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Tecnologic Development on *Pleurotus* Cultivation: Specific Practices Used in Brazil

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HIGHLIGHTS

- *P. ostreatus* and *P. sapidus* are the most productive species under the evaluated conditions.
- Different growing systems are suitable for the production of *P. ostreatus* var. Florida.
- Temperature control level affects differently the *P. ostreatus* var. Florida isolates.
- Environmental and strain factors affect yield and production parameters of *P. ostreatus* var. Florida.

Abstract: In Brazil, *Pleurotus* is the most important mushroom produced especially *P. ostreatus* var. Florida. In this country as in many others, the great potential for mushroom cultivation remains unexplored. Therefore, it is very important to develop new studies that allow optimizing its production. The aims of the manuscript were: i) to evaluate the productivity of six different species of *Pleurotus* (*P. citrinopileatus*; *P. djamor*; *P. ostreatus*; *P. ostreatus* var. Florida; *P. pulmonarius*; *P. sapidus*); ii) to measure the effect of three different environmental conditions during cultivation of three isolates of *P. ostreatus* var. Florida. As results, *P. ostreatus* and *P. sapidus* were the most productive isolates under the evaluated conditions. Different environments produced variable effects according to the *P. ostreatus* var. Florida isolates, being possible to observe a highly plastic strain (POF 02/18), a highly sensitive strain (POF 03/18) and a strain with variable responses (POF 01/18).

Keywords: Oyster mushrooms; edible mushrooms; food production; bioconversion; agro-industrial wastes.

INTRODUCTION

According to the growth projection scenario of the United Nations, the global population will reach 9.5 billion by 2050 [1] and in the next 50 years, the planet will need to produce more food than in the last 5,000 years, and the demand for protein will double according to the Food and Agriculture Organization of the United Nations [2]. Production of animal protein is very tasking on the environment and one reason for this is the efficiency (or inefficiency) of conversion of feed into animal tissue. In addition to land use, livestock production has an enormous role in soil degradation, water depletion and pollution, impact on biodiversity and a disturbance of the nitrogen and carbon cycles [3].

Looking at all these scenarios there is a need to focus on other available food supplementary that allows to survive the future conditions and avoid malnutrition, even in Brazil, which has always had a great tradition of animal production. For this purpose one of the best resources that exists are mushrooms. Mushrooms have the highest protein production per area and time unit i.e. 100 times more than the conventional agriculture and animal husbandry through their indoor cultivation in vertical space [4]. In Brazil, *Pleurotus* is the most important; more than 7475 tons are harvested annually representing a 48% of the total production [5] among the most cultivated species and *Pleurotus ostreatus* var. Florida stands out. However, the mushroom consumption in Brazil is still low compared to the European countries, where mushroom consumption is high and where families include mushroom cultivation as a domestic activity [6].

The price of mushrooms in the Brazilian market is very high, probably due to the lack of standardized technologies and consequent unstable production, in addition to other reasons. For many years, the mushroom cultivation technology used in Brazil was adapted from developed countries whose materials and climate were different from those of Brazil. In order to exploit the Brazilian potential for mushroom cultivation it is essential to develop cultivation technology for both, small growers and large mushroom farms [7]. Strain selection and temperature control during cultivation are among the major ecological factors that affect not only yield, but also morphological characteristics such as stalk height, stalk diameter and cap size [8]. Oyster mushroom can grow at moderate temperatures, ranging from 18 to 30 °C [9]. However, several authors have reported very different responses to temperature condition during fruiting body induction, depending on the cultivated species of *Pleurotus*, or even the strain [10,11]. The combination of the best air temperature, moisture, nutrient conditions as well as other variables, provides a synergistic effect optimizing the production of mushrooms, with a consequent increased production and cost reduction [12].

In order to provide information regarding the agronomic performance of some of the most widespread *Pleurotus* species in Brazil, and some specific isolates commonly used by local laboratories/companies, the aims of this work were: i) to evaluate the productivity and others agronomic parameters of six different species of *Pleurotus* (*P. citrinopileatus; P. djamor; P. ostreatus; P. ostreatus var. Florida; P. pulmonarius; P. sapidus*) and; ii) to measure the effect of the environmental condition given by different growing room technological levels on the performance of three isolates of *P. ostreatus* var. Florida.

