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Keywords: capsicum annuum, cunninghamella elegans, biological fertilizers, fungi chitosan, nutrient absorption, organic agriculture

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Influence of Bioprotector Produced by Interspecific Microbial Inoculation on Green Pepper Characteristics and Nutrient Uptake

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Abstract- The aim of this study was to evaluate PK rock biofertilizer mixed with earthworm compound inoculated with free-living diazotrophic bacteria (NPKB). The bioprotector achieved by the introduction of fungi chitosan by the addition of Cunninghamella elegans was also studied. In a field experiment were evaluated the effects of this biofertilizer and bioprotector (NPKB + C. elegans) on characteristics and nutrient uptake by green pepper (Capsicum annuum). The experiment was conducted at the Horticultural Experimental Station of the Agronomic Institute of Pernambuco - IBA during the rainy season (March-August, 2011). The study was a factorial (8x2) split plot design with eight fertilizer treatments and two sub treatments (with and without crustaceous chitosan applied to leaves) and with four replicates. The fertilizer treatments were as follows: NPKF mineral fertilizers applied at the recommended rate (RR); Biofertilizer - NPKB at 50% RR; NPKB at 100% RR; NPKB at 150% RR; NPKP (Bioprotector with fungi chitosan from C. elegans) at 50% RR; PNPK at 100% RR; PNPK at 150% RR; Control treatment (cow manure applied at 2.4 L plant⁻¹). The best fruit yield was obtained with the highest rate of PNPK and NPKB application. There were significant differences in nutrient uptake between the different fertilization treatments. In the experiment, no case of soft rot disease was observed; therefore, it was impossible to compare the treatments in this respect. The results indicate a great potential for PK rock biofertilizer with free-living diazotrophic bacteria (NPKB) and bioprotector with fungi chitosan (NPKB + C. elegans) as an alternative to NPK fertilization.

Keywords: capsicum annuum, cunninghamella elegans, biological fertilizers, fungi chitosan, nutrient absorption, organic agriculture.

I. INTRODUCTION

he growing world population and increased demands for fertilizers and pesticides have led to sensible changes in agricultural systems and the use of new techniques to maximize yields (Goy et al., 2009). Fertilization with NPK affects horticultural productivity and nutrient absorption; this increases yield and maximizes the productivity of the agricultural crop system (Stamford et al., 2008). Soluble fertilizers are important for plant yields; however, they are less available to low-income farmers due to their high prices. In modern and sustainable agriculture, the use of fertilizers can incrementally increase food production to meet economic criteria and increase biodiversity to minimize environmental damage (Stamford et al. 2008).

Biofertilizers produced from powdered P and K rocks are treated with elemental sulfur inoculated with Acidithiobacillus, which metabolically produces H₂SO₄. Such an arrangement increases the availability of the nutrients contained in the rocks. It is known that N is not supplied by powdered rocks in an amount sufficient to improve plant growth. To increase the N content in PK rock biofertilizer with low pH, it is necessary to add organic matter (OM) inoculated with free-living diazotrophic bacteria as proposed by Lima et al. (2010). In biological studies, crustaceous chitosan is frequently used to increase resistance to plant pathogens. Moreover, chitosan has chelating properties greater than those of other natural polymers due to the presence of amino groups, and it may release nutrients into the environment (Boonlertnirun et al. 2008; Goy et al., 2009). In this study, fungus biomass (Cunninghamella elegans) was applied to an NPKB biofertilizer for the production of a bioprotector that promoted chitin deacetylation through the acidity the oxidative promoted by sulfur bacteria The use of fungi chitosan has Acidithiobacillus. advantages over crustaceous chitosan, such as an independence from seasonal factors and the simultaneous extraction of chitin and chitosan (Franco et al., 2004).

