eISSN 1981-1829

Ciência e Agrotecnologia
Science and Agrotechnology

Shiitake mushroom cultivation in composted substrate: Is it possible?

Cultivo do cogumelo shiitake em substrato compostado: É possível?

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ABSTRACT

Lentinula edodes is a primary wood-decomposing fungus that can be cultivated on wood logs or via axenic culture on sawdust-based substrate supplemented with some type of bran. Currently, the axenic cultivation system is preferred because it is favorable for cultivation on an industrial scale. In this work, we evaluated the feasibility of using composted substrates with two formulations and different composting periods for shitake cultivation. It was possible to cultivate this mushroom in the composted substrates; however, the success of cultivation depended on the use of severe pasteurization. The composted substrates were favorable for fungal mycelial growth only when a temperature of 80 **°C** was used for pasteurization. Moreover, the productivity and biological efficiency of the composted substrate subjected to severe pasteurization were similar to those obtained for non composted substrates. The best results were obtained with 6 days composting followed by pasteurization for 12 h at 80 °C or composting for 4 days and autoclaving for 1 h.

Index terms: Fermented substrates; composting; *Lentinula edodes*; severe pasteurization.

RESUMO

Lentinula edodes é um fungo decompositor primário de madeira que pode ser cultivado em toras de madeira ou em cultivo axênico em substrato à base de serragem suplementado com algum tipo de farelo. Atualmente, o sistema de cultivo axênico é preferido porque é favorável ao cultivo em escala industrial. Neste trabalho, avaliamos a viabilidade do uso de substratos compostados com duas formulações e diferentes períodos de compostagem para o cultivo de shiitake. Foi possível cultivar este cogumelo nos substratos compostados; no entanto, o sucesso do cultivo dependeu do uso de pasteurização severa. Os substratos compostados foram favoráveis ao crescimento micelial fúngico apenas quando uma temperatura de 80 °C foi usada para pasteurização. Além disso, a produtividade e a eficiência biológica do substrato compostado submetido à pasteurização severa foram semelhantes às obtidas para substratos não compostados. Os melhores resultados foram obtidos com 6 dias de compostagem seguidos de pasteurização por 12 h a 80 °C ou compostagem por 4 dias e autoclavagem por 1 h.

Termos para indexação: Substratos fermentados; compostagem; *Lentinula edodes*; pasteurização severa.

Agricultural Sciences

Ciênc. Agrotec., 48:e013224, 2024
http://dx.doi.org/10.1590/1413-7054202448013224

Editor: Renato Paiva

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Received in June 6, 2024 and approved in October 18, 2024

Introduction

The shiitake mushroom [*Lentinula edodes* (Berk.) Pegler] is among the three most cultivated species in Brazil and has a great potential for growth in the Brazilian market (Associação Nacional dos Produtores de Cogumelos - ANPC, 2018). It is one of the most cultivated fungi in the world due to its gastronomic and medicinal properties, accounting for approximately 22% of the total edible mushroom production worldwide (Royse, Baars, & Tan, 2017; Kwon et al, 2016). For a long time, shiitake was grown in wooden logs, especially in oak and walnut logs in countries with a temperate climate and in eucalyptus logs in tropical countries such as Brazil (Sousa et al., 2019). However, over time, shiitake cultivation has been transitioned to the axenic cultivation system, using sawdust as the main ingredient (Gong et al., 2014).

In the last 20 years, some studies have addressed the partial or total replacement of sawdust with agro-industrial residues by-products or grasses for the mushroom cultivation. Sugarcane bagasse, in addition to other byproducts, is a material traditionally used in mushroom cultivation and can also be a good alternative for shiitake cultivation (Gao et al., 2020). However, sugarcane bagasse has become a valuable byproduct, mainly as fuel in alcohol and cachaça production plants. Therefore, other raw materials have also been evaluated for the mushroom cultivation. For instance, Mahdizadeh et al. (2021) obtained satisfactory results in axenic blocks of straw, sawdust, and rice bran to cultivation of various shiitake strains. According to Avila, Alves and Zied (2023), rice straw combined with 20% sawdust and 15% wheat bran is also an excellent alternative for growing this mushroom.

The expansion of shiitake cultivation in a continental country such as Brazil is one of the greatest bottlenecks to logistics for supplying mushroom substrates because few companies provide this service in the country. Therefore, it is important to develop alternative technologies so that mushroom cultivators in more distant regions can produce their own mushroom substrates. In this context, it is essential to use technology that does not require the use of autoclaves.