MATERIAL AND METHODS

Substrate production

Substrate production was the same for both experiments. *Pleurotus* substrate was prepared using a method of short composting, totaling 8 days of substrate preparation, 6 days for Phase I and 2 days for Phase II (semi-composted system). During Phase I process, 500 kg of *Brachiaria dictyoneura* and 500 kg of sugarcane bagasse (bulk material) were moistened for 2 days. On the 3th day, the pile was assembled and on the 4th day, the pile was turned. Then, the additional materials were added: rice and wheat bran (15 kg of each), calcitic limestone and gypsum (10 kg of each). Afterwards, two more turns were performed and at the 7th day the substrate was transferred to a pasteurization chamber (Phase II). The substrate was pasteurized at 68 °C during 24 hours and subsequently conditioned between 52 and 48 ºC for one day. After the Phase II process, the chemical characteristics of the substrate were analyzed (using three substrate samples for each nutrient).

Pleurotus species

The strains evaluated in the first experiment were the following: PCI 01/18 (*P. citrinopileatus*); PDJ 01/18 (*P. djamor*); POS 01/18 (*P. ostreatus*); POF 01/18 (*P. ostreatus* var. Florida); PPU 01/18 (*P. pulmonarius*) and PSA 01/18 (*P. sapidus*). In the second experiment POF 01/18, POF 02/18 and POF 03/18 isolates of *P. ostreatus* var. Florida were used. These isolates were obtained from different growers from Sao Paulo and Parana States (Brazil). They were deposited in the public culture collection of Sao Paulo State University, Dracena Campus, with open access to other researchers.

Inoculation

A number of 16 holes were made in all bags (1.8 cm of diameter) previous the substrate inoculation. After phase II process the spawn was thoroughly mixed with the substrate (2% of wet substrate). The mixture were packed into plastic bags (2 kg wet substrate) and for the first experiment substrate bags were incubated during 13 days in the mushroom house used specifically for *Pleurotus* cultivation, at 75 ± 5% relative humidity, and without ventilation. Under these conditions, the substrate temperature was kept at 26 ± 2 °C. For the second experiment, incubation was carried out in the growing systems (controlled, semi-controlled and low controlled)**,** which the environmental variables were influenced according to the technological degree of the cultivation environment.

Growing and harvest conditions

For both experiments, relative humidity during cropping period was 85 ± 10%. For the first experiment, temperature was 25 ± 3 °C. For the second experiment, temperature conditions variated according to the cultivation environment (controlled, semi-controlled and low controlled). Mushrooms were collected twice a day during each flush, weighed and counted for the analysis of the production parameters.

Evaluated parameters

The Automatic Meteorological Station recorded weather conditions during the cultivation period. The following production parameters were evaluated in both experiments: i) the yield calculated as 100 times the fresh weight (f.w.) of mushrooms divided by the f.w. of substrate, expressed as a percentage; and ii) the clusters mean weight calculated as weight of clusters harvested divided by the cluster number. Also, for the second experiment were measured: iii) the number of clusters harvested; iv) the mushroom number; v) mushroom mean weight calculated as total f. w. harvested during the cycle divided by the mushroom number; and vi) the production distribution (%) through the days of crop period.

Experimental details and statistical analyses

The first experiment was done in a completely randomized design with six replicates (bag with 2 kg of substrate). Six different species of *Pleurotus* (each species represented a treatment) were compared in terms of productivity. The second experiment was carried out using a double factorial completely randomized design, with three isolates of *P. ostreatus* var. Florida × three environmental conditions (total controlled system; semi-controlled system that provides less variation in temperature and humidity due to the thermal insulation of the chamber with Styrofoam; and finally plastic greenhouse with only partial control of relative humidity that provides large variation in the temperature during the day).

Experiments data were submitted to analysis of variance and the means were compared by Tukey test (5%). For each variable, the mean values and the MSD (minimum significant difference; Fisher's Least Significant Difference; alpha 0.05) were calculated.

RESULTS

Characteristics of the analyzed substrate were the following: nitrogen: 1.1 g kg^{-1}; organic matter 931 g kg^{-1}; organic carbon 83 g kg^{-1}; C/N ratio 75/1 and pH 7.7.

During the cultivation period, the average temperature outside recorded was 26°C with maximum and minimum peaks of 30°C and 23.2°C, respectively. The average relative humidity (RH) was 74.2% and the accumulated rainfall for this period was 181.5 mm.

