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Substrate for cupuaçu plantlets and the influence of cow urine as biofertilizer

Anapaula de Paula Cidade Coelho[1], Paulo Cesar Lima Marrocos[2], Marcelo Schramm Mielke[3], Martielly Santana dos Santos[4], Cibele Merched Gallo[5], Eduardo Gross[6*]

Abstract - The use of organic waste as components of substrate to produce fruit plantlets provides alternative materials, easily available and low cost. The aim of this work was to evaluate the effect of substrate and biofertilization with cow urine on the growth of cupuaçu plants (*Theobroma grandiflorum*) in an organic production system. A factorial experiment was conducted in a completely randomized design with 12 replications including four types of substrates (base mixture; base mixture + coconut fiber; base mixture + cocoa shell and base mixture + sand) with or without biofertilizer application totalizing 96 experimental units. Substrate and biofertilizer influenced the gas exchange and growth of *T. grandiflorum* plants. The interaction between these two factors significantly affected the dry matter, relative growth rate, net photosynthetic rate, and content of P, K and Mn in the leaves. Each factor, substrate and of cow urine application, independently, influenced the chlorophyll index, the leaf area, diameter, and height of the cupuaçu plants and the contents of N, Ca, Mg, Zn, Fe and Cu in the leaves. The base mixture substrate (composed by soil and organic compost) and fertilization with 1% of cow urine can be indicated to *T. grandiflorum* plantlets organic production.

Index terms: Nutrient content, Gas exchange, Growth rate, Organic fertilization; *Theobroma grandiflorum.*

Substrato para mudas de cupuaçuzeiro e influência da urina de vaca como biofertilizante

Resumo – A utilização de resíduos orgânicos como componentes de substrato para a produção de mudas de frutíferas provê materiais alternativos de fácil disponibilidade e de baixo custo. O objetivo deste trabalho foi avaliar o efeito do substrato e da biofertilização com urina de vaca no crescimento do cupuaçuzeiro (*Theobroma grandiflorum*), em sistema de produção orgânico. Foi conduzido um experimento fatorial em delineamento inteiramente casualizado, com 12 repetições, incluindo quatro tipos de substrato (mistura-base; mistura-base + fibra de coco; mistura-base + casca de cacau e mistura-base + areia), com ou sem aplicação de biofertilizante, totalizando 96 unidades experimentais. O substrato e o biofertilizante influenciaram as trocas gasosas e o crescimento das plantas de *T. grandiflorum*. A interação entre os dois fatores afetou significativamente a matéria seca, a taxa de crescimento relativo, a taxa fotossintética líquida e o conteúdo de P, K e Mn nas folhas. De forma independente, os fatores substrato e aplicação de urina de vaca influenciaram o índice de clorofila, a área foliar, o diâmetro e a altura das plantas de cupuaçu e os teores de N, Ca, Mg, Zn, Fe e Cu na parte aérea. A mistura-base do substrato (formada por solo e composto orgânico) e a fertilização com 1% de urina de vaca podem ser indicadas para a produção orgânica de mudas de *T. grandiflorum*.

Termos para indexação: Conteúdo de nutrientes, Trocas gasosas foliares, Taxa de crescimento, Fertilização orgânica; *Theobroma grandiflorum*.

Corresponding author:
egross@uesc.br

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[1]PhD student, Graduate Program in Crop Production, Universidade Estadual de Santa Cruz, Ilhéus-BA, Brazil. E-mail: anapaulaifbaiano@hotmail.com (ORCID 0000-0002-0593-4211)

[2]PhD in Crop Science, Comissão Executiva do Plano da Lavoura Cacaueira, Ilhéus-BA, Brazil. E-mail: marrocos@uesc.br (ORCID 0000-0003-3574-5152)

[3]PhD in Plant Physiology, Universidade Estadual de Santa Cruz, Ilhéus-BA, Brazil. E-mail: msmielke@uesc.br (ORCID 0000-0001-6930-2902)

