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# Use of Eucalyptus Charcoal Waste in the Formulation of Substrate for the Cultivation of Two Strains (LED 20/11 and LED 20/12) of *Lentinula edodes*

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**Abstract:** The shiitake mushroom (*Lentinula edodes*) is globally valued for its nutrition and medicinal properties. New technologies aim to increase production with less environmental impact, considering materials such as charcoal for substrate enrichment. This manuscript evaluated the effect of fine charcoal (FC) on the substrate formulation of two *L. edodes* strains (LED 20/11 and LED 20/12). The substrate consisted of 72% eucalyptus sawdust, 12.5% rice bran, 12.5% wheat bran, and 3% calcium carbonate (control treatment without charcoal). Treatments with FC proportionally reduced the use of sawdust, with doses of 1%, 2%, 4%, 8%, and 16% (relative to the substrate material). Yield, mushroom number, and mushroom weight were evaluated. The concentration of FC significantly affected the parameters analyzed, especially at the 4% dose. A negative correlation between mushroom number and weight was observed. For yield, the control treatment and the lowest dose of FC (1%) had the highest yields for the first harvest. Strain LED 20/12 showed lower yield variability due to the percentage of FC applied to the substrate. The incorporation of FC into the substrate for shiitake cultivation demonstrates efficacy; however, both the concentration and strain used are limiting factors for its applicability.

**Keywords:** shiitake; quality screening of mushroom; substrate formulation; strains; yield



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## 1. Introduction

The shiitake mushroom (*Lentinula edodes*) is widely recognized as a fungus of significant food and medicinal importance, ranking second among the most cultivated mushrooms globally [1,2]. This prominence is largely due to the abundance of health-beneficial biomolecules present in its composition, making it not only a nutritious food but also a functional food with a significant role in promoting health [3,4]. This mushroom has a long history of use in culinary and traditional medicine, especially in Asian countries where it is highly valued [5,6].

Since ancient times, mushroom production has been carried out using wood from forest species. Several records are found in the literature on the cultivation of shiitake (*Lentina edodes*) on oak (*Quercus* spp.), beech (*Fagus* spp.), pine (*Pinus* spp.), dalbergia (*Dalbergia* spp.), hestnut (*Castanopsis* spp.), or shii (*Pasania* spp.) trees (from which the mushroom derives its name). In the last decades, native forest species were replaced by exotic forest species, the main one being eucalyptus [7]. However, mushroom cultivation technologies have developed and wood cultivation was quickly replaced by sawdust substrate cultivation.

Sawdust substrate cultivation has several advantages, among which are shorter production time, constancy of crops yield, uniformity between flushes, greater biological

efficiency, and the possibility of using various forest and agricultural wastes as a constituent for a substrate formulation. Some biomass residues have been used at commercial levels (straws, sugarcane bagasse, etc.), but the most expressive is sawdust [8]. For the formulation of the substrate, sawdust is mixed with bran (wheat, rice, and corn), lime, and gypsum [9].

The bran plays a fundamental role in manipulating the carbon/nitrogen (C/N) ratio of the substrate, aiming to achieve ideal values between 70-40/1 [10]. Limestone is added to maintain the substrate pH within the optimal range between 5.5 and 6.5, providing favorable conditions for fungal growth. Gypsum, on the other hand, serves multiple functions by supplying calcium and sulfur essential for the fungus metabolism, while also contributing to substrate moisture reduction and increased aeration, creating a conducive environment for healthy mushroom development [11]. The selection of the ideal substrate for shiitake production takes into consideration not only economic cost but also the ability to provide high yields, considering qualitative aspects such as the number and size of harvested mushrooms.

Recently, several materials (beet pulp, corn cobs, sunflower seed husks, barley, composted fir bark, brewer's yeast, powdered whey, coconut pith, maguey tequila bagasse, peanut shells, and spent mushroom substrate) have been incorporated into substrate formulations as strategies to achieve these objectives [12–19]. In Brazil, producers have been incorporating small amounts of fine charcoal (FC) into their substrate formulations [20], aiming to reduce substrate production costs and provide macro (K and Ca) and micronutrients (Na) [21]. This practice has shown promise, not only in terms of cost savings but also in improving substrate quality and consequently mushroom performance. The addition of FC has been associated with increased moisture retention in the substrate, providing an ideal environment for mycelial growth and fruiting body formation. Therefore, the inclusion of FC in substrate formulations represents a promising strategy for mushroom producers, offering benefits both economically and productively.

