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Organic matter inoculated with diazotrophic bacterium *Beijerinckia indica* and *Cunninghamella elegans* fungus containing chitosan on banana “Williams” in field

Newton Pereira Stamford[*], Emmanuella Vila Nova da Silva, Wagner da Silva Oliveira, Marta Cristina Freitas da Silva, Marllon dos Santos Martins and Vinicius Santos Gomes da Silva

*Programa de Pós-graduação em Ciências do Solo, Departamento de Agronomia, Universidade Federal Rural de Pernambuco, Av. Dom Manoel de Medeiros, s/n, Dois Irmãos, Recife, Pernambuco, Brazil. *Author for correspondence. E-mail: newton.stamford@ufrpe.br*

ABSTRACT. The production of biofertilizers from rocks increases nutrients for plant nutrition without environmental pollution. The aim of this study was to evaluate the effectiveness of biofertilizers from phosphate and potassium rocks mixed with organic matter (earthworm compound) inoculated with free living diazotrophic bacteria (NFB 10001) and *Cunninghamella elegans* (fungus with chitosan) on yield, characteristics, and nutrient uptake of banana (cv. Williams), and attributes of a Red Yellow Argisoil of the rainforest Zone of Pernambuco, Brazil. The experimental design included two biofertilizers: (a) PK rock biofertilizers plus organic matter (NPKB) and (b) bioprotector (NPKP) applied at 50, 100 and 150% of the recommended rate for banana, which were compared with soluble mineral fertilizers (NPKF) applied at the recommended rate, and earthworm compound (20 ton ha^{-1}). The best results of the plant parameters were obtained with NPKB and NPKP applied at the highest rates (150% RR). A normal yield was produced when NPKB and NPKP were applied at the highest rates and NPKF at the recommended rate. The available P and K in the soil showed a significant fertilization effect, especially when NPKB and NPKP were applied at the highest rates. The biofertilizer and bioprotector may be alternatives to mineral soluble fertilizers.

Keywords: biotite, earthworm compost, fungi chitosan, organic fertilization, rocks with P and K.

Matéria orgânica inoculada com bactéria diazotrófica *Beijerinckia indica* e fungo *Cunninghamella elegans* contendo quitosana em banana no campo

RESUMO. A produção de biofertilizante de rochas aumenta a disponibilidade de nutrientes para as plantas, sem poluição ambiental. O objetivo foi verificar a efetividade de biofertilizante misto (BNPK) e bioprotetor (PNPK) com quitosana de fungo (*Cunninghamella elegans*), produzido com rocha fosfatada e potássica com enxofre elementar inoculado com *Acidithiobacillus,* mais matéria orgânica (húmus de minhoca) na produtividade, características e acumulação de nutrientes (N, P e K), e em atributos de um Argissolo Vermelho Amarelo da Zona da Mata de Pernambuco, Brasil. O delineamento experimental constou de duas fontes de biofertilizante: (a) biofertilizante (BNPK), (b) bioprotetor (PNPK) aplicadas em 3 doses (50, 100 e 150% da recomendação para banana), comparando com fertilizante solúvel (FNPK) na dose recomendada para banana, e com húmus de minhoca (20 t ha^{-1}). Para os parâmetros avaliados os melhores resultados foram com aplicação do BNPK e PNPK na dose mais elevada (150% RR). Na produtividade de frutos houve produção de frutos com aplicação de BNPK e PNPK e com FNPK. Os níveis de P e K disponível no solo mostraram a eficiência dos biofertilizantes especialmente com BNPK e PNPK nas doses mais elevadas. O biofertilizante e o bioprotetor mostraram ser alternativa para substituição a fertilizantes minerais solúveis.

Palavras chave: biotita, húmus de minhoca, quitosana fúngica, fertilização orgânica, rocha com P e com K.

Introduction

Banana was introduced in the rain forest region of Pernambuco State and used by farmers in conjunction with sugarcane harvest. Banana culture, in general, may increase job creation for people who work in agriculture, making it attractive and interesting to the regional economy (Borges & Junior, 2010).

Brazil is currently the second largest banana producer in the world; only India has greater production than Brazil (Matsuura, Costa, & Folegatti, 2004). In 2013, approximately 7,181 million tons of bananas were produced in Brazil. The State of Pernambuco possesses approximately 6% of the banana fruits that are produced in Brazil and represents the second greatest regional producer of banana (Instituto Brasileiro de Geografia e Estatística [IBGE], 2014).

The cultivar "Williams", known as "Giant Cavendish", belongs to the AAA group, sub-group "Cavendish", and is characterized as having a lower average height; the fruits are recovered with green skin and have a sweet taste in the final stage. This cultivar was chosen because it does not have specific temperature requirements, is resistant to yellow sigatoka disease, and requires large amounts of nutrients and water (Silva, 2000). The cultivar "Williams" also requires large amounts of nutrients, especially potassium, nitrogen and phosphorus, and in soils containing large available amounts of these nutrients, NPK fertilization is not necessary for normal productivity (Borges & Junior, 2010).

