Full Length Research Paper

Optimization of growth parameters for increased yield of the edible mushroom *Calocybe indica*

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This study was conducted to enhance the yield performance of *Calocybe indica* through optimization of the cultivation parameters by utilizing cheaper substrates that are available in Tamil Nadu, India. The total fresh mushroom yield obtained with a change in spawn substrate, spawn running bed substrate (SRBS), sterilization procedure and casing soil with different amendments were studied. Sorghum grains provided the highest yield of 1.58 ± 0.06 g fresh mycelia after 17 ± 0.75 days of incubation. The protein, fat, carbohydrate, dietary fiber, moisture and ash content of mushrooms were also analyzed. The maximum amount of nutritive value was obtained from the paddy straw and the lowest from sugarcane leaves as substrate. The fat, protein, carbohydrate, dietary fiber, moisture and ash (g/100 g dried matter) values of *C. indica* were 0.66 ± 0.02 g, 31.29 ± 1.56 g, 58.40 ± 1.75 g, 38.21 ± 1.91 g, 84.91% and 8.47 ± 0.25 g, respectively, when cultivated in paddy straw. Furthermore, the chemical sterilization yielded 1.16 kg of mushroom with a contamination rate of <8 of 30 bags. The different chemical compositions were used in the preparation of casing soil; however, it produced the highest yield of 1648 ± 49.44 g/kg (w/w) for paddy straw.

Key words: *Calocybe indica*, chemical sterilization, growth parameters, physical sterilization, substrate.

INTRODUCTION

Mushrooms have been favored as food by mankind for a long time. Mushrooms supply a rich addition to the diet in the form of protein, carbohydrates, valuable salts, minerals and vitamins. As food, the nutritional value of mushrooms deceits in between meat and vegetables. *Calocybe indica*, commonly known as milky white mushroom, grows during the summer and it is a tropical mushroom known for its nutritive value. Its robust size, sustainable yield, attractive colour, delicacy, long shelf-life and lucrative market value have attracted the attention of both mushroom consumers and prospective growers. *C. indica* is rich in protein, lipids, fiber, carbohydrates and vitamins and contains an abundant amount of essential amino acids and low fat product (Ruhul et al., 2010). These qualities make it suitable for food supplement in diet.

Commercial milky white mushroom growers are mostly confined to Tamil Nadu, India, particularly in Erode, Salem, Coimbatore, Trichy, Madurai and other districts (Krishnamoorthy, 2003). Among vital growth requirements, environmental factors play a major role in the growth and reproduction of edible fungi. Cultivation of *C. indica* is influenced by temperature and relative humidity for its yield. However, optimum conditions favorable for the growth of *C. indica* have not been clearly defined under controlled conditions. Growing edible mushrooms is the most efficient method of bioremediation of the large quantity of lignocellulosic wastes generated annually through agricultural and allied activities (Stamets, 2000).

Considering these facts, this research work was committed towards the optimization of the growth parameters to increase yield and to bioremediate agricultural wastes. Synthetic substrates using different cellulosic waste formulations are used to study their effect on the yield of mushroom. The aim was to investigate the effect of various substrates, casing materials and other supplements that can be used to optimize the growth conditions
Table 1. Design summary for optimizing the concentration of chemicals for sterilization of substrate.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-1</td>
</tr>
<tr>
<td>Formaldehyde (A)</td>
<td>ml/L</td>
<td>100</td>
</tr>
<tr>
<td>Carbendazim (B)</td>
<td>g/L</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 2. Experimental design and of \(2^2\) factorial design.

<table>
<thead>
<tr>
<th>No of Run</th>
<th>A: Formaldehyde (ppm)</th>
<th>B: Carbendazim (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>138.63</td>
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</tr>
<tr>
<td>13</td>
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<td>75</td>
</tr>
</tbody>
</table>

and enhance the yield of \(C.\) indica. Additionally, it was intended to compare the quality attributes of conventional methods of mushroom cultivation.

MATERIALS AND METHODS

The commercial edible mushroom, \(C.\) indica, procured from Tamil Nadu Agricultural University, Coimbatore was used for this study.

Preparation of spawn

Clean grains such as sorghum, wheat, bajra and rice husk were selected for spawn preparation.