FC is a large-scale residue generated after the combustion of eucalyptus wood in a closed reactor, with an absence of oxygen, and exhibits particle sizes ranging from powder to fragments of 4 mm. Fine charcoal or FC is a byproduct that poses a problem to the charcoal production chain, and its disposal remains one of the main challenges to be addressed [22,23]. Its chemical composition comprises 1.91 to 4.30% ash, 9.46 to 13.31% volatile matter, 96.38 to 91.65% carbon, 0.33 to 0.53% nitrogen, < 0.05% sulfur, and 2.30 to 2.42% hydrogen [24]. Viotto et al. [20] reported that the use of FC assists in moisture retention and oxygenation of the *L. edodes* substrate. The authors did not mention the amount of charcoal powder used in the substrate formulation.

In this sense, this manuscript aimed to evaluate different doses of FC in the production of *L. edodes* applied to the axenic substrate. In addition to the quality control of the substrate formulation, mycological studies suggest the importance of using more than one mushroom strain in order to reduce the variation of physiological responses [25,26]. So, productive parameters in two strains were also evaluated to provide greater reliability in the results obtained.

## 2. Materials and Methods

The experiment was carried out at the Centro de Estudos em Cogumelos, in Faculdade de Ciências Agrárias e Tecnológicas (FCAT/UNESP). A completely randomized block design was adopted in a 2 × 6 factorial scheme, with 10 replications (n = 120). The first factor corresponds to two strains of *L. edodes* (LED 20/11 and LED 20/12) and the second to different concentrations of fine charcoal (FC) added to the substrate formulation.

The ingredients used for substrate preparation were separated and weighed according to the following percentages: 72% eucalyptus sawdust, 12.5% rice bran, 12.5% wheat bran, and 3% calcium carbonate ($CaCO_3$). The treatments that had the addition of FC suffered a proportional reduction in sawdust, according to the following doses: 0%, 1%, 2%, 4%, 8%, and 16%, i.e., for the major dose the substrate was composed of 56% eucalyptus sawdust, 16% FC, 12.5% rice bran, 12.5% wheat bran, and 3% calcium carbonate ($CaCO_3$). Then,

the materials were mixed until becoming homogeneous; subsequently, the substrate was gradually moistened until reaching 62% moisture, and then it was deposited in 2 kg plastic bags and subsequently pressed in a hydraulic press. Next, they were autoclaved for 4 h at 121 °C. The chemical characteristics of the substrates used, the sawdust, and the fine charcoal are presented in Table 1.

**Table 1.** Chemical properties of substrate for *Lentinula edodes* production with varying concentrations of fine charcoal added to the substrate, as well as the materials used in substrate production.

| Substrate | N | $P_2O_5$ | K | Ca | Mg | S | O.M. | O.C. | Na | Cu | Fe | Mn | Zn | C/N Ratio | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | % | | | | | | mg/kg | | | | |
| 0% | 0.89 | 1.03 | 0.39 | 0.80 | 0.34 | 0.04 | 87.43 | 49.44 | 766.21 | 5.89 | 402.75 | 117.88 | 39.29 | 55/1 | 5.52 |
| 1% | 0.75 | 1.06 | 0.36 | 0.81 | 0.32 | 0.04 | 89.17 | 50.00 | 774.00 | 3.96 | 356.68 | 122.86 | 33.69 | 66/1 | 5.42 |
| 2% | 0.84 | 1.23 | 0.38 | 0.84 | 0.35 | 0.04 | 85.55 | 48.33 | 797.33 | 3.93 | 424.80 | 129.80 | 37.37 | 57/1 | 5.42 |
| 4% | 1.05 | 1.19 | 0.38 | 0.80 | 0.34 | 0.04 | 87.59 | 49.44 | 810.97 | 5.91 | 429.10 | 135.82 | 37.40 | 47/1 | 5.44 |
| 8% | 0.79 | 1.07 | 0.37 | 0.83 | 0.35 | 0.05 | 85.90 | 48.33 | 884.36 | 3.95 | 404.80 | 138.22 | 33.57 | 61/1 | 5.41 |
| 16% | 1.30 | 1.04 | 0.38 | 1.38 | 0.38 | 0.05 | 85.07 | 47.78 | 1081.33 | 1.98 | 401.60 | 168.16 | 35.61 | 36/1 | 5.35 |
| Sawdust * | 0.23 | 0.007 | 0.97 | 1.36 | 0.057 | - | - | 55.18 | 670 | 1.82 | - | - | 14.62 | 238/1 | 5.40 |
| Fine charcoal * | 0.21 | 0.025 | 2.32 | 1.83 | 0.067 | - | - | 55.18 | 7568 | 1.94 | - | - | 8.38 | 262/1 | 7.40 |