1st Experiment

The isolates tested were photographed during fruiting (Figure 1). The two variables analyzed (yield and cluster mean weight) showed statistically significant differences (Figure 2).

Figure 1. *Pleurotus* species (A: PCI 01/18; B: PDJ 01/18; C: POS 01/18; D: POF 01/18; E: PPU 01/18; and F: PSA 01/18).

Maximum yield was achieved by POS 01/18 and PSA 01/18 strains, with the values of 19.93 and 17.76%, respectively. The lowest yield was for PCI 01/18 (11.56%) and PDJ 01/18 strains (12.08%) (Figure 2). Respect to cluster mean weight PSA 01/18 strain obtained the highest registered weight (35.87 g), while PDJ 01/18, POF 01/18 and PPU 01/18 strains reached the lowest cluster mean weight (11.75, 18.19 and 19.82 g, respectively).

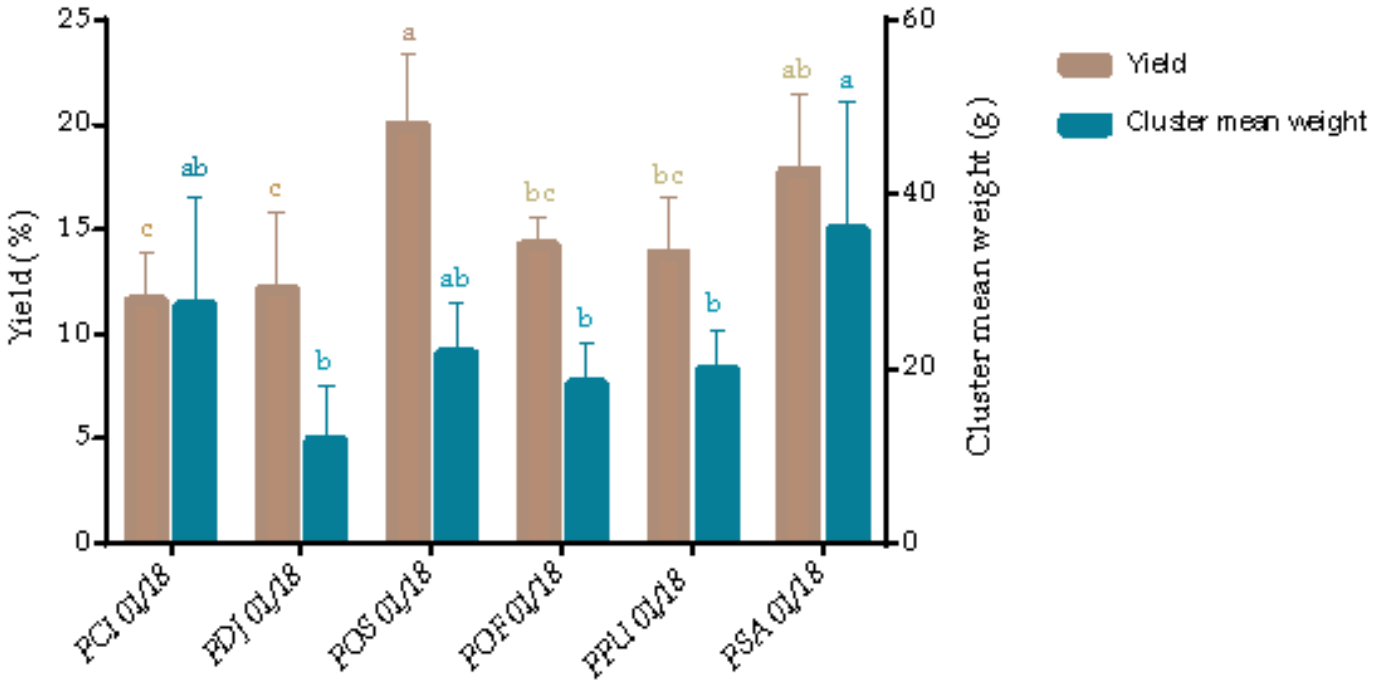


Figure 2. Yield (%) and cluster mean weight (g) of *Pleurotus* species. Different letters indicate significant differences at $p < 0.05$ according to Tukey's test, n = 6. Yield mean: 14.8 and yield DMS: 5.3. Cluster mean weight mean 22.41 and cluster mean weight DMS: 10.68.