The grains were washed and soaked for 6 to 8 h in cold water and then boiled for 40 min. The boiled grains were drained and supplemented with 2\% of calcium carbonate (CaCO\(_3\)), and then the excess moisture was reduced through air drying to 60\%. The treated grains (250 g) were packed in polypropylene bags (of size 200 x 300 mm in dimensions) with necks made from cut PVC pipes in place of the commercial plastic neck to hold the cotton plug and autoclaved at 121°C for about 45 min. The bags were taken out of the autoclave and allowed to cool for a day.

After sterilization and cooling, the bags were inoculated with pure culture of \(C.\) indica from the potato dextrose agar (PDA) Petri plates. The culture and grains were mixed by shaking to uniformly distribute the mycelium. Inoculated bags were incubated at 27 ± 2°C for mycelial growth without light for 17 to 23 days until the mycelium fully covered the grains.

Substrates for cultivation of \(C.\) indica

Coir pith, maize straw, paddy straw, sugarcane bagasse, sugarcane leaves and vettivera leaves were used as substrates for this study. All the substrates except coir pith were chopped into 2 to 3-inch pieces and soaked in water. The substrates were sterilized by physical and chemical methods.

Physical sterilization

The chopped substrates were soaked in water for 3 to 4 h. Steam sterilization was done by autoclaving the substrates at 121°C for various durations such as 15, 30, 45, 60, 75 and 90 min. Substrates were air-dried so that a moisture content of about 65% was allowed in the wet substrate prior to spawning.

Chemical sterilization

Statistical optimization of the concentration of chemicals by response surface methodology (RSM)

The optimum concentration of chemicals required for sterilization of the substrate was analyzed by RSM using the central composite design. The experiments were carried out by using Design-Expert 7.1.6 software package (Tables 1 and 2). RSM is a collection of statistical and mathematical techniques useful for developing, improving and optimizing the process. RSM defines the effects of the independent variables alone or in combination, on the process. In addition to analyzing the effects of the independent variables, this experimental methodology generates a mathematical model...
that accurately describes the overall process. It has been successfully applied to optimizing conditions in food, chemical and biological processes.

Experimental design of RSM for optimizing the concentration of chemicals for sterilization of substrate

The levels of two variables like temperature and pH were optimized for chemical sterilization of substrates. For that purpose, the response surface approach by using a set of experimental design (central composite design with five coded levels) was performed. The factors were at the level of 0. The axial distance, α, was chosen to be 1.68 to make this design orthogonal. A set of 13 experiments was done for two variables. The central values (0 level) chosen for experimental design were given as gram per liter (g/L).

In developing the regression equation, the test factors were coded according to the following equation:

\[ X_i = \frac{X_i - X_0}{\Delta X_i} \]  

Where \( X_i \) is the coded value of the \( i \)th independent variable, \( X_0 \) the natural value of the \( i \)th independent variable, \( X_i \) the natural value of the \( i \)th independent variable at the center point, and \( \Delta X_i \) the step change value of variables. For a two-factor system, the model equation is:

\[ y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \epsilon \]

Preparation of casing material

The casing material was prepared with various combinations of farm-yard manure. Different supplements were tried for the increased yield. Different combinations of supplements such as CaCO₃ and gypsum were mixed with the casing soil to promote growth of the mushroom. After mycelial colonization, the mouth of the spawn run bed was cut into two equal parts. Casing layer of half an inch thickness was laid over the spawn run open part of bed. After casing, the bags were transferred in to the cultivation sheds for fruiting at 32 to 37°C temperatures and 80 to 90% humidity.

Controlled environment such as temperature light and ventilation were maintained during the cropping period in the cultivation chamber. Water was also sprayed regularly to keep the surface of the substrate to maintain moisture. Fruiting bodies once after maturation were harvested from two flushes by twisting them slightly near the base and fresh weights were recorded immediately. Bits and pieces of harvested sporophores were not allowed to remain in the substrate. Biological efficiency was calculated as the ratio between the fresh weight of mushrooms and the dry weight of substrate per bag and was expressed as percent.

Proximate analysis

Estimation of the moisture content (Ranganna, 1977), crude fibre content (Maynard, 1970), total carbohydrates content (Anthrone method), ash content (Horsefall and Ayebaemi, 2004), protein content (Bradford method) and fat content (Soxhlet method), of the samples were done by the standard methods.