Chang and Miles (2004) reported the importance of working with substrates fermented by thermophilic microorganisms. The presence of these microorganisms could make the substrate less susceptible to contaminants and more selective for the mushroom, as occurs with species of the genera *Pleurotus* (Fr.) P. Kumm and *Agaricus* L. Due to the balance promoted by the microbial population in the substrate, it can simply be pasteurized, eliminating the need for sterilization in an autoclave. However, no study has reported attempts to advance research on this subject on shiitake mushroom.

Producing shiitake mushroom on composted substrates can be challenging. This mushroom is classified as a primary decomposer and is considered a white rot fungus capable of degrading cellulose, lignin and other macromolecules (Carvalho et al., 2016; Sousa et al., 2019). However, considering its nature as a primary decomposer, it is possible that the fungus does not grow well on substrates that have undergone prior fermentation. With this in mind, in this study, shiitake cultivation in composted substrate was attempted for the first time, seeking to also understand the effect of the presence of other microorganisms in the substrate on the growth of the shiitake mushroom.

Material and Methods

Spawn production

The *L. edodes* culture was reactivated in Potato Dextrose Agar (PDA) culture medium at 25 °C. Unhulled rice was used as a substrate for preparing the inoculum and was enriched with 2% wheat bran and 2% calcitic limestone. The rice was cooked for 20 min and then mixed with the other ingredients. The substrate was placed in glass flasks (400 g) and then autoclaved for 2 h at 121 °C, repeating the process once after 24 h. After the substrate cooled, the flasks were inoculated with 4 mycelial discs 5 mm diam. and incubated at 25 °C ± 3 °C until complete colonization.

Shiitake cultivation in composted substrate in polystyrene plastic container

Substrate formulation

A basic formulation was used for the initial composting trials, using *Eucaliptus* sawdust (76%) supplemented with 20% wheat bran and 4% calcitic limestone (S+WB). Finally, the formulation combining *Eucaliptus* sawdust (38%) and sugarcane bagasse (38%), also supplemented with 20% wheat bran and 4% calcitic limestone (S+SC+WB) was also evaluated. For each formulation tested, composting was conducted in triplicate to confirm the results.

Composting and pasteurization process

The composting process was carried out in 50 L polystyrene plastic container to allow the natural rise in temperature. Due to the high number of experiments, it was necessary to work with a smaller volume than normally used in composting experiments. The 50 L containers have a capacity for approximately 12 kg of dry substrate. In all experiments, the substrate moisture content was adjusted to a theoretical value of 65%, resulting in a total of approximately 34 kg in each container. The ingredients and water were mixed in a concrete mixer for 5 min to homogenize the material. After mixing, the substrates were transferred to the containers and covered to prevent the entry of flies. The process lasted up to 6 days, with samples taken at 4, 5 and 6 days to measure the pH and make the cultivation blocks. For these tests, the compost was turned every two days.

At each composting timepoint, the substrates were placed in high-density polyethylene (HDPE) bags suitable for shiitake cultivation, with 2 kg used for substrate with sugarcane bagasse and 2.5 kg for substrate without sugarcane bagasse. For pasteurization, temperatures of 60, 70 and 80 **°C** were tested for 12, 24 and 36 h, respectively, using autoclaving as a control (121 °C) for two periods of 2 h, with an interval of 24 h before the second period of autoclaving.

After the substrate was cooled, bags were inoculated with 2% spawn. The blocks were incubated at room temperature until the browning process was complete (3 months). The substrate spawn run was defined as the time required (days) for complete colonization of the substrate. Afterward, the plastic bag was removed, and the blocks were transferred to a cultivation room at 19 °C and 90% relative humidity. Cultivation was maintained until the third flush was obtained, with an interval of 15 days among flushes. After the first flush, the blocks were immersed in cold water for 12 h to induce fruiting in subsequent flushes. The harvested mushrooms were counted and weighed to determine their productivity and biological efficiency. Productivity was defined as P= [(mass of fresh mushrooms/wet mass of substrate) × 100]. Biological efficiency was defined as BE = [(mass of fresh mushrooms/dry mass of substrate) × 100]. The experiment was conducted in a completely randomized design, with 10 replications. The contamination rate was defined as CR= [(number of contaminated blocks/number of blocks for repletion) x 100].

Validation of the composting process for shiitake cultivation

This experiment aimed to validate the results of previous tests in which small volumes of substrate were used during composting. Each formulation contained 150 kg of dry substrate,

allowing the simulation of composting conditions on a normal scale. The sugarcane bagasse was ground until most of the material reached a particle size close to that of sawdust. A sample was taken and passed through sieves of different particle sizes (9-200 mesh) for evaluation and comparison with the particle size of the sawdust. The samples collected from each sieve were weighed, with 39% of the sugarcane bagasse showing fragments larger than 2.38 mm, reaching up to 10 mm in size; however, 61% of the material had a particle size ≤ 1.2 mm.

