

Biofertilizer produced by interactive microbial processes affects melon yield and nutrients availability in a Brazilian semiarid soil

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Abstract

Plant yield is improved by application of mineral fertilizers (NPKF) and organic materials, in general, are not effective to provide nutrients. Recent researches agree that biological fertilizers are important for a modern and sustainable agriculture. Rock biofertilizer (BPK) was produced from natural phosphate and biotite, mixed with sulfur inoculated with *Acidithiobacillus*. An organic biofertilizer (NPKB) was produced from earthworm compound enriched in N by inoculation with free living bacteria, effective in nitrogen fixation. The NPKB was inoculated with *Cunninghamella elegans* which contain chitosan in their cellular walls to produce the bioprotector (NPKP). An experiment was conducted in field conditions to compare NPKB and NPKP with mineral fertilizer (NPKF) on melon yield and in the soil nutrients. The treatments were: NPKP (4 t ha⁻¹), NPKP (8 t ha⁻¹), NPKP (12 t ha⁻¹), NPKB (8 t ha⁻¹), NPKB (12 t ha⁻¹), and (mineral Fertilizer - NPKF) in recommended rate (RR). Earthworm compound was added as control. Fruit yield was quantified and soil analyzed (pH, total N, available P and K, exchangeable Ca⁺² and Mg⁺²). The NPKB and NPKP significantly increased melon productivity and higher yield was achieved through applying NPKP (12 t ha⁻¹) and NPKF (RR). Comparing with the control treatment, available P and K increased when the NPKP (12 t ha⁻¹) was applied. The fertilizers treatments showed no significant effects in exchangeable Ca⁺² and Mg⁺². The NPKP and NPKB displayed the potential of the biofertilizer inoculated with diazotrophic bacteria and *C. elegans*, which may be an alternative for NPK fertilization favoring soil fertility.

Keywords: *Cucumis melon*, *Cunninghamella elegans*, bioprotector, free living diazotrophic bacteria, fungi chitosan, soil nutrients availability.

Abbreviations: NPKB_biofertilizer with NPK; NPKP_bioprotector with NPK; NPKF_soluble mineral fertilizers; RR_Recommended Rate.

Introduction

Fertilizers are very important to increase the yield of most crops, especially providing nitrogen, phosphorus and potassium. The high cost of the soluble fertilizers causes low income farmers to reduce the use of these products. Generally, the nutrients are not found in available forms in soils and the degradation of minerals needs to be processed by physical, chemical or biological reactions to promote their absorption by plants (van Straaten, 2007). Soluble fertilizers are recognized of great importance to plant yield but they are inaccessible by low income farmers due to the high cost, either the highly soluble mineral fertilizer penetrate into the soil and are leached to deeper layers (Moura et al., 2007). In a modern and sustainable agriculture, it is essential to use alternative products to increase food production, increase soil fertility and conservation of biodiversity, which minimizes environmental damage (Stamford et al., 2008; 2014). Despite all these well-recognized facts, in general, Brazilian soils contain low available P and renewable sources and natural phosphate are absolutely necessary and important for the rational use of these products in agriculture (Araújo et al., 2008). The high demand for fertilizers and the understanding of the people in reference to environmental problems and

especially the scarcity of primary material to produce soluble fertilizers increase the need to study other sources of fertilizer in sustainable agriculture (Lima et al., 2007; Moura et al., 2007; Stamford et al., 2009). Nitrogen is one of the most important nutrients for plant growth and yield due to its role in some chemical compounds as proteins, nucleic acids and many others components (Chien et al., 2008). However, the P and K rock biofertilizers do not release N to be used by plants and soil microorganisms. In agricultural systems, the free living diazotrophic bacteria such as *Azotobacter*, *Beijerinckia* and *Derxia*, have great potential for inoculation of organic wastes (Döbereiner, 1961), and many others organic matters, with high C:N ratio. On the other hand, the mixture of rock biofertilizers with organic matter, as earthworm compound, inoculated with free living diazotrophic bacteria effective in the process of biologic nitrogen fixation (BNF) may be important components to increase the soil fertilization and to release N for plant nutrition (Lima et al., 2010). The production of mixed biofertilizers despite to be alternative to provide nutrients for plants, is especially important when inoculated with fungi that contain chitosan in their biomass (cell walls) such as *Cunninghamella* (Franco et al., 2004).

