SLUDGE FROM RICE PARBOILING WASTEWATER TREATMENT PLANT AS ALTERNATIVE SUBSTRATE FOR THE PRODUCTION OF *Araucaria angustifolia* SEEDLINGS

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ABSTRACT – The sludge produced by rice parboiling wastewater treatment plants has substantial amounts of nutrients and organic matter, therefore requiring proper environmental disposal. One option is to use it as an alternative substrate for the production of forest species seedlings. This study aimed to assess the viability of using the sludge from rice parboiling wastewater as an alternative substrate for the production of *Araucaria angustifolia* (Bertol.) O. Kuntze seedlings. *A. angustifolia* seeds were kept in vermiculite until sprouting, then transferred to polyethylene bags filled with five different substrates (treatments): 100% sand (T1); 75% sand and 25% sludge (T2); 50% sand and 50% sludge (T3); 25% sand and 75% sludge (T4); 100% sludge (T5). Each treatment had 10 replicates (bag with one seedling). Shoot system growth, hypocotyl diameter, root system growth and biomass were evaluated at 94, 180, and 300 days of growth. Data were subjected to Analysis of Variance (ANOVA) followed by Tukey test with a significance level of 5%. Seedlings' growth was similar on treatments containing 25%, 50%, and 75% sludge, while 100% sand and 100% sludge resulted in less growth at 300 days. In conclusion, the sludge from rice parboiling wastewater is suitable as an alternative substrate, and the ratio of 25% sand + 75% sludge could be recommended, for exploit larger amounts of the residue.

Keywords: Biosolids; Industrial waste; Plant growth.

LODO DE ESTAÇÃO DE TRATAMENTO DE PARBOILIZAÇÃO DE ARROZ COMO SUBSTRATO ALTERNATIVO NA PRODUÇÃO DE MUDAS DE Araucaria angustifolia

RESUMO – O lodo produzido em estações de tratamentos de efluente de parboilização de arroz apresenta quantidades consideráveis de nutrientes e matéria orgânica, necessitando de uma destinação ambientalmente correta. Uma possibilidade é seu uso como substrato alternativo na produção de plantas de espécies florestais. O objetivo deste estudo foi avaliar a viabilidade do uso de lodo advindo do tratamento do efluente de parboilização de arroz como substrato alternativo na produção de mudas de ***Araucaria angustifolia*** *(Bertol.) O. Kuntze. Sementes de* ***A. angustifolia*** *foram colocadas em vermiculita até a germinação e, após, transplantadas para sacos de polietileno contendo cinco diferentes substratos (tratamentos): 100% de areia (T1); 75% de areia e 25% de lodo (T2); 50% areia e 50% de lodo (T3); 25% de areia e 75% de lodo (T4) e 100% de lodo (T5). Cada tratamento possuía dez unidades amostrais (saco com uma muda). Ao final de 94, 180 e 300 dias de cultivo, foram realizadas avaliações do crescimento da parte aérea, diâmetro do hipocótilo, avaliações do crescimento do sistema radicular e biomassa. Os dados foram submetidos à análise de variância (ANOVA), seguido do teste de Tukey ao nível de 5% de significância. O crescimento das plântulas foi similar nos tratamentos contendo 25%, 50% e 75% de lodo, enquanto 100% areia e 100% lodo proporcionaram menor desenvolvimento das plantas após 300 dias. Portanto, conclui-se que é viável o uso de lodo de tratamento de efluente de parboilização de arroz como substrato alternativo, sendo recomendado o uso de 25% de areia + 75% de lodo por destinar maior quantidade do resíduo.*

Palavras-Chave: Biossólidos, Crescimento de plantas, Rejeito industrial.

1. INTRODUCTION

About 25% of the rice consumed in Brazil comes from parboiling (Paraginski et al., 2014), a process that consists in pre-cooking the grains in potable water aiming to increase their yield and nutritive value (Volpe, 2014). Nevertheless, each kilogram of processed rice produces around four liters volume of wastewater (Nadaleti et al., 2018) with acidic pH and high levels of Biochemical Oxygen Demand, Chemical Oxygen Demand, and solids, that must be treated before returning to water bodies (Kumar et al., 2016). At the end of this process, a sludge waste is generated, whose composition includes organic substances and nutrients such as nitrogen and phosphorus (Faria et al., 2006).