For both compost formulations, the material was previously moistened to a moisture content of approximately 65%. Water was added gradually and, at each stage, a sample of the mixture was taken and squeezed between fingers to estimate the moisture content. Then, the supplements (wheat bran and limestone) were added, and the moisture content was estimated again, adding water as necessary. Finally, the compost windrows were assembled using a wooden structure measuring 1 m × 1 × 1 m (width × length × height). Composting was carried out for up to 8 days, with turnings carried out on days 2, 4, and 6. At each turning, the temperature was measured, and samples were taken for analysis of pH and moisture content. When necessary, the moisture was corrected in the subsequent turning, and water was added according to the preliminary results. At 4, 6, and 8 days of composting, blocks of 1.5 and 2 kg were prepared for substrates with and without sugarcane bagasse, respectively. The pasteurization processes were carried out as previously described, with durations of 12, 24, and 36 h evaluated. For autoclaving, durations of 1, 2, and 3 h were evaluated. The inoculation, incubation, cultivation and harvesting processes were conducted as previously described. During the colonization period, the contamination rate of the blocks and the time required for complete colonization of the substrate, in days, were evaluated. The experiment was conducted in accordance with a completely randomized design, with six replications.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the F test, and when differences were significant, the Scott–Knott test was used to compare means. Statistical analyses were carried out using the SISVAR statistical program (Ferreira, 2011).

Results and Discussion

Shiitake cultivation in composted substrate in containers

The pH ranged from 6.36 to 6.66 and 5.14 to 6.45 for S+SC+WB and S+WB substrates, respectively, while the temperature ranged from 45.4 to 46.5 and 46.7 to 49.2 for S+SC+WB and S+WB substrates, respectively (Table 1). The formulation with sugarcane bagasse was included in this experiment with the expectation that there would be an increase in temperature because the use of sawdust only as the main ingredient did not favor the increase in temperature, according to previous tests. However, even with the addition of sugarcane bagasse, the expected increase in temperature was not observed. It is possible that conducting the composting process inside the containers restricted aeration and, consequently, limited the growth of microorganisms (Ribeiro et al., 2017). Subsequently, we tested containers with drilled holes on the sides and bottom at 10 cm intervals, but the results did not change (data not showed).

Table 1: Physical parameters of composting with different substrate formulations and composting times. S+SC+WB: sawdust + sugarcane bagasse + wheat bran. S+WB: sawdust + wheat bran.

Time (days)	S+SC+WB	S+WB
	pH	
4	6.36	6.35
5	6.66	6.45
6	6.57	5.14
	Temperature (°C)	
4	45.4	46.7
5	46.5	49.6
6	46.1	49.2
	Moisture (%)	
4	67.4	55.0
5	66.4	58.0
6	65.5	62.5

The initial moisture content of the substrates was adjusted to a theoretical value of 65%. For the S+SC+B formulation, a small variation was observed in relation to the initially proposed value. However, for the S+B formulation, only composting for six days yielded compost that presented a moisture content close to the proposed theoretical value, while the 4- and 5-day composts had moisture contents below 60%. This probably occurred because sugarcane bagasse has a greater water absorption capacity than sawdust, and the moisture was measured as water was added by squeezing a small portion of the substrate between fingers. As sawdust absorbs less water than sugarcane bagasse, this formulation probably allows the release of water between the fingers when the moisture content is still below the desired level. However, moisture below 60% for shiitake cultivation could be beneficial because according to Shen et al. (2008), the best shiitake production results were obtained on substrates with 55% moisture compared to substrates with 50% and 60% moisture.

Contamination problems were observed only in the autoclaved substrates for the 5- and 6-day composting treatments (data not showed). The efficiency of the autoclaving process depends not only on time and volume but also on the microbial population present in the substrate (Chang & Wasser, 2017; Vieira & Pechia, 2018). However, autoclaved substrates are also more susceptible to contamination when some microbial cells survive the process or when external contamination occurs. On the other hand, pasteurization does not normally eliminate the entire microbial population present in the substrate, but aims to eliminate competing microorganisms, pathogens and pests (Buth, 2017). Therefore, after pasteurization, a large part of the microbial population survives and this remaining population can help control possible contaminants in the substrate (Souza et al., 2014). Composting is a fermentation process that basically depends on the action of several species of microorganisms, especially bacteria (Yang et al., 2022). During this process, therefore, the microbial community increases significantly, first with the community of mesophilic microorganisms and then with the community of thermophilic microorganisms (Souza et al., 2014).

The absence of contamination in the 4-day composting treatment may be associated with a smaller microbial population generated due to the shorter composting time. And, as the composting time increased, the microbial population naturally increased and, therefore, the heat treatment was not sufficient to eliminate all contaminants. However, it is important to emphasize that other uncontrolled factors may be involved in the contamination problem, which cannot be explained solely by the composting process. One of these factors is the possibility of contamination after the heat treatment, for example, during the substrate spawning.

