Iheringia
Série Botânica
ISSN ON-LINE 2446-8231

Jardim Botânico de Porto Alegre

Use of commercial biostimulator in the ex *situ* cultivation of a native medicinal plant of Cerrado: *Campomanesia adamantium*

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Received 21.I.2021
Accepted 17.VII.2023

DOI 10.21826/2446-82312023v78e2023018

ABSTRACT – *Campomanesia adamantium* (Camb.) O. Berg (‘guavira’) is a fruit plant native of ‘Cerrado’ with nutritional and medicinal properties with few studies related to its *ex situ* cultivation, especially with use of biostimulators of soil microbiota and nutrient availability for the plant. Thus, aimed to evaluate the effect of a biostimulator on chemical and microbiological attributes of the substrate, and on growth of ‘guavira’ plant *ex situ*. Six doses of the Penergetic® “K” biostimulator (0, 50, 100, 150, 200 and 250 g ha^{-1}) were studied. The chemical attributes showed the highest availability in the substrates without and with 50 g ha^{-1} of biostimulator. Microbial biomass carbon reached an increase of 41.2% at the dose of 200 g ha^{-1} compared to the substrate without biostimulator. The order of the nutrient content, regardless of dose, including without, in the shoot was N>Ca>K>Mg>P>Fe>Mn>Zn>Cu and root was N>Ca>K>P>Mg>Mn>Zn>Cu. The leaf number (26-leaf plant^{-1}) was greater in plants grown with 50 g ha^{-1} of biostimulator at 180 days after transplanting. The chlorophyll index decreased with increased doses. It can be concluded that for cultivation of ‘guavira’ in polyethylene bags the use of biostimulator is not necessary.

Keywords: chlorophyll-*a* fluorescence, ‘guavira’, microbial biomass, nutrients.

RESUMO – Uso de bioestimulador comercial no cultivo *ex situ* de planta medicinal nativa do Cerrado: *Campomanesia adamantium*. *Campomanesia adamantium* (Camb.) O. Berg (‘guavira’) é uma frutífera nativa do Cerrado com propriedades nutricionais e medicinais com poucos estudos relacionados ao seu cultivo *ex situ*, principalmente com uso de bioestimuladores da microbiota do solo e disponibilidade de nutrientes. Assim, objetivou-se avaliar o efeito de um bioestimulador nos atributos químicos e microbiológicos do substrato, e no crescimento *ex situ* da ‘guavira’. Foram estudadas seis doses do bioestimulador Penergetic® “K” (0, 50, 100, 150, 200 e 250 g ha^{-1}). Os atributos químicos apresentaram maior disponibilidade nos substratos sem e com 50 g ha^{-1} do bioestimulador. O carbono da biomassa microbiana atingiu aumento de 41,2% sob 200 g ha^{-1} em relação ao substrato sem bioestimulador. A ordem do teor de nutrientes, independentemente da dose, na parte aérea foi N>Ca>K>Mg>P>Fe>Mn>Zn>Cu e a raiz foi N>Ca>K>P>Mg>Mn>Zn>Cu. O número de folhas (26 folhas planta^{-1}) foi maior nas plantas cultivadas com 50 g ha^{-1} de bioestimulador aos 180 dias após o transplantio. O índice de clorofila diminuiu com o aumento das doses. Pode-se concluir que para o cultivo da ‘guavira’ em sacos de polietileno não é necessário o uso de bioestimulador.

Palavras-chave: biomassa microbiana, fluorescência da clorofila-a, ‘guavira’, nutrientes.

INTRODUCTION

Campomanesia adamantium (Cambess) O. Berg (Myrtaceae) is a fruit plant native of ‘Cerrado’ of Mato Grosso do Sul, Goiás, Minas Gerais and Santa Catarina (Lorenzi 2008), popularly known as ‘guavira’. It is a species that grows in a hot tropic climate, with a shrubs size and can reach 1.5 m in height, flowering occurs from August to October, with fruiting from November to December (Leão-Araújo *et al*. 2019). Propagation is by seed, which are recalcitrant and should be sown after harvest (Dresch *et al*. 2012).

Stands out due to its nutritional and medicinal value, like antimicrobial (Breda *et al*. 2016), anti-inflammatory, and antinociceptive (Viscardi *et al*. 2017), antioxidant and protective activity (Fernandes *et al*. 2015). The fruits are consumed *in natura* and by food and beverage industry, in the form of candies, ice cream and homemade liqueurs (Vallilo *et al*. 2006). Given its importance, the harvest is performed almost exclusively in native areas without management or appropriate cultivation (Carnevali *et al*. 2012), thus emerging concerns regarding the conservation of the species.

The production of seedlings in *ex situ* cultivation is a way to conserve the genotype of the species. The initial propagation of plants indirectly with transplantation allows seedlings to be obtained, which require care with the quality of the substrate. Therefore it is necessary to know the proportions and materials that compose the mixture, because they can affect the physical, chemical and microbiological properties of the substrate and consequently interfere with growth and development of the plant (Hartmann *et al*. 2008). The soils of the 'Cerrados' region where the species grows are characterized by being acidic and poor in nutrients under natural conditions (Abrahão *et al*. 2019), thus requiring new technologies to improve the quality of the substrate.

One of the ways is the use of biostimulators, which can improve substrate quality and conditions for seedlings cultivation, through stimulation of natural processes, contributing to absorption and nutrient use efficiency. Among the techniques currently employed, there is Penergetic® technology whose effect results from applying energized particles, according to the theories of Michael Faraday (1846) and James Clerk Maxwell (1864), of their products "K" and "P", to substrates or plants, which are from bentonite clay subjected to the application of electric fields, for the transfer of specific wave (Brito *et al*. 2012). The Penergetic® "K", applied to the soil, acts as biostimulator, enhancing and providing suitable conditions for microbiological activities and contributing to the acceleration of organic matter mineralization (Cobucci *et al*. 2015).

