Effect of Supplements on Growth and Nutritional Content of

*Pleurotus pulmonarius* Cultivated on Rice Straw

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1. INTRODUCTION

*Pleurotus pulmonarius* is one of the most preferred edible mushrooms being cultivated in the tropics and has gradually gained prominence in the subtropical and temperate zones of the world. *Pleurotus* spp are popular and widely cultivated throughout the world mostly in Asia, America and Europe because of their simple, low cost production technology and high biological efficiency (Mane et al., 2007). *P. pulmonarius*, commonly known as the Indian Oyster, Italian Oyster, Phoenix Mushroom, or the Lung Oyster, is a mushroom very similar to *Pleurotus ostreatus*, the pearl oyster, but with a few noticeable differences. The caps of *pulmonarius* are much paler and smaller than *ostreatus* and develop more of a stem. *P. pulmonarius* also prefers warmer weather than *P. ostreatus*. Otherwise, the taste and cultivation of the two species is generally described as largely the same (Stamet, 2000). In nature, *P. pulmonarius* is an active decomposer of wood and several other substrates. It has ability to colonise and digest several types of materials containing lignin, cellulose, starch, sugars and fermented proteins, due to its capacity of secreting a spectrum of enzymes.

Mushrooms are increasingly being recognized as important food products for their significant role in human health, nutrition and disease. Various substrates have different effects on the growth, yield and quality of mushrooms. This study was carried out to investigate the effect of rice straw substrate supplemented with different additives on the growth, yield and nutritional content of *Pleurotus pulmonarius*. The additives used as supplement were maize waste, Brewery waste and soya bean waste. The results obtained indicated that the growth and yield of *P. pulmonarius* varied widely with the various additives used. After the first and second flush, Brewery waste gave the highest mean number of fruiting bodies (25.5) and highest mean fresh weight (54.1g). The least mean number of fruiting bodies (6.5) and lowest fresh weight (22.4g) was obtained from the substrate without any additive. Fruiting body with highest length of stipe and pileus diameter was obtained from those grown on substrate supplemented with soya bean waste. The additives had effect on the nutritional value also, as highest protein content (26.92%) was observed on rice straw supplemented with Brewery waste and soya bean waste respectively. The lowest moisture content (8.51%), ash content (5.22%) and crude fibre (2.31%) were obtained from rice straw without additives. In the present study, substrate supplementation improved growth, yield and quality of *P. pulmonarius*. Due to high protein content of *P. pulmonarius*, it can be used as a substitute for expensive meat and fish in developing nations like Nigeria.

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Abstract

Mushrooms are increasingly being recognized as important food products for their significant role in human health, nutrition and disease. Various substrates have different effects on the growth, yield and quality of mushrooms. This study was carried out to investigate the effect of rice straw substrate supplemented with different additives on the growth, yield and nutritional content of *Pleurotus pulmonarius*. The additives used as supplement were maize waste, Brewery waste and soya bean waste. The results obtained indicated that the growth and yield of *P. pulmonarius* varied widely with the various additives used. After the first and second flush, Brewery waste gave the highest mean number of fruiting bodies (25.5) and highest mean fresh weight (54.1g). The least mean number of fruiting bodies (6.5) and lowest fresh weight (22.4g) was obtained from the substrate without any additive. Fruiting body with highest length of stipe and pileus diameter was obtained from those grown on substrate supplemented with soya bean waste. The additives had effect on the nutritional value also, as highest protein content (26.92%) was observed on rice straw supplemented with Brewery waste and soya bean waste respectively. The lowest moisture content (8.51%), ash content (5.22%) and crude fibre (2.31%) were obtained from rice straw without additives. In the present study, substrate supplementation improved growth, yield and quality of *P. pulmonarius*. Due to high protein content of *P. pulmonarius*, it can be used as a substitute for expensive meat and fish in developing nations like Nigeria.
extract of *P. pulmonarius* displayed anti-inflammatory and antitumor activity comparable to the standard reference drugs diclofenac and cisplatin respectively. An orally administered hot water extract of *P. pulmonarius* had a significant antihyperglycemic effect, halted the progression of diabetes and reduced the mortality of alloxan induced diabetic mice by approximately 50%. It showed a synergistic effect with the antidiabetic drug glibenclamide, supporting the possibility of effective combination therapy of glibenclamide and *P. pulmonarius* for diabetes (Badole, 2008). *P. pulmonarius* have antimicrobial properties and exhibit antioxidant activity also (Ramesh and Pattar, 2010). They have been observed to display excellent antitumor, anti-inflammatory and analgesic properties (Jose et al., 2002; Smiderle et al., 2008).

