Cultivation and nutrition analysis of *Pleurotus florida* (Oyster Mushroom) using banana leaves and paddy straw substrate

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Abstract

Present study deals with artificial cultivation of mushroom *Pleurotus florida*.,on two different agro waste material substrates under controlled conditions in the semi-arid climate. The main objective of the present investigation was to evaluate the best cellulosic waste substrate, which can be utilized for yielding maximum biomass -of *Pleurotus florida*. The present investigation was carried out on *Pleurotus florida*. using two different substrates, . paddy straw and banana leaves for its growth. Based on the data collected the cultivation period with the above said two substrates, it was found that the paddy straw is considered as best substrate to obtain the maximum yield of *Pleurotus florida***.** Oyster mushroom can be grown on cellulose rich material but are more sensitive to lignified substrates. The cultivation of *Pleurotus florida* on agricultural waste like Paddy straw and banana leaves gives very high yield as well as the nutrition contain protein, amino acid, carbohydrate and lipid were analysed.

Key words: biological efficiency, cellulosic waste, mushroom cultivation, nutrition analysis, *Pleurotus florida.*

INTRODUCTION

Mushrooms have been a widely used as food and food supplements for millennia. It is an important food item concerning human health, nutrition and disease prevention (Chang, 1996). Major medicinal properties attributed to mushrooms include anticancer, antibiotic, antiviral activities, immunity and blood lipid lowering effects. *Pleurotus* spp. are also rich in medicinal values. *Pleurotus florida* has antioxidant and antitumor activities (Nayana and Janardhanan, 2000; Manpreet *et al* ., 2004). Mushrooms are rich in protein, minerals and vitamins and they contain an abundance of essential amino acids. Moreover, nutritional composition is affected by many factors; these include differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting, specific portion of the fruiting bodies used for analysis (Benjamin, 1995).

Mushroom cultivation offers ample opportunities by turning agro industrial wastes into new forms of resources and protein-rich food by biodegradation, bioremediation, and biotransformation. This is because mushrooms are excellent converters of cheap cellulosic materials into valuable proteins. In fact, many studies have shown that agro industrial effluents are outstanding supplements that shorten crop period and increase mushroom productivities of oyster mushroom species (Sbhatu, *et al*., 2019). Nutritional attributes of the oyster mushroom is being increasingly realized in recent times because they are low in calories and high in protein as compared to rice, wheat, cabbage and milk. They are good sources of several vitamins including thiamine, riboflavin, niacin, biotin and ascorbic acids. The oyster mushrooms are good source of minerals and rich in carbohydrate and fibers as well (Benjamin 1995).

MATERIALS AND METHODS

Collection of sample

The oyster mushroom *Pleurotus florida* was collected from plant pathology department TNAU Aduthurai.

Spawn preparation

Spawn is eat grain referred to as the vegetative mycelium of the fungus, which is grown on cereal grains. Wheat grain spawn was prepared by the following method. Wheat grains were well washed in tap water and then half boiled in water. After that water from wheat grains was drained out. To remove excess water, wheat grins were spread over a tilted platform . This was followed by mixing of buggers $CaCO_3$ and $CaSO_4$ in 3:1 ratio (30 gm $CaCO_3$ and 10 gm $CaCO_3$ and 10 gm $CaSO_4$ per kg of half boiled wheat grains). The wheat grains were now half filled

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in bottles and plugged by cotton. The half filled bottles were autoclaved at the temperature 121°C and pressure 15 psi for 40 minutes then left for overnight followed buy inoculation of bottles by transferring inoculums of *P.florida* from cultures plate. Then bottles were incubated in BOD incubator at temperature 25° ±2°C. After 3-4 days of inoculation fungal mycelium started spreading in the grains. The mycelium is white net web like in appearance. The bottles were nearly half filled in 10-12 days and in 18-21 days these where completely filled with white mycelia growth.

Collection of substrate

'The mushroom can be cultivated using two types of agro wastes such as Paddy straw and Banana leaves. These substrates were collected from Panayakottai village, Thanjavur dt.,Tamilnadu, India.

Preparation of substrate

Mushroom beds were prepared using paddy straw, banana leaves trash used as substrates to find out the yield and quality of mushroom *P.florida*. One spawn bottle can be utilized for the preparation of two mushroom beds. In the present study three beds were prepared to each substrate size of each bed was 60× 30 cm.