However, the high cost of soluble NPK fertilizers normally contributes directly to the reduction of soluble NPK fertilizers applied by low farmers. In addition, soluble fertilizers, especially phosphorus and potassium, which are normally not found in available forms in soil, must be applied. In general the use of chemical, physical or microbiological processes to produce soluble fertilizer is necessary to increase the availability of nutrients in soil (Moura, Stamford, Duenhas, Santos, & Nunes, 2007).

The application of potassium fertilizers is very important and seems to be fundamental to Brazilian agriculture because K-soluble fertilizers are produced mainly by International Emprises (Canada, German, Russian and China), and approximately 90% of the K fertilizers that are necessary for application in Brazilian economic crops are obtained by exportation (Roberts, 2004).

The production of soluble fertilizers requires high energy consumption, and special and strategic processes are necessary to increase the use of P and K rocks. To increase the availability of nutrients that are contained in the rocks, one important alternative is biological solubility, which is possible with the sulfur oxidative bacteria *Acidithiobacillus* (Stamford et al., 2007). These oxidative bacteria use elemental sulfur to produce sulfuric acid and promote the availability of P and K contained in rocks through very high acidity and low pH (He, Baligar, Martens, Ritchey, & Kemper, 1996). This microbiological process promotes the transformation of elemental S into the soluble ion SO_4^{2-}, which is a plant nutrient, and the proton H^+ may be used for pH neutralization, especially in alkaline soils with high pH (Stamford, Santos, Silva Junior, Lira Junior, & Figueiredo, 2008).

However, rock biofertilizers do not introduce nitrogen into the soil, which is one of the most important nutrients for normal plant growth and productivity due to the lixiviation in the soil. Thus, nitrogen is normally found at low rates in soil. Organic wastes have significant effects because of increased soil physical conditions and have high biological activity but, in general, introduce low levels of nitrogen for plants (Lima et al., 2010).

Diazotrophic associative and symbiotic bacteria normally contribute to the soil ecosystem, but free-living diazotrophic bacteria have greater potential as alternatives for the enrichment of organic matter with N. Lima et al. (2010) reported an increase greater than 100% in N when using *Beijerinckia indica* to inoculate organic matter (earthworm compost) with 30 days of incubation.

Organic and sustainable agriculture also includes plant protection against pests and diseases and the application of chitosan, a natural biopolymer with peculiar characteristics that help plants to promote resistance against pests and diseases. Otha et al. (2000) reported that chitosan increases plant growth due to the introduction of a higher amount of N and to improved defense against microbial pathogens. Chitin and chitosan may be found in crustaceans, which are traditional sources of biopolymers, but the use of fungi of the order Mucorales, which produce chitin and chitosan in their cellular walls, may be an important alternative for bioprotector production (Stamford et al., 2008).

This study aims to evaluate the effectiveness of a biofertilizer and bioprotector, compared with soluble NPK fertilizers and earthworm compost with regard to banana yield, characteristics and absorption of nutrients in a field experiment. This study also aims to evaluate the effect of biofertilizers on soil chemical attributes.

Material and methods

Production of mixed biofertilizer and bioprotector

The biofertilizer (BNPK) that was used as an alternative source of NPK nutrients for plants was produced using rock biofertilizers mixed with sulfur and *Acidithiobacillus* following the methodology of Stamford et al. (2007) and mixed with organic matter (earthworm compost) enriched with N by inoculation with free-living diazotrophic bacteria (NFB 10001), in accordance with Lima et al. (2010). The bioprotector (NPKP) was produced by the addition of *Cunninghamella elegans,* a fungus that contains chitin and chitosan in its cell wall, to the biofertilizer (NPKB).

In the production of biofertilizer, the addition of a carbon source, such as ice cream waste, is necessary to promote the rapid growth of free-living bacteria. This organic matter was deposited at the UFRPE by the Unilever International Emprise. The results of the chemical analyses of the earthworm compost that was used to produce the NPK biofertilizer were as follows: pH 7.95; organic carbon (g kg^{-1}), 10.07; total N (g kg^{-1}), 7.5; total S (g kg^{-1}), 1.98; total P (g kg^{-1}), 1.1; and water content (%), 8.53. The results of the chemical analyses of the ice cream waste were as follows: pH 7.96, organic carbon, 14.4 (g kg^{-1}); and total N, 3.2 g kg^{-1}. Analyses of the products were performed at 10-day intervals, in accordance with Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA, 2009).

