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Effects of biofertilizers produced from rocks and organic matter, enriched by diazotrophic bacteria inoculation on growth and yield of sugarcane

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Abstract

Fertilizers improve nutrient absorption and plant growth. They are the most important factors to increase sugarcane yield. The aim of this study is to evaluate the effectiveness of biofertilizer produced from P and K rocks and organic matter (earthworm compound) (OM) enrich in N, and inoculation with free living diazotrophic bacteria on sugarcane yield and technological characteristics. The field experiment was conducted at the Santa Teresa sugarcane industry, located in the Brazilian Northeastern. The treatments were: Two sources of NPK (biofertilizer-NPKB and soluble fertilizers-NPKF) applied in three rates (NPKB 5000, 7500, 10000 kg ha^{-1}; NPKF 500, 750, 1000 kg ha^{-1}). Earthworm compound added as control, and the NPKB was inoculated with diazotrophic bacteria. The experiment was laid out as factorial (7×2) in randomized block design with four replicates. At harvest, the plant height, stalk diameter and yield, dry biomass (tops, leaves and stalks), soluble solids, sucrose, purity and total sugars were determined. The results showed that the biofertilizers increased sugarcane productivity. The best yield obtained when filter mud cake (FMC) along with NPKB were used, and NPKF applied in recommended rate (RR). The interaction between FMC and NPKB showed a great effect in plant characteristics. Based on results of this study, the NPKB is suggested as potential alternative for mineral fertilizers.

Keywords: *Saccharum* spp., earthworm compound, free-living diazotrophic bacteria, sustentable agriculture, organicmineral fertilization,
Abbreviations: FMC_filter mud cake; NPKB_biofertilizer with NPK; NPKF_soluble fertilizer with NPK; RR_Recommended Rate.

Introduction

Soluble fertilizers are of great importance to increase plant yield but their use by low-income farmers is difficult due to the high price and high solubility, which promotes percolation into the soil and plant damage (Van Straaten, 2007). In a modern and sustainable agriculture, the use of biofertilizers and soil amendments is eligible to increase plant production and to meet economic criteria to enhance soil fertility and to minimize environmental damage (Elsayed et al., 2008). In general, Brazilian soils present low available P content and renewable natural sources of phosphate. Therefore, it is necessary to carry out studies to determine the efficient use of these sources in agriculture (van Straaten, 2007). The high demands for fertilizers and efforts to reduce environmental problems and the scarcity of primary materials to produce soluble fertilizers have drawn attentions for efficient use of fertilizer sources in sustainable agriculture (Stamford et al., 2009). An alternative for effective and economic fertilization is the production of biofertilizers from phosphate and potash rocks mixing elemental sulfur inoculated with *Acidithiobacillus.* Sulfur-oxidizing bacteria are important in recycling nutrients in the soil and some species have relevant importance to release elements from rocks (Van Straaten, 2007; Stamford et al., 2006, 2008).

Brazil is currently the largest producer of sugarcane in the world with over 600 million tons of sugar processed every year, with an average yield of 60 t ha^{-1} (Stamford et al., 2008). To increase productivity, application of soluble fertilizers is a common agricultural practice in cultivation of sugarcane. However, the production of conventional NPK fertilizers has a rather high cost with large energy consumption, and only large companies perform the production processes.

In this context, production of biofertilizer from rocks mixed with organic matter, enriched in N by inoculation with free-living diazotrophic bacteria, could be an interesting alternative (Lima et al., 2010). The biofertilizer production is practical and can be processed with low energy consumption. The increase on nutrient availability minimizes the environmental impacts, because the release of nutrients for plants is realized gradually reducing the leaching to deeper layers and to the water table which may reduce environmental problems.

The effectiveness of phosphate and potash rock biofertilizers in various economic crops in different soil types in greenhouse conditions has already been described (Stamford et al., 2006, 2008, 2011). Moreover, in a previous laboratory study the potential of free-living diazotrophic bacteria to increase the total N content in organic matter has also been demonstrated (Lima et al., 2010).

