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In vitro cultivation technology and nutritional status of milky mushroom (*Calocybe indica*)

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The present study was conducted to evaluate the *in vitro* cultivation technology, proximate composition, mineral content and spectrum analysis of edible milky mushroom of *Calocybe indica*. Moisture, crude protein, carbohydrate, dietary fibre, total lipids, ash, ether extract, pH, nitrogen and carbon content in mushrooms were analysed. The results were found to be in 89, 14.9, 5.36, 8.02, 4.6, 7.05, 3.15, 5.4, 3.57 and 33.60% mg/100 g, respectively. The values of copper, manganese, zinc, iron, calcium, phosphorous, potassium and sodium content in mushrooms were found to be 0.44, 0.36, 0.05, 0.13, 0.51, 0.38, 1.35, 1.35 and 0.21 mg/100 g, respectively. Fourier transform infrared spectroscopy (FT-IR) spectrum of the mushroom indicated the presence of OH, COOH and NO_2 functional groups. The ultra-violet (UV) absorption showed at 294 nm with a shoulder at 321 and 379 nm indicating the presence of aromatic nature of the compounds. Data of this study suggests that mushrooms are rich in nutritional value.

Key words: *Calocybe indica*, Fourier transform Infrared Spectroscopy (FT-IR), UV Spectrometer, nutritional values.

INTRODUCTION

Mushrooms have been used as a part of regular diet for nutritional and medicinal values mostly by the ethnic group of Asian people from time immemorial. They contain minerals, vitamins and nutritive compounds, proteins, polysaccharide and have a low fat content (Khurshidul Zahid et al., 2004). The cultivation technology of mushroom is very simple, involves less cost and no special compost is needed. The main cultivation process of milky mushroom (*Calocybe indica*) is potentially new species to the world mushroom growers. It is a robust, fleshy, milky white, umbrella like mushroom, which resembles button mushroom. These species is suitable for hot humid climate and can be cultivated indoor in high temperature and high humidity areas. It grows well at a temperature range of 25-35°C and relative humidity more than 80%. It can be cultivated throughout the year in the entire plains of India and other countries. On an average, single mushroom weighs 55-60 g and mean yield is 356 g/bed, which accounts to 143% bio-efficiency. The milky mushroom have rich source of protein with content of 32.3% and fetches high market price compared to oyster mushrooms. It is highly suitable for drying, canning, soup powder preparation and pickle making. More than 80 edible mushrooms are considered for commercial

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exploitation, among these, milky mushroom has become the focal point of exploitation in India. This mushroom was first reported from India (Purkayastha et al., 1981). The Fourier-transform infrared spectroscopy is an analytical technique that enables the rapid, reagent less and high-throughput analysis of a diverse range of samples (Harrigan and Goodacre, 2003). Its importance lies in its ability to allow rapid and simultaneous characterization of different functional groups such as lipids, proteins, nucleic acids and polysaccharides in biological molecules and complex structures (Melin et al., 2004). Nutritional analysis of several mushroom species of different origins had been carried out in many laboratories in the world, but nutritional values of cultivated mushrooms remain speculative. Moreover, nutritional composition is affected by many factors; these include differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting and specific portion of the fruiting bodies used for analysis (Benjamin et al., 1995). Recently, *C. indica* have become an attractive functional food mainly because of their biochemical composition and antioxidant properties which have been reported to prevent oxidative damage by free radical and reactive oxygen species (ROS) and may prevent the occurrence of diseases like carcinogenesis, ageing, physical injury, infection and cardiovascular disease. Therefore, milky mushroom is considered as a better proxy for oyster mushroom notably in tropical regions with longer shelf life of three to four days. The present study was conducted to evaluate the *in vitro* cultivation technology and nutritional information of milky mushroom (*C. indica*).

MATERIALS AND METHODS

Collection of culture and maintenance

The pure culture of *C. indica* was obtained from Centre for Advanced study in Botany, University of Madras, Gundy Campus, and Chennai, India. The culture was maintained on potato dextrose agar slant and sub-cultured at regular monthly interval to sustain their fruiting vigour. They were preserved at 4°C temperature conditions. The above stock culture was used in further studies.

