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*Full Length Research*

# Cultivation of oyster mushroom on different agricultural wastes

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**ABSTRACT:** Oyster mushrooms are the best known due to their nutritional value and medicinal properties. In this study, *Pleurotus ostreatus* was cultivated on pretreated lignocellulosic agricultural wastes comprising of corn cobs, rice straw and ground nut shells. Agricultural waste conserves finite phosphate resources and embedded energy from industrial nitrogen fixation. It is also a way of sustainable food production. A dense white mass of mushroom mycelium colonized the substrate within 17 days of incubation at $30^0$C in dark phase. Subsequently, massive fructification appeared within 50 to 60 days in light phase after spawning. The study was further extended to repeated-harvest and maximum average yield of mushroom obtained was 197.8 g/Kg of dried corn cobs, followed by rice straw and ground nut shells. The yield was drastically reduced after third harvest and not noteworthy, which might be attributed to the exhaustion of nutritional constituents of substrates available for growing mushroom.

**Keywords:** Biological efficiency, proximate composition, substrates, yield.

## INTRODUCTION

The macro-fungi of basidiomycetes on earth estimated at 140,000, yet only 10% (approximately 40,000 normal species) are known till date. (Chang and Miles, 1981; Jong and Birmingham, 1993; Wasser and Weis, 1999; Van Griensven, 2001; Chang, 2001). A few of them have been studied in detail from the stand point of their commercial potential. Amongst, less than 15 species of mushrooms are accepted widely as food and even fewer have attained the status of items of commerce. According to mycological differentiation; the mushrooms are categorized as poisonous and non-poisonous or edible mushrooms. However, a very few are poisonous and a large number are consumed as edible source of nutrition. Edible oyster mushrooms have received considerable attention for their nutritional value, medicinal properties and biodegradation abilities due to various components and secondary metabolite identified from their mycelium and fruiting-bodies (Mandeel et al, 2005; Kumari and Atri 2014; Kües and Liu, 2000). The fruiting-bodies of mushrooms are complex structures, both morphologically and physiologically with undoubted variation in chemical composition corresponding to constituents of substrates (Kües and Liu, 2000).

The oyster mushrooms include *Pleurotus spp*. have been recognized as very good source of vitamins, particularly, thiamine and riboflavin. The proteinous matter mainly constitutes lysine and leucine, which is essentially required in human diet, which seriously lacking in most of the staple cereal foods. In addition, these oyster mushrooms contain sufficient amount of calcium, phosphorus and iron in association with certain antioxidants (Gunde-Cimerman, 1999; Philippoussis, 2009).

As far as nutritional and therapeutic importance, the present day mushroom industry is based on two main components: the application of traditional (although modernized techniques) methods for the production of fruiting-bodies (mushroom themselves) and the application of modern biotechnological techniques to produce mushroom derivatives; such as nutraceutical and dietary supplements (Chandra et al., 2013; Obodai et al., 2003; Adebayo and Martinez-Carrera, 2015; Kannahi and Sangeetha, 2015; Chang and Miles, 1989; Chang, 2006).

Moreover, mushroom cultivation technology is also

primarily based on the availability of lingo-cellulosic solid substrates to make cost benefits. In nature, a huge amount of agriculture wastes are available at very cheaper rate which could easily be utilized after appropriate pretreatments for colonization of mushroom mycelium yielding fermentable sugars as well as growth promoting factors required for the initiation of fruiting-bodies (Buswell et al., 1996, Chang et al., 2003, Obodai et al., 2003).

Therefore, the primary motivation behind these studies was to utilize some lingo-cellulosic agricultural wastes as solid substrates for eco-friendly cultivation of oyster mushroom, *Pleurotus ostreatus*, yielding fruiting-bodies and mycelium infested biomass left behind possibly be as protein supplement of animal feed.

## MATERIALS AND METHODS

### Organism and Spawn Preparation

A viable strain of *Pleurotus ostreatus* was obtained from stock pure culture of Microbial Culture Collection Unit of Food and Biotechnology Research Center, PCSIR Laboratories complex, Lahore, Pakistan; maintained on PDA/Potato Dextrose Agar slants/Petri dishes at 25°C in cooled incubator (Eyela, Mod, Japan).

