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Pleurotus ostreatus production using corn and legume husks

Producción del *Pleurotus ostreatus* utilizando cáscaras de maíz y leguminosas

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Abstract

Pleurotus ostreatus is an edible fungus that is produced on a wide variety of agricultural and agroindustrial wastes, due to its great capacity to colonize and degrade lignocellulosic substrates. The objective of this work was to evaluate the kinetics of radial growth, to obtain mycelium and the production of Pleurotus ostreatus in fresh state, using for its growth tender corn cob husk, legume husk (pea, bean and broad bean). In the first phase, mycelial growth was determined and in the second phase, production of the fungus Pleurotus ostreatus. For radial growth or mycelial growth, a completely randomized experimental design was used, with five treatments and five replicates. In the kinetics of radial growth, which was the obtaining of mycelium, the one that showed the best behavior in the growth of Pleurotus ostreatus was the culture medium Papa dextrose agar plus the inclusion of pea husk (Pisum sativum) 74 mm at 168 hours of growth, while the best harvest yield of Pleurotus

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ostreatus was fava bean husk (Vicia faba) 151.40 g of Pleurotus ostreatus, per kilogram of substrate in the first harvest. With these results, there is an alternative to use vegetable waste generated in the central market of the canton of Quevedo, Province of Los Ríos, Ecuador, to obtain a foodstuff and reduce the contamination that waste generates in the environment.
Keywords: Chocolate, tourism, entertainment

Resumen
Pleurotus ostreatus, es un hongo comestible que se produce en una gran variedad de residuos agrícolas y agroindustriales, debido a su gran capacidad de colonizar y degradar sustratos lignocelulósicos. El objetivo de este trabajo fue evaluar la cinética de crecimiento radial, para la obtención de micelio y la producción de Pleurotus ostreatus en estado fresco, utilizando para su crecimiento cáscara de mazorca de maíz tierno, cáscara de leguminosas (arveja, frejol y haba). En la primera fase se determinó el crecimiento micelial y en la segunda fase producción del hongo Pleurotus ostreatus. Para el crecimiento radial o crecimiento de micelio se utilizó un diseño experimental completamente al azar, con cinco tratamientos y 5 repeticiones. En la cinética de crecimiento radial, que fue la obtención de micelio el que mejor comportamiento demostró en el crecimiento de Pleurotus ostreatus fue el medio de cultivo Papa dextrosa agar más la inclusión de cáscara de arveja (Pisum sativum) 74 mm a las 168 horas de crecimiento, mientras que el mejor rendimiento de cosecha de Pleurotus ostreatus fue la cáscara de haba (Vicia faba) 151.40 g de Pleurotus ostreatus, por kilogramo de sustrato en la primera cosecha. Con estos resultados se tiene una alternativa de utilizar los residuos de hortalizas que se generan en el mercado central del cantón Quevedo, Provincia de Los Ríos, Ecuador, en la obtención de un alimento y disminuir la contaminación que los residuos generan en el medio ambiente.
Palabras clave: Chocolate, turismo, entretenimiento

Introduction

Fungi are the fruiting structures of specialized fungal species that have unique qualities to degrade organic substances and recycle nutrients in the soil. These are wonderful fungi that can convert agricultural wastes in rich in lignocellulosic materials into valuable protein-rich biomass having all essential nutrients. (Thakur, 2020). Mushroom production is emerging as an additional dimension in agricultural diversity; its cultivation lies in the utilization of agricultural residues rich in lignocellulosic materials. (Kumar et al., 2020).