The aim of this study is to evaluate the effects of PK rock biofertilizers mixed with earthworm compound, enriched in N, using inoculation with free-living diazotrophic bacteria (NPKB), on growth and yield characteristics of sugarcane. The experiment was conducted in field conditions, in which application of soluble mineral fertilizers (NPKF) at different rates, in presence and absence of filter mud cake (FMC) were compared with biofertilizers.

Results

The results showed that yield and shoot dry biomass were affected by the fertilizers treatments with and without FMC application (Table 1). The filter mud cake (FMC) showed significant effects for the evaluated parameters, in all fertilization treatments. For the fertilizer effects, increase may be observed between the sources and rates. The BNPK (150% RR) displayed the best results on yield and shoot dry biomass, when compared with the fertilizer treatments without FMC.

The biofertilizer (NPKB) applied in 150% RR promoted the best results (86.6 and 70.9 t ha^{-1}), greater than mineral fertilizer treatment (NPKF) with and without filter mud cake (FMC) application (64.5 and 67.7 t ha^{-1}). The biofertilizer in 150% RR showed significant difference on sugarcane yield with and without FMC, which promoted very great difference on sugarcane yield, comparing with the control treatment (earthworm compound) with and without FMC (53.6 and 49.3 t ha^{-1}). Thus, the effects of the FMC application was evident on sugarcane yield and shoot biomass that showed positive and significant response for the different fertilization treatments, with and without FMC application.

The results of sugarcane plant height and stalks diameter subjected to fertilization treatments and filter mud cake (FMC) were shown in Table 2. It shows increase of height by FMC application, especially in plants that received the higher rates of the different fertilization treatments. The maximum height achieved when NPKB applied in higher rates (225 cm). Furthermore, without FMC, significant increase in plant height was not observed by application of NPKB and NPKF in rates equivalent to 100 and 150% RR. However, when FMC applied, the NPKB treatment showed great increase on plant height, which significantly differed for NPKF in the used rates. For stalk diameter low response of the fertilization treatments were observed. No significant difference of FMC application was observed. The maximum stalk diameter was achieved, when NPKB and NPKF in higher rates applied. Application of earthworm compound (control) showed the low values with significant difference, compared with the others fertilization treatments ($p \leq 0.05$).

The data of total soluble solids (Brix) and apparent sucrose (Pol) in sugarcane analysis for the different fertilization treatments with and without filter mud cake (FMC) are shown in Table 3. In general, Brix and Pol displayed best results under treatments containing the sugarcane FMC. The results for Brix were greater, when biofertilizers applied in rates of 100 and 150% RR, especially in the treatment with mud cake application and without mud cake application. Low effect of the fertilization treatments observed on brix, but the best results obtained when NPKB and NPKF applied in higher rates (150% RR).

The results on Pol present greater effect of NPKB, especially when applied in the higher rate with FMC (14.15%). No significant difference for the NPKF treatment with FMC (13.70%) was observed on Pol.

The results for Purity and RTS, affected by the fertilization treatments with and without FMC, are present in Table 4. There were greater effect of FMC application for Purity in the control (68.5 and 76.8 with and without FMC) and RTS (90.7 and 102.9 with and without FMC). This represents low values. The NPKB application in rate of 150% RR showed some effects on Purity (84.9 and 83.5 with and without FMC), and for RST (116.2 and 120.0 with and without FMC), which are similar with effects of NPKF applied in 150% RR for Purity (.83.9 and 83.1 with and without FMC) and for RST (104.3 and 112.6 with and without FMC)

Discussion

Stamford et al. (2008) reported positive and significant effects of the PK rock biofertilizer inoculated with *Acidithiobacillus* and observed best effectiveness in relation with the soluble mineral NPK fertilizer in sugarcane shoot dry biomass.

The results are also in accordance with Santos et al. (2010) that found positive correlation between sugarcane yield and mud cake application in field conditions. In addition, Rossetto et al. (2008), described that filter mud cake (FMC) promoted increase in sugarcane yield and supported that the organic matters release phosphorus and calcium and others nutrients increasing nutrient uptake. These results are in accordance with Elsayed et al. (2008) that described interaction of organic and mineral fertilizers in the plant nutrient status.