* Source Brandão et al. (2003) [21].

The FC came from a commercial company that used eucalyptus wood (Carvão Explosão®, Junqueirópolis, São Paulo State, Brazil). The company had 35 ovens and the FC was removed every 2 or 3 days. The particle size of this material ranged from powder to 3 mm.

The spawn was produced in a sterile substrate, with all preparation and sterilization procedures performed according to Zied et al. [27]. Starting from the primary matrix, where fragments of mushrooms isolated from producers were added to a Petri dish with potato dextrose agar (PDA) culture medium, at the end of colonization, fragments of the culture medium with fungus were used to prepare more plates, this being the secondary matrix stage, which at the end of its total colonization, fragments were added to glass jars with a diameter of 8.6 cm and a height of 12.8 cm containing the same substrate formulation (without the addition of fine charcoal). Finally, after the total colonization of the substrate, the colonized substrate referring to the tertiary matrix was used for inoculum preparation. In all stages, incubation occurred at 26 ± 2 °C. It is worth noting that all steps were performed in a laminar flow chamber.

The bags with the substrates with and without FC were inoculated at 1.5% (*w*/*w*) in a laminar flow chamber and then incubated at 26 ± 2 °C for 80 days. The colonization period was divided into two stages, the spawn run lasting 25 days and the browning period lasting 55 days. Two strains of *L. edodes* were used, (i) LED 20/11, isolated from growers in the region of Juquitiba, São Paulo State, Brazil, and (ii) LED 20/12, isolated from growers in the region of Salto, São Paulo State, Brazil. The strains are currently deposited in the public collection of FCAT/UNESP, Câmpus Dracena.

After the browning (maturation), the bags were opened, and the blocks/substrates were washed in running water. Then, the substrate was taken to the cultivation chamber at a temperature of 20 ± 2 °C, a relative humidity of 90%, a $CO_2$ level < 1000 ppm, and a luminosity of 150–200 lux, with two irrigations a day. At the end of the production flushes, the substrate underwent water shock, where they were submerged in a tank with water for 8 h. Harvesting was performed manually 8 days after removing the blocks from the immersion. The total crop time was 160 days, and 4 flushes were harvested.

The agronomic behavior was evaluated through (i) yield, calculated through the fresh weight of mushrooms (g) divided by the fresh weight of substrate (g) ×100, expressed in a percentage; (ii) mushroom number, expressed in the unit(s) per bag; and (iii) weight of the mushrooms, calculated through the total fresh weight divided by the number of mushrooms, expressed in grams.

Through the software Sisvar® v. 5.6 (Universidade Federal de Lavras, Departamento de Estatística, Lavras, MG, Brazil), the data were submitted to analysis of variance and, when significant, the means were compared with each other using ScottKnot's test at 5% probability. For correlation analysis, the SAS statistical software, version 9.2, SAS Institute Inc., Cary, NC, USA) was used.

## 3. Results

According to Table 2, the strains factor significantly affected the number and weight of mushrooms. On the other hand, the yield was not significantly affected. In the 3rd and 4th harvest flush, the LED 20/12 strain provided a higher number of mushrooms than the LED 20/11 strain. For both strains, the highest number of mushrooms were harvested in the 4th flush and the lowest in the 2nd flush. A negative correlation was observed between the number of mushrooms and the weight of mushrooms, that is, flushes with a large number of mushrooms but with a smaller weight (Figure 1).