[4]PhD in Plant Physiology, Universidade Estadual de Santa Cruz, Ilhéus-BA, Brazil E-mail: martiellysantana@gmail.com (ORCID 0000-0002-0909-2664)

[5]PhD in Plant Physiology, Faculdade São Vicente de Pão de Açúcar, Pão de Açúcar-AL, Brazil. E-mail: cibele.gallo@hotmail.com (ORCID 0000-0002-0157-8504)

[6]PhD in Plant Sciences, Universidade Estadual de Santa Cruz, Ilhéus-BA, Brazil E-mail: egross@uesc.br (ORCID 0000-0002-1057-0432)

Introduction

Agroforestry systems for commercial and homegrown orchards have been adopted to increase the value of products from the Atlantic Forest and the Amazon, especially in the north and south of the state of Roraima and in the south and extreme south of Bahia (Marques et al., 2016). As an example, plantations of the cupuaçu tree (*Theobroma grandiflorum*) can be cited. The cupuaçu is a perennial fruit tree with spontaneous development in the western Amazon forests and currently distributed in all Brazilian states (CALZAVARA, 1987), and it is considered an important source of income and employment for producers in these regions (LIMA-PRIMO et al., 2018).

The cultivation system generally used for cupuaçu tree plantations is below productive potential, mainly due to inadequate management, beginning with the production of plantlets with low agronomic quality (LIMA-PRIMO et al., 2018). This production is influenced by internal factors, such as seed quality, and external factors, such as substrate and the fertilizer used (FERREIRA et al., 2009).

The substrate, which is made up of physical, chemical and biological fractions, is the environment where the roots grow and develop (KÄMPF, 2000). It consists of mineral and organic particles, containing pores occupied by water and/or air, influencing root absorption capacity, and, consequently, plantlets development (SUGUINO et al., 2011). The greater the diversity of materials used in the production of the substrate, the greater the available nutritional diversity for plant growth (VICENTINI et al., 2009), which enables plants to achieve their productive potential (SODRÉ et al., 2009). It is possible to obtain such conditions with the addition of conditioners to the substrate, which can be acquired from organic residues from agricultural industries (ARAÚJO NETO et al., 2015).

Regionally available agro-industrial wastes can be used as conditioners, as they have lower costs and contribute to reducing the environmental impact (TERRA et al., 2017). In this context, in the south of Bahia, two organic agro-industrial residues can be found with potential for use in substrates. These are the shell of the cocoa fruit, which is generated on farms through the harvest and separation of cocoa beans; and coconut shells, generated in the trade and industry of coconut water and coconut by-products.

The treatment and process of composting cocoa fruit shells transform these residues into an organic biofertilizer with low pollution potential (DOMÍNGUEZ et al., 2010) and high influence on the degree of colonization of arbuscular mycorrhizal fungi (GOMES JÚNIOR et al., 2018). Coconut shell fiber is generated by the agricultural industry of coconut water packing. The coconut shell represents 80% to 85% of the gross weight of the fruit and around 70% of all the waste generated on beaches along the entire Brazilian coast (Rosa et al., 2001). However, after triple washing (MATTOS et al., 2017), the shell presents favorable characteristics as a substrate conditioner for cultivated plants mainly for its physical structure, providing adequate aeration and water retention (KRAUSE et al., 2017). Associated with the use of these residues, washed sand can also be included in the substrate, as it has the advantage of low cost, chemical inactivity, structural stability and ease of cleaning and disinfection (Sodré, 2007). In addition, the use of washed sand as a substrate conditioner improves porosity, water drainage (BITENCOURT, et al., 2010) and aeration to the roots (TERRA et al., 2017).

Like the substrate, biofertilization is considered essential for the growth of organic plantlets. It is possible to use by-products of cattle rearing, such as cow urine, which is described as a disinfectant (Mohanty et al., 2014) with phytosanitary value, as it increases the resistance of plantlets to pests and diseases (BRASIL, 2018). Cow urine is rich in mineral elements, providing nutrients and other beneficial substances to plants at low cost to the farmers (Oliveira et al., 2010), and the efficiency of its use as biofertilizer influencing crops productivity has been proved (NÁPOLES et al., 2017; FREIRE et al., 2019).

