KAVAKA 60(3): 1-6 (2024) DOI: 10.36460/Kavaka/60/3/2024/1-6

# Cultivation of Oyster Mushroom (*Pleurotus djamor*) by Using Different Substrates in Laboratory Conditions

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(Submitted on January 01, 2024; Accepted on August 06, 2024)

## ABSTRACT

***Pleurotus djamor* (Oyster mushroom) is a type of fungus belonging to the class Basidiomycetes. It can be grown on different kinds of agricultural waste with high lignin and cellulosic contents. Mushroom production is a kind of sustainable development in which we use agricultural waste in mushroom production. On the other hand, agricultural waste left after mushroom production is used as compost. The main objective of this study was to see the production rate of mushrooms on different substrates. In the present study, different substrates like wheat straw alone, wheat straw with rice straw, wheat straw with leaves of *Madhuca indica* (mixed with 50%), *wheat* straw, with leaves of *Azadirachta indica* (mixed with 50%) were used as substrate was inoculated with *Pleurotus djamor* spawn and put it in the lab and maintained the temperature at 25 ± 3ºC and humidity at 80% for four weeks. It was observed that along with wheat and paddy, *Pleurotus djamor* can also be cultivated on the leaves of *Madhuca* spp. and *Azadirachta* spp. plants. The result shows the maximum yield on wheat straw. It is clear from the result that apart from wheat and paddy straw we can also grow oyster mushrooms on the waste leaves of forest trees. So, waste leaves can be used as an alternative substrate for the cultivation of *Pleurotus djamor*. The choice of substrate is a critical factor influencing the yield, growth rate, and overall health of the mushroom.**

**Key words:** Pink Oyster Mushroom, *Pleurotus djamor*, Growing Substrate, Mushroom Cultivation

## INTRODUCTION

Fungi are the group of most diverse heterotrophic organisms and the second largest living community after insects on earth (Singh and Singh, 2022). Fungi has broadly divided into two categories i.e., Macrofungi and Microfungi (Singh and Singh, 2022a). The most common macrofungal group is called mushroom. The macrofungal mushrooms are characterized by their distinct macroscopic fruiting bodies of underground mycelium of certain fungi belonging to the class Basidiomycetes and Ascomycetes (Singh and Singh, 2023b). They show a variety of habitats in terrestrial ecosystems such as parasitic, saprophytic, or symbiotic (Singh and Singh, 2022; Singh and Singh, 2023c). Mushrooms are important resources for food and medicine, they are rich in various nutrients and a wide range of health benefits such as boosting the immune system, promoting strong bones, providing an anti-cancer function as well as antidiabetic properties in humans (Kristine *et al*., 2021).

Mushrooms breed by spores and they germinate into hyphae (mycelium) under favorable conditions. Hyphae are filamentous and generally microscopic. Germinated hyphae form primary mycelium and then secondary mycelium through the process of plasmogamy (fusion of hyphae). They accumulate nutrients from the substrate and develop in the form of a colony. Based on certain environmental conditions (temperature, humidity, etc.), the mycelium is stimulated to develop fruiting bodies (sporocarp). The young fruiting body (sporocarp) is known as pins (bud) and it differentiates into a fully developed body with its distinct parts (structure). Spores are present in the gills that lie in the lower part of the pileus (cap) and

are responsible for new-generation development (Singh and Singh, 2022).

Mushroom is an accepted ideal food item and is also referred to as "Vegetarian Meat" due to their rich protein, low fat and carbohydrates, and high fiber which make them enriched food (Singh and Singh, 2023d). Mushrooms are referring as an important nutritional food because of their unique flavor, taste, and mineral constituents other than nutritional enrich quality (Singh and Singh, 2023d). Among all the qualities of mushrooms, become a region of cultivation to fulfill the requirements and needs.

Mushroomsalso perform an active role in the biodegradation of organic matter like dead and decaying wood, litter, straws, leaves, etc. (Singh and Singh, 2023c). Only a limited number of species are available in practice for cultivation and the remaining are wild (Singh and Singh, 2023d). The major cultivational mushroom species are *Agaricusbisporus* (Button Mushroom), *Pleurotus* sps. (Oyster Mushroom), *Volvariella volvacea* (Paddy Straw Mushroom) and *Calocybe indica* (Milky Mushroom) are reported in India (Singh and Singh, 2023c).

