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The application of the scratching technique has the same effect on *Pleurotus spp.*?

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HIGHLIGHTS

- The use of the scratching technique was studied in *Pleurotus spp*.
- The scratching increases the number of *Pleurotus djamor* mushrooms.
- The use of scratching is selective for different species of *Pleurotus*.

Abstract: The present work evaluated the agronomic behavior of species and varieties of *Pleurotus* submitted or not to the scratching technique. The cultivated species/varieties were *Pleurotus ostreatus* and *Pleurotus ostreatus* var. Florida, and *Pleurotus djamor*, where half of the treatments were scratched before the first and second flushes, while the rest were only scratched before the second flush. Yield (%), biological efficiency (%), weight of mushroom (g) and number of mushrooms (uni) were evaluated. It was observed that scratching harmed the mushroom weight for *P. ostreatus*, where the treatment without scratching produced significantly higher values, in the first harvest flush. On the other hand, scratching favored yield and the number of mushrooms for *P. djamor*. When the species are compared, it is noted that *P. ostreatus* obtained a lower yield, which was also reflected in the biological efficiency. *P. djamor* stood out with a large number of mushrooms, statistically higher than the other species, on the other hand, producing mushrooms of low weight of mushroom. The use of the scratched technique is not recommended for the conditions used in the work, due to the increase in labor and loss of mushroom weight.

Keywords: oyster mushroom, yield, screening of mushroom quality, mycelium breaking, cultivation technique.

INTRODUCTION

Brazil is one of the largest agricultural producers in the world, its sector has been growing in recent years and the expectation is that its production will continue to increase. The growth combined with the viability of intensive systems has provided the availability of lignocellulosic agricultural waste, which promotes environmental concerns, but also stimulates good business opportunities that generate economic or energy value [1].

In most cases, however, these agricultural wastes are not treated or reused [2]. The availability of nutrients in these materials provides suitable conditions for the microorganism's growth, such as edible mushrooms [3]. Using these wastes to mushrooms production through locally available technologies is a solution to turning them into a food source.

Since *Pleurotus spp.* can develop in tropical climates, besides being simple to produce and compatible with lignocellulosic substrates [4], their production potential is very high. This fact contributed to the increase in world production, occupying the second place of the most cultivated genus in the world, representing about 19% of total global production [5].

Pleurotus species have been adapted for cultivation in small spaces and require a short growth time compared to other mushrooms [6,7]. Cultivation methods are using sterilized substrate, stored in plastic bags [8,9] or glass or plastic pots [10,11].

The rapid colonization of the substrate by *Pleurotus* mycelium is an important characteristic in controlling contamination [7], but excessive mycelial formation in the upper part of the substrate is common in species of this genus, reducing the gas exchange capacity due to reduction of pores, which consequently impairs mushroom yields [12,13]. The absence of aeration in the substrate decreases the O2 concentration, initiating the fermentation process that converts nitrogen into ammonia, interrupting mycelial growth [14], as well as inhibiting the activity of hydrolytic enzymes, responsible for this process [15]. The cultivation of *Pleurotus* species still does not have concrete alternatives to solve this problem, exposing the need for studies aimed at adapting or creating technologies for this purpose.

The scratching technique is commonly used in some mushroom species to promote substrate gas exchange, this method consists of the mechanical removal of the first layer of mycelium together with the upper part of the aged, colonized substrate for uniform fruiting. The scratched surface is then lightly sprayed with water to stimulate formation of fruitbodies [16]. Although the application of this technique has also been reported in other species, such as *Calocybe indica*, *Flamulina velutipes*, *Hypsizygus marmoreus* and *Pholiota nameko* [16,17], no studies were found that report whether this practice involving species, varieties and application time, has some benefit on agronomic behavior. Thus, the aim of this work was to evaluate the effects of the scratching technique on the performance of *Pleurotus spp.*

MATERIAL AND METHODS

The experiment was carried out at the Centro de Estudo em Cogumelos of the Faculdade de Ciências Agrárias e Tecnológicas (FCAT/UNESP), Câmpus Dracena. The design was in completely randomized blocks, in a 3x2 factorial scheme, with 7 replications. The first factor corresponded to different species and varieties of Pleurotus (*P. ostreatus*, *P. ostreatus* var. Florida and *P. djamor*), and the second factor was the application or not of the scratching technique.

