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Effect of substrates on the cultivation of *Pleurotus ostreatus* and its nutritional analysis

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ABSTRACT

A mushroom is macro fungus having fleshy fruiting bodies with good source of protein, vitamins and minerals and very low fat and they require minimal sunlight for their growth. Cultivation of mushrooms becomes the most popular agro business worldwide. The growth and nutritional value of oyster mushroom (Pleurotus ostreatus) were determined using different substrates. Substrates such as topsoil(TS), areca nut husk(AH), areca palm leaves(AL), bamboo shoots(BS), and it's mixture with top soil were used for cultivation. The growth of mycelia on bamboo shoot was observed for a few days and then it was decayed. The highest mycelia growth was observed in topsoil followed by areca nut husk, areca palm leaves, mixture of areca nut husk and topsoil and very less in mixture of bamboo shoot and topsoil and mixture areca palm leaves and topsoil.

Keywords*:* Mushroom, *Pleurotus ostreatus*, topsoil, areca nut husk, and areca palm leaves, bamboo shoots, mycelia, nutritional value

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INTRODUCTION

Edible mushrooms which are saprophytic basidiomycetes have been successfully cultivated at commercial level worldwide using lignocelluloses wastes as substrates for their cultivation. The Pleurotus species was cultivated on different agro wastes. *Pleurotus eous* has the highest percentage of protein content [1]**.** Gibriel et al., stated that potato dextrose extract as liquid or solid media was the best medium tested for both rate and amount of fungal growth of *Pleurotus* [2]. The highest yield was observed for *P.sajor caju* and *P.columbinus* on rice straw. Sawdust was the second best organic substance tested followed by water hyacinth. Artificial substrates were screened for cultivation of *Pleurotus tuber regim.* [3]. *Pleurotus ostreatus* grown on different substrates are nutritious with high protein, fiber and low fat. Rice straw supplemented with 10% rice bran used as a control was found as the best substrate [4]. Cultivation on wheat with rice bran resulted in significantly faster mycelial growth as compared to other substrates followed by wheat with straw in *Volvariella volvaceae* and wheat with wheat bran in *Volvariella diplasia* [5]. Jackfruit resulted in faster mycelia growth when compared to other. Surprisingly, rice straw did not produce any fruiting bodies. The lowest biological and economic yields were found when culture was on Champ [6]. Sawdust-based oyster mushroom (*Pleurotus ostreatus*) spent substrate (OMSS) could be recycled after fermentation with three probiotic lactic acid bacteria (LAB) strains as a feed supplement for post-weaning calves, and fOMSS (fermented sawdust-based oyster mushroom spent substrate) has the beneficial effects of an alternative to antibiotics for a growth enhancer in dairy calves [7]. A mixture of river sand and fermented sawdust substrate is recommended as the best substrate for the production of *P.tuberregium* mushrooms while a mixture of corn waste and fermented sawdust substrate is recommended for sclerotial production [8].

MATERIALS AND METHODS

Selection of species
White oyster mushroom (*P.ostreatus*) was selected for study as they do not require strict environmental conditions. They are easy to grow and are readily available.

Collection of spawn
The spawns of Oyster mushroom were collected from Indian Mushroom Farm, Virugambakkam, Chennai, Tamil Nadu.

Substrate Preparation and Inoculation
The substrates were dried in sun. They are cut into small bits (1-2 cm long). All the four substrates (topsoil, areca nut husk, areca nut leaves, and bamboo shoot) were soaked overnight for 18-24 hours so that the moisture content in the substrate is approximately 70%. The water is then drained off and filled in autoclavable plastic bags. Seven different sets of substrates were prepared such as topsoil(TS), areca nut husk(AH), areca nut leaves(AL), bamboo shoot(BS), mixture areca nut husk and topsoil (AH+TS), mixture of areca nut leaves and topsoil(AL+TS), mixture of bamboo shoot and topsoil(BS+TS). The bags containing substrates were then sterilized for 90 minutes in an autoclave at 15 psi, 121^0C. The bags are allowed to cool after sterilization. Each bag is then inoculated with spawn and then tied the upper portion of the bags with rubber bands.

Incubation and harvest
The bags were incubated at 22-25^0C in dark room for spawn run and fructification. The pinholes were also made in the bags manually for exchange of gases. The humidity of the bags was maintained by spraying water thrice or twice a day using a spray bottle. Though carbon is essential for spawn running but at the time of fructification oxygen is essential for mushroom.

When primordia or pinheads developed, they were allowed to grow to mature fruiting bodies optimizing the temperature by 17-20^0C. The fully grown mushrooms were removed from the substrates and then the weight for analysis of the nutritional values of mushrooms obtained from each substrate.