The poly propylene bag method was chosen for mushroom cultivation process. Substrates (Paddy straw, banana leaves) were chopped into pieces of 2-3 inches length and soaked in water and then drained off from the paddy straw substrates. After words the substrates were sterilized using vertical autoclave at 15 1bs pressure for 20 min (Iqbal *et a*l.,2005). The sterilized substrates were placed on a wire mesh net for draining off excess water. Polythene bags in the size of 60 × 30 cm were procured and filled with the treated paddy straw as follows.

Mushroom bed preparation (Sivaprakasam 1985);

A polypropylene bag was tied at one end and sterilized substrates were filled through the open end for about 5 cm. A handful of spawn from the bottle was spread toward the periphery of this layer. Over the spawn some more paddy straw was put and pressed lightly. This process was repeated five times. The mouth of polypropylene bag was rolled and closed with tie threads. Holes were made over the polypropylene bags for aeration. One bottle of spawn was enough to inoculate two bags and they were kept in a ventilated dark chamber. After 15 days is was observed that the mycelia of *P.florida* had grown all over the substrates separately. Water was sprayed 3-4 times per day.

Cropping

After the completion of spawn run and pinhead appearance, the polythene bags were removed, exposing the total surface area of compact substrate and mycelia mass for fruit body development. For this they were transferred to the crop-running section of mushroom house. The compact mass of substrate and mycelium was maintained at 24±2°C and the humidity was maintained 90-95% by using a humidifier and cross were provided during the cropping period.

Harvesting

The matured fruit bodies of *P.florida* were harvested by hand pick up of clock wise or anti clock wise rotation before spraying of water. The harvested fruit body was weighed and recorded substrate wise individually. The same procedure was followed up to 2nd and 3rd harvesting. Then the total yield and percentage of biological efficiency were calculated. Finally harvested fruit body was used for further biochemical analysis.

Biological efficiency

Biological efficiency (B.E.) was calculated as the percentage conversion of dry substrates to fresh fruit bodies (Chang *et al*., 1981).

Nutrition analysis

$$\text{Biological Efficiency} = \frac{\text{Mushroom harvested per bag}}{\text{Substrate used per bag}} \times 100$$

Estimation of Total protein (Lowery *et al*., 1951)

To 500 mg of powdered sample of mushroom 5ml of 10 % TCA was added and centrifuged. From the supernatant, 0.1 ml and 0.2 ml of the two samples were pipetted. Then 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard sample were taken in their respective tubes. The volume was made up to 1ml with water. To each test tube, 5 ml of alkaline sodium carbonate was added and allowed to stand for 10 min. Then 0.5 ml of Copper sulphate was added and incubated at room temperature for 30 min. A blank solution was also maintained. Blue colour developed was read at 660nm. Standard graph was prepared by running standard protein and the results were expressed as mg protein g-1 of the sample. The amount of protein from the mushroom grown substrate was estimated.

Estimation of free amino acid (Jayaraman, 1981).

One hundred mg sample was taken with 80% ethanol in a pestle and mortar. The homogenate was centrifuged at 15,000 × g. The clear supernatant was made up to a known volume. From this 1ml was pipetted out into a test tube and diluted to 4ml with distilled water. To this 1ml of ninhydrin reagent was added and kept in boiling water bath for 15min. The tubes were then cooled and 1 ml of 50% ethanol was added. The purple colour developed was measured in spectrophotometer at 540 nm. Standard graph was

Table 1. Growth, Yield and Biological Efficiency of *Pleurotus florida* in agricultural waste substrate

Size	Substrate	Spawn Run (days)	Pin head formation (days)	Yield (g) per			Total Yield (kg/g)	Bio-conversion Efficiency
				I	II	III		
Entire	Paddy straw	22	26	134	121	70	324	64.8
	Banana leaf	20	23	130	112	69	312	62.4
2-6 cm long	Paddy straw	18	21	169	140	60	368	73.7
	Banana leaf	16	19	157	110	60	327	65.5
	Paddy straw	21	25	140	108	60	308	61.2
10-15 cm long	Banana leaf	20	23	138	100	58	296	59.3

Table 2. Estimation of nutrient content of *P.florida*

S.No	Nutrienta (mg/g)	Paddy	Banana
1	Amino acid	6.08 ± 0.01	5.02 ± 0.10
2	Carbohydrate	8.10 ± 0.30	7.00 ± 0.20
3	Lipid	3.00 ± 0.30	2.60 ± 0.20
4	Protein	23.6 ± 0.20	20.4 ± 0.10

made using a mixing of alanine, aspartic acid, tryptophan, proline and lysine. The results were expressed as mg / g of the sample.