Adequate management of organic plantlets provides better conditions for initial field growth, contributing to the phytosanitary aspects and reducing crop mortality (FERREIRA et al., 2009), facilitating medium and long-term crop handling (Oliveira, 2006). Thus, this study verified the impact of the substrate composition and cow urine application on the growth of cupuaçu plantlets analyzing their physiological, morphological and nutritional characteristics.

Material and methods

Experimental conditions

The experiment was conducted at the nursery of Instituto Floresta Viva (IFV), situated in the Serra Grande district, in municipality of Uruçuca, Bahia, Brazil (geographical coordinates -14,463020 /-39,045151, Datum SIRGAS 2000), between March and September 2019. The nursery where the experiment was installed is covered with black shading screens that allow 50% of the available solar radiation to pass when there is full sunlight. According to Köppen-Geiger, the experiment region has Af type climate, which is hot and humid, with a mean temperature of 24.4ºC, without a defined dry season, and mean monthly rainfall of 50 mm to 100 mm, which can reach up to 150 mm. These rainfall rates are typical of tropical forests (CLIMATE-DATA.ORG, 2020), so the plantlets were irrigated when there was no rain for three consecutive days, according to the nursery schedule.

Fruits from the matrices for plantlets production were obtained from the Instituto Federal Baiano (IF Baiano) of Uruçuca Campus. Following the determinations of MAPA normative instructions nº 38/2011, which establishes technical regulations for plantlets production in organic production systems, fruits were selected by size and shape, and they were manually pulped. The seeds were washed in running water and dried in the shade on paper towels for 24 h. To minimize the influence of genetic variability, 710 seeds were selected by size, shape, and weight (4.5 g each). They were then put to germinate for 30 days in a seedbed with washed sterilized sand.

The seedlings with similar development characteristics were selected for the experiment, namely those with 13 cm between the root-stem transition zone and the sprouting end of the main branch of the plantlets, 20 mm stem diameter and two expanded leaves. Pricking out was performed onto plastic bags 33 cm high and 21 cm wide.

Base mixture (BM) substrate was produced with soil (75%) and organic compost (25%) (Table 1), according to the methodology of Araújo Neto et al., (2015) for the cultivation of cupuaçu trees. The soil was obtained from the IFV area, where the organic compost was previously produced with cattle and chicken manures, at a proportion of 1:1, after 240 days of composting process. The organic compost was sieved (4 mm opening) before use. The coconut shell fiber was acquired from a coconut water factory in the municipality of Una (Bahia State). The cocoa shell compost came from Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC) area localized at Ilhéus municipality, and the washed sand was acquired from a building supplies store in the municipality of Uruçuca, (Bahia State). The chemical characteristics of the materials used in the composition of the substrates are shown in Table 1. The other three substrates were prepared using 70% of BM and 30% of each material: coconut fiber (BM-CF), cocoa shell (BM-CS) and sand (BM-S).

Table 1. Physical and chemical attributes of the substrate components before preparing the mixtures for treatments.

	pH	CE mS m^{-1}	% Humidity	g.kg^{-1}				mg.kg^{-1}					Total
				N	P	K	Ca	Mg	Fe	Zn	Cu	Mn	Na
S*	6.7	0.10	5.16	0	0	0	0	140	0	0	0	0	0
CF	5.9	3.15	0.56	1.25	0.11	0.44	0.30	0.14	224	20	8	2	0.14
CS	7.9	0.02	13.00	1.56	0.70	4.36	0.98	0.98	448	77	21	48	0.88
OC	7.4	0.03	46.57	0.70	2.46	1.37	0.70	0.70	315	305	190	503	0.53
Soil	4.6	3.16	13.55	0.10	0.06	0.01	0.01	0.01	380	22	17	0	0.03

*S = sand; CS = cocoa shell; OC = organic compost, CF = coconut fiber.