2nd Experiment

Regarding to mushroom production, the effect of environmental conditions was observed for POF 01/18 and POF 03/18 isolates (Figure 3). POF 01/18 reached higher yield values in controlled and low controlled systems (14.57% and 11.89%, respectively) than in semi-controlled system (7.24%). POF 03/18 reached the highest yield value in controlled system (9.23%) and the minimum yield value in low controlled systems (3.60%). The strain POF 02/18, on the contrary, stood out for not presenting significant differences in mushroom yield in the controlled system (8.00%), semi-controlled system (10.12%) or low controlled system (10.20%). Therefore, the effect of environmental conditions was dependent on the mushroom strain.

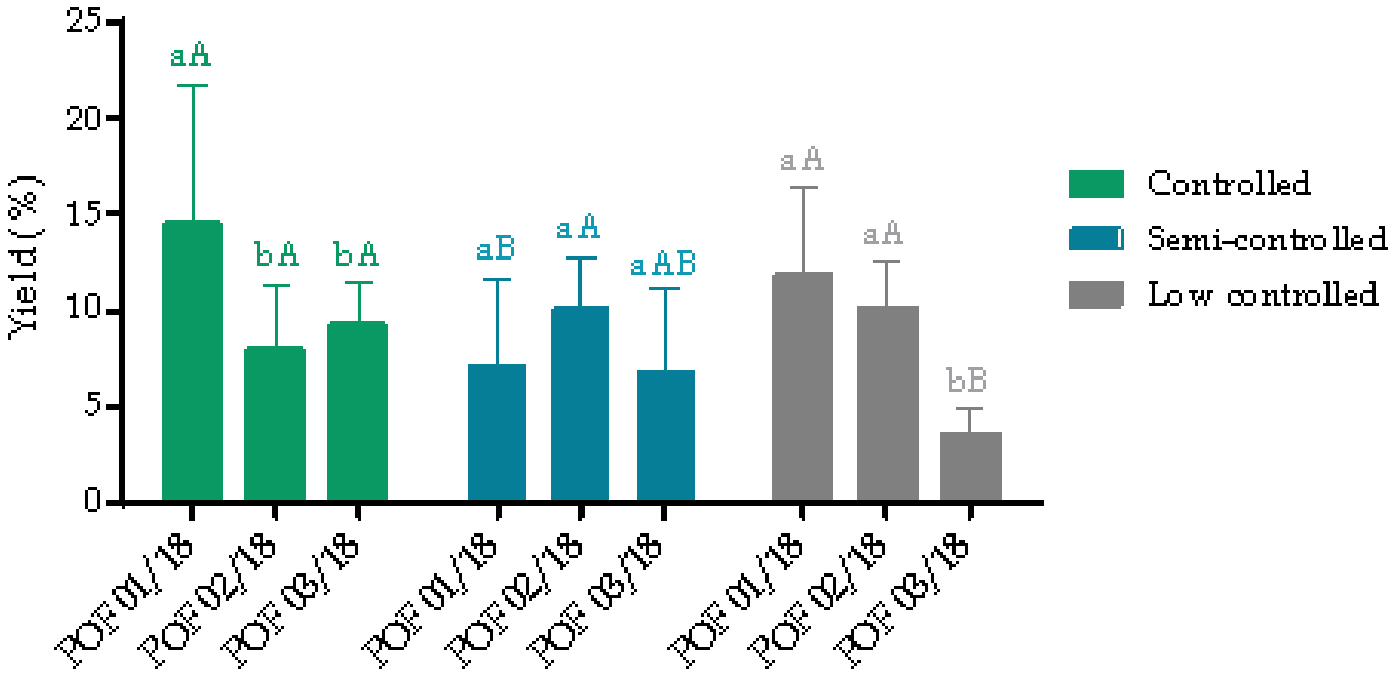


Figure 3. Yield (%) of *P. ostreatus* var. Florida isolates in different growing systems. Different lower case letters mean significant differences between the isolates and different upper case letters mean significant differences between the growing systems at $p < 0.05$ according to Tukey's test, n = 6. Mean: 9.10; DMS: 4.70.