Lately, there is a growing concern related to the organic and industrial waste disposal, which resulted in research that intends to exploit waste aiming to mitigate the environmental impacts that it could cause if inappropriate disposal occurred (Berilli et al., 2014). Then again there is greater demand for services and products related to the production of native forest species seedlings, whether to be used in degraded areas recovery, reforesting projects and/or environmental compensation (Caldeira et al., 2013). The production of seedlings must take into account the substrate, as plants will fix their roots in it and access nutrients and water, thus influencing the quality of the seedlings (Trigueiro and Guerrini, 2014; Siqueira et al., 2018).

To choose a substrate, their physical and chemical properties must be given consideration, so that it provides the plants a good development, but also economical aspects related to low cost and availability for acquisition (Delarmelina et al., 2014). Hence there is the possibility of using the sludge from rice parboiling wastewater treatment plants as substrate for the production of seedlings, as, according to Vieira et al. (2011), it contains minerals that may be used as nutrients by plants. Some studies use sewage sludge, or biosolid, as alternative substrate, which enables comparisons with that from rice industry (Abreu et al., 2019; Mendonça et al., 2019; Siqueira et al., 2018; Trigueiro and Guerrini, 2014; Caldeira et al., 2013).

A. angustifolia is a long-lived secondary species, commonly known as araucaria or Brazilian pine, naturally occurring in Brazil, spread throughout the states of Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS) (Gibinski and Krupek, 2016). According to Fritzsons et al. (2018), an area where the species occurs in Southern RS is Serra do Sudeste, which comprises the municipalities of Canguçu, Pelotas, Santana da Boa Vista and others. The species has great importance to biodiversity, in addition to the use of its seeds as food source by both fauna and human being. Due to its intensive wood exploitation, the species is now at extinction risk, classified as "in danger" by the Brazilian Endangered Flora List (BRASIL, 2014), and as critically endangered by the International Union for Conservation of Nature - IUCN (2018). In this way, it is legitimate the importance of research on the conservation and regeneration of *A. angustifolia*.

Considering the occurrence of this endangered native species in Serra do Sudeste area, where are also many rice industries, this study aimed to assess the viability of producing *A. angustifolia* seedlings using sludge from rice parboiling wastewater treatment at different proportion as substrate.

2. MATERIAL AND METHODS

The experiment was performed in a greenhouse at 31°42'56.2" S 52°18'40.0" W. The average temperature was 14±3ºC in the winter and 22±4ºC in the summer.

A. angustifolia seeds were collected at May 24, 2018, at Passo Fundo National Forest, RS, and kept refrigerated (4ºC). On June 12, 2018, the seeds were subjected to the immersion test in water, and those potentially nonviable (buoyant) were taken off. The remaining was disinfected with sodium hypochlorite at 2% for 20 minutes. Seeds were then scarified to partially remove the tegument, aiming to accelerate germination and increase its synchrony (Ferreira and Handro, 1979). In order to minimize sample lost, seeds were placed in germination trays containing vermiculite at June 13, 2018 and, 36 days after, when their rootlets were around 5 cm, they were individually transplanted to the experiment containers (polyethylene bags for seedlings, 6 cm diameter x 17 cm height).

Containers were previously filled with one of five treatment mix (v:v): 100% sand (washed, medium size) (T1); 75% sand 25% sludge (T2); 50% sand 50%

sludge (T3); 25% sand 75% sludge (T4); 100% sludge (T5). Sludge was obtained from a rice parboiling wastewater treatment plant in a rice processing company located at Pelotas, RS (Some information on the sludge chemical composition is presented in Table 1).

Experimental design consisted of three groups referring to the periods of evaluation: 94, 180 and 300 days of plant growth. Each group comprised five treatments with 10 plants (replicates) each, in a total of 150 plants. All the containers were placed on a bench in a greenhouse, watered to saturation when necessary and randomly repositioned every 15 days. According to Oliveira et al. (2016), repositioning the plants avoids eventual imbalanced competition for light, where some plants, due to their sizes, could be less exposed to light than others.

