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Pleurotus ostreatus mushroom productivity in different substrates of agro-industrial residues generated in the southern region of Tocantins, Brazil

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Abstract *— Some of the main agro-industrial residues available in the cerrado region in the south of the state of Tocantins were evaluated for the cultivation of the Pleurotus ostreatus mushroom. The following substrates, hay and andropogon grass, enriched with barley, organic compost, and corn cob, were tested. All substrates received 2% inoculant (Cal) and were incubated at 24°C. After colonization, the spawn were kept in an air-conditioned environment for 24 hours and humidity at 80%. The substrate andropogon grass increased with corn cob added with 2% lime showed the best result in mushroom production, with a biological efficiency of 39.08% and an average production of 381.07 g/Kg substrate in 25 days of follow-up.*

I. INTRODUCTION

With the increase in areas destined for agriculture in the North region, mainly in the Brazilian Cerrado biome, lignocellulosic residues of agricultural and forestry nature are available (SALES-CAMPOS; ANDRADE, 2011). These generated residues can contain great added value because many are rich in nutrients, or, due to the simple fact that reuse already adds more value, they are called residual plant biomass, generated mostly as a result of agricultural activity (BARBOSA et al., 2020; POPPE, 2021).

Some of these materials were tested in our work for the production of the P. ostreatus mushroom, as an alternative production to generate income for family agriculture and small producers, such as brewery residues (barley grain), crushed corn cob, and composting (organic compost). The conversion of these wastes into new products takes place with few resources, reduces environmental damage, and improves the circular economy.

An alternative for the use of these residues is bioconversion. In the cultivation of edible mushrooms, the recycling of these materials can be retransformed into foods

with high food potential, with proven properties such as anticancer, anticoagulant, known in the foreign market and with potential for expansion in the domestic market, rich in proteins, B vitamins, fibers , carbohydrates with low levels of lipids in addition to high levels of glutamate and low levels of sodium (HELENO et al., 2009; CHEUNG et al., 2012; BELUHAN & RANOGAJEC, 2011; BENTO; CASARIL, 2000). The interest of the international scientific community in mushrooms has increased due to these medicinal properties (EIRA, 2003)

II. THEORICAL REVIEW

In Brazil, the most produced and commercialized edible mushroom species are: Lentinula edodes (shiitake), Pleurotus spp. (shimeji, hiratake or houbitake) and Agaricus bisporus (Paris mushroom). The latter, the famous champignon, was the first to be cultivated in Brazil and is the most cultivated and consumed in the world (SANCHÉZ, 2009).

The basidiomycete Pleurotus ostreatus is a lignocellulosic fungus capable of degrading several materials, due to the production of extracellular enzymes, mainly lignolytic enzymes such as laccase (POPPE, 2005; SILVA et al., 2007, LECHNER; PAPINUTTI, 2006). This enzyme acts on lignocellulosic materials, allowing the use of agro-industry by-products, rich in lignin and cellulose, for mushroom cultivation. Thus, mushroom cultivation is a way of adding value to waste that would otherwise be discarded and producing biomass of therapeutic and nutritional interest (EIRA, 2003; ISHIKAWA et al., 2001; SHARMA; MADAN, 1993; SOUZA-PACCOLA et al., 2004).

The edible mushroom Pleurotus ostreatus belongs to the second largest group of mushrooms most produced and consumed worldwide, behind Agaricus bisporus (champignon) and followed by Lentinula edodes (shiitake). Its characteristics have very desirable biological properties, in addition to its delicious flavor and texture, it also has medicinal activities, such as: antitumor and antioxidant, prevents the increase in blood pressure in cases of hypertension and a hypocholesterolemic and immunomodulatory effect, in addition to antibacterial, antifungal and antibacterial activity. antiviral (ADEBAYO et al., 2012).

III. MATERIALS AND METHODS

Microorganism, Culture Media and Inoculum

The experiments were carried out in a cultivation house at the Bioactive Compounds Laboratory of the Federal University of Tocantins on the Gurupi Campus. The fungus Pleurotus ostreatus, (UFT PO Eguirra 2020), belonging to the mycoteca of UFT, Campus de Gurupi (TO) was maintained in the laboratory using PDA medium (potato, dextrose, agar) in Petri dishes, previously sterilized in an autoclave at 124°C for 1 hour.