Experimental design

Each experiment had three replicates. The following data were collected: mycelial growth in the different substrates, change in nutritive value while using different substrates, the number of contaminated bags after subjecting to different sterilization methods and variation, number of days required for the initiation of primordial in different substrates, the number of days required for total harvest, the number of effective fruiting bodies, economic yield, and biological efficiency and nutritional difference while using different combinations of casing soil.

Statistical analysis

All values were expressed as means ± standard deviation. The results were analyzed using one-way analysis of variance (ANOVA) and the differences among the treatments means were analyzed using the Tukey-Kramer multiple comparison test. \( P \) value<0.05 was considered as least significant. The software GraphPad InStat was employed for the statistical analysis.

RESULTS AND DISCUSSION

The effect of spawn grains on the rates of mycelial production of *C. indica*

The differences in the means due to grain were highly significant \((p<0.05)\) wet weight, and maximum mycelial growth was observed. The wet mycelial weight of *C. indica* was significantly increased in media containing sorghum. Wheat, rice husk and bajra induced lesser mean weights than sorghum. Sorghum was found to be

Preparation of cultivation bed

Substrates (500 g) were added to polypropylene bags (7 × 10" sizes) as different layers, spawns (100 g) were sowed over the six layers of substrate, and nearly 16.6 g of spawn was laid on each layer. The openings of the bags were plugged with cotton and secured with plastic rings. The spawned bags were incubated in dark under normal room temperature (25 to 35°C) for spawn run.

Cultivation chamber

Once after the spawn run and casing, the beds were incubated in the dome shaped partially sunken chamber lined with blue coloured sylphalene sheet which was used as the roofing material. The chamber’s pit walls were built with hollow blocks from the bottom, with smooth flooring and convenient steps near the entrance with good cross ventilation.

The skeleton structures of the chamber were made by waste polymer (Procured from Texnova, Chennai).
Table 3. Optimization of spawn on different substrate.

<table>
<thead>
<tr>
<th>Types of Substrate</th>
<th>Mycelial fresh weight (g)</th>
<th>Mycelia formation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>0.717 ± 0.035&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bajara</td>
<td>0.761 ± 0.041&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20 ± 0.86&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Paddy straw</td>
<td>0.844 ± 0.036&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23 ± 0.95&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maize straw</td>
<td>1.583 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17 ± 0.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values followed by the same letter are not significantly different (<i>P</i> < 0.05). Values represent the mean of triplicates with standard deviation.

Table 4. Nutritional analysis of <i>C. indica</i> grown on different substrates.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Moisture (%)</th>
<th>Protein (g/100g)</th>
<th>Fat (g/100g)</th>
<th>Carbohydrate (g/100g)</th>
<th>Fibre (g/100g)</th>
<th>Ash (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coir pith</td>
<td>79.2 ± 3.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.8 ± 1.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.85 ± 0.034&lt;sup&gt;d&lt;/sup&gt;</td>
<td>53.2 ± 1.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.6 ± 1.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.17 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maize straw</td>
<td>83.4 ± 4.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.5 ± 1.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.77 ± 0.0308&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54.5 ± 1.63&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>37.5 ± 1.87&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.3 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Paddy straw</td>
<td>84.9 ± 4.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.2 ± 1.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.66 ± 0.026&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.4 ± 1.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>38.2 ± 1.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.47 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sugarcane bagasse</td>
<td>78.6 ± 3.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.4 ± 1.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.72 ± 0.021&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55 ± 2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.3 ± 1.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.07 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sugarcane leaves</td>
<td>78 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.5 ± 1.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.74 ± 0.02&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>53.2 ± 2.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.1 ± 1.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.87 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vettivera leaves</td>
<td>81.3 ± 4.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.2 ± 1.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8 ± 0.024&lt;sup&gt;d&lt;/sup&gt;</td>
<td>55.6 ± 2.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.5 ± 1.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.97 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values followed by the same letter are not significantly different (<i>P</i> < 0.05). Values represent the mean of triplicates with standard deviation.

most favorable to mycelial extension of <i>C. indica</i>. The result of the analysis of variance (Table 3) show that the mycelial weight was 1.58 ± 0.06 g for <i>C. indica</i> on the 17th day of incubation when sorghum was used as substrate. Rice husk was next to sorghum with mycelial weight of 0.844 ± 0.036 g, although it obtained the yield on 23<sup>rd</sup> day. The substrates wheat and bajara least supported the growth of mycelia with average mycelial weight of 0.717 ± 0.035 g and 0.761 ± 0.041 g on the 18<sup>th</sup> and 20<sup>th</sup> day, respectively, after incubation. Nwanze et al. (2005) observed that in the case of <i>Lentinus squarrosulus</i>, wheat (1.17 g) and corn spawn (1.37 g) were similar and induced mycelial wet weights (Jiskani et al. 2007).

