

Initial development and nutrition of *Eugenia dysenterica* DC. on substrates formulated with sugarcane bagasse and filter cake

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Abstract

The *Eugenia dysenterica* DC. is a species native to Brazilian Cerrado (popularly known as cagaita), which presents many medicinal and nutritional properties. There is little information on about the propagation of this plant using seedlings. The sugarcane bagasse (SB) and filter cake (FC) can be an alternative for low-cost substrates formulation in regions with sugarcane production like the Cerrado region. This study aimed to investigate the use of SB and FC as substrates in the proportions of 100% SB, 75:25% SB:FC, 50:50% SB:FC, 25:75% SB:FC and 100% FC, along with a commercial substrate (Trimix[®]) for the initial development of *E. dysenterica*. The variables such particle density (PD), water availability (WA), remaining water (WR), aeration space (AS) and total porosity (TP) of the substrates were studied. The *E. dysenterica* seeds were sown in appropriate plastic containers filled with 288 cm³ of respective substrates. The seedlings were evaluated for plant height (H), stem diameter (\emptyset), leaf area (A), shoot, root and total dry matter, and Dickson Quality Index (DQI), along with macronutrients accumulation, 120 days after sowing. The treatment with combination of 25:75% SB:FC seemed to be a very appropriate substrate for production of *E. dysenterica* seedlings. This combination has shown adequate values for physical quality parameters. The reduction of SB proportion and increasing the FC caused increases in PD, WR and TP parameters and contrarily reduction in WA and AS. They promoted higher values for development of H, \emptyset , A, plant dry mass and DQI as well as the highest accumulation of N, P, K and Ca.

Keywords: Dickson Quality Index, substrate physic quality, macronutrients accumulation.

Abbreviations: PD_particle density; WA_water availability; WR_ remaining water; AS_aeration space; TP_total porosity; H_plant height; \emptyset _stem diameter; A_leaf area.

Introduction

The Cerrado (tropical savanna) is considered the second largest Brazilian biome and contains a great diversity of plant species (Paiva Sobrinho et al., 2010). The *Eugenia dysenterica* DC. is an endemic fruit species in the Cerrado (Souza et al., 2013), belonging to the Myrtaceae family and is popularly known as cagaita. The fruit tree can reach 10 to 15 m in height and has a tortuous, cylindrical, and exfoliating trunk with 20 and 40 cm diameter. The fruits, which are a berry type, are produced between September and November and exhibit thin skin and green color when young and pale yellow when ripen (Vieira and Scariot, 2006).

The cagaita's fruits and leaves have caught attention of many consumers and are being consumed in the world market as functional, medicinal and nutraceutical food. The fruits are considered an important source of vitamins C and A (Cardoso et al., 2011). The leaves are used to treat diarrhea, cardiac diseases, and for the reduction of blood cholesterol levels (Lima et al., 2011). The cagaita leaf extracts are also known for their antifungal properties (Costa et al., 2000). It has been also shown that the leaf extracts can inhibit α -amylase and α -glucosidase activities, which can be important in controlling diabetes (Souza et al., 2012) and can be a new natural cosmetic product (Moreira et al., 2017). Despite the

socioeconomic and ecologic importance of cagaita, (Bailão et al., 2015), there are few studies about the propagation of this species. The seedlings production is very important for the species maintenance in the Cerrado biome and for its world expansion.

The production of healthy, robust, well-nourished and high-quality seedlings is required for the successful of propagation of woody plant species after transplantation in the field. Substrate characteristics is a major factor affecting seedling quality and must adequately meet plant requirements in terms of its physical and chemical attributes, since it acts as a soil substitute during the seedling stage (Ferraz et al., 2005). Moreover, the substrate should exhibit slow decomposition, have a high cation-exchange capacity, be free of pathogens and seeds of undesired plants and should be available on the market at affordable prices (Dantas et al., 2009). To obtain these characteristics is often necessary to mix two or more components, which together will form a suitable substrate for the seedlings formation (Araújo Neto et al., 2009).

Several organic materials can be used as a substrate for seedling production; however, it is important to determine the most appropriate for each species (Castoldi et al., 2014). One important point to consider is the material availability in the

region, where the seedlings is produced, ensuring a constant supply and costs reduction (Mota et al., 2016). The production of organic waste is high and it is increasing in both rural and urban areas (Barros et al., 2017). Global sugar production in 2015, for example, was over 170 million mt of raw sugar, which resulted in over 300 million Mg of bagasse (USDA, 2015).