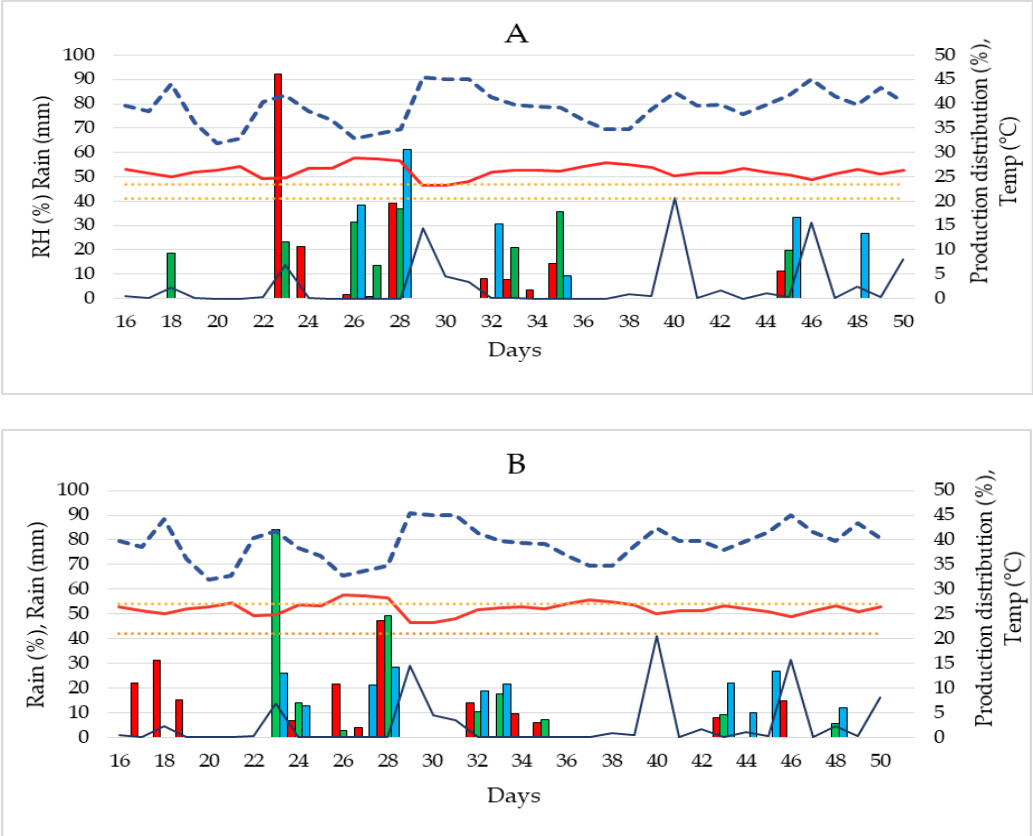
All the productive parameters showed significant differences. The strain factor influenced two analyzed variables (cluster number and mushroom number) while environmental factor influenced three variables analyzed (cluster mean weight, mushroom number and mushroom mean weight) (Table 1). For the cluster number, the influence of the strain factor was verified in the low controlled system, where POF 03/18 strain presented the lowest value registered and the POF 01/18 strain presented the highest cluster number value. For the cluster mean weight, the influence of the environment factor was verified on POF 01/18 strain, it shows the highest reported value in the controlled system and the lowest value was showed for semi-controlled system. Regarding to the mushroom number, the influence of the environment for POF 03/18 strain was verified, which presented the lowest value recorded in the low controlled system and highest to semi-controlled system. POF 01/18 strain showed the highest number of mushroom harvested comparing with the others isolates in the low controlled system. Regarding to the mushroom mean weight, the influence of the environment for POF 03/18 strain was shown. This strain presented the maximum and minimum values recorded for the low controlled and semi-controlled systems, respectively.

Table 1. Productive parameters of *P. ostreatus* var. Florida isolates in different growing systems.

Strain	Growing system					
	Controlled	Semi-controlled	Low control	Controlled	Semi-controlled	Low control
	Cluster number			Cluster mean weight		
POF 01/18	6.83 a A	4.67 a A	6.50 a A	60.24 a A	35.49 a B	39.18 aAB
POF 02/18	4.83 a A	5.67 a A	4.17 ab A	40.48 a A	35.57 a A	55.55 a A
POF 03/18	4.17 a A	4.50 a A	1.75 b A	50.45 a A	29.14 a A	46.02 a A
Mean		4.80			43.77	
DMS		3.49			21.17	
	Mushroom number			Mushroom mean weight		
POF 01/18	88.50 a A	59.16 a A	90.00 a A	4.10 a A	2.61 a A	4.35 a A
POF 02/18	50.33 a A	79.33 a A	55.00 b A	3.26 a A	2.45 a A	4.36 a A
POF 03/18	51.00 a AB	57.33 a A	15.75 b B	4.35 a AB	2.39 a B	7.31 a A
Mean		61.42			3.95	
DMS		42.48			3.67	

Values followed by different lower case letters within a column and upper case letters within a line are significantly different at $p < 0.05$, according to Tukey's test, $n = 6$.