Substrate preparation

For the tests, the fungi were inoculated in a substrate based on hay and andropogon grass, enriched with barley, organic compost, crushed corn cob 2% lime, with three replications each in two production flows until complete production/colonization with a space of 20 to 30 days between them, as specified in Table 1.

Table 1 - Substrate formulation

Base Substrate	Supplementation (added 2% lime)	Final substrate weight (g)
Hay	Barley (H+Bs)	102,0±3
Hay	Organic compound (H+Oc)	102,0±3
Hay	Corn cob (H+Cc)	102,0±3
Andropogon Grass	Barley (Ga+Bs)	102,0±3
Andropogon Grass	Organic compound (Ga+Oc)	102,0±3
Andropogon Grass	Corn cob (Ga+Cc)	102,0±3

Source: survey data.

To identify the experiments, we used the acronyms where H and Ga are the base substrates (Hay, Grass Andropogon) + the acronym for Barley supplementation (Bs), Crushed corn cob (Cc) and Organic compost (Oc).

Induction

Each residue was left to soak for at least two hours and then drained for 4 hours before packaging. The supplements were added after draining and the substrates obtained were packed in polypropylene bags with a capacity of 1 kg and autoclaved for 1 hour at 121°C. Each substrate bag received 20 g of inoculum, (2%), added to the top of the bag. Subsequently, the bags were partially closed, allowing the gas exchange necessary for mycelial growth.

The bags were incubated in a grow house with wooden shelves framed with fresh (untreated) bamboo under a black canvas cover to provide diffused light. The temperature and humidity were controlled with the help of a 5 liter air humidifier, maintaining a humidity around 90% and air conditioning at an average temperature of 23°C. The experiments were arranged in trays with two units each.

Determination of biological efficiency

All mushrooms harvested were subjected to a gravimetric evaluation to determine biological efficiency, using Equation 1.

$$EB = \frac{\text{Mushroom fresh weight}}{\text{Initial substrate weight}} x100 \quad (1)$$

The experimental design was completely randomized with six replications, using the Tukey test at 5% of significance to compare the means after the Analysis of Variance.

IV. RESULTS AND DISCUSSION

Despite the contaminations, there were important fructifications. Fruiting regularity was not taken into account, considering that each substrate fruited only once, with exceptions made to H+Oc and Ga+Cc substrates, with two and three fruiting moments, respectively. All fruiting mycelia were harvested at the end of the twenty-fifth day (Figure 1).

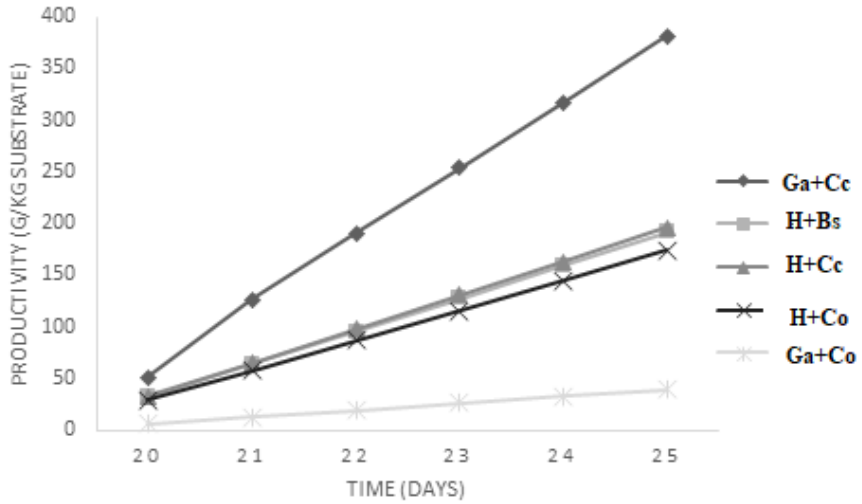

Fig.1. Mycelial growth of the Pleurotus ostreatus strain during 25 days in polyethylene plastic bags. Columns followed by the same letter did not differ significantly from each other by Tukey's test ($p<0.05$).

Source: survey data.

