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EVALUATION OF AGROINDUSTRIAL RESIDUES FROM THE SIERRA AND AMAZONIA IN THE MASS CONSERVATION *Pleurotus ostreatus* var. Florida.

Julia M. Escobar 1[a], Fernando Álvarez 2 [b], Jorge E. Lascano.

[a] Universidad Estatal Amazónica, mescobar@uea.edu.ec. Departamento de Ciencias de la Tierra, Carrera de Ingeniería Agroindustrial. Dirección: Km. 2. 1/2 vía Puyo a Tena (Paso Lateral) / 032-888-118 / 032-889-118

[b] Universidad Técnica de Ambato; mf.alvarez@uta.edu.ec. Facultad de Ciencia e Ingeniería en Alimentos, Carrera de Ingeniería en Alimentos. Dirección: Av. Colombia y Chile - Ambato, Ecuador - Teléfonos (032) 523039 - 521021 - 521081

Graphical Abstract	**Abstract.**
Insert grafical abstract figure here	The production and consumption of mushrooms of the genus *Pleurotus* in the present day has grown due to the fact they are a source of nutrients, are developed in a short time, and because of the diversity of substrates used in cultivation. The objective of the following work is to determine the best alternative of substrate for the cultivation of the mushroom ***Pleurotus ostreantus*** in agro-industrial waste of the sierra and the Ecuadorian Amazon (barley straw, wheat straw, bean peel, sugar cane bagasse, maize waste) in an individual form. An experimental design of a factor was used and variables were evaluated statistically such as the yield, the biological efficiency, precocity and the growth speed. The results statistically obtained indicate the barley straw as the best treatment, with a performance of 21.08%, biological efficiency of 84.83%, precocity of 18.6 days, growth speed of 5 days, pileus diameter of 4.9-5.9 cm, and protein content of 3.08%.

Introduction *(optional)*

The cultivation of edible fungi is a biotechnological industry that is continuously expanding, and which is receiving a growth in importance in the economic environment of many countries. The main producing

countries include China, The U.S., Holland, France, Spain, Poland, Italy, Canada, Ireland and The U.K. (Sanchez 2010). In Ecuador, edible fungi cultivation such as the *Pleurotus*, is found to be very lowly-developed and in the small scale at an artisanal level, cultivation uses sawdust in the province of Morona Santiago, in the Ecuadorian orient. (Ruilove M. 2014). The edible fungi of the genus *Pleurotus*, are organisms that can decompose lignocelluloses in an efficient manner without biological or chemical pre-treatment and grow easily under diverse artificial conditions, producing good yields in a large variety of lignocellulose waste, such as coffee pulp, straw, paper, corn cobs, sawdust, sugarcane bagasse, cocoa peel among others (Gaitan, 2005). The selection of substates for the cultivation of *Pleurotus* spp., should include the largest number possible of favorable properties such as: Good availability in quantity and continuity; known physico-chemical characteristics; regularity in their physic-chemical composition, advantageous acquisition price, convenient localization and close to the site of use and ease of transport. (Guzman *et al.* 2008). The indicators such as the biological efficiency, the yield, the frequency and percentage weight of each fruiting body are parameters that evaluate the effectivity and production of the substrates. (Garzon and Cuervo, 2008). The use of preventative waste from agro-industry for the cultivation of mushrooms is currently of a great importance, owing to the implementation of technologies for the production of food of nutritional quality acceptable for human consumption, at the same time contributing in a favorable manner to contamination problems.

Materials and Methods *(optional)*

The experimental development was carried out in the laboratories of the faculty of science and food engineering and from the Food Technology Investigations Unit owned by the Scientific Investigations Centre "CENI" from the "Universidad Tecnica de Ambato". During the experimentation, mushroom strains of *Pleurotus ostreastus* var. Florida, registered as CP-184, were generously given from the collection of the Centre of Industrial Biotechnology Studies (Universidad de Oriente, Cuba).

Lignocellulosic residues were used from sugarcane agricultural crops such as bagasse (Puyo), corn residues (such as leaves, stems, and cores), bean pods, and wheat and barley straws, which were analyzed proximally in the Polytechnic School of Chimborazo ESPOCH. The residuals that had a humidity greater than 12%, were proceeded to have the excess moisture in a tray dryer extracted and then stored in sacks in a cool and ventilated place, to be used during the experimentation. The substrates were cut into slices, pasteurized for 1 hour at 90 ° C, mixed homogeneously with the inoculum in a proportion between 8 and 10% of the wet weight of the substrate.

