Comparative study of five *Pleurotus* species cultivated in warm temperature on non-sterilized rice straw

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**ABSTRACT**

Five different *Pleurotus* species are cultivated on non-sterilized rice straw at 30 ± 2 °C. Smaller length of the substrate (1cm) showed better biological efficiency (BE) than the longer substrate (5cm). 20% spawn showed better efficiency than 10% spawn though spawn percentage has no effect on time of fruiting initiation. Length of the rice substrate much influenced the BE of *P. pulmonarius* whereas spawn percentage has more effect in *P. florida* than other species. The BE of *P. pulmonarius* and *P. florida* are comparable in first flush of production where 20% spawn and low substrate length are used. Early fruiting was observed in *P. ostreatus* followed by *P. pulmonarius* whereas *P. floridanus* showed more time requirement for initiation of fruiting. Morphometric data of fruiting body and basidium were generated. The protein, carbohydrate and polyphenol conc. were maximum in *P. ostreatus* and lowest in *P. florida*. Amount of moisture, crude fibre, ash, sodium, potassium contents are also determined in all the five species of oyster mushroom.

**Keywords:** Biological efficiency; Fruiting body; Morphometrics; Nutriceuticals; Oyster mushroom

**INTRODUCTION**

After Yeast fermentation, mushroom production has been considered as second most amongst the esteemed commercial microbial technologies (Sanchez, 2010). Due to the intrinsic tendency to grow upon a variety of substrates, mushrooms are considered to recycle organic wastes which unless are problematic for disposal (Croan, 2004). Cultivation of mushroom does not require fertile land as they can grow in the sheltered rooms degrading altogether various agro-residues (Gregori et al., 2007). Utilization of mushrooms as an alternative source of protein has been emphasized to be of great enthusiasm to the researchers for last few decades (Chang, 1999). Kurtzman (1976) considered mushroom protein as intermediate between that of animals and vegetables whereas Purkayastha and Nayak (1981) considered them as of superior qualities because of the presence of all the essential amino acids. Weinheim (2006) suggested that mushroom contains low calories and provide essential minerals thus regarded as a valuable health food. A number of edible mushroom species like *Agaricus, Auricularia, Calocybe, Flammulina, Lentinus Pleurotus, Volvariella*, etc. are commercially cultivated in different parts of the globe.

A number of mushrooms are considered not only as nutritionally rich food but also valuable from the viewpoint of medicinal purposes (Gregori et al, 2007; Novaes et al., 2007). Button mushroom (*Agaricus* spp) and shiitake (*Lentinus* spp) are widely accepted for commercial production. In recent days Oyster mushroom (*Pleurotus* spp.) are widely cultivated throughout the world due to their low cost production technology and higher biological efficiency (Mane et al. 2007). Oyster mushroom cultivation has now stepped up in second position after the button mushroom in terms of production turnover around the planet (Chang, 1999; Sanchez, 2010). Though oyster mushroom are initially cultivated in temperate countries like China, South Korea etc. but now-a-days its cultivation is flourishing also in tropical countries like India (Biswas, Datta & Ngachan, 2012).

In the present study five *Pleurotus* spp. are cultivated on non-sterilized rice straw in warm temperature (30°C) and optimal condition in respect to substrate length and spawn percentage are determined. Morphometric data, physiochemical analysis and centesimal composition of different mushrooms obtained from optimized condition are evaluated.

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MATERIALS AND METHODS

Mushroom species
Five Pleurotus spp. were used in the present study. Pleurotus flabellatus (MTCC 1799), Pleurotus ostreatus (MTCC 1802), Pleurotus pulmonarius (MTCC 1805) and Pleurotus floridanus (MTCC 6315) were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India whereas Pleurotus floridus (ITCC 3308) was obtained from Society for Rural Industrialization, Ranchi, India. All the species were maintained in PDA medium (Das and Mukherjee, 2007).

