



Research Article

VOLVARIELLA BOMBYCINA OF TAMIL NADU

**1,*MuthusamyKarnan, 2Pichai Tamilkani, 3Govindarajan Senthilkumar,
4Selvakumar Vijayalakshmi and 5Annamalai Panneerselvam**

^{1,2,4}Research Scholar, P.G and Research Dept. of Botany and Microbiology, A.V.V.M. Sri Puhpam College, Poondi, Thanjavur

³Assistant Professor, P.G and Research Dept. of Botany and Microbiology, A.V.V.M. Sri Puhpam College, Poondi, Thanjavur

⁵Head of the Department, P.G and Research Dept. of Botany and Microbiology, A.V.V.M. Sri Puhpam College, Poondi, Thanjavur

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The cultivation of the *Volvariella bombycina* was investigated. The specific medium of fungi MGYP broth and MGYPA were used for the culture and MGYP with 1% CMC, MGYP medium were used for enzyme production. Spawn was prepared using sorghum grains and paddy straw. The chemical analysis of mushrooms was as follows (percentage). Moisture 83.10 ± 0.57 , Proteins 54.07 ± 0.19 , Fiber 11.23 ± 0.31 , Fat 0.76 ± 0.27 , Carbohydrates 44.85 ± 0.07 , Ash 0.30 ± 0.63 and Energy (kcal) 384.01 ± 40.24 . The phytochemical analysis showed the presence of alkaloids, terpenoids, sugar, saponins, flavonoids, proteins and sterols. Phenols, tannins and quinines were absent in the above species. Phytochemical compounds were used for anticancer research, the present investigation will be useful for detection of new compounds against cancer cells.

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INTRODUCTION

Mushroom has long been valued as delicious and nutritional food in many countries. Mushroom is initially consumed for their flavour, now consumed because of their nutritional and medicinal properties (Mallavadhani *et al.*, 2006). *Volvariella bombycina* is commonly known as the silky sheath, silky rosegill, silver-silk straw mushroom, or tree mushroom, belongs to the family Pluteaceae. It is an uncommon but widespread species, having been reported from Asia, Australia, the Caribbean, Europe and North America. It is an edible (Zoberi, 1972; Dickinson & Lucas, 1979) and potential fungus for commercial cultivation (Huang & Wu, 1982; Elliott & Challen, 1985). Hayes (1977) described the materials used in the formulation of media for mushroom cultivation. These materials are as follows: (a) Vegetable-based materials which provide a reservoir of cellulose, hemicellulose and lignin. (b)

Supplements for activating growth; namely, manures which are used as a source of carbohydrates and nitrogen. (c) Available nutrients such as molasses, vegetable wastes for carbohydrates, urea, ammonium sulphate and nitrate for nitrogen. (d) Supplements designed to rectify mineral deficiencies such as gypsum, potash and superphosphate. *V. bombycina* were reported to have good antioxidant, antitumor and hypercholesterolemic effects (Badalyan and Suzanna, 2003). The present study was designed to investigate the phytochemical analysis and chemical composition of an edible mushroom *V. bombycina*. The phytochemical analysis includes alkaloids, terpenoids, sugar, saponins, flavonoids, proteins, sterols, phenols, tannins and quinines.

MATERIALS AND METHODS

Culture

The culture of *Volvariella bombycina* (MTCC No: 1345) was purchased from Microbial Type Culture Collection centre, Chandigarh.

*Corresponding author: MuthusamyKarnan,

Research Scholar, P.G and Research Dept. of Botany and Microbiology, A.V.V.M. Sri Puhpam College, Poondi, Thanjavur.

The specific medium of fungi MGYB broth and MGYPA were used and the culture was stored in Deep freezer at 4°C. MGYPS, MGYB with 1% CMC, MGYB medium were used for enzyme production.

Spawn preparation

Spawn of *V. bombycina* was produced using Sorghum grains and the substrate was half boiled after which air dried for 1h. Calcium carbonate (CaCO₃) was added along with the substrate at the concentration of 20% per Kg. Then the grains were packed using PP (poly propylene) cover size of 28 x 10cm, PVC neck and non absorbance cotton and sterilized in an autoclave at 121°C for 20min. The bags were cooled at room temperature atleast for 4 hrs. Bags were inoculated individually using the culture of *V. bombycina*. From a single mother spawn, 25 sub spawn bags were prepared.

Bed preparation

Paddy straw was collected selectively and soaked in water for 4-6 hrs and that straw was autoclaved at 121°C for 30min. Then the substrate and spawn were packed using 60x30cm size PP cover filled the bag 5 times in 5cm level intervals. The prepared bags were kept in the stretched bamboo frame with in restricting shed.