The substrate for production was prepared using: eucalyptus sawdust (75%), wheat bran (10%), cottonseed bran (10%) and soybean meal (5%). This formulation was hydrated with water until reaching 70% moisture and 2% calcium carbonate was added. The substrate was placed in plastic bags made of high-density polyethylene containing 500 g (length 26cm, width 10cm and height 19cm), then autoclaved at 121°C for 4 hours.

The inoculums were prepared following the methodology proposed by Vieira Junior and co-authors [18], using sorghum seeds that were boiled at 100°C for 30 minutes and then added with 2% limestone. To spawn poduction, the following steps were used: selection of mushroom and production of subculture; parent spawn; and finally grain spawn. The bags with the substrate were inoculated with 15g of inoculum (3% wet substrate) in a laminar flow chamber and then incubated for 18 days at 26 ± 2 °C. The strains used, POS 18/01 (*Pleurotus ostreatus*), POF 16/01 (*Pleurotus ostreatus* var. Florida) and PDJ 19/01 (*Pleurotus djamor*) were obtained from different growers from São Paulo and Paraná States (Brazil) and are deposited in the public collection of the Centro de Estudos em Cogumelos.

After incubation, the half of the bags were cut from their top surface, then the scratching technique was applied before the first and second flushes, where through the use of a spoon ~1 cm of the aged mycelium and substrate layer was removed (Figure 1).

Figure 1. (A) Before scratching; (B) scratching; (C) primordium formation in the first flush after scratching done at 18 days after inoculation; and finally (D) mushrooms at harvest point after scratching done at 18 days after inoculation.

On the other half of the bags, the scratching technique was applied before the second flush. Subsequently, the bags were taken to cultivation rooms with a temperature of 21± 2ºC, relative humidity of 75 ± 5%, CO_2 < 1200 ppm and 150-200 lux illumination, for fruiting [7,19]. The mushrooms were harvested twice a day, weighed and counted for analysis of production parameters [20]. Figure 2 shows the timeline of the entire crop, starting with the autoclaving of the substrate until the last day of harvest.