The inoculum production was carried out by sowing the strain of *Pleurotus Ostreatus* in Petri dishes with Maltose Agar, placing a small portion of the fungus in the center of the box, incubated at 20 ° C, for two to three weeks, until the box is completely covered with the mushroom mycelium. The primary inoculum was produced using barley grains, which were washed with abundant water and hydrated with cold water for 24 hours, after this time they were submerged in a Benomyl 0.02% solution for 10 minutes. Approximately 400 g of hydrated barley was placed in wide-mouthed jars with a capacity of half a liter and sterilized in an autoclave at 121 ° C for 15 minutes. Following that, the bottles were cooled, stirred to separate the grains from each other and favor homogeneous aeration and hydration. With the help of a sterile scalpel the mycelium was divided into four parts, these portions were deposited in jars with the barley grains, with a dissecting needle or sterile platinum handle. The bottles were incubated in a cabinet in darkness for 15 to 26 days, at a temperature of 28 to 30 ° C, until the mycelium completely covered the grains. These jars are called primary inocula and can be used to obtain more mycelium for a second generation of jars which are called secondary inocula, sewn in the same way.

The previously prepared substrates were placed together with the secondary inoculum in bioreactors (plastic bags), on a disinfected shelf one next to another, in darkness at a temperature of 24-28 ° C and a humidity of 75-90% for about a 12 - 20 day period in which the biomass would completely colonize. On the third day of incubation, perforations of a diameter of 1cm were made in the bags with needles, separated by 8cm, each one to permit the access of air. Once the bioreactors were covered by the mycelium, they were exposed to light in the humidification shelves and were kept in the fermentation room with an average relative humidity of 75-90%, temperature of 24-28 ° C and luminosity between 400-800 lux (measured by a digital luxmeter throughout the culture). In the fructification room, several ventilation processes were carried out with the purpose of eliminating CO2 produced in this stage and culminated with the harvest of the mushrooms that were made by cutting the bunches with a sharp instrument. During the development of the fruiting bodies and the harvest, the yield of the harvest was evaluated in percentage relation between the fresh weight of the mushroom and the weight of the wet substrate, the biological efficiency (EB) in percentage relation for each of the harvests using the following formula EB (%) = (Fresh mushroom weight / Dry substrate weight) * 100; precocity with respect to the time elapsed between the day of incubation and the day that the first carpotrophs or primordia appeared and the speed of growth of the pile diameter with respect to the time elapsed from the appearance of the carphophore to the harvest of the mushroom, expressed in cm /day.

Results and Discussion *(optional)*

Physical chemical composition of the residues

Table 1 shows the values of the proximal analysis of the substrates used in the production of *Pleurotus* mushrooms, the barley straw contains a moisture value of 8.3%, followed by the bean peel with 7.7% element that is harnessed by the mushrooms in their development and the synthesis of the protein available in the substrate. The nutrient most used by mushrooms is fiber, with wheat straw and barley (Table 1) having the most representative values with 50.6% and 46.0% respectively, this is split during the fermentation process and transformed into more simple compounds used during growth.

Table 1. Proximal analysis on dry basis of dry residues (%)

Component	BARLEY STRAW	WHEAT STRAW	SUGARCANE BAGASSE	BEAN PEEL	MAIZE		
					Leaves	Stem	Corn
Humidity*	8.3	4.6	6.6	7.7	6.8	7.4	6.7
Ashes	6.8	7.1	1.6	12.4	15.3	4.0	4.1
Ethereal Extract	3.1	4.5	7.9	3.1	0.5	1.1	1.2
Crude protein	3.2	2.1	2.4	8.3	16.9	3.9	3.5
Crude Fiber	46.0	50.6	28.3	38.7	21.0	23.2	74.9
Carbohydrates Totals	41.0	35.7	59.8	37.5	46.3	67.8	16.3

* Humidity presented n the substrate before the dry basis analysis.