The cow urine used in the experiment was collected from cows in the dairy herd of IF Baiano and was diluted to 1% in collected rainwater. The solution was applied (approximately 100 ml per plant) to the cupuaçu at the morning period, using a backpack sprayer, every 15 days, for four months, following the methodology adopted by IFV nursery to the production of native tree species. Cupuaçu plantlets without biofertilizer application received 100 mL of collected rainwater at each application date.

The experimental design was completely randomized with 12 replications (plantlets) in a 4x2 factorial scheme, the four different substrates (BM, BM-CF, BM-CS and BM-S) with and without cow urine application totalizing 96 sample units.

Growth analysis

At the beginning of the experiment, at the moment of plantlets pricking out onto plastic bags, ten seedlings were sampled for initial time (T1) evaluations of shoot height (H), diameter of the root-stem transition zone (D), number of leaves, leaf area (LA), total dry weight (TDW), dry weight of the roots (DWR), stem (DWS), leaves (DWL) and shoot (DWAP), followed by chemical analysis of the leaves were carried out. The LA of each seedling was determined using an LI-3100C, Li-Cor area meter (Li-Cor, inc. Lincoln, Nebraska, USA). To obtain the dry weights (DWR, DWS and DWL), ten seedlings were selected and taken to the Plant Physiology Laboratory of UESC, where they were washed, separated into root, stem, and leaves, and individually placed in identified paper bags and dried in a forced air oven at 65ºC until reaching constant weight. Total dry weight was calculated from the values for DWR, DWS and DWL (TDW=DWR+DWS+DWL).

The cupuaçu tree plantlets grew in the nursery for 152 days, and every 30 days, measurements were taken of shoot height (measured with a ruler), diameter at the root-stem transition zone (using a digital vernier caliper, taking two perpendicular measurements between each other at the base of the root-stem transition zone), number of leaves, and chlorophyll content estimate using a chlorophyll meter ClorofiLog (Falker, Porto Alegre, Brazil).

At the end of the experiment, all the plants were collected to obtain dry weight data, following the same methodology used for the plants at T1. The dry weight and leaf area data were then used to calculate: leaf area ratio: LAR=LA/TDW; leaf weight ratio: LWR=DWL/TDW; root weight ratio: RWR=DWR/TDW, relative growth rate: RGR=lnTDW2-lnTDW1/T2-T1), and liquid assimilation rate: LQAR=((TDW2–TDW1)/(T2–T1))x((lnLA2–lnLA1)/(LA2–LA1))), following Hunt (2016) procedures.

Leaf gas exchange measures

The net photosynthetic rate (*A*), stomatal conductance (*gs*) and the relationship between internal CO_2 concentration and that of the environment (Ci/Ce) were measured using an infrared gas analyzer, Li-6400 model (Li-Cor, USA) equipped with an artificial light source (6500-02B RedBlue). Measurements were carried out on completely expanded mature leaves, between 8 am and 11 am, under artificial photosynthetically active radiation (PAR) of 1,000 μmol photons m^{-2} s^{-1} at leaf level and around 390 ± 10 μmol CO_2 mol^{-1}. During the measurements, the temperature of the block was maintained at 28 ± 1 ºC and relative humidity at 65% ±5 in all the plantlets.

Nutrient analysis of the leaves

At the end of the growth period, the dried leaves of the plants from each treatment were sent to the laboratory of the Cocoa Research Center in CEPLAC for the leaf tissue analysis. The leaves were ground in a ball grinder (Tecnal, TE-3500 model) and a sample from each treatment was used for analysis of the macro and micronutrient content. Nitrogen (N) after sulfuric distillation was dosed for titration with HCl. Phosphorus (P) and potassium (K) concentrations were determined in the nitric-perchloric digestion extractor (Silva, 2009). P was determined using molecular absorption spectrophotometry and K was determined using flame photometry. For the micronutrients, the atomic absorption spectrophotometric method was used. Macro and micronutrient leaf concentration was multiplied by the dry leaf weight to obtain leaf nutrient content.