**Effect of different substrates on yield and yield-contributing characteristics**

The results reveal the yield, B.E. and nutrient content of the <i>C. indica</i> cultivated on different agro-wastes. The shortest time required to complete mycelial growth was observed in the paddy straw substrate (20 ± 2 days), followed by the Vettivera leaves (26 ± 2 days) and sugarcane bagasse (27 ± 2 days) substrates. The longest time (30 ± 2 days) required to complete mycelial growth was observed in the coconut coir substrate (Pani 2010). The minimum time for first flush was observed in paddy straw substrate (8 ± 1 days), which was statistically similar to that of the coconut coir, maize straw, and rice straw substrates. The longest time was recorded in sugarcane leaf substrate (14 ± 1 day). Alam et al. (2010) observed that 19.3 days was required for the primordial initiation of <i>C. indica</i> in a previous study conducted on paddy straw substrate. The biological yield for this study was 1.019 ± 5.1 g for paddy substrate subsequently maize substrate with 0.979 ± 4.9 g. The data is significantly more than the previous studies with substrates without supplementation. Work done by Pani (2010) revealed that paddy straw was the best among the substrates as it produced the maximum yield (0.712 kg) and biological efficiency (71.2% BE) of <i>C. indica</i>, and it took 30 days for pinhead appearance. Similarly Nuhu et al. (2010) observed 0.918 kg of biological yield per W/W of nutrient supplemented substrate.

**Nutritional analysis of <i>C. indica</i>**

The aim was to obtain the increased nutrient and yield of <i>C. indica</i>. The moisture content of the mushroom cultivated in paddy straw, maize straw, vettivera leaves and coir pith were found about 84.9, 83.4, 81.2 and 79.2%, respectively (Table 4). For the substrate paddy straw, 100 g of fresh <i>C. indica</i> contained 31.29 ± 1.56 g of proteins, 38.2 ± 1.91 g of fiber and 58.4 ± 1.75 g of carbohydrates. Least amount of protein was observed for the sugarcane bagasse with 27.4 ± 1.09 g. Moreover, 35.1 ± 1.05 g of fiber and 53.2 ± 2.12 g of carbohydrates was the least values found in sugarcane leaves. While comparing the fat content, mushrooms harvested from coir pith had elevated amount of 0.85 ± 0.03 g and the lowest was recorded in paddy straw with a value of 0.66 ± 0.02 g, which was a significant observation. According to Nuhu et al. (2008) <i>C. indica</i> contained 2.6 to 2.9 g of proteins, 0.6 to 0.7 g of lipids, 1.5 to 1.8 g of fiber and 6.3 to 7.3 g of carbohydrates. Based on the study results, it
Table 5. Effect of physical sterilization.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Sterilization temperature</th>
<th>Time of steaming (min)</th>
<th>Contamination (%)</th>
<th>pH</th>
<th>Temp °C</th>
<th>RH %</th>
<th>Total no. of flushes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paddy straw</td>
<td>Autoclaved at 121 °C</td>
<td>15</td>
<td>76 ± 3.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.5</td>
<td>~35</td>
<td>~85</td>
<td>9.7 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>60 ± 2.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.5</td>
<td>~35</td>
<td>~85</td>
<td>15 ± 0.62&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45</td>
<td>30 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.5</td>
<td>~35</td>
<td>~85</td>
<td>16.66 ± 0.94&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>0.6 ± 0.025&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5</td>
<td>~35</td>
<td>~85</td>
<td>15 ± 0.62&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>0.5 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5</td>
<td>~35</td>
<td>~85</td>
<td>16.7 ± 0.74&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
<td>0.5 ± 0.019&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5</td>
<td>~35</td>
<td>~85</td>
<td>13.65 ± 0.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values followed by the same letter are not significantly different (P<0.05). Values represent the mean of triplicates with standard deviation. RH, Relative humidity.

