Acta Iguazu
ISSN: 2316-4093

Alternative substrates and containers for *Ilex paraguariensis* seedlings production

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Artigo enviado em 13/11/2019, aceito em 24/07/2020

Abstract: Yerba mate is a Brazilian native species; its importance is due to cultural, environment, and economic factors. Species seedlings production needs to be improved, evaluating alternative substrates and containers. Therefore, we evaluated growth and quality of yerba mate seedlings produced on organic waste-based substrates in biodegradable and non-biodegradable containers. Commercial substrates based on pine bark (CS) and components based on sewage sludge (SS) and coal residue (granulometry from 1 to 3 mm - CR) were used. For each container, were prepared treatments with different substrate components: 75CS/25CR, 50CS/50CR, 25CS/75CR, 75CS/25SS, 50CS/50SS, 25CS/75SS, and CS. Substrates were submitted to physical and chemical analysis. The containers used were 110 cm^3 polypropylene tubes, 500 cm^3 polyethylene plastic bags, 460 cm^3 paperpot®, and 460 cm^3 TNT (non-woven fabric). Total height, stem diameter, shoot, and root dry biomass at the end of the seedlings production period were measured and total dry mass, Dickson quality index and height/diameter ratio were calculated. All containers used with commercial substrate provided satisfactory seedlings growth. The substrates showed distinct physical and chemical characteristics due to the addition of sewage sludge and coal residue, which resulted in higher pH and lower seedlings growth. Physical and chemical characteristics of substrates influenced growth and quality of yerba mate seedlings and the alternative substrates used in this study are not suitable to produce seedlings of the species on these conditions. The containers did not influence the variables used in this study.

Keywords: Organic residue, Seedling quality, Yerba mate.

Substratos alternativos e recipentes para produção de mudas de *Ilex paraguariensis*

Resumo: A erva-mate é uma espécie nativa do Brasil; sua importância relaciona-se a fatores culturais, ambientais e econômicos. A produção de mudas da espécie precisa ser melhorada, avaliando-se substratos e recipientes. Sendo assim, foram avaliados o crescimento e a qualidade de mudas de erva-mate produzidas em substratos a base de resíduos orgânicos em recipientes biodegradáveis e não biodegradáveis. Substrato comercial a base de casca de pinus (CS) e componentes a base de lodo de esgoto (SS) e resíduo de carvão (granulometria entre 1 e 3 mm – CR) foram usados neste experimento. Para cada recipiente, foram preparados 7 tratamentos com composições de substrato:

75CS/25CR, 50CS/50CR, 25CS/75CR, 75CS/25SS, 50CS/50SS, 25CS/75SS e CS. Os substratos foram submetidos a análises física e química. Os recipientes utilizados foram tubetes de polipropileno 110 cm^3, sacos plásticos 500 cm^3, paperpot® 460 cm^3 e TNT (tecido não tramado) 460 cm^3. Altura total, diâmetro do colo, biomassa seca aérea e radicial foram avaliadas ao final do período de produção, índice de qualidade de Dickson e relação altura/diâmetro foram calculados. Todos os recipientes usados com substrato comercial promoveram crescimento satisfatório das mudas. Os substratos apresentaram características físicas e químicas distintas conforme a adição de lodo de esgoto e carvão, resultando em maior pH e baixo crescimento das mudas. As características físicas e químicas dos substratos influenciaram no crescimento e qualidade das mudas de erva-mate e os substratos alternativos usados neste estudo não são adequados para a produção de mudas desta espécie nas condições propostas. Os recipientes não influenciaram nas variáveis avaliadas neste estudo.

Palavras-chave: Resíduo orgânico, qualidade de mudas, erva-mate.

Introduction

Yerba mate is a species widely spread by its consumption as beverages (Mireski et al., 2019) mainly in the southern region of Brazil. The state of Paraná accounts for 87% of the national production, with 393 000 tons of yerba-mate leaves produced on 77 731 hectares in 2018 (IBGE, 2019). Among the containers used in seedlings production, the most common are plastic bags and polyethylene tubes (Storck et al., 2016), but their manufacture depends on non-renewable resources. It is important to develop materials that are easy to degrade, which can replace containers based on petroleum polymers, to reduce the environmental impact, considering that they are also low cost and allow the proper growth of plants (Salto et al., 2016).