Soluble fertilizer (NPKF) was produced by mixing ammonium sulfate (20% N), simple superphosphate (20% P_2O_5) and potassium sulfate (50% K_2O), calculated based on soil analyses in accordance with the recommendations for banana (Instituto Pernambucano de Pesquisa Agropecuária [IPA], 2008). The fertilizer treatments were applied in the field at the moment of seedling transplantation.

Site and soil

A field experiment was conducted in a sugarcane field at the Experimental Station of the University Federal Rural of Pernambuco, located in the District of Carpina, Pernambuco, Brazil (07° 33' S and 35° 00' W; altitude 13 m). The soil is a sandy loam (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2006), representative of the Typic Fragiudult from the tableland rainforest region, with low levels of available P and K. The soil was chemically and physically analyzed using a compost sample that was collected from the experimental area (0-30 cm layer), and the results, using the methodology of Embrapa (2009), are shown in Table 1.

Table 1. Soil attributes of the Red Yellow Latosoil used in the field experiment.

Soil attributes	Results
Chemical analyses	
pH (Water) (1:2.5)	5.6
Ca^{2+} ($cmol_c$ dm^{-3})	1.4
Mg^{2+} ($cmol_c$ dm^{-3})	0.6
Al^{3+} ($cmol_c$ dm^{-3})	0.1
C org. (g kg^{-1})	6.5
Available K ($cmol_c$ dm^{-3})	3.2
Available P (mg kg^{-1})	5.9
Total N (g kg^{-1})	0.6
Physical analyses	
Sand (g kg^{-1})	788.0
Silt (g kg^{-1})	161.6
Clay (g kg^{-1})	50.4
Textural classification	Sandy loam

Field experiment

The soil was prepared by cutting and removing the natural vegetation in the experimental area, which had not been cultivated for five years, following conventional tillage with one plow and two disk operations. Then, furrows were simultaneously opened for the transplantation of banana seedlings, followed by fertilizer treatments. Each row with the NPK fertilizer treatments had 7 furrows (0.40 m long x 0.40 m large and 0.40 m deep), with the seedlings spaced 2.0 m apart and the rows, 2.5 m apart (rows 17.5 m long). The banana yield was determined using 10 plants from the two central rows, and the plant characteristics were determined from 4 plants for each fertilizer treatment.

Experimental design, determinations and statistical analyses

The experiment was conducted in randomized block design with four replicates. Eight fertilizer treatments were applied at different recommended rates (RR): (1) biofertilizer – NPKB at 50% RR, (2) NPKB 100% RR, and (3) NPKB 150% RR; (4) protector – NPKP at 50% RR, (5) NPKP 100% RR, and (6) NPKP 150% RR; (7) soluble fertilizer – NPKF at 100% RR; and (8) earthworm compost (20 ton ha^{-1}).

The soluble fertilizers contained ammonium sulfate (20% N), simple superphosphate (20% P_2O_5) and potassium sulfate (50% K_2O). The amount of mixed NPK soluble fertilizer (NPKF) was calculated based on the N, P and K contents in the simple fertilizers. For the biofertilizers, the NPK content that was obtained in the NPKB and NPKP was used, using the same amount for each corresponding NPK treatment in NPK soluble fertilizer. The amounts were calculated following the recommended rate (RR) for banana in Pernambuco State (IPA, 2008) and based on experimental results.

We determined the plant yields and banana characteristics (height, diameter, fresh and dry weight) for pseudostems and leaves (+3), and N, P and K absorption by banana at plant harvest. Soil chemical attributes (total C, total N, available P and K, and exchangeable Ca and Mg) were analyzed to test the effects of different fertilizer treatments after plant harvest.

Statistical analysis was performed with SAS (SAS Institute, 2011) version 11.0, using Tukey's test to compare the means ($p > 0.05$).

Results and discussion

Analyses of the biofertilizer and bioprotector

The results that were obtained during the final period of biofertilizer production when inoculated with free-living diazotrophic bacteria and fungi chitosan (*C. elegans*) are shown in Table 2.

Table 2. Chemical characteristics of the biofertilizer (NPKB) and of bioprotector (NPKP), at the final period of production in field conditions (mean of eight laboratorial analyzes). In the assay analyses were processed in samples collected in different positions at 10, 20 and 30 days after *C. elegans* inoculation.

Chemical characteristics	BNPK	PNPK	C.V.	LSD
pH (Water 1:2.5)	6.3^{a}	6.4^{a}	4.84	0.01
N (g kg^{-1})	17^{a}	15^{a}	10.25	1.53
Exchangeable Ca^{+2} (g kg^{-1})	4^{a}	4^{a}	12.56	0.08
Exchangeable Mg^{+2} (g kg^{-1})	0.4^{a}	0.4^{a}	14.69	0.01
Available P (g kg^{-1})	13^{a}	14^{a}	13.47	0.49
Available K^{+} (g kg^{-1})	13^{a}	13^{a}	14.58	0.57

[1]Means followed by the same letter are not significantly different by Tukey test ($p \leq 0.05$).

