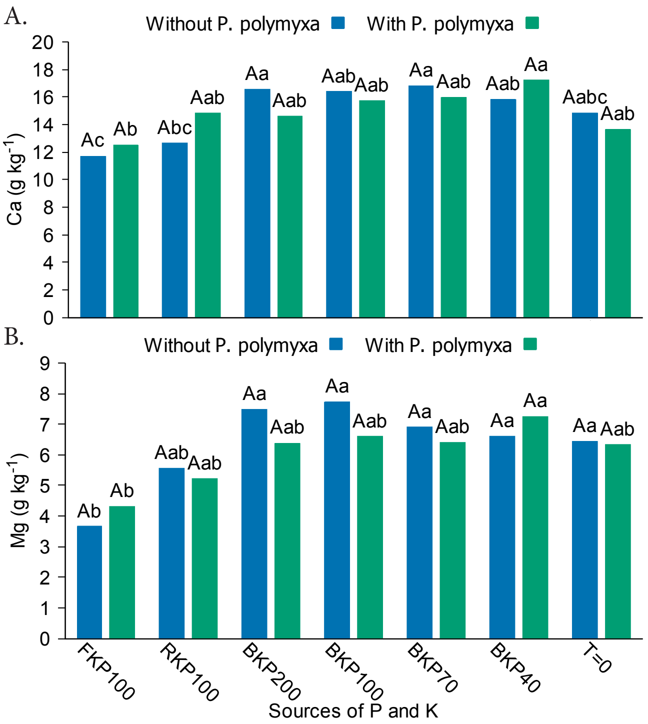
The N content in dry matter of cowpea bean (Figure 1B) was significantly higher in the treatment BKP100, compared to treatments RKP100 and FPK100, soil not inoculated with *P. polymyxa*. According to data cited by Freire Filho et al. (2005), the N content in shoot dry matter of cowpea bean are considered suitable for the proper development of this crop in all treatments in this study. Nitrogen promotes vigorous growth and it is essential for the production of amino acids, proteins and growth hormones.

Regarding phosphorus and potassium, Figures 2A and 2B, respectively, in the dry matter of cowpea bean shows that treatment with chemical fertilizer (FKP100) showed significantly higher values compared to the other treatments



Capital letters compare inoculation treatments within the same fertilization treatment. Lowercase letters compare fertilization treatments within the same inoculation treatment. Means with the same letter do not differ statistically from each other at 0.05 probability by Tukey test

Figure 2. Concentrations of P (A) and K (B) in shoots of cowpea bean in the studied treatments



Capital letters compare inoculation treatments within the same fertilization treatment. Lowercase letters compare fertilization treatments within the same inoculation treatment. Means with the same letter do not differ statistically from each other at 0.05 probability by Tukey test

Figure 3. Ca (A) and Mg (B) in the shoots of cowpea bean in the studied treatments

in soils inoculated and not inoculated with *P. polymyxa*. This result may be related to the fact meet these nutrients in a form readily assimilable soluble chemical fertilizers to the plants, encouraging more rapid absorption of these elements by the beans. Only FKP100 treatment showed levels of P and K considered suitable for the cultivation of cowpea bean (Freire Filho et al., 2005).

With regard to other macronutrients, Ca and Mg, Figures 3A and 3B, respectively, in general, treatments with biofertilizers showed significantly higher values compared to chemical fertilizer (FPK100) in soil not inoculated with *P. polymyxa*. In presence of bacteria in the soil, the Ca and Mg were higher in the treatment BPK40, also in relation to treatment with chemical fertilizer (FPK100). However, the values found for Ca are considered deficient for the proper development of this culture. The levels of Mg are considered suitable for the growth of cowpea bean, except for the treatment FPK100 (Freire Filho et al., 2005).

Overall, there was no significant effect ($p > 0.05$) inoculation of *P. polymyxa* absorption of macronutrients in shoot dry matter of cowpea bean in their respective treatment. However, Silva et al. (2006) found the efficiency of co-infection with bacteria of the genera *Paenibacillus* and *Bradyrhizobium* in the absorption of calcium, iron and phosphorus in cowpea bean under different methods of inoculation soluble fertilizer, on autoclaved. Minaxi et al. (2012) also noted that the *Bacillus* sp. positively influenced the growth and nutrient uptake of cowpea plants.