Source: ESPOCH, 2006

Elaboration: Julia Escobar; Mario Álvarez

Yield

Figure 1 shows the highest average value of 21.81%, for the wheat straw residue (RT) and 21.08% in the barley straw (RCB). Some authors mention that for the cultivation of *Pleurotus* spp., it is generally done on prepared substrates of wheat, rye or barley straw and production has been observed of 100 to 200 kg per ton of wheat straw, that is between 10 - 20% similar to the value obtained. (García, 1987, Yildiz *et al* 2002 and Salmones *et al*., 2005). The production of mushrooms in the substrate has certain limitations such as yield, where the greatest amount of mushrooms are obtained in the first harvest because the enzymes synthesize and take advantage of the fiber contained in the substrate during the development of the primordium until its final phase, while in the following harvests the mushroom uses the quantities that were left, reducing the production in half and in the third it is almost null.

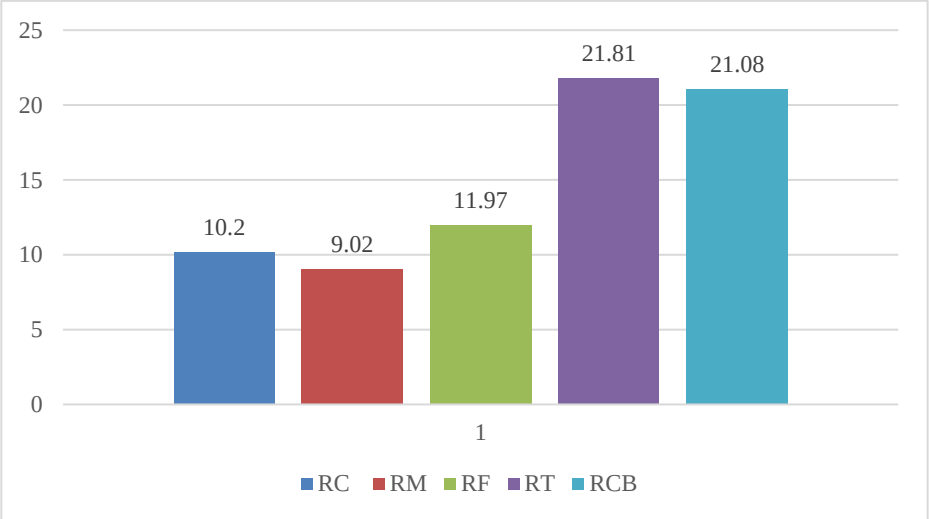

Figure 1. Yield of the three crops.

BIOLOGICAL EFFICIENCY

The results obtained can be seen in Figure 2, where greater biological efficiency occurs in the residues of barley, RCB, (84.83%) and wheat, RT, (71.78%) that have the highest values. The treatment that presents the lowest efficiency is for the corn residue (RM) and according to what was reported by Sánchez J. 2006, the productive quality of a substrate is perceived as acceptable from biological efficiencies of 50%, which compared to the data obtained is classified as suitable for commercial use. Philippoussis *et al.* 2001, presents values of 30.5% - 161.7% of biological efficiency in wheat residues with strains of P. *Ostreatus*. Other authors agree that wheat straw is the best substrate for its availability and economy (Kumari and Achal 2008).

