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Growth and nutritional status of maize plants in response to different doses and application frequencies of biofertilizer

Crescimento e estado nutricional de plantas de milho em resposta a diferentes doses e frequências de aplicação de biofertilizante

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Abstract

This study analyzed the effect of different doses and application frequencies of a dairy cattle-derived biofertilizer on the growth and nutritional status of maize cv. BRS Caatingueiro in a Yellow Latosol. The experiment was conducted in an open-air nursery at the Federal University of Vale do São Francisco, Juazeiro-BA. The experimental design was a randomized complete block design in a 6 x 2 factorial scheme, with six biofertilizer doses (0, 120, 240, 360, 480, and 600 mL per plant) and two fertigation frequencies (7 and 14 days), with five replicates. The seeds of maize cv. BRS Caatingueiro were planted in pots filled with Yellow Latosol. During the crop cycle (90 days), the following variables were evaluated: height, diameter, and number of leaves. During harvest, the variables evaluated were: root and shoot fresh and dry weight, root volume, chlorophyll index, and macro- and micro-nutrient contents of leaf and root tissue. Biofertilizer applications at 14-day frequencies promoted better plant growth compared to 7-day frequencies. However, despite the lower accumulation of some nutrients in relation to the application of larger doses, the dose of 360 mL biofertilizer promoted better root and leaf growth. Considering the uniformity and economy of the application of the product, it is recommended to apply the dose of 360 mL per plant every 14 days.

Additional keywords: liquid compound; nutrient recycling; *Zea mays* L.

Resumo

Este estudo teve como objetivo verificar o efeito de diferentes doses e intervalos da aplicação de biofertilizante, oriundo da bovinocultura leiteira, sobre o crescimento e o estado nutricional de plantas de milho BRS Caatingueiro, em Latossolo Amarelo. O experimento foi conduzido em viveiro aberto, localizado na Universidade Federal do Vale do São Francisco, Juazeiro-BA. O delineamento experimental utilizado foi o em blocos casualizados, com esquema fatorial 6 x 2, sendo seis doses de biofertilizante (0, 120, 240, 360, 480 e 600 mL por planta) e dois intervalos de aplicação da fertirrigação (7 e 14 dias), com cinco repetições. As sementes da cultivar de milho BRS Caatingueiro foram plantadas em vasos preenchidos com Latossolo Amarelo. Durante o ciclo da cultura (90 dias), foram avaliados a altura, o diâmetro, o número de folhas, e durante a colheita, as massas fresca e seca da parte aérea e da raiz, o volume de raiz, o índice de clorofila e os teores de macro e micronutrientes do tecido foliar e da raiz. As aplicações de biofertilizante, com frequência de 14 dias, promoveram melhor desenvolvimento das plantas, em relação à fertirrigação com sete dias. Entretanto, apesar de manter menor acúmulo de alguns nutrientes em relação às aplicações de doses maiores, a dose de 360 mL de biofertilizante promoveu melhor desenvolvimento de raiz e folhas das plantas. Considerando a uniformidade e a economia da aplicação do produto, recomenda-se a aplicação da dose de 360 mL por planta, a cada 14 dias.

Palavras-chave adicionais: composto líquido; reciclagem de nutrientes; *Zea mays* L.

Introduction

Liquid biofertilizers are extremely important when used to maximize plant growth, either as simple or enriched microbial fermenters, bringing together almost all macronutrients, micronutrients, and intermediary metabolites that act on cell metabolism and, consequently, on plant growth and development (Silva et al., 2007; Barros & Filho, 2008; Pinheiro & Barreto, 1996; Alves et al., 2009).

Biofertilizers result from fermentation, that is, from microbial synthesis on organic and mineral matter, with formation of sugars, lipids, amino acids, peptides, polypeptides, proteins (enzymes), vitamins, and others, dispersed in colloidal solution, acting on secondary metabolism and affecting plant health.

Biofertilizer production does not require a specific formula, consisting of a simple understanding of the fermentation process and its basic constituent elements, which depend on access and availability, being generally found in the surroundings of rural properties.