The use of fungi chitosan application in agriculture as a bioprotector does not appear in the literature. No published research on the use of *C. elegans* to produce biofertilizer with the addition of fungi chitosan was found. This study describes the production of a bioprotector and evaluates the stimulating effects of this biofertilizer and bioprotector with fungi chitosan on green pepper yield, nutrient uptake and in some commercial characteristics. The main objective of this study was to determine the feasibility of using PK rocks with organic matter enriched in N by free-living

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diazotrophic bacteria and with fungi chitosan as an alternative to conventional fertilizers. Furthermore, this product could potentially be used as a bioprotector against phytopathogenic microorganisms in further studies.

II. MATERIALS AND METHODS

a) The Production of Biofertilizers (NPKB) and Bioprotector (NPKP)

Biofertilizers from phosphate and potash rocks were produced at the Horticultural Experimental Station of the Federal University Rural of Pernambuco (UFRPE). Two furrows (each 10.0 m long, 1.0 m wide and 0.5 m deep) were constructed. For each biofertilizer, 4000 kg of natural phosphate with a total P of 240 g kg⁻¹ purchased from Irecê (Bahia), Brazil, and 4000 kg of potash rock (biotite) with a total K of 100 g kg⁻¹, purchased from Santa Luzia (Paraiba), Brazil, were mixed with 400 kg of elemental sulfur and inoculated with *Acidithiobacillus* bacteria; the biofertilizers were prepared following the procedure described by Stamford et al. (2007).

The sulfur oxidizing bacteria were grown in 2000 ml Erlenmeyer flasks that contained 1000 ml of a specific culture medium (El Tarabily et al., 2006). The media were sterilized for 30 min at 120 °C. The Erlenmeyer flasks were shaken (150 rev/min) for 5 days at 30 °C. The materials (phosphate and potash rocks mixed with elemental sulfur) were incubated for 60 days; the humidity was maintained at a level that was near the field holding capacity. To avoid the effects of excessive humidity due to rain and to increase the efficiency of the oxidizing bacteria, the furrows were covered daily using black plastic.

The analysis of the P and K rock biofertilizer (PKB) was performed with extraction by (A) Mehlich 1 solution and (B) citric acid, according to Embrapa (2009). This analysis yielded the following results: (P-biofertilizer)-pH = 3.8, the available P for (A) = 60 (g kg⁻¹) and for (B) = 48 (g kg⁻¹); (K biofertilizer-BK)-pH = 3.3, the available K for (A) = 10 (g kg⁻¹) and for (B) = 0.5 (g kg⁻¹).

The biofertilizer (NPKB) was produced under field conditions using PK rock biofertilizer (PKB) and organic matter (OM) obtained from sugar cane cake; these components were mixed in a proportion (PKB:OM) equivalent to (1:4) and inoculated with the free living bacteria (NFB 10001) selected in the previous assays. The diazotrophic bacteria were cultured in LG liquid media (50 ml) in 125 ml Erlenmeyer flasks and shaken (180 rpm) for 96 h at 28 \pm 5 °C temperature, according to the methodology described by Lima et al. (2010). After the inoculation, the material was incubated for 30 days following the process described above for the PK rock biofertilizer. The humidity was maintained near water holding capacity. Samples were collected; the total N was determined using the Kjeldhal method with a Kjeltec auto analyzer (1030 Model). The results of the chemical analysis of the mixed biofertilizer (NPKB) are as follows: pH was 6.90; the organic carbon equaled 120.7 g kg⁻¹; the total N equaled 19.8 g kg⁻¹; the total sulfur equaled 10.9 g kg⁻¹; the total P equaled 10.1 g kg⁻¹; and the total K equaled 15.1 g kg⁻¹.