Bagasse is the fibrous material remaining after removing the sucrose, water, and other extraneous material impurities from the delivered sugarcane (Webber, et al., 2016). The filter cake is a residue composed of the mixture of ground bagasse and mud/sludge from decantation, resulting from the sugar clarification process. For each ton of processed sugarcane, 30 to 40 kg of filter cake is produced (Adorna et al., 2013). These residues have already been successfully used in the production of seedlings of some Cerrado species, like *Pouteria garderiana* (Mota et al., 2016) and *Anacardium othonianum* (Dornelles et al., 2014). Thus, since little is known about the species initial development, this materials could be used for *E. dysenterica* seedlings production. In this scenario, the objective of this study was to evaluate the *E. Dysenterica* initial development and nutrition using substrates formulated with cane bagasse and filter cake.

Results and Discussion

Physical properties of substrates

Regarding the physical evaluations of the substrates, higher values of particle density (PD) were observed for the substrates SB:FC 25:75% and FC 100%, which were not statistically different, presenting averages of 1.14 and 1.25 g cm⁻³, respectively (Table 2). The particle density expresses the ratio between the mass of dry material and the actual volume occupied by these particles, not including the space occupied by the pores. Therefore, this characteristic is not affected by the granulometry of the substrates, but by their particles composition (Fermino and Kämpf, 2012). The lower the recipient used, the lower should be the density of the substrates (Fermino et al., 2010). In this study, the particle density varied from 0.61 g cm⁻³ to 1.25 g cm⁻³, increasing as a function of the increase in the FC proportion (Table 2). Webber et al. (2017) studied substrates based on SB and commercial organic substrate and verified particle density of 0.71 g cm⁻³ for the substrate containing 100% SB, close to that observed for the 100% SB substrate of the present study.

The commercial substrate (Trimix[®]) presented the highest mean (17.6 m³ m⁻³) for available water, while treatments SB:FC 25:75% and FC 100% presented the lowest mean values (8.95 and 8.65 m³ m⁻³) for this parameter, not differing between them (Table 2). It is important to consider that, when more water is available less energy is spent by plant to take advantage of it, which leads to water saving (Webber et al., 2016), resulting in lower costs of irrigation and fertilization. The frequent irrigation can strongly lead to nutrients leaching (Guerrini and Trigueiro, 2004).

However, the remaining water volume was higher in SB:FC 25:75% (42.7 m³ m⁻³) and 100% FC (44.7 m³ m⁻³) substrates, indicating that these substrates present higher percentage of micropores. Thus, the presence of particles with different sizes leads to an arrangement of the smaller particles among the larger ones, reducing the macropores, which are responsible for the aeration porosity. This is due to the fact that the substrates SB:FC 25:75% and FC 100% presented the lowest

values for the parameter of aeration space (23.3 and 19.0 m³ m⁻³), while the treatments SB 100% SB:FC 75:25% and Trimix presented the highest averages (Table 2) for this parameter (35.1, 31.0 and 32.6 m³ m⁻³, respectively). Substrates with higher aeration allow development of fine root hair and root branching, which increases the nutrients uptake from the substrate (Nemati et al., 2002).

For total porosity, it is known that the ideal substrate should have 75-85% of its pore volume (De Booot and Verdonck, 1972; Guerrini and Trigueiro, 2004). Considering this range, the substrates SB: FC 50:50%, SB: FC 25:75% and Trimix (which did not presented differences) were the ones with adequate total porosity (76.8, 75.0 and 79.9%, respectively), while for the other treatments the mean porosity was 71.7% (Table 2). This characteristic is important for plant growth, since higher roots concentration requires higher oxygen supply and rapid removal of the formed carbon dioxide (Fermino and Kämpf, 2012). Thus, the substrate must be sufficiently porous in order to allow efficient gas exchange, avoiding lack of oxygen for root respiration and for the microorganism's activity (Kratz et al., 2013).

Biometric measurements and Dickson Quality Index of the seedlings

The seedlings height was not different according to the treatments, ranging from 4.08 to 5.26 cm. The diameter was higher in the treatments SB:FC 50:50%, SB:FC 25:75% and FC 100% (which did not differ from each other), with averages of 1.39; 1.47 and 1.43 mm, respectively. The same three treatments resulted in higher values of leaf area (mean of 30.9 cm²), DMA (mean of 0.30 g) and DQI (mean of 1.0). The other treatments resulted in no differences, with a mean for diameter of 1.29 cm, 22.6 cm² for leaf area, 0.21g of DMA and 0.65 of DQI (Table 3).