The NPKB applied in 150% RR produced the best results, greater than the mineral fertilizer treatment and dependent of the sugarcane FMC, with very different sugarcane yield (86 and 25.5), with and without FMC application, respectively. The results are higher than the normal sugarcane yield (50 t ha^{-1}) obtained in the Pernambuco state (IPA, 2008).

The obtained results for yield and shoot dry biomass are in accordance with Stamford et al. (2008) who concluded that the PK rock biofertilizer inoculated with *Acidithiobacillus*, mixed with earthworm compound, may be applied as alternative for replacement of mineral fertilizer, in field conditions.

In greenhouse conditions, the application of P and K rock biofertilizers in tableland soil (Stamford et al., 2006) increased the sugarcane yield and showed effects on decrease of P and K in soil, especially when applied in the highest rate. El Tarabily et al. (2006) described the effects of sulfur oxidative bacteria increasing soil fertility and maize growth, when applied in calcareous soils in United Arab Emirates, especially in availability of Phosphorus. Ossom and Dlamini (2012) described the effects of filter mud cake on onion productivity and increase of nutrients.

Dario et al. (2003) reported the significant effects by application of sugarcane residues. Their results confirmed the increase in sugarcane plant height in field conditions. In addition, the sugarcane FMC combined with mineral fertilizers resulted in higher levels of N, P, K and organic carbon in soil (Kaur et al., 2005; Elsayed et al., 2008). Pereira et al. (2005) also described significant and positive effect of sugarcane FMC on cotton height. These results demonstrated the potential of the biofertilizer in sugarcane as reported by Stamford et al. (2006) for sugarcane in greenhouse experiment.

The effectiveness of PK rock biofertilizers inoculated with oxidative bacteria *Acidithiobacillus* reported by Stamford et al. (2008) demonstrated the positive and significant effects of biofertilizers in sugarcane, grown in the tablelands of the Brazilian rainforest region, especially when applied along with PK rock biofertilizer mixed with earthworm compound.

Nitrogen is one of the most important nutrients to increase plant growth and yield. It has vital roles in some chemical compounds such as proteins, nucleic acids and many other components necessary for all kind of life in the world. However, the P and K rock biofertilizers do not directly release N to be uptake by plants and microbial organisms in

Table 1. Sugarcane shoot dry biomass and stalk yield subjected to different source and rates of biofertilizer NPKB and mineral soluble fertilizer NPKF, with and without FMC.

Fertilization	Shoot Dry biomass (t ha^{-1})		Stalk yield (t ha^{-1})	
(% RR)[2]	Without FMC	With FMC	Without FMC	With FMC
Control	28,97±1,17Gb	30,92±1,12Ea	49.3±2.81Gb	53.6±2.86Fa
NPKB 50	46,28±0,80Db	59,79±0,80Da	59.2±2.76Eb	79.8±5.30Ca
NPKB 100	47,02±1,89Cb	60,07±0,65Ba	62.9±2.70Cb	80.8±0.77Ba
NPKB 150	49,78±0,99Ab	66,16±1,20Aa	70.9±1.56Ab	86.6±0.85Aa
NPKF 50	31,3, ±0,55Fb	45,03±3,70Da	57.8±1.21Fb	65.9±1.61Ea
NPKF 100	45,43±9,76Eb	53,02±0,65Ca	61.9±0.62Cb	67.6±2.66Ea
NPKF 150	48,49±1,03Bb	56,46±1,25Da	64.5±1.00Bb	67.7±5.80Da

Data followed by the same letter, upper case letter in columns and low case letters in rows, are not different by the Tukey's test ($p \leq 0.05$). C.V. (%) = shoot biomass = 9.76; yield = 9.25. [2] Percent of recommended rate for sugarcane in the state of Pernambuco (IPA, 2008). Control = earthworm compound (10 t ha^{-1}).