Thus, when applied to the substrate, the biostimulator can directly influence microbiological attributes, such as microbial biomass carbon (Cmic) and basal respiration (BSR). These attributes are used as indicators of changes in soil, since these are characteristics that are sensitive to alterations in soil quality, caused by changes of use and management practices, such as application of biostimulators (Trannin *et al*. 2007). The isolated analysis of these components may limit the soil analysis regarding microbial activity; thus, the metabolic quotient (qCO_2) and microbial (*q*MIC) along with these characteristics; provide more reliable information for the understanding of microbiological activity of the substrate (Alves *et al*. 2011).

Obtaining quality seedlings guarantees the definitive development in the field and can be achieved with morphological and physiological parameters assisting in the responses to the action of the biostimulator used as management in the cultivation of guavira seedlings. Therefore, biometric evaluations of the species' morphology guarantee a clearer and more evident understanding (Ajalla *et al*. 2014). The physiological, such as the study of change of chlorophyll fluorescence emission, arising mainly from the photosystem II (Panda *et al*. 2008), evaluate the photosynthetic performance of plants, in addition to being a non-invasive, highly sensitive and easy handling method, providing qualitative and quantitative information about the physiological condition of the photosynthetic apparatus in vivo (Baker *et al*. 2008).

Thus, the aim of this study was to assess the effect of different doses of a biostimulator on the chemical and microbiological attributes of the substrate and on the nutrition, physiology and growth of 'guavira' plant *ex-situ*.

MATERIAL AND METHODS

General conditions and obtaining of plants

The experiment was performed in the Medicinal Plant Garden – MPG (22° 11′ 43.7″ S, 54° 56′ 08.5″ W; 452 m above sea level), of Federal University of Grande Dourados (UFGD) in Dourados – MS, Brazil, in a protected environment with additional protection of 50% sombrite.

'Guavira' seeds were obtained from fruits of several matrices of 'Cerrado' (23° 32′ 30″ S, 55° 37′ 30″ W; 434 m above sea level) located in the municipality of Ponta Porã – MS. An exsiccate is deposited at the Herbarium DDMS (Dourados – MS), under number 4653. The seeds were manually removed from pulps and sown in 72-cell polystyrene trays, filled with commercial Bioplant® substrate composed of pine bark, peat, vermiculite, simple superphosphate, potassium nitrate and products formulated by third parties, with a pH of 5.8 and electrical conductivity between 0.5 and 2.0 mS cm^{-1}.

Cultivation conditions

When the plants reached average height of 4.0 cm they were transplanted to polyethylene bags of 1.3 L, filled with substrate composed of Oxisol (Santos *et al*. 2018), whose chemical attributes before cultivation (0-20 cm) are shown in Tab. 1, plus the addition of worm humus and decomposed leaves of cassava, in the proportion of 6:2:2 (v:v:v). The sample was determined the pH values (H_2O), potential acidity (H+Al), P and K contents [extracted with Mehlich-1 and determined in spectrophotometer (P) and fire photometer (k+)]; Ca^{2+}, Mg^{2+} and Al^{3+} [extracted with KCl 1,0 mol L^{-1} and determined in spectrophotometer of atomic absorption (Ca^{2+} and Mg^{2+}) and by the titration method (Al^{3+})]. All the chemical analyzes were performed according to the methodology proposed by Silva (2009). Posteriorly were calculated the sum of bases (SB), cations exchange capacity (CEC) and bases saturation (V%).

Experimental Design

The experimental design was randomized blocks with four replicates, comprised of six doses of the biostimulator Penergetic® "K" were studied (0, 50, 100, 150, 200 and 250 g ha^{-1}), applied on the substrate once before transplanting. Each pot contained 1 (one) plant each and 10 polyethylene bags per plot. Composition of the bioestimulator, according manufacture, in 2.5 kg it has 1.6% of nitrogen, 3.5% of potassium and 84% of substances organic (not mentioned) and pH value > 7 (Penergetic 2021).

Table 1. Analysis of the chemical attributes of the substrate, at the beginning of the cultivation cycle.

pH in H_2O		OM	P	Al	K	Ca	Mg
		g dm^{-3}	mg dm^{-3}	cmol$_c$ dm^{-3}			
5.33		114.24	7.50	0.07	1.07	5.8	1.3
Cu	Mn	Fe	Zn	H+Al	SB	CEC	V
mg dm^{-3}				cmol$_c$ dm^{-3}			%
10.63	108.7	413.3	31.39	2.0	8.17	10.1	50.3

Potential of hydrogen in water (pH in H_2O); Organic matter (OM); Phosphorus (P); Aluminum (Al); Potassium (K); Calcium (Ca); Magnesium (Mg); Copper (Cu); Manganese (Mn); Iron (Fe); Zinc (Zn); Potential acidity (H + Al); Sum of bases (SB); Cation exchange capacity (CEC); Base saturation (V%).

Measurements

The height of plants, collar diameter and leaf number per plant were assessed, from 60 to 180 days after transplanting (DAT), every 30 days. Considering that the species under study shows slow vegetative growth in the initial phase, the chlorophyll indexes were measured, as well as characteristics of chlorophyll-*a* fluorescence, when the seedlings were in the vegetative stage able to be transplanted to pots or field, i.e., at 180 DAT. Because after that period, according to Ajalla *et al*. 2014, seedlings tend to increase the growth and collar diameter.

Therefore, at 180 DAT the photochemical efficiency of photosystem II (F_v/F_m) and the absorbed energy conversion efficiency (F_v/F_o) were assessed, subjecting the third leaf, counted from the apex of the plant, for 30 minutes, to dark conditions using leaf clips. The readings were performed with the portable fluorometer model OS p 30 (OPTI-SCIENCES Chlorophyll Fluorometer). The contents of chlorophyll index and chlorophylls *a* and *b* were also determined, using portable clorofilometerFL-1030 clorofiLOG (Falker Automação Agrícola). Then, three whole plants were harvested at random from each plots, and evaluated for the length of the largest root, leaf and root areas were evaluated with area integrator (LI-COR, Model 3100 C – Area Meter). The total dry mass, and dry mass of leaves, stems and roots were weighed in a digital scale with precision of 0.001 g, after being placed in an oven with forced air flow (60 ± 5 °C) until obtaining constant mass. From the biomass data, height/diameter ratio, shoot, and root ratio data, the Dickson Quality Index (DQI) was calculated (Dickson *et al*. 1960), and the contents of macro and micronutrients of dry mass of shoot and root were quantified (Malavolta 2006).