The level of fungal growth development depends largely on environmental factors, as well as the type of substrate. Almost all agricultural wastes are available for fungal cultivation, as they contain lignocellulosic substances. The ability of the fungus to convert a high percentage of lignocellulosic substrates into fruiting bodies increases profitability. (Agba et al., 2021). Agricultural wastes include mainly cellulose, lignin and hemicellulose. The content of these chemical components in various types of crops is different. For example, the cellulose, lignin and hemicellulose contents of crop residues are 35-50%, 20-30% and 15-30%, respectively. (Mo et al., 2018)

One of the main problems faced worldwide is the disposal of both the large amount of agro-industrial waste and the residues derived from livestock activities. According to the Food Sustainability Index: a global study on nutrition, sustainable agriculture and food waste, which compiles data from 67 countries around the world: each year, the world population on average discards 37 kg of food per person. In general, high-income countries produce a higher amount of food waste compared to low-income countries. (Ritota & Manzi, 2019)

If these wastes are released to the environment without proper disposal procedure that may cause environmental pollution and harmful effects on human and animal health. Most of the agribusiness wastes are untreated and underutilized, therefore, in maximum reports it disposed either by burning, dumping or unplanned dumping. These untreated wastes create different problems with climate change by increasing a number of greenhouse gases. (Sadh et al., 2018)

Pleurotus mushrooms are considered healthy because of their richness in protein, fiber, vitamins and minerals. Pleurotus mushrooms are consumed as a functional food as they attract flavor and aroma, nutritional and medicinal value. Pleurotus species (oyster mushrooms) are commercially important edible and cultivated mushrooms worldwide. (Raman et al., 2020). This nutritious edible mushroom ranks second among commercially cultivated mushrooms. (Adebayo et al., 2021). The cultivation of edible mushrooms has grown from homemade techniques to a highly technical industry. (Guevara-Viejó et al., 2021). Mushroom consumption worldwide is steadily increasing as more people recognize mushrooms as a valuable source of healthy food; it is rich in carbohydrates, protein, minerals and vitamins, but low in calories and fat. (Sifat et al., 2020)

The objective of this work is the production and obtaining of mycelium of Pleurotus ostreatus, having an alternative to reduce environmental pollution generated by vegetable waste in the central market of the canton of Quevedo, Province of Los Ríos, Ecuador.

Methodology

The study was conducted at the experimental farm La María belonging to the State Technical University of Quevedo in June 2017. Pea (Pisum sativum); bean (Phaseolus vulgaris); fava bean (Vicia faba) and baby corn (Zea mays) husks were used, which were collected from the central market of Quevedo canton, Los Ríos Province, Ecuador. The pure culture of oyster mushrooms (Pleurotus ostreatus) was obtained from the microbiology area of the Rumiology laboratory, belonging to the Faculty of Livestock Sciences of the State Technical University of Quevedo.

To obtain the mycelium of the Pleurotus ostreatus fungus, PDA (Papa Dextrose Agar) culture medium was used at a dose of 39 g per liter of distilled and demineralized water, sterilized in autoclave at 121 ^{0}C for 30 minutes at 15 psi (pounds of pressure), sterilized glass petri dishes of 80 mm filled with 15 ml of PDA culture medium were used, where 4 mm of mycelium of the fungus Pleurotus ostreatus was taken with a stainless steel punch, deposited in the center of the petris boxes with culture medium for subsequent growth, the petris boxes were placed in an incubator (Memmert Schwabach, Germany) at 30^{0}C for 10 days until the mycelium of the fungus covers the entire diameter of the petri dish. 400 ml glass containers disinfected with chlorinated water were used and filled with wheat (Triticum) seed in an amount of 400 g and then autoclaved at 121 ^{0}C for 30 min at 15 psi (pounds of pressure), were left to cool at room temperature and then each container with the seed was inoculated with 40 mm of Pleurotus fungus mycelium and left in the oven (Memmert Schwabach, Germany) at 30 ^{0}C for 10 days, until total colonization was obtained. The shredded agricultural residues were weighed in an amount of 1 kilogram in plastic bags, then washed in running water and subsequent pasteurization at 100^{0}C for 45 minutes, the residues were allowed to cool to room temperature 25^{0}C, and seeded 100 g of wheat with Pleurotus ostreatus fungus mycelium per 1000 grams of pea, bean, broad bean and baby corn husks.

The shells inoculated with Pleurotus fungi were placed inside incubation chambers covered entirely with black plastic for 21 days, obtaining a total colonization of the shells, circular holes were made in the bags containing the shells with mycelium, Artificial light was provided to induce the fructification of the mushrooms, which with the help of a knife sterilized with 98% alcohol proceeded to cut the mushrooms to later weigh the production and carry out the physical and chemical analysis immediately.

