EFFECT OF PHOTOPERIODS AND PH ON MYCELIAL GROWTH AND FRUCTIFICATION OF *Pleurotus sajor-caju*

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ABSTRACT

*Pleurotus sajor-caju* (Fr) Singer was cultivated on substrates consisting of 86% cotton waste, 10% ricebran and 4% cassava peel powder. Fructification commenced four weeks after spawning and fruitbodies matured for harvesting in 3 days. The 24 hours darkness photoperiod treatment stimulated significantly highest mycelia growth of 13.3cm at P< 0.05. Optimal growth and fructification occurred at pH 7. The poorest yield of 51.6g was obtained from 24 hr. darkness treatment and pH 11. Highest mean number of fruitbodies produced per polypropylene bag was 23.0 for photoperiod’s treatment of 12 hours light and 12 hours darkness, and 43.0 for pH 7 treatment. Matured fruitbodies had pileus diameter of 4-12cm for pH 7 and 12 hours light, 12 hours darkness treatments. Matured mushroom spore print is white. The results of the study showed that 24hours darkness is the ideal condition for optimum mycelia growth, while 12hours light and 12hours darkness is the best condition for fruitbodies production.

**Key words:** *Pleurotus sajor-caju*, Cotton waste, Ricebran, Fruitbodies, Photoperiod

INTRODUCTION

Mushroom obtain their food directly from already made organic compound as they lack chlorophyll and hence cannot manufacture their own food directly from carbon-dioxide and water. Mushrooms are potential contributors to the world’s food supply since they have the ability to transform nutritionally worthless wastes into protein-rich food. Mushrooms are cultivated mainly for their high protein content, culinary value and aromatic flavor (Awan, 1983; Kadiri, 2005b). They are also low in dietary fat content, thus making them...
suitable for patients with cardiac diseases (Jonathan and Fasidi, 2001). Cellulosic residues of bleached kraft – pulp mill wastes (Muller et al., 1984, 1985) and crop residues such as waste cotton, stalks of maize and sorghum, sugarcane bagasse, sawdust and paper waste have been used as substrates for cultivating mushrooms (Kadiri, 2005a).

The properties of the substrate determine which fungi can grow in them (Oei, 1996). The environmental factors are also known to play important role in mushrooms cultivation (Rambelli, 1983; Bahl, 1988; Oei, 1996). Chandra and Purkayastha (1977) reported pH 5.5 as the optimal for the growth of A. campestus and Voluaniella volvcea. The fruitbodies are usually supplied with nutrient by the mycelia growing in the compost and following spore release, the mushroom enters senescence and cell death, presumably to prevent the mushroom from becoming a drain on the mycelium and the compost (Chang and Miles, 2004). Mushroom spawn is the mushroom mycelium growing on a given substrate. It serves as the planting material in mushroom cultivation. Mushroom or basidiocarps are fruits of the fungal plant and consists of two parts, cap and stipes. As the basidiocarp matures, the cap opens and gills are exposed. Mushroom spores are produced in the gills (Quimio and Chang, 1990).

In Nigeria, although edible mushrooms are consumed regularly especially by rural dwellers, their cultivation on a small or large scale is rarity. The present study is aimed at assessing the effect of photoperiods and pH on mycelial growth and fructification of Pleurotus sajor-caju using cotton waste as basal raw material.

**MATERIALS AND METHODS**

**Preparation of mycelia culture:** The mycelia culture of Pleurotus sajor-caju was established by tissue culture of the pileus on malt extract agar medium. This was regularly subcultured thereafter.