**Table 2.** *Lentinula edodes* productive parameters using two strains.

| Strain * | 1st Flush | 2nd Flush | 3rd Flush | 4th Flush |
|---|---|---|---|---|
| | Number of Mushrooms, u | | | |
| LED 20/11 | 5.5 ± 8.0 B | 2.9 ± 3.70 C | 5.1 ± 2.95 b B | 8.8 ± 2.79 b A |
| LED 20/12 | 7.7 ± 4.11 C | 3.3 ± 2.62 D | 11.0 ± 2.49 a B | 14.7 ± 3.41 a A |
| | Weight of mushroom, g | | | |
| LED 20/11 | 44.9 ± 13.95 A | 34.9 ± 21.31 B | 31.9 ± 14.37 a B | 15.9 ± 6.75 a C |
| LED 20/12 | 36.4 ± 43.46 A | 35.6 ± 16.07 A | 16.5 ± 7.58 b B | 9.9 ± 4.68 b B |
| | Yield, % | | | |
| LED 20/11 | 12.5 ± 3.91 A | 5.2 ± 2.11 C | 8.2 ± 3.79 B | 7.0 ± 5.17 B |
| LED 20/12 | 14.1 ± 10.91 A | 5.9 ± 2.94 C | 9.1 ± 6.92 B | 7.3 ± 8.43 C |

* Values for each lineage refer to the averages of all coal mill dosages. Means are followed by ± the standard deviation. Lowercase letters compare results in the same column and capital letters in the same line, for each parameter analyzed. Absence of letters indicates no statistical difference according to ScottKnot's test at 5% probability.

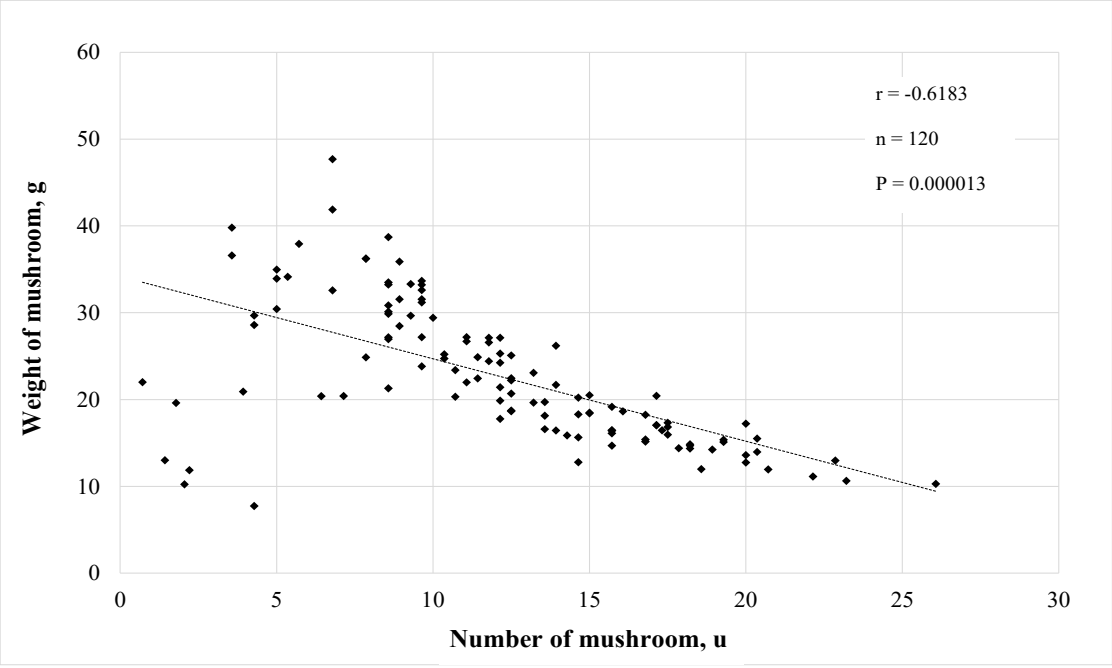


**Figure 1.** Correlation between weight and number of mushrooms, values referring to all experimental units.

The mushroom weight was influenced only in the last two flushes by the strains used, and LED 20/12 shows a significant reduction, compared to the 20/11 strain. However, both strains showed a higher weight/size of mushrooms in the 1st flush. A negative correlation was observed between the mushroom weight and the harvest time (Figure 2).