Spawn preparation
About 1000 gm of wheat grains has been taken for spawn production. The grains were boiled for half an hour then washed in flowing water. Excess water present was drained off and the grains were spread on the surface of clean blotting paper and air dried. 10 gm of calcium sulphate and 5 gm of calcium carbonate were mixed with the grains. Then about 100 gm of grain was placed in conical flask (250 ml) and sterilized in autoclave at 121˚C for 15 min and inoculated with the respective mushroom strains and kept at 30°C for 15 days (Das et al., 2010).

Substrate preparation
Dried rice straw has been collected from a local farm at Barasat, West Bengal, India. The chopped rice straws (1 cm/5 cm) are weighed and soaked in water for overnight. Excess water present in the substrate was drained off and the substrate is air dried for 15 min. There is no heat treatment of the substrate. About 750 gm wet substrate was mixed with either 10% or 20% spawn (wet wt./wet wt.). These spawned substrates were then put into 30 cm x 42 cm polythene bags. The bags were closed tightly with pin holes on the surfaces and kept in 30 ± 2 ºC (Das et al., 2010).

Biological efficiency (BE)
BE was calculated according to Das et al. (2010).

\[ \text{BE} \text{ (%)} = \frac{\text{fresh weight of mushroom} \times 100}{\text{dry weight of substrate}} \]

Microscopical studies
Microscopical studies and measurement were done using Carl Zeiss Microscope, Germany and measurement were done using Axio Vs 401E V4.3.0.101 (2004) software.

Preparation of fruiting body extract for determination of protein and carbohydrate
Fresh fruiting bodies (30 g) were disrupted by being crushed with acid washed sea sand in mortar and pestle. Then tissues were extracted with 100 ml of 20 mM imidazole buffer containing 1 mM EDTA, 2 mM PMSF (pH 7.8). Unbroken cells and cell debris were removed after centrifugation at 10000 rpm for 30 min and the supernatant was used for determination of protein and carbohydrate (Das and Mukherjee, 2007).

Protein determination
Protein concentration was determined by the method of Lowry et al. (1951) with slight modification. At first Lowry A (2% sodium carbonate mixed with 0.1 N NaOH solution) and Lowry B (1.56% copper sulphate solution mixed with 2.37% sodium potassium tartarate) reagents were prepared. Lowry C reagent was made by mixing 2 ml of (Lowry B) with 100 ml of (Lowry A). 20 µl mushroom extract was mixed with 980 µl of distilled water and incubated for 15 min after addition of 2 ml Lowry C reagent. After incubation 1 ml 1N Folin - Ciocalteau reagent solution (Diluted 2N commercial reagent with an equal volume of water on the day of use) was mixed and incubated for 30 min in dark condition. The OD values were taken at 660 nm and the protein values of mushroom samples were determined by comparing the standard curve of BSA.

Carbohydrate determination
Total carbohydrate was determined using anthrone reagent according to Pons et al. (1981) with some modifications. 100 µl of the sample was taken in a test tube and 5 ml of 2.5 N HCl was added with it. Hydrolyzed the mixture in a boiling water bath for three hours and neutralized it with solid sodium carbonate until the effervescence ceased. Made up the volume to 100 ml and centrifuged. Supernatant was collected. One ml of sample was mixed with 4 ml of anthrone reagent. The mixture was boiled for eight minutes in a boiling water bath. The OD values were taken at 630 nm after rapid cooling. The carbohydrate content of mushroom samples were determined by comparing the standard curve of glucose.

Polyphenol determination
Oven dried mushroom powder (500 mg) was mixed with 2 ml of 80% methanol. It was centrifuged at 10,000 rpm for 30 min. This process is repeated thrice by same way mixing with 80% methanol extract with pellet. Finally supernatant is collected and volume made up to 5 ml by adding 80% methanol. 2 ml supernatant was taken in a petriplate, evaporated to dryness then dissolved the residue in 2 ml distilled water. 1 ml sample has been taken and mixed with 2 ml Na2CO3 (2%), after 2 min incubation 1 ml Folin - Ciocalteau reagent is added. After 30 min incubation at room temperature OD720 has been taken and compared with gallic acid standard.
Determination of moisture content and dry matter of mushroom