This biopolymer may act with antifungal and fungistatic properties to protect plants against pathogens and increase nutrients availability, especially nitrogen and phosphorus (Franco et al., 2011; Stamford et al. 2014). The aim of this study is to evaluate the effects of Biofertilizer (NPKB) in melon using PK rocks plus elemental S with *Acidithiobacillus* mixed with organic matter (earthworm compound) enriched in N by inoculation with free living diazotrophic bacteria and bioprotector (NPKP) by addition of chitosan (*C. elegans*). This is an alternative to conventional soluble fertilizers (NPKF) to increase melon yield and some nutritional attributes in Argisol of the Southwestern Bahia, semiarid region of Brazil.

Results

Production and chemical analysis of the bioprotector (NPKP)

The bioprotector (NPKP) was produced in field conditions from the biofertilizer (NPKB) by addition of biomass of the fungi *C. elegans* which contain chitosan in their cell walls. The results of the chemical analysis at the final of the incubation period are shown in Table 1. The pH values were significantly different and the most effect of the incubation period was observed from 14 to 21 days. The effects of total N in biofertilizers were opposite of the pH values. The results showed a greater increase (20.4 g kg⁻¹) for the total N at the final of the incubation period, compared to initial time (9.6 g kg⁻¹). The earthworm compound presented total N= 8.0 (g kg⁻¹). Available P showed significant effect (68%) of microbial inoculation at the final of incubation. Available K and exchangeable Ca⁺² and Mg⁺² showed low effect on free living bacteria and *C. elegans*, comparing with the control treatment (earthworm compound). The NPKB presented: available P= 14 (g kg⁻¹), available K= 12 (g kg⁻¹); exchangeable Ca⁺²= 3.4 (g kg⁻¹) and Mg⁺² = 4.5 (g kg⁻¹).

Field experiment

Melon yield

The results for melon yield (t ha⁻¹) are shown in Fig. 1. There is no literature on effect of biofertilizers and bioprotector produced from powdered rocks with *Acidithiobacillus*, and organic matter obtained by interactive microbial processes yet (free living diazotrophic bacteria and fungi *C. elegans* that contain chitosan). A positive and significant response of the fertilization treatments were observed, especially when the mixed biofertilizer (NPKB) and the bioprotector (NPKP) applied in the highest rate (12 t ha⁻¹). It showed results similar to the mineral fertilizers (NPKF) which presented the best melon yields. The control treatment showed the lower melon yield and revealed the effect of organic and mineral fertilization on melon.

Soil pH after melon harvest

The soil pH showed significant changes after using fertilization sources. It showed significant effects of the fertilization treatments (Fig. 2). The bioprotector (NPKP) and the mixed biofertilizer (NPKB) followed by the control treatment showed the significant effects on soil pH.

Applications of commercial soluble fertilizers (NPKF) had slight influence on soil reaction. The effect of NPKP and NPKB increased the soil pH, which may be explained by the use of very high amount of organic matter that applied 4 times of PK rock biofertilizer (proportion 4:1). The organic (earthworm compound) and rock biofertilizers had pH 7.2 and 3.5, respectively.

Available P and K in soil

The results of available P and K in the soil after melon harvest were shown in the Fig. 3. Significant effects of NPKB and NPKP were observed in available P, comparing with mineral treatments and the control with earthworm compound. Higher amount of available P were obtained in soil with NPKP (8 t ha⁻¹). It was probably due to treatment with 12 t ha⁻¹, in which plants produced higher yield and removed from the soil large quantities of available P. Available K in the soil (Fig. 3) revealed significant increase when higher rate of the biofertilizer (NPKB) applied. Application of the bioprotector (NPKP) in higher amount reduced the available K in soil, due to the same reason reported for available P.

Exchangeable Ca⁺² and Mg⁺²

The results for exchangeable Ca⁺² and Mg⁺² in soil are shown in Fig. 4. For both exchangeable cations, the results were not significant due to fertilization treatments. The NPKP applied in rate 12 t ha⁻¹ presented the highest Ca⁺² content in soil. A slight increase observed for Mg⁺² in soil, which may be explained by the effect of sulphuric acid produced by *Acidithiobacillus* in the presence of sulfur.

