SCIENTIA
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Physic-chemical properties and substrate formulation for *Eucalyptus* seedlings production

Propriedades físico-quimicas e formulação de substratos para produção de mudas de *Eucalyptus*

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Resumo

Objetivou-se avaliar a viabilidade de uma série de materiais para formulação de substratos, com foco em resíduos agroindustriais, e a influência de suas propriedades físico-químicas na produção de mudas de *Eucalyptus benthamii,* e verificar a semelhança entre os componentes testados. Foram formulados 100 tratamentos. Realizou-se semeadura direta em tubetes de 55 cm³, acondicionados em bandejas plásticas, alocadas em estufa por 120 dias. Como resultados, em relação às propriedades físicas, formaram-se quatro grupos: Grupo 1- constituído por elementos de alta macroporosidade; Grupo 2- equilíbrio entre macro e microporos, Grupo 3- maior proporção de microporos e Grupo 4- alta densidade associada à alta microporosidade. Quanto às propriedades químicas, formaram 2 grupos: Grupo 2- biossólido e Grupo 1 - demais componentes. Verificou-se maior crescimento nos substratos com maior capacidade de retenção de água e os altos níveis de condutividade elétrica e pH não prejudicaram o crescimento das mudas. A casca de pinus semidecomposta e diferentes granulometrias de moinha de carvão proporcionaram redução no crescimento quando inseridas na formulação dos substratos. O resíduo regional, biossólido, apresentou o menor custo aliado ao bom crescimento das plantas e a utilização da moinha de carvão justifica-se a fim de reduzir os custos de produção.

Palavras-chave: Moinha de carvão, turfa, casca de pinus, casca de arroz carbonizada, biossólido, análise de agrupamento.

Abstract

Based on substrate importance for seedling production, we evaluated the feasibility of using a variety of materials, with a focus on agro-industrial residues, and the influence of their physic-chemical properties on *Eucalyptus benthamii* seedling production, as well as to check for similarities between the tested components. One hundred treatments were formulated. Direct seeding was performed in plastic tubes of 55 cm³, packed in plastic trays, and placed in a greenhouse for 120 days. According to physical properties, four groups were made: Group 1- elements with high macro-porosity; Group 2 - balance between macro-pores and micro-pores; Group 3 - higher proportion of micro-pores; and Group 4 - high density associated with high micro-porosity. Concerning chemical properties, two groups were formed: Group 2 - sewage sludge and Group 1 - other components. In general, we observed more growth in substrates with greater water-holding capacity. High levels of electric conductivity and pH did not hinder seedlings growth. Almost decomposed pine bark and different fine charcoal grain size provided reduction in growth when inserted in substrate formulation. Regional sewage sludge presented the lowest cost coupled with good plant growth, and the use of fine charcoal is justified in order to reduce production costs.

Keywords: Fine charcoal, peat moss, pine bark, carbonized rice husk, sewage sludge, cluster analysis.

INTRODUCTION

The area planted with *Eucalyptus* in Brazil has been growing year after year, due mainly to investments of paper and cellulose companies (IBA, 2014). This reflects on the increased demand for plus trees plantation, which is possible mainly through cloning. However, subtropical species cultivated in Brazil's southern region, have low levels of adventitious rootings (ASSIS; MAFIA, 2007; XAVIER et

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al., 2013). According to Brondani et al. (2014), the average percentage of *Eucalyptus benthamii* rooting is 43.5%, being considered a hard rooting species, thus justifying the production of seedlings by seed, the demand for plants of this species (SCHULTZ et al., 2012; KRATZ et al., 2013a).

Seedlings should have a good quality in order to avoid losses by mortality and low growth after planting, coupled the reduction of crop treatment applied to plantation, due to reduced competition from invasive plants, promoted by greater early growth of the crop (FIGUEIREDO et al., 2011). In this sense, many efforts have been made to improve the quality and reduce production costs. Among the factors of influence is the substrate, which needs to support the seedlings and supply water and nutrients needed for plant growth (HARTMANN et al., 2011). Intrinsic factors of substrates that affect plant growth are: their physicochemical properties, which vary depending on their origin, production method and components proportions (WENDLING et al., 2007; MELO et al., 2014).

The use of substrates with low cost coupled with formation of plants with a superior morphological quality, results in reduction of production cycle and lower economic spending (SIMÕES et al., 2012). Most commercial substrates are available only in specific locations in the country, increasing the costs when transported to more distant regions (KRATZ et al., 2013a). The use of regional waste substantially reduces the cost of substrate, although costs can vary depending on availability of each material (WENDLING et al., 2007; MELO et al., 2014).

Ready-to-use substrates may be found on the agricultural market; the main products being based on pine bark and peat moss. However, currently there is a low offer of pine bark for substrate manufacturing due to its increased use as an energy source for forestry industry boilers, as well as due to the reduction in the area planted with *Pinus* in Brazil (IBA, 2014). In the case of peat moss, its main disadvantage is the environmental impact, since it is a non-renewable material (CALDEIRA et al., 2011), together with temporary prohibition of its importation from Canada, the main supplier of the raw material (BRASIL, 2013).

Other wastes are already being used as substrate components, such as coconut fiber and carbonized rice husks; however, these are not found in all regions of the country, raising the cost of acquisition at sites distant from the production source. In contrast, there are residues found in all locations, such as sewage sludge, being technically feasible for seedling production (TRIGUEIRO; GUERRINI, 2004; GOMES et al, 2013; CALDEIRA et al., 2014), but not commonly used for this.

Considering the importance of using organic waste and the need to present new options of substrates, this study assessed the feasibility of using a range of materials, focusing on agro-industrial waste, and the influence of their physic-chemical properties *E. benthamii* seedling production, as well as checking the similarity between the tested components through cluster analysis.

MATERIAL AND METHODS

Preparation of substrates

The experiment began in December 2013, in the Laboratory of Forest Species Propagation, from Embrapa Florestas, located in Colombo, Paraná, Brazil (25°19'17" S and 49°09'39" W). The climate, according to Köppen, is Cfb (subtropical humid climate).

Two commercial substrates were used (based on peat moss and almost decomposed pine bark) and mixtures of different components (carbonized rice husk, fine charcoal, coconut fiber, almost decomposed pine bark, fine vermiculite, sewage sludge and peat moss) for formulation of 100 substrates (Table 1).

Charcoal from wood of *Pinus* and *Eucalyptus* was used to obtain two different grain sizes (3-5 mm and 1-3 mm). For this, three sieves with meshes of 5, 3 and 1 mm were used. Carbonized rice husk and sewage sludge were prepared according to the methodology described by Kratz et al. (2013a), and fine vermiculite (grain size around 0.71 to 2 mm), coconut fiber (grain size around 0.25 to 2 mm), Brazilian black peat moss, almost decomposed pine bark and commercial substrates were acquired from a substrates factory.

Materials were mixed manually along with basic fertilization (0.6 kg m³ ammonium sulfate [20% of N], 4 kg m³ simple superphosphate [20% of P_2O_5 and 14% of SO_4], 0.2 kg m³ potassium chloride [58% of K_2O] and 1.5 kg m³ FTE BR 12 [9% of Zn, 3% of Fe, 2% of Mn, 0.1% of Mo, 1.8% of B, 0.8% of Cu]).

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Tabela 1. Materials used (%) for substrate formulation (volume/volume).
Table 1. Materiais utilizados (%) na formulação dos substratos (volume/volume).