Table 6. Optimizing the concentration of chemicals for sterilization of substrate.

<table>
<thead>
<tr>
<th>No. of run</th>
<th>A: Formaldehyde [ppm]</th>
<th>B: Carbendazim [ppm]</th>
<th>Contamination %</th>
<th>Total yield %</th>
<th>Bio-efficiency %</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>500</td>
<td>75</td>
<td>5.5</td>
<td>1.11</td>
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<tr>
<td>2</td>
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<td>3.3</td>
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<td>120</td>
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<td>0.65</td>
<td>65</td>
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<tr>
<td>10</td>
<td>900</td>
<td>30</td>
<td>36.6</td>
<td>0.56</td>
<td>56</td>
</tr>
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<td>11</td>
<td>500</td>
<td>75</td>
<td>10</td>
<td>0.9</td>
<td>90</td>
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<tr>
<td>12</td>
<td>500</td>
<td>11.36</td>
<td>43.3</td>
<td>0.35</td>
<td>35</td>
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<tr>
<td>13</td>
<td>0</td>
<td>75</td>
<td>50</td>
<td>0.25</td>
<td>25</td>
</tr>
</tbody>
</table>

was concluded that paddy straw was the effective substrate for the cultivation of *C. indica* and it was used for further studies.

**Effects on sterilization methods on substrates for cultivation of *C. indica***

**Physical sterilization**

Current study explored that 6 h of soaking the substrate and steaming for 60 min yielded the maximum production with 0.6% contamination which was found to be economically viable (Table 5). The previous experiment done by Pathan (2009) revealed that the maximum percent yield was in case of soaking and boiling for 75 min (61.75%).

**Chemical sterilization**

The results of central composite design experiments for studying the effects of chemical sterilization of substrate on the contamination percentage are presented in Table 6. The F-value of 24.01 showed the model as significant. There was only chance of 0.01% variation in "model-F value" due to noise (Table 7). The values of "Prob > F" and less than 0.05 indicated that the model terms were significant. In this case, A<sup>2</sup> and B<sup>2</sup> are significant model terms. However, values greater than 0.1 indicated that the model terms are not significant. The "lack of fit F-value" of 9.26 was exhibited as significant and there was only slight chance of 0.03% variation in "Lack of Fit F-value" due to noise. Moreover, the lack of fit value was not significant, so the model has to be change to fit as significant.

The "Pred R-Squared" of 0.65 is in reasonable agreement with the "Adj R-Squared" of 0.905. "Adeq Precision" measures the signal to noise ratio and a ratio greater than 4 is desirable. The ratio of 13.486 indicates an adequate signal (Table 8). This model can be used to navigate the design space. The model coefficient was estimated by linear regression (Table 9). The following equations were used: Final Equation in terms of coded...
Table 7. F-test analysis (ANOVA for response surface quadratic model).

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F value</th>
<th>p-value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
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<td>1129.20</td>
<td>24.00</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>A-Formaldehyde</td>
<td>2160.77</td>
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<td>2160.77</td>
<td>45.94</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>B-carbendazim</td>
<td>1796.05</td>
<td>1</td>
<td>1796.05</td>
<td>38.19</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>2.72</td>
<td>1</td>
<td>2.72</td>
<td>0.05</td>
<td>0.8168</td>
<td></td>
</tr>
<tr>
<td>A^2</td>
<td>1152.03</td>
<td>1</td>
<td>1152.03</td>
<td>24.49</td>
<td>0.0017</td>
<td></td>
</tr>
<tr>
<td>B^2</td>
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<td>1</td>
<td>747.90</td>
<td>15.90</td>
<td>0.0053</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
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<td>7</td>
<td>47.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
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<td>3</td>
<td>95.92</td>
<td>0.05</td>
<td>0.8168</td>
<td></td>
</tr>
<tr>
<td>Pure Error</td>
<td>41.46</td>
<td>4</td>
<td>10.36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor Total</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Comparison of R^2 predicted and estimated.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std. Dev.</td>
<td>6.9</td>
<td>R-Squared</td>
<td>0.945</td>
</tr>
<tr>
<td>Mean</td>
<td>22.7</td>
<td>Adj R-Squared</td>
<td>0.905</td>
</tr>
<tr>
<td>C.V. %</td>
<td>30.21</td>
<td>Pred R-Squared</td>
<td>0.647</td>
</tr>
<tr>
<td>PRESS</td>
<td>2111.266</td>
<td>Adeq Precision</td>
<td>13.486</td>
</tr>
</tbody>
</table>