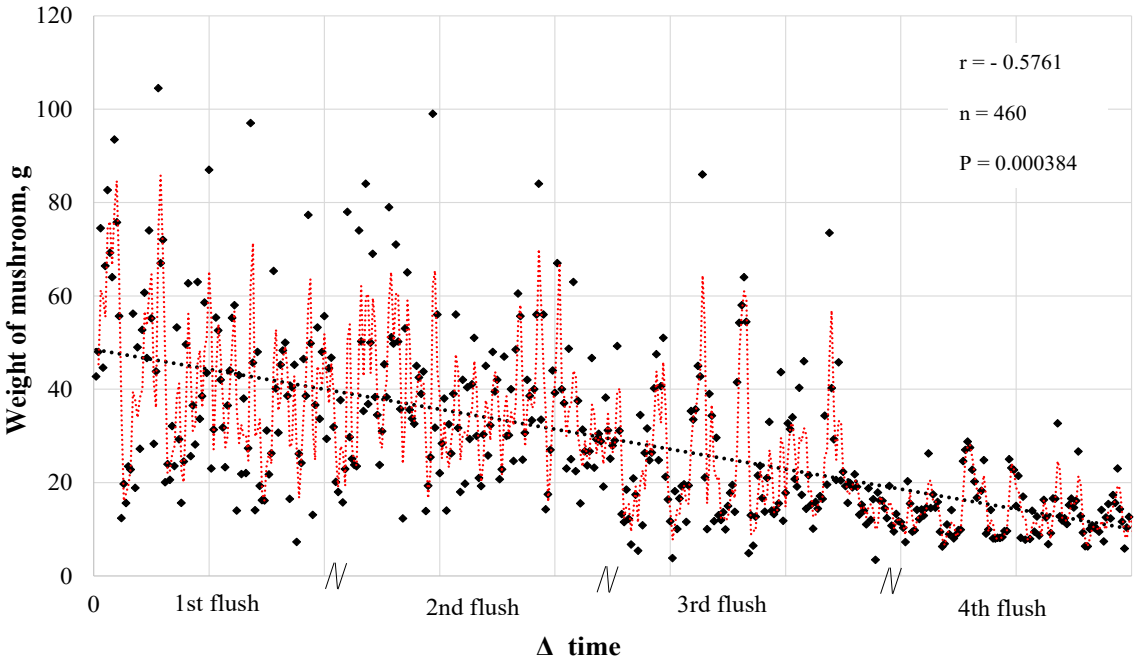


**Figure 2.** Correlation between weight of mushroom and production flushes, values referring to all experimental units (n = 120) in each harvest flush, with a total of 460 units.

According to Table 3, all mushroom parameters analyzed were significantly affected by the FC percentage factor. The lowest number of mushrooms obtained in the 3rd flush corresponds to the doses of 2 and 4% FC. Comparing harvested flushes, the 2nd flush presented the lowest number of mushrooms and consequently the lowest yield. This indicates that studies should be conducted aiming to improve yield in the 2nd flush, such as nutritional supplementation or an increase in the mycelial growth recovery interval between the 1st and 2nd flush.