At each period of evaluation (94, 180, 300 days), seedlings were removed from the plastic bags, had the substrate removed and their roots carefully washed with tap water. Seedlings were cut at the cotyledon insertion point, detached the shoot system from the radicular system, which was then separated into the main root and first order lateral roots. A metric ribbon was used to measure main shoot height, shoot system total length (main shoot height + lateral branches length), main root length, first order lateral roots average length, and root system total length; the number of lateral roots was also recorded, and hypocotyl diameter was measured with a pachymeter. Biomass was assessed through shoot system fresh matter, shoot system dry matter, main root fresh matter, main root dry matter, lateral roots fresh matter and lateral roots dry matter. Fresh roots and shoots were weighted in a semi-analytical scale, then dried in oven at 60ºC until reach constant weight.

Data were submitted to Shapiro-Wilk's normality test and Bartlett's homoscedasticity test. Once those conditions were fulfilled, one-way analysis of variance (ANOVA) was performed and mean values were compared by Tukey test with significance level at 5% ($p<0.05$). All statistical analyses were performed using R version 3.6.2 (R Core Team, 2019).

3. RESULTS

Considering main shoot height and shoot system total length at 180 days, seedlings grown in sand (T1) had the smallest values (19.73 cm and 29.03 cm, respectively), while treatments with sludge had greater results, although no significant difference was observed between them (Table 2). At 300 days, T2, T3, and T4 had no significant difference, but provided greater results in shoots and, as consequence, had greater shoot system total length. Thus, considering

Table 1 – Physicochemical characterization of sludge from rice parboiling wastewater treatment.
Tabela 1 – *Caracterização físico-química do lodo da estação de tratamento de efluente de parboilização de arroz.*

Physicochemical				
Assay	**R**	**U**	**LOQ**	**RV**
Total soluble phenols	0.05	mg/L	0.01	0.01
Free liquids in total mass	< 0.1	mL	0.1	-
Soluble nitrate (expressed as N)	1.0	mg/L	0.3	10.0
Oils and greases in total mass	< 10	mg/L	10.0	-
Solids	76.4	%	0.3	-
pH in total mass	6.25	-	0-14	2 to 12.5
Soluble sulphate (expressed as SO_4)	< 1.0	mg/L	1.0	250.0
Soluble surfactants	0.760	mg/L	0.4	0.5
Metals				
Assay	**R**	**U**	**LOQ**	**RV**
Soluble Al	< 0.2	mg/L	0.2	0.2
Soluble Cu	< 0.05	mg/L	0.05	2.0
Soluble Fe	8.5	mg/L	0.1	0.3
Soluble Na	43.7	mg/L	1.0	200.0
Soluble Zn	0.180	mg/L	0.1	5.0

R = result; U = unity; LOQ = limit of quantification; RV = reference value according to ABNT NBR 10004:2004. Source: adapted by the authors from the analysis report performed by EcoCerta – Laboratório de Análises Ambientais (Laboratory of Environmental Analysis), 2018.
R = resultado; U = unidade; LOG = limite de quantificação; RV = valor de referência de acordo com a ABNT NBR 10004:2004. Fonte: adaptado pelos autores a partir do relatório de análises realizadas pelo EcoCerta – Laboratório de Análises Ambientais, 2018.

Table 2 – Main shoot height (MSH), shoot system total length (SSTL), and hypocotyl diameter (HD) of *Araucaria angustifolia* seedlings at 94, 180 and 300 days of growth in different ratios of sand and sludge from rice parboiling wastewater treatment.
Tabela 2 *– Altura da parte aérea (MSH), comprimento total da parte aérea (SSTL), e diâmetro do hipocótilo (HD) das mudas de Araucaria angustifolia aos 94, 180 e 300 dias de cultivo em diferentes proporções de areia e lodo de estação de tratamento de efluente de parboilização de arroz.*

	MSH			SSTL			HD		
Treatment/Days	**94**	**180**	**300**	**94**	**180**	**300**	**94**	**180**	**300**
T1	15.09ab	19.73b	18.69c	15.09ab	29.03b	30.68c	0.60ns	0.60ns	0.60c
T2	16.01[a]	26.01a	33.86ab	16.01[a]	48.49a	116.07ab	0.61	0.64	0.85a
T3	14.73ab	25.87a	35.85a	14.73ab	40.74a	107.11ab	0.61	0.61	0.78ab
T4	15.94[a]	28.62a	34.25ab	15.94[a]	51.89a	131.17a	0.59	0.65	0.75ab
T5	13.00b	25.67a	29.08b	13.00b	45.60a	83.03b	0.55	0.64	0.73b

