

Cultivation of Oyster Mushroom (*Pleurotus ostreatus*) using Bio-fertilizers to Enhance Yield: A Review

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Abstract: The present review compiles the technology for cultivation of oyster mushroom, problems experienced during its cultivation and biofertilizer technology to combat the problems. Mushroom cultivation is a profitable agri-business. Incorporation of non-conventional crops in existing agricultural system can improve the economic status of the farmer. Mushrooms are the source of protein, vitamins and minerals and are anti-cancerous, anti-cholesterol, and anti-tumorous. Sawdust produced highest yield, biological efficiency and number of fruiting bodies, recommended as a best substrate for Oyster mushroom cultivation. Oyster mushroom proved to be one of the easiest to be grown, though it faces yield, nutrient, size issues. Biofertilizer includes certain bacteria such as Azotobacter, Rhizobia and Phosphate solubilizing Bacteria proved to yield better result.

Keywords: Bio-fertilizer, Cultivation, Oyster mushroom

Introduction

Mushroom cultivation technology, being relatively newer field and also because of unique way of raising the mushroom crop compare to cereals, pulses and vegetables, the role of microbial inoculants in it have not been studied much. Mushroom generally requires agro based substrate for their production. Microbial inoculants thrive well on these materials and hence chances of deriving benefits from microbial inoculants in mushroom cultivation technology are very high. In spite of previous research on microbial effect on mushroom production little information is available on different microbial community effect in the casing on mushroom sporophore formation. The presence of microbial population both in compost and casing soil plays vital role in *P. ostreatus* cultivation. The microbial biomass present in compost affects the mycelial spread during spawn run phase, while in casing soil it triggers the induction of reproductive phase of the *P. ostreatus* life cycle.

Review Literature

Oyster mushrooms (*Pleurotus* species), the third largest commercially produced mushroom in the world are found growing naturally on rotten wood material. Oyster mushroom (*Pleurotus* sp.) belonging to Class Basidiomycetes and Family Agaricaceae is popularly known as 'Dhingri' in India and grows naturally in the temperate and tropical forests on dead and decaying wooden logs or sometimes on dying trunks of deciduous or coniferous woods. It may also grow on decaying organic matter. And rotten wood material. It is rich in Vitamin C and B complex and the protein content varies between 1.6 to 2.5 percent & mineral salts required for the human body. The niacin content is about ten times higher than any other vegetables. Oyster mushroom can grow at moderate temperature ranging from 20 to 30 °C and humidity 55-70% for a period of 6 to 8 months in a year. The best growing season is during March/April to September/October and in the lower regions from September/October to March/April.

Oyster Mushroom Cultivation

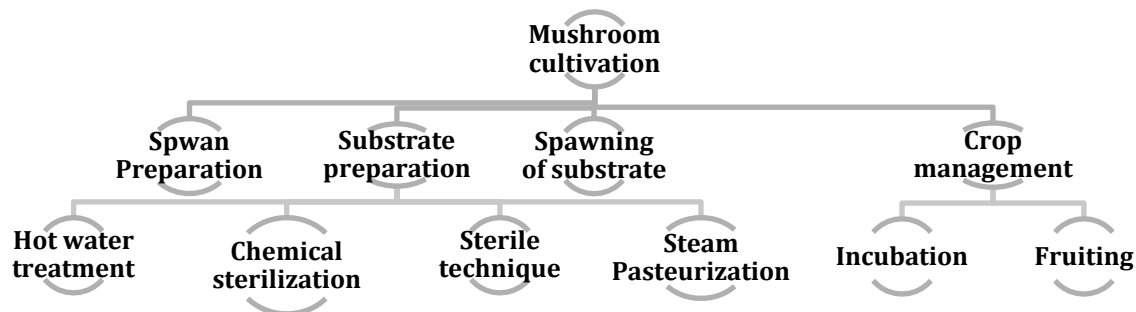
Oyster mushroom growing on straw can be fragmented into 4 segments:

1. Spawn Preparation

Substrates for oyster mushroom and their nutrition quality:

Agricultural-remains having cellulose and lignin which assists in more enzyme growth of cellulose that is corresponded to more yield. These include straw of wheat, ragi and paddy, leaves and stalk of maize, cotton

and millets used citronella leaf, saw dust, cotton waste, used tea leaf waste, sunflower stalks, dried grasses, dehulled corncobs, pea nut shells, and synthetic compost of button mushrooms etc.