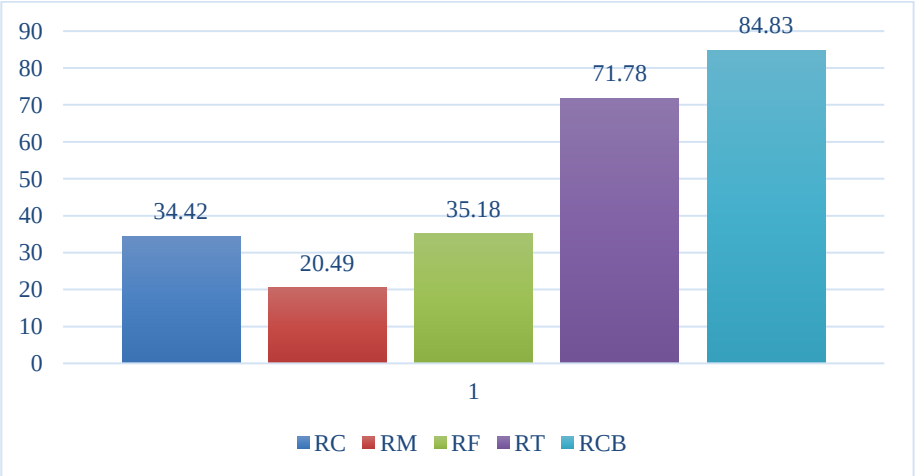

Figure 2. Biological efficiency of the three crops

PRECOSOTY

From the data obtained shown in Figure 3, a high value of days indicates the time it takes for the carpophores to appear from their incubation, that is, the shorter the number of days the faster the harvest will start, the lowest value from the residues of bean and a difference of 6 days between the substrates of barley straw and wheat (19 days), indicating that they offer a fast availability of mushrooms with respect to time. The results obtained in mushrooms *Pleurotus ostreatus* of sawdust-like substrate indicated precocity of 26 days (Martínez., 2008) and oil palm fiber as a substrate in different proportions presents 15 days of precocity. (Díaz C., 2014).

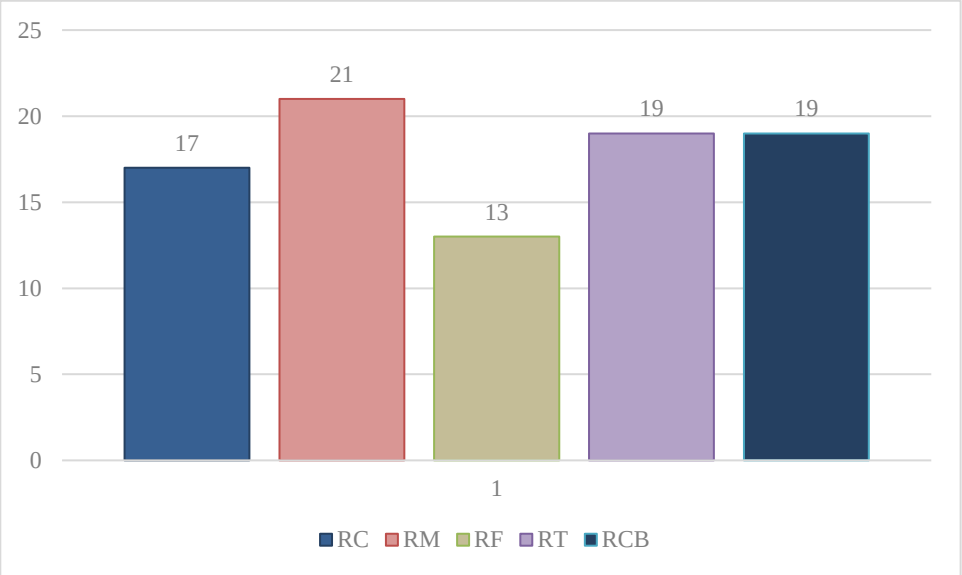

Figure 3. Precocity in the production of mushrooms

GROWTH RATE

The growth rate was taken from the day the primordium appears until the harvest of the mushroom, and is expressed in cm / day and whose data were adjusted to the curve that shows the growth of microorganisms. **Figure 4** presents the curve of growth of the pileus from wheat straw treatment + *Pleurotus ostreatus*. A typical growth curve can be observed of the microorganisms and presents an aquation of the third grade Y = -0.1157 X3 + 0.7684 X2 + 0.0954 X – 0.0421, with a correlation factor of 0.9990. The diameter of the largest size (5.5 cm) is obtained over 4 days and 12 hours, the optimum time to sew the mushroom, any longer and a decrease in size starts to show due to water loss, as sporulation starts. **Figure 5** expresses the growth curve of pileus in the treatment with barley residues + *Pleurotus ostreatus*, obtaining a correlation factor or 0.9992 and whose maximum development on day 5, for this it is recommended 4.5 days, time can be calculated through the equation that governs this process where Y is the diameter and X is the time in days, Y= -0.0844 X3 + 0.7105 X2 – 0.2632 X + 0.0419. The empiric equation presented can be used with the parameters that are indicated, where X represents the first derivative or latent time which is where the mycelium passes to primordium, the second derivative X2 explains the development of the mushroom where biological, biochemical and physiological changes occur and the third derivative says that X3 expresses the time of senescence where the mushroom begins sporulation, the loss of physical and sensory properties.