A large amount of agricultural waste viability and favorable climatic conditions provide great opportunities for growing oyster mushrooms. Its cultivation is a proposal for the bio-conventional conversion of cellulose waste into edible proteins. Traditionally, oyster mushrooms are mostly grown on rice and wheat straw, which is more expensive for many other purposes (Kumar *et al.*, 2004). The main nutrients in these substrates are less nitrogen and more carbon which supports the growth (Mandal *et al*., 2021).The common cultivable species of oyster mushrooms are *Pleurotus ostriatus*, *P. sajor-caju*, *P. florida*, and *P. djamor*. Agricultural waste is a sufficiently available conventional media that has not been used properly. In the present study, different agricultural waste and other substrateswere used for the cultivation of *P. djamor* and to evaluate their production.

## MATERIAL AND METHODS

### Preparation of Culture Media

To obtain a pure culture of oyster mushrooms, Potato Dextrose Agar (PDA) culture medium or tissue culture plating method was used. Initially,the pure culture of Oyster Mushroom was maintained on Potato dextrose agar (PDA) and malt extract agar (MEA) slant. One liter of PDA media was prepared by using of 250 grams peeled and sliced potato, 20 grams of dextrose, and 20 grams of agar,and it was made up to 1 liter by adding distilled water. The media was sterilized in an autoclave for 15 minutes at 121ºC with 1.5 kg/cm² pressure. Pouring of media was done on laminar airflow into Petri dishes having 9 cm. diameter. EachPetri dish was inoculated with Oyster Mushroom (*Pleurotus djamor)* culture by using the spatula and was incubated at 25 ± 3ºC. After 3 to 4 days, the media was covered with white mycelium (Kristine *et al*., 2021).

### Spawn Preparation

The development of mushroom production methodologies on agricultural waste like paddy straw and wheat straw gives very high yield (Sadhana and Siva Kumar, 2020). Spawn production is a highly technical operation and is generally done in the laboratory. Spawning is carried out aseptically, preferably using the same transfer chamber or the same inoculation room as is used in spawns' preparation (Mateen and Mumtaz, 2021). The mother culture substrate was prepared by using good quality wheat grains (1 kg), calcium-carbonate (5 grams), and gypsum (20 grams) and packed tightly in 25×18cm. polypropylene bag. These packets were sterilized in an autoclave for 1 hour at 121ºC and 1.5 kg/cm² atmospheric pressure and then these were kept 24 hours for cooling.

Then a piece of pure culture of mushroom mycelium with PDA measured 1×1 cm² was placed aseptically into the mouth of each mother culture packet of spawn and the packets were placed in the growth chamber at 24-26ºC in a dark place. After 15-20 days the mother culture became white due to the complete mycelium running and then it was ready for inoculating spawn packet. The prepared spawn should be used as soon as possible, otherwise, it becomes compact with time and makes spawning difficult.

**Preparation of Composting**

The variation in the nutritional makeup of mushrooms grown on various substrates could be caused by a variety of reasons (Sonali, 2012). Mushroomsgrow on substrates like paddy straw, wheat straw, forest leaves, etc. the different substrates along with wheat straw were used. The initial composition of leaves was obtained by various treatments. With a B-C ratio of 3.498, the mushroom was the most economically advantageous agricultural residue when it came to rice straw (Dubey *et al*., 2019).

Now the wheat straw along with different air-dried leaves was chopped into 3-4 cm pieces and soaked in water for 16-22 hours with the spreading of formalin (1.25% percent) on the three substrates. Excess water drained out and spread for 8-16 hours for evaporation of formalin content from these substrates.

**RESULTS AND DISCUSSION**

**Days for Completion of Spawn Running**

**Tables 1** and **2** present the various findings from the research effort. Three crucial stages of mushroom cultivation, spawn flowing, pinhead formation and fruiting body formation require the right humidity and temperature. Results were satisfactory at 25ºC for spawn running and 17-20ºC for fructification. The activate 2nd, 3rd, and 4th flush, covering the substrates with plastic is very important to make the substrate moist (Tesfaw *et al*., 2015).