The substrate is a very important factor for seedlings growth in restricted volume containers. An appropriate substrate needs to sustain seedlings and provides adequate conditions for its root system, and be exempt from invasive plants propagules, pathogenic pests, and fungi (Hartmann et al., 2011). It is known that substrates physical and chemical characteristics directly influence seed germination, root system development and nutritional status of plants, determining, at least partially, seedlings quality (Trazzi et al., 2014).

The use of alternative and renewable substrates has been the focus of several studies reusing waste such as sewage sludge, compounded pruning residues and coal residue (Anjos et al., 2018, Barros et al., 2019, Siqueira et al., 2019, Sá et al., 2020). However, it must provide all the physical, chemical, and biological characteristics necessary for the proper development of seedlings (Pascual et al., 2018). Many agricultural and urban wastes can be used as substrate components, as demonstrated by several studies developed in recent years (Trazzi et al., 2014; Kratz and Wendling 2016; Gabira et al., 2020). Sewage sludge from domestic sewage treatment process of urban centers and coal residue, generated in the production of charcoal, stand out as good options for this use. Both are wastes produced on a large scale and, when destined for landfills, generate environmental, social, and economic problems.

It is noted that environmental damage caused by anthropic activities is becoming critical and the application of new waste management practices and their use adequately in the agricultural activities can be viable alternatives to the disposal of these materials. Thus, this study aimed to evaluate the use of organic waste-based substrates in the growth and quality of yerba mate seedlings produced in different containers.

Material and methods

The experiment was carried out between October 2018 and July 2019 at the Laboratory of Forest Species Propagation of Embrapa Florestas, in Colombo – PR/Brazil (25º17'35'' S and 49º13'24'' O). The climate of the region is hot temperate, in whith the annual average temperature is 16.6 ºC, and significant rainfall throughout the year. The experiment was completely randomized designed in a 4×7 factorial scheme (containers x substrates), totaling 28 treatments with 4 replicates of 12 plants each.

The containers used were 110 cm^3 polypropylene tubes, 500 cm^3 polyethylene plastic bags, 460 cm^3 Paperpot®, and 460 cm^3 TNT (non-woven fabric). The substrates used were produced from mixtures of commercial substrate based on Pinus bark (CS), coal residue (CR) in granulometry from 1 to 3 mm and sewage sludge (SS) in granulometry up to 3 mm (Table 1).

Table 1. Substrates formulations used in the production of yerba mate seedlings.

Treatments	Materials (%)		
	CS	CR	SS
75CS/25CR	75.0	25.0	
50CS/50CR	50.0	50.0	
25CS/75CR	25.0	75.0	
75CS/25SS	75.0		25.0
50CS/50SS	50.0		50.0
25CS/75SS	25.0		75.0
CS	100.0		

CS - Pinus bark-based commercial substrate; CR - coal residue; SS - sewage sludge.

The coal residue was obtained from industrial charcoal production from pine and eucalyptus woods. Sewage sludge was obtained from a domestic sewage treatment factory resulted from an anaerobic reactor system (UASB) and complementary treatment with limestone. After formulation, the substrates were submitted to the evaluation of physical (density, macroporosity, water retention capacity, and total porosity) and chemical (electrical conductivity, hydrogenionic potential, and soluble salts) characteristics according to Normative Instruction No. 17 of the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA, 2007).

Yerba mate seeds were stratified into sowing according to the methodology described by Fowler and Sturion (2000). When germinated, seedlings were chopped into the containers and packed in a greenhouse with 50% shading, under irrigation via micro-sprinkler of 10 minutes every 1-hour and flow of 28.0 L $hour^{-1}$. Plants remained in these conditions for 10 days, until acclimatization occurred and, after this period, irrigation was adjusted to 5 minutes every 1-hour, thus remaining until the end of the growth of seedlings in the nursery.