The pH results were significantly different, and in both products, an effect of the time of incubation was observed, especially from 10 to 20 days. The reduction in pH values was evident in the biofertilizer NPKB upon inoculation of free-living bacteria (NFB 10001) and in the treatment with the addition of *C. elegans*. The ideal pH for banana normally is between 5.5 to 6.5, but the plants can withstand wide variation in pH values (Borges & Junior, 2010). The pH of the biofertilizers (BNPK and PNPK) is well established and may be considered ideal for banana in the field.

The results of both biofertilizer indicated a significant increase in the contents of N, P and K. N enrichment of the earthworm compound was similar to that observed by Lima et al. (2010), who reported an increase of up to 100% in total N content.

Regarding available P in the production of NPKB, there was a significant difference during the final period of incubation (Table 2). The highest available P was obtained with 30 days of incubation and showed an increase of up to 100% relative to the initial value. The increase in available K was significant, and for the NPKB biofertilizer, the highest values were obtained after 30 days of incubation.

The effects of chitosan application likely occurred because in the treatments with higher amounts of elemental sulfur inoculated with *Acidithiobacillus,* acid production increased the available P and K in the soil, as reported by Stamford, Lima, Santos, & Dias (2006); Stamford et al. (2007); Stamford et al. (2008); Stamford et al. (2014). These effects also occurred because chitosan increased the levels of N, P and K in the substrates, as described by Kowalski et al. (2006) and Goy et al. (2009).

The highest available P was obtained after 30 days of incubation and showed an increase of up to 100% relative to the initial value. The protector (NPKP) inoculated with *C. elegans* increased the available K up to 20% compared with that of the natural earthworm compound, likely via the release of this nutrient from the biotite rock and the organic matter.

The protector may release all of the macronutrients that are necessary for plant growth to increase yield. Due to N increases facilitated by nitrogen fixation carried out by free-living diazotrophic bacteria, and as reported by Kowalski, Terry, Herrera, & Peñalver (2006) and Goy, Britto, & Assis, (2009), chitosan may increase the levels of N, P and K in the substrates due to the formation of charged amino groups via chitosan deacetylation. Furthermore, *C. elegans* contains chitosan in its cell walls and also produces inorganic polyphosphate (Franco, Albuquerque, Stamford, Lima, & Takaki-Campos, 2011), increasing the solubility of P and other nutrients.

Yield of banana fruits in the field

The banana yield in the field experiment is shown in Table 3. The NPK biofertilizers and NPK soluble fertilizer were significantly effective, and the best yield was produced when applying NPKP at the highest rate ($NPKP_{150}$)

Table 3. Yield and characteristics of shoots and leaves (+3) of banana (cv. Williams) grown in an Argisoil and submitted to different fertilizer treatments.

Fertilizers	Yield	Shoot				Leaf (+3)			
		Fresh mass	Dry mass	Height	Diameter	Width	Length	Fresh mass	Dry mass
	ton ha^{-1}	kg		cm		cm		g	
Control [1]	-	$3.1^{d}\pm0.5$	$0.30^{d}\pm0.06$	$51^{c}\pm4.1$	$5.0^{b}\pm0.5$	$19.4^{c}\pm2.9$	$39.8^{d}\pm7.9$	$189^{d}\pm28$	$40^{c}\pm1.2$
Fertilizer NPK_{100}	$11.4^{b}\pm0.22$	$17.8^{b}\pm2.9$	$1.65^{b}\pm0.08$	$183^{b}\pm19.9$	$13.2^{a}\pm1.0$	$31.9^{b}\pm3.9.$	$71.4^{b}\pm9.1$	$392^{a}\pm47$	$70^{b}\pm1.7$
Biofertilizer $_{50}$	-	$13.4^{c}\pm2.4$	$1.35^{c}\pm0.06$	$96^{ab}\pm23.5$	$12.6^{a}\pm2.8$	$31.6^{b}\pm4.4$	$58.6^{c}\pm7.0$	$244^{c}\pm36$	$66^{b}\pm4.9$
Biofertilizer $_{100}$	$2.4^{d}\pm0.09$	$17.4^{b}\pm2.6$	$1.75^{b}\pm0.05$	$206^{a}\pm30.9$	$13.5^{a}\pm2.2$	$32.6^{b}\pm2.8$	$79.7^{b}\pm11.1$	$325^{b}\pm32$	$69^{b}\pm3.4$
Biofertilizer $_{150}$	$12.2^{b}\pm0.78$	$19.5^{a}\pm2.3$	$2.05^{a}\pm0.06$	$222^{a}\pm24.2$	$13.9^{a}\pm4.1$	$33.2^{b}\pm3.1$	$91.3^{a}\pm10.8$	$413^{a}\pm37$	$75^{b}\pm6.0$
Bioprotector $_{50}$	$4.4^{d}\pm0,46$	$14.7^{c}\pm2.6$	$1.55^{b}\pm0.09$	$198^{ab}\pm35.6$	$14.1^{a}\pm2.1$	$32.6^{b}\pm4.2$	$75.7^{b}\pm6.8.$	$380^{a}\pm34$	$72^{b}\pm4.8$
Bioprotector $_{100}$	$8.4^{c}\pm0.35$	$19.8^{a}\pm2.9$	$1.75^{b}\pm0.03$	$208^{a}\pm23.8$	$14.1^{a}\pm2.7$	$33.7^{b}\pm5.3$	$79.5^{b}\pm9.5$	$393^{a}\pm42$	$80^{a}\pm1.5$
Bioprotector $_{150}$	$17.8^{a}\pm0.47$	$21.1^{a}\pm2.1$	$2.15^{a}\pm0.06$	$219^{a}\pm26.6$	$14.6^{a}\pm1.9$	$39.4^{a}\pm4.1$	$97.9^{a}\pm10.7$	$407^{a}\pm52$	$84^{a}\pm2.9.$