**Effects of photoperiods on mycelia growth and fructification of P. sajor-caju**

The substrate used is a mixture of cotton waste, rice bran and cassava peel powder. The substrate constituents were soaked separately in boiling water for one hour and thereafter squeezed. The squeezed cotton waste was teased and the substrate constituents were combined together in the proportion of cotton waste (86%) + rice bran (10%) + cassava peel powder (4%). Large quantity of the substrate mixture was dispensed into autoclavable polypropylene bags at 1kg per bag. The bags were tied with rubber bands at their top and sterilized at 121°C for 30 minutes. The ensuing sterilized substrate in polypropylene bags was inoculated under a laminar air-flow chamber with a mycelia-ramified planting spawn of cotton waste (86%) + rice bran (10%) + cassava peel powder (4%). The planting spawn was obtained by inoculating
the above mixture with mycelia- ramified grain mother spawn of sorghum grain (92%) + CaSO₄ (8%), which in itself was obtained by inoculating the mixture with mycelia culture prepared based on the method used by Kadiri and Kehinde (1998). The inoculated bags were placed under the photoperiods of continuous light, continuous darkness, 8 hours light + 16 hours darkness and 12 hours light + 12 hours darkness at 6 bags per photoperiod at 30 ± 2°C, 70-85% relative humidity and 200-400 lux light intensity. At 26 days after incubation, the mycelial length was determined by placing a meter rule on the polypropylene bag and the length of the ensuing mycelium starting from the point of inoculation was taken. Mycelial density was obtained by visual observation. Two weeks later when primordial formation was observed, the bags were opened to allow for aeration and spray watering was carried out whenever needed. Thereafter, sprouting of fruit bodies was observed. The ensuing fruitbodies were counted and their weights and diameters determined. The fruitbodies maturation time, fruitbodies and spore colours were noted.

Effect of pH on mycelial growth and fructification of *P. sajor-caju*
The method employed was similar to that for the effect photoperiod on mycelial growth and fructification. The substrate constituents were cotton waste (86%) + rice bran (10%) + cassava peel powder (4%), which were prepared above. The substrate was divided into 6 portions, with each portion having 12kg weight. Using 1M HCl, 1M NaOH, pye unicam pH meter, five substrate portions were adjusted to pH 3, 5, 9, and 11 by dropwise addition of 1M HCl or 1M NaOH while the sixth portion, which is the control was found to have pH 7. Each portion was thereafter dispensed into 12 autoclavable bags at 1kg per bag and the bags sterilized at 121°C for 30 minutes. The contents of the bags were inoculated under a laminar air-flow chamber with a planting spawn as above. All the bags were incubated at 30 ± 2°C, 70-85% relative humidity (RH) and 200-400 lux light intensity. At 29 days after incubation, the mycelia length and density were taken using meter rule and visual observation as described earlier. Two weeks later full ramification of the substrate was noticed. The polypropylene bags were moved into a fruiting chamber with temperature of 27 ± 2°C, RH 75-90% and 200-350 lux light intensity. The bags were then opened up to allow for aeration and spray watering with water to be done whenever required. The emerging fruit bodies were counted and their weights and diameters determined. The fruit bodies maturation time, fruit bodies and spore print colours were noted.

Data analysis
The data collected were subjected to analysis of variance based on randomized complete block design and differences
between treatment means were separated using Duncan’s Multiple range Test (DMRT) at 5% probability level.

RESULTS

Effect of photoperiods

From the result obtained from the effect of photoperiods study, 24 hours darkness stimulated significantly highest mycelia growth of 13.3cm at P= 0.05% , this was closely followed by 8 hours light and 16 hours darkness (12.3cm). Twelve(12) hours light and 12 hour darkness stimulated a significantly higher mycelia growth of 11.7cm than the use of continuous light which stimulated the least mycelial growth of 10.0cm at P= 0.05% (Table 1).

Highest matured pileus range (4-12cm), stimulated significantly highest fruitbodies weight (92.0g) and production of 23.0 (at P = 0.05%) which were obtained from 12 hours light and 12 hours darkness. There were no significant difference at P= 0.05% in the mean number of fruitbodies produced from the use of 12 hours light and 12 hours darkness (23.0) compared to the use of continuous light (22.0). These were followed by 8 hours light and 16 hours darkness which produced 15.0 while the poorest Pileus range (3-4cm), fruit bodies weight (6.0g) and production (5.0) was recorded from 24 hours darkness (Table 2).