Fresh mushroom fruiting bodies were weighed and kept in an oven at 60 °C for overnight until the constant weight was gained. The moisture content and dry matter of *Pleurotus* spp. were calculated according the following formulas given by Khan et al. (2013).

\[
\text{Moisture content (\%) } = 100 \times \frac{(W_1 - W_2)}{W_1}
\]

\[
\text{Dry matter (\%) } = 100 \times \frac{W_2}{W_1}
\]

Where \(W_1\) = weight of fresh sample

\(W_2\) = weight of dry sample

Determination of ash content

One gram of oven dried sample was taken in cleaned and previously weighed china crucible. After ignition on a flame, crucible was placed in a muffle furnace at (550 ± 50) °C for three hours. After that crucible was cooled in desiccator and then weighed. Ash contents were calculated according to the formula of Khan et al. (2013).

\[
\text{Ash (\%) } = 100 \times \frac{(W_3 - W_4)}{\text{weight of sample}}
\]

Where \(W_3\) = weight of crucible

\(W_4\) = weight of crucible ± material

Determination of crude fiber content

The crude fibre content was determined according to Khan et al. (2013). Sample (10 g) was heated at simmering temperature (80 °C) with 200 ml H_2SO_4 and kept about half an hour. Through frequent addition of hot water the medium volume was kept constant. After addition of 500 ml cold water, the boiling was stopped. The content was filtered immediately under vacuumed condition. The residues were washed for several times with hot water and digested. The digested sample was then filtered until become neutral after addition of acetone. The residues were properly washed and transferred to crucible. The sample was then dried in constant weight. Crucible was placed in the muffle furnace at 65 °C for ignition. The ash and crude fibre was calculated according to the following formula

\[
\text{Crude fibre(\%) } = a - \left(\frac{b}{w}\right) \times 100
\]

Where \(a\) = Dry weight after digestion.

\(b\) = weight of ash

\(w\) = weight of sample

Determination of sodium and potassium in mushroom strains

Dried mushroom powder (0.5 g) was taken in 50 ml volumetric flask and 5 ml ternary acid (made by nitric acid, H_2SO_4 and perchloric acid in 9:4:1 ratio) was added with it. Then the mixture was heated on the hot plate until it turned colorless and transparent. After that volume was made up to 50 ml by distilled water in volumetric flask. Then calculate Na/K in flame photometer against standard solution.

Statistical analysis

All experiments were conducted in nine replicates (3 sets x 3 batches) and the parameters were given as mean ± standard deviation. Both mean and standard deviation were performed where appropriate, using the statistical package within Microsoft® Excel Version 2010. Graphs were drawn using GraphPad Prism V.5.

RESULTS

Effect of substrate length

All the tested five *Pleurotus* species are cultivated in non-sterilized rice straw at 30 ± 2 °C. In present experimental condition two types of rice straw are used. One of very small length about 1.0 cm and other of 5.0 cm length. The results show that the biological efficiencies are much better in tiny straw substrates (1.0 cm) than the straw with higher length (5.0 cm) (Fig. 1). 1805 shows highest BE in first flush using 1.0 cm rice straw as substrate whereas 1802 shows least BE in 2nd flush using 5.0 cm rice straw substrate. The time requirement for initiation of fruiting bodies are same or slightly less in smaller length of substrates (Table-1). Hence subsequently in all other experiments smaller length of rice straw substrate is used. 6315 requires about 24 ± 2 days for...
initiation of first fruiting flush whereas 1802 desires only 10 ± 1 day.

**Effect of spawn percentage**
The BE is better in 20% spawn than the 10% spawn in each experiments (Fig. 2) though the spawn % has no effect on time of fruiting initiation in present experimental condition (data not shown). Maximum increase of BE is found in 3308. 3308 and 1805 show more or less similar BE at first fruiting flush with 20% spawn follows by 1799. BE is lowest in 6315. Hence subsequent characterization, morphometric studies and biochemical analysis are done using fruiting bodies obtained from 20% spawn.

**Fruiting life**
The fruiting life (time span from initiation of primordia to deliquescence of fruiting body) varies 4-6 days in 1805, 4-5 days in 1802 and 6315 whereas only 3 days in 1799 and 3308.