S	CRH	C1	C2	CF	PB	FV	SS	PM	S	CRH	C1	C2	CF	PB	FV	SS	PM
1	100								51		90						10
2		100							52		70						30
3			100						53		50						50
4				100					54		30						70
5					100				55		10						90
6						100			56			90		10			
7							100		57			70		30			
8								100	58			50		50			
9	Commercial substrate based on peat moss								59			30		70			
10	Commercial substrate based on pine bark								60			10		90			
11	90			10					61			90			10		
12	70			30					62			70			30		
13	50			50					63			50			50		
14	30			70					64			30			70		
15	10			90					65			10			90		
16	90				10				66			90				10	
17	70				30				67			70				30	
18	50				50				68			50				50	
19	30				70				69			30				70	
20	10				90				70			10				90	
21	90					10			71			90					10
22	70					30			72			70					30
23	50					50			73			50					50
24	30					70			74			30					70
25	10					90			75			10					90
26	90						10		76				10	90			
27	70						30		77				30	70			
28	50						50		78				50	50			
29	30						70		79				70	30			
30	10						90		80				90	10			
31	90							10	81					90	10		
32	70							30	82					70	30		
33	50							50	83					50	50		
34	30							70	84					30	70		
35	10							90	85					10	90		
36		90			10				86					90		10	
37		70			30				87					70		30	
38		50			50				88					50		50	
39		30			70				89					30		70	
40		10			90				90					10		90	
41		90				10			91					90			10
42		70				30			92					70			30
43		50				50			93					50			50
44		30				70			94					30			70
45		10				90			95					10			90
46		90					10		96				90		10		
47		70					30		97				70		30		
48		50					50		98				50		50		
49		30					70		99				30		70		
50		10					90		100				10		90		

S- Substrate; CRH- Carbonized rice husk; C1- charcoal with grain size between 1-3 mm; C2- charcoal with grain size between 3-5 mm; CF- coconut fiber; PB- almost decomposed pine bark; FV- fine vermiculite; SS - sewage sludge; and PM- peat moss.

Substrate Analysis

Physical and chemical characterization of substrates was performed in the Watershed Management Laboratory, from Federal University of Paraná and Soil Chemistry Laboratory, Embrapa Florestas, respectively, according to the methodology described in the instruction No. 17 of Ministério da Agricultura, Pecuária e Abastecimento (BRASIL, 2007). Through this normative instruction the values of apparent density (Ad), water-holding capacity on a 10 cm tension (WHC 10) or micro-

-porosity (micro), potential of hydrogen (pH) and electrical conductivity (EC) were obtained. From these results, total porosity (Tp) and macro-porosity (Macro) were obtained. Tp corresponds to the volume of water retained in substrate at 0 hPa tension (fully saturated), while macro-porosity consists of difference between Pt and the volume of water held up at 10 hPa (WHC 10 or micro--porosity) (DE BOODT; VERDONCK, 1972).

Mineral nutrients phosphorus, potassium, calcium and magnesium were determined in their available form (NOGUEIRA; SOUZA, 2005), while carbon, nitrogen and sulfur in their total form were obtained using the CHNS Elemental Analyzer.

Seedling production

Direct seeding was performed in plastic tubes of 55 cm³ (packed in metal trays and separated at a spacing of cell tray) with *Eucalyptus benthamii* seeds from a Seed Production Area, located in Guarapuava, Paraná, Brazil. Manual seeder was used, in which every container received around 4 seeds, covered with a layer of 0.5 cm of fine vermiculite. After sowing, trays were placed in a greenhouse (4 daily irrigations of 10 min with flow rate of 144 L hour^{-1}) with monitored temperature (Table 2), where they remained for 120 days. Thirty days after sowing, thinning was done, leaving only one plant per tube.

Tabela 2. Minimal (T min.), maximal (T max.) and medium (T med.) air temperature inside greenhouse during the *E. benthamii* seedlings production period.
Table 2. Temperatura do ar mínima (T min.), máxima (T máx.), média (T méd.) no interior da estufa no período de produção das mudas de *E. benthamii*.

Month/2014	Morning T min.	Morning T max.	Morning T med.	Afternoon T min.	Afternoon T max.	Afternoon T med.
	°C ± SD			°C ± SD		
January	19.63±3.63	36.50±3.84	34.53±5.03	26.03±2.05	39.13±3.32	34.37±4.24
February	18.82±4.62	34.57±6.49	30.54±6.99	23.93±4.97	34.14±7.05	27.79±6.99
March	16.29±1.99	27.68±3.50	25.42±4.06	22.03±2.73	30.26±4.26	24.32±3.20
April	15.85±2.20	25.96±4.24	23.44±4.68	19.63±3.83	27.80±5.29	22.23±4.38

SD - standard deviation in relation to mean value.

On the 30th day, growth fertilization began (4 g L^{-1} of urea, 3 g L^{-1} of simple superphosphate, 0.25 g L^{-1} of FTE BR 10 (7% Zn, 4 % Fe, 4 % Mn, 0.1% Mo, 2.5 % B, 0.8% Cu) and 3 g L^{-1} of potassium chloride), applied every seven days until the 90th day. After this, rustication fertilization was performed (4 g L^{-1} of ammonium sulfate, 10 g L^{-1} of simple superphosphate, 4 g L^{-1} of potassium chloride, 1 g L^{-1} of FTE BR 10), which was also given every seven days until the 120th day. For fertilization preparation, the fertilizers were mixed with water and the resulting solution was applied to the seedlings with use of manual watering cans, applying approximately 5 ml per plant.

For the evaluation of seedling quality, the height of the aerial part was measured (ruler graduated in mm) and stem diameter (digital caliper) on the 90th and 120th days. On the 120th day, an evaluation of aerial and root part dry biomass (48 hours in oven at 65° C and weighing in a precision analytical balance of 0.001 g) of 100 seedlings of different height classes was made, in order to fit an equation to estimate the dry biomass of all seedlings.

Experimental design and statistical analysis

The applied experimental design was completely randomized with five replications of 10 plants and 100 treatments. In order to observe the influence of each component addition in different combinations, a regression analysis was performed. To verify which components used in the substrates formulation presented greater similarity concerning physic-chemical properties, a cluster analysis was performed by the nearest neighbor method. To determine groups, a 50% cut in the Euclidean distance was adopted (ALBUQUERQUE et al., 2006).

Cost

For evaluation of commercial components costs, the values of purchase were considered. Costs of fine charcoal and sewage sludge were obtained by summing the costs of transportation and of labor to prepare it, on the basis of a worker's wage employed in Farming, Forestry and Fishing Activities (PARANÁ, 2014).

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RESULTS AND DISCUSSION

Substrate Analysis

Cluster components analysis concerning physical properties, formed four substrates groups (G) in a 50% reduction of Euclidian distance: G1 - carbonized rice husk, G2- two grain sizes of charcoal, G3 - coconut fiber, fine vermiculite, peat moss and pine bark, and G4 - sewage sludge, being G1 and G4 the groups with greater differences regarding others (Figure 1A). From cluster analysis we found that G1 group had high macro-porosity, G2 had components that presented greater balance between macro and micro-porosity, G3 was formed by elements with higher proportion of micro-pores, and G4 had high density associated with high micro-porosity (Figures 1A and 2).

