

## SUBSTRATO EXAURIDO DE COGUMELOS PARA PRODUÇÃO DE MUDAS FLORESTAIS\*

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**RESUMO:** O objetivo deste trabalho foi avaliar o efeito do substrato exaurido de *Lentinula edodes* para a produção de mudas de *Eucalyptus grandis*. Foi utilizado um delineamento experimental 4x1, sendo 4 substratos (T1 (testemunha, 100% Carolina Soil II), T2 (25% substrato exaurido + 75% casca de arroz carbonizada), T3 (50% substrato exaurido + 50% casca de arroz carbonizada) e T4 (75% substrato exaurido + 25% casca de arroz carbonizada)), e uma espécie florestal (*E. grandis*). Avaliou-se o percentual de emergência, altura da parte aérea, diâmetro, número de folhas, massas secas aérea, radicular e total, índice de qualidade de Dickson, conformação do sistema radicular, lixiviado e acúmulo nutricional. Foi feita análise de variância e teste de Tukey (5%). O tratamento T4 foi o que mais se aproximou das características físicas do tratamento testemunha, sendo que os demais apresentaram baixa quantidade de microporos. Observa-se maiores teores de nutrientes nos tratamentos contendo substrato exaurido. O substrato exaurido afetou negativamente o percentual de emergência, mas não afetou o número de folhas. Quanto à altura, diâmetro do colo e índice de qualidade de Dickson, o T1 obteve valores estatisticamente superiores aos demais tratamentos, porém todos alcançaram parâmetros considerados aptos para o plantio. O acúmulo de nutrientes apresentou comportamentos distintos na parte aérea e no sistema radicular. A análise do lixiviado evidenciou menor perda de água e nutrientes no tratamento testemunha, que possuía maior capacidade de retenção. Os substratos contendo substrato exaurido produziram mudas viáveis, porém novos estudos são necessários utilizando diferentes manejos hídricos e adubações.

**Palavras-chave:** eucalipto, *Lentinula edodes*, viveiro.

## SPENT MUSHROOM SUBSTRATE FOR THE PRODUCTION OF FORESTRY SEEDLINGS

**ABSTRACT:** The aim of this study was to evaluate the effect of *Lentinula edodes* spent mushroom substrate for the production of *Eucalyptus grandis* seedlings. A 4x1 experimental design was used, with 4 substrates (T1 (control, 100% Carolina Soil II), T2 (25% spent substrate + 75% charred rice husk), T3 (50% spent substrate + 50% charred rice husk) and T4 (75% spent substrate + 25% charred rice husk)), and one forest species (*E. grandis*). Physical and chemical characterization were performed, the percentage of emergence, plant height, stem diameter, number of leaves, aerial, root and total dry mass, Dickson quality index, root system conformation, leachate and nutritional accumulation were evaluated. Analysis of variance and Tukey's test (5%) were performed. T4 treatment was the closest to the physical characteristics of the control treatment, and the others showed a low amount of micropores. Higher nutrient contents were observed on the treatments containing spent substrate. Spent substrate negatively affected the emergency percentage, but did not affect the number of leaves. As for height, stem diameter and Dickson quality index, T1 obtained statistically higher values than the other treatments, but all treatments reached parameters considered

suitable for planting. The accumulation of nutrients showed different behaviors in the aerial part and in the root system. The leachate analysis showed less loss of water and nutrients in the control treatment, which had a greater retention capacity. The treatments containing spent substrate produced viable seedlings, but further studies are needed using different water management and fertilization.

**Keywords:** eucalyptus, *Lentinula edodes*, nursery.

## 1 INTRODUCTION

In recent decades, the cultivation of edible mushrooms has expanded more than 30 times (ROYSE; BAARS; TAN, 2017) because of their flavor, nutritional value, medicinal properties, and potential for rust cultivation (SÁNCHEZ, 2010). According to Sun *et al.* (2020), more than 200 species of mushrooms are edible, and among these, *Agaricus* species stand out. *bisporus*, *Pleurotus ostreatus* and *Lentinula mushrooms* are the most widely cultivated species worldwide. Mushrooms are part of the human diet for millennia and are highly valued for their organoleptic characteristics. Recently, their demand has increased due to their high protein, fiber, vitamin, and mineral contents; low caloric count; and low lipid content (VALVERDE; HERNÁNDEZ-PÉREZ; PAREDES-LÓPEZ, 2015). Mushrooms can be cultivated on lignocellulosic materials from the agro-industry via simple infrastructure and rustic techniques. This promotes nutrient cycling, converting these materials into high-value-added food and potentially an important source of income for small producers. Therefore, this cultivation can be considered economically and environmentally sustainable (SPINOSA, 2008).

Although cultivating edible mushrooms has several advantages, it generates spent mushroom substrates or spent/postcultivation substrates, known in the literature as *SMS–Spent. Mushroom substrate*. According to Ma *et al.* (2014) reported that for every kilogram of fresh mushrooms, between 5 and 6 kilograms of SMS (shared material) are generated. The reuse of SMS is important for the transition to a circular economy, in which agro-industrial waste is considered a resource that should be reused in other biotechnological processes, aiming at the efficiency of these processes and sustainability (GRIMM; WÖSTEN, 2018). This nutritionally rich material can be used in

bioremediation processes such as air, water, and soil; pesticide removal; mushroom cultivation; plant cultivation in open environments or greenhouses; soil conditioning; animal feed; and as a source of renewable energy (RINKER, 2017). Despite these uses, the importance of SMS reuse is relatively new, so further studies evaluating its use should be carried out. In this context, the absence of research on the use of this material for the production of *Eucalyptus grandis seedlings*, a species of great economic and environmental importance, in the literature was noted. Considering the extensive literature confirming the efficiency of SMS in the cultivation of other plants, it becomes relevant to study the potential of using SMS for the production of forest seedlings, as this rich material may become viable in silviculture.