There are several sources of organic and mineral elements that can be used in biofertilizer production. However, the following are generally used for this purpose: fresh bovine manure (bacterial inoculant), a source of energy to feed the bacteria (molasses, milk, among others), mineral elements for compound enrichment (ashes, ground rock), and water (non-chlorinated, with sourcing from a river or rainwater) for dilution (Pinheiro & Barreto, 1996; Medeiros et al., 2007).

The fermentation process can be either anaerobic (no oxygen) or aerobic (with the presence of oxygen), the result being a liquid compound that can be used in foliar and soil fertilization or directly in the irrigation system (Kiehl, 1985; Araújo et al., 2007; Medeiros et al., 2011).

The time of preparation of the biofertilizer is related to the end of the fermentation process. For regions where the temperature is higher, such as the semiarid region, this period lasts around 15 to 30 days. For regions with lower temperatures, in turn, this period increases to 45 days.

Organic solutes from the biofertilizer promote chemical and physical improvements in the soil, and increase microbial activity and nutrient availability, which favors cell elongation and plant growth (Freire et al., 2010; Pereira et al., 2010). According to Barros & Filho (2008), liquid biofertilizer application stands out because of the faster assimilation by the plant, being very useful for short-cycle crops with high nutrient demand.

The use of biofertilizers in the form of enriched microbial fermenters has been one of the most used processes in trophobiotic management of crops, contributing to increased natural resistance of plants to the attack of pests and pathogens, besides exerting a direct action on phytoparasites (Alves et al., 2009).

According to Pinheiro & Barreto (1996), the

biofertilizer concentration of 5% provides nutrients and intermediate metabolites to the soil, which favor nutrient availability by the action of microorganisms.

The use of phosphate and potassium biofertilizers produced from rocks mixed with organic matter such as earthworm humus has shown high efficiency in research with different soils and crops (Lima et al., 2010).

Mesquita et al. (2010) verified that the application of biofertilizer enriched with protein mixture and macro- and micronutrients promoted the accumulation of sodium and macro and micronutrients in the leaves of plants and affected the export of the respective chemical elements at fruit harvest, at all levels of input application to the soil.

According to reports evidenced in maize research, biofertilizer application has been very important for plant growth (Bezerra et al., 2008; Lima, 2012; Sousa et al., 2012). Lima et al. (2012), in an experiment carried out in the field, obtained increased plant height, stem diameter, and dry matter yield of maize after sixty days of bovine biofertilizer application.

In this context, this work evaluates the effect of doses and application frequencies of biofertilizer on the growth and nutrient content of maize plants.

Material and methods

The experiment was conducted in an open-air nursery at the experimental area of the Federal University of Vale do São Francisco (UNIVASF), in Juazeiro-BA, from December to January 18, at the following geographic coordinates: 09°25'00"S, 40°30'00"W, and altitude of 371 m. Annual temperature and rainfall averages are 24.2 °C and 430 mm, respectively (CPRM, 2005). According to Köppen classification, the climate is type BSw. The experimental design was randomized blocks in a 6 x 2 factorial scheme, with five replicates. The treatments consisted of six different doses of biofertilizer: 0; 120, 240, 360, 480, and 600 mL plant⁻¹; applied by means of fertigation, at two application frequencies (7 and 14 days), during the initial 90 days of the cycle of maize (*Zea mays* L.) cv. BRS Caatingueiro.

The climatic conditions during the experiment are shown in Figure 1, with the monthly averages of maximum temperature, minimum temperature, mean temperature, and rainfall.

The experiment was carried out using 12-liter pots filled with soil collected at the 0-20 cm depth layer of a Yellow Latosol with no history of cultivation. Initial chemical characterization was performed according to Embrapa (2009), as shown in Table 1.

After the soil was sieved, portions of 11 dm³ received 500 g of earthworm humus as a soil conditioner. The soil portions were transferred to the pots, followed by sowing of the cultivar BRS Caatingueiro using two seeds per pot, at a depth of about 2/3 of the seed size. After emergence, thinning was performed maintaining one plant per pot.