Regarding the root dry matter (RDM), while the Trimix[®] treatment presented the highest mean (0.51 g), the FC 100% treatment presented the lowest (0.36 g), with no differences among them for the other treatments (mean of 0.43 g), with values between Trimix[®] and 100% FC. It is known that Ca is important for the development of the plant root system (Ritchey et al., 1982). However, although the 100% FC substrate presents five times more Ca when compared to the Trimix[®] treatment, its RDM was 30% lower than the observed for the Trimix[®] treatment. It is probably due to the aeration space in the 100% FC substrate, which was 41% smaller than Trimix[®] (Table 3) and may have resulted in less space for root development.

The total dry matter (TDM) was not different according to the treatment used in this study, ranging from 0.65 to 0.75 g (Table 3). However, considering the DQI value is more important than analyzing plants dry matter separately. This index is considered a promising integrated measure of morphological traits, being a good indicator of seedling quality as well as its robustness and biomass distribution, considering several important parameters (Fonseca et al., 2002). In the present study, the DQI ranged from 0.65 to 1.08, after treatments with SB:FC 50: 50%, SB:FC 25:75% and FC 100%, presenting the highest results for FC 100%. Hunt (1990) suggested that index ≥ 0.20 is the standard to indicate better quality of seedlings. Considering this value, all studied treatments are within the quality standard (Table 3).

Table 1. pH value, carbon:nitrogen ratio (C:N), organic matter content (OM), organic carbon (OC), nitrogen (N), phosphorus (P), sulfur (S), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) in a commercial substrate (Trimix[®]) and in substrates obtained from sugarcane bagasse (SB) and sugarcane filter cake (FC).

Substrate	pH ²	C:N	OM	OC	N	P	S
	----- g 100g ⁻¹ -----						
SB 100%	6.2	61.7	85.2	49.4	0.8	0.23	0.04
SB:FC 75:25%	7.2	29.4	65.9	38.2	1.3	1.28	0.16
SB:FC 50:50%	7.2	40.7	56.3	32.6	0.8	1.97	0.60
SB:FC 25:75%	7.4	19.2	53.1	30.8	1.6	1.78	0.44
FC 100%	7.4	15.6	51.2	29.7	1.9	2.15	0.37
Trimix [®]	6.3	30.0	31.1	18.0	0.6	0.45	0.06

Substrate	K	Ca	Mg	Cu	Fe	Mn	Zn
	----- g 100g ⁻¹ -----			----- mg kg ⁻¹ -----			
SB 100%	0.1	0.36	0.12	57	595	283	68
SB:FC 75:25%	0.2	1.47	0.17	100	615	409	116
SB:FC 50:50%	0.2	2.00	0.20	115	710	495	146
SB:FC 25:75%	0.2	2.09	0.21	190	640	505	170
FC 100%	0.2	2.24	0.21	123	660	536	158
Trimix [®]	0.7	0.41	0.40	48	590	503	107

Note. ²pH measured in H₂O.

Table 2. Dry density (DD), wet density (WD), particle density (PD), water available (WA), water remaining (WR), aeration space (AS) and total porosity (TP) of a commercial substrate (Trimix[®]) and of substrates obtained from sugarcane bagasse (SB) and filter cake (FC).

Substrate	DD	WD	PD	WA	WR	AS	TP
	--- kg m ⁻³ ---		g cm ⁻³	----- m ³ m ⁻³ -----			
SB 100%	248.6 d	343.4 d	0.61 d	11.3 b	24.2 d	35.1 a	70.7 c
SB:FC 75:25%	291.9 c	445.5 c	0.73 d	11.0 bc	30.3 c	31.0 ab	72.2 ab
SB:FC 50:50%	360.4 b	600.7 b	1.00 bc	10.4 bc	38.5 b	27.8 bc	76.8 ab
SB:FC 25:75%	405.0 a	727.2 a	1.14 bc	8.95 bc	42.7 a	23.3 cd	75.0 ab
FC 100%	393.4 a	743.9 a	1.25 a	8.65 c	44.7 a	19.0 d	72.4 b
Trimix [®]	401.8 a	599.7 b	0.81 cd	17.6 a	29.8 c	32.6 ab	79.9 a

Note. Means in a column followed by the same lowercase letter are not significantly different by Tukey test ($p \leq 0.05$).

Table 3. Height (H), stem diameter (ϕ), foliar area (A), dry matter of aerial part (DMA), root dry matter (RDM), total dry matter (TDM) and Dickson Quality Index (DQI) of guapeva (*Eugenia dysenterica* Mart. DC) seedlings growth in a commercial substrate (Trimix[®]) and in substrates obtained from sugarcane bagasse (SB) and filter cake (FC).