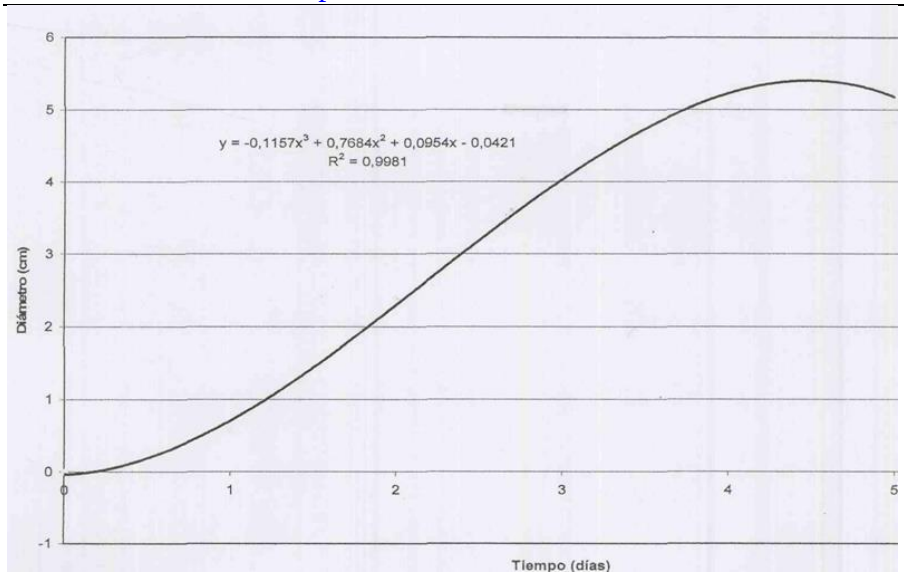

Figure 4. Growth curve of mushroom pileus on wheat straw.

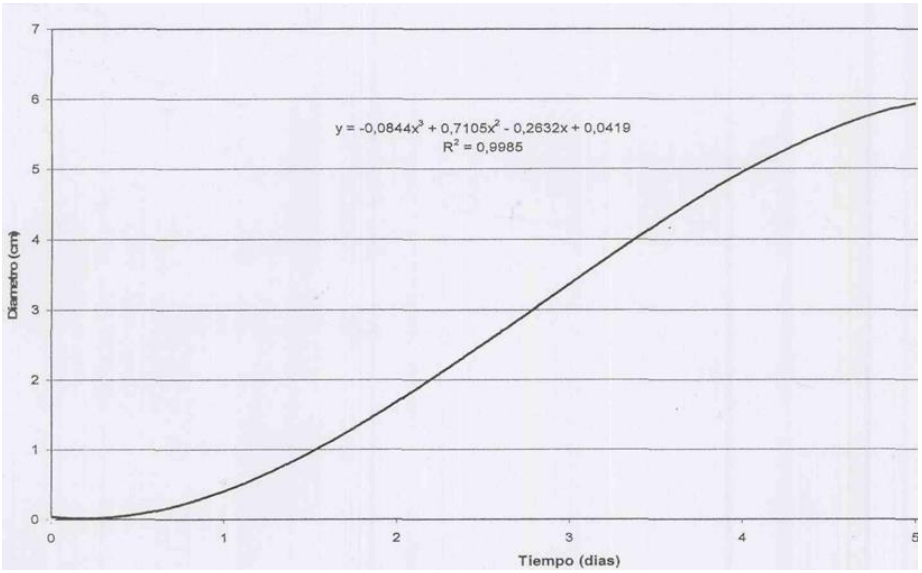

Figure 5. Growing curve of mushroom pileus on wheat straw.

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Conclusions *(optional)*

The indicators of mushroom production *Pleurotus ostreatus* var. Florida, through solid fermentation, show that wheat straw and barley straw have high amounts of moisture, crude protein and raw fiber, essential components for the development of mushrooms affecting both the performance and biological efficiency, parameters that determine the viability for large-scale production and in comparison with values reported by Martínez and Carrera (1989) that mention biological efficiency (EB) values of 96% for the cultivation of *Pleurotus* in barley residues, the data obtained can be improved by means of the combination of lignocellulosic residues that contain a high content of crude protein and usable fiber.

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