Significant (p= 0.005) differences were observed in the substrate spawn run (SR) as a function of composting time and formulation (Table 2). For the S+WB formulation the 6 -day composting treatment (CT) showed the slowest SR using pasteurization as HT (46.6 days), followed by the substrate composted for four days using autoclaving as HT (40 days). The differences in SR for the other treatments were not significant. Considering the effect of the HT, significant differences were observed only for 6 -day CT where the autoclaved substrate showed shorter SR. For the S+SC+B formulation, the best SR was observed in the substrate subjected to four days of composting using pasteurization as HT (30.8 days), which was significantly better than 4 -day and 6 -day CT, both pasteurized. Moreover, the autoclaved treatments for this formulation did not show significant differences in SR. Considering the effect of the HT, significant differences were observed only between the 4 -day CT substrates where pasteurization showed the best result of SR.

The SR exceeded that established by Royse (2009), according to which a time of 18 to 23 days guarantees optimal production results. Nevertheless, the time required for complete colonization also depends, in addition to the quality of the substrate, on the substrate volume, quantity of inoculum, distribution efficiency, and quality of the inoculum (Gaitán-Hernández & Cortés, 2014). For the present work, a factor that seems to greatly affect the colonization of the *L. edodes* cultivation substrate, in addition to nutritional quality, is the microbiota population that survives the heat treatment in association with the composting time.

Table 2: Effect of composting time (CT) and heat treatment (HT) on the contamination rate (CR) and substrate spawn run (SR). A: autoclaving; Past: pasteurization; S+WB: substrate based on sawdust and wheat bran; S+SC+WB: substrate based on sawdust, sugarcane bagasse and wheat bran.

CT (days)	HT	SR (days)	
		S+B	S+SC+B
4	A	40.0 aB	40.8 aAβ
4	Past	34.8 aA	30.8 aAα
5	A	31.2 aA	40.2 bA
5	Past	36.0 aA	39.2 aB
6	A	33.4 aAα	39.2 aA
6	Past	46.6 bBβ	37.0 aB
CV (%)		13.04	

Lower case letters compare the means in the rows. Upper case letters compare the means 1, 3 and 5 in the columns, corresponding to the different composting time for autoclaved substrates. Underlined upper case letters compare the means 2, 4 and 6 in the columns, corresponding to the different composting time for pasteurized substrates). Greek letters compare the means in the columns at each composting time in function of the heat treatment (autoclaving and pasteurization). The absence of letters indicates non-significance by the Scott-Knott test (p > 0.05).

In the present work, all blocks formed the brown film within the expected timeframe (data not showed). The brown film formation precedes the primordia initiation and, subsequently, fruiting. In general, mushroom cultivators open the blocks within 90 days. The formation of a brown film is considered key to the good productivity of the shiitake block. Strains that fail to form a brown film or exhibit late formation do not produce mushrooms or are less productive than brown coated blocks (Zied et al., 2016; Sousa et al., 2019).

The highest P was obtained in the S+B substrate composted for five days and autoclaved (17.4%), followed by the substrate of 4 -day CT (14%) and 5 -day CT (12.5%) both pasteurized (Table 3). For the S+SC+B formulation, the results were very similar, except for 5 -day CT autoclaved substrate, which showed a very low P. Regarding biological efficiency, the results followed a similar pattern for the S+WB formulation, while for the other formulation the differences were not significant for all treatments. Considering the effect of HT the differences were significant only for 5-day CT where the autoclaved substrate showed a higher P (17.4%) compared to the pasteurized substrate (12.5%) for S+WB and

the opposite for S+SC+WB where the 5-day CT and pasteurized substrate was much better than the autoclaved substrate.

Importantly, the blocks of the S+SC+WB substrate began to fall apart when immersed in water during the second production flush. This was because the sugarcane bagasse used was only crushed, compromising the proper compaction of the block due to the presence of very large fragments. This aspect will be addressed later in this work.

These results indicated that it is viable to include sugarcane bagasse in the formulation of the shiitake cultivation substrate, replacing some of the sawdust, as long as the sugarcane bagasse is ground to obtain fragments smaller than 1 cm. Other studies have shown that it is possible to partially replace sawdust with other materials, as reported by Avila et al. (2023), who used rice straw as the main ingredient in the shiitake cultivation substrate.