[1]Control - Earthworm compost applied in rate (20 ton ha^{-1}) recommended rate (RR) for banana (IPA, 2008); $FNPK_{100}$ - soluble fertilizer in recommended rate (RR) – IPA (2008); BNPK = biofertilizer applied in 50, 100 and 150 % RR; PNPK - Bioprotector applied in 50, 100 and 150% RR. Means followed by different letters are significantly different by Tukey test ($p \leq 0.05$).

Similar results were reported by Stamford et al. (2011) in a field experiment with table grape in the Brazilian semiarid region (San Francisco Valley) and by Oliveira et al. (2014) regarding the growth of melon in the Brazilian semiarid region (Bahia Southwestern). In a greenhouse study that was performed to evaluate the agronomic effectiveness of NPKB and NPKP biofertilizers compared with NPKF in the cowpea legume, Berger et al. (2013) found that these treatments increased the yield similarly to the present study with banana under field conditions.

Moura et al. (2007) evaluated some characteristics of melon in the San Francisco Valley and Brazilian semiarid region, and Lima, Stamford, Santos, Dias (2007) evaluated lettuce in the Cariri region, (Ceará State, Brazil). In a greenhouse experiment growing sugarcane in a soil from the Brazilian tableland rain forest region, Stamford et al. (2006) reported positive and significant effects of phosphate and potash rock biofertilizers, showing an evident increase in the industrial characteristics and shoot dry matter of sugarcane compared with the soluble NPK fertilizer. The authors observed decreased soil pH when applying higher rates of biofertilizers, likely due to the acidity promoted by the *Acidithiobacillus* bacteria when the species was applied to produce P and K rock biofertilizers.

Characteristics of banana leaves and pseudostems

The results pertaining to weight and other characteristics in leaves (leaf +3) and to the pseudostems of banana as affected by application of fertilizer treatments under field conditions are presented in Table 3. The fertilizer treatments displayed slight significant differences in the weight of banana leaves, and a significant increase in the characteristics of pseudostems was observed, especially when applying greater rates of NPKF, NPKB and NPKP compared with the control treatment with earthworm compost (2.4 L $plant^{-1}$).

These results agree with Stamford et al. (2016), who found a correlation between sugarcane yield and fertilizer application. Moreover, this author found that organic matter promoted a higher yield in sugarcane, likely because the organic matter released P, Ca and other nutrients that are necessary for plant nutrition. Similarly, these results agree with the greenhouse studies of Stamford et al. (2008). These authors concluded that a PK rock biofertilizer mixed with earthworm compost may be an alternative to mineral-soluble fertilizers. Furthermore, residual fertilizer effects may be observed, especially when applying PK rock biofertilizer.

Nutrient accumulation in leaves

The results of nutrient accumulation in the leaves (leaf 3+) in banana when submitted to different fertilizer treatments are presented in Table 4. A positive and significant response was observed when applying soluble fertilizer (NPKF) at the recommended rate (RR) and biofertilizer (NPKB) and protector (NPKP) at the highest rates compared with the control treatment with earthworm compost (2.4 L $plant^{-1}$).

The total N that accumulated in the banana leaves with the application of biofertilizers and mineral fertilizers was significant compared with the control treatment with earthworm compost. The biofertilizer treatment (NPKB and NPKP) applied at the highest rates showed a significant and evident effect, likely due to the positive interaction between the P in the substrate and the nitrogen absorbed by the banana leaves.