The chemical attributes of the substrates were determined according to Silva (2009); the microbiological attributes, microbial biomass carbon (Cmic) by fumigation-extraction method (Vance *et al*. 1987) and the basal respiration (BSR) by fumigation-incubation method (Jenkinson & Powlson 1976). The qCO_2 was determined from the ratio BSR/Cmic and the *q*MIC, defined by the ratio Cmic/C-organic total (Anderson & Domsch 2010).

Statistical analysis

Data were subjected to the Shapiro-wilk normality test and only number of leaves were transformed to square root prior analysis of variance and, when significant to the F test, the means were subjected to regression analysis in function of the doses of biostimulator ($p < 0.05$). Data taken over the cycle were analyzed as plots subdivided in time and subjected to analysis of variance and regression ($p < 0.05$). As supplementary analysis, the means in relation to chemical and microbiological attributes of the substrate; macro and micronutrients from the shoot and root; and the dry mass were subjected to variance and covariance matrices to perform a Principal Component Analysis (PCA).

RESULTS

The maximum content of P was 32.6 mg dm^{-3} with application of 90.9 g ha^{-1} of biostimulator (Tab. 2). In relation to Ca in the substrate, the maximum content of 7.4 cmol$_c$ dm^{-3} occurred with application of 12.7 g ha^{-1} of biostimulator (Tab. 2). The same trend was observed for SB and CEC, whose maximum values of 12.1 and 18.7 cmol$_c$ dm^{-3}, respectively, occurred with 31.7 and 58.7 g ha^{-1} of biostimulator, respectively (Tab. 2). The minimum content of Fe (358.6 mg dm^{-3}) occurred with the application of 97.7 g ha^{-1} of biostimulator, and the maximum content of 675 mg dm^{-3} with 250 g ha^{-1} (Tab. 2). The content of Zn in the substrate decreased with the addition of the biostimulator, and the lowest content (11.2 mg dm^{-3}) occurred with 250 g ha^{-1} (Tab. 2).

Regarding the microbiological attributes of the substrate, the mathematical models of regression tested did not adjust to the data of microbial biomass carbon (Cmic) and basal respiration (BSR) of the substrate, obtaining means of 252.5 µg C g^{-1} and 36.9 µg C-CO_2 g^{-1}, respectively (Tab. 2). However, the application of biostimulator increased the amount of Cmic, such increase reached 41.2 % at the dose of 200 g ha^{-1} (335.6 µg C g^{-1}) compared to the substrate without the biostimulator (197.3 µg C g^{-1}) (Tab. 2), which showed lower amount. Although it did not fit the model employed, BSR was higher at initial doses (0 and 50 g ha^{-1}), reaching increase of 24.06% and 23.03%, respectively, compared to the substrate with the greatest application (250 g ha^{-1}) (Tab. 2).

Table 2. Chemical and microbiological substrate attributes of samples with six levels of the biostimulator.

Chemical attributes	Equation	Maximum value	Biostimulator (g ha^{-1})	R^2
pH water	$\hat{y} = \bar{y} = 5.8$	-	-	N/adj
OM (g dm^{-3})	$\hat{y} = \bar{y} = 61.4$	-	-	N/adj
P (mg dm^{-3})	$\hat{y} = 29.5053 + 0.0681{*}x - 0.0003{*}x^2$	32.6	90.9	0.86
K ($cmol_c$ dm^{-3})	$\hat{y} = \bar{y} = 0.93$	-	-	ns
Ca ($cmol_c$ dm^{-3})	$\hat{y} = 7.4592 + 0.00079x - 0.000031{*}x^2$	7.4	12.7	0.80
Mg ($cmol_c$ dm^{-3})	$\hat{y} = \bar{y} = 3.5$	-	-	N/adj
Cu (mg dm^{-3})	$\hat{y} = \bar{y} = 11.4$	-	-	N/adj
Mn (mg dm^{-3})	$\hat{y} = \bar{y} = 147.0$	-	-	N/adj
Fe (mg dm^{-3})	$\hat{y} = 469.1832 - 2.2606x + 0.0115{*}x^2$	675	250	0.72
Zn (mg dm^{-3})	$\hat{y} = 16.0082 -1.5735{*}x$	16.2	-	0.87
H+Al ($cmol_c$ dm^{-3})	$\hat{y} = \bar{y} = 6.7$	-	-	N/adj
SB ($cmol_c$ dm^{-3})	$\hat{y} = 12.0729 + 0.0029x - 0.000046{*}x^2$	12.1	31.7	0.76
CEC ($cmol_c$ dm^{-3})	$\hat{y} = 18.5939 + 0.0066x - 0.000057{*}x^2$	18.7	58.7	0.72
V (%)	$\hat{y} = \bar{y} = 62.9$	-	-	N/adj
Microbiological attributes				
Cmic (µg C g^{-1})	$\hat{y} = \bar{y} = 252.5$	-	-	N/adj
BSR (µg C-CO_2 g^{-1})	$\hat{y} = \bar{y} = 36.9$	-	-	N/Adj
qCO_2 (%)	$\hat{y} = \bar{y} = 64.9$	-	-	ns
*q*MIC (%)	$\hat{y} = \bar{y} = 0.6$	-	-	ns

Potential of hydrogen in water (pH in H_2O); Organic matter (OM); Phosphorus (P); Aluminum (Al); Potassium (K); Calcium (Ca); Magnesium (Mg); Copper (Cu); Manganese (Mn); Iron (Fe); Zinc (Zn); Potential acidity (H + Al); Sum of bases (SB); Cation exchange capacity (CEC); Base saturation (V%); microbial biomass carbon (Cmic); Basal respiration (BSR); Metabolic (qCO_2) and microbial (*q*MIC) quocient; N/adj – indicates not adjust; ns – indicates non-significant; *indicates significant difference ($p < 0.05$) between biostimulator; ȳ – means average; ŷ – means y-axis value calculated from the equation for each characteristic.