The results obtained thus far allowed us to conclude that the composting process could be carried out for at least four days and that pasteurization for 36 h provided production results similar to those of the autoclaving process. There are no reports of composted substrates being used for shiitake production (Chang & Miles, 2004), contrary to what is already known for *Agaricus* mushrooms (Chang & Wasser, 2017) and for some *Pleurotus* species (Vieira & Andrade, 2016; Vieira & Pechia, 2018; Yang et al., 2022). As a result, Chang and Miles (2004) expressed the importance of studying the use of thermophilic microorganisms for the production of shiitake cultivation substrates. However, since then, there have been no studies using this approach, which could indicates the difficulty of growing shiitake on composted substrates. Therefore, to our knowledge, this is the first work using composted substrates for the cultivation of shiitake.

However, pasteurization of the substrate at 60 °C was not efficient. In addition to contamination problems, it was observed that the shiitake strain did not grow well even before the contamination established itself in the substrate. Afterward, the pasteurization temperature was increased to 70 °C; however, this attempt was not successful, although the performance of substrate pasteurized at this temperature was better than that of the substrate pasteurized at 60 °C. Morales and Sánchez (2017) tested a pasteurization process performed over 6 h at 60 °C for the cultivation of different species of mushrooms. According to the authors, only *L. edodes* and *Ganoderma lucidum* (Curtis) Karst produced no mushrooms on the pasteurized substrates, which corroborates the results observed in the present work.

The pasteurization temperature was increased to 80 °C, and the pasteurization time was also increased. For subsequent experiments, 36 h of pasteurization was used to avoid the risk of contamination. The heat treatment time was again evaluated in the final validation experiment and the results of which will be discussed later.

As shown in Table 3, the lowest mushroom production was observed in substrates composted for six days for all formulation and heat treatment groups. Nevertheless, colonization and formation of the brown coat occurred normally on these substrates. However, it is important to highlight that the composting tests were conducted inside containers, where aeration was limited.

As previously discussed, the pasteurization process at 80 °C for 36 h was as efficient as autoclaving in controlling contamination, in addition to providing good mushroom yields. The best productivity result was obtained in the autoclaved substrate after five days of composting (17.4%) for the S+B formulation. The S+SC+WB formulation tended to present similar yields with the S+WB formulation, except when the substrate was autoclaved after five days of composting. Zied et al. (2016) reported a productivity of 20.9% in axenic cultivation using a substrate with 80% *Eucaliptus* sawdust and 20% wheat bran.

Table 3: Effect of composting time (CT) and heat treatment (HT) on the productivity (P) and biological efficiency (BE) of the two substrate formulations. A: autoclaving; Past: pasteurization; S+WB: substrate based on sawdust and bran; S+SC+WB: substrate based on sawdust, sugarcane bagasse and bran.

CT (days)	HT	P (%)		BE (%)	
		S+WB	S+SC+WB	S+WB	S+SC+WB
4	A	11.1 aB	13.0 aA	24.6 aB	30.6 aA
4	Past	14.0 aA	11.8 aA	31.2 aA	27.9 aA
5	A	17.4 aAα	7.8 bBβ	41.4 aAα	21.3 bA
5	Past	12.5 aAβ	12.5 aAα	29.8 aAβ	34.4 aA
6	A	8.1 aB	6.8 aB	21.4 aB	19.9 aA
6	Past	8.7 aB	8.4 aA	24.6 aA	22.8 aA
CV (%)			30.31		

Lower case letters compare the means in the rows. Upper case letters compare the means 1, 3 and 5 in the columns, corresponding to the different composting time for autoclaved substrates. Underlined upper case letters compare the means 2, 4 and 6 in the columns, corresponding to the different composting time for pasteurized substrates). Greek letters compare the means in the columns at each composting time in function of the heat treatment (autoclaving and pasteurization). The absence of letters indicates non-significance by the Scott-Knott test ($p > 0.05$).

Validation of the composting process for shiitake cultivation

The composting temperature increased significantly when the S+SC+WB formulation was used, contrary to what was observed in the process carried out inside containers for the same formulation (Figure 1). The temperature increased to above 50 °C in the first four days of composting and was above 60 °C after six days of composting, peaking at 64 °C after eight days of composting. These results confirmed, therefore, that the use of greater volume and greater aeration of the substrate were decisive in allowing better conditions for the evolution of the thermophilic microbiota of the compost, as previously pointed out by Chang and Wasser (2017), Vieira and Pechia (2018) and Ribeiro et al. (2017).

The highest contamination rates (Figure 1) were observed in blocks prepared with the composted substrate for up to four days. Even with the autoclaving treatment, a high contamination rate was observed after 1 and 2 h, while after 3 h of autoclaving, a contamination rate of more than 10% was still observed. In the case of pasteurization, a contamination rate of 66.6% was observed for the 12 -h period and less than 10% for the 24 -h period. Only after 36 h of pasteurization a zero contamination rate was obtained for the substrate composted for four days. These data suggest that, with this composting time, the substrate still maintained a very high mesophilic population, with a longer time required for its elimination.

