BIOCONVERSION OF LIGNOCELLULOSIC AGRI-WASTE INTO EDIBLE PROTEIN BY MUSHROOM CULTIVATION AND EVALUATION OF YIELD ON DIFFERENT AGRI-RESIDUES WITH BIOCHEMICAL COMPOSITION

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ABSTRACT

Pleurotus ostreatus (Oyster mushroom), a saprophytic macroscopic fungus, can grow on several lignocellulosic substances by degradation activity supported by several enzymes secreted by them. The mushroom is widely consumed for its delicious flavour in cuisines and medicinal purposes. The present study was focused on its cultivation on three different residues viz. paddy straw (Oryza sativa), wheat straw (Triticum aestivum) and garden grass (Cynodon dactylon). The research further focused on evaluating the biochemical composition including moisture, lipids, proteins and phenolic contents. All three substrates were compared based on total yield, cropping days and biological efficiencies. Maximum yield (458.97g/5kg substrate) and biological efficiency (91.79%) were observed in paddy straw, whereas the early growth was observed on garden grass. This study concludes the presence of high proteins and low lipids content in mushrooms, which is ideal for human consumption.

Keywords: Agricultural wastes, Mushroom, Cynodon dactylon, Oryza sativa, Pleurotus ostreatus, Triticum aestivum

Introduction

Pleurotus ostreatus (Oyster mushroom) was initially classified under the well-defined order Agaricales and division Basidiomycota. The mushroom possesses an eccentric stalk and a caudad ‘gilled’ cap resembling the shape of an oyster. It is also referred as ‘tree oyster mushroom’. P. ostreatus is an edible mushroom consumed worldwide for its high protein, fibre and carbohydrate content with low fats, sodium and calories (Cohen et al., 2002). Low levels of sodium and high phosphorus and
Potassium levels in *Pleurotus* are valuable health food for the hypertensive and diabetic patients (Manzi *et al.*, 1999; Chang & Wasser, 2012). It is also a good source of minerals such as calcium, magnesium, potassium, iron, selenium, zinc, manganese, copper, cobalt, chromium, molybdenum, sodium, and various vitamins such as riboflavin, biotin and thiamine (Chang & Buswell, 1996; McDowell, 2003). The presence of polysaccharide β-glucans or polysaccharide-protein complexes content in medicinal mushrooms offers a wide therapeutic application on human health due to their anti-diabetic, anti-carcinogenic, immunomodulatory, anti-inflammatory, cardiovascular, anti-microbial, hypcholesterema and hepatoprotective nature (Kim *et al.*, 2007; Synytsya *et al.*, 2009; Chaturvedi *et al.*, 2018). Polysaccharides of the 1,3-β-glucans family also induce an anti-tumour effect in any part by activating certain immune cells, make it more useful in medicinal perspective (Wisbeck *et al.*, 2017; Chaturvedi *et al.*, 2018).

The agronomically and economically important oyster mushroom is the world’s second most cultivated mushroom after *Agaricus bisporus*. It can be easily grown on various lignocellulosic substrates other than their natural habitats such as urban wastes (i.e. dry grass, pea pods, tea leaves, etc.), agricultural residues, domestic wastes, wood, straw and municipal waste etc. (Garg *et al.*, 2017). Mushroom cultivation could be a profitable agribusiness in India due to the abundance of agri-waste generated in fields. The technology of artificial cultivation of mushrooms helps to improve the economic status of small farmers.

The conversion of agri-residue into highly nutritious food by mushroom cultivation increases the scope of utilization of the otherwise harmful wastes which causes air pollution if burnt. *P. ostreatus* has been successfully employed in bioremediation (mycoremediation) of contaminated soil in situ and ex-situ in Nigeria (Adenipekun & Lawal, 2012). Keeping in the mind the versatile importance of Oyster mushroom in medicinal purpose and treatment of biohazards via bioremediation, this piece of research was carried out with the objective to cultivate *P. ostreatus* on paddy straw, wheat straw and garden grass and further determine the effect of substrates on the yield, biological efficiency and their nutritional values.