In the Principal Component Analysis (PCA) of chemical and microbiological attributes of the substrate, axis 1 explains 55.5 % (PC1) and axis 2, 22.9 % (PC2) of the original information of the data (Fig. 1). The OM, Ca, Mg, Mn, Zn, CEC and SB were similar in the substrate with the lowest doses (0 and 50 g ha^{-1}) of biostimulator being correlated with the contents of basal respiration (BSR) observed in these doses (Fig. 1). The P, pH, V% were similar with 150 and 200 g ha^{-1} of biostimulator and explain the contents of Cmic (Fig. 1). While the mean efficiency by qCO_2 was correlated to the contents of Cu and Fe on the substrate with application of the highest dose 250 g ha^{-1} (Fig. 1).

The nutrient contents of shoots of 'guavira' plants, regardless of dose of biostimulator and without (control) presented the following decreasing order N (14.9 g kg^{-1}) > Ca (11.9 g kg^{-1}) > K (3.5 g kg^{-1}) > Mg (3.2 g kg^{-1}) > P (2.0 g kg^{-1}) > Fe (315.3 mg kg^{-1}) > Mn (154.9 mg kg^{-1}) > Zn (29.5 mg kg^{-1}) > Cu (8.4 mg kg^{-1}), and only P and Ca were significantly affected (Tab. 3). The highest contents of P (2.2 g kg^{-1}) and Ca (14.1 g kg^{-1}) occurred in plants grown without biostimulator in the substrate (Tab. 3). While in the roots in decreasing order N (11.4 g kg^{-1}) > Ca (7.0 g kg^{-1}) > K (6.4 g kg^{-1}) > P (1.9 g kg^{-1}) > Mg (1.8 g kg^{-1}) > Mn (46.8 mg kg^{-1}) > Zn (27.9 mg kg^{-1}) > Cu (12.9 mg kg^{-1}). and only P were significantly affected (Tab. 3). The highest contents of P (2.3 g kg^{-1}) were observed without biostimulator (Tab. 3).

Photochemical efficiency of photosystem II (F_v/F_m) was not influenced by the doses of biostimulator, obtaining an average of 0.792 (Fig. 2A). In relation to the F_v/F_0, the same trend followed, with the maximum value (3.9) being contacted with the application of 84.3 g ha^{-1} of biostimulator ($\hat{y}=3.8754+0.002868x-0.000017{*}x^2$, $R^2=0.81$) and the values decreased, with the lowest value (3.4) in 'guavira' plants cultivated with application of 250 g ha^{-1} (Fig. 2B). There was linear reduction in total chlorophyll index (Fig. 2C) and chlorophyll *b* (Fig. 2D) of 'guavira' leaves with the increase in the dose of biostimulator.

During cultivation cycle, the largest plant height (21.8 cm) and collar diameter (2.7 mm) were recorded at 180 DAT. However, the mathematical models of regression tested, did not adjust to the data of plant height and collar diameter in function of doses of biostimulator. The largest maximum leaf number occurred in plants grown in substrate with 50 g ha^{-1} of biostimulator (plant with 26 leaves) ($\hat{y}=15.4825-0.1766{*}x+0.0013{*}x^2$; $R^2 = 0.96$), but close to that grown in substrate without biostimulator (plant with 24 leaves) at 180 DAT ($\hat{y}=20.5085-0.3158{*}x+0.0018{*}x^2$; $R^2 = 0.91$) (Fig. 3). The smallest maximum leaf number (plant with 16 leaves) occurred with 100 g ha^{-1} of biostimulator, which featured pattern similar to those grown in substrates with 150, 200 and 250 g ha^{-1} (Fig. 3).

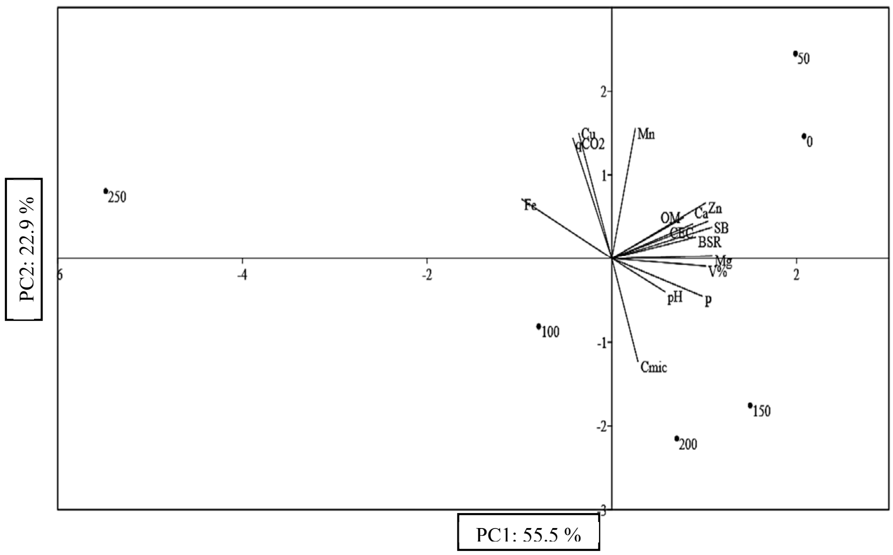

Figure 1. Two-dimensional graphic of the chemical and microbiological attributes in different doses of biostimulator. PC1 and PC2 correspond to the Principal Components Potential of hydrogen (pH); Organic matter (OM); Phosphorus (P); Calcium (Ca); Magnesium (Mg); Copper (Cu); Manganese (Mn); Iron (Fe); Zinc (Zn); Sum of bases (SB); Cation exchange capacity (CEC); Base saturation (V%); microbial biomass carbon (Cmic); Basal respiration (BSR); Metabolic quocient (qCO_2).