After six days of composting, the contamination rates decreased drastically. For the 1- h and 2 -h autoclaving treatments, the contamination rates were greater than 10%; however, no contamination was observed after 3 h of autoclaving. Notably, no contamination was observed for the samples pasteurized for 12 h and 24 h. Interestingly, for the 36 -h treatment, 6% contamination was observed, which may be associated with external contamination factors and not with the effectiveness of the process itself.

For the substrate composted for eight days, all the treatments showed contamination, except for the 36 -h pasteurization treatment. The temperature increased slightly more after eight days of composting than after six days of composting, with the temperature increasing from 62.4 to 64 °C. This temperature favors the growth of thermophilic microorganisms, which could be considered beneficial for the quality of the compost (Souza et al., 2014). However, this was not confirmed because the results were inferior to those at six days of composting.

The pH of the compost varied from 5.4 to 5.9 during the process. These values are lower than those obtained with substrates composted for longer times (Ribeiro et al., 2017); however, they are within the range considered ideal for shiitake production (Chang & Miles, 2004). The moisture content of the substrate was 62%, 64% and 67% on days 4, 6 and 8 of composting, respectively. In all turnings, which took place on the second, fourth and sixth days, water was added with the aim of reaching a moisture content of approximately 65%. Therefore, increasing moisture was observed from the 4th to the 8th day of composting. The concern with correcting moisture throughout composting is justified due to the loss of water through evaporation, especially that caused by high temperatures during the thermophilic phase (Yang et al., 2022).

For the S+WB formulation, it was decided that only the treatments with the best results obtained with the S+SC+WB formulation would be carried out. Therefore, composting was only carried out for up to six days, and for this composting time, the substrates were pasteurized for only 12 h, which were the best treatment conditions observed in the previous experiment.

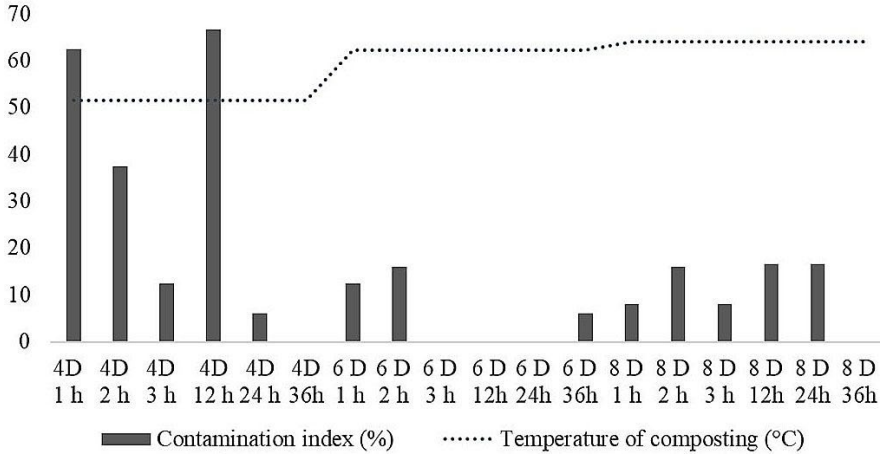

Figure 1: Contamination index and temperature of the S+SC+WB substrate over several days of composting and heat treatment. Bars with numerical values above represent the contamination rate. The dotted line represents the temperature during the composting process. Legend: 1 h, 2 h and 3 h- autoclaving time; 12 h, 24 h and 36 h- pasteurization time; 4 D- 4 days of composting; 6 D- 6 days of composting; 8 D- 8 days of composting.

Contrary to what was observed for the S+SC+WB formulation, practically no contamination was observed in the blocks with the S+WB substrate. The only treatment that showed contamination was the substrate with six days of composting and 2 h of autoclaving, with a contamination rate of just 2%, which may be associated with external contamination too.

As expected, the temperature of the compost without sugarcane bagasse did not increase, as observed for the formulation with bagasse (data not showed). Nevertheless, the temperature reached values higher than those observed in composting conducted in closed containers (41 °C to 46 °C). These results demonstrate, therefore, that conventional composting in greater volume, in fact, presents more favorable conditions for the development of thermophilic microorganisms, even for formulations without sugarcane bagasse, as previously pointed out by Ribeiro et al. (2017).

The great absence of contamination in the S+WB substrate can probably be explained by the lower microbial load present in the sawdust than in the sugarcane bagasse. It is possible that even after the sugarcane juice is extracted, the resulting bagasse still contains traces of sugar that may favor the development of various mesophilic microorganisms. Nevertheless, it was expected that the greater increase in temperature in the compost with sugarcane bagasse could promote greater elimination of mesophilic contaminants. However, unfortunately, this was not observed.