Methods of substrate preparation: The mycelial growth can take place on a simple water treated straw but there are number of other cellulolytic molds already present on straw, which compete with *Pleurotus* mycelium during spawn run and also secrete toxic metabolites hampering its growth. The popular methods of substrate preparation are as follows. The accepted practices of substrate preparation are:

i. Steam pasteurization

Soaked substrate is loaded in wooden trays and then kept in a pasteurization room at 58-62°C for 4 hours. Temperature of the pasteurization room is changed by means of the steam through a boiler.

ii. Hot water treatment

The substrate after cutting (3-5 cm) is wetted in hot water for Wheat straw: 1 to 2 hours at 80°C and for Paddy straw: 85°C for 30- 45 minutes. After draining, spawn is added at room temperature. This treatment enables mycelial growth takes place easily.

iii. Chemical sterilization technique

Various species of *Gliocladium*, *Penicillium*, *Doratomyces*, *Aspergillus* and *Trichoderma*, spp. are the common fungi on the substrate during oyster mushroom production. The presence of the fungi does not allow the mycelium to grow on the substrate. In order to kill or suppress for 25-40 days after spawning avoid the mould growth the substrate is treated with steeping in a chemical solution of carbendazim 50% WP (37.5 ppm) and formaldehyde (500 ppm) for a period of 16-18 h. The technique, which was standardized at DMR, Solan by Vijay and Sohi in 1987, follows galvanized drum with 90 liters of water was taken out of 200 liters capacity. Approximately about 10 to 12 kgs of substrate is soaked in water. In another plastic container, Bavistin 7.5 g and 125 ml formaldehyde (37-40%) is dissolved in water and slowly added to the drum which contains the substrate. Straw is balled and coated with a polythene sheet. After completion of 15 to 18 h the substrate is removed from drum and surplus water is drained.

iv. Sterile technique

The substrate was cut into small pieces which was soaked into water and after removing of excess water, the straw is placed in the polythene cover which is heat resistant and sterilized in an autoclave at 20 p.s.i. for 1-2 hours followed by addition of spawn under sterile conditions.

3. Spawning of substrate

Freshly prepared (20-30 days old) grain spawn is best for spawning. The complete procedure is done in the sterile conditions which is previously treated with 2% formaldehyde before 48 hours. The spawn 2 to 3% of the wet wt. of the substrate spawn is required. 300 gm of spawn is required for about 8-12 kg of wet substrate

or 2 to 3 kg of dry substrate. Spawn can be mixed thoroughly or mixed in layers. Spawning is done in polythene bags (60 x 45 cm) of 125-150 gauge thickness. Small holes are done in all the sides of the cover including bottom so that the excess water can drain. Punctured bags give more and fast crop (4-6 days) than non-punctured bags because of accumulation of high CO₂, which reduces fruiting.

4. Crop Management

(A) Incubation

The polythene bags which are spawned are moved to dark room for mycelium growth, the growth of the mycelium takes place between 10 to 33°C, but it grows maximum at 22 to 26°C.

(B) Fruiting

The last stage of mushroom cultivation is fruiting, this happens after the fully growth of mycelium on the substrate, if any contaminated bags are observed should be removed and the half-colonized bags kept for more days for complete growth. The size of the fruit depends on the humidity of the substrate, the fruits are bigger in size with more humidity (85-90%) and the fruits are smaller in size at the humidity (65-70%) and the concentration of CO₂ during harvesting should be less than 600 ppm. or 0.6%. Proper ventilation has to be provided during fruiting. Currently, high biofuel prices have caused an increase in food prices and food scarcity in many countries (World Bank, 2008).