T1 = 100% sand; T2 = 75% sand 25% sludge; T3 = 50% sand 50% sludge; T4 = 25% sand 75% sludge; T5 = 100% sludge. Different letters in the column indicate significant differences according to Tukey test at 5% probability. Columns with no letters do not differ statistically (ns).
T1 = 100% areia; T2 = 75% areia 25% lodo; T3 = 50% areia 50% lodo; T4 = 25% areia 75% lodo; T5 = 100% lodo. Letras diferentes na coluna indicam diferenças significativas de acordo com o teste de Tukey a 5% de probabilidade. Colunas sem letras não possuem diferença significativa (ns).

these variables, the longer the period of development, the more promising for *A. angustifolia* shoot growth are the mixtures of sludge and sand. In this study, at both 94 and 180 days, values of hypocotyl diameter had no significant differences. At 300 days, values for hypocotyl diameter ranged from 0.60 cm to 0.85 cm, which the smallest value was observed in T1, and the greatest, in T2, T3 and T4 (Table 2).

No significant differences were observed for main root length at 94 and 180 days (Table 3). At 300 days, T4 had the lowest value (23.5 cm) for this avaliation, which was significantly different from T2 (32.93 cm), the greatest value. Despite of the size constraint in the bags used for cultivation, no coiling of the main root was observed.

The highest values for the number of lateral roots at 180 and 300 days were observed in T1 and T2, both treatments with the highest proportion of sand (100% and 75%, respectively) (Table 3). The values for lateral roots average length at 94 and 180 days were not significantly different between the treatments, yet, at 300 days, T1, T2, and T3 provided the highest values, ranging from 6.96 cm and 7.91 cm (Table 3).

The results for main root length, number of lateral roots, and lateral roots average length corroborate those found for root system total length (Table 3). On the whole, the treatments with higher proportion of sludge (ranging from 50% to 100%; T3, T4 and T5) provided the lowest values for root system total length in the periods of evaluation, as there were fewer and shorter lateral roots, especially in the last period of evaluation.

Only sand as substrate (T1) provided the lowest shoot fresh matter (Table 4) and shoot dry matter at 300 days (Table 5), which corroborate the results found for shoot length and shoot system total length (Table 2). Substrates containing sludge showed values ranging between 25.5 g and 37.01 g for fresh matter, and 7.04 g and 10.62 g for dry matter.

Table 3 – Main root length (MRL), number of lateral roots (NLR), lateral roots average length (LRAL), and root system total length (RSTL) of *Araucaria angustifolia* seedlings at 94, 180 and 300 days of growth in different ratios of sand and sludge from rice parboiling wastewater treatment.
Tabela 3 *– Comprimento da raiz principal (MRL), número de raízes laterais (NLR), comprimento médio das raízes laterais (LRAL) e comprimento total do sistema radicular (RSTL) de mudas de* ***Araucaria angustifolia*** *aos 94, 180 e 300 dias de cultivo em diferentes proporções de areia e lodo de estação de tratamento de efluente de parboilização de arroz.*

Treatment/ Days	**MRL**			**NRL**			**LRAL**			**RSTL**		
	94	**180**	**300**	**94**	**180**	**300**	**94**	**180**	**300**	**94**	**180**	**300**
T1	15.37ns	29.25ns	31.83ab	20.60ns	50.4a	52.70a	8.10ns	6.44ns	7.39ab	154.10a	343.54a	418.85a
T2	17.90	29.89	32.93a	23.10	42.8ab	53.10a	7.39	7.15	7.91a	159.16a	337.21ab	434.93a
T3	14.78	27.20	26.38ab	16.0	30.8c	34.40b	4.87	6.30	6.96ab	90.60b	218.51c	260.62b
T4	16.59	23.65	23.50b	17.0	38.4bc	37.50b	4.61	5.73	5.60b	91.15b	241.67bc	232.71b
T5	20.51	30.36	26.33ab	19.10	28.4c	34.0b	4.91	6.51	5.60b	110.14ab	209.42c	216.29b