The SR data in the blocks with the different treatments are shown in Tables 4 and 5. The shortest SR was observed for the S+WB substrate in the 4 -day CT autoclaved for 1 and 2 h and pasteurized for 12h, as well as in the 6 -day CT autoclaved for 1, 2 and 3h and pasteurized for 12 h. Therefore, the pasteurization proved to be an efficient HT for the 4 and 6 -day CT substrates in terms of SR. This result is very important since a lower pasteurization time is important for preparing substrates for mushroom cultivation, reducing the energy cost of the process. For the S+WB formulation, the best results were obtained with 4 -day CT using 1 and 2h of autoclaving and with 6- day CT using 12h of pasteurization. Only 16 days were needed for complete substrate colonization in these treatments. These results are very good and are much better than that obtained with the S+SC+WB substrate. This SR is even better than that proposed by Royse (2009), which was 18 to 23 days. Therefore, for this experiment, complete success was observed in the time required to complete the substrate colonization (16 to 24 days). Importantly, for this purpose, the inoculum was thoroughly mixed with the substrate instead of just being added to the top of it, which was possible with a reduction from 2.5 to 2 kg of substrate in each bag.

The speed of mycelial growth may be related to the substrate formulation and, consequently, to its nutritional quality (Mahdizadeh et al., 2021). However, it is possible that the greater speed of colonization in this substrate may also be associated with a lower microbial load generated during composting. The lower temperature during composting indicates that there was also less microbial growth, resulting in a lower microbial biomass (L**ó**pes-González et al., 2015). This lower microbial biomass and corresponding concentration of microbial metabolites may also have led to a weaker inhibitory effect on the growth of *L. edodes*.

Table 4: Effect of composting time (CT) and heat treatment on the spawn run (SR) of the S+SC+WB substrate. A: Autoclaving; Past: Pasteurization.

CT	SR					
	A			Past		
	1	2	3	12	24	36
4	37 aA	37 aA	40 aB	36 aA	43 bA	44 bA
6	36 aA	37 aA	36 aA	36 aA	40 bA	49 cA
8	61 aB	61 aB	61 aC	62 aB	63 aB	63 aB
CV (%)	4.35					

Lower case letters compare the means in the rows for autoclaved substrates (A) and underlined lower case letters compare the means in the rows for pasteurized substrates (Past). Upper case letters compare the means in the columns. For a same variable, means followed by the same letter do not differ by the Scott-Knott test ($p > 0.05$).

Table 5: Effect of composting time (CT) and heat treatment (HT) on the spawn run (SR) of the S+WB substrate. Legend: T- Time; A- Autoclaving; Past- Pasteurization.

CT	SR			
	A			Past
	1	2	3	12
4	16 aB	16 aA	20 bA	-
6	20 cAγ	18 bBβ	24 dBδ	16 α
CV	5.82			

Lower case letters compare the means in the rows and upper case letters compare the means in the columns. Greek letters compare the means in the row for 6- days composting time in function of the heat treatment (autoclaving and pasteurization). The absence of letters indicates non-significance by the Scott-Knott test ($p > 0.05$).

Productivity and biological efficiency

According to the data presented in Table 6, the highest mushroom yield was obtained in the S+B substrate composted for four days and autoclaved for 1 h (28.3%), followed by the S+B substrate composted for six days pasteurized for 12 h (25.3%) and autoclaved for 1 h (20.3%). These results did not differ statistically ($p= 0.05$) from each other. For the S+SC+B formulation, the highest yield was obtained on the substrate composted for six days and autoclaved for 2 h (14.17%). However, the differences between all treatments were not significant.

Table 6: Effect of composting time and heat treatment on the productivity of the S+SC+WB and S+WB substrates. CT- composting time; HT- heat treatment; T- Time of heat treatment; A- Autoclaving; Past- Pasteurization.

Compost	TC (days)	T P/A	P (%)	EB (%)	CV
S+FT	4	1 h/A	28.3 aα	46.5 aα	23.38
	6	12 h/Past	25.3 α	41.1 α	
	6	1 h/A	20.3 aα	32.3 aα	
	4	2 h/A	16.4 bβ	27.0 bβ	
	4	3 h/A	15.8 bβ	26.0 bβ	
	6	2 h/A	14.2 bβ	23.5 bβ	
S+BC+FT	6	2 h/A	14.2 a̲α̲	29.5 a̲α̲	45.19
	8	36 h/Past	12.0 α̲	17.9 α̲	
	6	36 h/Past	10.9 α̲	22.7 α̲	
	6	12 h/Past	10.6 α̲	22.2 α̲	
	6	1 h/A	8.5 a̲α̲	17.8 a̲α̲	
	6	24 h/Past	8.5 α̲	17.8 α̲	
	6	3 h/A	8.4 a̲α̲	17.5 a̲α̲	
	4	24 h/Past	7.9 α̲	17.0 α̲	
	8	1 h/A	10.2 a̲α̲	15.2 a̲α̲	
	8	2 h/A	9.4 a̲α̲	14.0 a̲α̲	
	4	36 h/P	6.2 α̲	13.4 α̲	
	8	3 h/A	6.9 a̲α̲	10.4 a̲α̲	
	8	24 h/P	4.5 α̲	6.6 α̲	