**Table 3.** *Lentinula edodes* productive parameters using different fine charcoal percentages.

| Dose, % * | 1st Flush | 2nd Flush | 3rd Flush | 4th Flush |
|---|---|---|---|---|
| | Number of Mushroom, u | | | |
| 0 | 6.9 ± 11.24 B | 3.1 ± 2.88 B | 9.1 ± 10.54 a A | 11.7 ± 6.03 A |
| 1 | 6.8 ± 5.37 B | 2.8 ± 1.66 C | 7.1 ± 3.92 a B | 11.4 ± 5.42 A |
| 2 | 4.8 ± 4.74 B | 2.9 ± 1.31 B | 6.5 ± 7.10 b A | 12.0 ± 9.65 A |
| 4 | 4.9 ± 7.32 B | 2.8 ± 1.59 B | 4.5 ± 4.37 b B | 12.5 ± 5.71 A |
| 8 | 6.4 ± 14.07 B | 4.0 ± 2.27 B | 7.8 ± 4.30 a B | 11.5 ± 9.36 A |
| 16 | 6.3 ± 6.86 B | 3.3 ± 4.49 C | 7.9 ± 3.11 a B | 10.9 ± 6.65 A |
| | Weight of mushroom, g | | | |
| 0 | 48.1 ± 21.77 A | 37.0 ± 24.0 b A | 23.0 ± 10.48 B | 12.8 ± 4.15 a B |
| 1 | 44.8 ± 19.36 A | 50.1 ± 17.85 a A | 24.0 ± 10.96 B | 13.5 ± 3.05 a B |
| 2 | 38.6 ± 19.98 A | 28.3 ± 19.49 b B | 26.9 ± 13.32 B | 11.3 ± 8.89 b C |
| 4 | 32.9 ± 24.84 A | 25.2 ± 11.16 b A | 24.0 ± 20.39 A | 8.3 ± 7.95 b B |
| 8 | 47.9 ± 16.01 A | 37.8 ± 17.79 b A | 22.7 ± 12.57 B | 14.7 ± 8.55 a B |
| 16 | 31.8 ± 15.14 A | 33.4 ± 18.04 b A | 24.6 ± 17.43 A | 13.1 ± 4.67 a B |

**Table 3.** *Cont.*

| Dose, % * | 1st Flush | 2nd Flush | 3rd Flush | 4th Flush |
|---|---|---|---|---|
| | Number of Mushroom, u | | | |
| | Yield, % | | | |
| 0 | 16.5 ± 4.93 a A | 5.7 ± 4.37 a C | 10.5 ± 2.59 a B | 7.5 ± 2.71 C |
| 1 | 15.3 ± 6.07 a A | 7.0 ± 3.54 a B | 8.5 ± 2.59 a B | 7.7 ± 2.7 B |
| 2 | 9.4 ± 5.55 b A | 4.1 ± 2.13 b B | 8.7 ± 3.51 a A | 6.8 ± 3.33 A |
| 4 | 10.6 ± 5.95 b A | 3.6 ± 2.21 b C | 5.4 ± 2.55 b B | 5.2 ± 3.37 B |
| 8 | 12.1 ± 4.24 b A | 7.5 ± 2.09 a B | 9.0 ± 1.99 a B | 8.5 ± 2.90 B |
| 16 | 12.8 ± 6.43 b A | 5.6 ± 3.36 a C | 9.7 ± 1.85 a B | 7.2 ± 3.84 C |

* Referring to the average of the two strains. Means are followed by ± the standard deviation. Lowercase letters compare results in the same column and capital letters in the same line, for each parameter analyzed. Absence of letters indicates no statistical difference according to ScottKnot's test at 5% probability.

The total yield obtained in the present manuscript was high, with values ranging from 38.7 to 41.5% in treatments without the addition of FC and 16.3 to 43.8% in treatments that received different percentages of FC (Figure 3). The LED 20/12 strain showed lower yield variability (great plasticity) as a function of the percentage of FC added to the substrate. The greatest variations in yield comparing the strains were verified at the dose of 4%, with a range of 47.1%. On the other hand, the greatest variations in yield comparing the percentage of FC were between the doses of 4 and 8%, with a range of 53.3%.