The protector (PNPK) was the biofertilizer (NPKB) with the addition of the mycelial biomass of the fungus Cunninghamella elegans (UCP 542). This fungus contains a considerable amount (7-8%) of chitosan in its cell wall. The fungus C. elegans was purified in Petri dishes on PDA medium and grown for 10 days at 28 °C. The monosporic C. elegans culture was obtained from the Mucorales fungus in Potato-Dextrose (BD) medium as recommended by Franco et al. (2004). Erlenmeyer flasks with volumes of 2000 mL were shaken (180 rotations per minute) at 28 °C for 96 h. The mycelial biomass was diluted (1 L culture per 10 L of distilled water) and added to the substrate by manual application. The mixture was incubated for 35 days. Samples were collected weekly for chemical analyses (pH, total N, and available P and K) as described for NPKB production.

b) Experimental conditions and soil analyses in the field experiment

A field experiment with sweet pepper (cv. All Big) was carried out at the IPA Experimental Station located in the rainforest region of Pernambuco State, Brazil. The District of Vitoria de Santo Antão is situated at 8° 8' 00' S and 35° 22' 00" W at an altitude of 146 m.

During the course of the field experiment (December 2010–March 2011), the photoperiod remained close to 12 h of darkness and 12 h of light. The temperature oscillated between 28°C and 36°C, and the relative humidity was 60-80%. The soil was prepared for the crop by cutting and removing all of the vegetation from the experimental area. Soil was prepared by conventional tillage with one plowing and two diskings; the rows were then opened to transplant the green pepper seedlings. The rows were made systematically to maintain a declivity of approximately 0.2-0.5% to avoid soil run-off.

Seeds were pre-germinated in trays (128 cells per tray) and transplanted in the field 38 days after the seeds were initially planted. The fertilizers were mixed with the surface soil (10 cm deep) before the seedlings were planted. The subplots (8.4 m²) measured 2.8 m long and 3 m wide. Plants were placed with a spacing of 1.0 m x 0.40 m. To estimate yield, 10 plants were collected from the central rows of each sub plot. Plants were collected weekly for four harvests. The total fruit yield, the number of fruits and nutrients in fruits were analyzed.

The NPKF fertilizer mixed with ammonium sulfate, simple superphosphate, and potassium sulfate

was prepared based on the recommended rate (RR) following soil analyses and the recommendations for irrigated green pepper in the state of Pernambuco, Brazil (IPA, 2008). NPKF fertilizers were applied at seedling transplantation. For N and K, ammonium sulfate and potassium sulfate were applied. The NPKB and NPKP treatments were as follows: 50, 100 and 150 (g plant⁻¹), which correspond to 50% RR, 100% RR and 150% RR, were applied at seedling transplantation and in the two fertilization dressings. In the control treatment, farmyard manure (2.4 L plant⁻¹) was applied at seedling transplantation, and the same amount was applied in the two fertilization dressings.

The soil used was classified as Red Yellow Latosol (Embrapa, 2006) and was located in the rainforest region in Pernambuco state, Brazil. The soil analysis (Embrapa, 2009) revealed the following: pH $(H_2O) = 6.1$; total N = 0.55 g kg⁻¹, available P = 2.7 mg dm⁻³, available K = 10.4 mg dm⁻³, and the exchangeable cations (mmol_c dm⁻³) Ca = 16 and Mg = 4.1.

c) Experimental design and statistical analyses

The field experiment was set up in a factorial (8x2), randomized split plot design with 4 replicates. The treatments were as follows: (1) NPKF soluble fertilizers applied at the recommended rate - RR; (2) NPKB at 50% RR; (3) NPKB at 100% RR; (4) NPKB at 150% RR; (5) NPKP at 50% RR; (6) NPKP at 100% RR; (7) NPKP at 150% RR; and (8) the control treatment (farmyard manure). All fertilizer treatments were applied with or without shrimp chitosan (90% purity, 95% deacetylation) purchased from Sigma Industry. Shrimp chitosan was applied to the leaves at seven days after seedling transplantation. The natural occurrence of root pathogenic fungi was observed.

The statistical calculations for the production of the bioprotector and for the field experiment parameters were achieved using the Program SAS software version 9.2 (SAS Institute 2011). Analyses of variance and averages were compared using the Tukey test at a probability of p < 0.05.