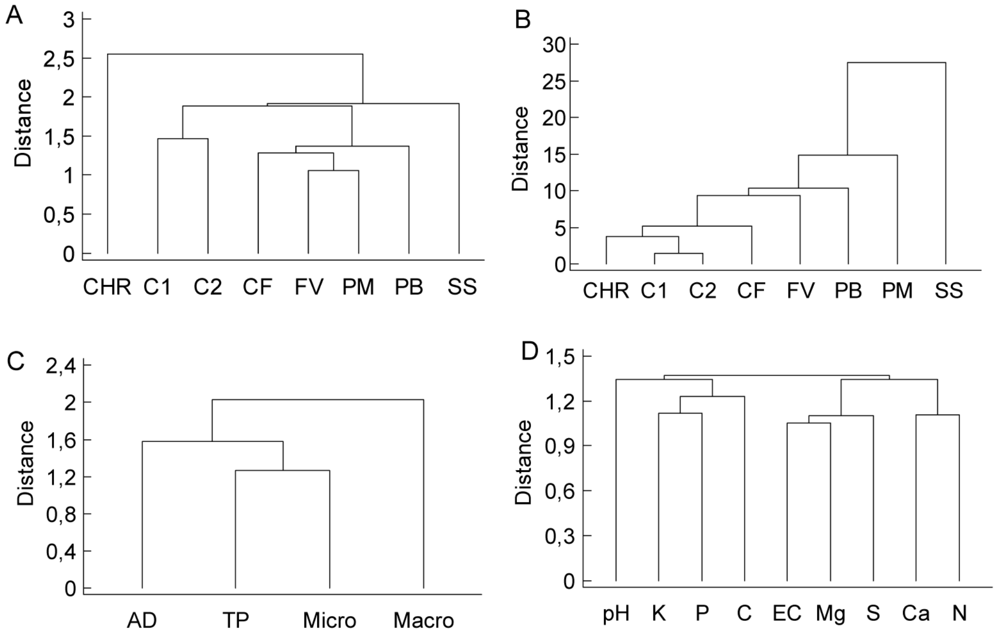

Figura 1. Cluster analysis of the components against the physical properties (A) and Chemical properties (B). Cluster analysis of the physical properties (C) and chemical properties (D).

Figure 1. Análise de agrupamentos pelo método do vizinho mais próximo: dos componentes em relação as propriedades físicas (A) e químicas (B); propriedades físicas (C) e químicas (D).

Among physical properties, total porosity and micro-porosity presented greater similarity, followed by apparent density and macro-porosity, similar in cluster analysis result of components, in which carbonized rice husk, followed by sewage sludge, were the substances with higher difference compared to the others (Figure 1 C and A). In spite of presenting similarities with charcoal-based components, carbonized rice husk differentiates itself due to its high macro-porosity, a feature specific to this component (Figure 2 D), and sewage sludge due to its high micro-porosity and apparent density (Figure 2 A).

The addition of G1 (carbonized rice husk) to G3 (coconut fiber, fine vermiculite, peat moss and pine bark) and G4 (sewage sludge) groups resulted in a decrease of apparent density and micro-porosity and increase of porosity and macro-porosity values. On the other hand, G2 (different grain size of charcoal) when combined with fine vermiculite (G3) led to an increased density and reduction of total porosity and micro-porosity. When combined with peat moss and pine bark (G3) or sewage sludge (G4), it provided a reduction in density, porosity and micro-porosity (Table 3).

Results from cluster analysis show that carbonized rice husk and sewage sludge cannot be replaced by any of studied elements, due to high macro-porosity of carbonized rice husk (56.9%) and high micro-porosity associated with apparent density of sewage sludge (56.3%; 583.3 Kg m^{-3}). Results indicate that it is necessary to combine elements of G1 and G2 to G3 and G4, to improve its physical characteristics, obtaining as a result substrates with greater balance between macro- and micro-pores. Despite not being in the same group, charcoal based components should not be combined with carbonized rice husk, because all components feature low water retention capacity and micro-porosity (Figure 2 C).

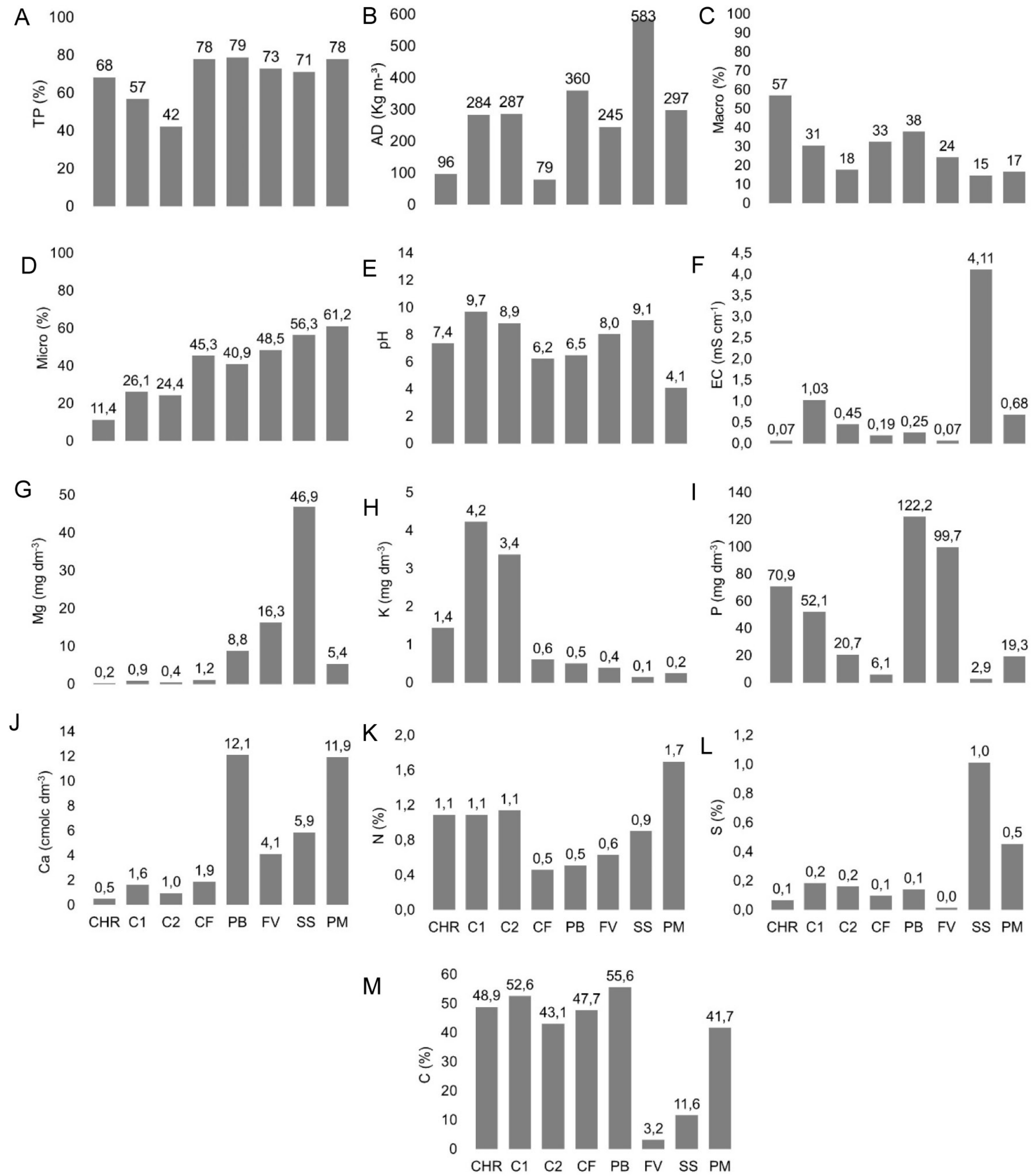

Figura 2. Physicochemical properties of components used in substrate formulation: AD - apparent density (A); TP - total porosity (B); Macro – macro-porosity (C); Micro – micro-porosity (D); pH - potential of hydrogen (E); EC - electrical conductivity (F); Mg - magnesium (G); K - potassium (H); P - phosphorous (I); Ca - calcium (J); N - nitrogen (K); S - sulfur (L); and C - carbon (M).