In this paper, possibly the harvest period of cowpea bean (45 days) may have relevance in the interference of a better performance of the bacterium *P. polymyxa* possible mechanisms in use (Lal & Tabacchioni, 2009) for absorption of minerals timely harvest of cowpea bean. This implies that the improvement of this process also requires an evaluation of the adequacy of the best time of hatching biofertilizer added to the soil and the incubation conditions. Since the availability of nutrients may vary with the conditions of the environment as highly competitive microbial activity in the rhizosphere, soil type, species and age of the plant (Odunfa & Oso, 1978), temperature, pH and rock type, since according to Nahas & Assis (1992), in their study, found that phosphate rocks from different backgrounds influence on the ability of solubilizing fungus *Aspergillus niger*.

With respect to soil pH (Table 2), the values obtained by the treatments showed significant reduction in the pH of the control (6.2) to 4.1, 4.0 and 3.9 at the higher doses of biofertilizer BPK70, BPK100 and BPK 200, respectively, in soil not inoculated with *P. polymyxa*. The pH fertilized with crushed rock showed the significantly higher value (6.7), compared to other treatments. The same trend occurred in soil inoculated with *P. polymyxa*, where soils fertilized with BPK70, BPK100 and BPK200 showed pH values significantly lower compared to the other treatments. Stamford et al. (2006) also found a reduction of pH of the soil under cowpea bean biofertilizer rock when applied in higher doses, emphasizing the effect of *Acidithiobacillus* and sulfur used in the production of biofertilizer, which results in the generation of sulfuric acid and subsequent reduction of the soil pH.

There was no significant difference in all fertilization treatments studied in the pH of the soil inoculated with *P. polymyxa* compared to uninoculated, except the control.

Table 2. Effect of treatments on soil pH, at 75 days after planting

Inoculation	Fertilization						
	FPK100	RPK100	BPK 200	BPK 100	BPK 70	BPK 40	T=0
Without *P. polymyxa*	5,4 Ca	6,7 Aa	3,9 Ea	4,0 Ea	4,1 Ea	4,6 Da	6,2 Bb
With *P. polymyxa*	5,3 Ba	6,5 Aa	3,8 Da	4,0 Da	4,2 CDa	4,5 Ca	6,5 Aa

Lowercase letters compare inoculation treatments within the same fertilization treatment; Capital letters compares fertilization treatments within the same inoculation treatment. Means followed by the same letter do not differ statistically from each other at 0.05 probability by Tukey test

The pH variation in the treatments, however, did not affect the uptake of macronutrients in the dry biomass (Figures 1B to 3B), taking into account the values of T=0 (pH 6.2) and RPK100 (pH 6.7) that would be more available in this range (Malavolta et al., 1997). This result did not affect the development of cowpea bean, as reported by the biomass (Figure 1A). The development of plants which result from the action of plant growth promoting rhizobacteria is measured, among other variables, by the biomass (Lucy et al., 2004). Similar results were found by Stamford et al. (2004) which showed that cowpea bean was tolerant to low soil pH, by the action of *Acidithiobacillus* present in rock biofertilizers produced without reducing crop yield.

In the present study, the lack of negative response of cowpea bean to low soil pH possibly occurred due to a combination of factors such as the increase of available P in the soil due to the application of fertilizers and biofertilizers and adaptation of cowpea bean to moderately acidic soils (Stamford et al., 2006).

Regarding the available nutrients in the soil, significant differences between treatments was observed. As data presented in Table 3, the P content, in the control was 12.3 $cmol_c$ dm^{-3} into the soil without *P. polymyxa*, reached values very expressive in all treatments, especially BPK200 showing values significantly higher (868.8 $cmol_c$ dm^{-3}; 605% above the control group), compared to other treatments. With the exception of soil fertilized with BPK40, all other fertilized treatments showed values statistically superior to control soil (T = O). This trend occurred in soils inoculated and not inoculated with *P. polymyxa*. The values of available P in soil fertilized treatments are regarded as high in terms of soil fertility (IPA, 2008).

The exchangeable K increased significantly ($p < 0.05$) in soil fertilized with chemical fertilizers compared to other treatments. However, there have also improved for biofertilizer at its highest level (BPK200) and the rocks (RPK100) compared to non-fertilized soil (T = 0). According to IPA (2008), K contents less than 0.12 and greater than 0.23 $cmol_c$ dm^{-3} soil are considered low and high, respectively, for the cultivation of cowpea bean. According to Melo et al. (2005), the amount of potassium considered critical for the normal development of the cowpea bean is between 20 and 40 kg ha^{-1} and cowpea bean rarely responds to potassium fertilization. However, Oliveira et al. (2009) observed in cowpea bean yields with values above 150 kg ha^{-1} in the soil. In this study 60 kg ha^{-1} was added, according to fertilizer recommendation. Therefore, the low values of K in the soil is probably due to the lowering of the pH of the soil, limiting the availability of the element.