*L. edodes* SMS in the formulation of substrates for the cultivation of *E. grandis seedlings*, as well as its impact on the morphophysiological characteristics of the plants.

## 2 MATERIALS AND METHODS

The experiment was conducted at the Forest Seedling Production Nursery of the Faculty of Agronomic Sciences (FCA) of UNESP, in the municipality of Botucatu – SP (geographic location: Latitude 22°51'17.9"S Longitude 48°25'58.2"W), from August 2021 to September 2022. Four different formulations were used for the cultivation of *Eucalyptus grandis* (Table 1). For each treatment, 352 tubes were prepared. Four plots per treatment were subsequently set up with 12 useful central seedlings for evaluation, totaling 48 useful seedlings per treatment.

**Table 1.** Proportions of inputs in the substrate formulations

	<b>Carolina Soil II</b>	<b>SMS from <i>L. edodes</i></b>	<b>CAC</b>
Treatment 1	100%	–	–
Treatment 2	–	25%	75%
Treatment 3	–	50%	50%
Treatment 4	–	75%	25%

SMS – *Spent Mushroom Substrate*; CAC = carbonized rice husk.

The formulations were subjected to physical characterization of the substrate according to Silva (1998) to determine total porosity, macropores, micropores, retention, compacted density, pH and electrical conductivity.

The conventional substrate (Carolina soil – control) and carbonized rice husk were purchased from a store at Botucatu – SP. The *L. edodes* SMS was supplied by the company Fungibras of Botucatu – SP (geographic location: latitude 22°51'01.1"S longitude 48°29'27.0"W). The *L. edodes* SMS blocks were sun-dried for 48 hours, manually fragmented, and sieved through a 2 mm mesh steel sieve. Four formulations were then prepared (Table 1) via a concrete mixer. Water was added manually to the formulations until an average moisture content of 60% was reached. For each formulation, 352 tubes were prepared, which were filled with the substrates and compacted. For sowing, seeds of *Eucalyptus grandis* W. Hill ex Maiden, cultivar LCFA001, from IPEF – Instituto de Pesquisas e Estudos Florestais, Piracicaba - SP, were used. Sowing was carried out manually via a squeeze bottle with an injector. The trays were covered with a thin layer of substrate and irrigated.

The trays were placed in a shade house with a microsprinkler irrigation system, with a flow rate of 200 L h<sup>-1</sup> per nozzle, automatically activated by an electrical panel for 20 seconds every 30 minutes, from 9 am to 4 pm. After germination, the trays were moved to a mini-tunnel greenhouse, where their spacing was altered to 50% occupancy of the tray. After the initial growth of the seedlings, thinning was carried out, leaving only one seedling per tube, prioritizing those with greater vigor and better positioning in the tube. Before being moved to the full-sun area, the height of the seedlings was selected to ensure that all the replicates within

the same substrate had the same average height for this variable.

The irrigation water for the nursery comes from SABESP (São Paulo State Basic Sanitation Company), with a pH of approximately 7.0 and an average electrical conductivity of 0.047 mS m<sup>-1</sup>. A water depth of 17 mm was used because of the high temperature and sun exposure of the plants during the experiment. Standard fertilization was carried out for all individuals via fertigation three times a week. For the macronutrient solution, the following fertilizers were used: calcium nitrate, purified monoammonium phosphate (MAP), potassium chloride, ammonium sulfate, urea, magnesium sulfate, and 13% iron at concentrations of 0.87, 0.12, 0.40, 0.15, 0.54, 0.52, and 0.03 g/L, respectively. The micronutrient solution was composed of boric acid, manganese sulfate, zinc sulfate, copper sulfate, and sodium molybdate at concentrations of 6.0, 6.0, 1.4, 0.6, and 0.16 g/L, respectively. The electrical conductivity of the solution was 3.04 mS, and the pH was 5.93. During the hardening-off period, the nutrient mixture consisted of only potassium chloride at a concentration of 600 mg L<sup>-1</sup> of K.

For the 352 tubes prepared for each treatment, the number of seedlings that developed was counted to calculate the percentage of emergence. The variables shoot height (cm) and stem diameter (mm) were evaluated for the 12 useful seedlings from each plot. Shoot height was measured with a millimeter ruler from the edge of the tube to the apical bud that gave rise to the last leaf. The stem diameter (mm) was measured flush with the edge of the tube via a digital caliper. To measure the shoot (g) and root dry mass of the seedlings (g), the stem was sectioned close to the substrate and divided into two parts. The number of leaves on each seedling was counted.

The root systems were washed in running water over a sieve. Then, the shoots and roots were separately placed in paper bags and placed in an oven at 70 °C until they reached constant mass, which was measured on a precision electronic

$$IQD = \frac{Massa\ seca\ total}{\left(\frac{Altura\ da\ parte\ aérea}{Diâmetro\ do\ colo}\right) + \left(\frac{Massa\ seca\ da\ parte\ aérea}{Massa\ seca\ radicular}\right)} \quad (1)$$

The root system conformation was evaluated according to Simões, Silva and Silva (2012). In this evaluation, the qualitative concepts "suitable and unsuitable for planting" were assigned. The concept "suitable for planting" (%) was assigned to the root system formed by a clod with little or no flexibility (qualification "excellent" and "good") and with the presence of new roots. The concept "unsuitable for planting" (%) was assigned to the disintegrated root system with an absence of new roots (qualification "poor").