**Morphometric studies**
The diameter of pileus and stipe is maximum in 1805 whereas diameter of pileus is lowest in 3308. In 6315, 1802 and 1799 diameter of pileus and stipe slightly varies. Length of stipe is maximum in 1802 and minimum in 1799 (Table-2). Pileus thickness of 1805 is more than other four species. 1802 has moderate thickness whereas 6315 showed least thickness of pileus (Table-2).

The fresh weight of 1805 fruiting body is more than the other strains (Table 3). 1802 shows moderate weight of fruiting bodies. Whereas 6315 and 3308 have less weight of fruiting bodies. The total number of fruiting bodies per bag in present experimental condition is higher in 3308 and lower in 6315 (Table 3).

The measurement of basidium and basidiospores of all the five strains are also taken (Table 4). The length of basidium is maximum in 3308 and minimum in 1799, breadth of basidium is maximum in 1805 and minimum in 6315, average area of basidiospore is maximum in 3308 and minimum in 1805. The no. of basidium present per unit area (1000 sq. µm) in 6315 is 8 but 4 in 3308.

Moisture content and dry matter of all the mushroom strains are calculated. Moisture content is maximum in 6315 strain and minimum in 1805 and obviously dry matter is maximum in 1805 strain but minimum in 6315 (Table 5). 3308 has highest percentage of ash followed by 1802 whereas the ash percentage of 1805 is less than half of 3308. 1799 and 6315 showed similar amount of ash percentage (Table 5). 1805 contains highest percentage (10.2) of crude fibre followed by 1799. The other strains show very less amount of crude fibre. 3308 shows the least amount of fibre (1.4%) content (Table 5).

**Measurement of protein, carbohydrate and polyphenols**
The protein, carbohydrate and polyphenol conc. of all the Pleurotus strains are measured (Table 5). The protein conc. is maximum in 1802 strain moderate in 6315 and 1805 whereas minimum protein concis found in 3308 in present experimental condition. The carbohydrate quantity is maximum in 1802 and 6315 strains and minimum carbohydrate contents are present in 1805. 1802 and 1805 strains show maximum amount of polyphenols than other species. 1799 shows moderate amount of polyphenols.
Table 3: Measurement* of the weight of individual fruiting body* of different Pleurotus sp. grown on 30±2 °C

<table>
<thead>
<tr>
<th>Name of the strains</th>
<th>wt. of pileus (gm)</th>
<th>wt. of stipe (gm)</th>
<th>wt. of fruiting body (gm)</th>
<th>No. of fruiting body (1st flush)</th>
<th>No. of fruiting body (2nd flush)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1799</td>
<td>2.9±0.20</td>
<td>0.8±0.11</td>
<td>3.7±0.15</td>
<td>41±14</td>
<td>Nd</td>
</tr>
<tr>
<td>1802</td>
<td>3.6±0.22</td>
<td>0.9±0.12</td>
<td>4.5±0.17</td>
<td>24±8</td>
<td>12±3</td>
</tr>
<tr>
<td>1805</td>
<td>5.4±2.00</td>
<td>1.1±0.22</td>
<td>6.6±2.10</td>
<td>23±9</td>
<td>14±4</td>
</tr>
<tr>
<td>3308</td>
<td>1.7±0.10</td>
<td>0.9±0.11</td>
<td>2.6±0.10</td>
<td>58±4</td>
<td>48±4</td>
</tr>
<tr>
<td>6315</td>
<td>1.8±0.10</td>
<td>1.1±0.14</td>
<td>2.9±0.12</td>
<td>18±5</td>
<td>Nd</td>
</tr>
</tbody>
</table>

Nd: Not detected. *Means±SD, *fruiting bodies were obtained from first flush using 20% spawn and non-sterilized rice straw (1 cm) as substrate at 30±2 °C

Table 4: Morphometric data* of basidium and basidiospores of different Pleurotus strains* grown on 30±2 °C