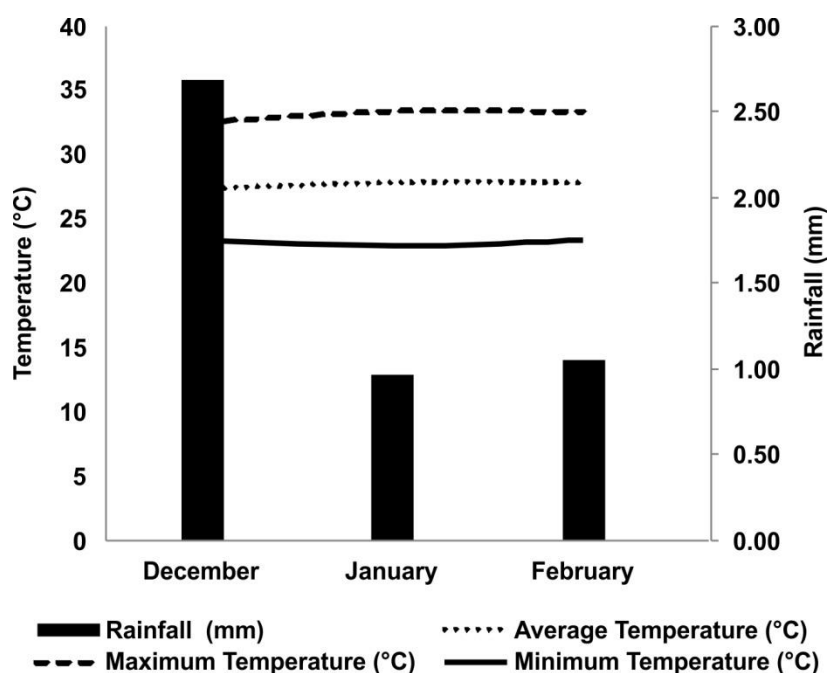


Figure 1 - Monthly averages of temperature, and rainfall referring to the crop cycles. Collected data in the weather station near experiment.

Table 1 - Chemical characterization of the soil before start of the experiment.

EC (dS m ⁻¹)	pH	OM (g kg ⁻¹)	P (mg dm ⁻³)	V (%)	Cu ----- (mg dm ⁻³)	Fe ----- (mg dm ⁻³)	Mn -----	Zn -----
0.55	6.3	8.0	5.0	67	4.2	42.3	141	2.0
K	Na	Ca	Mg	Al	H+AL ----- (cmol _c dm ⁻³)	SB -----	CTC -----	
0.60	0.08	3.1	1.5	0	2.6	5.3	7.8	

EC - electrical conductivity of the saturation extract; OM.- organic matter; P - available phosphorous extracted by Mehlich¹; Ca²⁺- exchangeable calcium; Mg²⁺ - exchangeable magnesium; Na⁺ - exchangeable sodium; K⁺ - exchangeable potassium; Al³⁺ - exchangeable acidity; CTC - cation exchange capacity; V - base saturation; Fe - iron available; Mn - manganese; Cu - copper; Zn - zinc. Micronutrients extracted by Mehlich¹

The biofertilizer was produced in a 500-L plastic water tank covered with a lid, which was closed to generate aerobic conditions and positioned so as to avoid sunlight. The container was closed for 30 days, so that the anaerobic microorganisms could ferment the material and produce the biofertilizer, whose composition can be observed in Table 2.

A biofertilizer sample was filtered, frozen, and sent for microbiological analysis to assess the quality of the product. Biological analyses for the separation of polypeptide substances were carried out at the Chemistry and Environmental Laboratory of Porto Alegre, RS, by means of electrophoresis, according to Pfeiffer's Chromatography adapted by Rivera & Pinheiro (2011). Table 2 shows the results of the analyses.

The biofertilizer was applied through hermetically sealed bypass injectors, in which the diluted solution was injected into the irrigation system by pressure difference. A pressure gauge was installed on the tubing as an aid to maintain optimum operating pressure

for drippers. The applications started after sowing until harvest (90 days).

Drip irrigation was performed using the TDR 100 equipment to maintain soil water balance. PVC tubes were used to calibrate the equipment. The tubes had 0.15 m in diameter and 0.15 m in length, being filled with the same soil used in the experiment where the reading probes were coupled. The soil was saturated and set to drain and dry naturally, in which constant moisture readings were carried out by TDR 100 and gravimetry, the containers being weighed in the latter. Data were tabulated and soil field capacity was determined. Then, a mathematical model was generated to fit the moisture content provided by the TDR 100. In practice, soil moisture readings were taken with TDR 100 before irrigation, calculating the amount of water needed for the soil to return to field capacity.