**Materials and Methods**

The study was carried out at Plant Biotechnology, Rajiv Gandhi South Campus (RGSC), Banaras Hindu University (BHU), Barkachha, Mirzapur, Uttar Pradesh between January – June, 2019.

**Experimental design**

The whole experiment was planned in a completely randomized design (CRD). Mushrooms were cultivated on the three different substrates in 5 kg plastic bags, with two replicas of each substrate. Total yield was obtained in three flushes per bag.

**Collection of samples**

Dried agri-waste residues i.e., paddy straw, wheat straw and garden grass were
collected from wheat and paddy fields and Malviya Park, RGSC (BHU), respectively. These residues were treated with 4% formaldehyde solution and autoclaved separately.

**Culture of Pleurotus ostreatus**

The seeds of *P. ostreatus* were collected from the National Horticultural Research and Development Foundation, Janakpuri, New Delhi. The cultures were maintained on potato dextrose agar (PDA) slants at 24±2°C in BOD incubator. Subculturing was done in every 14-15 days.

**Spawn preparation**

Spawns were prepared in polythene bags. 1 kg of wheat grains were boiled (10-15 min on water bath) and mixed with 2% (w/w) of CuSO$_4$ and 4% (w/w) of CaCO$_3$. The bags were inoculated with the log phase culture of *P. ostreatus* from PDA slants. Prepared spawn bags were incubated at (27±2) °C for 12-15 days until the grains are fully covered with the thick layer of mycelium.

**Cultivation of Oyster mushroom**

Paddy straw, wheat straw and garden grass were used as substrates for mushroom cultivation. Dried and chopped substrates were chemically sterilized by soaking in 4% formaldehyde solution overnight. Excess water was drained off and the substrate was autoclaved. Polythene bags (5 kg) were filled with the four alternate layers of substrate and spawn. Around 200 gm of spawn was used in 5 kg substrate. Small holes were made for aeration and the mouth of the bag was sealed (Fig. 1a). These bags were incubated in a dark-isolated room for next 15 days. Next, the cylindrical mushroom beds were obtained by vertically cutting the bags, without damaging the bed, when a thick layer of mycelium was developed over the substrate (Fig. 1b-c). Watering was done thrice a day using hand sprayer. Fruiting bodies were developed after 10-12 days (Fig. 1d-e). Harvesting was done after 12-15 days by gently twisting them around their stipes and the mushrooms were collected in three flushes. Watering was stopped a day before harvesting of mushrooms. Harvested mushrooms were weighed and stored in a sealed polybag (Fig. 1f).

**Biochemical profile**

**Biological efficiency:**

Biological efficiency (BE) was calculated to compare all the three substrates used in cultivation based on its capability to support the growth of the mushrooms (average total yield was calculated). BE was calculated using the following formula:

\[
\text{Biological efficiency} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100
\]

**Moisture content:**

Moisture content was measured quantitatively by taking 5.0 gm of the mushroom sample in a crucible and transferring into hot air oven at 100-105°C until the constant weight was achieved. The moisture (%) can easily be calculated as described by Raghuramulu *et al.* (2003).

\[
\text{Moisture content(%) =} \frac{\text{Moisture content}}{\text{Weight of the mushroom taken}} \times 100
\]

Where, Moisture content = weight of mushrooms before drying - weight of mushrooms after drying.
Figure 1: (a) spawning stage, (b) mycelium stage, (c) hyphae stage, (d) budding fruiting body, (e) mature fruiting bodies, (f) harvesting

**Phenolic content:**

The methanolic extract was prepared by crushing 5 gm sample in 50 ml of 80% methanol into a homogeniser and then filtering through a muslin cloth. About 0.5 ml of this methanol extract was taken into a fresh test tube and 125µl Folin-ciocalteau reagent was added and the mixture was then kept for 10 min at room temperature. Then, 125µl of 20% (w/v) sodium carbonate was added and mixed gently by stirring and left
Figure 2: Nutritional values of harvested fruiting bodies of *P. ostreatus* cultivated on different substrates

for another 60 min at room temperature. Finally, the absorbance was recorded at 670 nm using UV-Vis spectrophotometer. The phenolics content was calculated from a standard calibration curve of Gallic acid (Amin et al., 2006).