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Length of basidium (µm)</th>
<th>Breadth of basidium (µm)</th>
<th>No. of basidium per 1000 sq. µm</th>
<th>Area of basidium (sq. µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1799</td>
<td>16.4±2.0</td>
<td>4.7±0.8</td>
<td>6</td>
<td>11.37±2.3</td>
</tr>
<tr>
<td>1802</td>
<td>20.3±2.5</td>
<td>3.3±0.8</td>
<td>5</td>
<td>7.65±1.2</td>
</tr>
<tr>
<td>1805</td>
<td>22.3±2.2</td>
<td>6.2±1.1</td>
<td>6</td>
<td>6.20±1.2</td>
</tr>
<tr>
<td>6315</td>
<td>26.5±3.8</td>
<td>2.9±0.4</td>
<td>8</td>
<td>8.95±1.6</td>
</tr>
<tr>
<td>3308</td>
<td>29.2±3.4</td>
<td>4.1±1.0</td>
<td>4</td>
<td>15.68±2.2</td>
</tr>
</tbody>
</table>

*Means±SD, *fruiting bodies were obtained from first flush using 20% spawn and non-sterilized rice straw (1 cm) as substrate at 30±2 °C

Measurement of sodium and potassium content

Na and K contents in different Pleurotus strains are measured from the dried mushroom powder (Table 6). Potassium content of all the oyster mushroom strains are superior than their sodium content. The potassium content varies from 18.00 to 23.43 ppm whereas sodium content varies from 5.81 to 10.56 ppm.

DISCUSSION

In comparison to button mushroom cultivation of oyster mushroom (Pleurotus spp.) is very much simple and the agroclimatic condition of India is very much encouraging for their farming. Though Pleurotus cultivation has been accomplished very efficiently within the temperature range of 22-27 °C (Asraf et al, 2013) but the present investigation has been carried out in slightly higher temperature range i.e. (30±2) °C. Oyster mushroom can be cultivated on a large no of lignocellulosic substrates like wood log, straw, vegetable peels even on weeds (Gregori et al, 2007). Pavlik (2005) reported that the biological efficiency of P. ostreatus varies from substrate to substrate. Different types of straw are by and large used for commercial production of Pleurotus. Though for commercial purposes pasteurized or composted straw are used but nonpasteurized straw are also used for cultivation of oyster mushroom (Das et al, 2007, 2010).

In the present investigation two types of rice straw are used. One of very small length about 1.0 cm another of 5.0 cm length. The results show that the biological efficiencies are much better in the straw with lower length than the straw with higher length (Fig. 1). For primordia initiation, the time requirement are same or slightly less in smaller length of substrates. (Table-1). Possibly the fungi availed more surface area for colonization in tiny substrates. Zhang et al. (2002) reported similar result where ground straw showed better efficiency than chopped straw. The BE is better in 20% spawn than the 10% spawn (Fig. 2). Zhang et al (2002) reported higher spawn percentage enhanced mushroom yield. Here the spawn % has no effect on time of fruiting initiation. BE is more or less comparable in P. florida (3308) and P. pulmonarius (1805) followed by P. flabellatus (1799) and P. ostreatus (1802). BE is lowest in P. floridanus. 3308 showed about 73% increase in BE of first flush by increasing the spawn percentage from 10 to 20 (Fig. 2). Asraf et al (2013) showed highest yield in P. ostreatus than P. sajorcaju and P. djamor. However, Obozai et al (2000) have been found lower biological efficiency in P. ostreatus. The time for primordia initiation is more in P. floridanus and less in P. ostreatus (Table 1). Many workers showed that initiation of fruiting body is dependent on type and quality of substrates (Gregori et al, 2007). The length of stipe, diameter of pileus etc are some unique characters of mushroom which varies from species to species. Here the length of stipe is maximum in P. ostreatus (1802) (Table 2). The fresh weight of individual fruiting body is more in P. pulmonarius than other tested strains (Table 3). P. ostreatus shows moderate weight of fruiting bodies. Whereas P. floridanus and P. florida have less weight of fruiting bodies. Neelam and Singh (2013) showed that the fresh wt. of P. flora as 1.34-1.55g and P. ostreatus as 1.32-1.47g. The total number of fruiting bodies per bag in present experimental condition is lower in P. floridanus and higher in P. florida (Table 3).