Figure 2. Propriedades físico-químicas dos componentes usados na formulação dos substratos. Densidade aparente (A), porosidade total (B), macroporosidade (C), microporosidade (D); potencial hidrogeniônico (E); condutividade elétrica (F); magnésio (G); potássio (H); fósforo (I); cálcio (J); nitrogênio (K); enxofre (L) e carbono (M).

Materials with low density raise the mixture's macro-porosity and reduce water retention capacity of substrates (CALDEIRA et al., 2014), as was verified with increased proportion of carbonized rice husk and different charcoal grain sizes. On the other hand, the lightweight materials such as coconut fiber and fine vermiculite caused an increase in water holding capacity, due to presence of internal pores (SIMÕES et al., 2012; KRATZ et al., 2013a). Different water retention results indicate the need for water management for each substrate composition (SIMÕES et al., 2012). In seedlings of *E. grandis* produced in two commercial substrates subjected to different irrigation regimes, growth is associated with substrate and irrigation scheme applied by it, as noted by Lopes et al. (2007).

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Tabela 3. Physic-chemical analysis results. Apparent density (AD), total porosity (TP), micro-porosity (Micro), macro-porosity (Macro), potential of hydrogen (pH), electric conductivity (EC), magnesium (Mg), potassium (K), phosphorus (P), calcium (Ca), nitrogen (N), carbon (C) and sulfur (S).

Table 3. Resultados da análise física e química. Densidade aparente (AD), porosidade total (TP), microporosidade (Micro), macroporosidade (Macro), potencial hidrogeniônico (pH), condutividade elétrica (EC), magnésio (Mg), potássio (K), fósforo (P), cálcio (Ca), nitrogênio (N), carbono (C) e enxofre (S).

Substrate	AD	TP	Micro	Macro	pH	EC	Mg	K	P	Ca	N	C	S
	kg m^{-3}		%			mS cm^{-1}		mg dm^{-3}		cmolc dm^{-3}		%	
CS1	315.34	77.29	50.37	26.93	5.86	0.57	8.62	0.35	55.22	24.50	1.84	44.04	0.55
CS2	193.09	69.06	45.06	24	5.26	0.93	4.05	1.67	39.04	3.44	0.26	25.94	0.06
90 CRH/ 10 CF	86.30	69.48	16.31	53.17	7.21	0.09	0.48	1.36	53.70	0.43	1.03	47.06	0.06
70 CRH/ 30 CF	86.65	75.37	25.44	49.94	6.92	0.09	0.58	1.23	53.15	0.71	0.89	44.58	0.08
50 CRH/ 50 CF	78.26	76.58	31.91	44.67	6.72	0.10	0.55	1.14	37.45	1.14	0.85	43.32	0.11
30 CRH/ 70 CF	73.86	80.02	40.02	39.99	6.68	0.10	0.90	0.95	20.80	1.73	0.76	42.98	0.11
10 CRH/ 90 CF	63.82	79.05	50.24	28.81	6.58	0.12	0.93	0.73	10.05	1.94	0.44	40.73	0.12
90 CRH/ 10 CF	125.77	70.99	16.53	54.46	7.20	0.12	1.69	1.51	99.45	2.44	1.04	42.18	0.10
70 CRH/ 30 CF	165.11	72.65	22.02	50.62	6.90	0.11	2.75	1.29	118.50	5.06	0.94	37.46	0.08
50 CRH/ 50 CF	204.45	73.08	26.49	46.59	6.69	0.13	4.44	0.96	112.75	7.25	0.61	30.8	0.06
30 CRH/ 70 CF	286.82	77.89	33.84	44.05	6.59	0.14	7.86	0.79	123.35	9.63	0.56	29.97	0.07
10 CRH/ 90 CF	339.57	79.33	39.70	39.63	6.53	0.17	9.55	0.65	121.70	11.69	0.51	27.66	0.07
90 CRH/ 10 CF	116.8	70.62	18.82	51.8	7.38	0.07	1.80	1.13	62.75	0.73	0.94	37.91	0.02
70 CRH/ 30 CF	139.75	69.61	25.71	43.9	7.37	0.05	3.06	1.00	62.00	1.63	0.69	27.96	0.03
50 CRH/ 50 CF	160.60	69.00	33.38	33.44	7.49	0.04	5.31	0.65	53.95	1.75	0.42	16.19	0.01
30 CRH/ 70 CF	179.53	68.39	38.59	29.81	7.79	0.05	8.06	0.54	62.85	2.50	0.18	7.21	0.00
10 CRH/ 90 CF	172.3	69.57	41.80	27.19	7.39	0.02	8.06	0.38	59.90	2.50	0.11	2.47	0.00
90 CRH/ 10 SS	168.10	72.43	16.93	55.5	9.15	1.08	8.17	0.71	46.64	2.75	1.15	34.52	1.10
70 CRH/ 30 SS	263.98	72.72	26.00	46.76	9.16	2.24	15.04	0.46	2.17	4.00	1.02	21.77	0.97
50 CRH/ 50 SS	387.65	72.16	37.41	34.75	9.12	3.62	29.37	0.27	4.35	4.69	0.97	15.13	1.23
30 CRH/ 70 SS	472.56	72.79	44.55	28.24	9.11	4.10	38.76	0.23	2.20	6.19	0.99	14.11	1.87
10 CRH/ 90 SS	542.33	74.19	50.78	23.42	9.09	4.26	44.30	0.15	2.07	5.69	0.98	12.57	2.12
90 CRH/ 10 SS	120.59	74.41	17.94	55.5	6.33	0.09	0.69	1.46	73.70	2.06	1.33	47.33	0.12
70 CRH/ 30 SS	156.68	72.93	22.71	49.08	5.06	0.20	1.81	1.33	64.90	4.81	1.51	46.19	0.23
50 CRH/ 50 SS	208.29	73.61	39.41	38.34	4.49	0.32	2.31	1.08	50.35	7.25	1.61	44.33	0.30
30 CRH/ 70 SS	244.79	75.45	51.1	24.35	4.19	0.52	3.56	0.66	38.00	9.31	1.67	43.37	0.36
10 CRH/ 90 SS	282.24	75.53	55.16	20.37	4.12	0.57	4.88	0.42	28.70	11.19	1.71	42.62	0.39
90 C1/ 10 PB	284.22	58.39	28.33	30.06	8.87	0.55	2.00	4.41	67.95	3.44	1.14	57.64	0.08
90 C1/ 10 PB	292.15	62.33	29.74	32.58	8.39	0.56	3.25	4.46	94.65	4.38	0.94	49.22	0.08
50 C1/ 50 PB	311.50	66.54	32.81	33.73	7.56	0.29	4.65	2.90	99.20	7.19	0.81	43.67	0.08
30 C1/ 70 PB	324.32	70.51	35.8	34.71	7.22	0.26	5.89	2.58	115.75	9.13	0.7	37	0.07
10 C1/ 90 PB	343.70	73.86	39.00	34.86	6.66	0.18	8.09	1.19	115.85	11.25	0.52	30.31	0.08
90 C1/ 10 FV	280.38	59.29	30.69	27.77	9.49	0.60	1.43	4.87	59.60	1.53	1.31	64.65	0.07
70 C1/ 30 FV	232.00	56.04	29.80	25.77	9.27	0.43	2.25	2.88	30.40	1.56	1.13	57.24	0.06
50 C1/50 FV	208.86	59.92	35.07	24.85	9.13	0.40	3.63	2.41	19.10	1.75	0.99	48.96	0.05
30 C1/70 FV	179.68	59.22	36.69	22.53	8.63	0.23	8.07	1.45	11.50	1.68	0.63	30.41	0.03
10 C1/90 FV	169.00	65.58	41.23	24.36	8.07	0.10	10.48	0.43	5.35	2.00	0.36	16.64	0.02
90 C1/10 SS	322.84	57.29	28.16	29.13	9.18	1.99	3.78	3.85	62.94	2.45	1.20	55.33	0.34
70 C1/30 SS	386.70	54.82	34.02	20.8	9.26	2.54	7.46	2.46	36.47	3.13	1.16	42.52	0.71
50 C1/50 SS	492.70	61.74	43.67	18.07	9.22	2.88	22.92	1.60	23.45	5.19	1.03	26.78	0.97
30 C1/ 70 SS	539.06	64.68	47.92	16.75	8.98	3.55	27.24	0.95	5.30	5.56	1.01	19.74	1.29
10 C1/90 SS	594.71	66.06	55.26	10.8	9.04	3.78	32.20	0.21	2.17	6.50	1.01	13.27	1.33
90 C1/10 PM	143.88	60.38	33.93	26.45	7.88	0.82	1.50	4.46	45.80	3.25	1.44	65.23	0.29
70 C1/30 PM	169.13	62.34	36.55	25.79	6.62	0.44	2.00	3.49	30.60	4.50	1.39	61.76	0.37
50 C1/50 PM	197.47	63.77	38.88	24.89	5.60	0.59	2.63	3.15	32.75	6.94	1.53	55.9	0.42
30 C1/70 PM	223.31	64.69	46.98	17.71	4.87	0.55	3.00	2.92	33.10	9.06	1.66	51.86	0.43
10 C1/90 PM	250.01	63.08	51.53	11.55	4.32	0.61	4.75	1.36	21.50	11.69	1.76	45.86	0.52
90 C2/10 PB	281.05	47.32	28.02	19.30	8.42	0.35	1.43	3.59	52.15	1.95	1.31	65.33	0.08
70 C2/30 PB	310.15	56.34	31.80	24.54	8.24	0.23	1.75	3.28	65.45	2.81	1.03	57.61	0.07
50 C2/50 PB	341.56	59.70	34.11	25.58	7.43	0.23	4.06	2.63	102.10	6.38	0.85	47.76	0.08
30 C2/70 PB	347.75	64.65	35.06	29.59	6.97	0.24	5.67	2.26	131.05	8.69	0.68	38.60	0.08
10 C2/90 PB	353.94	75.23	40.63	34.60	6.62	0.23	8.12	1.12	125.35	11.25	0.60	32.21	0.08
90 C2/10 FV	273.38	41.17	24.98	16.19	8.81	0.38	0.70	2.95	16.65	0.83	1.29	68.78	0.06