**Lipids:**

Lipid profile was determined using Folch *et al.* (1957) method with slight modifications. About 5.0 gm sample was crushed and mixed with 2:1 ratio (v/v) of 50 ml of chloroform:methanol mixture and kept it undisturbed for 3 days and then centrifuge the solution at 1000 rpm after filtering through a muslin cloth. The upper layer of methanol was separated with Pasteur pipette and the second layer of chloroform was evaporated on water bath at 70°C; the last turbid layer containing lipid was weighed on weighing balance. The lipid content was calculated by the following formula:

\[
\text{Lipids (\%)} = \frac{\text{Lipid content}}{\text{Weight of mushroom taken}} \times 100
\]

**Protein estimation:**

About 2 gm of the sample was minced with 10 ml phosphate buffer extract and the mixture was homogenised and filtered. Protein estimation was done using the standard bovine serum albumin (BSA) curve method using spectrophotometry (Lowry *et al*., 1951). Different dilutions of BSA protein solutions were prepared by mixing BSA (1 mg/ml) and water. The final volume of each test tube was made up to 2 ml. Then, 2 ml of alkaline copper sulphate was added, mixed thoroughly and incubated the solution at room temperature for 10 min. Then, added 0.2 ml of the Folin-ciocalteau reagent to each test tube and incubated at room temperature for the next 30 min. Readings were taken at 660 nm in UV-Vis spectrophotometer.
Table 1: Yield of *P. ostreatus* on different substrates viz. paddy, wheat and garden grass

| Sl. No. | Substrate       | Yield (I flush) g | Yield (II flush) g | Yield (III flush) g | Total yield (g) *
|---------|-----------------|-------------------|-------------------|--------------------|------------------|
| 1.      | Paddy straw     | 282.355           | 135.495           | 41.12              | 458.97           |
| 2.      | Wheat straw     | 135.49            | 72.8450           | 33.78              | 242.12           |
| 3.      | Garden grass    | 296.03            | 99.10             | 37.35              | 432.49           |

*Mean for 2 trials; expressed in g/5kg substrate

Table 2: Harvesting total yield, days of spawn run and biological efficiency of *P. ostreatus* on different substrates viz. paddy, wheat and garden grass

| Sl. No. | Substrate       | Spawn run (days) | Total Yield (g/5kg substrate) | B.E (%) *
|---------|-----------------|------------------|-------------------------------|-----------|
| 1.      | Paddy straw     | 15               | 458.97                        | 91.79     |
| 2.      | Wheat straw     | 18               | 242.12                        | 52.66     |
| 3.      | Garden grass    | 14               | 432.49                        | 89.19     |

Table 3: Biochemical profile of *P. ostreatus* on different substrates viz. paddy, wheat and garden grass

| Sl. No. | Substrate       | Moisture (%)   | Phenolics (mg GAE/g) | Proteins (mg/100ml) | Lipids (%) |
|---------|-----------------|----------------|---------------------|---------------------|------------|
| 1.      | Paddy straw     | 80.52±0.23     | 2.16±0.02           | 5.03±0.05           | 0.47±0.01  |
| 2.      | Wheat straw     | 80.90±0.08     | 2.24±0.03           | 5.70±0.02           | 0.35±0.05  |
| 3.      | Garden grass    | 84.29±0.02     | 2.23±0.03           | 4.08±0.005          | 0.40±0.02  |

*Mean of 3 tests taken; represented in value ±S.E.

Results and Discussion

**Evaluation of different substrates for the cultivation of mushroom**

While comparing the three substrates, various parameters such as yield, cropping time and biological efficiencies were taken into consideration. The highest yield of *Pleurotus* was recorded on paddy straw as shown in Table 1. Our findings are supported by the published work of Porselvi & Vijayakumar (2019) and Mamiro & Mamiro (2011), who recommended the use of paddy straw for higher yield. Nasreen et al. (2016) suggested the use of 1.25% acid - or base along with the substrates for better yield. Zhang et al. (2011) reported approximately 10% more yield on paddy straw than that on wheat straw.