We weighed 100 g of pea husk; 100 g of bean husk; 100 g of bean husk and 100 g of baby corn husk; for the four culture media. The 100 g of each of the samples (pea, bean, bean and baby corn husks), chopped and washed, were placed in each aluminum container, and then 1 L of distilled water was added for each sample.

It was taken to the fire and it was left to boil for 30 minutes, it was covered to avoid excessive losses by evaporation. It was filtered with the help of gauze and cotton to avoid the passage of any impurity, it was placed in flasks containing 20 g of agar and 20 g of dextrose, then these solutions of the different peels were dissolved with the help of magnetic agitators and heaters. To prepare the PDA (potato, dextrose, agar), 200 g of potato (Solanum tuberosum) (peeled in squares) were used, these pieces of potato were boiled to obtain a solution which was transferred to a flask containing 20 g of agar and 20 g of dextrose, then dissolved with the use of magnetic stirrers and heaters.

The five prepared solutions were heated to uniformly dilute the agar and dextrose by boiling for 30 minutes. They were autoclaved at 121 °C and 15 psi for 30 minutes. A total of five culture media were obtained: PDA (potato dextrose agar); PDACA (potato dextrose agar

+ pea peel); PDACF (potato dextrose agar + bean peel); PDACH (potato dextrose agar + bean peel); PDACMT (potato dextrose agar + baby corn peel), in the biosafety cabinet 15 mL of each medium was deposited in the petri dishes and allowed to solidify.

The mushrooms were harvested with the help of a knife disinfected with 96% alcohol and the mushrooms were cut and weighed on a Sartorius digital scale.

A 4 mm diameter of PDA invaded by the mycelium of the fungus *Pleurotus ostreatu*s under study was cut with a punch and sown in the center of an 80 mm petri dish, which contained 15 mL of culture medium and was incubated in a Memmert Schwabach incubator, Germany at 29 °C. With the help of a calibrator, measurements were taken every 24 hours of the growth diameter of the mycelium of the *Pleurotus ostreatus strain*. Two measurements were taken in different directions from the second to the seventh day of incubation, which was the total invasion of the petri dish.

In the first phase, mycelial growth was determined and in the second phase, production of the fungus *Pleurotus ostreatus*. For radial growth or mycelial growth, a completely randomized experimental design was used, with five treatments and five replicates. For the harvest yield of *Pleurotus ostreatus* mushroom, a completely randomized design was used with four treatments and five replicates, and Tukey's multiple range test was used ($p<0.05$), and the data were analyzed in the SAS version 9 program.

Results

Table 1 shows the results of radial growth of *Pleurotus ostreatus* mycelium in various culture media, it can be seen that there are statistical differences between treatments, with better growth in PDACA, compared to the PDA control treatment and the other culture media prepared with corn and legume husks. These results may be due to the chemical composition of the pea husk in its low hygroscopic moisture content (3.44%) and also to the neutral detergent fiber content (58.25%). Agricultural, agro-industrial and urban residues of vegetable origin have a high content of cellulose, a glucose polymer, which is the main component of the cell wall of plants. (Díaz Muñoz et al., 2019). P*leurotus ostreatus* is a saprophyte, with high flexibility to grow by bioconversion in a wide range of lignocellulosic materials. (Fayssal et al., 2021).

According to (Mahadevan & Shanmugasundaram, 2018), reported similar values, in the Comparative effect of different culture media on mycelial growth performance of *Pleurotus sapidus* mycelial growth, 7.72 cm, on Yeast Malt Agar medium, at 8 days of radial growth kinetics. Likewise (Hoa & Wang, 2015)published higher radial growths of 9.00 cm in *Pleurotus ostreatus*, at 8 days of growth, in different culture media, these results may be due to the use of pure fungal culture media. (Phadke Monika et al., 2020)In the case of *Pleurotus ostreatus,* lower mycelial growths were obtained between 36.11 mm to 45.28 mm at 8 days of inoculation, in different culture media. While, (Bankole & Salami, 2017)reported growths of 8.50 cm on Papa dextrose agar and 5% sucrose corncob agar media, at 5 days of inoculation. (Maftoun et al., 2017)In addition, they used additives in PDA medium to increase the growth of *Pleurotus mycelium,* obtaining 10 cm of mycelium in a culture medium composed of PDA + yeast extract + malt extract + sodium nitrate, 12 days after inoculation.