Nutritional analysis

The samples were analysed for chemical composition (moisture, proteins, fat, carbohydrates and ash) using the AOAC procedures (AOAC, 1995). The crude protein content ($N \times 4.38$) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15 °C. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation:

$$\text{Energy (kcal)} = 4 \times (\text{g protein}) + 3.75 \times (\text{g carbohydrate}) + 9 \times (\text{g fat}).$$

Phytochemical analysis (Harborne et al., 1998)

Biochemical test were performed to analysis the primary and secondary compounds. Proteins and common sugars are included in primary constituents and secondary compounds are terpenoids, alkaloids and phenolic compounds.

Production of Enzyme

Production of enzyme involves amylase, cellulase and laccase enzymes. These enzymes were taken in the study and their methods for production were given below,

Amylase production

In 1ml of enzyme extract added with 1ml of 1% soluble starch in citrate-phosphate buffer (pH 7.0) and incubated in a water bath at 40°C for 30 minutes. Blank consisting of 2ml of the enzyme extract that was boiled for 20 min (boiling inactivates the enzyme) and starch solution was added and treated with the same reagent using the experimental tubes.

The reaction was stopped by adding 2ml of DNS reagent (1.0 g of 3, 5, dinitrosalicylic acid, 20 ml of NaOH and 30g of sodium potassium tartarate in 100ml) and boiled for 5 min at 80°C. After cooling 20 ml of distilled water was added and the absorbance was read at 540 nm.

Cellulase production

The filtrates of each fungus were assayed for Cellulase using the modified dinitrosalicylic acid (DNSA) reagent method of Zhou et al., 2009. The amount of reducing sugar that was released was determined by adding 1 mL of DNSA to 1 mL of filtrate-starch-reaction mixture, and the absorbance was read at 540 nm using a spectrophotometer. Cellulase activity in the filtrate was determined by the method of Jahangeer et al., 2005. The assay medium contained 0.55% carboxymethyl cellulose (CMC) in 0.55M acetate buffer (pH 6.8), and the reducing sugars released were measured by the DNSA reagent method of Parra et al., 2005.

Laccase production

Laccase activity was measured spectrophotometrically using Guaiacol as a substrate with an absorbance coefficient value of 6800 M⁻¹cm⁻¹ at 470 nm (Collins and Dobson, 1997). The reaction mixture consisted of 3 mL of 100 mM of guaiacol dissolved in 10% acetone (v/v) in sodium acetate buffer (100 mM, pH 5.0), and 1 mL culture filtrate. The mixture was incubated for 15 min and the absorbance was read at 470 nm. One unit (U) of laccase activity was defined as the amount of enzyme catalyzing the production of one micromole of coloured product per min per mL.

RESULTS

The purchased culture was stored in deep freezer at 4°C aseptically. Sorghum grains were used for spawn preparation (Figure 1).

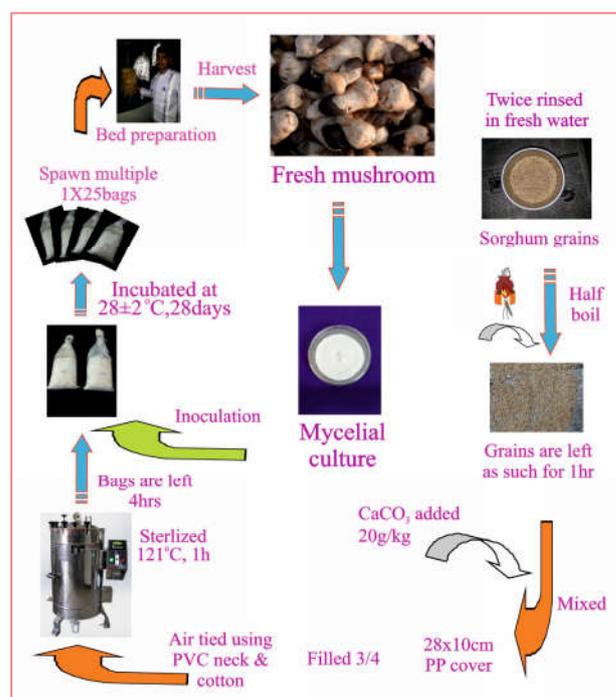


Figure1. Sorghum grains prepared using spawn bottle

Table 1. Biochemical compounds detected in the mushroom

Species	Alkaloids	Terpenoids	Phenol & Tannins	Sugar	Saponins	Flavonoids	Quinines	Protein	Steroids
<i>V. bombycina</i>	+	+	-	+	+	+	-	+	+

These prepared spawns were used to cultivate the *V. bombycina* by using paddy straw as a substratum. Paddy straw, an agro waste material is easily available in Thanjavur district, this technique is the oldest and commonest technique. Mushrooms were harvested at three times within 35 – 40 days for one time spawn preparation and their yield was calculated. 51% yield was recorded in first harvest (15 – 20 days), 31% in second harvest (24 - 28 days) and 18% was recorded in third harvest (30 – 35 days) (Figure 3).