Spawn was prepared by using grains of Sorghum and Barley purchased from local market. These grains were individually cleaned in chamber facilitated with a pressurized dry air blower and 2 mm perforated dish onto which these grains were placed. The dust and undesirable material were collected in filter cloth bag. The air cleaned grains were thoroughly washed with soft water and subsequently blanched for 20 minutes in a steam fitted trays with canopy to protect heat loss. After completion of blanching cycle, the soft grains of Sorghum and Barley were transferred to mixing unit to add $CaCO_3$ (1.0%) and $CaSO_4$ at the rate of 0.1% on dry weight basis of grains. Theses salts were added to maintain the pH near 6.0 and to prevent adhesion of grains with the surface of plastic bags, respectively (Jain and Vyas, 2002). The white mycelium of *P. ostreatus* were transferred aseptically from petri dishes to the grains as inoculant and incubated at 25°C in an incubator.

### Cultivation experiments

The selected lingo-cellulosic substrates such as corn cobs, rice straw and ground nut shells were individually dried in sunlight and chopped into 1 to 2 cm and soaked overnight in tap water at ambient temperature in a 50 L stainless steel jacketed kettle connected with steam boiler. Thereafter, the temperature of soaking water was increased to boiling point by circulating the steam in the jacket of Kettle. After steeping, the water was decanted and substrate(s) were partially dried in a hot air oven (Gas Operated).

Following the pretreatment, the wet substrate weighing 1 Kg was placed in Polypropylene bags of 25 x 25 cm size as described by Chang and Miles (1989). The bags were sealed by using cotton wool plugs in conduit/poly vinyl chloride pipes (3 x 10 cm) by tying a rubber band around the neck. The bags were autoclaved at 121°C, 15 psi for 30 minutes and sterilized bags were allowed to cool for 5 hours to attain room temperature. The spawning was done at the rate of 2% wet weight basis of substrate by thorough mixing. These bags were transferred to Climatic Chamber equipped with temperature, humidity and light intensity control systems. The temperature and relative humidity (RH) were maintained between 25 to 26°C and 80 to 90%, respectively.

After appearance of dense colonization of white Mycilial mass which inter-wovened the whole substrate and emergence of pin-head of mushroom, bags were cut with knife vertically from upper to downward and placed in white light for the growth of fruiting-bodies. Mushrooms were harvested as the fruiting-bodies developed and attained full size over the surface of substrate after 50 days of spawning. All experiments were conducted in triplicate according to the methods recommended by Chang and Miles (1989).

### Calculation of production parameters

Biological Efficiency (BE) was calculated as proposed by Chandra et al. (2013). Yield was determined as described by Chang and Miles (1989) and productivity was obtained by relationship between the fruiting-bodies dry weight and cultivation time.

$$\%\ \text{Bioefficiency (BE)} = \frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrate}} \text{ x } 100$$

### Determination of chemical composition

The chemical composition of mushrooms and substrates were determined. Dry Matter (DM) was determined by drying the samples at 105°C overnight and the ash was determined by igniting the sample in muffle furnace at 550°C for 6 hours. Organic matter (OM) was calculated by subtracting ash from DM (AOAC, 2002). Nitrogen (N) content was estimated by Kjeldahal Method and crude protein (CP) was calculated as per N x 6.25.

## RESULTS AND DISCUSSION

Edible oyster mushrooms include *Pleurotus ostreatus* have been recognized as mushroom with dual function to humans; both as food and medicine (Jong and Birmingham, 1993; Wasse and Weis, 1999; Chang, 2001; Van Griensven, 2001) and attained the status of item of

**Table 1.** Relationship of harvest of mushroom grown on different substrates and biological efficiency.

| Substrates | Harvest period (days) | Biological Efficiency (%) | | | Total B.E (%) | Average B.E (%) |
|---|---|---|---|---|---|---|
| | | 1st Harvest | 2nd Harvest | 3rd Harvest | | |
| Corn cobs | 55 | 25.0 | 19.2 | 15.0 | 59.2 | 19.73 |
| Rice straw | 50 | 23.0 | 18.0 | 13.0 | 59.0 | 19.66 |
| Ground nut shells | 60 | 20.0 | 15.0 | 4.5 | 39.5 | 13.00 |

**Table 2.** Protein contents of fresh and dried mushrooms grown on different substrates.

| Fruiting-bodies | Substrates | | |
|---|---|---|---|
| | Rice straw | Corn cobs | Ground nut shells |
| Fresh | 3.40 | 13.43 | 9.37 |
| Dried | 21.52 | 47.18 | 31.56 |