Table 3. Macro and micronutrient contents of dry mas of shoots and roots with six levels of the biostimulator.

Nutrients	Equation	Maximum value	Biostimulator ($g\ ha^{-1}$)	R^2
		Dry mass of shoot		
N ($g\ kg^{-1}$)	$\hat{y} = \bar{y} = 14.9$	-	-	N/adj
P ($g\ kg^{-1}$)	$\hat{y} = 2.3215 – 0.6177^*x + 0.2170^*x^2$	2.2	0	0.89
K ($g\ kg^{-1}$)	$\hat{y} = \bar{y} = 3.5$	-	-	ns
Ca ($g\ kg^{-1}$)	$\hat{y} = 13.7529 – 2.7385^*x + 0.7086^*x^2$	14.1	0	0.89
Mg ($g\ kg^{-1}$)	$\hat{y} = \bar{y} = 3.2$	-	-	N/adj
Cu ($mg\ kg^{-1}$)	$\hat{y} = \bar{y} = 8.4$	-	-	N/adj
Mn ($mg\ kg^{-1}$)	$\hat{y} = \bar{y} = 154.9$	-	-	N/adj
Fe ($mg\ kg^{-1}$)	$\hat{y} = \bar{y} = 315.3$	-	-	ns
Zn ($mg\ kg^{-1}$)	$\hat{y} = \bar{y} = 29.5$	-	-	N/adj
		Dry mass of root		
N ($g\ kg^{-1}$)	$\hat{y} = \bar{y} = 11.4$	-	-	ns
P ($g\ kg^{-1}$)	$\hat{y} = 2.3745 – 0.6423^*x + 0.1830^*x^2$	2.3	0	0.75
K ($g\ kg^{-1}$)	$\hat{y} = \bar{y} = 6.4$	-	-	N/adj
Ca ($g\ kg^{-1}$)	$\hat{y} = \bar{y} = 7.0$	-	-	N/adj
Mg ($g\ kg^{-1}$)	$\hat{y} = \bar{y} = 1.8$	-	-	ns
Cu ($mg\ kg^{-1}$)	$\hat{y} = \bar{y} = 12.9$	-	-	N/adj
Mn ($mg\ kg^{-1}$)	$\hat{y} = \bar{y} = 46.8$	-	-	N/adj
Zn ($mg\ kg^{-1}$)	$\hat{y} = \bar{y} = 27.9$	-	-	N/adj

Nitrogen (N); Phosphorus (P); Potassium (K); Calcium (Ca); Magnesium (Mg); Copper (Cu); Manganese (Mn); Iron (Fe); Zinc (Zn); N/adj – indicates not adjust; ns – indicates non-significant; *indicates significant difference ($p < 0.05$) between biostimulator; $\bar{y}$ – means average; $\hat{y}$ – means y-axis value calculated from the equation for each characteristic.

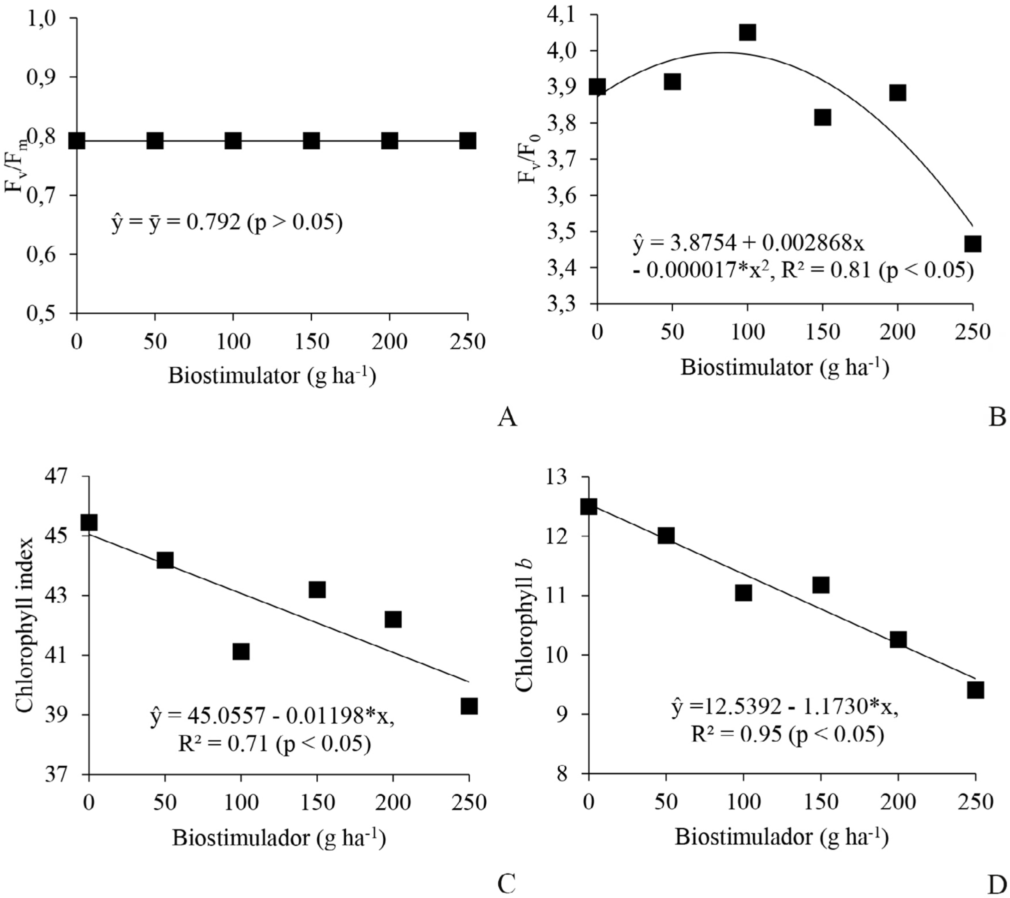

Figure 2. Photosynthetic activity of 'guavira' plants. **A.** Photochemical efficiency of photosystem II (F_v/F_m); **B.** absorbed energy conversion efficiency (F_v/F_0); **C.** chlorophyll index and **D.** chlorophyll *b* of 'guavira' plants grown on substrate with different doses of biostimulator, *indicates significant difference ($p < 0.05$) between biostimulator.