Statistical analysis

The obtained data were analyzed using factorial analysis of variance (ANOVA), followed by t-test for the fertilization factor and Tukey's test for the substrate factor, at a 5% level of significance using the Statistica 8 program.

Results and discussion

The substrates and the biofertilizer application (cow urine at 1%) influenced the growth of the cupuaçu plantlets, promoting significant responses in leaf gas exchanges (Table 2 and 3), in growth and in mineral nutrition (Table 2, 3 and 4).

Table 2. Net photosynthetic rate (*A*), total dry weight (TDW), relative growth rate (RGR), and leaves content of P, K and Mn of cupuaçu plantlets cultivated on different substrates, fertilized or not with cow urine (1%) after 152 days of growth.

Variable	Fertilization	Substrates			
		BM	BM-CF	BM-CS	BM-S
A (μmol m^{-2} s^{-1})	Without	3.2±0.19bA*	4.4±0.15±aB	4.4±0.34aA	5.3±0.09aA
	With	3.6±0.09cA	5.9±0.11aA	4.5±0.10bA	2.9±0.17cB
TDW (g)	Without	9.6±0.17aB	8.1±0.24abB	7.2±0.47bA	7.7±0.41bB
	With	14.2±0.59aA	9.4±0.34bA	7.7±0.36cA	9.5±0.17bA
RGR (mg g^{-1} day^{-1})	Without	17.6±0.12aB	16.5±0.19abB	15.6±0.46bA	16.1±0.36bB
	With	20.1±0.28aA	17.5±0.23bA	16.1±0.30cA	17.4±0.12bA
P (g)	Without	9.1±aB	8.3±bB	10.8±aA	8.4±aA
	With	16.8±aA	12.6±bA	12.2±bA	11.3±bA
K (g)	Without	5.5±aA	4.7±aB	5.0±aB	5.4±aA
	With	7.7±aA	7.2±aA	8.2±aA	6.6±aA
Mn (mg)	Without	51,9±aA	49,4±aA	38,8±bA	65,1±aB
	With	62,4±abA	75,4±aA	64,8±aA	94,5±aA

* Mean values ± standard error. Means followed by the same lower-case letter in the rows and by same upper-case letter in the column do not differ by Tukey's test and t-test at 5% probability for each variable. BM = base mixture (100%), BM-S = base mixture + sand; BM-CF = base mixture + coconut fiber; BM-CS = base mixture + cocoa shell.

Table 3. Relationship of internal and environmental concentration of CO_2 (Ci/Ca), chlorophyll index (CI), leaf area (LA), diameter (D), height (H), liquid assimilation rate (LQAR), leaf area ratio (LAR), and leaves content of nitrogen (N), calcium (Ca) and zinc (Zn) of cupuaçu plantlets cultivated on different substrates after 152 days of growth.

Variable	Substrate (S)			
	BM	BM-CF	BM-CS	BM-S
Ci/Ca	1.0±0.64b*	1.5±0.71a	1.2±0.66ab	1.2±0.71ab
CI	20.19±0.43ab	20.62±0.50a	18.73±0.52b	20.17±0.56ab
LA (cm^2)	748.5±46.4a	585.7±36.1b	598.0±51.0b	643.0±47.1ab
D (mm)	7.2±0.19a	6.4±0.17b	6.4±0.24b	6.4±0.19b
H (cm)	36.1±1.53a	30.7±1.25b	25.5±1.14b	28.9±1.58ab
LQAR (mg cm^{-1} day^{-1})	0.23±0.01a	0.20±0.01b	0.17±0.01b	0.18±0.01ab
LAR (dm^2 g^{-1})	80.3±6.21a	68.1±4.31ab	65.7±4.13b	74.5±4.08ab
N (g)	58.3±3.58a	58.6±a	56.0±ab	49.0±2.70b
Ca (g)	59.3±5.05a	47.0±ab	36.8±b	42.1±4.30b
Zn (mg)	280.3±29.98a	224.8±ab	185.4±b	161.3±12.64b

* Mean values ± standard error. Means followed by the same letter in the rows do not differ by Tukey's test at 5% probability. BM = base mixture (100%), BM-S = base mixture + sand; BM-CF = base mixture + coconut fiber; BM-CS = base mixture + cocoa shell.