Fertigation was performed every 7 days during the first 90 days. The fertigation solution consisted of 0.15 g L^{-1} ammonium sulfate, 2.3 g Yoorin MG L^{-1} [18.0% P, 18.0% Ca, 7.0% Mg, 10.0% Si], 1.6 g L^{-1} potassium chloride and 0.25 g FTE BR 10 L^{-1} [7.0% Zn, 4.0% Fe, 4.0% Mn, 0.1% Mo, 2.5% B, 0.8% Cu]. After this period, the fertigation solution was adjusted to 4 g L^{-1} of urea, 3 g L^{-1} of Yoorin MG, 3 g L^{-1} potassium chloride and 0.25 g L^{-1} of FTE BR 10, applied every 7 days, during the remaining period of seedlings growth. In both stages of fertigation, 4 ml of solution was used for each seedling in each application.

To evaluate seedlings quality, we measured shoot height and stem diameter of all seedlings of each repetition using, a millimeter ruler and a digital caliper. After the end of the of seedlings production in the nursery (180 days), five plants of each repetition were used to perform destructive analysis. For dry biomass, seedlings were separated into shoot and root parts, which were cleaned from substrate residues, packed in identified paper bags and dried in an oven at 65 ºC until constant weight. From the data obtained the Dickson Quality Index (DQI) (Equation 1) and the height/diameter ratio (H/D) were calculated.

$$\mathrm{DQI}=\frac{\mathrm{TDM}}{{}^{\mathrm{H}}/_{\mathrm{SD}}+{}^{\mathrm{SDM}}/_{\mathrm{RDM}}} \qquad (1)$$

SDM = shoot dry mass; RDM = root dry mass; TDM = total dry mass; SD = stem diameter; H = height.

The data were submitted to Shapiro-Wilk test to verify normality. Subsequently, ANOVA was applied to verify influence of treatments on the variables and, when the F-value was significant, Scott-Knot test ($p < 0.05$) was applied to detect differences between means of treatments. The software R (R Core Team, 2019) was used to perform all analyses.

Results and discussion

The materials added to the commercial substrates changed its physical characteristics in different ways (Table 2). When compared to commercial substrate (CS), treatments with coal residue did not cause significant changes in substrate density. In those with the addition of sewage sludge, there was a significant increase in density of as the proportion of sludge increased.

Table 2. Bulk density (D), Macroporosity (Macro), Water Holding Capacity (WHC), Total Porosity (TP), Electrical Conductivity (CE), Hidrogenionic Potential (pH) e Soluble Salt Content (SSC) of the materials used as substrates to produce yerba mate seedlings.

Substrate	Physical characteristics				Chemical characteristics		
	D (kg m^{-3})	Macro (%)	WHC (%)	TP (%)	EC (dS cm^{-1})	pH	SSC (g L^{-1})
75CS/25CR	492.20 c	39.48 a	38.27 c	77.75 a	0.55c	8.0 c	15.24 d
50CS/50CR	483.14 c	41.21 a	35.31 c	76.53 a	0.68 c	8.4 c	18.50 d
25CS/75CR	431.21 c	38.87 a	28.77 d	67.64 c	0.89 c	9.2 b	21.61 d
75CS/25SS	611.29 b	26.44 c	49.50 a	75.94 a	2.06 b	9.5 ab	70.91 c
50CS/50SS	640.18 b	20.83 c	51.75 a	72.58 b	2.91 ab	9.9 ab	104.91 b
25CS/75SS	705.18 a	15.40 d	51.09 a	66.49 c	3.71 a	10.1 a	147.32 a
CS	459.13 c	31.54 b	41.76 b	73.30 b	0.20 d	6.7 d	5.17 e

CS - Pinus bark-based commercial substrate; CR - coal residue; SS - sewage sludge. Means followed by the same letter in the column did not differ significantly by the Tukey test ($p < 0.05$).