III. Results

a) Biofertilizer and Protector with diazotrophic bacteria and C. elegans

The chemical analyses of the products (pH, total N, available P and available K) are shown in Table 1. The pH results demonstrated significant differences. In both products, an effect of the time of incubation, especially from 10 to 20 days, was observed. A reduction in pH was evident in the biofertilizer NPKB treatment with the inoculation of free-living bacteria (NFB 10001) and in treatments with the addition of *C. elegans*. The effects on total N, available P and available K by the biofertilizer were inversely proportional to the observed pH values (Table 1). The best results for the biofertilizer NPKB were obtained when the substrate was

incubated for 28 days. The pH stabilized between 6.0-6.5, the total N was 10 (g kg⁻¹), the available P was 1.39 (g kg⁻¹), the available K was 1.2 (g kg⁻¹), the exchangeable Ca^{+2} was 0.34 (g kg⁻¹) and the exchangeable Mg⁺² was 0.45 (g kg⁻¹).

The available P contained in NPKB was significantly different depending on the period of incubation (Table 1). The highest available P was obtained at 30 days of incubation; an increase of up to 100% compared to the initial measurement was observed. The increase in available K was significant. In the NPKB biofertilizer, the highest available K values were obtained after 30 days of incubation. However, the bioprotector with *C. elegans* increased the available K by up to 20% more than obtained in the NPKB biofertilizer.

b) Green pepper productivity

Rock biofertilizers mixed with an earthworm compound, inoculated with diazotrophic bacteria (NPKB) or mixed with *C. elegans* (NPKP) were more effective than the conventional fertilizer (NPKF) and increased the sweet pepper yield (Table 2). The fertilization with NPKP (150% RR) resulted in a greater green pepper fruit yield of 21.36 t ha⁻¹. The fruit yields with NPKP (100% RR) and NPKB (150% RR) were 19.14 and 19.07 t ha⁻¹, respectively. The treatment with soluble fertilizer (FNPK) produced a yield of 17.38 t ha⁻¹. The control treatment (farmyard manure applied at 2.4 L plant⁻¹) had the lowest fruit yield (15.65 t ha⁻¹). Interestingly, all of the fertilizer treatments applied showed yields greater than the average yield of irrigated green pepper for the state of Pernambuco (15 t ha⁻¹).

c) Fruit commercial characteristics

The green pepper fruit commercial characteristics are presented in Table 3. The yields of green pepper increased with the application of different fertilizer treatments. The best results were obtained when the bioprotector (NPKP) was applied at the highest rate (150% RR). In this case, green pepper vield increased by 28% and 19% compared with the control treatment and the fertilizer (NPKF) treatment, respectively. The largest number of fruits was observed when NPKB was applied at the highest rate (68,000 fruits ha⁻¹) and when NPKP was used (67,500 fruits ha⁻¹). The smallest number of fruits was observed for the control (29,250 fruits ha⁻¹).

The effects of the different fertilizer treatments on the commercial characteristics of green pepper are outlined in Table 3. The commercial characteristics of green pepper fruits and the yield and number of green peppers showed the same pattern among the different fertilization treatments. The effects of the fertilization treatments on the length, diameter and thickness of the green pepper fruits were greatest when NPKB (150% RR) and NPKP (150% RR) were applied. 2014

d) Nutrients in the green pepper fruits

The nutrients contained in the green pepper fruits are presented in Table 4. The greatest increase in the amount of total N in the leaves was observed when NPKP was applied at the highest rate (150% RR), followed by NPKB (150% RR), and NPKF (100% RR). When NPKB (150% RR) was applied, the response was not significantly different from the response observed in the NPKF application; these treatments are not significantly different than the NPKP (100% RR) and NPKB (100% RR) treatments. Plants grown under the control treatment, NPKB and NPKP applied at 50% RR had the lowest amount of total N in the leaves. An effect of the inoculation with free-living diazotrophic bacteria (NFB 10001) was observed; the microorganisms caused an increase in the N content of the biofertilizer NPKB, especially when the product was applied at higher rates. The total P found in the green pepper fruits revealed significant differences based on the fertilizer treatment applied (Table 4). The highest total P values were

observed when NPKP was applied at 150% RR, followed by NPKB applied at 150% RR (Table 4). The total K in green pepper revealed that the NPKP treatment at application rates of 150% and 100% RR resulted in significant differences compared with the other fertilization treatments. The total P found in fruits under the control treatment and the NPKF treatment were not significantly different. Thus, the NPKB and NPKP treatments resulted in the best nutrient status in green pepper fruits.