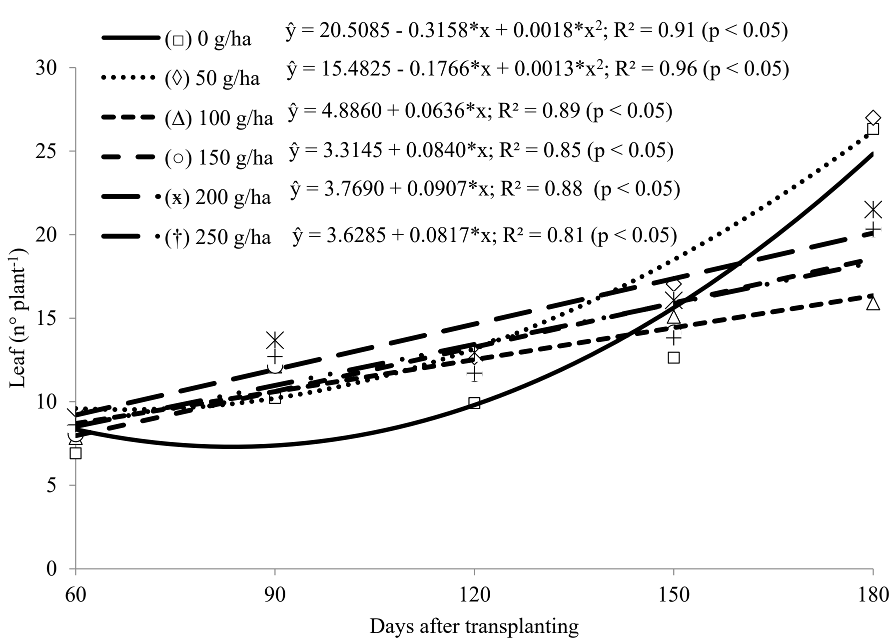

Figure 3. Number of leaves of 'guavira' plants according to doses of the biostimulator and epochs of evaluation, * indicates significant difference ($p < 0.05$).

The greatest leaf area (LA) (252.8 cm² plant^{-1}) occurred in 'guavira' plants cultivated with application of 250 g ha^{-1} of biostimulator (Tab. 4). The regression models tested did not adjust to root length (RL); Dickson quality index (DQI); Leaf (LDM), Stem (SDM) and total (TDM) dry mass mean of 15.1 cm; 3.2%; 1.6, 0.4 and 2.8 g plant^{-1}, respectively (Tab. 4).

The data concerning production (dry mass, leaf area, root length and Dickson quality index) were compared in relation to the chemical attributes of the substrate and macro and micronutrients of leaf area by Principal Components Analysis, in which the main component (PC1) explained 45.4 % and axis 2 (PC2) explained 29.2 % of original information (Fig. 4).

Table 4. Morphological characteristics and dry mass of 'guavira' plants grown on substrate with different doses of biostimulator.

	Biostimulator (g ha^{-1})	
Morphological Characteristics and DQI	Equation	R^2
LA (cm² plant^{-1})	$\hat{y} = 181.3813 – 0.2173x + 0.0018^*x^2$	0.63
RA (cm² plant^{-1})	$\hat{y} = \bar{y} = 16.9$	ns
RL (cm)	$\hat{y} = \bar{y} = 15.1$	N/adj
DQI (%)	$\hat{y} = \bar{y} = 3.2$	N/adj
Dry Mass	Equation	R^2
LDM (g plant^{-1})	$\hat{y} = \bar{y} = 1.6$	N/adj
RDM (g plant^{-1})	$\hat{y} = \bar{y} = 0.7$	ns
SDM (g plant^{-1})	$\hat{y} = \bar{y} = 0.4$	N/adj
TDM (g plant^{-1})	$\hat{y} = \bar{y} = 2.8$	N/adj

Leaf (LA) and Root (RA) area; Root length (RL); Dickson quality index (DQI); Leaf (LDM), Root (RDM), Stem (SDM) and total (TDM) dry mass, N/adj – indicates not adjust; ns – indicates non-significant; * indicates significant difference ($p < 0.05$) between biostimulator; $\bar{y}$ – means average; $\hat{y}$ – means y-axis value calculated from the equation for each characteristic.