Coal residue addition, when compared to pure commercial substrate, resulted in increased macroporosity in all proportions (75CS/25CR, 50CS/50CR, and 25CS/75CR) associated with gradual reduction of micropores, according to increase in the amount of this component. Sewage sludge caused an inverse effect on substrates, gradually reducing percentage of macropores and increasing micropores when compared to other substrates. Substrates total porosity increased with proportions of coal residue and sewage sludge of 3:1, and coal residue 2:1 (substrates 75CS/25CR, 50CS/50CR and 50CS/50SS). Also, was higher than that of pure commercial substrates, while the addition of higher proportions of these materials (25CS/75CR and 25CS/75SS) reduced total porosity.

Substrates physical characteristics influence in different ways each of the variables related to seedlings production, as observed by Wendling et al. (2007) when testing substrates composed of bovine manure, sawdust, and residues of yerba mate to produce yerba mate seedlings. Substrates used in that study presented different characteristics according to proportions of components added to commercial substrate. Therefore, the characteristic of particles that compose them, such as size and shape, was one of the main factors that directly interfered with the arrangement of mixtures (Fermino et al., 2018).

Regarding substrates chemical characteristics, both coal residue and sewage sludge provided an increase in electrical conductivity and pH as their proportions increased. It was the most significant difference in substrates with sewage sludge. By increasing the proportion of sewage sludge in substrate composition, there was an increase in soluble salt content and pure commercial substrate showed the lowest concentration of this variable.

Seedlings growth analysis showed that commercial substrate (CS) provided an increase for height and diameter parameters in all containers (Table 3). Exception was found for diameter in substrate 75CS/25CR in TNT container, which did not present a significant difference for the CS. The H/D ratio also showed higher values in seedlings produced in SC substrate, except for Paperpot, where H/D ratio did not differ in any of the different substrates. Substrates 75CS/25CR and 50CS/50CR of tubes and 75CS/25CR and 75CS/25SS of TNT also showed no significant difference. Evaluating only the containers, we observed little influence on growth in height and stem diameter. This difference was better noticed when looking for H/D ratio, with one substrate differing for lower value in tubes and TNT, and three on plastic bags and Paperpot. We found the lowest values in the plastic bag and Paperpot containers.

These results can be explained by reduction of macroporosity and increased density, which, according to Pascual et al. (2018), are common characteristics to this type of residue. These characteristics reduced drainage and aeration, providing an environment not suitable for root system growth (Guerrini and Trigueiro, 2004). It is also recommended that total porosity must be bigger than 85% (Pascual et al., 2018) and none of the substrates used in this study reached this value.

The considerable increase in pH, electrical conductivity and soluble salt content of substrates containing sewage sludge may be the result of the addition of limestone for its stabilization after treatment. The high value of pH and CE is not a desirable characteristic for seedlings production. Adequate substrates must have the pH between 5.0 and 6.5 and electrical conductivity below 1.0 mS cm^{-1} (Gonçalves et al., 2000). The pH of the substrate directly affects the mobility and availability of ions and, when outside the indicated range, nutrient deficiency or toxicity may occur (Pascual et al., 2018). In addition, the species present different levels of tolerance to physicochemical characteristics of substrates and, given the natural distribution of yerba mate in acidic soils (Pandolfo et al., 2003), it is likely that the high values of pH negatively influenced seedlings growth.

Table 3. Height, stem diameter and H/SD ratio of yerba mate seedlings 180 days after planting.