Substrate	H	ϕ	A	DMA	RDM	TDM	DQI
	cm	mm	cm ²	g	g	g	-
SB 100%	4.24 a	1.30 d	20.1 a	0.21 b	0.46 ab	0.68 a	0.65 b
SB:FC 75:25%	5.19 a	1.27 d	25.4 a	0.21 b	0.44 ab	0.65 a	0.65 b
SB:FC 50:50%	5.26 a	1.39 abc	30.3 a	0.32 a	0.42 ab	0.75 a	0.97 a
SB:FC 25:75%	4.93 a	1.47 a	26.8 a	0.29 a	0.40 ab	0.70 a	0.95 a
FC 100%	4.92 a	1.43 ab	28.9 a	0.29 a	0.36 b	0.66 a	1.08 a
Trimix [®]	4.08 a	1.33 bcd	23.3 a	0.21 b	0.51 a	0.73 a	0.64 b

Note. Means in a column followed by the same lowercase letter are not significantly different by Tukey test ($p \leq 0.05$).

Table 4. Total accumulation of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) in guapeva (*Eugenia dysenterica* Mart. DC) seedlings growth in a commercial substrate (Trimix[®]) and in substrates obtained from sugarcane bagasse (SB) and filter cake (FC).

Substrate	N	P	K	Ca	Mg	S
	----- mg per seedling -----					
SB 100%	9.0 b	1.0 b	2.7 c	3.7 d	2.1	0.7 c
SB:FC 75:25%	10.7 b	1.1 b	3.3 b	7.8 c	1.7	4.1 a
SB:FC 50:50%	13.5 a	1.3 b	3.9 b	12.1 a	2.1	1.4 b
SB:FC 25:75%	14.4 a	3.8 a	4.3 a	11.9 a	1.9	1.3 b
FC 100%	12.8 ab	1.2 b	3.8 b	9.9 b	1.8	0.2 c
Trimix [®]	12.7 ab	1.2 b	4.7 a	3.5 d	2.4	0.2 c

Note. Means in a column followed by the same lowercase letter are not significantly different by Tukey test ($p \leq 0.05$).

However, the higher the value above 0.20, the better the final quality for the seedlings to go to the field (Bernardino et al., 2005).

Nutrient accumulation by seedlings

Among the substrates that promoted the highest DQI value (Table 3), SB:FC 25:75% also presented highest macronutrient accumulation (Table 4). This accumulation followed the order of N > Ca > K > P > S > Mg, corresponding to 14.4; 11.9; 4.3; 3.8; 2.1 and 2.1 mg per seedling, respectively. For N accumulation, the SB:FC 25:75% treatment did not differ from the SB:FC 50:50% treatment but resulted in values 1.1 times higher than seedlings from the 100% FC and Trimix treatments (which did not differ from each other), and 1.6 and 1.3 times higher than SB 100% and SB:FC 75:25%, respectively, which did not differ among them (Table 4).

It is important to indicate that, in all treatments, N was the nutrient with higher accumulation by the seedlings, probably due to its higher requirement during the first stages of plant development (Malavolta et al., 1997). The N restriction can lead to reduced growth, since this nutrient, besides being part of the structure of amino acids, proteins, nitrogen bases, nucleic acids, enzymes, vitamins and pigments, participates in processes such as ionic absorption, photosynthesis, respiration, cell multiplication and differentiation (Marschner, 1995). The Nitrogen stimulates the plant vegetative growth (Malavolta et al., 1997) and is the most accumulated element by the *Eugenia dysenterica* seedlings, especially for the treatments SB:FC 50:50%, SB:FC 25:75% and FC 100%. This explains the fact that the seedlings that grown in these treatments also presented the highest mean values for leaf area (Table 3).

Treatment SB: FC 25:75% accumulated approximately 3.4 times more P (Table 4), when compared to others (that did not differ among themselves). The main function of P in plants is the energy accumulation and transfer (Malavolta, 2006). Therefore, limitations on P availability at the beginning of the crop cycle may result in restrictions on its development, with no recovery during the next stages, even increasing the P supply at appropriate levels (Roggatz et al., 1999). For the K accumulation, the plants grown in SB:FC 25:75% did not present differences, when the Trimix treatment was applied (mean of 4.7 mg per seedling), resulting in an accumulation approximately 1.6 times higher than the SB 100% treatment (which presented the lower accumulation of these elements) and 1.3 times higher than the other treatments (which did not differ from each other) (Table 4). It is important to remember that K is especially important for fruits, because this element acts in the transportation of phloem photoassimilates, being an important nutrient for the fruit formation (Marschner, 1995).