Tabela 3 - Continuação. Physic-chemical analysis results. Apparent density (AD), total porosity (TP), micro-porosity (Micro), macro-porosity (Macro), potential of hydrogen (pH), electric conductivity (EC), magnesium (Mg), potassium (K), phosphorus (P), calcium (Ca), nitrogen (N), carbon (C) and sulfur (S).

Table 3 - Continuation. Resultados da análise física e química. Densidade aparente (AD), porosidade total (TP), microporosidade (Micro), macroporosidade (Macro), potencial hidrogeniônico (pH), condutividade elétrica (EC), magnésio (Mg), potássio (K), fósforo (P), cálcio (Ca), nitrogênio (N), carbono (C) e enxofre (S).

Substrate	AD	TP	Micro	Macro	pH	EC	Mg	K	P	Ca	N	C	S
	kg m^{-3}		%			mS cm^{-1}		mg dm^{-3}		cmolc dm^{-3}		%	
70 C2/30 FV	252.63	52.32	33.06	19.26	8.76	0.22	2.69	2.62	16.32	1.63	1.1	60.39	0.05
50 C2/50 FV	228.91	55.85	35.45	20.4	8.53	0.22	5.77	1.90	11.46	1.48	0.87	46.56	0.04
30 C2/70 FV	171.92	59.52	36.99	22.53	8.19	0.19	7.79	0.99	17.06	1.20	0.51	30.85	0.02
10 C2/90 FV	167.17	66.64	43.08	23.57	7.63	0.04	9.04	0.40	16.60	1.45	0.24	12.41	0.01
90 C2/10 SS	349.37	44.95	28.42	16.53	9.32	0.94	2.50	2.86	17.26	1.50	1.14	63.94	0.25
70 C2/30 SS	432.97	53.88	36.69	17.19	9.13	2.75	5.62	2.46	17.66	2.50	1.16	49.06	0.49
50 C2/50 SS	463.62	56.27	40.73	15.54	9.13	2.45	18.55	1.26	2.29	4.63	1.00	27.6	0.54
30 C2/70 SS	513.83	58.01	43.06	14.95	9.07	3.57	19.03	0.76	1.44	5.88	1.03	25.49	0.63
10 C2/90 SS	572.91	67.53	49.8	17.73	9.05	4.06	32.49	0.19	2.77	7.50	1.06	22.99	1.20
90 C2/ 10 PM	298.85	49.41	31.8	17.61	7.38	0.45	0.50	2.59	25.91	1.00	1.42	71.87	0.21
70 C2/ 30 PM	307.89	55.72	38.11	17.61	5.75	0.75	0.23	3.05	21.09	1.65	1.43	66.26	0.19
50 C2/ 50 PM	312.03	58.87	43.28	15.6	5.03	0.62	2.63	2.95	19.21	7.56	1.6	57.14	0.33
30 C2/ 70 PM	290.66	70.34	44.51	25.83	6.08	0.97	6.08	2.56	20.79	17.06	1.66	50.98	0.41
10 C2/ 90 PM	285.66	71.97	48.19	23.77	5.75	1.12	8.56	1.28	21.01	22.56	1.8	46.44	0.49
90 PB/10 CF	267.00	76.39	41.12	35.27	6.48	0.13	7.81	0.55	123.40	12.63	0.45	28.63	0.22
70 PB/30 CF	232.16	75.88	42.6	33.28	6.42	0.17	7.13	0.57	121.40	11.38	0.45	29.11	0.45
50 PB/50 CF	234.72	75.28	44.84	30.46	6.42	0.14	4.94	0.59	107.20	9.88	0.44	31.13	0.69
30 PB/70 CF	176.78	74.69	47.08	27.63	6.38	0.14	2.77	0.60	72.60	6.56	0.46	34.57	0.75
10 PB/90 CF	118.85	72.74	48.32	24.43	6.51	0.13	2.14	0.62	41.45	4.75	0.42	34.83	0.92
90 PB/10 FV	297.15	76.88	40.24	36.64	6.49	0.19	9.06	0.52	123.85	12.56	0.46	26.17	0.08
70 PB/30 FV	305.67	70.90	37.79	33.11	6.61	0.15	9.63	0.47	121.10	11.56	0.37	22.91	0.07
50 PB/50 FV	283.45	70.10	41.71	28.40	6.72	0.10	9.84	0.37	93.65	10.38	0.33	18.98	0.06
30 PB/70 FV	237.08	67.98	44.23	23.75	6.88	0.09	11.46	0.28	68.60	7.63	0.26	14.13	0.04
10 PB/90 FV	207.27	64.68	42.29	22.39	7.14	0.05	12.47	0.24	41.45	5.69	0.11	5.97	0.02
90 PB/ 10 SS	376.58	76.3	42.13	34.17	8.11	1.37	18.98	0.46	36.05	11.63	0.55	25.43	0.26
70 PB/ 30 SS	414.39	71.76	41.87	29.89	8.53	2.63	30.20	0.31	3.83	9.19	0.71	20.08	0.64
50 PB/ 50 SS	470.84	71.59	48.99	22.6	8.77	3.38	37.06	0.25	4.31	7.94	0.82	16.88	0.88
30 PB/ 70 SS	518.67	70.92	51.09	19.83	8.90	3.51	36.82	0.18	1.67	6.63	0.89	13.97	1.16
10 PB/ 90 SS	547.31	71.38	52.47	18.91	9.00	4.08	41.60	0.14	1.83	6.63	0.96	13.12	1.39
90 PB/10 PM	355.06	78.04	41.18	36.86	6.36	0.19	8.25	0.49	112.20	12.38	0.57	29.06	0.15
70 PB/30 PM	342.67	76.05	44.41	31.64	5.72	0.24	7.80	0.44	100.55	12.19	0.86	32.32	0.19
50 PB/50 PM	336.09	73.80	47.46	26.33	5.07	0.39	5.56	0.41	88.80	11.06	1.07	35.25	0.27
30 PB/70 PM	305.28	73.05	52.81	20.24	4.49	0.62	5.18	0.33	69.35	11.63	1.32	38.89	0.35
10 PB/90 PM	303.74	72.25	58.39	13.86	4.18	0.63	4.13	0.26	37.55	12.88	1.60	42.01	0.44
90 CF/ 10 FV	84.28	79.32	49.36	29.96	6.36	0.09	2.37	0.49	7.79	1.75	0.31	33.66	0.14
70 CF/ 30 FV	109.72	72.88	46.59	26.29	6.89	0.09	3.69	0.34	9.49	2.56	0.24	22.79	0.08
50 CF/ 50 FV	120.26	67.50	40.98	26.52	6.55	0.06	5.22	0.29	14.33	2.56	0.16	15.61	0.06
30 CF/ 70 FV	135.56	68.11	47.01	21.10	7.10	0.05	6.89	0.23	34.15	2.44	0.10	7.45	0.03
10 CF/ 90 FV	150.87	68.70	43.10	25.60	7.03	0.02	7.81	0.29	16.95	2.50	0.06	2.69	0.02
