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In vitro cultivation of *Pleurotus ostreatus* and *Lentinula edodes* in lignocellulosic residues from Amazon

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The mycelial growth speed of *Pleurotus ostreatus* (POS 09/100) and *Lentinula edodes* (LED 96/13) was evaluated in four substrates based on *Simarouba amara* sawdust, *Anacardium giganteum* sawdust, *Euterpe precatoria* seeds and *Musa* sp. AAB stems. The loss of organic matter of the substrates and the mycelial vigor of the strains studied were also evaluated. The greatest mycelial growth for *P. ostreatus* occurred in the substrates formulated with *S. amara* sawdust (29.45 cm^3/day) and *A. giganteum* (27.58 cm^3/day). The best performance for *L. edodes* occurred in the substrate of *A. giganteum* (13.22 cm^3/day), followed by the *S. amara* (11.30 cm^3/day). The most intense rates of vigor were presented in the *E. precatoria* substrate which was added with brans. The most significant loss of organic matter for both mushrooms occurred in the substrate formulated with a supplement of *A. giganteum* (54% for *P. ostreatus* and 61% for *L. edodes*). There was no mycelial growth in the formulation prepared with banana stem in both mushrooms tested.

Key words: Edible fungi, mycelial growth, alternative substrates.

INTRODUCTION

The importance of mushrooms cultivation is significant because it represents an efficient choice to enable the use of organic matter for bioconversion into high- value added products, such as edible mushrooms (Sales-Campos et al., 2010). *Pleurotus ostreatus* and *Lentinula edodes* are among the species with a great economic importance for cultivation. *L. edodes* is high quality food, full of proteins, vitamins and minerals, and low fat and calories, thus being widely suggested for cultivation and consumption (Andrade et al., 2008). According to the study of Bononi et al. (1999), its worldwide consumption has increased significantly, and some European and American countries have been importing this mushroom more and more. There is a potential consumer market in Brazil, although there are only some small producers, despite the presence of a huge Asian colony in the country.

On the other hand, the consumption of *P. ostreatus* has increased significantly in the last years because of its outstanding taste and great availability in the market, occupying the fourth position in the world production of edible mushrooms (Bononi et al., 1999). The *Pleurotus* gender fungi compete with the other edible fungi due to their growth speed and ability to adapt to a series of sub-

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Abbreviations: BOD, Biological oxygen demand; **POS,** *Pleurotus ostreatus*; **LED,** *Lentinula edodes*.

strates (Vieira et al., 2013), which happens because of an enzymatic complex composed of cellulases, ligninases, laccases, xylanases, peroxidases and hemicellulases (Elisashvili et al. 2008). The behavior of the mycelial growth may vary according to a series of factors, such as the fungi species, genetic characteristics of the strain, temperature, type of substrate, supplementation, carbon/ nitrogen ratio, and others (Andrade et al., 2008; Sales-Campos et al., 2008). Several steps must be taken in the production of edible mushrooms from the obtention of the inoculum to the sale of the final product, as stated by Donini et al. (2005).

Studying the conditions of the use of wood in industries from Amazonas, Sales-Campos et al. (2000) verified a loss of up to 60% of this raw material due to lack of technology and appropriate handling, thus generating a great amount of waste with an underestimated potential. The residues generated by this sector in the Amazon region have caused environmental pollution, and some alternatives have been found for making good use of these residues (Vianez and Barbosa, 2003). However, little has been done to soothe this matter. Sales-Campos et al. (2010) showed the use of different wood and agro-industrial residues of the region for the cultivation of edible fungi. Nevertheless, the author reports the need of testing the different substrates in relation to the fungus to be cultivated. Therefore, the objective of the present study was to test the viability of regional residues, aiming at their future use in the cultivation of edible mushrooms, by evaluating the mycelial growth speed of *P. ostreatus* and *L. edodes* in substrates formulated with regional lignocellulosic residues.

MATERIALS AND METHODS

The present study was carried out at the Laboratory of Edible Fungi Cultivation of the Coordination of Technology and Innovation (CTI) of the National Institute for Amazon Research (INPA), Manaus, Amazonas, Brazil. The residues were selected according to the production of local residues. *Simarouba amara* Aubl.(marupá) sawdust and *Anacardium giganteum* Hanc. Ex. Engl. (cajuí) sawdust, obtained from researchers at the CTI/INPA, were used as wood residues. The materials were processed at the CTI saw-mill, dried in solar dryer and stored in plastic bags for the formulation of the substrates. The agro-industrial residues were *Musa* sp. AAB cv. Pacova stalks (pacovã banana) and *Euterpe precatoria* Mart. (açaí), which were ground in a DPM4 forrage grinder, following the same drying and storage processes used for the wood residues.