For chemical characterization of the substrates before and after cultivation, filtrates were obtained following the Dutch 1:1.5 (v:v) method. For each treatment, 100 mL of substrate was added to 150 mL of distilled water and filtered. Fifty milliliters of filtrate was obtained from each treatment and was used for chemical characterization of the substrate. For chemical characterization of plant nutrients, the dry matter of the aerial and root parts of each treatment was separately ground in a Wiley-type mill. The chemical characterizations were performed at the Chemical Analysis Laboratory of Fertilizers and Soil Amendments, belonging to the Department of Forest Science, Soils and Environment – FCA/UNESP, Botucatu, SP, geographical location 22°85'04.54"S and 48°43'16.41"). The levels of macronutrients (N, P2O5, K2O, Ca, Mg, S, C, C/N and pH),

balance with two decimal places. The total dry mass (g) was determined from the sum of the shoot and root dry masses, and subsequently, the Dickson quality index (DQI) was calculated according to the following formula:

micronutrients (Na, B, Cu, Fe, Mn, Zn), pH and electrical conductivity were determined. To collect the leached solutions, plastic bags were secured with elastic bands before the first growth fertigation of the day, with 10 seedlings from each treatment. After fertigation, the pH and electrical conductivity of these solutions were measured. This procedure was repeated with hardening-off fertigation. The volume of the leached solution from these stages was measured in a graduated cylinder.

For comparisons between treatments, the statistical program Sisvar was used, where the means were compared via Tukey's test at the 5% significance level. Boxplots for data visualization were generated via the Python programming language, version 3.10, with the numpy, pandas, and matplotlib libraries in the Google Colab integrated development environment. To compare the effects of the physical and chemical characteristics of the substrates on nutrient accumulation in plants, Pearson's correlation coefficient was used.

### 3. RESULTS AND DISCUSSION

The results of the physical characterization of the substrates are shown in Table 2, where it is possible to observe differences in these parameters between the substrates.

**Table 2.** Physical characterization of the substrates

	T1	T2	T3	T4
Total porosity (%)	80.29	85.18	85.26	83.92
Macropores (%)	25.32	54.42	51.47	42.74
Micropores (%)	54.97	30.76	33.79	41.18
Retention (mL/55 cm <sup>3</sup> )	28.02	15.70	17.23	21.07
Density (kg/m <sup>3</sup> )	182.50	296.71	343.83	452.23
pH	6.27	5.79	5.67	5.57
EC (mS/cm)	0.66	0.61	1.04	1.18

T1 = 100% Carolina Soil II; T2 = 25% depleted *Lentinula substrate*; T3 = 50% depleted substrate of *L. edodes* + 75% carbonized rice husk; T4 = 50% depleted substrate of *L. edodes* + 50% carbonized rice husk; T5 = 75% depleted substrate of *L. edodes* + 25% carbonized rice husk; EC = electrical conductivity. Average of 12 repetitions.

The total porosity of the SMS + CAC-based treatments was greater than the porosity of the control treatment (T1 – Carolina Soil). In addition to the higher total porosity, these treatments also presented a greater quantity of macropores than did the control treatment. These results are corroborated by Guerrini and Trigueiro (2004), who explained that carbonized rice husk is a lightweight material that is inert to hydration, increasing the substrate porosity as the amount of this material in the mixture increases. According to Gonçalves and Poggiani (1996), the recommended value for total porosity is 75–85%, so all the treatments obtained adequate values for this parameter. Additionally, according to the same authors, the macroporosity should be between 35% and 45%, a value reached only by substrate T4. With respect to micropores and retention, an inversely proportional relationship was observed between the amount of CAC used and the values obtained for these parameters, and the control treatment resulted in higher values than did the other treatments. According to Guerrini and Trigueiro (2004), the addition of CAC results in increased macroporosity but decreased microporosity. The authors add that there is a direct relationship between microporosity and the substrate's retention capacity. The literature recommends that microporosity values be between 45% and 55% (GONÇALVES; POGGIANI, 1996), which was only obtained by the control treatment. The retention should be between 20 and 30 mL<sup>/50 cm<sup>3</sup></sup> (DELAMELINA *et al.*, 2014), achieved by treatments T1 and T4.

The literature recommends a substrate density between 250 and 500 kg/m<sup>3</sup> (KRATZ *et*

*al.*, 2013), so the SMS + CAC-based treatments obtained values within the recommended range, unlike the control treatment, which obtained lower values. However, Lopes *et al.* (2008), when evaluating the impact of the physical attributes of two different substrates on the production of eucalyptus seedlings, values lower than the recommended values were obtained, which did not affect seedling development. Although some substrates did not reach the values recommended by the literature for different parameters, Silva *et al.* (2021), when evaluating the effects of the physical properties of different substrates on the quality of clonal eucalyptus seedlings, values that deviated from the recommended range were also obtained. The authors explain that the recommended values do not apply to all species, container types, propagation methods, water and nutritional management, or materials used in substrate formulation; therefore, values different from those recommended in the literature may still be adequate. With respect to pH, the greater the amount of SMS present in the substrate was, the lower the pH. Values between 5.57 and 6.27 were obtained, so all values are within the range recommended (5.0–6.5) by Kratz. *et al.* (2013). With respect to electrical conductivity, Santos *et al.* (2014) recommended a value of up to 1.0 mS/cm, so only treatments T3 and T4 were slightly above that recommended by this author.

Table 3 contains the chemical characterization of the different treatments before cultivation, as well as of SMS and individually carbonized rice husk, for comparison. In the samples containing SMS, pure or in different combinations, it was not

possible to obtain the phosphorus, sulfur, and boron contents.