Few of the reported pharmacological actions with their chemical constituents are reported in the Table 1.

Table 1: Pharmacological effects and chemical constituents of Pleurotus

Pharmacological effect	Chemical constituents	References
Anticancer	Water soluble protein (or) polysaccharides	Jedinak A <i>et. al</i> (2010) Wu <i>et. al</i> (2011) De Silva DD <i>et. al</i> (2012) [56,57,58] Bokek P & Galbavy S (2001) Wang H & Ng TB (2000)
Antioxidant	β-D Glucan (pleuran) Lectin	Zhang YX <i>et al</i> (2012) Mitra P <i>et al</i> (2013) [59,60,61,62]
Antitumor	β-D Glucan (pleuran) Glycopeptides Proteoglycans	Bokek P & Galbavy S (2001) Li <i>et al</i> (1994) Sarangi I <i>et al</i> (2006) Silva S. <i>et al</i> (2012) Devi KSP <i>et al</i> (2013) [59,63,64,65,66]
Antiviral	Ubiquitin-like protein	Wang H & Ng TB (2000) Ei-Fakharany <i>et al</i> (2010) [60,67]
Antibacterial	β-D Glucan (pleuran), silver nanoparticles (AgNPs)	Karacsonyi S & Kuniak L (1994) Mirunalini S <i>et al</i> (2012) Vamanu E <i>et al</i> (2012), [68,69,70]
Antidiabetic	Unspecified bioactive	Krishna S & Usha PTA (2009) Ghaly <i>et al</i> (2011) [71,72]

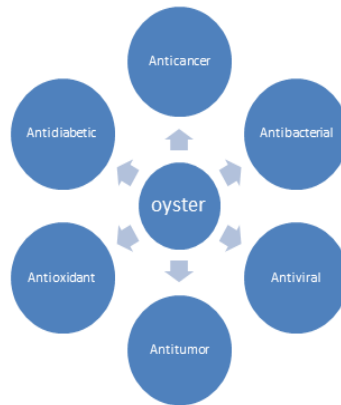


Figure 1 Pharmacological application

Oyster Mushroom Cultivation Problems

Problems associated with the violation of substrate preparation technology, climatic and sanitary incubation parameters etc., are as follows:

1. The block has brown streaks from the perforations,
2. The second flush gives a very low yield or no yield at all. Such problems are observed when the substrate moisture is insufficient.
3. As Fungi cannot sufficiently absorb the nutrients due to lack of moisture. On the remains of the clusters, moisture begins to accumulate and a secondary bacterial infection develops, therefore they surprisingly look wet, not dry.
4. Mold in the block – green (trichoderma) or black (mucor)
5. The appearance of green mold in the slots of blocks during the overgrowth.
6. Condensate and water-logged substrate condensation can occur due to the sudden changes in temperature of the room where the blocks are overgrowing.
7. Abnormal development of primordia, the presence of “cones” Indicates temperature and humidity swings during incubation process before the development of primordia, then – gradual increase to 85-90%.
8. Long stems caused by high CO_2 levels in the room they are in.

Technology to Combat Problem

Biofertilizers are new advancements to combat the described issues for mushroom cultivation.

1. Role of Biofertilizers in Mushroom Cultivation

Microwave vacuum pyrolysis of waste palm shell (WPS) was performed to produce biochar, which was then tested as bio-fertilizer in growing Oyster mushroom (*Pleurotus ostreatus*). The pyrolysis approach generated a biochar containing a highly porous structure with a high BET surface area (up to 1250 m^2/g) and a low moisture content (≤ 10 wt%), exhibiting desirable adsorption properties to be used as bio-fertilizer since it can act as a housing that provides many sites on which living microorganisms (mycelium or plant-growth promoting bacteria) and organic nutrients can be attached or adsorbed onto. This could in turn stimulate plant growth by increasing the availability and supply of nutrients to the targeted host plant. The results from growing Oyster mushroom using the biochar record an impressive growth rate and a monthly production of up to about 550 g of mushroom. The shorter time for mycelium growth on whole bag log (30 days) and the highest yield of Oyster mushroom (550 g) was obtained from the cultivation medium added with 20 g of biochar. Our results demonstrate that the biochar-based bio-fertilizer produce from microwave vacuum pyrolysis of WPS show exceptional promise as an alternative growing substrate for mushroom cultivation.