The preparation of the primary matrix (inoculum) was carried out by transferring small pieces of the mycelium of the fungus (stored inside test tubes) to Petri's dishes containing BDA medium (Bononi et al., 1999). Next, the dishes were incubated inside a biological oxygen demand (BOD) at 25°C for the mycelial growth of the fungus until it reached about three quarter of the dishes. Then, they were kept in cold storage at 4°C to stop the mycelial growth until the experiment was assembled. The *P. ostretus* strain used was POS 09/100 and the *L. edodes* was LED 96/13, which were obtained from the collection of the School of Agronomic Sciences of the São Paulo State University (FCA/UNESP). The substrates used in this experiment followed the methodology adopted by Sales-Campos et al. (2010), with the ratios presented in Table 1. $CaCO_3$ was used for the pH adjustment of the substrates. The substrates were homogenized, moistened for around 75% of humidity and disposed in 500 ml bottles, and each bottle was filled with 200 g of the substrate. Next, they were sterilized at 121°C, under the pressure of 1 atm. Soon after sterilization, they were cooled up to the environmental temperature and taken to a previously sterilized laminar flow chamber in order to avoid contamination and, thus, receive the inoculum from the previously prepared primary matrixes.

The mycelial growth was measured by four millimetric tapes, which were placed equidistantly outside the bottles. The bottles were then stored in a BOD at 25°C, which is considered the optimal temperature for the fungal growth (Bononi et al., 1999). The measurement was performed daily to the point of total colonization of the substrates by the fungus. To compare the growth speed (cm^3/day), data from the 15^{th} day was collected, when one of the fungi reached the bottom of the bottle. The averages of the volumetric mycelial growth were obtained by calculating the cylindrical volume of the colonized solid medium ($B \times r^2 \times H$) and the average speed was evaluated by the ratio between the average volume of growth and the time spent for such growth (Gonçalves, 2002). A subjective scale was used to explain the results of the analysis of the mycelial vigor of the fungi in the different substrates: *, weak; **, regular; ***, good; ****, intense. For calculating the percentage of organic matter loss, the colonized substrates were daily dried and weighed until they obtained constant weight in order to be compared with the weights of the substrates that were not colonized by the fungi (control). The following formula was used:

$$PMO\ (\%) = \frac{\text{Mass of initial substrate} - \text{mass of residual}}{\text{Mass of initial substrate}} \times 100$$

The design of the present experiment was totally randomized 8 x 2 (types of substrates x strains) with 5 repetitions each, totalizing 80 bottles. The experimental results were submitted to parametric variance analysis by the Tukey test at the level of 5% of significance, using the Sisvar 4.2 software. The Origin 3.0 software by MicroCal was used to compare the parameters of the polynomial regression model.

RESULTS AND DISCUSSION

When comparing the different substrates analyzed, the mycelial growth of POS 09/100 and LED 96/13 showed a significant difference in all the treatments, except in the treatments formulated with banana stalks, in which neither of the strains tested grow (Table 2). The mycelial growth for *P. ostreatus* was reached in the SIA-MA SUPL treatment, although it was not statistically different from the SIA-MA N SUPL, the SIA-CA N SUPL and the SIA-AÇA SUPL.

The best result for *L. edodes* occurred for the substrate SIA-CA SUPL, which showed no statistical difference from the SIA-MA SUPL. It was also observed, in a general way, that the supplementation also favored the growing rate of the fungus in the substrate based on marupá (SAI-MA SUPL), as Sales-Campos et al. (2008) evaluated when they analyzed the mycelial kinetic of *P. ostreatus* grown in *S. amara* sawdust to determine the

Table 1. Mixing ratio of ingredients for each substrate formulation.

Substrate	Ingredients %					
	Marupá sawdust	Cajuí sawdust	Açaí seed	Banana marc	Bran mixture	$CaCO_3$
MA-SUPL	80				18	2
CA-SUPL		80			18	2
AÇA-SUPL			80		18	2
BAN-SUPL				80	18	2
MA N SUPL	98					2
CA N SUPL		98				2
AÇA N SUPL			98			2
BAN N SUPL				98		2
Mixture of bran cereal, rice, corn and wheat in proportions of 7:2.5:0.5.						
Represents the SUPL supplemented substrates and N SUPL not supplemented.						