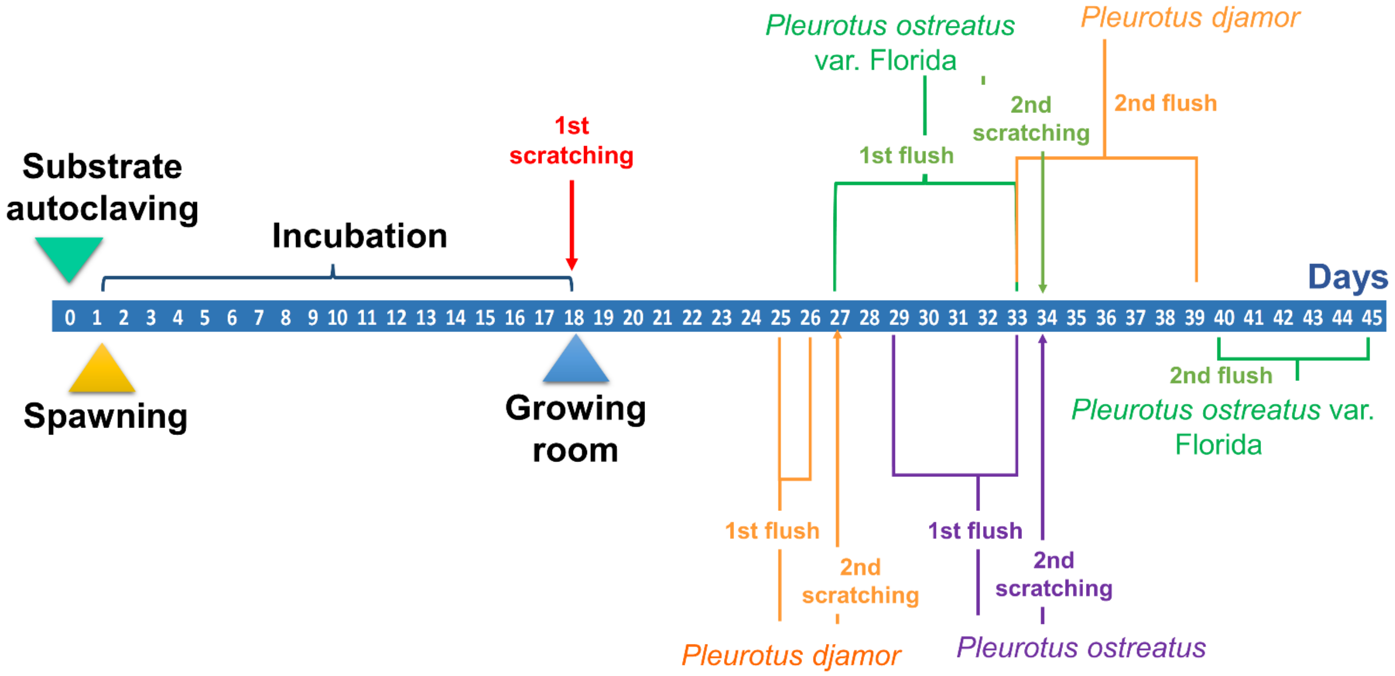

Figure 2. Crop period schedule.

The agronomic behavior was evaluated through: i) yield, calculated through the fresh mass of mushrooms (g) divided by the weight of the initial wet substrate (g) x 100, expressed in percentage; ii) mushroom number, expressed in unit(s); iii) weight of mushrooms, calculated through the total fresh weight divided by the number of mushrooms, expressed in grams; and iv) biological efficiency, calculated through the fresh weight of mushrooms by the dry weight of the initial substrate x 100, expressed in percentage [21, 22].

Data were submitted to analysis of variance and when significant, Tukey's mean comparison test was applied, both at 5% probability. For analysis and preparation of the heat map through the Euclidean distance, Pearson correlation and dispersion analysis, the R 4.1.0 software (R Foundation for Statistical Computing, Vienna, Austria) was used.

RESULTS

The species *P. djamor* and *P. ostreatus* var. Florida had two production flushes, with a total cycle of 39 and 42 days, respectively. *P. ostreatus* had only one production flush, with a total production cycle of 33 days. In order to verify the isolated action of the scratching technique, Table 1 presents the agronomic parameters only for the first flush, where the technique was applied in the half of the production bags. The other half of production bags were not applied scratching technique before the 1st flush.

The influence of the technique was significant in the species/varieties studied, being statistically affected the number of mushrooms for *P. djamor* (38.5 with and 23.8 u without scratching) and weight of mushroom for *P. ostreatus* (4.5 with and 5.8 g without). The species with the highest yield and biological efficiency was *P. ostreatus* var. Florida, with of 8.3% and 27.7% using the scratching technique (Table 1).

Table 1. Agronomic behavior of the first flush with and without scratching technique.

	Species/varieties		
Scratching	*P. ostreatus*	*P. ostreatus var. Florida*	*P. djamor*
	Yield (%)		
With	6.2 b	8.3 a	6.6 b
Without	6.0	7.4	6.5
CV (%)	18.43		
	Number of mushroom (u)		
With	7.2 b	8.1 b	38.5 a A
Without	5.2 b	6.7 b	23.8 a B
CV (%)	48.89		
	Weight of mushroom (g)		
With	4.55 a B	5.25 a	1.07 b
Without	5.80 a A	5.65 a	1.54 b
CV (%)	20.19		
	Biological efficiency (%)		
With	20.85 b	27.71 a	22.28 b
Without	20.28	24.76	22.26
CV (%)	18.43		

Means with different letters (lowercase between lines and capital letters between columns) differed by Tukey's test at 5% probability. Absence of letters, there was no statistical difference.

The highest total yield was obtained by *P. ostreatus* var. Florida (13.2 with scratching before 1st and 2nd flushes and 12.8% without scratching before 2nd flush) and the lowest by *P. ostreatus* (6.2% with scratching before 1st and 2nd flushes and 6.08% without scratching before 2nd flush). *P. djamor* presented the highest number of mushrooms and the species *P. ostreatus* the lowest, the weight of mushroom behaved in an inverse way, through mushrooms with greater weight for *P. ostreatus* and lesser for *P. djamor* (Table 2).

Table 2. Agronomic behavior of different of *Pleurotus* with the application of the scratching technique at different times of cultivation.