In a study carried out with melon, Oliveira et al. (2014) observed a positive and significant effect of biofertilizer produced from phosphate and potash rocks inoculated with *Acidithiobacillus* oxidative bacteria compared with P and K soluble fertilizer. Andrade et al. (2013) described similar results with cowpea in greenhouse and field experiments, but in this study, the authors observed an effect of inoculation with arbuscular mycorrhizal fungi.

Table 4. Nutrient accumulation (NPK) in leaves (+3) of banana (cv. Williams) grown in a Red Yellow Argisoil in field conditions (means of eight replicates).

Fertilizer treatments	Nutrients accumulation in banana leaves (+3)		
	Total N	Total P	Total K
	mg $leaf^{-1}$		
Control (earthworm compost)	317^{c} ±39.9	163^{c} ±27.1	832^{c} ±48.8
Soluble fertilizer $FNPK_{100}$	1904^{a} ±299.4	334^{a} ±57.6	1397^{a} ±205.3
Biofertilizer $BNPK_{50}$	1452^{b} ±195.2	259^{b} ±41.8.	1341^{b} ±216.7
Biofertilizer $BNPK_{100}$	1664^{b} ±200.6	273^{b} ±56.2	1374^{b} ±217.0
Biofertilizer $BNPK_{150}$	1927^{a} ±208.2	370^{a} ±60.0.	1404^{a} ±206.2
Bioprotector $PNPK_{50}$	1490^{b} ±177.4	266^{b} ±45.2.	1295^{b} ±181.3
Bioprotetor $PNPK_{100}$	1908^{a} ±244.0	293^{b} ±50.8.	1377^{a} ±220.9
Bioprotetor $PNPK_{150}$	2001^{a} ±212.3	398^{a} ±70.9	1407^{a} ±168.8
C.V.(%)	12.6	17.9	15.4

[1]Control - Earthworm compost applied in rate (20 ton ha^{-1}) recommended rate (RR) for banana (IPA, 2008); $FNPK_{100}$ - soluble fertilizer in recommended rate (RR) – IPA (2008); BNPK = biofertilizer applied in 50, 100 and 150 % RR; PNPK - bioprotector applied in 50, 100 and 150 % RR. Means followed by different letters are significantly different by Tukey test ($p \leq 0.05$).

In addition, table 3 shows that the total P in banana leaves under treatment with NPKB150 was similar to that in leaves under soluble fertilizer (NPKF100) treatment. However, the total P that accumulated in banana leaves under NPKB150 treatment was the highest, superior even to NPKF100.

The total K that accumulated in banana leaves when $BNPK_{150}$ and $NPKP_{150}$ were applied was similar to that which accumulated when $NPKF_{100}$ was applied. These results demonstrate that the availability of N, P and K increases when applying biofertilizer at higher rates.

Soil chemical attributes

The results of the soil pH analyses after the banana harvest showed the significant effects of the fertilizer treatments (Table 5). The NPKB and NPKP biofertilizers, especially at the highest rates, presented approximately neutral pH values (pH 6.5), which may explain the nutrient availability. When applying the control treatment (earthworm compost – 2.4 L $plant^{-1}$), an increase in soil pH was observed because the organic matter had a very high pH (near pH 8.0), whereas the soluble fertilizer (NPKF) decreased soil pH, likely due to the use of sulfate fertilizers, especially ammonium sulfate (Chien, Gearhart, & Collamerm, 2008).

The available nutrients that were in the soil (total N, available P and K) after the banana harvest are presented in Table 5. Total N displayed very high values when NPKB and NPKP were applied at the highest rates, and in general, a significant reduction in total N was observed, likely as a function of the increased N absorption by banana in the field harvest.

In general, the PK rock biofertilizer exhibited better plant parameters, likely due to the effects of nutrient availability in the soil. The biofertilizer and the soluble fertilizer, when applied at the highest rate, exhibited the best results compared with the other fertilizer treatments. Stamford et al. (2008) reported significant effects of PK rock biofertilizers inoculated with *Acidithiobacillus* on some characteristics of sugarcane and observed the best effectiveness compared with mineral NPK fertilizer.

Several studies have reported the effects of PK rock biofertilizers on soil pH, especially when applied at high doses. These effects are due to the sulfuric acid that is produced by the oxidative bacteria *Acidithiobacillus* and because the biofertilizer has a low pH (approximately 3.0-3.5). However, our PK rock biofertilizer was mixed with sugarcane mud cake in a proportion of OM: BP+BK, equivalent to 3.0: 0.5+0.5, and the OM (earthworm compost) had a pH of approximately 7.9, which neutralized PK rock fertilizer acidity. Stamford et al. (2006) described the effects of a mixed biofertilizer on soil pH reduction when applied at higher doses.