The values of Ca (Table 4) were significantly higher in exchangeable treatments FPK100 (6.55 $cmol_c$ dm^{-3}) and RPK100 (1.60 $cmol_c$ dm^{-3}) were not inoculated with *P. polymyxa*, compared to the other treatments, with the level of Ca in the soil considered high in the treatment FPK100 and medium in the treatment RPK100 (IPA, 2008). The same trend occurred in soil inoculated with *P. polymyxa*, with values statistically higher in Ca treatments FPK100 (5.40 $cmol_c$ dm^{-3}) and RPK100 (1.67 $cmol_c$ dm^{-3}), in relation to others.

Regarding the levels of Mg in soil (Table 4), treatments RPK100 and T = 0 showed significantly superior to the other studied treatments, soil inoculated and non-inoculated with *P. polymyxa*. The values of these Mg soil treatments, however,

Table 3. Effect of treatments on P concentration (mg dm^{-3} pot^{-1}) and K ($cmol_c$ dm^{-3} pot^{-1}) in soil at 75 days after planting

Inoculation	Fertilization						
	FPK100	RPK100	BPK 200	BPK 100	BPK 70	BPK 40	T=0
				P			
Without *P. polymyxa*	618 Ba	388 Ca	868,8 Aa	466,5 BCa	286,5 Da	160,8 DEa	12,3 Ea
With *P. polymyxa*	523,5 Ba	460,3 Ba	803,3 Aa	398,8 Ba	380,3 Ba	179 Ca	18,8 Ca
				K			
Without *P. polymyxa*	0,30 Aa	0,08 Ba	0,06 Ba	0,06 Ba	0,06 Ba	0,05 Ba	0,06 Ba
With *P. polymyxa*	0,28 Aa	0,08 Ba	0,07 Ba	0,06 Ba	0,05 Ba	0,05 Ba	0,05 Ba

Lowercase letters compare inoculation treatments within the same fertilization treatment; Capital letters compare fertilization treatments within the same inoculation treatment. Means followed by the same letter do not differ statistically from each other at 0.05 probability by Tukey test

Table 4. Effect of treatments on the content of Ca and Mg ($cmol_c$ dm^{-3} pot^{-1}) in soil at 75 days after planting

Inoculation	Fertilization						
	FPK100	RPK100	BPK 200	BPK 100	BPK 70	BPK 40	T=0
				Ca			
Without *P. polymyxa*	6,55 Aa	1,60 Ba	0,99 CDa	0,74 Da	0,76 Da	0,85 CDa	1,31 BCa
With *P. polymyxa*	5,40 Ab	1,67 Ba	1,01 CDa	0,78 Da	0,76 Da	0,91 CDa	1,35 BCa
				Mg			
Without *P. polymyxa*	0,23 Ba	0,87 Aa	0,20 Ba	0,17 Ba	0,17 Ba	0,25 Ba	0,85 Aa
With *P. polymyxa*	0,21 Ba	0,88 Aa	0,20 Ba	0,15 Ba	0,16 Ba	0,24 Ba	0,84 Aa

Lowercase letters compare inoculation treatments within the same fertilization treatment; Capital letters compare fertilization treatments within the same inoculation treatment. Means followed by the same letter do not differ statistically from each other at 0.05 probability by Tukey test are considered mean level to effect soil fertility in plants (IPA, 2008). Although cowpea bean is a culture quite efficient in soils of low fertility, this culture needs Ca and Mg in pH close to neutral, what happens to the treatments (T = 0, RPK and FPK) with higher pH .

With respect to the inoculation of bacteria *P. polymyxa* there was no significant difference between the values of macronutrients soil inoculated or not. In some cases, the amounts of nutrients in the soil not inoculated were higher than in soil inoculated with *P. polymyxa*, probably due to the need for immobilization of nutrients to the bacterial metabolism. Microorganisms are responsible for immobilization processes of nutrient for microbial biomass, which can reach values equivalent to 100 kg of N, 80 kg of P, 70 kg of K and 11 kg of Ca per hectare. As the biomass of microorganisms is recycled 10 times faster than the fraction of dead organic soil, is that the amount of nutrients present in the cells of the microorganisms is very significant before nutrient cycling around the ecosystem (Andreola & Fernandes, 2007).

Conclusions

1. The study showed that the studied rock and the biofertilizers can adequately meet the need of nutrients that plants need for their development.

2. The inoculation with bacteria *P. polymyxa*, in general, did not significantly influence the values of macronutrients in shoot dry matter of cowpea bean and soil as well as soil pH, fertilization in relation to the study.

3. There were improvements in soil chemical properties with the addition of pure rock, and biofertilizers especially for P at the highest level compared to soluble fertilizer and control.

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