**Table 3.** Chemical characterization of substrates (macro- and micronutrients) before cultivation.

Nutrients (mg L <sup>-1</sup> )	Substrates					
	T1	T2	T3	T4	SMS	CAC
N	20	24	28	27	20	14
P	29	-	-	-	-	39
K	104	186	157	149	333	138
Here	34	154	136	212	364	39
Mg	47	132	157	186	610	35
S	66.6	-	-	-	-	15.1
In	12.50	14.40	19,20	19,20	38.80	6.80
B	0.08	-	-	-	-	0.05
Ass	0.09	0.09	0.07	0.08	0.13	0.06
Faith	0.60	1.60	0.75	2.24	1.19	0.05
Mn	0.37	5.71	1.72	5.46	2.43	0.16
Zn	0.16	0.25	0.07	0.27	0.20	0.02

T1 = 100% Carolina Soil II, T2 = 25% depleted *Lentinula substrate* T3 = 50% exhausted substrate of *L. edodes* + 75% carbonized rice husk, T4 = 75% exhausted substrate of *L. edodes* + 25% carbonized rice husk, SMS = 100% spent substrate of *L. edodes*, CAC = 100% carbonized rice husk.

The nitrogen content was greater in the treatments with SMS than in the control treatment. Antônio *et al.* (2010) evaluated the viability of using brewery biosolids as a substrate for eucalyptus production and reported an increase in the nitrogen content of the substrate as a function of the increase in biosolids. The authors reported that the greater nutrient content in the substrates resulted in plants that were statistically greater in height than plants that were grown in the control substrate. Similarly, D'Ávila (2008) reported better results in terms of the morphological characteristics of eucalyptus seedlings in substrates supplemented with nitrogen.

With respect to the phosphorus content, it was not possible to obtain the content of this nutrient in the SMS-based treatments. In the control treatment, a value of 29 mg/L<sup>-1</sup> was obtained, which was above the range of 6--10 mg/L<sup>-1</sup> recommended by Lopes *et al.* (2008). The potassium values ranged from 104 to 186 mg/L<sup>-1</sup>, with the lowest value in the control treatment and the highest value in substrate T2. Silva *et al.* (2014), when evaluating the production of *E. grandis* in alternative organic substrates, obtained K values between 120 and 310 mg/L<sup>-1</sup>, which are close to those reported in the present study. With respect to calcium, the control treatment resulted in a value of 34

mg/L<sup>-1</sup>, whereas the other treatments resulted in values between 136 and 212 mg/L<sup>-1</sup>. Lopes *et al.* (2008) recommended that calcium values in substrates for plant cultivation be between 150 and 249 mg/L<sup>-1</sup>; therefore, the concentrations of the treatments containing SMS, with the exception of T3, are within the recommended range according to these authors. The control treatment resulted in a magnesium value of 47 mg/L<sup>-1</sup>. The addition of SMS resulted in a gradual increase in this micronutrient in the substrates, with values between 132 and 186 mg/L<sup>-1</sup>. However, all treatments are still below the level recommended by Lopes *et al.* (2008), above 200 mg/L<sup>-1</sup>. With respect to sulfur, it was also not possible to obtain the content of this nutrient in the SMS-based treatments. In the control treatment, a value of 66.6 mg/L<sup>-1</sup> was obtained.

The addition of SMS to the substrate also resulted in higher sodium levels, especially in treatments T3 and T4. Bitencourt, Deknes, and Laura (2022) evaluated the use of tannery sludge in the production of eucalyptus seedlings and reported that the addition of this material resulted in an increase in sodium in the substrate, leading to increased electrical conductivity and salinity stress, which negatively impacted plant height, root length, and root dry mass. The authors explained that

the presence of sodium hinders magnesium absorption and the availability of iron, manganese, copper, and zinc. The boron content in the control treatment was  $0.08 \text{ mg/L}^{-1}$  and it was also not possible to obtain the values of this nutrient in the other treatments. The value is within the range recommended by Lopes *et al.* (2008), from  $0.005$  to  $0.5 \text{ mg/L}^{-1}$ . The copper values were similar across all the substrates, ranging from  $0.07$  to  $0.09 \text{ mg/L}^{-1}$ . Lopes *et al.* (2008) suggest copper values between  $0.001$  and  $0.5$ , so all substrates are within the range recommended by the literature.

Lopes *et al.* (2008) recommended that iron values be above  $70 \text{ mg/L}^{-1}$  which was not achieved by any of the substrates. Iron is important for electron transfer during photosynthesis and for the biosynthesis of metalloporphyrin, which impacts the formation of chlorophyll and some enzymes; therefore, iron deficiency results in slow plant growth and decreased productivity (NAVAS; NUNES; VASCONCELLOS JUNIOR, 2016). For manganese, the literature suggests levels between  $0.3$  and  $3.0 \text{ mg/L}^{-1}$  (LOPES *et al.*, 2008), which were achieved in treatments T1 and T3, whereas those in treatments T2 and T4 were above the recommended level. Excess manganese is potentially phytotoxic, limiting plant growth, distorting the leaf blade, inducing necrosis in the leaves and stem, and causing leaf tip burn (MAIA, 2019). With respect to zinc, Lopes *et al.* (2008) recommended values between  $0.3$  and  $3.0 \text{ mg/L}^{-1}$  which were not reached by any of the treatments, although those of T2 and T4 were similar ( $0.25$  and  $0.27 \text{ mg/L}^{-1}$ , respectively).