Table 9. Model coefficient estimated by linear regression.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient estimate</th>
<th>df</th>
<th>Standard error</th>
<th>95% CI Low</th>
<th>95% CI High</th>
<th>VIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>8.4</td>
<td>1</td>
<td>3.06</td>
<td>1.147479</td>
<td>15.65</td>
<td></td>
</tr>
<tr>
<td>A-Formaldehyde</td>
<td>-16.43</td>
<td>1</td>
<td>2.42</td>
<td>-22.16</td>
<td>-10.70</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>-0.82</td>
<td>1</td>
<td>3.42</td>
<td>-8.93</td>
<td>7.28</td>
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</tr>
<tr>
<td>A^2</td>
<td>12.86</td>
<td>1</td>
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<td>6.72</td>
<td>19.01</td>
<td></td>
</tr>
<tr>
<td>B^2</td>
<td>10.36</td>
<td>1</td>
<td>2.60</td>
<td>4.22</td>
<td>16.51</td>
<td></td>
</tr>
</tbody>
</table>

df, Degree of freedom;

Factors:

Contamination = +8.40 -16.43 * A -14.98 * B -0.8 * A * B +12.87 * A^2 +10.37 * B^2

Final equation in terms of actual factors:

Contamination = +101.10663 -0.11808 * Formaldehyde -1.07811 * Carbendazim -4.58333E-005 * Formaldehyde + carbendazim +8.04297E-005 * Formaldehyde^2 +5.12037E-003 * Carbendazim^2

Effect of chemical sterilization on total yield

The results of central composite design experiments for studying the effects of chemical sterilization of substrate on the contamination percentage are presented in Table 6. The F-value of 12.12 showed the model is significant. There is only a chance of 0.24% variation in "model-F value" due to noise (Table 10). The values of "Prob > F" and less than 0.05 indicated that the model terms are significant. In this case, A^2 and B^2 are significant model terms. However, values greater than 0.1 indicated that the model terms are not significant.

The "Lack of Fit F-value" of 9.26 indicated significance and there is only slight chance of 0.08% variation in "Lack of Fit F-value" due to noise. The lack of fit value was not significant, so the model has to be change to fit as significant. The "Pred R-Squared" of 0.46 was in reasonable agreement with the "Adj R-Squared" of 0.82. "Adeq Precision" measures the signal to noise ratio and a ratio greater than 4 is desirable. The ratio of 8.58 indicated an adequate signal (Table 11). This model can be used to navigate the design space. The model coefficient was estimated by linear regression (Table 12) using the following equations: final equation in terms of coded...
Table 10. F-test analysis (ANOVA for Response Surface Quadratic Model).

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
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<td>5</td>
<td>0.2</td>
<td>12.11</td>
<td>0.0024</td>
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<tr>
<td>A-Formaldehyde</td>
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<td>1</td>
<td>0.02</td>
<td>1.81</td>
<td>0.2198</td>
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<tr>
<td>B-carbendazim</td>
<td>0.03</td>
<td>1</td>
<td>0.03</td>
<td>2.22</td>
<td>0.1792</td>
</tr>
<tr>
<td>AB</td>
<td>0.02</td>
<td>1</td>
<td>0.024</td>
<td>1.45</td>
<td>0.2670</td>
</tr>
<tr>
<td>A^2</td>
<td>0.56</td>
<td>1</td>
<td>0.56</td>
<td>34.26</td>
<td>0.0006</td>
</tr>
<tr>
<td>B^2</td>
<td>0.46</td>
<td>1</td>
<td>0.46</td>
<td>27.97</td>
<td>0.0011</td>
</tr>
<tr>
<td>Residual</td>
<td>0.11</td>
<td>7</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>0.077</td>
<td>3</td>
<td>0.025</td>
<td>2.61</td>
<td>0.1882</td>
</tr>
<tr>
<td>Pure Error</td>
<td>0.03</td>
<td>4</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor Total</td>
<td>1.11</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 11. Comparison of R² predicted and estimated.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std. Dev.</td>
<td>0.128</td>
<td>R-Squared</td>
<td>0.896</td>
</tr>
<tr>
<td>Mean</td>
<td>0.713</td>
<td>Adj R-Squared</td>
<td>0.822</td>
</tr>
<tr>
<td>C.V. %</td>
<td>18.005</td>
<td>Pred R-Squared</td>
<td>0.457</td>
</tr>
<tr>
<td>PRESS</td>
<td>0.605</td>
<td>Adeq Precision</td>
<td>8.58</td>
</tr>
</tbody>
</table>