In Figure 4 it is possible to verify that for the controlled system the start and the end of production were on the 18th and 48th, respectively. In the semi-controlled system, production began on the 16th and ended on the 48th. For the low controlled system, a lower precocity was verified since the start of production was on the 23rd and the crop cycle ended 50 days, i.e. it lasted two more days compared to the other systems. For POF 01/18 strain in controlled and low controlled systems, this strain reached 94% of its production in 35 days in the controlled system and 68% for the same period in low controlled system. Concerning to POF 02/18 strain, it showed low variation in the production times since, in 35 days it reached a production of 90% for controlled system, 93% in the semi-controlled system and 85% in the low controlled system.



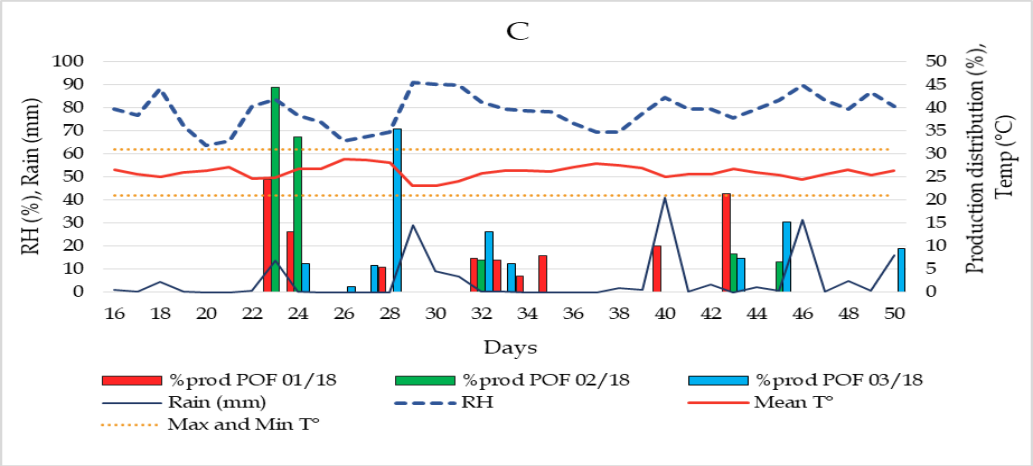


Figure 4. Production distribution (%), weather conditions (temperature, relative humidity and rainfall) and maximum and minimum temperature range in growing system with total environmental control (A); growing system with semi environmental control (B) and growing system with low environmental control (C).

DISCUSSION

The results obtained provide information regarding the yield and the main productive characteristics of some of the most commonly *Pleurotus* species used in Brazil by mushroom companies and local laboratories.

1st Experiment

The greater performance of *P. ostreatus* is in agreement with many previous works where performance of several species of the genus *Pleurotus* were compared [17,18]. However, there are also many works where the results were different [19,20,21]. From the literature, yields of cultivated mushrooms always differ between fungal species, type or size of substrate, supplementation of substrates and other varying growth factors [17].

P. ostreatus mushroom yield (17.8%) was similar to that obtained by [22] which used a sugarcane bagasse-based compost with *Brachiaria* and supplements (17%). *P. sapidus* also reached a yield of 17.8%, which is an intermediate value among those reported by [23,24] who reported 239 g and 156g for each kg of wet substrate, whose formulation used sisal leaf waste mixed with *Panicum coloratum* [23] and whole cattail weed [24]. These values correspond to a yield of 23.9 % and 15.6% according to the equation used in this work.

P. pulmonarius reached a yield of 13.78%, which is slightly higher than those reported by [25] on maize stalk supplemented with maize flour residues (11%). The yield of *P. djamor* (13.36%) was slightly lower than the reported value [26] who obtained 156 g of fruit bodies in 750g of sugarcane bagasse-based substrate, which would be equivalent to a yield of 20.8%, while [27] reported 4.68% in the same substrate. In this work, *P. ostreatus* var. Florida reached a yield of 14.2% whereas Biswas and coauthors [28] reported 218 g of fresh mushrooms in 4.66 kg of sugarcane bagasse-based substrate, which is equivalent to a yield of 4.67%. The yield of *P. citrinopileatus* (11.5%) was lower than that reported by Atila [29] (19.99%) on oak sawdust-based substrate supplemented with wheat bran and by [30] who obtained a yield of 33% on a wheat straw-based substrate.