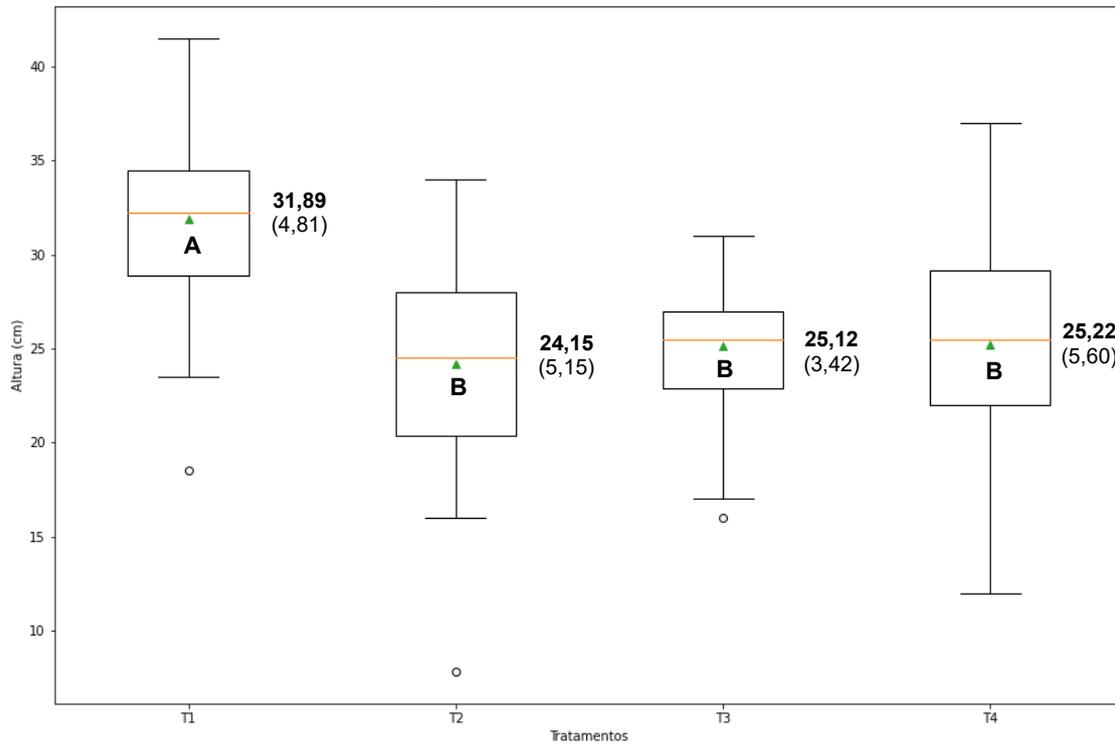
In terms of emergence percentage, the control treatment resulted in the highest value, with a value of  $96.59\%$ . The T4 treatment resulted in  $65.90\%$  yield, and the T2 treatment

resulted in  $60.22\%$  yield. The worst result,  $36.07\%$ , was obtained with treatment T3. Although the emergence percentage in the treatments containing SMS was considerably lower than that in the control treatment, these still obtained superior results to those of some authors. Da Ros *et al.* (2015) evaluated the emergence percentage of *E. grandis* sown in different organic substrates and reported averages of  $43.8$  to  $78.1\%$ , with the control treatment resulting in  $50\%$  emergence. The authors explained that the physical characteristics of the substrates affect the germination percentage, with density affecting oxygen availability, which compromises seed germination, and that the water absorption capacity of the seeds is influenced by water retention and substrate aeration, which are affected by substrate porosity, especially in seedling production systems in small containers. In the present experiment, the highest emergence percentages were obtained for substrates T1 and T4, which had greater numbers of micropores and retention capacities.

Although the results obtained in the SMS treatments were superior to those obtained by some authors, they are still lower than those reported in other studies. Melo *et al.* (2014) evaluated the emergence percentage of *E. grandis* in different substrates (based on cattle manure, carbonized rice husk, coconut fiber, and vermiculite, in different proportions), obtaining values close to  $100\%$  in all substrates. In view of the above, it is necessary to investigate the physical and chemical factors that influence the emergence percentage of *E. grandis* seeds in SMS-based substrates.

Figure 1 shows the average height of *E. grandis* in the different treatments.

**Figure 1.** Effect of substrate on the height (cm) of *Eucalyptus grandis* seedlings. The standard deviations of the means are in parentheses.



T1 = 100% Carolina Soil II, T2 = 25% depleted *Lentinula* substrate T3 = 50% exhausted substrate of *L. edodes* + 75% carbonized rice husk, T4 = 75% exhausted substrate of *L. edodes* + 50% carbonized rice husk, T5 = 75% exhausted substrate of *L. edodes* + 25% carbonized rice husk. Means preceded by the same letters do not differ statistically by Tukey's test at the 5% probability level ( $p < 0.05$ ).

The average height in the control treatment was 31.89 cm, which was significantly greater than that in the other treatments. Treatments T2, T3, and T4 had average heights of 24.15, 25.12, and 25.22 cm, respectively, which did not significantly differ from each other. Although the control treatment was statistically superior to the other treatments, all the treatments reached the height recommended by Gomes *et al.* (2003), who reported that seedlings with heights between 20 and 35 cm are considered suitable for planting. Furthermore, Wendling *et al.* (2021) considered the ideal planting height to be between 15 and 25 cm.