Table 2. Plant height and stalk diameter of sugarcane with biofertilizer (NPKB) and mineral fertilizer (NPKF) application, subjected to FMC addition[1]

Fertilization/	Plant height (cm)		Stalk diameter (mm)	
(RR-%)[2]	Without FMC	With FMC	Without FMC	With FMC
Control	104.50±0.58Ca	113.25±0.5Ca	17.45C	17.39C
NPKB 50	149.00±0.82Ab	207.63±19.5Aa	18.39BC	18.27BC
NPKB 100	154.00±1.63Ab	211.50±1.9Aa	22.17A	22.00A
NPKB 150	162.75±3.77Ab	225.25±0.96Aa	22.30A	22.20A
NPKF 50	128.25±5.68BCb	164.00±20.74Ba	19.63B	19.00B
NPKF 100	148.00±.2.52Aa	152.50±0.58Ba	21.95A	21.70A
NPKF 150	151.50±0.58Aa	153.50±17.94Ba	22.38A	22.25A

[1]Data with the same low case letter in rows and up case letter in the different fertilizers treatments were not different by the Tukey test ($p \leq 0.05$). C.V. (%) = height = 6.94; diameter = 6.72. [2] Percent of recommended rate for sugarcane in the state of Pernambuco (IPA, 2008). Control = earthworm compound (10 t ha^{-1}).

soil are necessary to release this nutrients. On the other hand, the mixture of rock biofertilizers with organic matter, such as earthworm compound, inoculated with free-living diazotrophic bacteria showed effectiveness in biological nitrogen fixation (BNF) (Lima et al., 2010). This process may be important to increment soil fertility.

Caione et al. (2011) demonstrate the effectiveness of FMC application, especially by increasing the phosphorus availability. They found significant effect on sugarcane's stalk diameter due to P application. In addition, they reported no effects of the fertilization treatments with and without FMC application.

There is no literature on effect of biofertilizer in Brix and Pol on sugarcane. Brix and Pol displayed highest values under mud cake treatments. The data are in agreement with Santos et al. (2010) that found greater values of Brix and Pol in sugarcane when sugarcane residues applied.

Santos et al. (2010), studied the influence of mud cake and phosphorus application on sugarcane, and obtained excessive values of Brix and Pol when higher rates of phosphorus applied (Stamford et al., 2009). Stamford et al. (2008), in a greenhouse study also verified positive and significant effect of PK rock biofertilizer inoculated with *Acidithiobacillus* mixed with earthworm compound in increasing soil nutrients availability, compared with application of mineral fertilizers.
The effects of the NPKB inoculated with the free living diazotrophic bacteria were significant. Furthermore, there were greater effect for FMC application on Purity and ATR. These results are in accordance with Santos et al. (2010) who also obtained greater values of Purity and ATR when sugarcane residues applied. The effects of BNPK biofertilizer in rate of 150% RR were greater than the different sources and rates of other fertilization treatments, with and without FMC application.

Materials and Methods

Site and soil description

A field experiment was conducted in the Santa Tereza sugarcane Industry, located at the Goiana District, Pernambuco state, Brazil, with the geographical coordinates 07 º 33 'S and 35 º 00' W and altitude 13 m. The soil classified as Spodosol Humiluvic Ortic (Embrapa, 2006), representing the characteristic of the tableland from the Brazilian rainforest region.

The sugarcane (*Saccharum* spp.–variety RB92579), recommended for the rainforest region by the Sugarcane Experimental Station of Carpina (SESC/UFRPE), was cropped during August 2010 to December 2011.

The soil samples were collected in deep 0-30 cm, before filter treatments application. The chemical and physical analysis were processed (Embrapa, 2009) and presented the following results: pH water (1:2,5) = 6.0; Al = 0.1 (cmol$_c$ dm^{-3}); total N = 0.3 (g kg^{-1}); available P = 12 (mg dm^{-3}); exchangeable cations (cmol$_c$ dm^{-3}) - K = 0.05; Ca = 2.54; Mg = 0.94; CTC = 3.63 (cmol$_c$ dm^{-3}); Density = 2.65 g kg^{1}; granulometric analysis (g kg^{1}); coarse sand = 740; fine sand= 210; silt = 10; clay= 40.