For the determination of total nitrogen and crude protein, leaf and root tissue samples were subjected to sulfur digestion and quantified by the Kjeldahl method (Embrapa, 2006).

Table 2 - Characteristics of the biofertilizer produced in aerobic condition. Identification of biological and microbiological composition.

Materials used in the preparation of the biofertilizer			
Composition	Origin	Unity	Quantity
Water	River	L	300
Fresh manure	Lactating cows	L	80
Seaweed	<i>Lithothamnium</i>	kg	4
Molasses	Dehydrated	kg	6
Honey	Bees	L	2
Rock powder 1	MB-4	kg	5
Rock powder 2	Phosphate	kg	5
Composição biológica			
Parameter	Type	Method	Result
Hydrophilic oligopeptides	Bacillysin	Electrophoresis	Present
	Chlorotetain	Electrophoresis	Present
	Rhizocticin	Electrophoresis	Present
Lipopeptidantibiotic	Surfactin	Electrophoresis	Present
	Fengymycin	Electrophoresis	Present
	Iturin A	Electrophoresis	Present
Cyclopeptide	Mycobacilin	Electrophoresis	Present
Microbiological composition			
Parameter	Method	Result	Unity
Heterotrophic bacteria	Standard Methods 9215 B	8.7 x 10 ³	UFC/mL
Fecal coliforms (<i>E. coli</i>)	Standard Methods 9223 B	<1.8	NMP/100mL
<i>P. aeruginosa</i>	Pour Plate	<1.8	UFC/mL
<i>Salmonella</i> sp.	Pour Plate	Absent	UFC/25 ml
<i>Staphylococcus aureus</i>	Pour Plate	<1.0	UFC/mL

For the determination of P, K, Ca, Mg, S, Cu, Fe, Mn, Zn, B, and Na in leaf and root tissue, the methodology of Embrapa (2009) was used.

Chlorophyll indexes a and b were determined from readings performed on mature leaves located in the middle third of the plants, selecting the medium leaflet. A chlorophyll meter ClorofiLOG CFL 1030 was used for measurements (Carvalho et al., 2012).

At ninety days after sowing, the following analyses were performed: plant height (measured using a 30 cm ruler), stem diameter (measuring the plant collection area using a digital caliper), and number of leaves. To determine root volume and root fresh and dry weight at the end of the experiment, the roots were removed from the soil and separated from the shoots.

For biomass determination, all plant components (root, stem, panicle, and leaf) were harvested and weighed separately. Both main and fine roots were removed from the pots by means of running water. After complete removal from the soil, the roots were weighed for biomass determination. Then, the roots were placed in test tubes with water, whose increase in volume corresponded to the volume of roots. Samples comprising the stem, panicle, leaves, and roots were dried at 50 °C in a forced-air ventilation oven until constant weight for dry biomass determination.

The results were submitted to analysis of variance and the means compared by the Tukey test at 5% probability and regression analysis. The analyses were performed with the aid of the statistical program SISVAR - System for Analysis of Variance (Ferreira, 2003).

Results and discussion

Biofertilizer application affected ($P < 0.5$) root volume and leaf dry mass, with interaction between doses and application frequencies of biofertilizer. However, only the applied dose of 360 mL significantly affected root volume, while the dose of 600 mL was significant for leaf dry matter production, with the best results being obtained with the 14-day frequency (Figure 2).

Leaf dry weight (Figure 2B) was significantly affected by the interaction between doses and application frequencies, showing a value of 54.7%, corresponding to 36.16 g plant⁻¹, for the dose of 600 mL, at the 14-day frequency.

The dose of 600 mL applied every 14 days has probably promoted greater accumulation of organic and/or inorganic compounds from leaf-forming tissues when compared to the 7-day frequency. Galbiatti et al. (2011) evaluated the development of common bean (*Phaseolus vulgaris* L.) and found that the use of biofertilizer and mineral fertilization influenced plant growth, dry weight of leaves, stems, petioles and shoots, and leaf area. The treatments using biofertilizer presented higher average values than those that did not use, with the best yield being reached in the treatments that received bovine manure-based biogas effluent.