Spawn preparation

Wheat grain spawn of *C. indica* was prepared in glass bottles as described (Garcha et al., 1981). The wheat grain was semi boiled then 2% calcium carbonate and 0.2% gypsum was added to 1 kg of semi boiled wheat grain. The mother culture was prepared in glass bottles filling them ⅓ full and the working spawn was prepared in plastic bags capped with cotton plugs by rubber bands. The mother culture was grown on potato dextrose agar a medium that is first supplemented to the mother spawn and at full growth it was transferred to the working spawn bags of *C. indica.*

Preparation of bed and harvesting

The paddy straws were collected from local farmers of the Tuticorin district, Tamil Nadu, India. The straw was used as a substrate for cultivation. The substrate was soaked in cold water for 4 h. After draining excess water, the materials were treated in hot water (80°C) for 60 min and dried in shade. For the bed preparation, polythene bags of 60 x 30 cm size and 100 gauge thickness was used and cylindrical beds was prepared using 0.5 kg of substrate (dry weight) per bed. The filled paddy grain spawn of *C. indica* was used at 6% level to the wet weight of the substrate and the beds were spawned following layer method of spawning (Baskaran et al., 1978). After 10 to 15 days, when the beds were fully colonized by the mushroom fungus, they were cut into two equal halves and applied with casing soil to a height of 2 cm over the spawn run substrate in each of the half bed. The beds were uniformly and regularly sprayed with water until the last harvest. In total, three crops were harvested at intervals of three to five days.

Nutritional status of *C. indica*

The collected fresh mushrooms, shade dried and coarse powder was analysed for nutrients namely moisture, crude protein, fat, ash, crude fibre, minerals using FTIR and UV spectrometer.

Crude protein

Five grams of ground mushroom was taken with 50 ml 0.1 N NaOH and boiled for 30 min. The solution was cool to room temperature and centrifuged at 1000 rpm. The supernatant was collected and total protein content measured (Folch et al., 1957).

Crude lipid

The total lipid was determined by slight modified method (Lowry et al., 1951). The five grams of ground mushroom was suspended in 50 ml of chloroform: methanol (2:1 v/v) mixture then mixed thoroughly and let to stand for three days. The solution was filtered and centrifuged at 1000 *g* by a table centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid.

Crude fiber

The total fibre was determined (Raghuramulu et al., 2003). Ten grams of moisture and fat-free sample was taken in a beaker and 200 ml of boiling 0.255 N H_2SO_4 was added. The mixture was boiled for 30 min keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker, and 200 ml of boiling 0.313 N NaOH added. After boiling for 30 min, the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80 to 100°C and weighed (We) in an electric balance. The crucible was heated in a muffle furnace at 600°C for 5-6 h, cooled and weighed again.

Total ash

One gram of the sample was weighed accurately into a crucible (Raghuramulu et al., 2003). 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 h at 600°C. It was then cooled in desiccators and weighed.

To ensure completion of ash, the crucible was then heated in the muffle furnace for 1 h, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or greyish white in colour.

Mineral analysis

Total ash was taken for the analysis of mineral contents. Two millilitres of concentration HNO_3 was added to the ash and heated for 2 min. One drop of hydrogen peroxide was added into the solution. The solution was then transferred into a volumetric flask and total volume was made to 50 ml by adding deionised distilled water. This was then used to analyze the contents of calcium (Ca), iron (Fe), manganese (Mn), magnesium (mg), zinc (Zn), selenium (Se) and arsenic (As) by flame and graphite method with atomic absorption spectrophotometer (Perkin Elmer: AS 80).

Fourier transform infrared spectroscopy (FT-IR) spectral analysis

The lyophilized samples of *C. indica* (10 mg) were mixed with 100 mg of dried potassium bromide (kBr) and compressed to prepare a salt disc. The disc was then read spectrophotometrically (FTIR-8400S, SHIMADZU, Japan). The frequencies of different components present in each sample were analyzed (Line et al., 2008).

UV spectrophotometer

UV spectrum for the test powder was recorded in SHIMADZU-UVVIS-160 spectrophotometer, Japan. 1 mg of sample was dissolved in 3 ml of high performance liquid chromatography (HPLC) grade methanol and the mix was recorded between 200 and 500 nm where the extracts exhibited absorption maxima.

RESULTS

Figure 1 shows *C. indica* grown on paddy straw and were analysed for nutrients such as moisture: moisture content was found to be 8.9%. The hundred grams of dry *C. indica* contains of moisture, proteins, carbohydrate, fibre, fat, ash, ether extract, pH, total nitrogen and total carbon were found to be 89, 14.09, 13.09, 5.63, 8.02, 4.6, 7.05, 3.57, 33.60 and 5.4 mg/100 g, respectively (Table 1). The minerals content of copper, manganese, zinc, iron, calcium, phosphorous, potassium and sodium were found to be 0.44, 0.036, 0.05, 0.13, 0.51, 0.38, 1.35 and 0.21, respectively (Table 2).