	Species/varieties		
Scratching	*P. ostreatus*	*P. ostreatus* var. Florida	*P. djamor*
	Yield (%)		
1st and 2nd flush	6.25 c	13.2 a	11.17 b A
2nd flush	6.08 c	12.8 a	9.54 b B
CV (%)	14.37		
	Number of mushroom (u)		
1st and 2nd flush	7.28 c	18.71 b	50.71 a A
2nd flush	5.28 c	18.57 b	29.71 a B
CV (%)	37.78		
	Weight of mushroom (g)		
1st and 2nd flush	4.55 a B	3.61 b	1.24 c
2nd flush	5.80 a A	3.47 b	1.72 c
CV (%)	19.68		
	Biological efficiency (%)		
1st and 2nd flush	20.85 c	44.00 a	37.23 b A
2nd flush	20.28 c	42.66 a	31.80 b B
CV (%)	14.37		

Means with different letters (lowercase between lines and capital letters between columns) differed by Tukey's test at 5% probability. Absence of letters, there was no statistical difference.

Significant differences in yield using the scratching technique were only observed in *P. djamor* species, with 11.1%. Higher values were also found in the number of mushrooms and biological efficiency for *P. djamor*. In *P. ostreatus* the technique affected the weight of mushroom.

To demonstrate the similarity between the treatments and the correlation between the variables, a heat map was constructed (Figure 3). By Ward's method (left grouping), the *P. ostreatus* was separately from the other species, due to differences in their agronomic behavior (shade of different colors, ranging from 1.5 to -1.0). This indicates that the research conditions (substrate formulation or management of environmental variables) were not efficient for *P. ostreatus*.