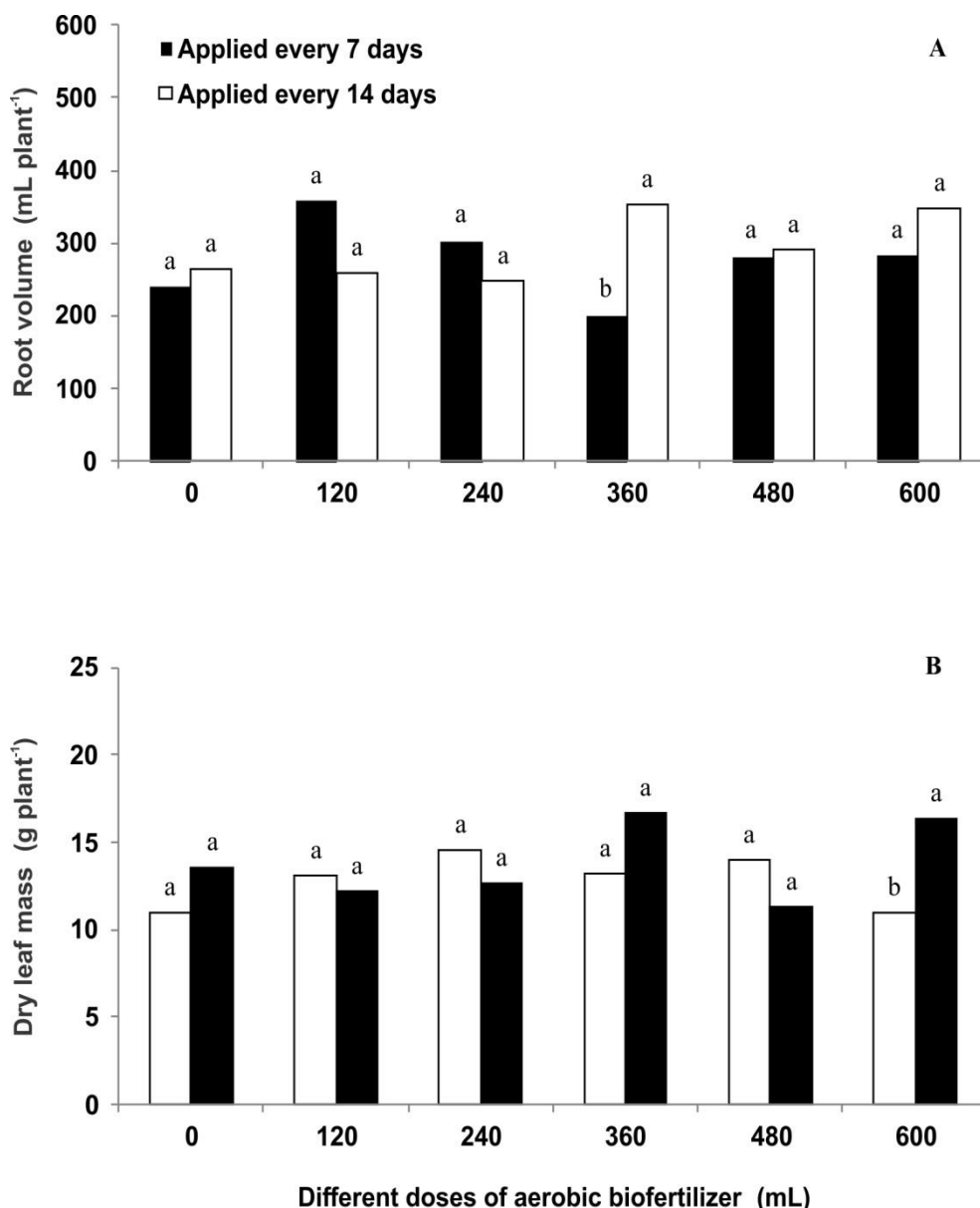


Figure 2 - Root volume (A) and dry leaf mass (B), in 'Caatingueiro' corn plants at different rates and frequency of application of aerobic biofertilizer.

Lima et al. (2012), studying the growth and development of maize plants as a function of different dilution levels of bovine biofertilizer, verified that the biofertilizer promoted increased leaf dry matter, stem diameter, and shoot biomass after 60 days of cultivation. Other studies have also reported the same effect, for instance, Araújo et al. (2007) in coffee leaves under concentrations of the biofertilizer Supermagro.