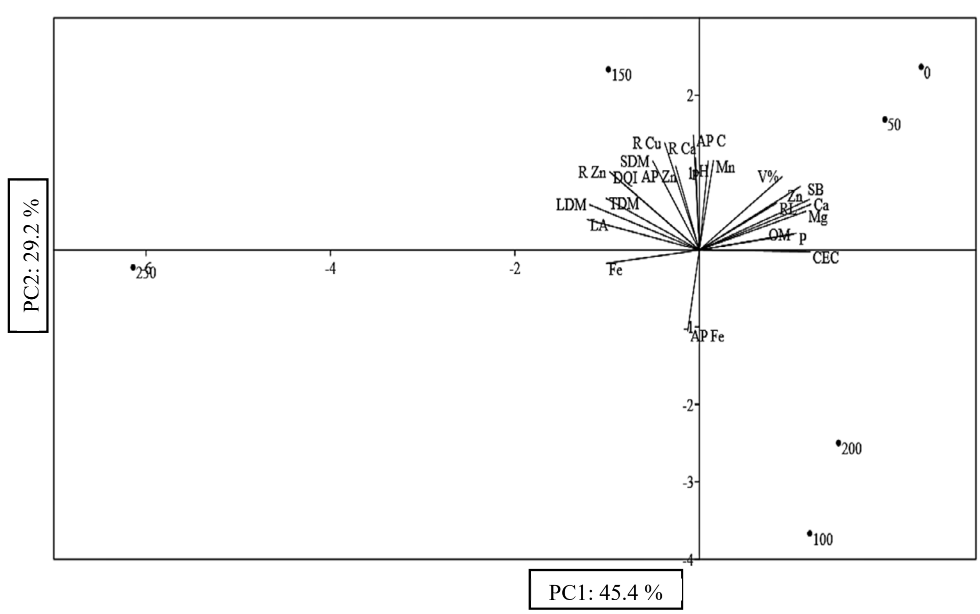

Figure 4. Two-dimensional graphic of the production data, chemical attributes of substrate, macro and micronutrients of dry mass of aerial parts in different doses of biostimulator. PC1 and PC2 correspond to the Principal Components Leaf dry mass (LDM); Stem dry mass (SDM); Total dry mass (TDM); Leaf area (LA); Dickson quality index (DQI); Root length (RL); Potential of hydrogen (pH); Organic matter (OM); Sum of bases (SB); Cation exchange capacity (CEC); Base saturation (V%); Phosphorus of substrate (P); Calcium of substrate (Ca); Calcium of root (R Ca); Magnesium of substrate (Mg); Copper of substrate (Cu); Copper of shoot (AP Cu); Copper of root (R Cu); Manganese of substrate (Mn); Iron of substrate (Fe); Iron of shoot (AP Fe); Zinc of substrate (Zn); Zinc of shoot (AP Zn) and Zinc of root (R Zn).

DISCUSSION

Chemical attributes P, Ca, Mg, SB, CEC, Cu, Mn, Fe and Zn of substrates, regardless of dose of biostimulator (Tab. 2) can be considered high according Sousa & Lobato (2004), This was because possibly occurred due to the worm humus and cassava leaves, components of the substrate used in the experiment, being great sources of organic matter, which contribute to nutrient availability, after mineralization Sousa & Lobato (2004). Another factor that might have contributed was the pH of 5.7 and 6.0 that the substrates presented at the end of the cultivation cycle, which are considered adequate, as it is the range that contributes for greater availability of nutrients for plants (Malavolta 2006). The high P content is an interesting factor because 'guavira' demands N and P for its growth (Vieira *et al*. 2011). SB indicated availability of ions Ca, Mg and K, and CEC values are influenced by variations of these nutrients and indicate soil capacity to adsorb exchangeable cations (Costa & Zocche 2009). The importance of these characteristics relies in the fact that for the ex-situ cultivation of native species, one of the first features to be standardized is the proper correction of the soil, which induces increased contents of Ca and Mg; neutralizes the acidity of the substrate; reduces the solubility of Fe and Al and increases the activity of beneficial microorganisms in the substrate, thus accelerating the decomposition of organic matter, making available N and P (Auler *et al*. 2019). The application of biostimulator possibly contributed to the increased availability of Fe causing decrease in the other nutrients of the substrates. That is because Fe in excess can be toxic to the plant, as well as decrease the absorption capacity of P, K, Ca, Mg, Mn and Zn (Malavolta 2006).

The Cmic values of this experiment were lower than 541 µg C g^{-1} observed by Carneiro *et al*. (2009) in native 'Cerrado' of Mato Grosso do Sul, however, was higher in relation to millet cultivation systems under conventional tillage (106 µg C g^{-1}), turnip under no-tillage (149 µg C g^{-1}) and close to those of sorghum under no-tillage (236 µg C g^{-1}). Considering that no-tillage increases Cmic and that in our study these values were close, then the type of substrate used may have contributed to balance the Cmic. Whereas the BSR was higher, regardless of the dose of biostimulator, than those found by Carneiro *et al*. (2009) in all systems, including the native 'Cerrado' of MS which was 7.9 µg C-CO_2, probably due to the fact that our experiment was held in plastic bags and not in native and/or management system, causing acceleration in microbial activity.

The OM and other chemical attributes (Ca, Mg, Mn, Zn, CEC and SB) are related to the release of C-CO_2, because microorganisms decompose organic matter that is available on the soil and release carbon dioxide. The higher respiratory rate is desirable, when the goal is to increase nutrient availability, because this indicates the rapid transformation of organic wastes into nutrients available to plants (Cunha *et al*. 2011). The P, pH and V% are related to Cmic, because they indicate the amount of carbon fixed in microorganisms cells, then the higher the Cmic the greater the ability of decomposition of organic matter of the substrate due to microbial activity (Calegari *et al*. 2014), thus favoring P release, which is a complex organic matter making it more available for plant absorption. It is possible to observe that the contents of Cu and Fe were important to determine microbial efficiency, which is measured by metabolic quotient (qCO_2), because the higher the value the lower the biomass efficiency, since this ratio is a measure of how much CO_2 is being released by respiration and incorporation of C to microbial biomass (Anderson & Domsch 2010). Elevated values of qCO_2 are indicators of microbial communities subjected to stress conditions, such as nutrient deficiency, acidity, and water deficit (Pezarico *et al*. 2013).

Our results of macro and micronutrients of shoots and roots were lower than those found by Melo et al. (2019) in *C. adamantium* cultivated with liming and different substrate texture, with higher content of N (23.5 g kg^{-1}), Ca (12.4 g kg^{-1}), K (11.8 g kg^{-1}), Mg (2.8 g kg^{-1}), P (6.9 g kg^{-1}) in the shoots e N (22.2 g kg^{-1}), Ca (6.5 g kg^{-1}), K (7.9 g kg^{-1}), Mg (1.8 g kg^{-1}), P (6.6 g kg^{-1}) in the roots, both with the addition of 5 Mg ha^{-1} of limestone in the Oxisol. However, as observed, the addition of the biostimulator affected the nutrition of 'guavira' seedlings. Similarly, higher values of N (18.2 g kg^{-1}) and P (5.1 g kg^{-1}) than those found in this study were observed by Vieira *et al*. (2011), that studied the early development of 'guavira' under protected cultivation with 50% luminosity and addition of mineral fertilizers in the substrate at doses of 2.5 mg kg^{-1} of N and 158.3 mg kg^{-1} of P_2O_5, causing greater absorption of these nutrients by the plant.