T1 = 100% sand; T2 = 75% sand 25% sludge; T3 = 50% sand 50% sludge; T4 = 25% sand 75% sludge; T5 = 100% sludge. Different letters in the column indicate significant differences according to Tukey test at 5% probability. Columns with no letters do not differ statistically (ns).
T1 = 100% areia; T2 = 75% areia 25% lodo; T3 = 50% areia 50% lodo; T4 = 25% areia 75% lodo; T5 = 100% lodo. Letras diferentes na coluna indicam diferenças significativas de acordo com o teste de Tukey a 5% de probabilidade. Colunas sem letras não possuem diferença significativa (ns).

Table 4 – Shoots fresh matter (SFM), main root fresh matter (MRFM), and lateral roots fresh matter (LRFM), for *Araucaria angustifolia* seedlings at 94, 180 and 300 days of growth in different ratios of sand and sludge from rice parboiling wastewater treatment.
Tabela 4 *– Massa fresca da parte aérea (SFM), massa fresca da raiz principal (MRFM), e massa fresca das raízes laterais (LRFM), de mudas de* ***Araucaria angustifolia*** *aos 94, 180 e 300 dias de cultivo em diferentes proporções de areia e lodo de estação de tratamento de efluente de parboilização de arroz.*

	SFM (g)			MRFM (g)			LRFM (g)		
Treatment/Days	**94**	**180**	**300**	**94**	**180**	**300**	**94**	**180**	**300**
T1	3.88b	8.24b	7.91c	2.00ns	3.75ns	4.89b	1.25ns	3.32ns	4.53b
T2	5.62a	22.47a	36.62a	1.42	4.1	8.42a	1.10	4.67	12.42a
T3	4.71ab	19.16a	35.12ab	1.38	3.14	6.36ab	0.92	3.35	6.49b
T4	5.27ab	25.65a	37.01a	1.39	3.32	4.95b	0.70	3.51	5.06b
T5	3.71b	20.24a	25.5b	1.45	3.73	5.57b	0.70	2.94	4.12b

T1 = 100% sand; T2 = 75% sand 25% sludge; T3 = 50% sand 50% sludge; T4 = 25% sand 75% sludge; T5 = 100% sludge. Different letters in the column indicate significant differences according to Tukey test at 5% probability. Columns with no letters do not differ statistically (ns).
T1 = 100% areia; T2 = 75% areia 25% lodo; T3 = 50% areia 50% lodo; T4 = 25% areia 75% lodo; T5 = 100% lodo. Letras diferentes na coluna indicam diferenças significativas de acordo com o teste de Tukey a 5% de probabilidade. Colunas sem letras não possuem diferença significativa (ns).

No significant difference between treatments was found for main root fresh matter (Table 4) and main root dry matter (Table 5) at 94 days, whereas, at 300 days, T2 provided the highest values, 8.42 g for main root fresh matter and 2.89 g for main root dry matter. T2 also provided the highest values for lateral roots fresh matter (12.42 g; Table 4) and lateral roots dry matter (2.27 g; Table 5) at 300 days, which was significantly different from other treatments.

4. DISCUSSION

Data related to the characterization and use of sludge from rice parboiling wastewater treatment plants as alternative substrate for the production of tree species seedlings are scarce in the literature, whereas most of those publications focus on the wastewater treatment system itself but do not mention its sludge disposal. Thus, this work uses as reference studies related to the use of sludge from sewage wastewater and other substrates, many of them on the production of angiosperm species.