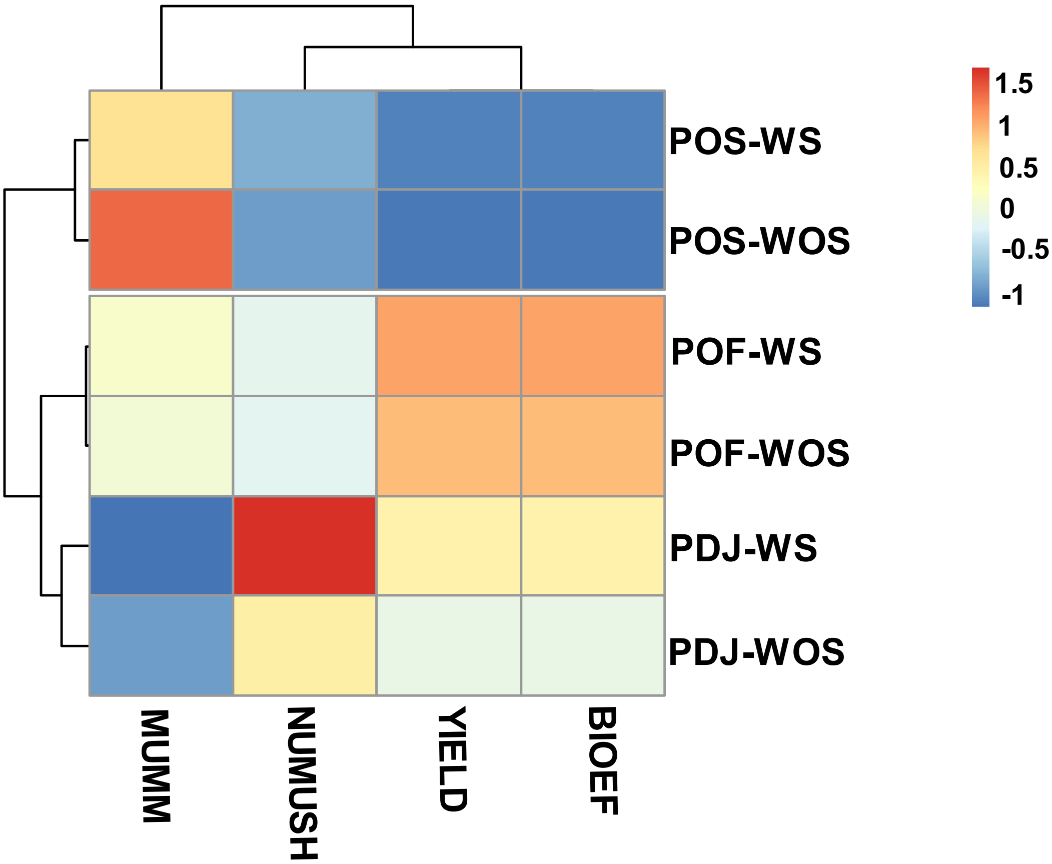

Figure 3. Heat map based on mean Euclidean distance and Ward's hierarchical clustering method, in the cultivation of *Pleurotus*. In the upper part, it indicates grouping in relation to agronomic parameters. On the left side, grouping according to species/varieties and scratching technique. The color scale represents the values of agronomic parameters, being intense red color represents high value, and intense blue color represents low value, where: POS (*Pleurotus ostreatus*), POF (*Pleurotus ostreatus* var. Florida) and PDJ (*Pleurotus djamor*), submitted (ws) or not (wos) to scratching technique. Agronomic parameters indicated by mean mushroom weight (Mmw), number of mushrooms (Nmush), yield (Yield) and biological efficiency (Bioef).

Another important point refers to the Euclidean distance of the clusters on the left, which the furthest being *P. djamor* and the closest being *P. ostreatus* var. Florida, which denotes the great influence of the scratching technique for *P. djamor* and the smallest influence for *P. ostreatus* var. Florida, in relation to the values of yield, number and weight of mushroom, and biological efficiency obtained (agronomic behavior).

In relation to the above grouping, from the analyzed variables (yield, number and weight of mushroom, and biological efficiency), it is evident that the mushroom number, productivity and biological efficiency are dependent, while in another direction was the mushroom weight, showing a negative Pearson correlation (r = -0,928), as shown in Figure 4.

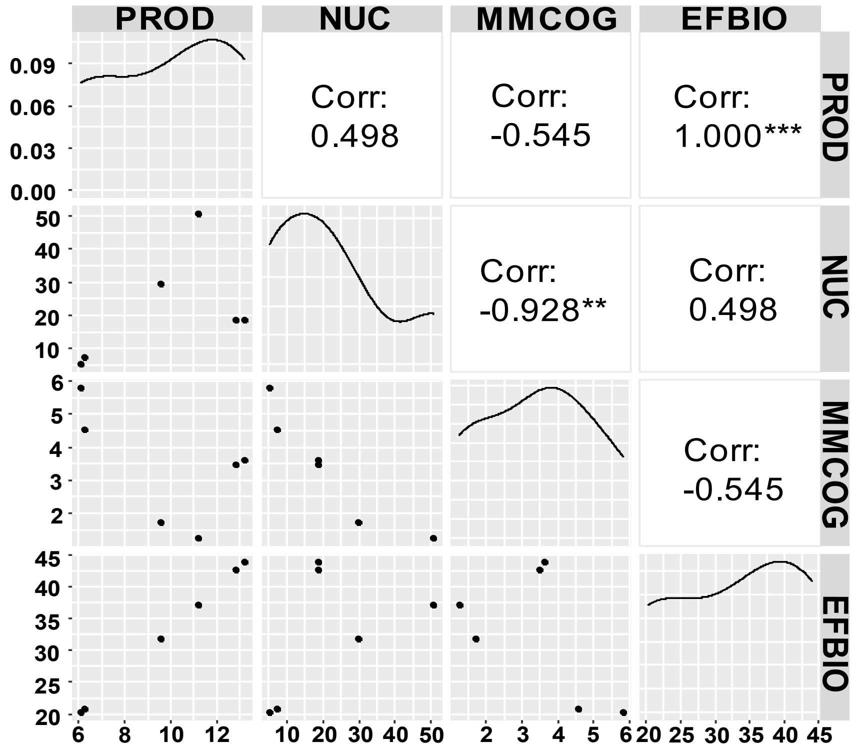

Figure 4. Scatter plot and Pearson's correlation coefficient matrix for comparisons between agronomic behavior data *Pleurotus* species/varieties, where: Numush: number of mushrooms, Mmw: weight of mushroom, and Bioef: biological efficiency. *Indicate statistical difference (p<0.05), ** difference with negative correlation, and *** difference with positive correlation.

According to Figure 5, it can be noted that for *P. ostreatus* var Florida, scratching done before the first flush tended to increase the yield in this flush, decreasing it in the second. On the other hand, *P. djamor* with scratching just done before the second flush reduced the yield in this flush. The scratching technique does not change the precocity in the studied species/varieties.