Table 2. Mean speed of mycelial growth (cm^3 / day) strains POS 09/100 and LED 96/13 on different substrates based on regional lignocellulosic residues, after 15 days of incubation.

Lineage	Substrate							
	SIA-MA SUPL	SIA-MA Ñ SUPL	SIA-CA SUPL	SIA-CA Ñ SUPL	SIA-AÇA SUPL	SIA-AÇA Ñ SUPL	SIA-BAN SUPL	SIA-BAN Ñ SUPL
POS 09/100	29.45 Aa	27.30 Aab	24.57 Ab	27.58 Aa	26.75 Aab	19.12 Ac	0.00 Ad	0.00 Ad
LED 96/13	11.30 Ba	1.40 Bcd	13.22 Ba	3.71 Bc	1.47 Bcd	7.43 Bb	0.00 Ad	0.00 Ad

Means followed by the same letters, lowercase and uppercase on each line in each column do not differ by Tukey test at the 5% level of significance ($p < 0.05$).

Table 3. Subjective scale of force mycelial growth on different substrates based on regional lignocellulosic residues.

Treatment	Vigor/substrate	
	POS 09/100	LED 96/13
SIA-MA SUPL	***	**
SIA-MA N SUPL	*	*
SIA-CA SUPL	***	***
SIA-CA N SUPL	**	*
SIA-AÇA SUPL	****	****
SIA-AÇA N SUPL	***	***

*, Weak; **, regular; ***, good; ****, intense.

optimal condition for the mycelial growth. Through the experiment, we verified that there was no growth in the substrates formulated with banana stalks, even with the supplementation of cereal brans. Motato et al. (2006) evaluated the capacity of mycelial growth of *Pleurotus djamor* in banana (*Musa paradisiaca*) agroindustrial residues and abarco (*Cariniana pyriformis*) sawdust under different temperatures and also verified that there was no growth in the substrates formulated with banana stalks, concluding that the pure banana stalks or their mixture with sawdust was not appropriate for the cultivation of this fungus. The probable cause for the restraint in the fungal growth in substrates based on stalks (BAN SUPL and BAN N SUPL) is the presence of a high amount of hydrolysable tannins.

In the evaluation made by Soffner (2001) about the potential use of stem of *Musa sp* for pulp production, approximately 46.75% of extractives were found in their chemical composition, which are included in this category of hydrolysable tannins. However, when conducting the processes of washing and pre-aqueous extraction of stem for the purpose of reducing the content of the extractives soluble in water, the author found the washing procedure, followed by pre-extraction aqueous, removed 46.45% of the constitution of the material with an extractive removal efficiency of both fungi showing different vigor for each substrate as shown in Table 3. The results show a relationship between the bran supplementation and the mycelial vigor, where the supplementation promotes the intensity of force in both fungal lineages tested. Observing the two lineages (POS 09/100 and LED 96/13), a greater vigor occurred in the SIA-ACA SUPL substrate, however, it showed a lower growth rate compared to other substrates, by approximately