Discussion

The effect of the NPKP inoculated with the free living bacteria and *C. elegans* at the final stage of incubation compared with the earthworm compound (pH 7.2) was significant. A reduction was observed when the substrate incubated for 28 days, which showed pH values around 6.3-6.4. This may not be harmful on growth and yield, especially in tropical plants. The effect of the inoculation with free living diazotrophic bacteria was significant and proved the evident increase in total N by the process of nitrogen fixation. The two biofertilizers maintained the same total N content with no significant effect by the addition of *C. elegans*. The highest available P was observed after 28 days of incubation, by which increase up to 60% observed compared to initial period of biofertilizer production. The available K maintained the same values during the period of incubation. The bioprotector with *C. elegans* increased the exchangeable Mg⁺² up to 20%, compared to NPKB biofertilizer at the final stage of incubation. This is probably due to the solubilization of the nutrient contained in the biotite rock. The effects of biofertilizer and bioprotector was reported by Stamford et al. (2014). They applied NPKB and NPKP for grape (*Vitis labrusca* cv Isabel), a long cycle crop, grown in a Brazilian soil of the San Francisco Valley. Applying biofertilizers from PK rocks mixed with earthworm compound showed effectiveness on yield of grape (*Vitis vinifera* cv. Italia) at the San Francisco Valley, Pernambuco state, semiarid region of the Brazilian Northeastern (Stamford et al., 2011).

Table 1. pH values, total N, available P and K and exchangeable Ca⁺² and Mg⁺² in the period for production of bioprotector (NPKP) in the field assay (35 days), analyzed weekly using the Embrapa (2009) methodology.

Incubation (days)	pH H ₂ O _(1:2.5)	Total N	Available		Exchangeable Ca ⁺²	Mg ⁺²
			P	K		
g kg ⁻¹						
NPKP ₀	6.04 ^B ± 0.01	9.6 ^C ± 0.1	8.2 ^B ± 0.6	10 ^B ± 0.4	3.5 ^A ± 0.3	3.9 ^B ± 0.6
NPKP ₇	6.28 ^A ± 0.02	11.2 ^C ± 0.1	12.3 ^A ± 1.6	12 ^A ± 1.5	3.6 ^A ± 0.4	4.1 ^{AB} ± 0.1
NPKP ₁₄	6.29 ^A ± 0.01	14.4 ^B ± 0.3	13.6 ^A ± 0.9	12 ^A ± 1.7	3.4 ^A ± 0.1	4.5 ^A ± 0.6
NPKP ₂₁	6.36 ^A ± 0.02	17.0 ^{AB} ± 0.3	13.7 ^A ± 1.8	12 ^A ± 1.7	3.6 ^A ± 0.1	4.6 ^A ± 0.4
NPKP ₂₈	6.40 ^A ± 0.01	20.6 ^A ± 0.4	13.9 ^A ± 1.2	12 ^A ± 0.5	3.4 ^A ± 0.4	4.5 ^A ± 0.1
NPKP ₃₅	6.39 ^A ± 0.02	20.4 ^A ± 0.3	13.8 ^A ± 1.2	12 ^A ± 0.8	3.4 ^A ± 0.3	4.5 ^A ± 0.3

Means followed by the same capital letter are not significant (Tukey test, $p \leq 0.05$).

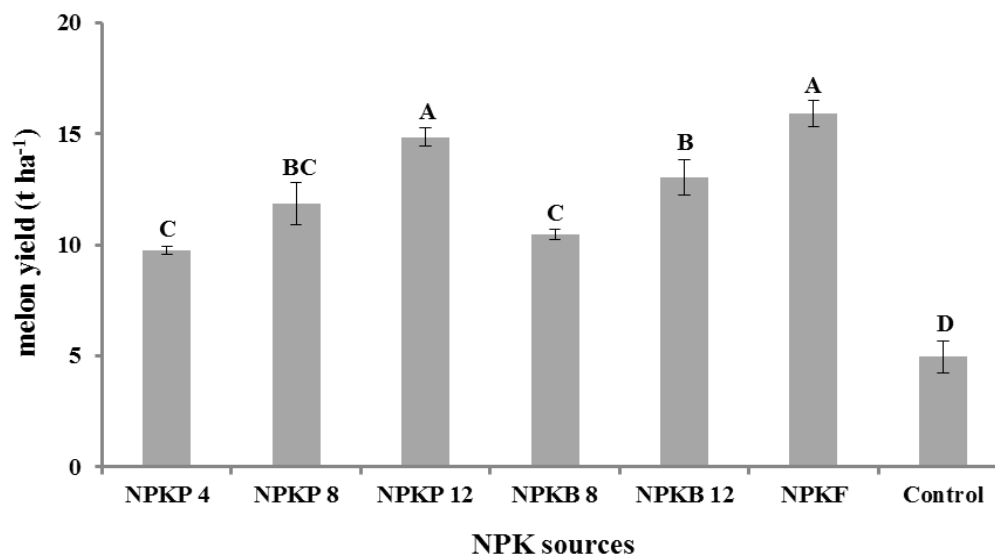


Fig 1. Effects of bioprotector – NPKP (t ha⁻¹) and biofertilizer – NPKB (t ha⁻¹) on melon yield compared with conventional fertilizers - NPKF (t ha⁻¹) and control treatment (earthworm compound 2.4 L plant⁻¹) grown in a Red Yellow Argisol of Southwestern Bahia. C.V. (%) = 6.18. Means followed by the same capital letter are not significant (Tukey test, $p \leq 0.05$).