Substrate	Container			
	Tube	TNT	Plastic Bag	Paperpot®
		Height (cm)		
75CS/25CR	5.4 Ba	5.8 Ba	2.4 Bb	3.1 Bb
50CS/50CR	3.2 Ca	2.9 Ca	3.0 Ba	2.3 Ba
25CS/75CR	2.2 Ca	2.3 Ca	2.3 Ba	2.0 Ba
75CS/25SS	2.3 Ca	2.2 Ca	1.9 Ba	2.0 Ba
50CS/50SS	2.4 Ca	2.1 Ca	1.9 Ba	2.0 Ba
25CS/75SS	2.3 Ca	2.0 Ca	1.9 Ba	2.1 Ba
CS	7.9 Aa	8.1 Aa	7.5 Aa	5.0 Ab
		Stem Diameter (mm)		
75CS/25CR	2.10 Ba	2.35 Aa	1.15 Bb	1.87 Ba
50CS/50CR	1.37 Ba	1.42 Ba	1.50 Ba	1.45 Ba
25CS/75CR	1.22 Ca	1.30 Ba	1.27 Ba	1.17 Ca
75CS/25SS	1.32 Ca	0.92 Ba	1.17 Ba	1.02 Ca
50CS/50SS	1.12 Ca	1.20 Ba	0.97 Ba	1.00 Ca
25CS/75SS	1.10 Ca	1.10 Ba	1.50 Ba	0.90 Ca
CS	2.57 Aa	2.95 Aa	2.72 Aa	2.62 Aa
		H/SD ratio		
75CS/25CR	2.46 Aa	2.46 Aa	2.05 Bb	1.69 Ab
50CS/50CR	2.40 Aa	2.01 Ba	2.04 Ba	1.64 Aa
25CS/75CR	1.87 Ba	1.82 Ba	1.78 Ba	1.73 Aa
75CS/25SS	1.81 Bb	2.51 Aa	1.70 Bb	1.96 Ab
50CS/50SS	2.18 Ba	1.89 Ba	2.07 Ba	2.14 Aa
25CS/75SS	2.08 Ba	1.81 Bb	1.26 Bb	2.33 Aa
CS	3.05 Aa	2.78 Aa	2.71 Aa	1.89 Ab

CS - Pinus bark-based commercial substrate; CR - coal residue; SS - sewage sludge. Means followed by the same letter in the column did not differ statist significantly by the Tukey test ($p < 0.05$).

Seedlings shoot, root, total dry biomass, and Dickson quality index were higher in CS, followed by 75CS/25CR, in all containers (Table 4). For these characteristics, little difference was found between seedlings produced in each of the containers.

Kratz et al. (2017) recommended the use of sewage sludge to produce *Eucalyptus benthamii* seedling with reduced cost. Gonzaga et al. (2018) also found that sewage sludge improved growth and morphological qualities of *Eucalyptus grandis* seedlings. However, in our study, it was observed that the addition of this component is not recommended to produce yerba mate seedlings. This result was also obtained by Kratz et al. (2013) for of *Mimosa scabrella* seedlings production, not recommending the use of this residue in its production. The authors associated the negative result with high pH, since the species occurs in acid pH soils, and should be less tolerant to alkaline soils, such as yerba mate.

Table 4. Shoot, root, total dry mass and Dickson Quality Index of yerba mate seedlings 180 days after planting.