The effect of the 360 mL dose on root volume (Figure 2A) was not sufficient to affect leaf dry weight accumulation in plants (Figure 2B). This means that despite the 14-day frequency of biofertilizer application showing superior results when compared to the 7-day frequency, this better development of the root system did not promote an increase in leaf dry weight, and consequently did not affect the accumulation of organic

matter (photoassimilates) and inorganic compounds in the leaves.

Diniz et al. (2011), analyzing the effect of biofertilizer application on the production and growth of leaf biomass of yellow passion fruit, obtained with the maximum dose, that is, 100% biofertilizer applied without adding water, the value of 314.78 g plant⁻¹. According to the same authors, fermented liquid cattle manure stimulated the growth and biomass production of roots and branches of the plants.

Figure 3 shows that regardless of the frequencies of biostimulant application (120, 240, 480, and 600 mL), root formation remains the same. The same result was obtained in relation to root volume. The applied dose of 360 mL with the 7-day frequency showed the lowest result for root fresh weight.

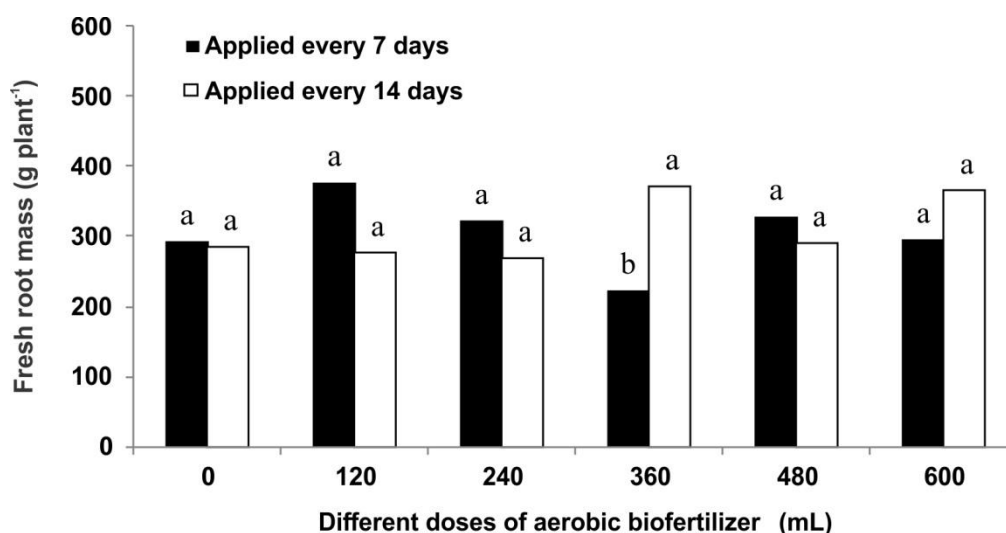


Figure 3 - Fresh root mass evaluated in 'Caatingueiro' corn plants at different doses and frequency of application of aerobic biofertilizer.

For the variables height, diameter, dry weight of roots, stems and panicles, there was no significant difference for the evaluated factors.

Several studies have reported that biofertilizer application increases biomass production and shoot and root growth in several plant species (Bezerra et al., 2008; Galbiatti et al., 2011; Souza et al., 2012). Others report that the biofertilizer activity in the soil works as a factor to improve physical and chemical conditions,

favoring the increase of its nutrient release capacity, thus increasing the availability of minerals for absorption and transport by plants (Silva Júnior et al., 2009; Mesquita et al., 2010).

Shoot (stem and panicle) fresh weight showed significance for all factors, accounting, on average, for 38% of the variation as a function of biofertilizer applications (Table 3).

Table 3 - Variance analysis for doses of biofertilizer and application frequencies in 'Caatingueiro' corn plants.