Lower case letters compare the means in the columns for the S+WB substrate and underlined lower case letters compare the means in the columns for the S+SC+WB substrate, both for autoclaved treatments. Greek letters compare the means between autoclaved and pasteurized treatments in the columns. For a same variable, means followed by the same letter do not differ by the Scott-Knott test ($p > 0.05$).

The treatments that resulted in high levels of contamination did not provide sufficient blocks for the number of repetitions needed for statistical tests. However, in general, all the treatments involving sugarcane bagasse achieved lower P and BE compared to the use of substrates based only on sawdust. The coefficient of variation (CV) in the experiment using sugarcane bagasse was almost twice compared to the experiment without sugarcane bagasse., indicating a difficulty in producing a uniform substrate for shiitake cultivation using this formulation. This strongly indicates that favoring the composting process with an increase in the thermophilic population does not necessarily favor agronomic performance of the substrate by *L. edodes*. Even though these microorganisms were eliminated by heat treatment (pasteurization or autoclaving), the presence of this biomass and, consequently, of several metabolites still inhibited the growth of the fungus. Although the grinding of sugarcane bagasse allowed excellent compaction and structuring and formation of a brown film in the blocks, this management approach did not influence the P of the blocks. However, it allowed the production cycle to be carried out without the blocks falling apart, mainly through management by immersion in water.

Interestingly, the productivity of substrates based on sugarcane bagasse did not drastically increase compared to that of the containers treatments in the previous test. Even though the S+SC+WB substrate composted for six days and pasteurized for 12 h had a zero-contamination rate (Figure 1), the mushroom yield was significantly equal to that of the treatments with the lowest values (10.90% and 22.70% P and BE, respectively). This shows that the zero-contamination rate was not sufficient to guarantee higher productivity of *L. edodes* on the S+SC+WB substrate.

Therefore, the inclusion of sugarcane bagasse in large-scale composting and natural ventilation of the substrate does not seem to provide high shiitake productivity. Villar et al. (2016) reported that the choice of substrate has a great influence on the dynamics of the microbiota in compost. Optimizing the substrate may be one of the approaches by which the performance of shiitake on the S+WB substrate can be approved. One explanation for the low contamination rate and high productivity in this substrate is that sawdust contains a level of lignin and cellulose suitable for the growth of *L. edodes* and has phenolic compounds that are oxidized by laccase during the browning process (Santos et al., 2011; Yang et al., 2016). Mata et al. (1998) also reported that

phenolic compounds can inhibit substrate contaminants. On the other hand, sugarcane bagasse favors the growth of contaminants most likely due to the presence of remaining sugars.

Therefore, the best treatment, considering the availability of an autoclave, the simplicity of the method, the efficiency of the colonization of the blocks and the lack of contamination data, is four days of composting and 1 h of autoclaving. In this system, the production of the mushroom substrate would include the composting step for four days, without turning, but it would also allow the substrate autoclaving time to be reduced. However, for a production system without the use of an autoclave, the system with six days of composting and 12 h of pasteurization provided similar P and BE values and is therefore more suitable for producers with lower investment capacity.

Despite the apparent negative effect of the presence of the microbiota generated during composting, further studies must still be carried out with the aim of evaluating the inoculation of the substrate with different species of thermophilic microorganisms, with the potential to stimulate the mycelial growth of *L. edodes*.

Conclusions

The use of composting to produce shiitake mushroom substrates has proven to be viable for substrates based on sawdust and wheat bran, as long as composting is carried out in six days, with pasteurization for 12 h at 80 °C or composting for four days and autoclaving for 1 h.

Author Contribution

Conceptual idea: Dias, E.S; Methodology design: Dias, E.S.; Zied, D.C.; Siqueira, F.G.; Data collection: Castro, C.P.; Abreu, C.G.; Moraes, T.S.J.; Data analysis and interpretation: Dias, E.S.; Zied, D.C.; Siqueira, F.G.; Castro, C.P.; Abreu, C.G.; Moraes, T.S.J.; and Writing and editing: Dias, E.S; Castro, C.P.

Acknowledgments

The FAPEMIG (Fundação de Amparo à Pesquisado Estado de Minas Gerais), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) are acknowledged for financial support.

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