Substrate	Container			
	Tube	TNT	Plastic Bag	Paperpot®
Shoot dry mass (g seedling^{-1})				
75CS/25CR	1.4167 Ba	2.2285 Ba	0.2096 Bb	1.3006 Ba
50CS/50CR	0.4686 Ca	0.5208 Ca	0.5129 Ba	0.4810 Ca
25CS/75CR	0.2496 Ca	0.2370 Ca	0.4828 Ba	0.2815 Ca
75CS/25SS	0.1119 Ca	0.2113 Ca	0.1007 Ba	0.0922 Ca
50CS/50SS	0.0605 Ca	0.0284 Ca	0.0447 Ba	0.0956 Ca
25CS/75SS	0.0462 Ca	0.0515 Ca	0.0461 Ba	0.0406 Ca
CS	3.8277 Ab	4.0749 Aa	2.9564 Ac	2.3439 Ac
Root dry mass (g seedling $^{-1}$)				
75CS/25CR	0.3854 Bb	0.8809 Aa	0.0798 Bc	0.3480 Bb
50CS/50CR	0.1783 Ba	0.2357 Ba	0.1687 Ba	0.1995 Ba
25CS/75CR	00881 Ca	0.1279 Ba	0.1650 Ba	0.0975 Ca
75CS/25SS	00375 Ca	0.0239 Ba	0.0215 Ba	0.0226 Ca
50CS/50SS	0.0357 Ca	0.0078 Ba	0.0173 Ba	0.0181 Ca
25CS/75SS	0.0091 Ca	0.0147 Ba	0.0117 Ba	0.0113 Ca
CS	1.0275 Ab	1.1776 Aa	0.8390 Ac	0.5695 Ac
Total dry mass (g seedling $^{-1}$)				
75CS/25CR	1.8020 Bb	3.1094 Ba	0.2894 Bc	1.6486 Bb
50CS/50CR	0.6469 Ca	0.7564 Ca	0.6816 Ba	0.6805 Ca
25CS/75CR	0.3377 Ca	0.3649 Ca	0.5678 Ba	0.3790 Ca
75CS/25SS	0.1494 Ca	0.1452 Ca	0.1222 Ba	0.1148 Ca
50CS/50SS	0.0962 Ca	0.0362 Ca	0.0620 Ba	0.1136 Ca
25CS/75SS	0.0553 Ca	0.0662 Ca	0.0578 Ba	0.0519 Ca
CS	4.8552 Aa	5.2525 Aa	3.7954 Ab	2.9133 Ac
Dickson Quality Index				
75CS/25CR	0.26 Bb	0.30 Bb	0.68 Aa	0.07 Bb
50CS/50CR	0.14 Ba	0.14 Ba	0.18 Ba	0.15 Ba
25CS/75CR	0.08 Ba	0.07 Ba	0.10 Ba	0.13 Ba
75CS/25SS	0.02 Ca	0.03 Ba	0.02 Ba	0.02 Ba
50CS/50SS	0.01 Ca	0.03 Ba	0.01 Ba	0.01 Ba
25CS/75SS	0.01 Ca	0.01 Ba	0.01 Ba	0.01 Ba
CS	0.40 Ab	0.76 Aa	0.85 Aa	0.70 Aa

CS - Pinus bark-based commercial substrate; CR - coal residue; SS - sewage sludge. Means followed by the same letter in the column did not differ significantly by the Tukey test ($p < 0.05$).

Production of yerba mate seedlings is made mostly in containers such as plastic bags and polyethylene tubes (Wendling et al., 2006). Salto et al. (2016) point out that physical and chemical properties of substrates are also related to the dimensions and shape of containers. It influences seedlings growth since larger containers have greater water retention and greater space for root system development. In our study, there were no significant differences in seedlings growth between the different containers, indicating that, in this case, the critical factor was the substrate. Still, it was observed that plastic tubes and TNT showed better growth compared to plastic bags and Paperpot.

Another factor that should be considered is the ease of handling the containers. In our study, plastic tubes provided the best handling for filling with substrate and transporting seedlings in the nursery. TNT and Paperpot caused difficulty during filling and transport because nursery did not have adequate equipment and structure. Plastic bags are easy to fill, but its handling in nursery is not ergonomic. Despite the difficulties of filling and handling Paperpot and TNT containers, it is important to point out that, because there is no need to remove seedlings from the containers in the planting, their establishment and initial growth in the field is faster (Viégas et al., 2018).

Although our results do not indicate a beneficial effect of the use of coal residue and sewage sludge for yerba mate, we observed the possibility of replacing some traditional inputs seedlings production. It is necessary to develop studies using the same materials, but with lower pH values, as well as, with greater coverage of materials, also evaluating seedlings after field planting.

Conclusion

Physical and chemical characteristics of substrates influence growth and quality of yerba mate seedlings. Among the evaluated substrates, the commercial presented best results in all containers; substrate compositions based on sewage sludge and coal residue are not indicated for yerba mate seedlings production because of high pH. Containers did not provide significant differences for yerba mate seedlings growth.

Acknowledgments

To the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) and the Brazilian Agricultural Research Company - Embrapa Florestas.

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