**Table 1:** Periods of different developmental stages of fruiting body on different substrates.

| S.No. | Name of substrates | Days of completion of spawn running | Days for Pin-head formation | Days for Fruiting Body Formation |
|---|---|---|---|---|
| 1 | Wheat straw | 12 days | 17 days | 22-25 days |
| 2 | Rice straw and wheat straw | 15 days | 20 days | 29 days |
| 3 | Leaves of *Madhuca indica* and wheat straw | 16 days | 22 days | 28 days |
| 4 | Leaves of *Azadirachta indica* and wheat straw | 13 days | 16 days | 22 Days |

**Table 2:** Yields of mushrooms on different substrates with different flushes (crops).

| S.No. | Yield | 1st Flush | 2nd Flush | 3rd Flush |
|---|---|---|---|---|
| 1 | Wheat straw | 210 grams | 345 grams | 369 grams |
| 2 | Rice straw and wheat straw | 190 grams | 285grams | 290 grams |
| 3 | Leaves of *Azadirachta indica* and wheat straw | 205 grams | 267 grams | 284 grams |
| 4 | Leaves of *Madhuca indica* and wheat straw | 210 grams | 280 grams | 250grams |

**Spawning progression**

**Table 1** shows that the spawning cycle lasted 2 to 3 weeks after inoculation. The substrates were inoculated on the same day. These results are consistent with the results of Tan (1981) who reported that after inoculation spawninglasted three weeks and that the fruiting bodies appeared after 2-3 days.

**Pinhead formation**

Pinhead formation is the second stage of mycelium growth during mushroom cultivation. Small pinhead-shaped structures have been observed, these pinheads are formed 6 to 7 days after spawning starts. These results are consistent with those of Ahmad (1986) who found that *Pleurotus ostreatus* completed spawning in 17-20 days on various media and that the time to pinhead formation was 23-27 days. Pin-head formation (primordium initiation) was observed following the invasion of substrates by mycelia growth (Girmay *et al*., 2016).

**Figure 1**: Mushroom Pinheads.

**Fruiting bodies formation**

This is the third and final stage of growing a mushroom. Fruiting bodies appeared 3-6 weeks after pinhead formation and appeared 27-34 days after inoculation. These results agree with Quimio (1976,1978) who reported that fruiting bodies appear 3-4 weeks after inoculation. The highest number of fruiting bodies (8.52 bags) was found in wheat: paddy straw compost and the lowest (4.5 bags) was produced in decomposed cow dung (Uddin *et al*., 2012).

**Figure 2:** Mushroom fruiting body

**Yield of Oyster Mushrooms**

The crop of Oyster Mushrooms was harvested in three harvests. The maximum coverage was achieved in the first flush followed by the second and third flush. The fruiting bodies of the button mushrooms were better in quality based on the stipe and cap parameters (Shivrattan *et al*., 2022). The maximum mean yield of wheat straw was estimated at 924 grams. Therefore, is recommended as the best substrate for growing oyster mushrooms, which agrees with the conclusions of Hami (1990), who studied the cultivation of oyster mushrooms on wheat straw of different types of wood and concluded that *Pleurotus ostreatus* lends itself to the highest yields

**Table 3**: Total yield of Oyster Mushroom on different substrates.

| Substrates | Total yield (in grams) |
|---|---|
| Wheat straw | 924 grams |
| Rice straw and wheat straw | 765 grams |
| *Madhuca indica* leaves and wheat straw | 815 grams |
| *Azadirachta indica* leaves and wheat straw | 756 grams |

## CONCLUSION

Growing mushrooms is an effective way to recycle waste. *P. ostreatus* grown on a variety of substrates is high in protein, and fiber and low in fat. It can also provide economic incentives for the agri-food sector to explore these residues as valuable resources and develop new businesses to use them to manufacture nutritious mushroom products. Mushroom cultivation can thus become one of the most profitable agro-businesses that could produce food from different substrates and help to dispose of them in an environment-friendly way. Almost all growers use wheat straw for the production of *P. ostreatus*, which is also one of the best substrates in this study. Therefore, different substrates also have to be used, although the yield is lower than wheat straw. Other substrates such as rice straw with wheat straw, *Azadirachta* leaves with wheat straw and *Madhuca* leaves with wheat straw can be used as alternative substrates with the cultivation of *P. djamor* (Oyster Mushroom).

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