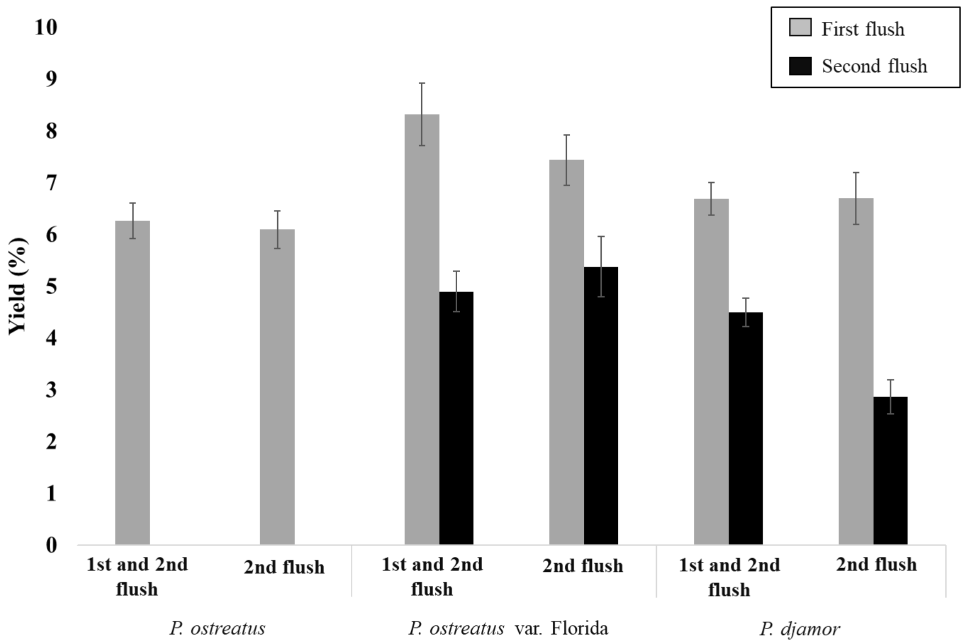

Figure 5. Yield of *Pleurotus* according to harvest flushes, with scratching done in the first and second flush and scratching done only in the second flush.

DISCUSSION

Oliveira and Naozuka [23], studying *P. ostreatus* and *P. djamor* in sugarcane bagasse-based substrate, obtained 21 and 26% of biological efficiency, respectively, which is in agreement with the results of this research, since that *P. djamor* showed biological efficiency superior to *P. ostreatus* in both treatments, with and without application of scratching. Likewise, Atila [24] obtained yield and biological efficiency with values even higher than those found in this research cultivating *P. djamor*, which highlights the importance of using lignocellulosic residues, such as sawdust, for oyster cultivation.

Among the species studied, *P. ostreatus* had the lowest biological efficiency, with 20.85% and 20.28% for the treatment with and without scratching in only one flush. These values are considered low, since Curvetto and coauthors [25] reached values of biological efficiency between 37.2% and 73.6%. *P. ostreatus* has the ability to adapt to different temperatures, being able to be cultivated between 20 and 25ºC [7], so this factor was not limiting in the yield of the species in this work, where the cultivation was conducted at 21± 2ºC.

On the other hand, it is observed that *P. ostreatus* demands low temperatures for fruiting induction, ranging from 10 to 15ºC [26,27], unlike the other species, where induction occurs between 18 and 25ºC (*P. djamor*) and around 26±5ºC for *P. ostreatus* var. Florida [7, 28]. In this case, the absence of ideal conditions for induction of primordia may be reduced the efficiency of *P. ostreatus* in these cultivation conditions, since the temperature remained stable.

P. ostreatus and *P. djamor* species have a common method of substrate treatment, which is autoclaving and larger range of fruiting temperatures. On the other hand, *P. ostreatus* var. Florida has different treatment methods, and a smaller temperature variation (Table 3). So, the first factor that appears to influence *Pleurotus* yields is the substrate formulation process, and second the cultivation conditions. The number of flushes and production time is also important factor that be considerate due biological efficiency influence.

Table 3. Comparison between biological efficiency of *Pleurotus spp.* according to the cultivation conditions.