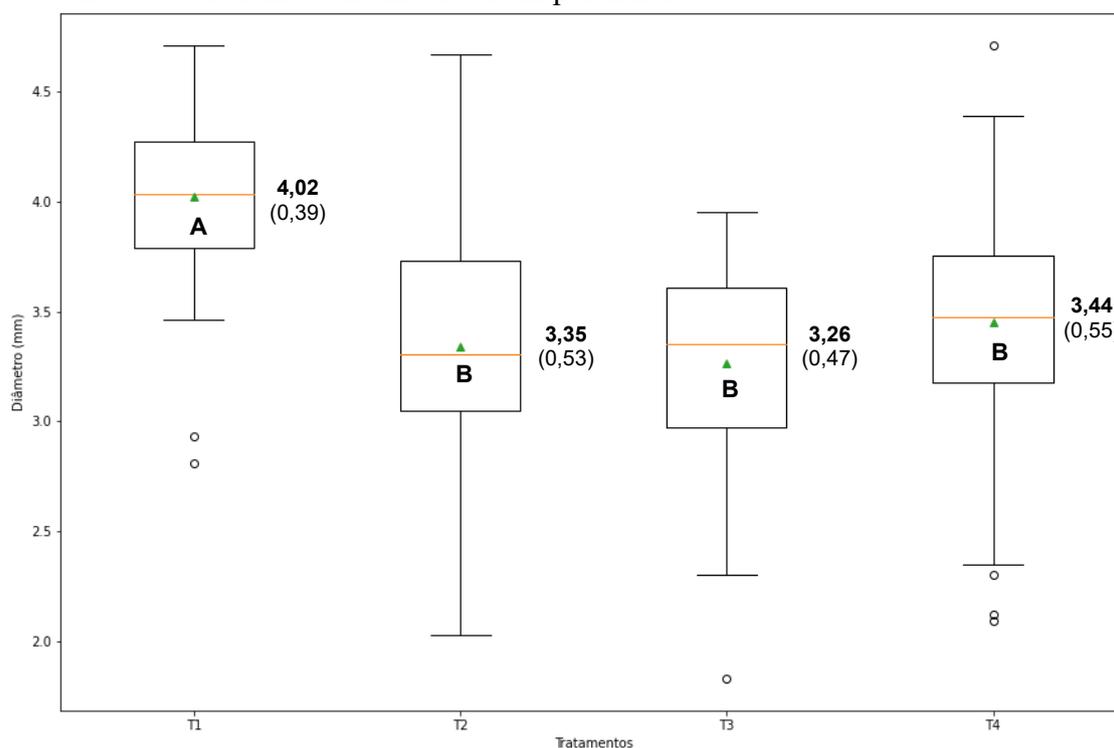
The values obtained are in agreement with those reported by other authors who evaluated different substrates for eucalyptus cultivation. Toledo *et al.* (2015) used compost based on paper and cellulose manufacturing waste in different proportions for the cultivation of *E. urograndis* and reported

average heights between 18.73 and 37.31 cm. Silva *et al.* (2018) evaluated the viability of sewage sludge and peach palm husk in different proportions for the cultivation of *E. urograndis* and reported averages between 19.3 and 36.3 cm. Similarly, Lobo, Oliveira and Silva (2020) used sewage sludge in different proportions for the cultivation of a hybrid clone of *E. grandis* × *E. urophylla*, reaching average heights between 21.87 and 31.62 cm.

Figure *et al.* (2019) evaluated the correlation between different morphological parameters of eucalyptus seedlings in nurseries and their survival in the field at 3, 6, 12, and 24 months and reported a significant correlation between seedling height and survival in the months evaluated. Therefore, seedling height is an important indicator of plant success.

Figure 2 shows the average neck diameter of *E. grandis* in the different treatments.

**Figure 2.** Effect of substrate on the stem diameter (mm) of *Eucalyptus grandis* seedlings. The standard deviations of the means are in parentheses.



T1 = 100% Carolina Soil II, T2 = 25% depleted *Lentinula* substrate T3 = 50% exhausted substrate of *L. edodes* + 75% carbonized rice husk, T4 = 75% exhausted substrate of *L. edodes* + 50% carbonized rice husk, T5 = 75% exhausted substrate of *L. edodes* + 25% carbonized rice husk. Means preceded by the same letters do not differ statistically by Tukey's test at the 5% probability level ( $p < 0.05$ ).

In terms of height, the control treatment resulted in a statistically greater average diameter than did the other treatments, with a value of 4.02 mm. Treatments T2, T3, and T4 resulted in averages of 3.35, 3.26, and 3.44 mm, respectively, which did not significantly differ from each other. All the treatments achieved a considerable diameter that was viable for planting ( $> 2$  mm) according to the Wendling method. *et al.* (2021).

The results obtained are consistent with, and in some cases superior to, those reported by authors who evaluated organic waste for eucalyptus production. Toledo *et al.* (2015) reported average diameters between 2.50 and 3.41 mm for *E. urograndis* grown in substrates supplemented with organic compost based on waste from paper and pulp manufacturing. In the study by Silva *et al.* (2018), average diameters between 2.1 and 3.0 mm were obtained for *E. urograndis* grown

in substrates based on sewage sludge and peach palm bark. Lobo, Oliveira and Silva (2020) reported average diameters between 2.0 and 2.7 mm for a hybrid of *E. grandis* x *E. urophylla* in substrates supplemented with sewage sludge. Lower values were reported by Silva *et al.* (2014) for *E. grandis* grown in alternative substrates, with averages between 1.51 and 1.90 mm.

There was no significant difference in the number of leaves among the treatments, with averages of 55.22, 55.39, 57.16, and 63.62 for treatments T1, T2, T3, and T4, respectively. This result is positive because, according to Figueiredo *et al.* (2019), the number of leaves correlated positively with the growth of eucalyptus seedlings after planting.

Table 4 shows the average values for the shoot, root, and total dry mass of *E. grandis* in the different substrates.

**Table 4.** Shoot dry mass, root dry mass, and total dry mass of *Eucalyptus grandis* in the different treatments. The standard deviations of the means are in parentheses.

Treatment	Mass aerial part	root mass	Total mass
T1	3.25 (0.77) A	0.92 (0.2) A	4.17 (0.88) A
T2	1.97 (0.81) B	0.61 (0.23) C	2.58 (1.03) B
T3	2.26 (0.67) B	0.68 (0.18) BC	2.94 (0.81) B
T4	2.27 (0.89) B	0.77 (0.25) B	3.04 (1.11) B

T1 = 100% Carolina Soil II, T2 = 25% depleted *Lentinula substrate* T3 = 50% exhausted substrate of *L. edodes* + 75% carbonized rice husk, T4 = 75% exhausted substrate of *L. edodes* + 50% carbonized rice husk, T5 = 75% exhausted substrate of *L. edodes* + 25% carbonized rice husk. Means followed by the same letter in the same column do not differ statistically according to Tukey's test at the 5% probability level ( $p < 0.05$ ).