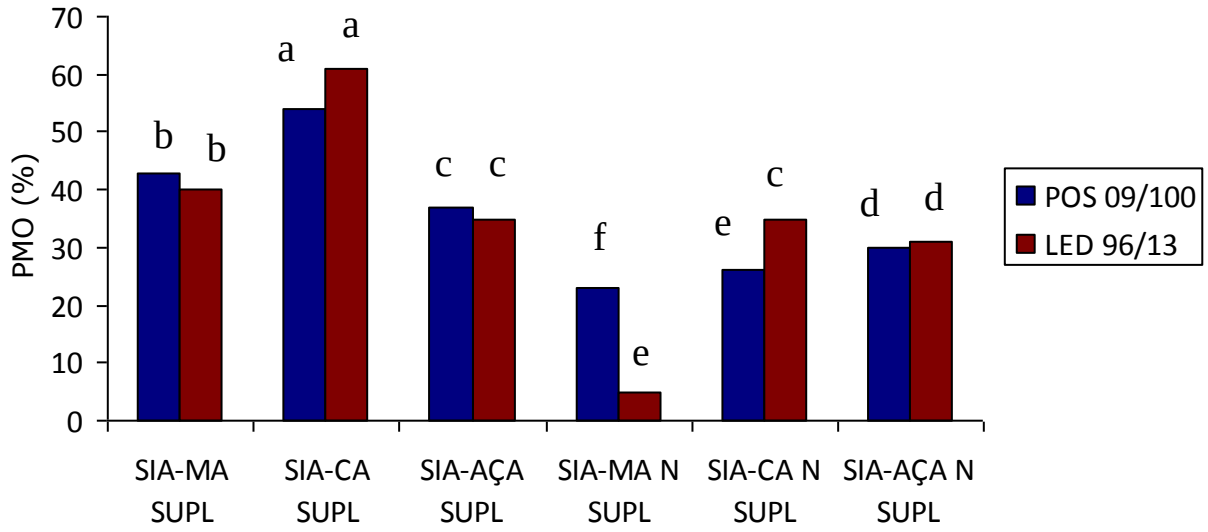

Figure 1. Loss of organic matter (PMO) (%) obtained with *Pleurotus ostreatus* (POS 09/100) and *Lentinula edodes* (LED 96/13) in regional lignocellulosic residues. Means comparison of treatments in each line with the same letter do not differ by Tukey test at 5% significance.

89.7%. Both fungi showed different vigor for each substrate as shown in Table 3. The results show a relationship between the supplementation with bran and mycelial vigor, where the supplementation promotes the intensity of force in both fungal lineages tested. By observing the two lineages (POS 09/100 and LED 96/13), a greater vigor occurs on the SIA-ACA SUPL substrate; however, it showed a lower growth rate compared to other substrates.

Pedra and Marino (2006), during the evaluation of the mycelial growth, induction of primordia precocity and productivity of mushrooms *Pleurotus* spp. in sawdust coconut shell (supplemented with bran) as an alternative substrate for the production of edible mushrooms, found that supplementation with 20 or 40% wheat bran and rice increased the speed and mycelial vigor growth of three strains of *Pleurotus* spp. The present study found similar results, where supplementation improved the rate of mycelial growth and vigor of both lineages.

In the analysis of loss of organic matter (PMO) of the substrates used in the cultivation of POS 09/100, LED 96/13, it was observed that treatment SUPL CIS-CA had the highest amount of loss in both strains (54 and 61%, respectively) differing from the other treatments (Figure 1). The study of Furlan et al. (2000) addressing the adaptation of the production technology of edible mushrooms of the genus *Pleurotus* waste of irrigated rice, supplemented by determining the substrate that best promotes the production, indicated that supplementation had no effect on the rate of PMO where the greatest loss was attributed to treatment Y1 supplemented with a 1% yeast extract (68%), an increase of approximately 9% compared to the control, which received no supplementation. In the present study, the greatest PMO also occured on substrates that received supplementation, although no significant difference was found between strains. This explains why supplementation in the right dose promotes colonization and subsequent decomposition of the substrate, since the fungus removes greater amounts of nutrients for production.

Figure 2 shows a volumetric growth rate (cm3/day) of edible fungus *P. ostreatus* (POS 09/100) and *L. edodes* (LED 96/13) on different substrates. The results of the polynomial regression curves show a very wide instantaneous daily growth for each species, with different peaks being indicatied for each day of incubation (not understood). The mycelial growth on substrates started only from the 3rd day of incubation, and showed an oscillation in its speed over the days.

By analyzing the POS 09/100, polynomial regression curves show a fairly homogeneous growth rate of the fungus in each substrate examined, however, the treatment MA SUPL SIA achieved the best average speed at the outset of colonization, which then remained at a steady speed. The treatment CIS-ACA SUPL grew very slowly on the seventh day of incubation. However, it showed a very high peak on the eighth day and decreased again, re-turning to grow slowly during the day. In the analysis of lineage LED 96/13, there was a variation in speed between the substrates analyzed where we could ob-serve that the highest growth rate occurred with the treatment SIA CA-SUPL. As for the SIA-CA N SUPL, which showed a speed of decreasing colonization, one can observe that, from the fourth day of