Effect of pH

Results obtained from pH studies showed that pH 7 stimulated the highest fruitbodies production (43.0), significantly (P= 0.05%) highest weight of 89.2g , mycelia density (5+) and highest range of matured pileus diameter of 4-12cm (Table 3). pH 5.0 stimulated

Table 1: Effect of photoperiods on mycelial growth and density using cotton waste as basal material.

<table>
<thead>
<tr>
<th>Photoperiods</th>
<th>Mean linear mycelia extension (cm) at 26days of incubation</th>
<th>*Mycelial Density (Degree of whiteness)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 hours light and 16 hours darkness</td>
<td>12.3b</td>
<td>4+</td>
</tr>
<tr>
<td>24 hours darkness</td>
<td>13.3a</td>
<td>4+</td>
</tr>
<tr>
<td>Continuous light</td>
<td>10.0d</td>
<td>3+</td>
</tr>
<tr>
<td>12 hours light and 12 hours darkness</td>
<td>11.7c</td>
<td>4+</td>
</tr>
</tbody>
</table>

Means followed the same letter in a vertical column are not significantly different at P=0.05 according to Duncan’s multiple Range Test.

*Mycelial density was estimated on visual scale 3+ to 4+, with 3+ being the least dense.
Table 2: Effect of photoperiods on fructification and production of P. sajor-caju using cotton waste as basal material.

<table>
<thead>
<tr>
<th>Photoperiods</th>
<th>Range of matured pileus diameter (cm)</th>
<th>Mean weight of fruit bodies (g)</th>
<th>Mean number of fruitbodies produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 hours light and 16 hours darkness</td>
<td>4- 10</td>
<td>45.6c</td>
<td>15.0b</td>
</tr>
<tr>
<td>24 hours darkness</td>
<td>3- 4</td>
<td>6.0d</td>
<td>5.0d</td>
</tr>
<tr>
<td>Continuous light</td>
<td>4- 10</td>
<td>51.8b</td>
<td>22.0a</td>
</tr>
<tr>
<td>12 hours light and 12 hours darkness</td>
<td>4- 12</td>
<td>92.0a</td>
<td>23.0a</td>
</tr>
</tbody>
</table>

Means followed the same letter in a column are not significantly different at P=0.05 according to Duncan’s multiple Range Test.

*Mycelial density was estimated on visual scale 3+ to 4+, with 3+ being the least dense.

DISCUSSION

Highest mycelial growth was obtained from 24 hours darkness, while the highest fructification was obtained from 12 hours light and 12 hours darkness. These may be due to the fact that darkness stimulates rapid mycelial growth. This is similar to a past worker who obtained the highest spawn run of Stropharia rugosa-annulata in total darkness condition (Oei, 1996).

The high production and weight of fruit bodies obtained from 12 hours light, 12 hours darkness, and continuous light may be due to proper aeration, presence of light and ventilation of the substrates as oxygen and light are necessary for the production of fruiting bodies of mushrooms. This is related to the findings of past worker who discovered that light is needed for proper development of the cap of all oyster mushrooms and for high cropping of Stropharia rugosa-annulata (Oei, 1996).

The elongated stalk with narrow cap and low production of fruitbodies obtained from 24 hours darkness may be due to

significantly highest mycelia extension of 13.0 while the poorest was recorded from pH 11.0 (9.5). However, there were no significant differences in the number of fruitbodies produced by the use of pH 3.0 & 5.0 P= 0.05%. At pH tending towards alkalinity, mycelia growth was observed to be retarded (Table 3).
Table 3: Effect of pH on mycelial growth, density, fructification, and production of *P. sajor-caju* using cotton waste as basal material.