Table 12. Model coefficient estimated by linear regression.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient estimate</th>
<th>df</th>
<th>Standard error</th>
<th>95% CI low</th>
<th>95% CI high</th>
<th>VIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.04</td>
<td>1</td>
<td>0.057</td>
<td>0.912</td>
<td>1.183</td>
<td></td>
</tr>
<tr>
<td>A-Formaldehyde</td>
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<td>1</td>
<td>0.045</td>
<td>-0.046</td>
<td>0.168</td>
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</tr>
<tr>
<td>B-carbendazim</td>
<td>0.067</td>
<td>1</td>
<td>0.045</td>
<td>-0.039</td>
<td>0.175</td>
<td>1</td>
</tr>
<tr>
<td>AB</td>
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<td>1</td>
<td>0.064</td>
<td>-0.229</td>
<td>0.074</td>
<td>1</td>
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<tr>
<td>A^2</td>
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<td>1</td>
<td>0.0487</td>
<td>-0.400</td>
<td>-0.170</td>
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<tr>
<td>B^2</td>
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<td>1</td>
<td>0.0487</td>
<td>-0.37298</td>
<td>-0.142</td>
<td>1.017</td>
</tr>
</tbody>
</table>

Factors:

R² Total Yield in Kg = +1.05 +0.06 * A +0.068 * B -0.078 * A * B 0.29 * A² -0.26 * B²

Final equation in terms of actual factors:

R² Total Yield in Kg = -0.46470+2.25880E-003*Formaldehyde+0.02275*carbendazim-4.30556E-006*Formaldehyde*carbendazim-1.78281E-006

*Formaldehyde² - 1.27284E-004*carbendazim²

Improved total yield, when compared to single factor analysis. Physical and chemical sterilizations produced the best and similar results. Comparing the cost effectiveness and least usage of chemical sterilizers, the chemical sterilization could be considered as a method for sterlizing substrates.

Effect of different casing materials along with supplements on yield and yield-contributing characteristics

Farm-yard manure, red soil and sand were used as casing materials to evaluate the yield and yield-contributing characteristics of C. indica. Maximum biological efficiency was recorded in farm-yard manure, red soil and sand at the proportion of (1:3:1) which yielded 145.1% and followed by the farm-yard manure
and red soil (3:1) with yield 128% and farm-yard manure and sand (3:1) 104.1% casing materials (Figure 3). Previous studies conducted by Ruhul et al. (2010) indicated that a maximum biological efficiency was recorded in cow dung and soil (62.94%) followed by the farm-yard manure (62.64%), spent mushroom substrate.
(61.66%), and soil and sand (61.46%) casing materials. Hence, farm-yard manure, red soil and sand at the proportion of (131) with 2% CaCO₃ and 1.75% of gypsum brought a significant yield of 1.64 ± 49.4 Kg (Figures 4 and 5) of mushroom per dry weight of substrate. Previous studies done by Kassim et al. (1990) stated that the number and yield of mushrooms was increased when the supplements were added to the casing soil. The yield was significant when nutrient supplements were added to the casing material. Days of primordial formation, total cropping days gets reduced and BE increases as a result of amendment.
Effect of casing soil on the nutritive value

Casing soil amendments supported the yield significantly, porous loam texture coupled with farmyard manure, CaCO₃ and gypsum contributed for the yield. 31.42 ± 1.57 g of proteins, 37.68 ± 1.50 g of fiber, 0.74 ± 0.03 g of fat, 8.75 ± 0.35 g and 56.73 ± 2.83 g of carbohydrates was observed. Studies conducted earlier described that the casing layer is an essential component for the artificial cultivation of *C. indica*. According to Sassine et al. (2010) the casing layer must be very loose; otherwise, the primordia cannot penetrate from the bottom to the top of the casing layer.

ACKNOWLEDGEMENTS

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REFERENCES