According to Gonçalves et al. (2014), main shoot height is a feature commonly used in seedlings evaluation, which allows to estimate plant growth in the field. As stated by Gomes et al. (2012) and Neto (2015), plant height can be influenced by factors such as nutritional levels where, for example, an increased supply of nitrogen favors growth in height. Although nitrogen levels in the substrate mixtures were not measured in this work, the sludge chemical characterization (Table 1) shows that nitrate is present, which may have contributed for *A. angustifolia* seedlings growth. Nevertheless, Garbin and Dillenburg (2008) attested that this species assimilates inorganic nitrogen preferentially as ammonium. Vieira et al. (2011) noticed that the sludge from rice parboiling wastewater treatment is rich in nitrogen (23.5 g kg^{-1}) and phosphorus (42.4 g kg^{-1}), which may support plant growth. In a study with angiosperms, Trigueiro and Guerrini (2014) assigned a gain in height in *Schinus terebinthifolia* Raddi seedlings to the nitrogen present in the sewage sludge used as substrate. According

Table 5 – Shoots dry matter (SDM), main root dry matter (MRDM), and lateral roots dry matter (LRDM) for *Araucaria angustifolia* seedlings at 94, 180 and 300 days of growth in different ratios of sand and sludge from rice parboiling wastewater treatment.
Tabela 5 *– Massa seca da parte aérea (SDM), massa seca da raiz principal (MRDM), e massa seca das raízes laterais (LRDM) de mudas de* ***Araucaria angustifolia*** *aos 94, 180 e 300 dias de cultivo em diferentes proporções de areia e lodo de estação de tratamento de efluente de parboilização de arroz.*

	SDM (g)			MRDM (g)			LRDM (g)		
Treatment/Days	**94**	**180**	**300**	**94**	**180**	**300**	**94**	**180**	**300**
T1	0.97ab	2.87b	2.81c	0.45ns	1.19a	1.74b	0.13a	0.69ns	0.88b
T2	1.21a	5.89a	10.62a	0.27	1.09ab	2.89a	0.09ab	0.7	2.27a
T3	0.99ab	4.71a	9.82ab	0.38	0.94ab	1.91b	0.05ab	0.6	1.13b
T4	1.12ab	6.4a	9.89ab	0.27	0.89b	1.59b	0.02b	0.58	0.95b
T5	0.79b	5.01a	7.04b	0.25	0.98ab	1.57b	0.03b	0.49	0.72b

T1 = 100% sand; T2 = 75% sand 25% sludge; T3 = 50% sand 50% sludge; T4 = 25% sand 75% sludge; T5 = 100% sludge. Different letters in the column indicate significant differences according to Tukey test at 5% probability. Columns with no letters do not differ statistically (ns).
T1 = 100% areia; T2 = 75% areia 25% lodo; T3 = 50% areia 50% lodo; T4 = 25% areia 75% lodo; T5 = 100% lodo. Letras diferentes na coluna indicam diferenças significativas de acordo com o teste de Tukey a 5% de probabilidade. Colunas sem letras não possuem diferença significativa (ns).

to Abreu et al. (2019), treatments with higher levels of biosolids and, consequently, greater amounts of nutrients, showed better results for *S. terebinthifolia* seedlings main shoot height.

The sludge supplies organic matter, increasing water and nutrients retainment. According to Gomes et al. (2012), sewage sludge associated to commercial substrate and subsoil provided greater height and root dry matter in *Tectona grandis* L.f. seedlings. For *Chamaecrista desvauxii* (Collad.) Killip seedlings, the combination of 60% sewage sludge + 20% carbonized rice peel + 20% in natura coffee straw provided greater growth (Caldeira et al., 2013). In the assessment of *Psidium cattleyanum* Sabine plantlets growth, Marques et al. (2018) observed higher seedlings were produced with 40% and 60% sewage sludge combined with organic compost. Treatments containing 20% sewage sludge + 80% sand sediment, and 60% sewage sludge + 40% sand sediment provided greater average height, average stem diameter and average number of leaves in *S. terebinthifolia* (Mendonça et al., 2019).

In this study, the sludge from rice parboiling wastewater treatment provided better results when mixed with sand. Sand may have benefited plantlets growth by enabling better water draining and improving organic matter and nutrient supply from the sludge. According to Delarmelina et al. (2014), to support and facilitate plant growth, the substrate should enable appropriate aeration, and proper water draining and retainment balance, without causing soaking. However, *A. angustifolia* plants did not show satisfactory growth when the substrate was only sand. Corroborating this study, Garcia et al. (2012) noticed that the ideal growth of *Bactris gasipaes* Kunth seedlings was not achieved using only sand as substrate, and the authors emphasized its use combined with other materials. According to Alvino and Rayol (2007), sand is poor at maintaining moisture, has low water retention and distribution, besides being nutritionally poor, which may impair plant growth in height. According to Wendling and Delgado (2008), *A. angustifolia* seedlings are ready to use when they reach between 13 and 30 cm height, which varies depending on the vessel used for cultivation. In this study, seedlings grown in substrate containing sludge were over 25 cm height at 180 days.