**Table 3.** Average weight of mushroom on repeated harvest grown on different substrates.

| Substrates | Harvest Period (Days) | Flush weight (g/Kg) | | | Average harvest (g) |
|---|---|---|---|---|---|
| | | 1st harvest | 2nd harvest | 3rd harvest | |
| Corn cobs | 55 | 250.0 | 192.2 | 150.02 | 197.33 |
| Rice straw | 50 | 230.0 | 180.0 | 130.002 | 180.0 |
| Ground nut shells | 60 | 200.0 | 150.0 | 45.0 | 131.66 |

commerce. These significant factors stimulated to focus the studies on solid state cultivation process for the production of fruiting bodies with the expense of lingo-cellulosic components of corn cobs, rice straw and ground nut shells as substrates. However, these substrates were individually used after appropriate pretreatments to remove certain growth inhibitors and non-biological material. The pretreated substrate retained moisture with promising water activity (a*w*) near 0.8, hence facilitated inter-fibrous gaseous exchange and enhanced mycelia colonization, either embedded or on the surface of substrate as indicated in Table 3.

The data of Table 1 indicates certain intrinsic factors affecting Mycilial growth, yield of fruiting bodies associated with physiological and morphological activities resulted in the release of fermentable carbohydrates necessary for the growth during the course of fungal morphogenesis. In addition, a significant effect of inoculum size, composition of each substrate, and incubation temperature was observed during mushroom cultivation. These studies also showed that typical morphological changes occurred over 15 days on solid substrates. White fungal hyphae bound tightly and penetrated the substrate which led to the formation of compact fungal biomass. In light provided by florescent tubes, a phase of primordial growth initiated with subsequent fruiting-bodies of *Pleurotus ostreatus* within the span of 50 to 60 days at 25$^{o}$C. Furthermore, *P. ostreatus* preferentially utilized corn cob and ground nut shells and produced abundant mycelial mass, hence occupied maximum surface area within 17 days in the semi dark phase after spawning with mycelia infested barley grain. The nutritional composition of substrate play a crucial role in Mycilial growth initiation and further development of mushroom primordia as shown in Table 4.

The study also focused on repeated harvest of fruiting-bodies and their yield on the basis of dry substrate. The average yield of fruiting-bodies in first, second and third harvest indicated 97.3, 180.0 and 131.6 g/Kg on corn cobs, rice straw and ground nut shells, respectively. It can also be seen that the average values of Biological Efficiency and yield of mushroom remained almost same by using corn cobs and rice straw. The Biological Efficiency is good standard of comparison for evaluating the efficiency of substrate conversion in mushroom biomass. These findings also revealed that the average yield of oyster mushroom, highest growth yield and affected biological efficiency (BE) corresponding to the pretreatment of substrate (Oseni et al., 2012).

From these results it can further be compared that corn cobs might release a mixture of fermentable carbohydrates and necessary growth promoting compounds as a function of lingo-cellulolytic enzymes secreted by *P. ostreatus.* However, an increased amount of protein (47.18%) and crude fibers (11.0%) was found in the fruiting-bodies of mushroom grown on corn cobs.

These studies also provided information that after repeated–harvest of fruiting-bodies, the nutritional contents of exhausted substrate left behind showed a considerable amount due to the residual effect of mycelia infestation (Nasehi et al., 2017).

**Table 4.** Proximate composition of substrates used for the cultivation of *Pleurotus ostreatus*.

| Solid substrates | Proximate composition (%) | | | | |
|---|---|---|---|---|---|
| | Moisture | Ash | Crude fat | Crude fiber | Crude protein |
| Corn cobs | 4.48 | 1.60 | 0.35 | 12.10 | 1.40 |
| Ground nut shells | 7.75 | 3.50 | 0.15 | 71.50 | 1.875 |
| Rice straw | 9.00 | 18.70 | 1.58 | 32.56 | 2.60 |

## Conclusion

This study concluded that fungal Mycilial activities in the perspective of substrate preference and yield of fruiting-bodies, the micro-morphology might exert direct effects on the metabolic pathways through the co-regulation of genes. These cytogenetic events led to the hyper production of ligno-cellulolytic enzymes with their synergetic effect influenced the hydrolysis and efficient release of carbohydrates, proteins, and growth promoting constituents, hence, resulted in the massive fructification of mushroom. However, actual phenomenon is still obscure and in view of micro-morphogenesis and cytogenetic events integrated with metabolomics of *P. ostreatus* need comprehensive studies to improve the nutraceutical properties of such oyster mushrooms.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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