The nutritional and chemical compositions of mushroom are responsible for their medicinal values (Lindequeist et al., 2005). However, nutritional composition of mushroom is affected by many factors among which the composition of growth substrate and the method of cultivation are of major importance (Belewu, 2003). Nutritional supplementation of cultivation substrate is an important cultural practice of mushroom cultivation (Ayodele and Okhuoya, 2007). Most of the growth, yield and quality parameters varied significantly when mushrooms were cultivated with different levels of supplementation (Mahbuba et al., 2010). Substrate supplementation with various additives including nitrogen sources has been reported to improve growth, yield and quality of mushrooms (Khare et al., 2010; Onyango et al., 2011). They usually change the decomposition rate and the sequence of decomposition of substrate components (Zadrazil, 1993).

Mushroom cultivation is also a useful method of environmental waste management and waste disposal. Many agricultural and industrial by-products can find uses, as substrates, in mushroom production (Chinda, and Chinda, 2007). Some of these materials litter and sometimes pollute our environment. In view of the importance of *Pleurotus pulmonarius* (use as food condiment and in medicine), it became necessary to study the simplest and cheapest substrate that would give the highest weight yield and nutritional content for its production. The aim of this research was to investigate the effect of rice straw substrate, supplemented with maize waste, Brewery waste and soybean waste on the growth, yield and nutritional content of *P. pulmonarius*.

### 2. MATERIALS AND METHOD

#### 2.1 Source of Culture

Pure culture of *P. pulmonarius* (Fries) Quel. was obtained from the Biotechnology Laboratory, of the National Root Crops Research Institute, Umudike, Abia state.

#### 2.2 Culture Preparation

For the propagation of the main culture, 2.0% malt extract agar was used. MEA plates were inoculated under sterile conditions, with a mycelium/agar plug (6mm diameter) of a young actively growing margin of the colony. Prior to its use as an inoculum for grain spawn, a mycelium/agar plug was inoculated at the center of the plate and incubated at 25°C in the dark for seven days, until the surface of the plate was completely covered. Sub-culturing was carried out in order to get more cultures.

### 2.3 Preparation of Grain Spawn

Guinea corn (*Sorghum bicolor*) was used as grain substrate for planting spawn. This was bought from the seed market. The grains were soaked in water and thoroughly washed. They were sieved and filled half way into heat resistant bottle that were thoroughly pre-washed. The bottles were covered and autoclaved at 121°C for 20 mins. After autoclaving, the bottles were allowed to cool and the sterile grains were inoculated with 10mm mycelial discs and incubated at 25°C in full darkness for two weeks to enable the mycelia to permeate.

#### 2.4 Preparation of Substrates for Cultivation

The main substrate used for spawn run was rice straw. The rice straw was chopped up into small pieces of about 7-10 cm long and soaked overnight in water. The rice straw as a main substrate was supplemented with brewery waste, soya-bean waste and maize waste respectively as additives. The supplements (additives) were soaked separately for about 12 hours. An amount, 500 g. of substrate-supplement mixtures was filled in a 4 litre transparent white polypropylene bucket, which were uniformly perforated from the mid-point to the upper part of the bucket, individually for each treatment. Twenty-five grams of each supplement was weighed out and added to the substrate. The non-supplemented substrate (without additive) served as the control.

The substrate-supplement thoroughly mixed, were covered and then pasteurized at 80°C for 2 hours in a drum. After pasteurization, the substrates were allowed to cool in the culture room. On cooling, 50 g of the grain spawn were inoculated into each experimental bucket and mixed thoroughly to ensure good contact with the substrates, then covered. The covering was necessary to reduce contamination, minimize the escape of water vapour and also to trap carbon dioxide, which helps to stimulate vegetative growth (Zadrazil, 1978.) The doors and the windows of the growth room were shut and the floor was watered once every day from the day of inoculation, to maintain the humidity of the room. After 11 days when the mycelia had fully colonized the substrate, the buckets were opened to improve aeration and allow the flushing of the fruit body. The windows were opened at intervals to increase ventilation and lower the temperature.

#### 2.5 Harvesting

Harvesting was carried out when the fruiting bodies were matured. The process of harvesting involves the removal with scalpel of the matured fruiting bodies from their substrate without any destruction on the substrate bag. The mature mushroom was held on their stipe below the pileus and close to the substrate level and was gradually detached. All fruiting bodies of a particular substrate bag were harvested at the same time since each bag had to be watered after harvest. Watering was done by immersing the bags in a bowl of water for 5 secs. This is to enable the substrate to have moisture that enables fruiting to occur again for harvest.
2.6 Data Collection

The yield of *P. pulmonarius* on the different substrate supplementation were determined by recording the number, weight and size of the fruit bodies after sprouting. The measurements from the various replicates were added and their mean value calculated. The following parameters of growth / yield were measured.