The total N observed in the green pepper fruits was highest when the NPKP fertilizer was applied. Compared to the mineral fertilizer (NPKF) treatment, the application of mixed biofertilizer (NPKB) applied at the highest rate (150% RR) increased the total N content up to 50%, and NPKP applied at 150% RR increased the total N content by 100% (Table 4).

The best results were observed when NPKP was applied at the highest rate (150% RR), followed by NPKB (150% RR) and NPKF (100% RR). When NPKB (150% RR) was applied, the response was not significantly different than that observed for the NPKF application; these treatments were not significantly different than the NPKP (100% RR) and NPKB (100% RR) treatments. The lowest total N values were observed with the control treatment and the NPKB and NPKP treatments applied at 50% RR.

The total P observed in the green pepper fruits was significantly affected by the fertilizer treatment (Table 4). The highest total P value was observed when NPKP was applied at a rate of 150% RR, this was followed by NPKB application at a rate of 150% RR. Significant differences in the total K in the green pepper fruits were observed in the PNPK application at rates of 150% and 100% RR compared to other fertilizer treatments. The control and NPKF treatments did not

These results suggest that the biofertilizer obtained from PK rocks with the addition of the earthworm compound and the bioprotector inoculated with *C. elegans* chitosan may be applied to soil with low nutrient content as an alternative fertilization method for increment green pepper productivity.

IV. Discussion

a) Production of the biofertilizer-bioprotector

A reduction in pH values was observed when the biofertilizer NPKB was applied along with an inoculation of the free-living bacteria (NFB 10001) and the addition of *C. elegans*. The P and K rock biofertilizers present with pH values of 3.0 and 3.5, respectively. However, the observed pH values in the NPKB and NPKP treatments were satisfactory for tropical plants and mostly likely do not result in harmful effects.

As a result of biofertilizer and bioprotector production, both substrates showed substantial increases in the N, P and K contents. The enrichment in N content in the earthworm compound was similar to that observed by Lima et al. (2010), who obtained an increase in total N content of up to 100%.

The highest available P content was obtained after 30 days of incubation; the P content increased up to 100% relative to the initial time point. The bioprotector (NPKP) inoculated with *C. elegans* increased the available K up to 20% compared with the natural earthworm compound; this was likely due to the release of this nutrient from the biotite rock and the organic matter.

The biofertilizer-bioprotector may release all of the macronutrients necessary for plant growth and increased yield. Free-living diazotrophic bacteria increase the N content through the effectiveness of the process of nitrogen fixation. As reported by Kowalski et al. (2006) and Goy et al. (2009), chitosan may increase the levels of N, P and K in the substrates due to the formation of charged amino groups due to chitosan deacetylation. Furthermore, C. elegans contains chitosan in the cellular wall and produces polyphosphates (Franco et al., 2011) that increase the solubility of P and others nutrients.

The nutrients P and Ca are liberated from the P and K rocks by the oxidative bacteria *Acidithiobacillus*. These bacteria act on the natural P rocks that contain high P and Ca content and, in the same manner, release the nutrients K and Mg from the biotite mineral. In the production of the PK rock biofertilizers, the oxidative bacteria *Acidithiobacillus* use the elemental sulfur and produces sulfuric acid through a metabolic reaction; the soluble $S-SO_4^{-2}$ released during this process may be utilized for plant nutrition.

Furthermore, the interactive processes carried out by these microbial organisms release micronutrients contained in the earthworm compound. In this way, a complete biofertilizer is produced. In addition, the chitin and chitosan from *C. elegans* may protect plants against damage by inhibiting pathogenic microorganisms (Berger et al., 2011).

b) Green pepper productivity

Moura et al. (2007) obtained similar results on melon yield comparing P and K rock biofertilizers and organic matter (earthworm compound) with P and K mineral fertilizers in a Brazilian Argisol. Oliveira et al. (2010) applied organic matter to castor bean (10 t ha⁻¹) and observed an increase in melon yield, reporting that this effect occurred because the organic matter increases nutrient solubilization.