Moura et al. (2007) reported the effect of PK rock biofertilizer supplemented with organic matter (earthworm compound) on melon yield, grown in an Argisol of Petrolina, Brazil. The present research showed the possibility of the use of biofertilizers from apatite and biotite plus elemental sulfur inoculated with *Acidithiobacillus*, mixed with organic matter enriched in N by inoculation with free living diazotrophic bacteria and *C. elegans*, fungi that contain chitosan, due to the improvement of melon yield and by the increase in availability of nutrients in the soil.

Changes in soil pH after melon cultivation

In the field experiment, the soil pH showed no significant effects after application of the fertilization treatments. A slight pH reduction was observed when the mineral fertilizer (NPKF) applied. This effect was likely due to the addition of ammonium sulfate (N mineral fertilizer) which can increase acidity as described by Chien et al. (2008). Berger et al. (2010) observed similar effect in soil pH in a study applying rock biofertilizer mixed with organic matter in cowpea legume. Silva et al. (2011) grew melon in two soils of Rio Grande do Norte State and used three sources of P (triple superphosphate, P rock biofertilizer, and mixed triple superphosphate plus phosphate rock). They observed a slight increase in soil pH when applied P rock biofertilizer in a red Yellow Latosol. Lima et al. (2007) verified the effect of P

and K rock biofertilizers, produced with P and K rocks inoculated with *Acidithiobacillus* and mixed with earthworm compound, in two consecutive harvests of lettuce. They observed that the pH was not affected by the fertilization treatments. Oliveira et al. (2010), evaluated the agronomic effectiveness of castor bean residues in soil attributes and observed a linear reduction in soil pH, applying organic matter in different rates and the pH values varied from 6.0 to 5.0. Stamford et al. (2004, 2006, 2009) showed the effect of P and K rock biofertilizers (produced by addition of elemental sulfur inoculated with *Acidithiobacillus* bacteria) in reduction of soil pH. The authors reported that the acidity effect was produced by the metabolic H₂SO₄ produced by the oxidative bacteria. However, it is important to note that in these experiments the P and K biofertilizer was not applied in mixture with organic matter, unlike what was done in the present study. The effect of the NPKP increasing soil pH may be explained by the use of large amount of organic matter (earthworm compound) during the biofertilizer production.

Change in soil's available P

The effectiveness of biofertilizers (NPKB and NPKP) to generate available P in the soil was analyzed. The results indicated that the inoculation with free living diazotrophic bacteria and *C. elegans* have positive and significant effects.

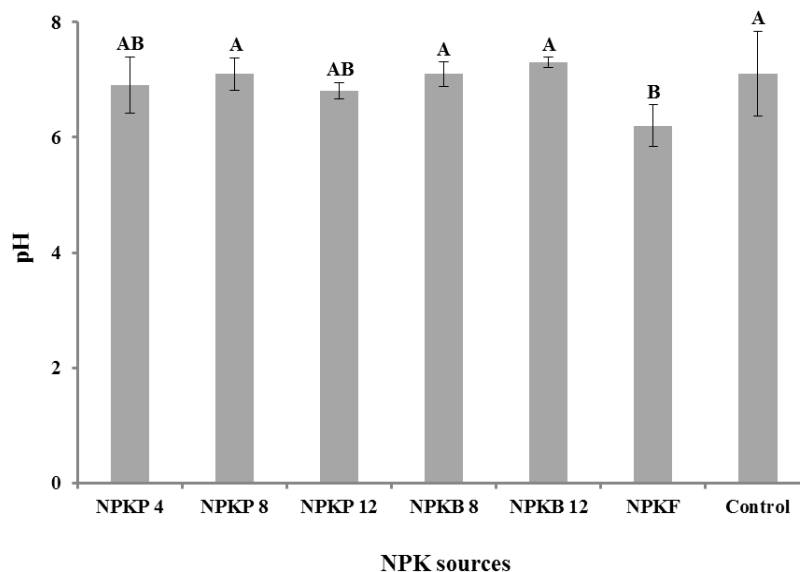


Fig 2. Effects of protector – NPKP ($t\ ha^{-1}$), biofertilizer – NPKB ($t\ ha^{-1}$) on soil pH compared with conventional fertilizers - NPKF ($t\ ha^{-1}$) and control treatment (earthworm compound - $2.4\ L\ plant^{-1}$) after melon harvesting, grown in a Red Yellow Argisol of Southwestern, Bahia). Coefficient of variation - C.V. (%) = 5.62. Means followed by the same capital letter are not significant (Tukey test, $p \leq 0.05$).