Mean	264.16	67.80	39.10	28.68	7.34	0.82	8.09	1.49	46.38	5.60	0.90	38.37	0.33
Standard Deviation	123.69	8.45	9.44	10.26	1.42	1.16	10.05	1.23	39.03	4.69	0.44	18.09	0.42
VC (%)	46.82	12.47	24.14	35.77	19.38	142.40	124.20	82.69	84.15	83.82	48.79	47.15	128.61

CS1 - Commercial substrate based on peat moss; CS2 - Commercial substrate based on pine bark; CRH - Carbonized rice husk; C1- charcoal with grain size between 1-3 mm; C2 - charcoal with grain size between 3-5 mm; CF- coconut fiber; PB- almost decomposed pine bark; FV- fine vermiculite; SS- sewage sludge; and PM- peat moss; VC - Variation coefficient.

For chemical properties, the components were separated into 2 groups, at distance of 50%: G1 - carbonized rice husk, two different charcoal grain sizes, coconut fiber, fine vermiculite, pine bark and peat moss; and G2 - sewage sludge, in ascending order of fertility (Figure 1B). Concerning chemical properties, groups were formed from a distance of 80%: G1 - carbon, G2 - phosphorus and potassium, G3 - potential of hydrogen - pH, G4 - calcium and nitrogen, G5 - electrical conductivity, magnesium and sulfur (Figure 1 D).

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Among evaluated components, coconut fiber was the most inert, with low concentration of all macronutrients examined, together with a pH within appropriate range and low electrical conductivity. Incinerated materials presented greater contents of carbon, phosphorus and potassium, associated with high pH and low values of nitrogen, sulfur, calcium, magnesium and electrical conductivity; and sewage sludge, with higher concentrations of magnesium, sulfur and high values of pH and electrical conductivity; peat moss with higher levels of nitrogen and calcium; and pine bark of phosphorus and calcium (Figure 2).

As for chemical properties, the division of substrates in just two groups is justified by the fact that sewage sludge presented major dissimilarity in a greater number of chemical properties, compared to other components, resulting in high values of magnesium, sulfur and electric conductivity (Figure 3). According to Caldeira et al. (2014), the high electrical conductivity of sewage sludge is linked to its high salt concentration, corroborating Guerrini and Trigueiro (2004) and Kratz et al. (2013b).

Substrates properties and growth

The use of peat moss, sewage sludge and coconut fiber as pure substrates provided greater growth, when compared with its use as mixtures component. On the other hand, carbonized rice bark, fine vermiculite, two different grain sizes of charcoal and pine bark presented the need to be combined with other elements in order to obtain a greater increase in growth (Figures 3 and 4).

A trend in seedlings growth was observed with the increase in proportion of one component in relation to other, however, only 7 of 18 combinations evaluated presented significant value ($\alpha<0.05$): PB/PM, PB/SS, PB/CF, CRH/PM, CRH/CF, C1/PM and C2/FV (Figure 3).

Among the significant results, linear growth of plants was observed with increasing proportion of coconut fiber and peat moss as opposed to carbonized rice husk. Similar results were verified by adding coconut fiber, sewage sludge and peat moss to pine bark; peat moss to charcoal with grain sizes between 1-3 mm and fine vermiculite to charcoal with grain sizes between 3-5 mm. In general, it was observed among the evaluated components; that the addition of pine bark and charcoal to other components provided a reduction in plant growth. When combined with each other, there was no significant difference in growth (Figure 3).

It is possible to check that addition of peat moss, coconut fiber and sewage sludge to carbonized rice husk provided increased seedling growth, while pine bark had the opposite effect; doses above 70% made growth unsatisfactory (below 25 cm on the 120^{th} day). As for coconut fiber, the use up to 70% is recommended, combined with carbonized rice husk (Figure 3 B).