The cropping time was affected by different substrates. The fastest growth was observed when garden grass was used as the substrate. The BE or simply the effectiveness of the mushroom strain was obtained to be highest (91.79%) on paddy straw (Table 2). Sharma et al. (2013) reported the highest yield and BE values (381.85 gm & 95.46% respectively) of mushrooms cultivated on paddy straw.
**Evaluation of biochemical composition of mushrooms grown on three different substrates (Figure 2, Table 3)**

**Estimation of moisture and lipids content:**

The highest moisture content (84.29%) in the present study was observed in mushrooms cultivated on garden grass (Table 3). The moisture content of fresh mushroom was reported in other studies ranging between 85-88% (Alam et al., 2008; Deepalakshmi & Mirunalini, 2014). Whereas, typically high moisture content up to 89.69% was reported by Kajendran et al. (2018).

The amount of lipids is comparatively low in mushrooms. Kwon & Uhm (1984) and Paul et al. (2016) reported 0.5% and 4.2% lipids in *P. ostreatus* mushroom. A significant decrease in the concentration of TLC (total lipid content) from 0.36% in the first flush to 0.07% in the third flush was detected by Abeer et al. (2013). The amount of lipid contained is enumerated in the Table 3. *P. ostreatus* cultivated on paddy straw was found to have the highest lipid content (0.47%) whereas the lowest lipid content (0.33%) was observed in mushrooms cultivated on wheat straw.

**Estimation of phenolics and protein content:**

The total phenolics and protein content were comparable in all substrates. Total phenolic content (TPC) in mushroom cultivated on garden grass (2.225 mg GAE/g) were notably higher than that in wheat straw (2.243mg GAE/g*) and paddy straw (2.162mg GAE/g†). On the other hand, the estimated total protein contents were significantly higher in mushrooms grown on wheat straw (5.70 mg/100ml**) than that in paddy straw (5.034 mg/100ml***) and garden grass (4.082 mg/100ml***) (Table 3).

The results obtained in the present study exhibits similarity with the findings of Yilmaz et al. (2017) who quantified the value of TPC=2.67 mg GAE/g in *P. ostreatus* cultivated on walnut tree saw dust. Whereas, the substrates were found to have significant effect on total phenolic contents which are evident from previous studies. Yildiz et al. (2017) reported TPC= 1.77 mg GAE/g in *P. ostreatus* cultivated on 100% chestnut (*Castanea sativa*) saw dust. Yilmaz et al. (2016) reported TPC= 1.51 mg GAE g⁻¹ in *P. ostreatus* cultivated on *Tilia tomentosa* leaves. Yim et al. (2010) quantified the TPC in commercially cultivated mushrooms found 7.98 mg GAE g⁻¹.

The results obtained for total protein content present in mushrooms cultivated on paddy straw (5.034 mg/100ml) were quite closer to the results (5.27 mg/100ml) attained by Kajendran et al. (2018). Whereas, protein content reported in our study was much higher than content 3.4 mg /100 ml obtained by Alam et al. (2008).

**Conclusion**

In conclusion, three substrates were found to have significant effects on the growth pattern and nutritional value of the mushrooms cultivated on them. Good

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*Results were expressed in mg GAE/g of extract  
**Results were expressed in mg/100ml of extract  
†Results were expressed in mg GAE/g of extract  
**Results were expressed in mg GAE/g of extract  
**Results were expressed in mg/100ml of extract
amounts of protein, phenolics, moisture and low lipid levels were found in *P. ostreatus*, which also indicates the good nutritive values for the human being. The data reported above reveals the implementation of paddy straw as a substrate for high yield and biological efficiency and *C. dactylon* (garden grass) for early growth. Garden grass was able to retain relatively high percentage of moisture, which was an important intrinsic factor for optimum growth. High protein content in mushrooms indicates its potential use as a protein supplement. From an economic point of view, the cultivation of protein-rich mushroom species *P. ostreatus* enables the farmer to get extra income and more protein harvest from his agri-waste.

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