Causes of variation	Average squares				
	SPMF	LFM	LDM	RFM	RV
Frequencies (F)	350.90*	100.88 ^{ns}	15.63*	87.36 ^{ns}	3402.05*
Doses (D)	1378.54*	1841.72*	9.77*	2924.10 ^{ns}	5578.48*
F x D	856.18*	1323.43*	27.00*	20650.16*	19290.80*
Residue	540.75	659.02	10.64	8570.33	6561.89
CV (%)	19.60	17.92	24.53	30.0	28.33

* p < 0.05; ns - not significant; CV - coefficient of variation; SPMF - Stem and panicle fresh mass; LFM - Leaves fresh mass; LDM - Laves dry mass; RFM - Root fresh mass; RV - Root volume.

When evaluating the influence of biofertilizer doses and application frequencies on leaf chlorophyll content and the accumulation of P, Ca, Mg, S, Cu, Fe, Mn, Zn, and Na, both in roots and shoots, no significant differences were observed. Thus, for maize cv. BRS Caatingueiro, the different doses and application frequencies did not interfere in chlorophyll biosynthesis and did not alter the absorption and accumulation pattern of macro- and micronutrients in leaf tissues.

Similar results were found by Inoue et al. (2011), who found no differences in dry matter yield and N, P, and K concentrations in the tissue of plants that received different types and doses of biofertilizer. According to these authors, the viability of using biofertilizer will depend on the crop type, as well as on the characteristics of the soil that will receive this effluent, based on the nutritional needs of crops. In addition, excess concentration of biofertilizer elements in the soil must be avoided.

Although there was no interaction between doses and application frequencies of biofertilizer for N, K, and B in the shoots, and for N and B in the roots of plants, leaf N content responded in isolation to the frequency, being influenced significantly by the 7-day frequency.

Boron content in maize roots (Figure 4) showed a significant response to the 14-day frequency. Thus, it is possible to infer that the potential of absorption of some nutrients by the plants can show a time limit as to the capacity of transport by the plasma membrane. This rate of transfer of nutrients from the soil to the cell cytoplasm may require a variable amount and time of transport for each macro- and micronutrient. According to Dechen et al. (1991), root development is provided by the action of boron, one of the main functions of this micronutrient being the synthesis of phytonutrients such as auxin, which can act in cell wall formation and cell division in several plant species.

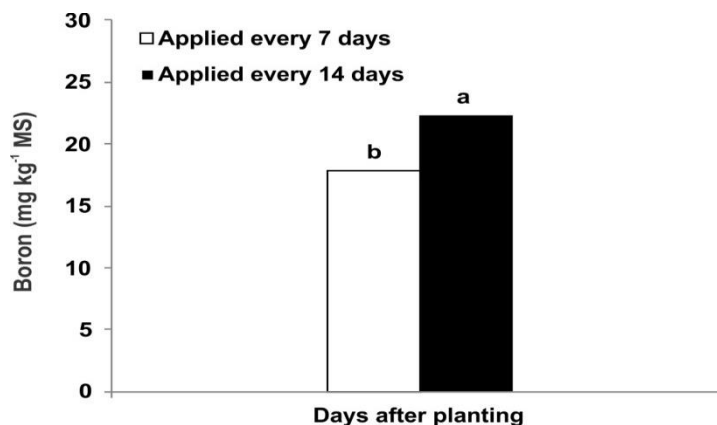


Figure 4 - Boron content in corn roots as a function of the biofertilizer application range.

Some studies verified isolated effects for the increase of nutrients in plant tissues. Alves et al. (2009) did not observe a significant effect of the interactions between types of biofertilizers and calcium doses for nitrogen, phosphorus, and potassium contents in the dry matter of sweet pepper leaves. However, statistical superiority of the pure biofertilizer was observed for sulfur content.

Studies on the export of nutrients from “Baixinho de Santa Amália” papaya, treated with biofertilizers, found that mineral-enriched biofertilizer was more efficient in relation to the pure one in the accumulation of sodium and macro- and micronutrients

in the leaves of plants, and in the export of their nutrients. At the beginning of flowering, the plants had adequate levels of nitrogen, potassium, sulfur, boron, copper, and zinc; and were deficient in phosphorus, calcium, magnesium, iron, and manganese. Potassium (among macronutrients) and iron (among micronutrients) were the most exported at fruit harvest. Thus, the need for absorption of a given nutrient varies from species to species as a function of the phenological stage of plants.

Figure 5 presents the effects of the application of biofertilizer doses on leaf nitrogen, potassium, and boron contents.