Spectrum analysis

FT-IR vibrational frequencies obtained at 3315 cm^{-1} inferred the O-H stretching (hydrogen bonded intermolecular), the bands of at 2490 cm^{-1}, the bands at 2242 and 2074 cm^{-1} corresponded to N-O bending, C=O stretching at 1870 cm^{-1}, C=O symmetric carboxylate stretching at 1413 cm^{-1}. The C-O stretching appeared at 1116 cm^{-1}. O-C=C asymmetric stretching was at 1150 cm^{-1} and a sharp C-X out of plane bending at 603 cm^{-1}. All these characteristic bands of the FT-IR spectrum of mushroom indicated the presence of OH, COOH and NO_2 groups (Figure 3). The UV absorption maxima appeared at 294 nm with a shoulder at 321 and 379 nm indicating the aromatic nature of the compounds (Figure 2).

DISCUSSION

Mushrooms are generally classified into four groups: edible mushrooms, medicinal mushrooms, poisonous mushrooms and magic or hallucinogenic mushrooms. Edible mushrooms are ideal healthy foods. They may contribute enormously to the supply of both macro and micro nutrients in our diet. They are considered to be the potential source of carbohydrates, proteins, fat, and minerals. All of which contribute to the food value. Cultivated *C. indica* using 49 different substrates including various plant products, crop residues and leaves recorded the higher yield from paddy straw supplemented with 5% maize meal (Mahesh and Yadav, 2006). In the present study, paddy straw was used for the cultivation of *C. indica*. The crude protein, fat and total carbohydrate contents of *C. indica* and *P. sajor - caju* analysed at various growth stages exhibited strikingly different results (Sivapraksam and Ramaraj, 1997). In the present study, protein, carbohydrate, amino acids and lipid contents of *C. Indica* was analyzed (Table 2).The total fat content was greater in *C. indica* which is significant to *P. sajor-caju* and *P. florida.* It was also significantly richer in carbohydrates than the three species of *Pleurotus* (Nuhu et al., 2008). On the other hand the fiber content in *C. indica* is significantly lower about 8% than that in *Pleurotus* spp. Mushroom are also rich in mineral contents. The total ash content found was 7.5. In the present study, the FTIR spectrum of mushroom indicated the presence of OH, COOH and NO_2 groups. Similarly in the previous study of FT-IR spectra of *Pleurotus* spp. straw powder, a broad stretching band was observed at 3416 $cm^{-}1$ due to the presence of OH and NH groups (Ranjani et al., 2013). The biosynthesis of *C. indica* extract was shown at UV 200 to 600 nm which was observed in the presence of silver nanoparticles (Sujath et al., 2013) similar to the present study of UV absorption maxima which appeared at 294 nm with a shoulder at 321 and 379 nm indicating the aromatic nature of the compounds. In conclusion, the chemical compositions of edible mushrooms determine their nutritional value and sensory properties as also mentioned (Shah et al., 1997; Manzi et al., 2001). These data suggest that dietary *C. indica* is a good source of nutrients specially protein and fibre. Mushrooms are rich in protein, edible fibre and minerals but lipid content is low. These results also indicate that the studied mushrooms have good nutritive value for human.