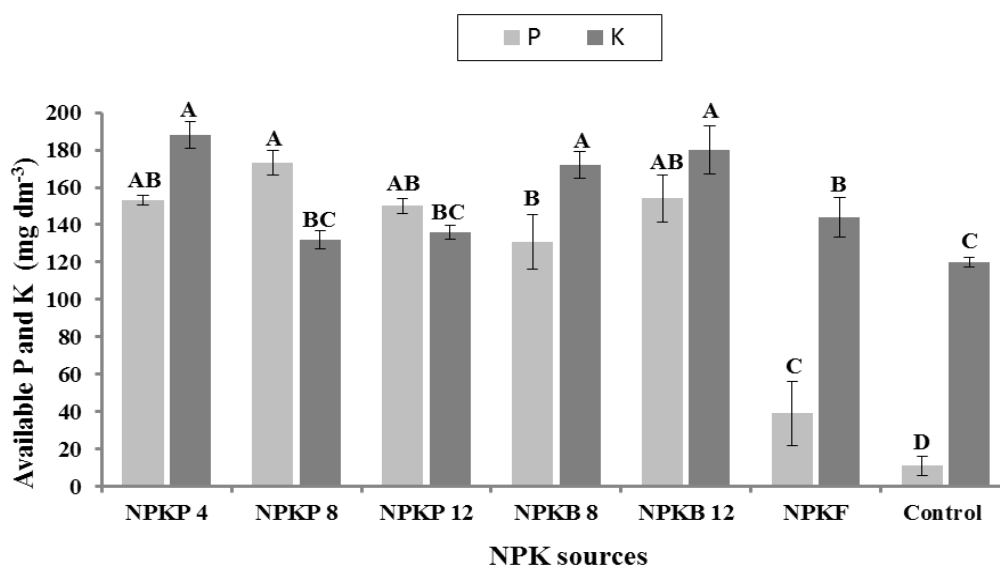


Fig 3. Effects of protector fertilizer – NPKP ($t\ ha^{-1}$) and biofertilizer – NPKB ($t\ ha^{-1}$) on available P and K in the soil compared with conventional fertilizers - NPKF ($t\ ha^{-1}$) and control treatment (earthworm compound - $2.4\ L\ plant^{-1}$) after melon harvesting, grown in a Red Yellow Argisol of Southwestern, Bahia. C.V. (%): available P = 9.26; Available K = 5.24. Means followed by the same capital letter are not significant (Tukey test, $p \leq 0.05$).

These results agree with Stamford et al. (2014) in grape (*Vitis labrusca*) and with Oliveira et al. (2010) evaluating the available P in soil during the melon growth. They reported that the effect may occur due to the increases of organic matter and nutrient solubilization, which promote the balance between K and Ca and results in increase in phosphate availability. Silva et al. (2011) applied different P sources on melon and concluded the positive effects of P rock biofertilizer that produced the highest content of available P in soil. Stamford et al. (2009) also observed significant effect of PK rock biofertilizers on melon growth and in available P in Argisol soil of the semiarid region (San Francisco Valley), compared with conventional soluble fertilizers. Lima et al. (2007) evaluated the effectiveness of biofertilizers from P

and K rocks plus elemental sulfur inoculated with *Acidithiobacillus* mixed with earthworm compound, in two consecutive crops with lettuce in a Yellow Latosol of the Cariri region, compared with mineral fertilizers. They observed higher residual power of the biofertilizers in P availability. The effect of NPKP and NPKB in available P in soil may be attributed to the other native bacteria that exist in soil besides *Acidithiobacillus*. It also can be associated with the fungi that produce phosphatases and chitosan (Franco et al., 2011) and can participate on solubility of P and others nutrients in minerals of soil and PK rocks. Kowalski et al. (2006) and Goy et al. (2009) proposed that chitosan can increase the content of N, P and K in the substrate. Stamford et al. (2014) showed significant effect of NPKB and NPKP in