For Ritchie et al. (2010), the hypocotyl diameter is an important morphologic trait to estimate plant growth and predict its performance at field, depending on the variations to which each species is exposed to. Dillenburg et al. (2010) noticed that while *A. angustifolia* seeds were attached to the plants, the hypocotyl mass increased, followed by a decrease, confirming that the hypocotyl is a drain for the seed stocks, regardless of the presence or absence of light. In their work with the species, Franco and Dillenburg (2007) observed that the plant retained a bond with the seed for 80 to 120 days, then changing from a dependence state to autonomy. That is, the plantlets were attached to the seeds and part of their reserves were transferred to the hypocotyls. As no difference in the variable was observed at 94 and 180 day, it is assumed that, until this time, the stocks that have moved from the seed to the hypocotyl have had no effect on its diameter. The ideal hypocotyl diameter for forest species ranges from 0.5 to 1.0 cm (Gonçalves et al., 2000).

According to Trigueiro and Guerrini (2014), and Siqueira et al. (2018), substrates should have physical and chemical properties that enable proper plant development, including a fine root system structure. In general, this study showed greater root growth in substrates containing greater sand ratio. These results may be due to the fact that sand is more porous and has low nutrients content (Picolotto et al., 2007), which requires roots to extend in search of nutrients, including T2 and T3, where the higher sand ratio may have influenced increased lateral roots growth (considering number and length of lateral roots). In a study by Mendonça et al. (2019), *S. terebinthifolia* plantlets cultivated in 100% sandy sediment showed greater results in roots rather than in height or stem diameter, compared to substrates made of sewage sludge and sandy sediment. Tillmann et al. (1994) say that there is a trend of increased root length in a substrate with higher porosity, aeration, and water availability. As presented in the results, T2 showed greater shoot growth when compared to T1. Negreiros et al. (2004) state that sand might enable greater plant fitness when mixed with other substrates. The results from the present work corroborate those found by Trigueiro and Guerrini (2014) for *S. terebinthifolia*, where there was reduced root system development in treatments with higher ratio of sewage sludge, which may be due to the substrate's high density, which reduces the draining and, consequently, the aeration.

In a study with *Sesbania virgata* (Cav.) Pers., the substrate that produced the higher shoot system dry matter at 150 days (2.11 g) was a mix of 60% sewage sludge and 40% vermiculite (Delarmelina et al., 2014). In one of the few studies using the sludge from rice parboiling wastewater treatment, Vieira et al. (2011) tested the addition of 2.15 g Kg^{-1} sludge in maize plants cultivated in plastic vessels containing 4 Kg of soil and observed a significant increase in plants dry matter after 41 days. Substrates composed by 60% or 20% sewage sludge combined with carbonized rice peel and in natura coffee straw provided better results for root dry matter of *Chamaecrista desvauxii* (Collad.) Killip plants cultivated for 150 days (Caldeira et al., 2013). In another study, *Eucalyptus grandis* W. Hill seedlings grown in 80% sewage sludge and 20% coffee straw showed higher values of root dry matter (Caldeira et al., 2014).

5. CONCLUSION

Although plants cultivated in 100% sand (T1) had greater root growth, they produced lower values for shoot growth variables, like main shoot height and hypocotyl diameter, in comparison with sludge from rice parboiling wastewater treatment plants in different proportions.

Seedlings growing in 100% sludge (T5) provided slow growth, both for shoots and root system.

The combination of sand and sludge from rice parboiling wastewater treatment plants provided the best overall results, considering shoot and root system variables at 300 days. Furthermore, the ratio 25% sand + 75% sludge (T4) could be recommended for the production of *A. angustifolia* seedlings as it exploits a larger amount of sludge.

6. AUTHOR CONTRIBUTIONS

F.F.C. and E.S. conceived the ideas and designed the methodology, V.N.B. and F.F.C. performed the experiment and collected the data, F.K.P. analyzed the data. All authors contributed to the writing.

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