Regarding the Mg accumulation, which is a fundamental element for the chlorophyll ring formation (Taiz and Zeiger, 2006), no difference between the treatments was observed, with a mean of 1.9 mg per plant (Table 4). For S, the seedlings grown with SB:FC 25:75% accumulated 1.6 times more S than those produced using SB:FC 75:25% and SB:FC 50:50% (which did not differ from each other and presented an average accumulation of 1.4 mg per seedlings); and 5.4 times more than the other treatments (which did not differ among themselves and presented a mean of accumulation of 0.45 mg per seedlings) (Table 4). It is important to emphasize that S plays an important role in the structure of plant proteins (Taiz and Zeiger, 2006).

Materials and Methods

Location, experimental design and treatments

The experiment was conducted under greenhouse conditions in Plant Tissue Culture Laboratory (PTCL) at the Federal Institute of Goiás, Rio Verde, GO, Brazil (with geographical coordinates of 17° 48' 16" S and 50° 54' 19" W). A randomized block design was used with six treatments (substrates) and five repetitions. A commercial substrate Trimix® was used as standard for the production of forestry seedlings and five alternative substrates produced from residues of the sugar-alcohol industry were tested. The alternative substrates were obtained from the mixture of sugarcane bagasse (SB) and sugarcane filter cake (FC), in the following proportions (vol vol⁻¹): SB 100%; SB:FC 75:25%; SB:FC 50:50%; SB:FC 75:25%; and FC 100%.

Substrates characterization

The chemical characterization of the six tested substrates is shown in Table 1. The physical analysis was performed in four samples of each substrate according to Zorzeto et al. (2014). Dry density (DD), wet density (WD), particle density (PD), available water (WA), remaining water (WR), aeration space (AS) and total porosity (TP) were evaluated (Table 2).

Plant material and seeding

The *Eugenia dysenterica* Mart. DC seeds were obtained from ripe fruits harvested in an area belonging to the municipality of Montes Claros de Goiás, Go, Brazil (with geographical coordinates of 16° 06' 20" S and 51° 17' 11" W, and 592 m above sea level). The fruits were manually pulped at the PTCL and the seeds were carefully washed in running water.

The seeding was performed on plastic tubes (appropriate containers for seedlings production) with capacity of 288 cm³ and filled with the substrates. One seed was sown in each plastic tube (at depth of 1.5 cm) and each experimental unit (replicate) consisted of five tubes.

After seeding, the plastic tubes were allocated in suspended grids in the greenhouse. Irrigation by sprinkling was automatically performed four times a day. During the 120 days of the trial conduction, the average temperature inside the greenhouse corresponded to 27.6°C and the average air humidity to 69%.

Biometric measurements and Dickson Quality Index

Biometric measurements were performed at 120 days after seeding. Height (H) and stem diameter (Ø) of seedlings were measured using a millimetric ruler and precision digital caliper. The leaf area (A) was obtained by integrating the images of the leaves into an image-handling software. Seedlings were also divided into leaves, stem and roots and the materials were washed and placed to be dried in an air-forced circulation oven at 65 °C to obtain the dry mass. The Dickson Quality Index (DQI) was obtained according to the following equation (Dickson et al., 1960):

$$QDI = TDM / ((H / \varnothing) + (SDM / RDM)) \quad (1)$$

Where, TDM: Total Dry matter (g); SDM: Shoot Dry Matter (g); RDM: Root Dry Matter (g); H: height (cm); and Ø: stem diameter (mm).

Macro and micro nutrient analysis

The plant samples were individually ground in a Willey mill, and the N, P, K, Ca, Mg, S, Fe, Cu, Mn, Zn and B contents were determined according proceedings described by Malavolta et al. (1997). The content of each nutrient was multiplied by the root or shoot dry matter and the total accumulated in the seedlings was obtained by the sum of both and then division by five (number of seedlings of each experimental unit).

Data analysis

All data were submitted to ANOVA and means were compared by Tukey's test ($p \leq 0.05$) using the software SISVAR (Ferreira, 2011).

Conclusion

This research indicated that the substrate containing 25% of cane bagasse (SB) and 75% of filter cake (FC) can be used in the production of *Eugenia dysenterica* seedlings. The SB: FC 25:75% combination resulted in adequate physic quality parameters of the substrate. Reducing the SB proportion and increasing the FC proportion, it was possible to observe an increase in particle density, water remaining and total porosity and a reduction of water availability and aeration space. The substrate SB:FC 25:75% also provided adequate development in height, diameter, leaf area, dry matter production and Dickson quality index of the plants, besides promoting the higher macronutrients accumulation in the seedlings, which followed the order N> Ca> K> P> S> Mg.

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