Biofertilizer production

The P and K rock biofertilizers were produced at the University Federal Rural of Pernambuco, using 4,000 kg of natural phosphate (11 % total P, purchased from Irecê Bahia), Brazil) and 4,000 kg of biotite (10 % total K), from Santa Luzia (Paraíba), Brazil, following the procedure described by Stamford et al. (2008).

Analysis of the P and K rock biofertilizer were: (P-biofertilizer)-pH= 3.8, available P = 60 (g kg^{-1}); (K-biofertilizer)- pH = 3.3, available K = 10 (g kg^{-1}). The mixed biofertilizer (BNPK) from PK rock biofertilizers plus earthworm compound organic matter (OM) inoculated with the selected free-living bacteria (NFB 1001) cultured in LG liquid media according to Lima et al. (2010). The BNPK was produced mixing 4 m^{3} (OM) with BPK biofertilizers (1 dm^{3}). The chemical analysis of the earthworm compound showed: pH 7.15; organic carbon (100.7 g/kg); total N (8.6 g/kg); total S (2.98 g/kg); available P (11.2 g/kg). The Biofertilizer at the final period present (Embrapa, 2009): pH (H_2O) = 6.9; total

Table 3. Total soluble solids (Brix) and apparent sucrose (Pol) for sugarcane submitted to fertilization treatments with and without sugarcane mud cake (FMC).

Fertilization / (%RR)	Brix (%)		Pol (%)	
	Without FMC	With FMC	Without FMC	With FMC
Control	13.59±0,59Cb	15.06±0,01Da	9.32±0,11Eb	11.58±0,55Ca
NPKB 50%	14.22±0,42Bb	16.61±0,06Aa	11.17±0,15Cb	13.01±0,59Ba
NPKB 100%	15.47±0,38Ab	16.84±0,13Aa	12.35±0,51Bb	13,82±0,42Aa
NPKB 150%	15.87±0,39Ab	16.95±0,19Aa	13.47±0,35Ab	14.15±0,43Aa
NPKF 50%	14.39±0,02Ba	15.25±0,26Ca	10.11±0,18Db	12.14±0,32Ca
NPKF 100%	14.43±0,04Bb	15.31±0,08BCa	11.96±0,03Ba	12.40±0,28Ca
NPKF 150%	15.41±0,01Ab	16.48±0,04Ba	12.94±0,06ABb	13.70±0,10Aa

Data with by the same letter (upper case letter in columns and low case letters in rows), are not different by the Tukey's test ($p \leq 0.05$). C.V. (%) Brix = 3.42 and Pol = 4.15. [2] Percent of recommended rate for sugarcane in the state of Pernambuco (IPA, 2008). Control = earthworm compound (10 t ha^{-1}).

Table 4. Purity and Recoverable Total Sugar (RTS) for sugarcane submitted to the different sources and rates of fertilizers with and without sugarcane mud cake FMC.

Treatment /RR	Purity (%)		RTS (kg t^{-1})	
	Without FMC	With FMC	Without FMC	With FMC
Control	68.5±0.18Fb	76.8±0.69Ea	90.7±0.28Fb	102.9±0.34Ga
NPKB 50%	78.5±0.58Da	79.3±0.37Da	98.7±0.58Db	109.9±0.60Da
NPKB 100%	79.8±0.13Cb	82.0±0.58Ca	109.1±0.69Bb	116.9±0.65Ba
NPKB 150%	84.9±0.10Aa	83.5±0.30Aa	116.2±0.78Ab	120.0±0.58Aa
NPKF 50%	70.2±0.04Eb	79.6±0.57Da	92.1±0.59Eb	105.7±0.58Fa
NPKF 100%	82.9±0.06CDb	80.9±0.24Ca	98.9±0.18Db	108.4±0.14Ea
NPKF 150%	83.9±0.54Bb	83.1±0.71Ba	104.3±0.28Cb	112.6±0.20Ca