For commercial substrates, there has been more growth in *E. benthamii* seedlings using substrates based on peat moss. Similarly to pine bark, commercial substrate based on this substance alone provided less plant growth (23.8 cm), when compared with commercial substrate based on peat moss (31.8 cm) (Figure 3 A).

Similarity between physical properties correlated with results observed in seedling growth. Apart from pine bark, substrates with greater water retention capacity presented more seedling growth, denoting water requirement of the species, which can be justified by high temperatures observed in the production period, associated with the water irrigation program (Table 1). Results indicate that pine bark, when used in high proportions, is not suitable for *E. benthamii* seedling production, due to the lower growth observed with the use of commercial substrate based on this element (23.8 cm), when compared to commercial substrate based on peat moss (31.8 cm). These results agree with other research work with *Eucalyptus* (KRATZ et al., 2013b; KRATZ; WENDLING, 2013).

The use of residual charcoal proved to be inadequate as a component of substrates, regardless of associated element, due to reduction in seedlings growth when substrate was added (Figure 3 C and D). This result can be due to its low water retention capacity associated with high pH (Figure 2).

On the other hand, the use of organic substrates was shown to be feasible, which in accordance to Guerrini and Trigueiro (2004), is related to increased water and nutrient retention capacity. This is confirmed by results observed with use of sewage sludge on *E. grandis* and *E. benthamii* (TRIGUEIRO; GUERRINI, 2004; KRATZ et al., 2013 b), with organic compounds of agro-industrial residues in *E. grandis* seedlings (SILVA et al., 2014) and with substrates containing matured cattle manure, in seedlings of *E. grandis* with carbonized rice husk, coconut fiber and vermiculite (MELO et al., 2014).

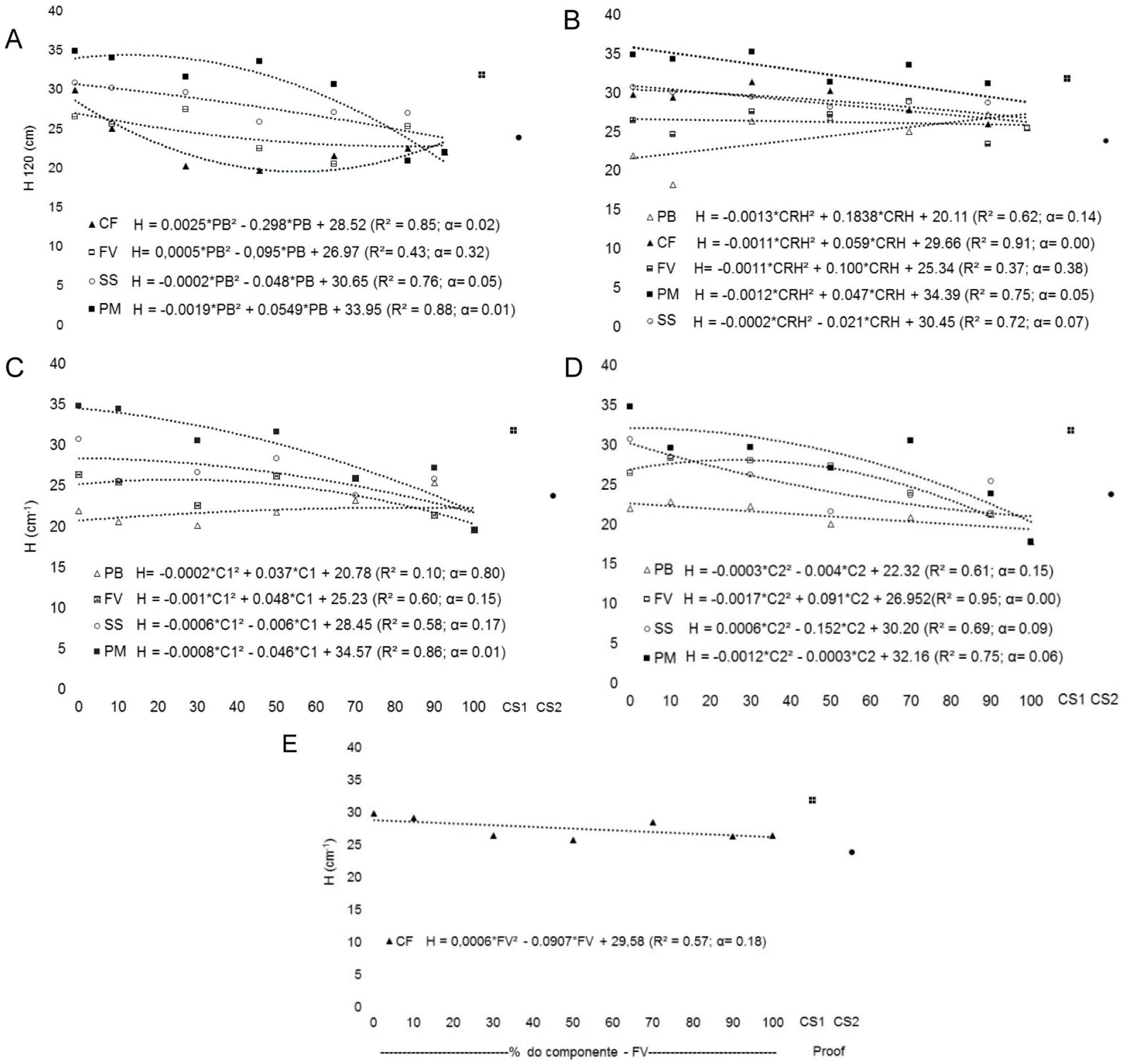

Figura 3. Polynomial regression for *E. benthamii* seedlings height on 120th day, produced on substrates made from two components (volume/volume) in different proportions. Pine bark (CP), carbonized rice husk (CAC), charcoal with grain size between 1-3 mm (C1), charcoal with grain size between 3-5 mm (C2), coconut fiber (FC), fine vermiculite (VF), peat moss (TF) and sewage sludge (BIO). CP/TF, CP/BIO, CP/BIO, CP/FC, CP/VF (A); CAC/ TF, CAC/BIO, CAC/FC, CAC/FC, CAC/FC, CAC/VF, CAC/CP (B); C1/TF, C1/BIO, C1/VF, C1/ CP (C); C2/TF, C2/BIO, C2/VF, C2/TF (D); FC/VF (E). Proof treatments: commercial substrate based on peat moss (SC1) and commercial substrate based on almost decomposed pine bark (SC2).

Figure 3. Regressão polinomial para a altura aos 120 dias de mudas de *E. benthamii* produzidas em substratos formulados a partir de dois componentes (volume/volume) em diferentes proporções. Casca de pinus (CP), casca de arroz carbonizada (CAC), carvão vegetal com granulometria entre 1-3 mm (C1), carvão com granulometria entre 3-5 mm (C2), fibra de coco (FC), vermiculita fina (VF), turfa (TF) e biossólido (BIO). CP/TF, CP/BIO, CP/BIO, CP/FC, CP/VF (A); CAC/ TF, CAC/BIO, CAC/FC, CAC/FC, CAC/FC, CAC/VF, CAC/CP (B); C1/TF, C1/BIO, C1/VF, C1/CP (C); C2/TF, C2/BIO, C2/VF, C2/TF (D); FC/VF (E). Tratamentos testemunha: substrato comercial a base de turfa (SC1) e substrato comercial a base de casca de pinus semidecomposta (SC2).