As the genus Pleurotus belongs to subdivision basidiomycota so characters of basidium and basidiospores are important. Table 4 shows some of these characters which might be important for taxonomical studies.

Moisture content and dry matter of any living matter is reciprocal to each other. Moisture content is maximum
Das, et al.: Comparative study of five Pleurotus spp. in warm temperature 30±2 °C using non sterilized rice straw as the substrate.

**Table 5: Determination**a of various physiochemical parameters of different Pleurotus spp. grown on 30±2 °C

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Moisture (%)</th>
<th>Dry matter (%)</th>
<th>Crude fibre (%)</th>
<th>Ash (%)</th>
<th>Protein (µg/g tissue)</th>
<th>Carbohydrate (µg/g tissue)</th>
<th>Polyphenol (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1799</td>
<td>87.5±0.21</td>
<td>12.5±0.21</td>
<td>6.6±0.19</td>
<td>5.9±0.09</td>
<td>392±13</td>
<td>216±12</td>
<td>53.75±7.8</td>
</tr>
<tr>
<td>1802</td>
<td>88.2±0.15</td>
<td>11.8±0.15</td>
<td>3.1±0.06</td>
<td>8.7±0.19</td>
<td>856±29</td>
<td>260±18</td>
<td>67.85±9.7</td>
</tr>
<tr>
<td>1805</td>
<td>85.0±0.18</td>
<td>15.0±0.18</td>
<td>10.2±0.25</td>
<td>4.8±0.06</td>
<td>417±17</td>
<td>208±14</td>
<td>65.40±9.4</td>
</tr>
<tr>
<td>3308</td>
<td>88.0±0.16</td>
<td>12.0±0.16</td>
<td>1.4±0.08</td>
<td>10.6±0.16</td>
<td>294±21</td>
<td>228±17</td>
<td>18.90±3.3</td>
</tr>
<tr>
<td>6315</td>
<td>91.1±0.19</td>
<td>8.9±0.19</td>
<td>2.8±0.09</td>
<td>6.1±0.12</td>
<td>448±23</td>
<td>255±21</td>
<td>16.40±2.9</td>
</tr>
</tbody>
</table>

*aMean±SD, *fruiting bodies were obtained from first flush using 20% spawn and non-sterilized rice straw (1cm) as substrate at 30±2 °C.

As Pleurotus is one of the most important edible mushroom so the biochemical characterization is very much needed to conclude its nutritional values (Table 5). In the present study the protein concentration is highest in *P. ostreatus*, moderate in *P. flabellatus* and *P. pulmonarius* whereas minimum protein concentration is found in *P. floridanus*. The amount of carbohydrate is maximum in *P. ostreatus and P. floridanus* strains and minimum carbohydrate is present in *P. pulmonarius* (Table 5). Dundar et al. (2008) showed that the carbohydrate content of *P. ostreatus, P. sajor-caju* and *P. eryngii* as 37.87, 37.72 and 39.85 g/100 g dry material, respectively. *P. ostreatus* and *P. pulmonarius* strains show maximum quantity of polyphenols than other species. *P. flabellatus* shows moderate amount of polyphenols whereas *P. floridanus* and *P. florida* show least amount of polyphenols (Table 5).

CONCLUSION

Temperature plays a critical role in fruiting body development in different mushroom including *Pleurotus* spp. In the present investigation the BE, morphometrics of fruiting body and basidium and chemical characterization of fruiting bodies of five different *Pleurotus* spp. were done using rice straw as substrates at (30±2) °C. The BE not only varies from species to species but also on quality of substrates (length) and spawn percentage. Protein, carbohydrate, polyphenol, sodium potassium contents of different strains of *Pleurotus* spp are also studied and compared with other available literatures. This is possibly the first report of cultivation of such a number *Pleurotus* spp. in warm temperature 30±2 °C using non sterilized rice straw as the substrate.

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Authors’ contributions

ND and NCK developed the concept and designed the experiments. SM and LB done the experiments. ND wrote the manuscript and overall supervised the work.

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