Estimation of Total carbohydrate (Dubois *et al.*, 1956)

One hundred mg of powdered sample of mushroom was taken in a test tube and hydrolyzed with 2 ml of 96 per cent conc-$H2SO_4$ from 30 minutes at 100°C. To 5 ml of hydrolysate 1 ml of 5 per cent phenol and 5 ml of $H2SO_4$ were added and mixed thoroughly. The colour developed was measured at 490nm in spectronic 20. Glucose was used as standard. The standard graph was prepared by running standard glucose (conc 10 µg to 100 µg). The amount of carbohydrate was calculated using standard graph and the results were expressed as mg carbohydrate g-1 of the sample. The carbohydrate from the mushroom grown on paddy substrates was estimated.

Estimation of lipid (Sato, 1988).

One hundred mg of samples on dry weight basis was homogenized in a pestle and mortar with extraction solvent A and filtered through filter paper. The filtrate was vortexed with sodium sulphate to remove moisture. Then it was taken in a pre weighed bottle and dried by a steam of nitrogen. The dried extract was weighed and the total lipids were estimated by subtract the initial weight from the final weight. The amount of total lipid was expressed as mg/g by weight.

RESULTS AND DISCUSSION

The results indicated that the spawn running was completed in the bags 10 to 14 days and pinheads appeared on the 19th – 25th day. Pinheads turned into leaf like on 23rd day and the first harvest was made at about 26 – 28 (Table 1) days. The second harvest was in another 4 or 5 days. In the present investigation, *P. florida* was found to grow well on all the three substrates and formed mycelium. *P. florida* had fastest spawn run on paddy straw substrate (13th day) followed by sugarcane trash (17th day). Deepak *et al* (2019) found the pin head formation in paddy straw substrate on 18th day followed by sugarcane trash 21st day. Studies by Bulti *et al.,*(2021) revealed that oyster mushrooms (*Pleurotus ostreatus*) could grow on corncob, finger millet straw, bamboo waste, and their combination with varying growth performances. Amitesh *et al.,*(2020) recorded the highest yield in wheat straw (1380 g/kg substrates) followed by paddy straw (1240 g/kg substrates) and sugarcane bagasse (1140 g/kg substrates).

In the present study, the substrates like paddy straw and Banana Leaves, were used for bed preparation. After bed preparation process over, the fruit bodies will be developed during first week. The fully developed fruit bodies were harvested after 20 days. The second and third harvests were also done after first harvesting. In the first harvesting (after 20 days of bed preparation) the maximum fruit bodies (500gm) were harvested in paddy straw substrate using bed followed by Banana Leaves (200gm).

After first harvesting is over the second harvest were also done after 25 days of bed preparation. In this period, the maximum fruit bodies were harvested in paddy straw substrate using prepared bed (300gm) followed by Banana leaves (100gm) . After second harvesting is over, the third harvest of the fruit bodies was done after 30days of the bed preparation. In this period the maximum fruit bodies were harvested in paddy substrate using prepared bed (150gm) followed by Banana trash leaves (50gm) (Table 1).

In the present study the biochemical compounds *viz.*,amino acid, carbohydrate, lipid and protein were estimated. The maximum content of amino acid (6.08 ± 0.01), carbohydrate (8.10 ± 0.30), lipid (3.00 ± 0.30) protein (23.6 ± 0.20) was observed when paddy straw wasused as a substrate. The lower content of amino acid (5.02 ± 0.10), carbohydrate (7.00 ± 0.20), lipid (2.60 ± 0.20) and protein (20.4 ± 0.10) were recorded, when banana leaf was used as a substrate (Table 2).

In a study by Deepak *et al.*(2019). the maximum content of protein (23.1 mg/g), carbohydrate (11.5 mg/g), amino acid (8.9 mg/g) and lipid (3.9 mg/g) was observed in paddy straw used as a substrate, while the lower contents of protein (19.1 mg/g), carbohydrate (7.2 mg/g), lipid (0.5 mg/g) were recorded when sugarcane trash used as a substrate. On the other hand, Suraj *et al.*(,2020) concluded that the wheat straw was a suitable and one of the best residue for oyster mushroom (*P. florida*)

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