Hoa and coauthors [31] associated the differences in terms of yield to the differences on substrate types, which include cellulose/lignin ratio, mineral contents, pH, electrical conductivity and carbon nitrogen ratio (C/N). Bellettini and coauthors [12] lists a large number of extrinsic factors such as substrate heat treatment, cultivation room temperature, humidity and CO_2 content, which affect in the performance of the *Pleurotus* spp.

Although *P. ostratus* var. Florida is the most cultivated species in Brazil, as it allows cultivation at high temperatures (supporting up to 31 °C), it is essential to highlight the importance of this study to look for alternatives of *Pleurotus* species for winter cultivation. Thus, the species *P. ostreatus* (POS 01/18) and *P. sapidus* (PSA 01/18) can be indicated for cultivation with temperature control about 25 ± 3 °C. It should be emphasized that the average weight of the cluster is a variable that is proportionally associated with the mushroom number per cluster and the mushroom weight, but also depends on the morphology of each species.

2nd Experiment

The three growing systems evaluated were suitable for the production of different isolates of *Pleurotus var*. Florida. Even in the rustic plastic greenhouse, the versatility of oyster mushrooms allows their cultivation with a minimal technological investment [13], reported that *Pleurotus* spp. are the easiest, quickest and least expensive species to grow since it requires low cultivation technology. However, the three isolates evaluated showed different responses for both yield and some variables analyzed.

POF 03/18 isolate showed a high variation according to the cultivation system with the worst performance in the low controlled system. On the contrary, POF 02/18 strain showed no significant differences between environmental factors, which indicates a great plasticity to the different growing systems and thermal amplitude.

The results reported here emphasize the importance of adjusting the mushroom cultivation technology with the most appropriate isolates for those growing conditions. Therefore, the best isolates could be selected according to each mushroom grower, environmental conditions and mushroom facility.

It was notable that in the semi-controlled system three isolates showed the lowest values of average mushroom weight and average cluster weight, which could indicate that systems with temperatures of 24 ± 3 °C could be a good strategy to use in the production of small mushrooms of *P. ostreatus* var. Florida. Small fruit bodies tend to be more palatable because of their lower fiber content. Moreover, the large sized fruit bodies are considered as an inferior quality since such fruit bodies tend to break during packaging and decreases the shelf life, thus reducing their quality [14].

P. ostreatus var. Florida is a variety developed for high temperature cultivation [15]. However, a shorter time for the first harvest was observed in the controlled and semi-controlled systems for two isolates. Probably, the lower temperature may have acted as a factor in pinning [16]. This induction was absent in the low controlled system and fruiting began after registering a decrease in external temperature (Figure 2C). Similar results were reported by [10] when compared the production of *P. florida* in two different culture systems: a concrete room with 20-30 °C and a regular thatched method with 25-30°C, where they observed 18 and 26 days for the first harvest, respectively.

The highest yield was obtained from POF 01/18 strain in the controlled and low controlled systems. However, it is important to consider the cultivation cycle, since the highest percentage of total production in the shortest time was obtained in controlled system. In the case POF 02/18 strain, although it was not the most productive strain, it could be successfully cultivated in mushroom farms with low technological level since it was not affected either in yield or in cultivation cycles in any of three evaluated systems. Finally, POF 03/18 strain could be successfully cultivated only in systems with temperature control.

CONCLUSION

According to results of the present study in first experiment under the evaluated conditions, the most productive species were *P. ostreatus* and *P. sapidus*, which could be diversification alternatives to the cultivation of *P. ostreatus* var. Florida. In second experiment, the different environments produce very different effects according to the isolate of *P. ostreatus* var. Florida used, being possible to observe a highly plastic isolate (POF 02/18), a highly sensitive (POF 03/18) and a isolate with variable responses (POF 01/18). These results suggest a genetic variability between the commercial isolates from *Pleurotus ostreatus* var. Florida. Thus, it is essential to select the strain that best suits the growing conditions of each mushroom growing establishment.

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Conflicts of Interest: Declaration of interest statement Authors declare that they have no conflict of interest.

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