Figure 2: Cultivation of *Volvariella bombycina* using circular bed method

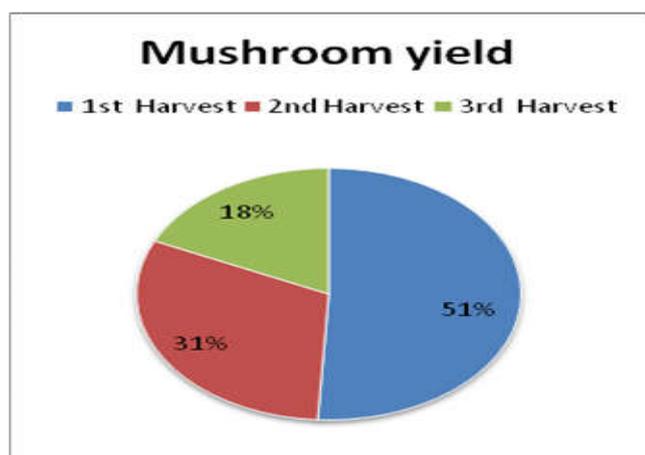


Figure 3. Yield rate of *Volvariella bombycina*

Among various methods used circular method was a good method and gave best yield than others in this study (Figure 2). Chemical composition of the mushroom sample were analysed and tabulated. Moisture 83.10 ± 0.57 , Proteins 54.07 ± 0.19 , Fiber 11.23 ± 0.31 , Fat 06.76 ± 0.27 , Carbohydrates 44.85 ± 0.07 , Ash 05.30 ± 0.63 and Energy (kcal) 384.01 ± 40.24 were analysed (Figure 4). Based on the phytochemical analysis, the fungus showed the presence of alkaloids, terpenoids, sugar, saponins, flavonoids, proteins and sterols. Phenols, tannins and quinines were absent in the above species (Table 1).

V. bombycina produced 0.074 unit of amylase and 0.092 unit of cellulase and 0.624 unit of laccase. The laccase enzyme was produced in the higher quantity. All parameters except moisture were presented for dried matter

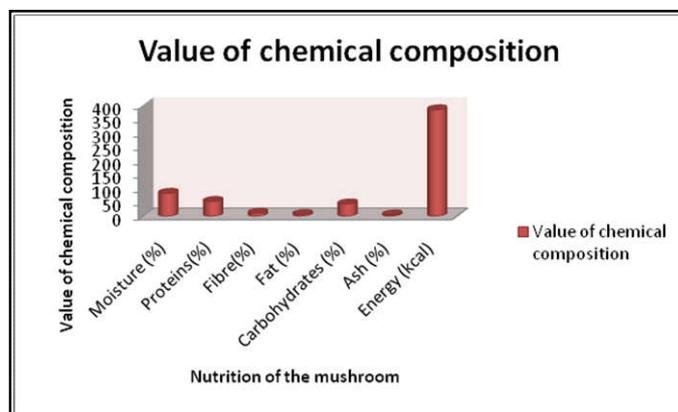


Figure 4. Value of chemical composition

DISCUSSION

Mushrooms have rich nutritional value with high content of proteins, vitamins, minerals, fibers, trace elements and low or no calories and cholesterol (AgaharMurugkar and Subbulakshmi, 2005; Wani *et al.*, 2010). Many of them have been used in folk medicine for thousands of years. Some of them are nutraceuticals (natural food having potential value in maintaining good health and boosting immune system of the human body) while others can produce potent nutraceuticals (compounds that have medicinal and nutritional attributes and are consumed as medicines in the form of capsules or tablets but not as food) (Elmastas *et al.*, 2007; Ribeiro *et al.*, 2007). Mushrooms are known to be rich sources of various bioactive substances like antibacterial, antifungal, antiviral, antiparasitic, antioxidant, antiinflammatory, antiproliferative, anticancer, antitumour, cytotoxic, anti-HIV, hypocholesterolemic, antidiabetic, anticoagulant, hepatoprotective compounds, among others (Wasser and Weis, 1999; Lindequist *et al.*, 2005; Ajith and Janardhanan, 2007). Several bioactive secondary metabolites have been isolated and identified from *V. bombycina* fruit bodies, mycelium or pure culture. The compounds ergosta-4, 6, 8(14), 22-tetraene-3-one, ergosterol peroxide, indole-3-carboxaldehyde, and indazole were found in liquid culture (Xu *et al.*, 2010). In 2009, the novel compound isodeoxyhelicobasidin was identified from culture broth; this compound inhibits the enzyme human elastase (Xu *et al.*, 2009). The fungus also produces compounds that have antioxidative activity. (Badalyan., 2003). Jagadeesh *et al.* (2010) reported that 1.15 and 2.72% lipid contents were present in mycelia and fruit body of *V. bombycina*, respectively.

Conclusion

V. bombycina are rich in proteins with low lipid content. The results from present study indicated the *V. bombycina* mushroom as ideal food. This edible fungus provide two main

benefits to people, they are a source of food and income. These have a greater potential in both health and wealth for rural people who cultivate paddy-straw as part of integrated farming systems.

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