Table 4. Leaf area ratio (LAR), liquid assimilation rate (LQAR), leaf weight ratio (LWR), root weight ratio (RWR), height (H), diameter (D), and leaves content of N, Ca, Mg, Fe, Cu and Zn of cupuaçu plantlets fertilized or not with cow urine (1%) after 152 days of growth.

Variable	Without biofertilizer	With biofertilizer
LAR (dm^2 g^{-1})	78.3±5.89a*	65.6±3.48b
LQAR (mg cm^{-1} day^{-1})	0.18±0.01b	0.21±0.01a
LWR (dm^2 g^{-1})	0.41±0.02a	0.35±0.02b
RWR (dm^2 g^{-1})	0.3±0.02b	0.4±0.02a
H (cm)	28.9±1.23b	31.7±1.52a
D (mm)	6.3±0.19b	6.9±0.20a
N (g)	44.3±2.19b	61.7±1.68a
Ca (g)	33.6±3.10b	58.9±3.24a
Mg (g)	6.4±0.39b	9.2±0.42a
Fe (mg)	1041.1±61.77b	1572.5±67.69a
Cu (mg)	17.4±1.79b	26.8±3.2a
Zn (mg)	173.0±15.8b	252.9±23.8a

* Mean values ± standard error. Means followed by the same letter in the rows do not differ between each other by t-test at 5% probability. BM = base mixture (100%), BM-S = base mixture + sand; BM-CF = base mixture + coconut fiber; BM-CS = base mixture + cocoa shell.

Biofertilization had an impact on growth and survival of the plantlets after the 152 days of the experiment. This can be demonstrated by the death of some plants in the treatments without cow urine application. In total, the death of 10 plantlets was observed, six being in the BM-CS substrate. The high mortality observed in this substrate without biofertilization may be explained by the high pH (7.9) of the cocoa shell used in its composition, thus compromising the quality and resistance of the plantlets to the attack of pathogens (Table 1). Similar results were reported by Araújo Neto et al., (2015) in cupuaçu plantlets, using a substrate containing *Ceiba pentandra* (L.) Gaertn. residue, which presented pH 8.1.

No deaths were observed in the biofertilized treatments, reinforcing the importance of cow urine as an organic fertilizer and biopesticide since it promoted an increase in nutrient availability and resistance to the attack of pathogens on the plants. This finding is reinforced by the trophobiosis theory (COSTA et al., 2015) and corroborated by Freire et al., (2019). The nutritional status was detected by chemically analysis of cupuaçu plantlets leaves, which presented a significant increase in N (10%), Ca (75.5%), Zn (46%), Mg (44%), Fe (51%) and Cu (54%) when biofertilized with cow urine (Table 4). Freire et al., (2019) when analyzing chemically cow urine, obtained means of 4.20 $g.kg^{-1}$ of N, 0.63 $g.kg^{-1}$ of Ca; 4.00 $mg.kg^{-1}$ of Zn; 0.26 $g.kg^{-1}$ of Mg; 21.00 $mg.kg^{-1}$ of Fe and 1.00 $mg.kg^{-1}$ of Cu, which may justify the results for nutritional status of cupuaçu plantlets. Cow urine as a liquid fertilizer to the plants has been encouraged in organic production system (LANGMEIER et al., 2002), considering that is a product with high viability of use as a nutritional source (FREIRE at al., 2019).