The difference observed in green pepper productivity when the bioprotector was applied is due to the effect of adding the fungus *C. elegans* to the biofertilizer (NPKP); the fungus produced inorganic polyphosphate and increased P and N due to the amino acid changes observed in the deacetylation of chitosan (Franco et al. 2011). The large amount of N in chitosan (6.9 to 8.7%) may increase both vegetative and reproductive plant growth. Such growth is consistent with reports by Otha et al. (2004) and Rabea et al. (2003). These authors observed that when chitosan was applied to the soil as a mixed fertilizer, the resulting high levels of nitrogen, phosphorus, and potassium increased plant growth compared with the control treatments.

Boonlertnirun et al. (2008) reported that the period of chitosan availability in the soil may increase when it is applied to the biopolymer at the shoot; prolonged contact of the plant root and the soil favored the interaction between the positive charges of the chitosan and the negative charges of the nutrients contained in the soil, which may influence nutrient absorption by plants and contribute to increased plant yield.

c) Green pepper characteristics

Oliveira (2010) observed significant effects of PK rock biofertilizers mixed with an earthworm compound on the characteristics of melon. Lima et al. (2007) reported that PK rock biofertilizers affected the commercial characteristics of lettuce grown in an Argisol soil in Ceará state, Brazil. However, the locus number of the fruits did not differ significantly, as this is a genetic characteristic that varied with species and is not influenced by environmental effects such as fertilization treatments.

The greater effects of the bioprotector with fungi chitosan application (NPKP) most likely occurred

because the treatments with PK rock biofertilizers plus elemental sulfur are inoculated with *Acidithiobacillus*. The oxidative bacteria produce sulfuric acid, which increases the release of available P and K from the rocks, as proposed by Stamford et al. (2006; 2007; 2008). Chitosan also increases the levels of N, P and K in the substrates, as proposed by Kowalski et al. (2006) and Goy *et al.* (2009).

In an Argisol from a semiarid region (the San Francisco Valley), Stamford et al. (2009) observed that P and K rock biofertilizers had significant effects on melon characteristics compared with conventional soluble fertilizers. Lima et al. (2007) evaluated the effectiveness of biofertilizers from P and K rocks with elemental S and inoculated with *Acidithiobacillus* bacteria. The authors reported that the rock biofertilizers mixed with an earthworm compound improved yield and promoted higher residual effects on two consecutive lettuce crops compared with conventional fertilizers in a Yellow Latosol.

d) Nutrients uptake

The effects of the bioprotector with fungi chitosan application (NPKP) in nutrients uptake probably occurred because in the treatments with higher amounts of PK rock biofertilizers plus elemental sulfur inoculated with *Acidithiobacillus* the oxidative bacteria produced sulfuric acid which increase the release of available P and K from the rocks, as proposed by Stamford et al. (2006; 2007; 2008). In the other hands, chitosan also increase the levels of N, P and K in the substrates as proposed by Kowalski et al. (2006) and Goy et al. (2009). The results suggest that the biofertilizer from PK rocks plus earthworm compound and the bioprotector inoculated with *C. elegans* produced chitosan and applied in soil with low nutrients content may be alternative for green pepper fertilization.

The results in nutrients uptake were in accord with Lima et al. (2010) who observed an increase in total N up to 100% when applied the free living diazotrophic bacteria (NFB 10001) inoculating the earthworm compound. It was verified the effect of the bioprotector due to the inoculation with free living diazotrophic bacteria (NFB 10001) because the microorganism increment N in the biofertilizer NPKB, especially when applied the product in higher rates, and the addition of chitosan increase N content as described by Oliveira et al. (2010).