Despite the decrease, the photosynthetic system of 'guavira' plants were intact, regardless of dose of biostimulator, considering Suassuna *et al*. (2010) which report that in order to justify the intact photosynthetic system, the ratio F_v/F_m must vary between 0.750 and 0.850 ($\bar{y}$ = 0.792). Zanandrea *et al*. (2006) suggest that values between 4 and 6 of F_v/F_0 are considered normal, but those lower or greater than these indicate that the plant is under stress. Based on that information, only plants grown with 100 g ha^{-1} of biostimulator would have values considered normal (4.05), indicating greater efficiency in the conversion of absorbed energy.

These results should have relation with the lower contents of P and Mg and excess of Fe in the substrate. That is because phosphorus is important in the formation of ATP and NADP, main energy sources used in photoassimilate transport, storage and transfer of energy (Viégas *et al*. 2013), and later will participate in the biochemical processes of Calvin cycle. Mg and Fe together participate in the synthesis of chlorophyll, since Mg is the central atom of chlorophyll molecule (Pareek *et al*. 2017, Taiz *et al*. 2017) and Fe is essential for chlorophyll synthesis, cytochrome pathway, which are chargers containing Fe (Heredia Zárate & Vieira 2018).

The height and collar diameter data did not adjust can be justified, because 'guavirais' a native plant and is not involved in improving processes, so there is great genetic variability in seedlings (Miranda *et al*. 2016). After 150 DAT, seedlings grown without the biostimulator and with the lowest dose (50 g ha^{-1}), started to emit new leaves, with larger numbers of leaves being found after 180 DAT (Fig. 3). Despite the leaf number of plants grown with 50 g ha^{-1} of biostimulator, the difference for leaves without the biostimulator is small (Fig. 3) and it is possible to observe that in the other doses affected the emergence of new leaves, indicating the the bioestimulator did not is indicated for this function. This is because leaf number produced by plants of 'guavira' is an important factor, because their leaves have several medicinal properties (Fernandes *et al*. 2015, Breda *et al*. 2016, Viscard *et al*. 2017).

The leaf area, regardless of dose, included without, were higher than those found by Carnevali *et al*. (2014), who observed greater LA (66.2 cm^2 $plant^{-1}$) of 'guavira' at the dose of 16 Mg ha^{-1} of limestone, after 200 DAT. Probably, this difference might be due to the substrate used, since worm humus and cassava leaves were added to the mixture, which may have contributed to better performance of the plants. Lower values de DQI were obtained by Ajalla *et al*. (2014) from 'guavira' grown in dRL (100%), regardless of level of shading, which found DQI of 2.6% after 275 DAT. The difference may be related to the sources of substrate used, since in our study worm humus and cassava leaves were added, which probably contributed for greater nutrient availability due to mineralization of organic matter, leading to greater DQI and consequently greater quality of seedlings (Caldeira *et al*. 2012).

The production of LMD, SDM, TDM and LA are explained by the contents of Cu and Zn in aerial parts observed at the dose of 150 g ha^{-1}, since they participate in many enzymatic processes such as electron transport, phosphorylation of glucose in starch production (Chaves *et al*. 2010). This is because Cu is associated to the activation of several enzymes of the plant, one of them plastocyanine, which is involved in electron transport, in addition to being a crucial micronutrient for process of oxidation and reduction (Malavolta 2006). Whereas Zn participates in synthesis of the amino acid tryptophan, which is the precursor of the hormone indolecetic acid (IAA), from the auxin class (Taiz *et al*. 2017, Casanova-Sáez *et al*. 2021). This hormone is the crucial regulator for plant growth, since its main function is the expansion of cells, causing cell elongation, consequently contributing for growth of plant organs, resulting in greater mass production and further expansion of leaves.

The OM, P, Ca, Mg, Mn, Zn, SB and CEC were similar in the substrate with the lowest doses (0 and 50 g ha^{-1}), being correlated with the length of the largest root (RL) observed in these doses, because the chemical attributes contribute to the elongation of roots, especially those related to base saturation, such as Ca, which is directly related to development, acting on formation and integrity of cell wall membranes, causing root elongation (Dong *et al*. 2021).

Fe content in the substrate and shoots might have caused toxicity, since the Principal Components Analysis showed that its presence negatively affected the relation of leaf area with 250 g ha^{-1} of biostimulator, in addition to dry mass, which were explained with 150 g ha^{-1}. In guava (myrtaceae) plants, a species close to our study, the indicated for good development is the leaf content between 50-150 mg kg^{-1} (Natale *et al*. 2002, Santana *et al*. 2016), our result showed an average of 315 mg kg^{-1}, indicating a very marked excess. That is because Fe in excess can trigger several damage to plants, such as anatomical changes, rupture of root cells and disruption of cellular components; photosynthetic damage and less chlorophyll content (Chatterjee *et al*. 2006), affecting plant development.

CONCLUSION

Therefore, it can be concluded that the best responses regarding chemical attributes were observed at the dose of 50 g ha^{-1} of the biostimulator and without the biostimulator. Microbiological attributes do not change with the biostimulator. However, the biostimulator affects the chlorophyll index, chlorophyll *b*, and the absorbed energy conversion efficiency, although, has no influence on the nutrients in shoots and roots of 'guavira', in order of the nutrient content, regardless of the dose of the biostimulator, in shoots view of 'guavira' plants was N>Ca>K>Mg>P>Fe>Mn>Zn>Cu, and at the root it was N>Ca>K>P>Mg>Mn>Zn>Cu. In the cultivation of 'guavira' there is no need to use a biostimulator to obtain a greater number of leaves/plant that are based on the medicinal use of the plant.

ACKNOWLEDGMENTS

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for granting scholarships, and to Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) for financial support.

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