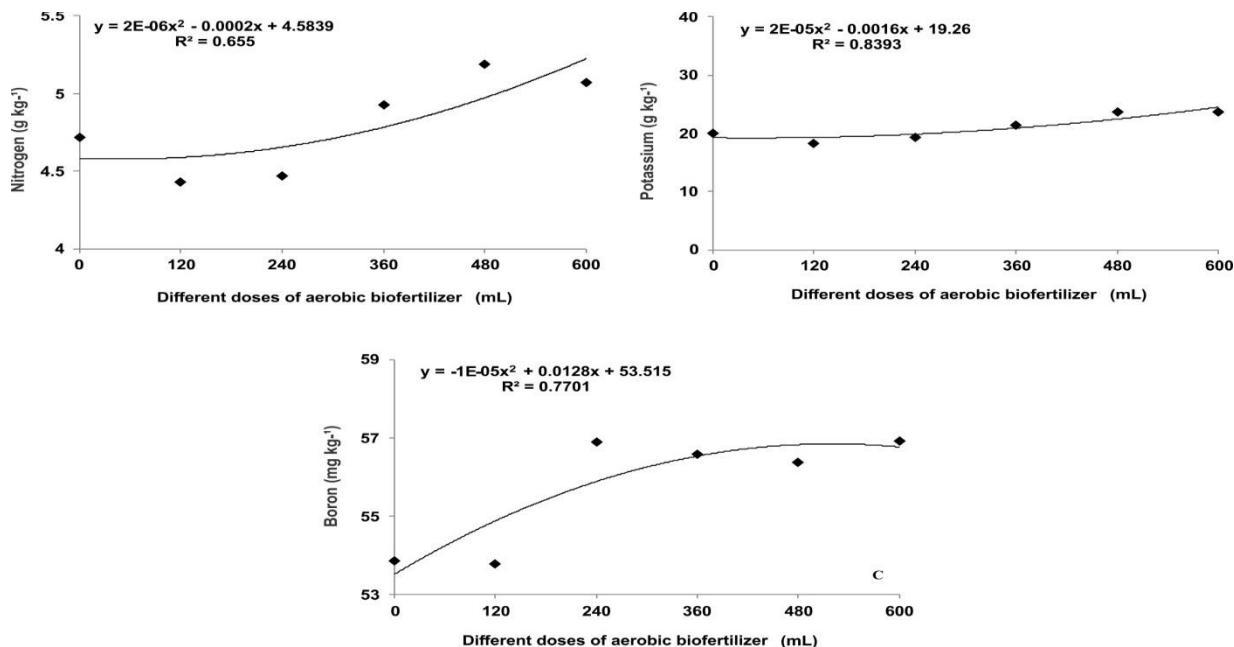


Figure 5 - Leaf nitrogen (A), potassium (B), and boron (C) content of 'Caatingueiro' corn as a function of aerobic biofertilizer doses and application frequencies.

An increase in leaf N content is observed as a function of increasing doses of biofertilizer (Figure 5A). Probably this increase occurred due to higher absorption and transport of nitrogen to the leaves. For K content (Figure 5B), successive increases were observed

from the 360 mL dose of the biostimulant per plant. The maximum biofertilizer concentrations yielded 23.7 g kg⁻¹ potassium in leaf tissues, or 18.7% of the total of the macronutrient in comparison to the control treatment.

For boron (Figure 5C), the highest amounts stored in the leaf tissue were obtained from the 240 mL dose of biofertilizer. Notwithstanding, the 600 mL dose promoted the highest accumulation of this micronutrient in foliar tissues, reaching 17.02 mg kg⁻¹, which corresponds to 56.9% of this micronutrient in comparison to the control treatment.

Similarly, Araújo et al. (2007) found that with increasing compound doses, leaf N, K, and Mg contents increased, while P, Ca, B, Cu, Fe, and Mn contents decreased. Only Mn presented leaf content below the critical levels as a function of the compound. Magrini et al. (2008) found that this increase in N content is partly explained by its high mobility within the plant.

Conclusions

According to the studies carried out on the effect of different doses and application frequencies of biofertilizer, the dose of 360 mL per plant, associated to the 14-day frequency of biofertilizer application, is recommended for the cultivation of maize cv. BRS Caatingueiro in Yellow Latosol.

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