V. Conclusions

The use of biofertilizers from PK rocks mixed with an earthworm compound (NPKB) and enriched in N content by inoculation with diazotrophic bacteria (NFB 1001) and the NPKB inoculated with *C. elegans* (NPKP) increased fruit yield compared with mineral fertilizers. The use of NPKB and NPKP resulted in positive increases in some important commercial characteristics of green pepper and its nutrient status; the nutrient availability in the soil also increased. We conclude that NPKB and NPKP are a possible alternative to NPK mineral fertilizers applied to green pepper.

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Period	рН Н О	Total N	Available		Exchangeable	
(days) -	1120	g kg ⁻¹				
Po	$6.04^{b} \pm 0.01$	$4.8^{\text{b}}\pm0.01$	$0.82^{\text{b}}\pm0.06$	$0.8^{\text{b}}\pm0.04$	$0.35^a\pm0.03$	$0.39^{\text{b}}\pm0.06$
P 7	$6.28^{\text{a}}\pm0.02$	$5.6^{\text{b}}\pm0.01$	$1.23^{a} \pm 0.16$	$1.2^{a} \pm 0.15$	$0.36^{a}\pm0.04$	$0.41^{a} \pm 0.01$
P ₁₄	$6.29^{a}\pm0.01$	$7.2^{ab}\pm0.03$	$1.36^{a}\pm0.09$	$1.2^{a} \pm 0.17$	$0.34^{a}\pm0.01$	$0.45^a\pm0.06$
P ₂₁	$6.36^{\text{a}}\pm0.02$	$8.5^{a}\pm0.03$	$1.37^{a} \pm 0.18$	$1.2^{a} \pm 0.17$	$0.36^a\pm0.01$	$0.46^{a}\pm0.04$
P ₂₈	$6.40^a\pm0.01$	$10.3^{a}\pm0.04$	$1.39^{a} \pm 0.12$	$1.2^{a}\pm0.05$	$0.34^a\pm0.04$	$0.45^{\text{a}}\pm0.01$

Table 1 : pH values, total N and available P and K in the production of biofertilizer (NPKB) in a previous assay,
incubated during 28 days in field conditions ^(a) .

^(a) Means with the same letter are not different by the Tukey test ($p \le 0,05$).

Table 2 : Green pepper productivity and number of green pepper fruits, as affected by the different fertilization treatments ^(a)

	Fruits of green pepper	
Fertilization treatments ^(b)	Productivity	Number
	t ha-1	Unit ha ⁻¹
NPKB (50% RR)	16.42 ± 1.12^{cd}	53750 ± 9.07^{ab}
NPKB (100% RR)	17.98±2.03°	51750 ± 9.13^{ab}
NPKB (150% RR)	$19.07 \pm 1.55^{\circ}$	68000 ± 5.83^{a}
NPKP (50% RR)	16.32±1.97°	51750 ± 9.12^{ab}
NPKP (100% RR)	19.14±2.21 ^b	67500 ± 15.24^{a}
NPKP (150% RR)	21.36±2.00 ^a	60000 ± 5.28^{a}
NPKF (100% RR)	17.38±2.50°	51250 ± 10.16^{ab}
Control	15.65 ± 1.90^{d}	29250±7.73 ^b
CV (%)	13	3

^(a) Means with the same letter are not different by the Tukey test ($p \le 0,05$).

^(b) NPKB = Organic matter (earthworm compound) plus PK rocks biofertilizer inoculated with free living diazotrophic bacteria; PNPK = Protector (NPKB with fungi chitosan from C. elegans); FNPK = soluble fertilizers (RR recommended rate), control (earthworm compound 2.4 kg plant¹). CV= Coefficient of Variation.