The control treatment resulted in the highest values for shoot and total dry mass (3.25 and 4.17 g, respectively), which differed statistically from those of the other treatments. The T2, T3, and T4 treatments resulted in shoot dry masses of 1.97, 2.26, and 2.27 g, respectively, and total masses of 2.58, 2.94, and 3.04 g, respectively, which did not significantly differ from each other. In terms of root dry mass, the control treatment resulted in a greater mass than did the other treatments, differing significantly from them, while treatment T4 did not differ from T3 but differed from T1 and T2. Treatment T3 did not differ from T4 or T2 but differed from T1.

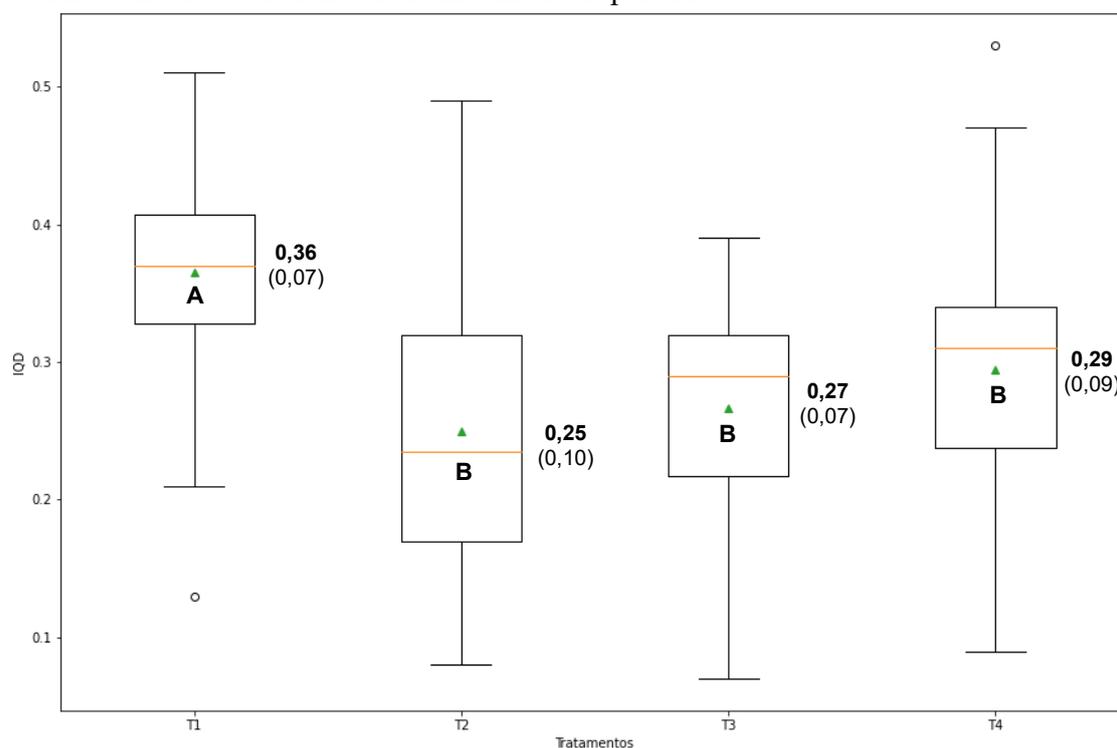
Compared with those of other authors, the values obtained are higher than those described by Silva *et al.* (2018), who reported shoot dry mass values between 0.58 and 0.97 g and root dry mass values between 0.27 and 0.48 g. However, the height and diameter values reported by these authors are lower than those reported in the present study, which impacts these values. Similarly, the values described by Toledo *et al.* (2015) were lower, between 0.82 and 1.52 g for the shoot and between 0.47 and

0.78 g for the root, although the highest values were obtained in the treatments with heights and diameters similar to those of the present experiment. Higher values were obtained by Lobo, Oliveira and Silva (2020), although the height values were similar to those of the present experiment, with a lower diameter. The authors obtained shoot dry masses between 3.04 and 6.76 g and root dry masses between 1.36 and 4.32 g, resulting in total masses between 4.40 and 11.08 g.

With respect to the relationships between dry mass variables and other morphological variables, Silva *et al.* (2021) reported a significant positive correlation between shoot and root mass with respect to height, diameter, the Dickson quality index, and optimal root system conformation, as well as a significant negative correlation between shoot and root mass with respect to poor root system conformation in seedlings. Therefore, height, diameter, and root quality influence the obtained mass values, which in turn influence the Dickson quality index.

Figure 3 shows the Dickson quality index for *E. grandis* in the different treatments.

**Figure 3.** Effect of substrate on the Dickson quality index (DQI) of *Eucalyptus grandis* seedlings. The standard deviations of the means are in parentheses.



T1 = 100% Carolina Soil II, T2 = 25% depleted *Lentinula* substrate T3 = 50% exhausted substrate of *L. edodes* + 75% carbonized rice husk, T4 = 75% exhausted substrate of *L. edodes* + 50% carbonized rice husk, T5 = 75% exhausted substrate of *L. edodes* + 25% carbonized rice husk. Means preceded by the same letters do not differ statistically by Tukey's test at the 5% probability level ( $p < 0.05$ ).

The control treatment resulted in the highest IQD value, 0.36, which differed statistically from the other treatments. Treatments T2, T3, and T4 resulted in IQD values of 0.25, 0.27, and 0.29, respectively, which did not significantly differ from each other. The increase in the proportion of SMS in these treatments caused a gradual increase in IQD. Although the control treatment differed from the other treatments, all the treatments exceeded the minimum IQD value of 0.20 recommended by Gomes and Paiva (2004). The values obtained are above those described by Silva *et al.* (2014), who reported that the use of alternative organic residues in the cultivation of *E. grandis* negatively affected the IQD, resulting in values between 0.032 and 0.056. Similarly, Kratz and Wendling (2013) obtained IQD values between 0.01 and 0.06 for *E. dunnii* grown on different substrates, considering values outside the ideal range.