For the cupuaçu, the cow urine also improved morphophysiological conditions, as observed in the net photosynthetic rate, in growth (Table 2 and 4) and in the allocation of biomass to the root system (Table 4). Biofertilized plantlets presented increases of 19% and 35% for LQAR and RWR, respectively, revealing the influence of cow urine on increased total dry weight in function of leaf area (LQAR), with greater allocation of biomass to the root system, which guarantees plantlets resistance to environmental stress and increases the survival rate in the field (GOMES JÚNIOR et al., 2018). The indirect effect on plantlets growth through rooting was also observed by Gadelha et al., (2009) in pineapple plantlets.

Similarly, the application of biofertilizer increased 10% the height (H) and diameter (D) of the cupuaçu plantlets. These significant results for these variables were also observed by Véras et al. (2014) when studied the use of cow urine at 1% and 5% as biofertilizer in *Tamarindus indica*. The H and D values observed in cupuaçu plantlets are even higher (115% and 150%, respectively) than those found by Nascimento et al. (2017) when using biofertilizers produced with cow manure and organic material on cupuaçu, after seedling emergence, with one application per week for 72 days.

The beneficial influence of coconut fiber as a substrate conditioner was shown in the net photosynthetic rate (*A*) of biofertilized plantlets, with an increase of up to 104% when compared with the other treatments, and the lowest value was observed in plantlets cultivated in BM-S substrate (Table 2). This result may be associated with the 10% increase observed in the chlorophyll index (CI), which, in turn, was influenced by the greater increase in N (20%) found in the BM-CF substrate (Table 3). This fact is justified, as N is present in the structure of chlorophyll, which is an important pigment in the photochemical stage of photosynthesis (CHAPMAN et al., 1997), and its presence in the leaf indicates foliar activity, resulting in an increase in the photosynthesis rate, in the metabolism, in the formation of structural components and in the allocation of biomass (Wright et al., 2000), contributing to the development of seedlings (KRAUSE et al., 2017). Similar results in the photosynthetic rate were observed by Silva et al., (2009) in *Hancornia speciosa*, when evaluating substrates with different proportions of compost with cow manure.

Although significant differences in stomatal conductance (gs) were not found, the higher value of *A* in BM-CF may also be related to the greater availability of CO_2 in the intercellular spaces due to the higher Ci/Ce ratio (50%) found in this substrate (Table 3). Through the Ci/Ce ratio it is possible to verify the efficiency or not in the carbon fixation reactions, being considered an appropriate indicator for the stomatal limitation of photosynthesis, responsible for the changes in stomatal opening and resistance to the influx of CO_2 (GUERRA et al., 2017), which, in this case, favored the biochemical stage of photosynthesis.

The effect of the combination of the substrate type and the biofertilizer were observed for TDW and RGR in cupuaçu plantlets, demonstrating significance in the BM substrate and in biofertilization, with an increase of up to 98% and 29%, respectively, when compared to the other treatments, which did not differ between each other (Table 2). Considering that the nutritional conditions of the plant influence the increase in the RGR value (LAMBERS et al., 1992), and that RGR is related to the increase in biomass from pre-existing biomass (HUNT, 2016), the results observed in the present study are justified by the increase in mineral nutrient absorption in these treatments, resulting in physiological responses in the plantlets, with greater biomass production (TDW), which, in turn, influenced the increase in RGR (ZULFIQAR et al., 2019).

Furthermore, the combination between the factors showed significant results for P and K content in the leaves of cupuaçu plantlets. The highest values were observed in the treatments with biofertilization, reinforcing the nutritional importance of cow urine for the plantlets. Regarding the substrate, however, the best results were in BM for P and BM-CS for K, compared with the other treatments, which did not present differences between each other (Table 2). This result was attributed to the higher content of these ions observed in the respective components of these substrates. BM substrate contains 7.5% more organic compost than the other substrates, with a P content 3.5 times greater than the cocoa shell compost used in the BM-CS substrate, which, in turn, presented K content 3.2 times greater than organic compost. The P and K values of the coconut shell fiber and sand conditioners were up to 10 times lower when compared to the organic compost and the cocoa shell (Table 1).