Table1. *Radial growth of Pleurotus ostreatus mycelium inoculated in different culture media prepared with corn husk and legumes.*

GROWTH IN (MM)

HOURS OF GROWTH	PDA	PDACA	PDACF	PDACH	PDACMT	P<
	1.00 c[1/]	3.80 a	2.60 b	2.60 b	2.00 b	**<.0001**
	2.00 c	7.80 a	4.60 b	4.20 b	4.20 b	**<.0001**
	3.40 d	11.00 a	6.80 b	7.60 b	7.60 bc	**<.0001**
	6.00 c	13.20 a	8.60 c	11.60 c	11.60 a	**<.0001**
	10.80 c	21.40 a	11.00 c	15.00 b	15.00 b	**<.0001**
	34.00 b	47.00 a	43.00 ab	34.00 b	34.00 b	**<.0001**
	58.00 B	**74.00 A**	**59.00 B**	**59.00 B**	**47.00 C**	<.0001

PDA = potato dextrose agar

PDACA = potato dextrose agar + pea husk (Pisum sativum)

PDACF = potato dextrose agar + bean husk (Phaseolus vulgaris)

PDACH = potato dextrose agar + bean husk (Vicia faba)

PDACMT = potato dextrose agar + baby corn husk (Zea mays)

1/ Means with equal letters do not differ statistically, according to Tukey ($p<0.05$).

Table 2, shows the yield of *Pleurotus ostreatus* fungus, harvested on corn and legume husks, with statistical differences among the treatments, presenting a better yield of fresh hogos the fava bean husk with 151.40 grams per kilogram of fava bean husk. These results are similar to those reported by (Roblero Mejía et al., 2021)The results are similar to those reported by the study of *Pleurotus ostreatus*, with yields of 152.80 grams of *Pleurotus ostreatus* mushrooms in a mixture of pangola grass, coffee pulp and corn stover. (Pokhrel et al., 2013)In the same study, the authors of the study, who obtained lower yields in pea residues 137.51 grams and in corn stalks, obtained similar yields of 158.96 grams in the first harvest of *Pleurotus ostreatus* mushrooms. In addition (Chukwurah et al., 2012) reported higher yields of *Pleurotus ostreatus* mushrooms in a substrate composed of corn straw, sawdust, lime and water, obtaining a production of 220 grams in the first harvest and 270.50 grams in the sixth harvest of *Pleurotus*. However, they harvested lower yields of (Fufa et al., 2021)However, they harvested lower yields of 144.18 grams of *Pleurotus* on corn cob residues. Also (Nasir et al., 2021) obtained, the lowest yield was recorded for the first flush (95 g), second flush (88 g), third flush (80 g) and total yield (263 g) in the case of Treatment-T5 (100 % corn cob residues).

Table 2. *Yield of Pleurotus ostreatus mushroom, harvested on corn husks and legumes.*

	PEA SHELLS	BEAN SHELLS	BEAN SHELLS	BABY CORN HUSK	P<
PERFORMANCE	72.00 B1/	80.00 B	151.40 A	72.00 B	0.0001

1/ Means with equal letters do not differ statistically, according to Tukey ($p<0.05$).

Yield of fresh mushrooms per 1000 g of soft corn husks and legumes, in a single harvest.

Conclusions

In the production of Pleurotus ostreatus, bean husk showed the best performance in edible mushroom harvest, while in the production of mycelium, the medium Papa Dextrose Agar plus the inclusion of pea husk had the best performance in radial growth kinetics, demonstrating that these legume residues can be used in the production of *Pleurotus ostreatus.*

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