Toledo *et al.* (2015), although they obtained diameter and height values similar to those of the present experiment, they reported

lower mass values, resulting in an IQD between 0.13 and 0.17. Lobo, Oliveira and Silva (2020) reported a positive effect of substrate supplementation with organic residue, obtaining IQD values between 0.33 and 0.84, with the lowest value in the control treatment, and the IQD increased proportionally to the amount of supplementation in the substrate. The values reported by these authors were greater than those reported in the present study, except for those reported in the control treatment.

As observed by Silva *et al.* (2021), the IQD is significantly positively correlated with height, diameter, shoot dry mass, root dry mass, and optimal root system conformation, whereas a significant negative correlation is observed between the IQD and poor root system conformation.

The root system of all the seedlings in the control treatment was considered suitable for planting, with 82% of the seedlings classified as "excellent" and 18% as "good". In treatment T2, a greater number of seedlings

with a root system were considered "poor", with 15% of the seedlings in this classification; 43% were classified as "excellent", and 42% were classified as "good". In treatment T3, only 2% of the seedlings were classified as having a "poor" root system, with 75% of the remaining seedlings classified as "excellent" and 23% classified as "good". For treatment T4, 4% of the seedlings presented a "poor" root system, 58% presented "excellent" and 38% presented "good" root system. The latter presented a greater substrate density, which may have hindered root development. According to Wendling *et al.* (2021), one of the parameters for a seedling to be considered suitable for planting is the quality of the root system, which must occupy the entire internal area of the tube, with the presence of new roots. In this context, seedlings with an "optimal" or "good" root system are considered suitable for planting. Similar results were obtained by Gabira (2018) in seedlings of a hybrid clone of *E. grandis* × *E. urophylla* grown in a substrate based on sewage sludge and sugarcane bagasse.

With respect to nutrient accumulation in the aerial part, macroporosity and total porosity were positively correlated with potassium and negatively correlated with magnesium and

copper. Microporosity and retention were negatively correlated with potassium and positively correlated with copper. Density was positively correlated with only zinc. pH was negatively correlated with manganese and zinc. The electrical conductivity was positively correlated with only the calcium content. In the root system, macroporosity and total porosity were negatively correlated with magnesium, but no correlation was found with copper, as in the aerial part. Microporosity and retention were negatively correlated only with iron. Density was positively correlated with boron and zinc. pH was negatively correlated with nitrogen, boron, and zinc. The electrical conductivity, as in the aerial part, was positively correlated with the calcium content. A difference in nutrient accumulation in the aerial part and in the root system was also reported by Gabira (2018) when the correlations between nutrient accumulation and the physical and chemical characteristics of substrates in the cultivation of *E. grandis* × *E. urophylla* clones were evaluated.

Table 5 shows the volume, pH, and EC of the fertigation leachates from the growth and hardening treatments.

**Table 5.** Volume, pH, and electrical conductivity of the leachate solution after fertigation for growth and hardening of *Eucalyptus grandis* seedlings in the different treatments.

Fertigation for growth				
Treatment	Average (mL)	volume	pH	EC (mS)
T1	93		6.58	0.64
T2	100		6.57	2.0
T3	175		6.34	1.1
T4	198		6.32	1.3
Fertigation for hardening				
Treatment	Average (mL)	volume	pH	EC (mS)
T1	139		6.71	0.71
T2	160		6.53	0.58
T3	183		6.58	0.69
T4	205		6.88	0.59

T1 = 100% Carolina Soil II, T2 = 25% depleted *Lentinula* substrate T3 = 50% exhausted substrate of *L. edodes* + 75% carbonized rice husk, T4 = 75% exhausted substrate of *L. edodes* + 50% carbonized rice husk, T5 = 75% exhausted substrate of *L. edodes* + 25% carbonized rice husk.

The lowest volume and electrical conductivity were obtained in the control

treatment, possibly because of its greater retention. The use of SMS resulted in a larger

volume of leachate, with treatment T2 resulting in the highest EC value, which may mean that this substrate was not as efficient in nutrient absorption. Similar to the growth fertigation analysis, the use of SMS resulted in a larger volume of leachate, whereas the control treatment resulted in the smallest volume, possibly due to its retention. The pH and electrical conductivity of all the treatments during the hardening-off stage were similar, indicating similar potassium absorption by the seedlings on the different substrates.

Considering the leachate volume and electrical conductivity results along with the physical characterization of the substrates, SMS (solid waste material) combined with carbonized rice husk does not provide adequate micropore content, affecting the retention capacity of the substrate. Therefore, the viability of this material in conjunction with another residue with a relatively high micropore content should be investigated, as should different water management and fertilization practices.

#### 4 CONCLUSIONS

The use of exhausted mushroom substrate (spent mushroom substrate (SMS)) *Lentinula* in different proportions in the formulation of substrates for the cultivation of *Eucalyptus grandis* seedlings affected the physical and chemical characteristics of the substrates.

The emergence percentage was negatively affected by the use of SMS. The height, stem diameter, and Dickson quality index of the seedlings, although lower than those with conventional substrate, obtained values considered adequate for planting. The root system conformation of some seedlings was negatively affected by SMS.

The accumulation of nutrients and their correlations with the physical and chemical characteristics of the substrates differed in the aerial part of the plant and in the root system. In the leachate solution, greater water and nutrient loss was observed in the SMS treatments than in the other treatments due to the lower retention capacity of these nutrients.

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