Data followed by the same letter, upper case letter in columns and low case letters in rows, are not different by the Tukey test ($p \leq 0.05$). C.V. (%) Purity = 2.7 and RTS = 4.15. [2]Fertilization in function of the recommendation for sugarcane in Pernambuco state, by IPA (2008). Control = earthworm compound (10 t ha^{-1}).

total N (21 g/kg) available P (20 g/kg) and available K (19 g/kg). To prepare the mixed mineral fertilizer (NPKF) we used: ammonium sulphate (20% N); simples superphosphate (20% P_2O_5) and potassium sulphate (50% K_2O). The amount for application was in accord with the soil analysis and the recommended rate for sugarcane for the Pernambuco state (IPA, 2008).

Soil preparation and treatments

The soil was prepared by cutting and removing all of the vegetation from the experimental area. Soil was prepared by the conventional tillage processing, one plowing and two disking, and the rows opened to plantation. The rows were prepared systematically to maintain declivity around 0.2-0.5% to avoid soil run off.

The experiment was set up in a factorial (7 x 2) in randomized block design with four replicates. The fertilization treatments used two sources of NPK (biofertilizer and soluble fertilizer) applied in three rates (50, 100 and 150% recommended rate for sugarcane in the Pernambuco state, Brazil). Additionally, the treatments were conducted with sugarcane filter mud cake (FMC) (60 t ha^{-1}) and also No-filter mud (without filter mud cake). At harvest, the following characteristics were determined: plant height; Tirar diameter and yield; dry biomass (tops, stalk and leaves); soluble solids, sucrose, purity and total sugars.

The treatments with sugarcane FMC (60 t ha^{-1}) and without sugarcane FMC were applied in a factorial using the following fertilization treatments: (1) Biofertilizer (BNPK) and (2) Mineral Fertilizer (FNPK), applied in 3 rates: BNPK rate 1 (5000 kg ha^{-1}); BNPK rate 2 (7500 kg ha^{-1}); BNPK rate 3 (10000 kg ha^{-1}); FNPK rate 1 (500 kg ha^{-1}); FNPK rate 2 (750 kg ha^{-1}); FNPK rate (1000 kg ha^{-1}). A control treatment with earthworm compound (20 t ha^{-1}) was applied for comparative purpose (as control). Each plot contained four rows with 10 m long and 1 m between rows. For calculation, 10 m^2 from the 2 central rows was considered.

Determinations and statistical analyzes

The sugarcane height (cm) was determined in ten plants of each plot. The height data was calculated from the soil level to the intersection of the leave +1, in 40 plants per the fertilization treatments. The medial diameter (mm) was determined in the plant base (15 cm from the soil surface), and the sugarcane yield was estimated in plant harvest, using a dynamometer equipment used by the sugarcane industry. Plant biomass of sugarcane was determined by wet and dry weight of leaves, stalks and up shoots (pointers) separately and followed calculating the total plant biomass.

Sugarcane technological characteristics were evaluated by analyzes of soluble solid concentration (Brix), apparent sucrose (pol), purity (pur) and total recoverable sugars (TRS). The technological analyzes were processed in the Santa Tereza sugarcane industry, in accordance with Consecana (2006).

The statistical calculations for the field experiment parameters were achieved using analysis of variance, in which the effects of fertilization and the FMC interactions were calculated by SAS software Program 11.0 version (SAS Institute, 2011). Analyzes of variance and averages were compared by the Tukey's test at probability ($p \leq 0.05$). All parameters analyzed were normally distributed.

Conclusions

The present study showed that biofertilizer (NPKB) produced from PK rock inoculated with *Acidithiobacillus* bacteria mixed with organic matter (earthworm compound), enriched the N by inoculation with diazotrophic bacteria may be used as biofertilizer to increase sugarcane yield and some plant and industrial parameters. The biofertilizer (NPKB) may be used as alternative to soluble mineral fertilizers. The FMC application showed positive and significant effects in the sugarcane parameters and had interactive effects with the fertilization treatments.

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