2.6.1 Number of fruit bodies: This was done by directly counting the number of fruit bodies on each substrate.

2.6.2 Height of fruit bodies: The height was measured in centimetres using transparent ruler from the base of the stipe to the pileus.

2.6.3 Diameter of the pileus: This was also measured in centimetres with ruler from one edge of the pileus across the stripe to the other edge.

2.6.4 Fresh weight of fruit bodies: This was done using an electrical weighing balance.

2.7 Proximate Analysis

Proximate Analysis was carried out on mushroom grown on each of the substrate treatment.

Nutrients like carbohydrates, protein, fat, ash, moisture and crude fibre contents were determined by using the methods outlined in the AOAC (1990). Protein determination was carried out using the Kjedahl method. Fat determination was carried out using a Soxhlet apparatus. Also, determination of fibre content was done according to the enzymatic gravimetric method.

**Determination of total ash**

One gram of the sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5 to 6 hr at 600°C. It was then cooled in a dessicator and weighed. To ensure completion of ashing, the crucible was then heated in the muffle furnace for 1 hr, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or grayish white in color.

Then total ash was calculated as: Ash content (g/100 g sample) = Weight of ash × 100/Weight of sample taken (Raghuramulu et al., 2003).

**Total carbohydrate estimation**

The content of the available carbohydrate was determined by the following equation: Carbohydrate (g/100 g sample) = 100 − [(moisture + fat + protein + ash + crude fiber) g/100 g] (Raghuramulu et al., 2003).

2.8 Statistical Analysis

Using SPSS (IBM SPSS Statistics 19), one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) were done. Statistical significance was set at P<0.05.

3. Results

Table 1 shows the effect of rice straw supplementation with brewery waste, soya bean waste and maize waste on growth and yield of *P. pulmonarius*. Results obtained from this study showed that Brewery waste gave the highest number of fruiting body (25.5) and highest weight of fruiting body (54.1g), followed by soyabean waste (15.5) and (37.4g) respectively. The substrate without any supplement (control) gave the lowest number of fruiting body and lowest weight of fruiting body.

Length of stipe and diameter of pileus was highest in *P. pulmonarius* grown on substrate supplemented with soyabean waste, followed by that of Brewery waste (Table 1). The least length of stipe and pileus diameter was obtained from substrate without additives (control).

Table 2 shows the effect of supplements on proximate composition of *P. pulmonarius* fruiting body. The supplements had significant effect on the nutritional value of *P. pulmonarius* fruit body as shown in Table 2.

Table 1: Effect of supplement on growth and yield of *P. pulmonarius*.

<table>
<thead>
<tr>
<th>Substrate+ supplement</th>
<th>No. of fruiting body</th>
<th>Weight of fruiting body (g)</th>
<th>Length of stipe (cm)</th>
<th>Diameter of pileus (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R+ Brewery waste</td>
<td>25.5</td>
<td>54.1</td>
<td>3.17</td>
<td>7.50</td>
</tr>
<tr>
<td>R+ Soya bean waste</td>
<td>15.5</td>
<td>37.4</td>
<td>3.67</td>
<td>8.00</td>
</tr>
<tr>
<td>R+ Maize waste</td>
<td>17.0</td>
<td>28.8</td>
<td>2.63</td>
<td>7.00</td>
</tr>
<tr>
<td>Control</td>
<td>6.5</td>
<td>22.4</td>
<td>2.10</td>
<td>6.93</td>
</tr>
</tbody>
</table>

Values are means of 3 replicates. R: Rice straw.

Table 2: Effect of supplements on proximate composition (%) of *P. pulmonarius* fruiting body.

<table>
<thead>
<tr>
<th>Substrate+ supplement</th>
<th>MC</th>
<th>Ash</th>
<th>Fibre</th>
<th>Fat</th>
<th>Protein</th>
<th>CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.51c</td>
<td>5.22c</td>
<td>2.31c</td>
<td>4.73c</td>
<td>20.33b</td>
<td>58.87b</td>
</tr>
<tr>
<td>R+ Soya bean waste</td>
<td>9.10b</td>
<td>5.27a</td>
<td>3.22a</td>
<td>4.68b</td>
<td>26.92a</td>
<td>56.7b</td>
</tr>
<tr>
<td>R+ Brewery waste</td>
<td>8.76bc</td>
<td>6.27a</td>
<td>2.77a</td>
<td>5.22a</td>
<td>26.92a</td>
<td>51.3c</td>
</tr>
<tr>
<td>R+ Maize waste</td>
<td>8.93b</td>
<td>5.78b</td>
<td>2.16b</td>
<td>4.91b</td>
<td>12.12c</td>
<td>62.68a</td>
</tr>
</tbody>
</table>