The kinetics show uniform growth between treatments over time, with similar growth rates (Figure 1), except for Andropogon Grass with corn cob (Ga+Cc) substrates. For Ga+M, the average productivity was 63.5 g/kg of substrate/day and for the Ga+Oc substrate it was only 6.53 g/kg of substrate/day, which showed the lowest productivity during the tests. Table 2 shows data on initial and final weight, fresh weight and dry weight of the mushrooms harvested, mycelial development and the number of days the substrate had to express its data.

Table 2 - Experimental cycle data

WEIGHT (g) +2% lime	H+Bs	H+Cc	H+Co	Ga+Bs	Ga+Cc	Ga+Co
initial substrate	102.00	102.00	102.00	102.00	102.00	102.00
final substrate	88.14	75.25	99.83	--	116.52	89.90
fresh mushroom	19.49	20.00	17.73	--	39.87	4.00
mushroom dry	1.50	2.20	3.13	--	4.35	0.40
Production days	23 days	24 days	13 days	--	20 days	23 days

Source: survey data.

The Ga+Bs formulation had substrate contamination not allowing Pleurotus colonization after inoculation, the experimental unit was excluded from the tests. Several attempts were made to reduce substrate moisture, but due to the hygroscopic characteristics of both, the expected results were not obtained. The hay

supplemented with Barley and corn cob had a good efficiency, around 20%, with an average production around 190 g/Kg of substrate (Table 3). In addition to presenting a fast mycelial growth with spontaneous fruiting.

Table 3 – P. ostreatus mushroom production results under different agricultural residues enriched as follows.

Basis	Suplementation	Time (days)	Average Production (g/Kg substrate fresch)	Biological Efficiency (%)
Hay	Barley	23	191.07±0.01[a]	19.06[a]
Hay	Organic compound	13	173.82±0.03[b]	17.38[b]
Hay	Corn cob	24	196.07±0.02[a]	19.60[a]
Andropogon Grass	Barley	-	-	-
Andropogon Grass	Organic compound	23	39.21±0.05[c]	3.92[c]
Andropogon Grass	Corn cob	20	381.07±0.03[d]	39.08[d]

Values are expressed as mean ± SD. Means inthe same row with diferente superscipts were significantly diferente ($p \leq 0.05$). Source: survey data.

The use of hay with organic compost had a lower biological efficiency (17.76%), with a yield of 173.82 g/Kg of substrate. However, its production period was only 13 days, the shortest of all experiments. A slower micellization was observed compared to the others despite having fructification when exposed to light, but with many primordia aborted in the course of mushroom development. After harvest, there was a high rate of contamination of the substrate, it was removed from the experiment after fruiting. The substrate appeared dry and possibly depleted of nutrients.

Andropogon Grass showed not to have a good biological efficiency. Substrate Andropogon Grass suplemented with Organic compost (Ga+Oc) had many pins, however, did not develop, not reaching large fructifications. There was no contamination in the substrate. The substrate was healthy, despite being dry, with little mycelial vigor and low biological efficiency. However, the grass had a good result when complemented with corn cob (34.21%) and a better yield in this work (381.07 g/Kg of substrate). It produced two large sized mushrooms, both weighing over 15g each. There were other pins, but they didn't develop.

This result leads to the understanding that substrates that contain a supplement with corn residues have a tendency to produce better yields. This result corroborates with Dias et al. (2003), who, when using pure corn husk in their studies, obtained a biological efficiency of 51.1% with an average production of 85 g of mushroom per kilogram of fresh substrate.

At the end of the experiment, all substrates showed a dry appearance, with superficial green mold contamination. In the mushroom induction and production phase, physical factors such as temperature, luminosity, gas exchange, water availability in the compost, relative humidity and induction methods are aspects that influence mushroom production and quality (ZADRAZIL; GRABBE, 1983). The superficial contamination covered some points of the white mycelium, leaving it dry, and without future fruiting.

For the North region, one of the possibilities would be the use of agro-industrial residues based on corn in a greater proportion, combining with the others as a way of reducing the cost.

V. CONCLUSION

We seek to demonstrate the feasibility of the enterprise, for its cost-effectiveness, overcoming the difficulties of acclimatization of the Cerrado biome with the development of mushroom strains that are more adaptable to local climatic conditions, such as high temperature and low humidity, in addition to reducing the contamination factor that remains active in the experiments precisely because of these conditions.

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