Table 3 : Green pepper characteristics: Fruit length (FL), fruit diameter (FD) and skin thickness (ST) affected by fertilization with biofertilizer (NPKB) and bioprotector (NPKP) in three rates, mineral fertilizer (NPKF) in recommended rate (RR) and the control treatment (earthworm compound – 2.4 kg plant⁻¹) ^(a)

Fertilization Green pepper Commercial Characteristics ^(a)						
Treatments ^(b)	Fruit length (FL)	Fruit diameter (FD)	Skin thickness (ST)			
cm						
NPKB (50% RR)	88.1 ± 4.16^{bc}	71.2±3.41 ^b	$5.1 \pm 0.25^{ m b}$			
NPKB (100% RR)	91.1 ± 6.95^{ab}	74.7 ± 1.78^{ab}	5.6 ± 0.45^{ab}			
NPKB (150% RR)	93.7 ± 4.30^{a}	76.8 ± 1.61^{a}	5.9 ± 0.26^{a}			
NPKP (50% RR)	86.7±4.11 ^b	71.6±2.34 ^b	5.4 ± 0.37^{ab}			
NPKP (100% RR)	93.0±2.61 ^a	74.6 ± 2.53^{ab}	5.7 ± 0.35^{ab}			
NPKP (150% RR)	93.7 ± 3.25^{a}	76.9 ± 3.13^{a}	6.0 ± 0.14^{a}			
NPKF (100% RR)	84.5±5.75 ^c	71.4 ± 2.72^{b}	5.5 ± 0.44^{ab}			
Control	75.6 ± 5.86^{d}	70.0±2.63°	4.5 ± 0.36^{b}			
CV (%)	3 3	7				

^(a) Means with the same letter are not different by the Tukey test ($p \le 0.05$).

^(b) NPKB = Organic matter (earthworm compound) plus PK rocks biofertilizer inoculated with free living diazotrophic bacteria; PNPK = Bioprotector (NPKB with fungi chitosan from C. elegans); control (earthworm compound 2.4 kg plant⁻¹). CV = Coefficient of Variation.

Fertilization treatments ^(b)	Nutrient in green pepper leaves ⁽¹⁾			
	Total N	Total P	Total K	
		kg ha ⁻¹		
NPKB ₅₀ (50% RR)	$23,7^{c} \pm 2.21$	2,27 ^b ±0.31	8,62 ^b ±1.11	
NPKB ₁₀₀ (100% RR)	26,5 ^b ±2.54	$2,49^{b} \pm 0.25$	$10,20^{ m b}\pm1.52$	
NPKB ₁₅₀ (150% RR)	$28,0^{ab}\pm 2.55$	2,65 ^{ab} ±0.14	$11,18^{ab} \pm 1.24$	
NPKP ₅₀ (50% RR)	$23,4^{\circ}\pm2.38$	$2,08^{b} \pm 0.20$	$9,10^{\rm b}\pm1.31$	
NPKP ₁₀₀ (100% RR)	28,2 ^{ab} ±2.44	$2,54^{\rm b}\pm 0.31$	$12,03^{a}\pm1.42$	
NPKP ₁₅₀ (150% RR)	31,5 ^a ±2.33	$3,06^{a} \pm 0.28$	$13,53^{a} \pm 1.26$	
NPKF ₁₀₀ (100% RR)	$28,4^{ab}\pm 2.71$	$2,38^{b}\pm0.29$	$10,08^{b} \pm 1.20$	
Control (farmyard manure)	$22,4^{c}\pm 2.84$	2,08 ^c ±0.31	$8,04^{b} \pm 1.55$	
CV (%)	9	13	18	

Table 4: Total N, P and K in green pepper fruits as affected by fertilization with biofertilizer (NPKB), bioprotector (NPKP) applied in three rates, mineral fertilizer (NPKF) in recommended rate and the control treatment (earthworm compound – 2,4 kg plant⁻¹) ^(a)

^(a) Means with the same letter are not different by the Tukey test ($p \le 0.05$).

^(b) NPKB = Organic matter (earthworm compound) plus PK rocks biofertilizer inoculated with free living diazotrophic bacteria; PNPK = Protector (NPKB with fungi chitosan from C. elegans); FNPK = soluble fertilizers (RR recommended rate), control (earthworm compound 2.4 kg plant¹). CV= Coefficient of Variation.