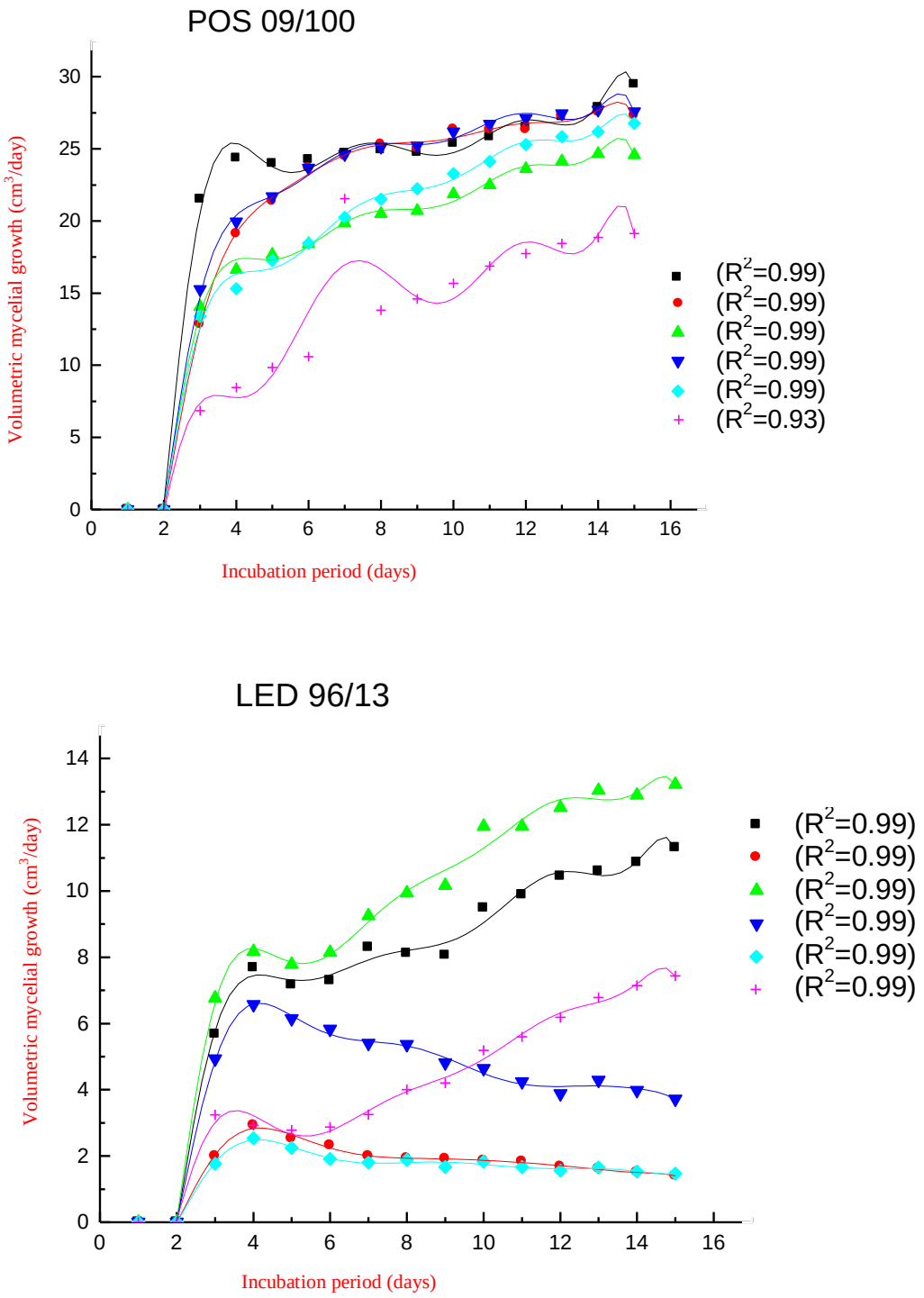

Figure 2. Instant volumetric mycelial growth (cm³/day) from the edible fungus *Pleurotus ostreatus* (POS 09/100) and *Lentinula edodes* (LED 96/13) in different substrates.

incubation, its speed began to decrease gradually. Rossi et al. (2000) argue, in assessing the rate of mycelial growth of *L. edodes*, the effect of the depth of the bottle used and the substrate supplementation with rice bran and molasses from sugar cane, the mycelium of *L. edodes* grows faster when it is nearer the surface of the

substrate, since higher gas exchange occurs, allowing an increased oxygen supply, lignocelulolítica higher activity and thus a greater growth rate.

Conclusion

There was a positive effect on supplementation of cereal bran in all treatments, which allowed a higher growth rate and vigor of mycelial strains. The substrates formulated based on banana stem were not presented as an efficient alternative for growing mushrooms, but there is need for further study on the use of stem to identify ways to make its use in biotechnology mushrooms viable. The substrates source timber had a higher viability for growing mushrooms of the genera *Pleurotus* and *Lentinula*, since the fungi have provided the best indices to ensure a good yield.

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