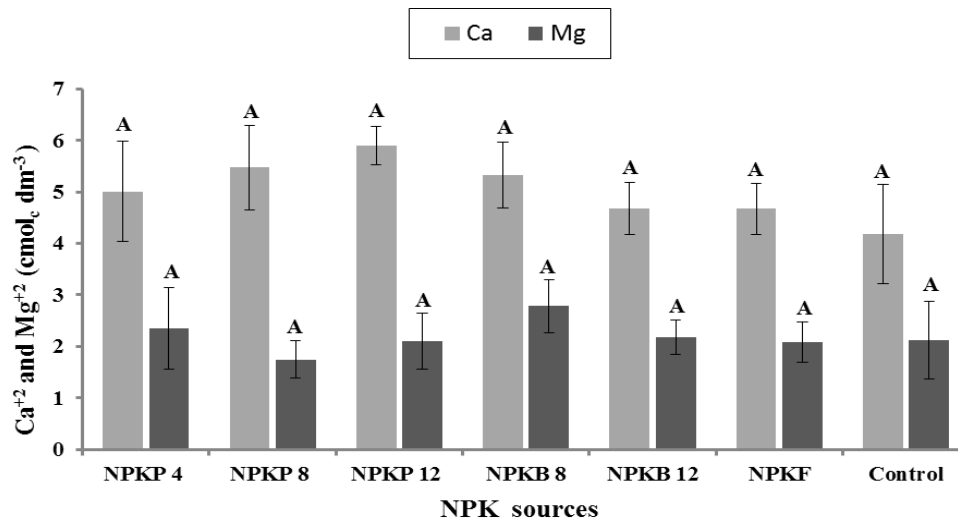


Fig 4. Effects of protector – NPKP, biofertilizer – NPKB (t ha⁻¹) in exchangeable Ca⁺² and Mg⁺² in the soil compared with conventional fertilizers - NPKF (t ha⁻¹) and control treatment (earthworm compound - 2.4 L plant⁻¹) after melon harvesting, grown in a Red Yellow Argisol of Southwestern, Bahia. C.V. (%): Ca⁺² = 28.71; Mg⁺² = 25.29. Means followed by the same capital letter are not significant (Tukey test, $p \leq 0.05$).

soil available P, applied in two depths (0-20 and 20-40 cm), in a soil of the San Francisco Valley grown with grape (*Vitis labrusca*).

Changes in available K

It is important to know that there are not many references about application of K biofertilizers produced from powdered rocks. In the same way described for P, the available K in soil increases when soluble mineral fertilizers (NPKF) are applied, probably due to the higher concentration of K in the soluble fertilizer, and similar results were found with NPKB applied in the higher rate. In soils of the coastal tableland region of Pernambuco State, grown with sugar cane, Stamford et al. (2006) described great increase in available K in soil grown with sugar cane when applied K rock biofertilizer plus elemental sulfur inoculated with *Acidithiobacillus*, and similar results were found in the present study with melon. Lima et al. (2007) verified positive and significant effect of P and K fertilization in available K in soil, after two consecutive harvests of lettuce in the region of Cariri, Brazil. Stamford et al. (2014) reported significant effect of NPKB and NPKP in available K when applied at two depths (0-20 and 20-40 cm), in a soil of the San Francisco Valley grown with grape (*Vitis labrusca*). In an Argisol of the San Francisco valley Stamford et al. (2009) showed increment in the available K in soil when applied mineral fertilizer (NPKF) and PK biofertilizer in the highest rate (160 kg ha⁻¹). Callegari (2009), in a study to evaluate the content of soil nutrients during the melon growth observed a K decrease 26 days after seedling transplantation and reported that the effect may be due to the higher use of K by plants because this nutrient is vital in the photosynthesis process, especially during the growth stage.

Exchangeable Ca⁺² and Mg⁺²

There are not many references that report the effects of biofertilizers in exchangeable Ca⁺² and Mg⁺² in soils. Stamford et al.