2. Rhizobia as biofertilizers for mushroom cultivation.

In Iran, rhizobia can be used as biofertilizers for increasing the harvest and nutritional quality of cultivated mushrooms more efficiently. To prove this, processed wheat grains were incubated separately with pure mycelium alone of American oyster mushroom (*Pleurotus ostreatus*), together with a 2×2 cm yeast mannitol agar slab containing *Bradyrhizobium elkanii*, a rhizobial strain used for soybean biofertilizers. It was found that pure mycelium together with *B. elkanii* increased ash, Ca, P, K and protein contents by 128, 16, 3, 17 and 24%, respectively. It also increased biological efficiency and dry matter content of mushroom by 10 and 30%, respectively, and decreased time to primordial initiation by about 7 h. Therefore, the use of rhizobia as biofertilizers in mushroom cultivation seems to be a promising method of producing a higher yield of mineral and protein-rich mushroom more efficiently, which should be further developed for other types of mushrooms.

3 Effect of Liquid Biofertilizers on Button Mushroom Yield

Present research work has been carried out to understand the effect of liquid biofertilizers on button mushroom yield. The application of at the time of casing gave slightly better result followed by application of biofertilizers at the time of spawning. All the treatments of biofertilizers i.e. Azotobacter and PSB either alone or in combination were found significantly effective in improving growth and yield of *A. bisporus* as well as effect on parameters of compost and casing like nitrogen and phosphorus content. However, the phosphate solubilizing bacteria either alone or in combination performed better as compared to Azotobacter alone. The reduction in time taken for pinhead initiation and button formation (2-5) days after casing was observed in *A. bisporus* due to application of biofertilizers. Significantly increased in number of fruits per bed as well as average weight of fruit body due to use of biofertilizers as compared to uninoculated treatments. The treatments of microbial inoculants showed an increase of 17.47 per cent to 64.19 per cent in yield of button mushroom and recorded biological efficiency of 19.97 per cent to 27.92 per cent. Nitrogen and phosphorus content in spent compost showed decline over initial value. Low reduction of N-content was observed in Azotobacter inoculated treatments and low phosphorus content in PSB inoculated bags. Highest contamination was recorded in the control treatment. There was decline in population of Azotobacter and phosphate solubilizing bacteria in compost and casing after harvest of mushroom over initial standard population. Among the different treatments tested, the treatment of Azotobacter + PSB @ 25 ml/kg casing, was found to be very effective because it showed significant increase in number of fruits per bed (185.75); improvement in average weight of fruit body (15.02 g), increased in yield 2.792 g/10 kg of compost i.e. 64.19 per cent higher and 27.93 per cent higher biological efficiency. Highest N- content in casing 0.17 per cent. PSB (*B. megaterium*) @ 25 ml/kg of casing showed significant reduction in days required for pinhead initiation and days required for button formation i.e. 12.25 and 16.25 days respectively.

Conclusion

The key to success in mushroom growing is the careful observance of the technological regulations of the enterprise. And the key components of this success are a qualitative sterile mycelium, properly selected technologies for mushroom back raw materials processing (classical hydrothermal treatment for small enterprises and tunnel pasteurization for medium and large ones) and the availability of quality microclimate systems in the growing rooms. Besides biofertilizers proves to be the most important technology for oyster mushroom cultivation. It plays key role in increasing yield and combating peculiar problems faced during the cultivation of Oyster mushroom.

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