Stamford et al. (2011), in a study applying rock biofertilizer mixed with organic matter in grape, observed a similar effect on soil pH. Silva et al. (2011) evaluated melon growth (in two soils of Rio Grande do Norte State) using three sources of P (triple superphosphate, P rock biofertilizer, and mixed triple superphosphate plus phosphate rock) and observed a slight increase in soil pH when applying P rock biofertilizer in a red Yellow Latosoil. Lima et al. (2007) verified the effect of P and K rock biofertilizers produced with P and K rocks with elemental rock inoculated with *Acidithiobacillus* oxidative bacteria mixed with earthworm compound in two crops of lettuce and observed that, in general, pH was not affected by the fertilizer treatments.

Oliveira et al. (2010), in a study evaluating the agronomic effectiveness of castor bean residues in soil attributes, observed a linear reduction in soil pH with organic matter rates and promoted variation in pH values from 6.0 to 5.0. Stamford et al. (2004; 2006; 2009) and Moura et al. (2007) reported that P and K biofertilizers decreased the soil pH after the application of P and K rock biofertilizers plus elemental sulfur inoculated with *Acidithiobacillus*. The authors attributed the effects to the acidity of the metabolic H_2SO_4 produced by oxidative bacteria. However, the P and K biofertilizer was mixed with organic matter, in contrast to the present study, where NPK nutrients were released by biofertilizers that were produced with rock biofertilizers mixed with organic matter (earthworm compost) enriched with N by microbial inoculation.

Table 5. Soil chemical analyzes (pH, total C and N, available P and K), after the first harvest of banana (cv Williams) in the field experiment (means of eight replications).

Fertilizer treatments	pH H_2O	Total C	Total N	Available P	Available K
	1.0:2.5	-------- g kg^{-1} -----------		----------- mg kg^{-1} --------	
Control- earthworm compost	$5.6^a \pm 0.09$	$20^e \pm 2.5$	$1.0^c \pm 0.08$	$40^f \pm 4.33$	$16.9^d \pm 2.32$
Soluble fertilizer $FNPK_{100}$	$5.1^c \pm 0.32$	$33^d \pm 2.8$	$1.6^b \pm 0.12$	$66^e \pm 3.30$	$38.0^{ab} \pm 2.68$
Biofertilizer - $BNPK_{50}$	$5.4^b \pm 0.30$	$55^c \pm 3.5$	$1.9^a \pm 0.17$	$85^d \pm 6.18$	$27.5^c \pm 28.05$
Biofertilizer - $BNPK_{100}$	$5.4^b \pm 0.34$	$111^a \pm 8.6$	$1.9^a \pm 0.14$	$207^a \pm 16.51$	$41.7^a \pm 4.05$
Biofertilizer - $BNPK_{150}$	$5.3^b \pm 0.32$	$128^a \pm 10.0$	$2.1^a \pm 0.27$	$295^a \pm 14.81$	$46.7^a \pm 4.39$
Bioprotetor - $PNPK_{50}$	$5.2^b \pm 0.07$	$68^c \pm 10.6$	$1.6^b \pm 0.19$	$137^c \pm 10.72$	$27.7^c \pm 4.17$
Bioprotetor - $PNPK_{100}$	$5.5^a \pm 0.24$	$87^b \pm 9.8$	$1.6^b \pm 0.24$	$175^b \pm 16.56$	$34.2^b \pm 4.67$
Bioprotetor - $PNPK_{150}$	$5.5^a \pm 0.18$	$87^b \pm 10.7$	$1.7^b \pm 0.21$	$176^b \pm 14.00$	$40.5^a \pm 4.63$.
C.V. (%)	2.4	8.5	12.9	10.3	14.5

[1]Control - Earthworm compost applied in rate (20 ton ha^{-1}) recommended rate (RR) for banana (IPA, 2008); FNPK100 - soluble fertilizer in recommended rate (RR) – IPA (2008); BNPK = biofertilizer applied in 50, 100 and 150 % RR; PNPK - Bioprotector applied in 50, 100 and 150% RR. Means followed by different letters are significantly different by Tukey test ($p \leq 0.05$).

Available P and K in the soil showed a significant effect when we applied the PK biofertilizer and mineral soluble fertilizer (Table 5). It is important to describe the effect of the PK sources, especially the biofertilizers (NPKB and NPKP) applied at the highest rates, which yielded significant amounts of available P and K after the banana harvest.

The application of P and K biofertilizers in tableland soils (Stamford et al., 2006) increased the sugarcane stalk yield and affected several soil chemical attributes, especially when applied at the recommended rates. The available K in the soil increased when NPK soluble fertilizers were applied, likely due to the higher K concentration in the soluble fertilizer, followed by treatment with NPKB applied at a higher rate (150% RR).

Similar results were reported by Oliveira et al. (2014) when applying PK rock biofertilizers supplemented with earthworm compost to melon grown in an Argisoil from Petrolina District, Pernambuco, Brazil, and in grape (*Vitis labrusca* – cv. Isabel) in the region of irrigated fruits (Botticelli viniculture) in the Valley of San Francisco (Stamford et al., 2014; 2015).