The high K value found in the composition of the BM-CS substrate possibly provoked luxury consumption of this element by the cupuaçu plantlets cultivated in this substrate, causing an antagonistic effect in K/Ca (MENARD et al., 1962), reducing the leaf content of Ca through competitive inhibition of absorption (TAIZ et al., 2017). This can be confirmed by the lower value for Ca observed in BM-CS (36.8 g per leaves). A similar result was reported by Sodré et al., (2012) in *Theobroma cacao* plantlets, and by Souza et al., (2002) in *Ananas comosus* plantlets, when analyzing the effect of potassium fertilization. This is because, potassium plays a crucial role in several physiological processes, acting on the regulation of stomatal opening and limiting water loss (CAVALCANTE et al., 2018).

When analyzing the results obtained for leaf nutrient content in cupuaçu plantlets as a set, the best responses were observed in biofertilized plantlets grown in BM substrate. When comparing these values (Table 2, 3 and 4) with those presented by Frazão et al. (2006), when they characterized the visual symptoms of macronutrient deficiency in cupuaçu plantlets, and, by Almeida et al., (2021), when evaluating the omission of nutrients in the development and mineral composition of young cupuaçuzeiro plants, it can be stated that the use of cow urine as biofertilizer and BM substrate enables the maintenance of nutritional values of the shoot of the plantlets, above the critical range for the presentation of visual symptoms of deficiencies.

The growth variables analyzed in the cupuaçu plantlets with different responses to type of substrate were H, D, LA, LQAR and LAR (Table 3). All of these variables obtained higher values in BM substrate, which demonstrates the importance of the quantity of organic compost (30%) used in the preparation of the substrate, without the addition of conditioners. Studies carried out by other authors demonstrate that organic composts in substrates significantly affect plant growth, as in the example of research carried out by Araújo et al., (2013) in *Carica papaya* L. plantlets, and by Araújo Neto et al., (2015) and Santos et al., (2014) when testing organic compost in substrate for the growth of cupuaçu plantlets.

The H and D variables contributed to the evaluation of cupuaçu plantlets quality, which also increases the estimate for survival in the field (GOMES et al., 2002). Plantlets cultivated in BM substrate show an increase of up to 41.5% and 12%, respectively, in comparison to the other treatments, which did not differ between each other (Table 3). After 152 days of treatment, the plantlets reached a height of 36.1 cm and a diameter of 7.2 mm and were ready for planting in the field (LIMA-PRIMO, 2018).

The highest LA increase was observed in BM substrate, which was around 27.8% higher than the other treatments, which did not differ between each other. LA represents the raw material for photosynthesis and, as such, is of great importance in the evaluation of plant growth (PEIXOTO et al., 2011), which, in turn, is related to LQAR, a variable that reflects the capacity of the plant to increase its phytomass in function of the assimilatory surface, indicating the efficiency of the plant in the production of dry material (LUCCHESI, 1984). This also explains the higher value of this variable in BM substrate (35%), when compared to the other substrates, which did not show a difference between each other.

LAR is a growth characteristic that represents the size of the assimilatory surface in relation to total dry biomass. As a variable considered to be a parameter that expresses the useful LA for photosynthesis (HUNT, 2016), in this study, a higher value was also observed (22.3%) in BM substrate, when compared to the other substrates, which did not show a difference between each other.

Conclusion

Different substrate composition and the use of 1% cow urine as a biofertilizer influenced the development of cupuaçu plantlets. Biofertilization contributed to the survival and growth of plants, improving nutritional and morphophysiological conditions.

The coconut fiber as a conditioner substrate influenced leaf gas exchange, however, among the tested substrates, the most suitable to produce cupuaçu plantlets with higher growth, nutritional increasing and improvement in morphophysiological conditions was the mixture base composed by soil and organic compost. On the other hand, the use of cocoa shell as part of substrate should be careful considered for cupuaçu plantlets production.

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