(2014) reported significant effect of NPKB and NPKP applied at two depths (0-20 and 20-40 cm), in a soil of the San Francisco Valley grown with grape (*Vitis labrusca*). The effect on exchangeable Ca⁺² was greatest and evident, especially when applied P biofertilizer in higher rate. The soluble mineral fertilizer (NPKF) and the control treatment showed low effect in exchangeable Ca⁺² in soil. The exchangeable Ca⁺² increased in a considerable amount in comparison with the values observed in the soil analyzed before the start of the experiment. The effect prior fertilization is due to the solubilization of Ca⁺² contained in the phosphate rock. The results are similarly to those found by Stamford et al. (2006) in a soil of the coastal tableland of the Pernambuco State, Brazil, grown with sugar cane, and higher values of exchangeable Ca⁺² were obtained when applied PK rock biofertilizer. Stamford et al. (2014) observed increase in Ca⁺² when applied the biofertilizer (NPKB) and the bioprotector (NPKP) in grape (*Vitis labrusca*) in a soil of the San Francisco Valley. Despite no significant effect of the fertilization treatments can be observed that exchangeable Mg⁺² had a substantial increase compared to the Mg in soil analyzed before the treatments application. Stamford et al. (2014) showed significant effect in Mg⁺² availability when applied NPKB and NPKP in two depths (0-20 and 20-40 cm), in a soil of the San Francisco Valley grown with grape (*Vitis labrusca*). The highest values of exchangeable Mg⁺² in soil, compared with the soil analyzed before melon crop, may be explained by the solubilization of Mg⁺² contained in the mineral (biotite) used to produce the K rock biofertilizer, probably by the effect of the sulphuric acid produced metabolically by *Acidithiobacillus* in the presence of elemental sulfur. Similar results were obtained by Stamford et al (2006) in a tableland soil of the humid region of Pernambuco state grown with sugar cane, and by Stamford et al. (2009) with melon in the San Francisco Valley, semiarid region of Pernambuco state. The authors showed high amount of exchangeable Mg⁺² in soil when applied P and K rock biofertilizer. Oliveira et al. (2010) reported significant effect in exchangeable Mg⁺² when applied organic matter (10 t ha⁻¹) compared with the control treatment without organic matter application.

Material and methods

Production of biofertilizers (BNPK) and bioprotector (PNPK)

Prior to production of biofertilizer BNPK and PNPk were obtained the PK rock biofertilizers mixing powdered rocks plus elemental sulfur inoculated with the oxidative bacteria *Acidithiobacillus thiooxidans*. PK rock biofertilizer was produced at University Federal Rural 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 natural phosphate (240 g kg⁻¹ total P), purchased from Irecê (Bahia), Brazil, were applied with 4,000 kg of potash (biotite) containing 100 g kg⁻¹ total K (purchased from Santa Luzia (Paraíba), Brazil, following the procedure described by Stamford et al. (2007). The sulfur-oxidizing bacteria were grown in 2,000-mL Erlenmeyer flasks that contained 1,000 mL of culture medium 9K (El Tarabily et al., 2006) sterilized for 30 min at 120 °C. The Erlenmeyer flasks were shaken (150 rev min⁻¹) for 5 days at 30 °C. The materials (phosphate and potash powdered rocks that were mixed with elemental sulfur) were incubated for 60 days. Daily, the humidity was maintained at a level that was near the 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 P and K rock biofertilizer, using methodology (A) Mehlich 1 and (B) extraction with citric acid, according to Embrapa (2009), yielded the following results: (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) = 5 (g kg⁻¹). The organic biofertilizer (BNPK) was obtained by mixing the PK rock biofertilizer (BPK) with organic matter (earthworm compound) in proportion (BPK: OM) equivalent to 1:3. The analysis of the earthworm compound purchase from Febras Company showed the following results: pH 7.25; organic carbon 120.7 g kg⁻¹; total N 8.6 g kg⁻¹; total sulfur 2.98 g kg⁻¹; total P 11.2 g kg⁻¹. The organic biofertilizer with earthworm compound enriched in N was produced in field conditions with selected free living bacteria (NFB 10001) cultured in LG liquid media (50 ml) in 125 ml Erlenmeyer flasks, shaken (180 rpm) for 96 h at 28 ± 5 °C, according to the methodology of Lima et al. (2010). After inoculation, the material was incubated for 35 days following the same process described above for the PK rock biofertilizer, and the humidity was maintained near water holding capacity. Samples were collected, and the total N determined by the Kjeldhal method, using the Kjeltex auto analyzer (1030 Model). At the final time of incubation the results of the chemical analysis of the mixed biofertilizer (BNPK) are as follows: pH 6.55; organic carbon 90.7 g kg⁻¹; total N 20.6 g kg⁻¹; total sulfur 12.9 g kg⁻¹; available P 12.2 g kg⁻¹; available K 10.1 g kg⁻¹. The protector (PNPK) represents the biofertilizer (NPKB) with addition of micelial biomass of the fungus *Cunninghamella elegans* (UCP 542), which contains a considerable amount (7-8%) of chitosan in the cellular wall. The fungus *C. elegans* was purified in Petri dishes on medium PDA grown 10 days at 28 °C. The monospore culture of the *C. elegans* was obtained grown the Mucorales fungus in Potato - Dextrose (BD) medium as recommended by Franco et al. (2004), using 2000 mL Erlenmeyer flasks kept under shaking (180 rotations per minute) at 28 °C by 96 h. The micelial biomass was diluted (1 L culture per 10 L of distilled water) and added to the substrate by manual application, and then incubated for 35 days. Weekly samples were collected for chemical analyses

(pH, total N, available P and K) as described in the NPKB production.