Values are means of 3 replicates and values within the same column bearing different letters are significantly different (P<0.05). MC: Moisture content, CHO: Carbohydrate, R: Rice straw.
The highest protein content (26.92%) was observed on fruit body grown from substrate supplemented with soya bean waste and brewery waste while minimum (12.12%) was obtained from substrate supplemented with maize waste. Maximum carbohydrate content (62.68%) was got from maize waste, followed by control (58.87%) while the minimum (51.35%) was from brewery waste supplement. The fat content was low compared to protein and carbohydrate, with the lowest (4.68%) from soya bean waste while the highest (5.22%) was from brewery waste. The crude fibre ranged from 2.16% in maize waste to 3.22% in soya bean waste and the ash content ranged from 5.22% in unsupplemented substrate to 6.27% in Brewery waste. Moisture content was highest in soya bean waste, but lowest in control set up. As regards nutritional quality, substrates supplemented with Brewery waste and soya bean waste respectively, gave better results than maize waste.

4. Discussion

In this study, all the supplements viz: soyabean waste, brewery waste and maize waste supported the mycelia growth of *P. pulmonarius* on rice straw. Brewery waste stimulated the highest mycelia extension followed by soya bean waste, while the least was from maize waste. The results obtained indicate that the growth and yield of *P. pulmonarius* varied with the various supplements added to the main substrate (rice straw). Brewery waste had the highest number of fruiting body and weight of fruiting body, whereas the substrate without any supplement gave the lowest number of fruiting body and fresh weight. Soya bean waste had greatest influence on stipe length and pileus diameter, while the substrate without supplement gave the lowest. Finding of this study is in line with the report of Rossi et al. (2003) who reported that any amount of rice bran added to the substrate increased the number of fruiting body especially at 25% and 30% level. The increased productivity of *P. pulmonarius* supplemented with Brewers waste, soyabean and maize waste can be attributed to the carbohydrates, amino acids and mineral elements present in the supplements. According to Royse (2001), different supplements, such as wheat bran, rice bran, millet, rye and maize powder are suggested to add to saw dust to serve as major nutrients to provide optimum growth medium. These supplements improve the production, quality, flavor, and shelf life of cultivated mushrooms (Okhuoya et al., 2005).

The results of the moisture, ash, fat, fibre, protein and carbohydrate of *P. pulmonarius* fruit bodies cultivated on rice straw supplemented with brewery waste, maize waste and soya-bean waste differ significantly (p<0.05). Carbohydrate and protein were the most abundant nutrient in the fruit body of *P. pulmonarius* with the highest content obtained from substrate with additives. The result of this study is similar to Sarker et al. (2009) who reported that the addition of any amount of supplements to substrate showed an improvement in mushroom quality.

The relative high percentage of carbohydrate and protein in the mushroom fruit bodies cultivated on the substrates supplementation conforms to the work of Nwoko et al., (2017). The high protein contents of the *P. pulmonarius* fruit bodies cultivated on the various substrates confirms the assertion by several workers that mushroom protein is intermediate between that of animals and vegetables, but superior to most other foods, including milk and contains all the nine essential amino acids required by man (Chang and Miles, 2004; Kurtzman, 2009). Protein is an important nutritional component and protein deficiency, the world’s most serious human nutrition problem, especially in third world countries like Nigeria. Therefore *P. pulmonarius* with its high protein content is a promising food that may be substituted for meat to overcome protein malnutrition problem and mineral deficiency. Low fat content of *P. pulmonarius* shows that the mushroom could be good for people with cardiac problems. This is in line with the reports of Okwulehie and Odunze (2004) who maintained that mushrooms generally contain low oil and fat, and because of that, they are recommended as good supplements for patients with cardiac problems.

Nutritional composition of mushroom is affected by many factors among which the composition of the substrate is of major importance. The nutritional composition of *P. pulmonarius* obtained in this study may have been contributed by the different substrate composition used. This can be supported by the findings of Shah et al. (2004) where *P. florda* and *P. ostreatus* on sawdust, gave a higher significant nutritional value than that cultivated on corn cobs.

CONCLUSION

With the results obtained, it is clear that the supplementation of rice straw with additives in the fructification of *P. pulmonarius* improved their growth, yield and nutritional quality. The finding of this study also shows that brewers waste and soya bean waste may be very effective for a higher yield and better quality *P. pulmonarius*. The results on nutritional analysis of *P. pulmonarius* indicate that the studied oyster mushroom species have good nutritive value for human. The fruitbodies can be eaten as a protein source or an alternative to fish and meat in rural and urban areas where these items are expensive. This study is very important considering its potential contribution to the agricultural production, research, pollution control, waste management, medicine, economy, poverty alleviation and its potentials as cheap protein source. Hence, cultivation of *P. pulmonarius* should be encouraged in Nigeria because agro-based substrates used for its cultivation are in abundance. It could also help in recycling and elimination of agricultural and industrial waste materials.

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