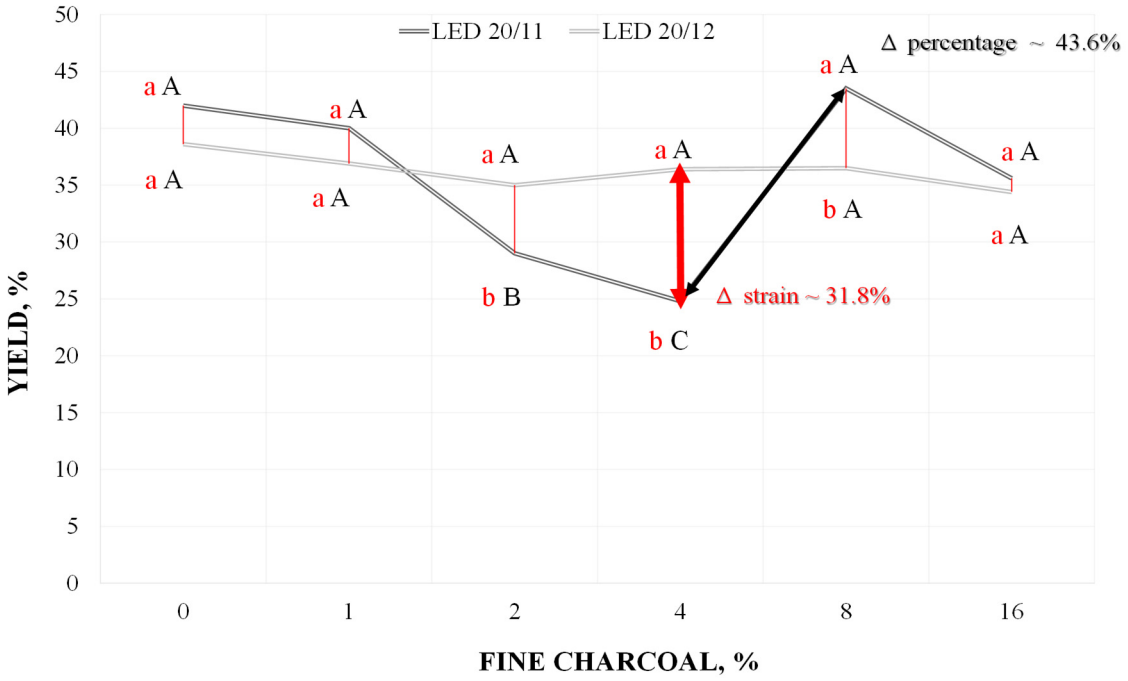


**Figure 3.** Total yield (sum of 1st, 2nd, 3rd, and 4th flush) of the strains with different fine charcoal percentages. Lowercase letters compare results between strains and uppercase between percentages of charcoal using ScottKnott's test at 5% probability. Black arrows mean the greatest difference between the applications of fine charcoal at a dose of 4 to 8%. Red arrows mean the greatest difference between strains at the 4% dose.

## 4. Discussion

The search for mushroom quality is increasing by consumers [28], and the data verified in this manuscript were important, which points to a reduction in the quality of mushrooms in the final harvest flushes. However, it is noted that the 3rd and 4th flushes have an intermediate yield, even higher than the 2nd flush (Table 2). In this sense, mushrooms

of lower size can be sliced or directed to the industry (manufacture of soups, pasta, pate, preserves, etc.). Some strains and new cultivars have been developed to reduce the impacts of biotic and abiotic factors on the production of *L. edodes* [29,30]. Xiong et al. [31] showed that the production in the 1st flush varied between 60–75% of the total three flushes; however, the authors did not mention what happened with the number and weight of mushrooms between the flushes. Few studies have characterized in detail the variability of agronomic parameters throughout the crop, aiming at the selection of strains for possible genetic improvement.

Sousa et al. [32], studying the effect on yield of six strains, found that five of them had the lowest yield in the 2nd flush, similar to the results in this manuscript. The authors obtained a good precocity with the UFLA-LE4 strain, with the total harvest being, practically, in the 1st and 2nd flushes. Atila [8], studying different substrate formulations without additional supplementation with bran (wheat, rice, and others), found the highest yield in the 2nd flush in the substrate with oak sawdust, which seems to indicate that the addition of a material rich in nitrogen favors the production on the 1st flush.

The percentage of FC influenced the mushroom weight differently; the intermediate doses (2 and 4%) were the ones with the lowest mushroom weight. As for the strain factor, the percentage of the FC factor also verified a significant reduction in the mushroom weight in the 4th flush (Figure 2).