Field experiment

Soil site and analyses

The field experiment was undertaken at Itapetinga, Bahia State. The soil classified as “Red Yellow Argisol medium texture” (Embrapa 2006) is a characteristic soil with low available P and K. The climate in accord with Köppen classification is of Aw type. The chemical and physical analyzes of soil samples collected before the fertilization treatments application, at 0-20 cm deep, showed the following chemical attributes: pH (H₂O) = 5.6; Organic matter (g kg⁻¹) = 12.3; Electrical conductivity (dS m⁻¹) = 0.15; P (Mehlich 1) = 4 mg dm⁻³; exchangeable cations (cmolc dm⁻³) K = 0.26; Ca = 1.3; Mg = 0.60; Na = 0.05; Al = 0.05 and H+Al = 1.65; S = 2.18; T = 3.83 cation saturation = 57%. Physical attributes: particle density (g cm⁻³) = 2.62; bulk density (g cm⁻³) = 1.66; sand (g kg⁻¹) = 90; lime (g kg⁻¹) = 7 and clay (g kg⁻¹) = 3.

Experimental conditions

One month before carried out the field experiment the seedlings of melon (Siemens hybrid “10.00”) were produced in polypropylene trays (450 cells) with the commercial substrate “Vivatto Slim”. The seedlings were sown at the “IF Baiano” Campus (February 02, 2011) and the seedlings were transplanted manually, 10 days after sown (DAS). The soil was prepared for melon cultivation cutting and removing all vegetation of the experimental area and following conventional tillage with one plowing and two disking. Followed the rows were open to melon seedlings plantation, and at the same time were applied the respective fertilization treatments. Melon was grown spaced 2.0 m x 0.5 m, in plots with four rows 10 m long and 8 m large corresponding to a total area of 80 m² with 80 plants, and 36 plants were harvested to evaluate the experimental yield. The irrigation was realized based in the tensiometers methodology, installed in the soil at 20 cm deep, and 10 cm distance of the sprinkler unit (water drops), in accord to Leão et al. (2007). The water tension in the soil was applied to maintain the moisture near field holding capacity. The fertilizers at the planting date were applied in rows 10 m long and 10 cm deep. The dressed fertilization was realized 5 days after seedling transplantation. After the fruits harvest, soil samples were collected at 0-20 cm deep, to analyze the chemical attributes: pH, available P and K (Mehlich 1), exchangeable sodium, calcium and magnesium, in accord to Embrapa (2009) methodology.

Treatments experimental design and statistical analysis

The field experiment was set up in a completely randomized block design, with four replicates. The fertilization treatments were: T1 = NPKP rate 4 t ha⁻¹; T2 = NPKP rate 8 t ha⁻¹; T3 = NPKP rate 12 t ha⁻¹; T4 = NPKB rate 8 t ha⁻¹; T5 = NPKB rate 12 t ha⁻¹; T6 = NPKF mineral fertilizer and T7 = organic compound 2.4 kg plant⁻¹ (control treatment). The fertilization treatments followed the recommendation for irrigated melon in Pernambuco State (IPA, 2008). The statistical calculations for the field experiment parameters were achieved using analysis of variance, which included the effects of fertilization treatments, using SAS software Program 9.2 version (SAS Institute, 2011). Analyzes of variance and

averages were compared by the Tukey's test at probability $p < 0.05$. All parameters analyzed were normally distributed.

Conclusions

The present study show that biofertilizer (NPKP) produced from PK rock inoculated with *Acidithiobacillus* bacteria mixed with organic matter (earthworm compound) enriched in N by inoculation with diazotrophic bacteria and *C. elegans* may be used as source of nutrients to increment plant yield and increase soil nutrients (total N, available P, available K, exchangeable Ca^{+2} and exchangeable Mg^{+2}). The NPKP produced with PK rocks mixed with organic matter (earthworm compound) inoculated with diazotrophic bacteria and addition of fungi chitosan *C. elegans* may be alternative for replacement of soluble mineral fertilizers. The NPKP and NPKB supply nutrients to the soil, which may be used to improve plant yield and showed great power to maintain successive yields.

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