<table>
<thead>
<tr>
<th>pH</th>
<th>Mean linear mycelial extension (cm) 41 days after incubation</th>
<th><em>Density</em> of mycelial extension (degree of whiteness)</th>
<th>Range of matured pileus diameter (cm)</th>
<th>Mean weight of fruit body (g)</th>
<th>Mean number of fruit bodies produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 3.0</td>
<td>10.5c</td>
<td>3+</td>
<td>4-9</td>
<td>79.8c</td>
<td>41.0b</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>13.0a</td>
<td>4+</td>
<td>4-11</td>
<td>87.1b</td>
<td>41.0b</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>11.3b</td>
<td>5+</td>
<td>4-12</td>
<td>89.2a</td>
<td>43.0a</td>
</tr>
<tr>
<td>pH 9.0</td>
<td>10.5c</td>
<td>3+</td>
<td>4-10</td>
<td>77.4d</td>
<td>32.0c</td>
</tr>
<tr>
<td>pH 11.0</td>
<td>9.5d</td>
<td>2+</td>
<td>3-10</td>
<td>51.6e</td>
<td>31.0c</td>
</tr>
</tbody>
</table>

Means followed the same letter in a column are not significantly different at P=0.05 according to Duncan’s multiple Range Test.

*Mycelial density was estimated on visual scale 3+ to 4+, with 3+ being the least dense.*

High concentration of carbon dioxide due to insufficient aeration. This is in line with past workers who discovered that carbon dioxide level affects stipe elongation and pileus expansion in *Agaricus bisporus* (Zadrazil and Kurtzman, 1982). Fruitbodies of *P. Sajor-caju* attained maturity and white spore print in 3 days. This is similar to the findings of Kadiri (1998) who obtained fruitbodies maturation and white spore print of *P. Sajor-caju* in 2 days, which is also similar to the result of Fasidi and Kadiri (1993) who obtained maturation and white spore print of *Lentinus subnudus* in 3 days.

Matured fruitbodies had widest pileus diameter of 4-12 cm for both photoperiod and pH treatments. This result is in line with past workers who obtained pileus diameter of 5-8 cm of *P. sajor-caju* (Kadiri, 1998), and pileus diameter of 4-6 cm of *Lentinus subnudus* (Fasidi and Kadiri, 1993). Highest mean number of matured fruitbodies produced per polypropylene bag was 23 for photoperiod treatments and 43 for pH treatments. This result is comparable to the one obtained by Kadiri (1998) who observed the mean number of matured fruitbody of *P. sajor-caju* produced per compost bag to be 13.
The significance of the findings from photoperiodic study is that mushroom mycelia grow rapidly in dark environment (Ingold, 1984; Oei, 1996) while oxygen and light are essential for fruitbodies production (Oei, 1996).

It was observed that pH 7 supported the highest fructification while pH 5.0 supported the highest mycelia growth followed by pH 7. This may be due to the fact that *P. sajor caju* thrives best at slightly acidic to neutral pH. This is in line with past worker who obtained optimum growth of *P. sajor caju* at pH 6. (Kadiri, 1998). This is also in line with Oso (1977) who obtained optimum mycelial growth of *Pleurotus – tuberegium* at pH 6.

The significance of the findings from pH studies is that slightly acidic to neutral pH favours optimum mycelia growth and fructification (Kadiri, 1998) while at pH tending towards alkalinity, mycelia growth is retarded. Alkalinity is a known hibitor to mycelia growth (Quimio and Chang, 1990).

From the results of the present study, it can be concluded that 24 hours darkness is the ideal condition for optimum mycelia growth, while 12 hours light and 12 hours darkness is the best condition for fruit bodies production. However, pH 7 obtained from this study and slightly acidic pH 6 obtained by past worker (Kadiri, 1998) is the ideal pH for optimum mycelia growth and fructification of *P. sajor caju*.

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