Nutritional analysis
Moisture analysis:
Twenty gram of fresh mushroom was weighed and dried in an oven at 100~105 ^{0}C and cooled in a dessicator. The process of heating and cooling was repeated till a constant weight was achieved. The moisture content was calculated as following equation:

Determination of total protein
Five gram of grinded mushroom was taken with 50 ml of 0.1 N NaOH and boiled for 30 min. The solution was cooled in room temperature and centrifuged at 6000 rpm. The supernatant was collected and total protein content was measured according to the method of Lowry et al. (1951). For the determination of protein content from fresh mushroom, 5 g was taken with 50 ml phosphate buffer and homogenized with a tissue homogenizer. Five millilitre of homogenized was taken with 50 ml of 0.1 N NaOH and protein content was determined as mentioned above.

Determination of total lipid
Total lipid was determined by slight modified method of Folch et al. (1957). Five gram of grinded mushroom was suspended in 50 ml of chloroform: methanol (2: 1 v/v) mixture then mixed thoroughly and let stand for 3 days. The solution was filtrated and centrifuged at 1000 g by a table centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid. For the determination of total lipid from fresh mushroom, 5 g was taken with 50 ml phosphate buffer and homogenized with a tissue homogenizer. Five ml of homogenized was taken with 50 ml of chloroform: methanol (2: 1 v/v) mixture and lipid content was determined as mentioned above.

Determination of crude fiber
Ten grams of moisture and fat-free sample was taken in a beaker and 200 ml of boiling 0.255 N H2SO4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The

material was then transferred to the same beaker, and 200 ml of boiling 0.313 N NaOH added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80~100^{0}C and weighed (We). The crucible was heated in a muffle furnace at 600^{0}C for 5~6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber.

Determination of total ash

One gram of the sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5~6 hours at 600^{0}C. It was then cooled in a dessicator and weighed. To ensure completion of ashing, the crucible was then heated in the muffle furnace for 1 h, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or greyish white in colour.

Biological Efficiency (BE)

Biological Efficiency (%) =dry weight of harvested sclerotium/dry weight of substrates x 100

RESULTS AND DISCUSSION

The effect in the nutritional values of mushroom is due to the different substrates that are used for cultivation. After 3-4 days the mycelia formation was observed. Pinheads could be seen after 18 days of inoculation in topsoil substrate, 20 days for areca palm leaves and 21 days for areca nut husk substrate. In the mixture of areca nut husk and topsoil, mixture of areca palm leaves and topsoil, and mixture of bamboo shoot and topsoil, pinheads were observed after four weeks. Topsoil was found to be the best for mycelia growth. However, the fruiting bodies produced were more in areca palm straw as compared to other substrates used.

The temperature was maintained at17-20^{0}C during fructification of mushroom to avoid loss in moisture content and shrinkage of mushroom. Excess water hindered the growth of mushroom. The bag containing bamboo shoot as a sole substrate showed no mycelia growth of mushroom instead it was decayed and worms started to grow. However, there was growth in mycelia when bamboo shoot is mixed with topsoil (Table.1&2).

Table 1: Effect of substrates on the production of *P.ostreatus* mushroom

Substrates	Spawn running started (days)	Fruiting initiation(days)	Biological efficiency (%)
Topsoil	3	18	76
Areca nut husk	3	21	57.5
Areca palm leaves	3	20	60
Areca nut husk + topsoil	5	28	68
Areca palm leaves + topsoil	9	34	54
Bamboo shoot + topsoil	7	30	48

Table 2: Proximate composition of *P.ostreatus* mushroom

Total content (%)	Topsoil	Areca nut husk	Areca palm leaves	Areca nut husk + topsoil	Areca palm leaves + topsoil	Bamboo shoot + topsoil
Moisture content	84.28	81.5	83.86	84.97	85.15	85.76
Ash content	3	3.2	2.2	2.8	2.6	3.4
Crude fiber	2.4	2.6	1.8	2.4	2	2.8
Crude protein	10.07	10.17	10.09	10.03	10.025	10.15
Crude lipid	0.5	0.5	1	0.25	0.5	0.25

The growth of mycelia on bamboo shoot was observed for a few days and then it was decayed. The highest mycelia growth was observed in topsoil followed by areca nut husk, areca palm leaves, mixture of areca nut husk and topsoil and very less in mixture of bamboo shoot and topsoil and mixture areca palm leaves and topsoil. The protein content of mushroom was highest in areca nut husk(10.17%) followed by the mixture of bamboo shoot and topsoil(10.15), areca palm leaves(10.09%), topsoil(10.07%), mixture of areca nut husk and topsoil(10.03%) and mixture areca palm leaves and topsoil(10.025%). The biological efficiency was highest in topsoil (76%) and lowest in the mixture of bamboo shoot and topsoil (48%).

Fig.1 Primordia or Pin heads

Fig.2 Fruiting clusters

Fig.3 Matured Oyster

CONCLUSION

For the maximum yield of oyster mushroom, areca palm leaves can be used as a substrate followed by topsoil in compilation with other substrates. The main advantage of growing oyster mushrooms is the ease in the cultivation technique as well as the fast growth. Also mushrooms are high in protein which is vital in the maintenance of body tissue, including development and repair. They are not easily attacked by diseases and pests, so they can be grown in rural areas where strict environmental conditions are not to be followed. Further studies may be carried out to increase the protein content and other nutritional values of mushroom.

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