Electrical conductivity of substrate shall not exceed the limit of 1.0 $mS.cm^{-1}$ (GONÇALVES et al., 2000), with just sewage sludge being non-standard (Figure 2 F). However, among evaluated components, it takes second position concerning growth (Figure 3), indicating that *E. benthamii* tolerates high levels of salinity, as verified by Kratz et al. (2013 b), in which substrates with EC ranging from 0.1 to 2.6 mS/cm provided adequate plant growth. Similarly, as verified by Mendonça et al. (2010), *E. camaldulensis*, *E. tereticornis* and *E. robusta* seedlings showed to be resistant to salinity until electrical conductivity of 8.33 dS m^{-1}, and it was noticeable that it did not cause reduction in chlorophyll content, which is a major factor in plants sensitive to salinity.

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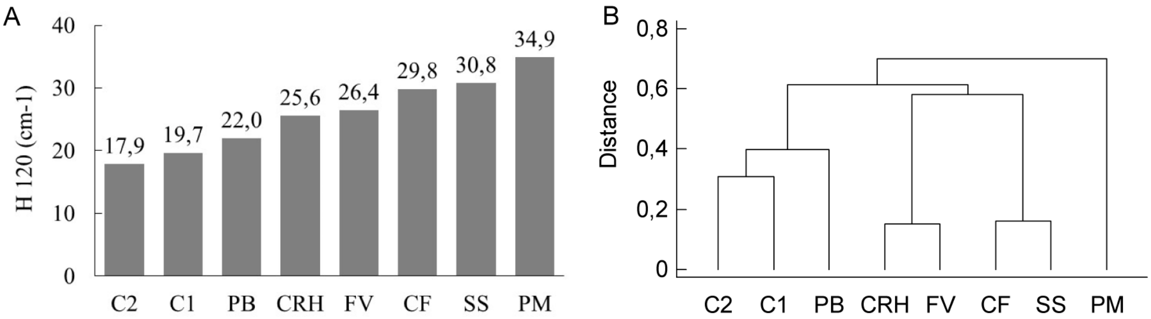

Figura 4. Height of *E. benthamii* seedlings on 120th day (A) and cluster analysis by nearest substrate neighbor method based on growth (B). CAC- Carbonized rice husk; C1- charcoal with grain size between 1-3 mm; C2- charcoal with grain size between 3-5 mm; FC- coconut fiber; CP- almost decomposed pine bark; VF- fine vermiculite; BIO- sewage sludge; and TF - peat moss.

Figure 4. Altura de mudas de *E. benthamii* aos 120 dias (A) e análise de agrupamento pelo método do vizinho mais próximo dos substratos com base no crescimento (B). CHR - casca de arroz carbonizada; C1- carvão vegetal com granulometria entre 1- 3 mm, C2-carvão com granulometria entre 3- 5 mm, CF- fibra de coco, PB- casca de pinus semidecomposta, FV-vermiculita fina, SS- biossólido e PM- turfa.

The pH showed a negative correlation with growth (R = -0.48**), confirming the lower growth observed in substrates based on fine charcoal (pH = 9.7; 8.9) and carbonized rice husk (pH = 7.4). However, sewage sludge with alkaline pH (9.1) provided adequate growth of plants, which means that it is not possible to infer plant growth based on only this property. In general, substrates with pH below 5.0 can cause deficiency of nitrogen, potassium, calcium, magnesium and boron to plants, while in pH above 6.5 deficiencies of phosphorus, iron, manganese, zinc and copper are expected (VALERI; CORRADINI, 2000). However, species from the *Eucalyptus* genus showed variable tolerance to this factor, as seen in seedlings of *E. benthamii* (KRATZ et al., 2013 b) and *E. urophylla* (OLIVEIRA JUNIOR et al., 2011), produced in substrates with different pH values, providing adequate growth.

Obtaining cost

The regional waste, charcoal chaff and sewage sludge, presented lowest cost, given that they were donated by producing companies. The shipping and preparation cost were responsibilities of the grower. Coconut fiber and pine bark, which in the past were considered as wastes, are currently marketed, due to their technical feasibility, combined with use of bark for energy generation. Fine vermiculite presented higher production cost, followed by commercial substrates (Table 4).

Tabela 4. Purchase price, transport, preparation and total cost of components used for substrate formulation for *E. benthamii* seedlings production in metropolitan region of Curitiba, in 2014.

Table 4. Valor de compra, custo de transporte, preparo e total dos componentes utilizados para formulação dos substratos empregados na produção de mudas de *E. benthamii* na região metropolitana de Curitiba, em 2014.

Substrate	Purchase value (BRL/m³)	Transport (BRL/m³)	Preparation (Man/day)*	Total cost (m³)
CC1	-	50.0	62.0	112.0
SS2	-	20.0	62.0	82.0
PB	150.0	-	-	150.0
PM	153.8	-	-	153.8
CRH	165.0	-	-	165.0
CS1	250.0	-	-	250.0
CS2	217.0	-	-	217.0
CF	260.0	-	-	260.0
FV	280.0	-	-	280.0

* Paraná, 2014; [1] Considering distance of 200 km; [2] Considering distance of 50 km. (Dollar at 2.45); SS - sewage sludge; CC - fine charcoal; PB - almost decompose pine bark; PM - peat moss. CHR - carbonized rice husk; CS1 - commercial substrate based on peat moss; CS2 - commercial substrate based on pine bark; CF - coconut fiber; FV - fine vermiculite.

Higher cost of commercial substrates in relation to components leads to the possibility of formulating substrates in nurseries to reduce costs, albeit with the need to carry out trials aimed at adapting formulation of different species and growing conditions. The use of substrates with low cost combined with formation of seedling with better morphological quality results in reduction of production cycle and a lower cost (SIMÕES et al., 2012). It is worth noting that the use of regional waste substantially reduces substrate cost (MELO et al., 2014; WENDLING et al., 2007), although the costs can vary depending on the availability of each material (WENDLING et al., 2007).

Among combinations that showed no significant influence ($\alpha > 0.05$) it is appropriate to use charcoal-based components for substrates formulation to reduce costs, especially when combined with vermiculite and peat moss. In spite of showing lower growth, plants reached the minimum height indicated for planting (15 cm) (WENDLING; DUTRA, 2010). However, higher seedlings result in a higher field initial start-up, reducing competition with invasive plants, also promoting a cost reduction (FIGUEIREDO et al., 2011).

CONCLUSIONS

The substrates were grouped into four groups by chemical properties: G1 - elements of high macro-porosity (carbonized rice husk); G2 - balance between macro-pores and micro-pores (two different grain sizes of charcoal); G3 - higher proportion of micro-pores (coconut fiber, fine vermiculite, peat moss and pine bark); and G4 - high density associated with high micro-porosity (sewage sludge).

The substrates were grouped into two groups by chemical properties: G1 - lower fertility (carbonized rice husk, two different grain sizes of charcoal, coconut fiber, fine vermiculite, pine bark and peat moss) and G2 - greater fertility (sewage sludge).

Different materials and formulations of substrates can be adopted for *E. benthamii* seedlings production; better are those with greater water retention capacity, peat moss, sewage sludge and coconut fiber.

Sewage sludge waste presented the lowest cost coupled with good plant growth and the use of fine charcoal is justified to reduce production costs.

Almost decomposed pine bark and different grain sizes of chaff charcoal provided a reduction in growth when inserted into the substrate formulation.

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