The yield results showed that the control treatment (without FC) and the one with the lowest dose (1%) had the highest yields in the 1st flush. In the 2nd flush, the substrate with 1, 8, and 16% FC was equivalent to the control, and in the 3rd flush, the substrate with 1, 2, 8, and 16% FC was also equivalent to the control. In the 4th flush, the addition of FC did not influence significantly the yield.

This can happen due to the presence of certain chemical compounds in FC. Roz et al. [33] details the formation of FC with parallel events, the first one is the formation of partially carbonized material occurring between 120 and 300 °C, and the second one is the formation of tar, methane, and phenolic compounds occurring at 300 and 600 °C due to the breakdown of holocellulose. Pyrolysis takes place at temperatures below 700 °C and involves thermal decomposition of the material [34]. Hilscher et al. [35] emphasize that pyrogenic carbon contains highly aromatic structures, not being readily accessible to microorganisms as an energy source.

Furthermore, the aromatic structure of FC possesses hydrophobic characteristics, which can reduce water penetration into the porous spaces of the substrate [36]. This can promote water drainage to the bottom of the substrate within pockets, leading to anaerobic diseases. In the cultivation of *L. edodes*, the formation of exudates from its metabolism is natural, which typically results in additional liquid accumulation inside the bags. Therefore, the interaction between the FC structure and the metabolic processes of mushrooms can significantly influence the hydrological and microbiological dynamics of the substrate, requiring careful management to ensure optimal cultivation conditions and to prevent issues with opportunistic microorganisms.

The great variability in total yield concerns the use of FC in the production of *L. edodes*. The chemical constitution of FC depends on its structure, the material of origin, and the conditions of its formation, such as temperature and burning time, moisture of the plant material, and availability of oxygen, among others [35]. In the present manuscript, the levels of P, K, Ca, Na, and Mn increased with the application of FC; on the other hand, the content of Zn decreased. Rodriguez and Royse [37] reported that low Mn levels in the substrate favor the production of *Pleurotus eryngii*. Zied et al. [38] verified a negative correlation between *Agaricus subrufescens* yield and the content of K, P, and Mg. Finally, the content of soluble salts directly affects the productivity of mushrooms, increasing the electrical conductivity of the medium [39].

The pressing need to advance innovative technologies aimed at mushroom cultivation manifests as an undeniable requirement, aligned with contemporary dynamics. In this context, the imperative for healthy eating intensifies, driven by the significant growth of

the global population [40,41]. As agricultural and food sectors expand their horizons, the exponential production of waste in considerable volumes is observed. These wastes are often disposed of in landfills or subjected to incineration, resulting in a series of adverse environmental impacts [42,43]. Faced with this complex panorama, it becomes essential to explore innovative solutions that not only meet the increasing demand for food but also effectively address the environmental challenges faced by modern society.

On the other hand, the raw materials traditionally employed in production are becoming scarce due to the intensification of mushroom production [41], particularly concerning *L. edodes*, one of the most cultivated mushrooms worldwide [1,2], resulting in increased prices for these materials, reducing profitability. This highlights the urgency of research into alternative materials aimed at reducing production costs.

In this regard, the utilization of alternative waste, such as FC, emerges as a crucial aspect for enabling sustainable agriculture practices. However, to achieve this goal, the development of innovative research is essential to demonstrate the effectiveness of this method in various situations.

Through the advancement of knowledge about the use of FC in mushroom production, several environmental gains can be achieved. As FC has cyclic C chains, it has a significant potential to reduce atmospheric $CO_2$ [44]. It is known that $CO_2$ is one of the greenhouse gases that cause global warming. To prevent this warming from being intensified, the implementation of negative emission technologies is desired. Negative emissions are technologies that result in the net removal of $CO_2$/GHG from the atmosphere [45]. As interest in manipulating the C cycle grows, increasing substrate C content through FC production, it becomes important to better understand, and ultimately be able to predict, its mineralization [46].

## 5. Conclusions

Different strains of *Lentinula edodes* exert distinct influences on agronomic parameters. The incorporation of fine charcoal proves effective as an additive to the substrate; however, high concentrations result in reduced yield in the initial production stages. Thus, the inclusion of fine charcoal as a substrate component in shiitake production is recommended, although prior investigations into its compatibility with the strain and concentration are necessary.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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