However, Maia, Botelho, Faria, and Leite (2009) reported that chitosan applied to grape leaves did not promote a significant increase in plant characteristics. Mazaro, Gouvea, Wagner Junior, and Citadin (2012), when applying chitosan to leaves, observed a similar response in strawberry yield and fruit weight. These authors agree that chitosan has only a slight effect on plant yield because the polymer promoting the resistance induction and syntheses of defense composts may interfere with nutrient absorption and plant yield.

The results for exchangeable Ca^{+2} and Mg^{+2} following different fertilizer treatments are presented in Table 6. The data in the soil showed a significant effect of the fertilizer treatments, especially when the NPKP was applied at the highest rate (150% RR), obtaining the highest amount of exchangeable Ca^{+2} in soil, followed by NPKP at a rate of 100% RR. The soluble mineral fertilizer (NPKF) and control treatment showed low exchangeable Ca^{+2}. Furthermore, the Ca^{+2} content in the soil increased considerably compared with the values observed in the analyzed soil before banana seedling transplantation in the field experiment.

The nutrients P and Ca are liberated from P and K rocks by the oxidative bacteria *Acidithiobacillus* acting on natural P rocks with significant P and Ca content, and similarly, the nutrients K and Mg are released from the biotite mineral. In the production of PK rock biofertilizers, the oxidative bacteria *Acidithiobacillus* use elemental sulfur and produce sulfuric acid metabolically, and the soluble $S\text{-}SO_4^{-2}$ that is released in this process may be used in plant nutrition (El-Tarabily, Soaud, Saleh, & Matsumoto, 2006).

The results for exchangeable Mg^{+2} were significant for the fertilizer treatments. However, a significant effect of the fertilizer treatments was observed, and the Mg^{+2} content increased compared with the Mg^{+2} content in the soil analyzed before the seedling transplantation to the field.

The highest values of exchangeable Mg^{+2} in soil may be explained by the solubilization of nutrients contained in the mineral (biotite) that was used to produce the K rock biofertilizer (BK), and the increase was likely promoted by the sulfuric acid effect that was produced metabolically by *Acidithiobacillus* in the presence of elemental S. The results show the effect of the sulfur oxidative bacteria *Acidithiobacillus* on the solubilization of minerals contained in the P and K rocks, as described by Stamford et al. (2006; 2007; 2011).

Table 6. Soil chemical analyzes (exchangeable Ca^{2+} and Mg^{2+}), after the first harvest of banana (cv Williams) in the field experiment (means of eight replications).

Fertilizer treatments	Exchangeable Ca^{+2}	Exchangeable Mg^{+2}
	$cmol_c\ dm^{-3}$	
Control (Earthworm compost)	$7.8^c \pm 1.59$	$0.78^c \pm 0.19$
Fertilizer $NPKF_{100}$	$8.9^b \pm 1.08$	$0.96^{ab} \pm 0.39$
Biofertilizer $NPKB_{50}$	$8.2^c \pm 1.87$	$0.79^c \pm 0.21$
Biofertilizer $NPKB_{100}$	$9.4^b \pm 1.31$	$0.94^{ab} \pm 0.35$
Biofertilizer $NPKB_{150}$	$10.2^a \pm 1.65$	$1.06^a \pm 0.61$
Bioprotector $NPKP_{50}$	$8.7^b \pm 1.95$	$0.79^c \pm 0.46$
Bioprotector $NPKP_{100}$	$9.8^a \pm 1.53$	$0.90^b \pm 0.80$
Bioprotector $NPKP_{150}$	$10.5^a \pm 1.90$	$1.04^a \pm 0.41$
CV (%)	15.7	17.4

[1]Control - Earthworm compost applied in rate (20 ton ha^{-1}) recommended rate (RR) for banana (IPA, 2008); $FNPK_{100}$ - soluble fertilizer in recommended rate (RR) – IPA (2008); BNPK = biofertilizer applied in 50, 100 and 150% RR; PNPK - bioprotector applied in 50, 100 and 150 % RR. Means followed by different letters are significantly different by Tukey test ($p \leq 0.05$).

Conclusion

We conclude that the time of incubation affects biofertilizer production and that, in general, the best period for incubation of P and K rocks is approximately 60 days, whereas that of organic matter (earthworm compost) is 28 to 30 days. The present study demonstrated that biofertilizer (NPKB) produced from PK rock inoculated with *Acidithiobacillus* bacteria mixed with organic matter (earthworm compound) enriched with N by inoculation with diazotrophic bacteria and fungi chitosan may be used as biofertilizer to increase banana yield and certain plant characteristics. The protector (NPKP) and biofertilizer (NPKB) may be alternatives to soluble mineral fertilizer.

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