References	Base substrate	Substrate treatment	Biological efficiency (%)	Number of flushes	Cultivation conditions	
					Temp (°C)	MSTR (%)
			Pleurotus ostreatus			
This Work	***Eucalyptus* sawdust**	**Autoclaving**	**20.85***	**1**	**21 ± 2**	**75 ± 5**
[23]	Sugarcane bagasse	Autoclaving	21	1	24 ± 1	80
[11]	Bamboo sawdust	Autoclaving	44.09	-	14 ± 1	90 ± 5
[29]	*T. scleroxylon* sawdust	Autoclaving	50.93	6	28 ± 3	90 ± 5
			Pleurotus ostreatus var. Florida			
This Work	***Eucalyptus* sawdust**	**Autoclaving**	**43.33***	**2**	**21 ± 2**	**75 ± 5**
[30]	Wheat straw	Chemical disinfection	13	5	25 ± 3	77.5 ± 2.5
[25]	*B. dictyoneura*	Pasteurization	138.7	4	26 ± 5	80 ± 10
[21]	*Andropogon* straw	Pasteurization	12.3	3	-	-
			Pleurotus djamor			
This Work	***Eucalyptus* sawdust**	**Autoclaving**	**37.23***	**2**	**21 ± 2**	**75 ± 5**
[23]	Sugarcane bagasse	Autoclaving	26	1	24 ± 1	80
[24]	Stem of *Phaseolus*	Autoclaving	78.2	3	17 ± 1	85
[31]	Sugarcane bagasse	Autoclaving	50.59	5	25 ± 3	77.5 ± 2.5

*Value corresponding to the average of 1st and 2nd flush. Temp (Temperature) and MSTR (Moisture).

Regarding the number of mushrooms, Shubhra and Jaitly [30] studying *P. ostreatus* var. Florida and *P. djamor* in wheat straw, produced 6.33 and 8.67 mushrooms per 0.5 kg of substrate, values below those found by this research, demonstrating that it is possible to produce a high number of mushrooms using the scratching technique. Shubhra and Jaitly [30], cultivating *P. ostreatus* var. Florida in wheat straw, obtained mushroom greater than that of *P. djamor*, similar our results.

Victor and Ifeanyi [32], producing *P. ostreatus* in sawdust supplemented with 10% wheat bran, similar to the formulation studied here, reached a yield of 10%. Yamauchi and coauthors [11] cultivating *P. ostreatus* on bamboo sawdust and rice bran, applied the scratching technique on opened substrate bags, with the aim of breaking the surface layer of mycelium. Although the authors did not compare the application or not of scratching, they reached high values of mushroom number (33.6 mushrooms/0.6 kg of substrate) and productivity (97.9 g/0.6 kg of substrate).

Taking into account that the first flush is usually the most productive for this genus, the results of this research follow the results obtained by Atila [24], Ashraf and coauthors [33], Obodai and Vowotor [29], and Ali and coauthors [34], where all had the highest yield in the first flush and lower in the subsequent flush.

One of the biggest costs in mushroom production is labor. Araújo and coauthors [35] found that 10.03% of the cost for the production of substrate in the production of *Agaricus bisporus* is related to labor, and in cultivation this percentage rises to 56.29%. In this sense, proposing another process in the production chain requires an expressive response in yields, which justify its addition. Therefore, no significant increases in yield were observed for the species *of P. ostreatus* and *P. ostreatus* var. Florida that supports the use of the scratching technique. In the same way, the species of *P. djamor* obtained a short increase in productivity (1.63%), which does not allow the use of the technique, even due to the increase in the number of mushrooms that reflects a lower weight of mushroom, which affects the commercial value [36,37] and harvest time.

As already discussed, different materials in the formulation of substrates can provide higher or lower yields (Table 3). It is important to point out that a better agronomic behavior can be achieved through the adoption of other technologies and cultivation characteristics, highlighting the importance of future studies on the scratching technique in *Pleurotus* that address other conditions and cultivation techniques.

CONCLUSION

The effectiveness of the scratching technique can be selective for different species/varieties of *Pleurotus*, been *P. djamor* higher yields and *P. ostreatus* had losses in weight of mushrooms. The technique is not indicated for the species studied here, as it promotes an increase in labor during cultivation and, on the other hand, offers little or no gain in yield.

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Conflicts of Interest: The authors declare no conflict of interest.

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