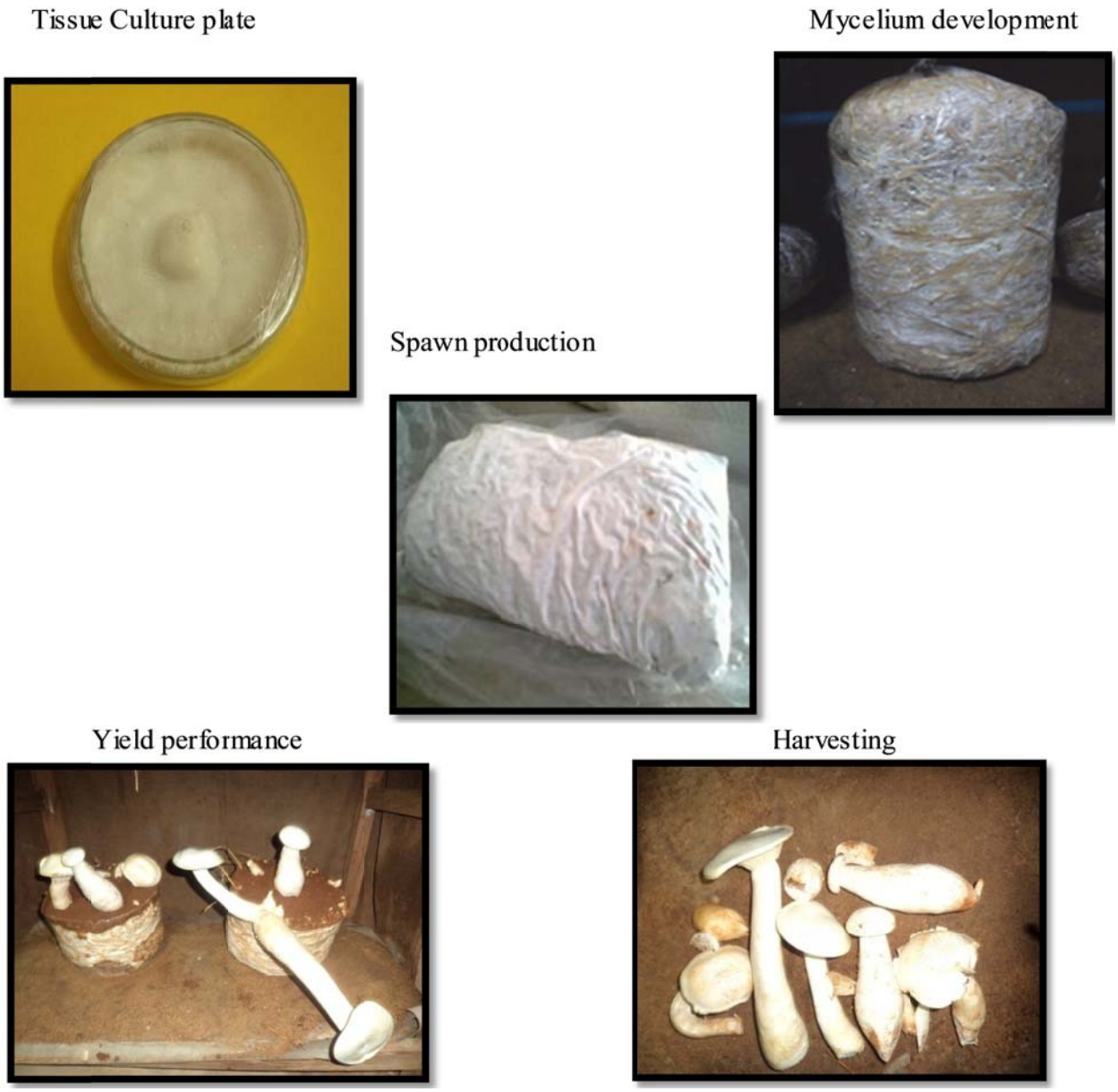

Figure 1. Cultivation technology of *Calocybe indica.*

Table 1. Proximate analysis of *Calocybe indica.*

Nutritional parameters g/100 g	Value (%)
Moisture	89
Crude protein	14.09
Carbohydrate	5.63
Crude fibre	8.02
Lipid	4.6
Ash	7.05
Ether extract	3.15
(pH) 5% solution	5.4
Total nitrogen	3.57
Organic carbon	33.60

Table 2. Micro nutrition analysis of *Calocybe indica.*

Micro nutrient analysis	Value (mg/g)
Copper	0.44
Manganese	0.36
Zinc	0.05
Iron	0.13
Calcium	0.51
Phosphorous	0.38
Potassium	1.35
Sodium	0.21

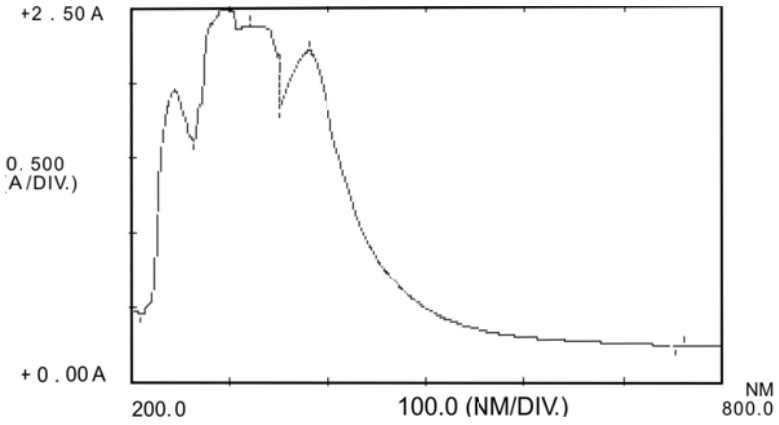

Figure 2. UV spectrum of *Calocybe indica.*

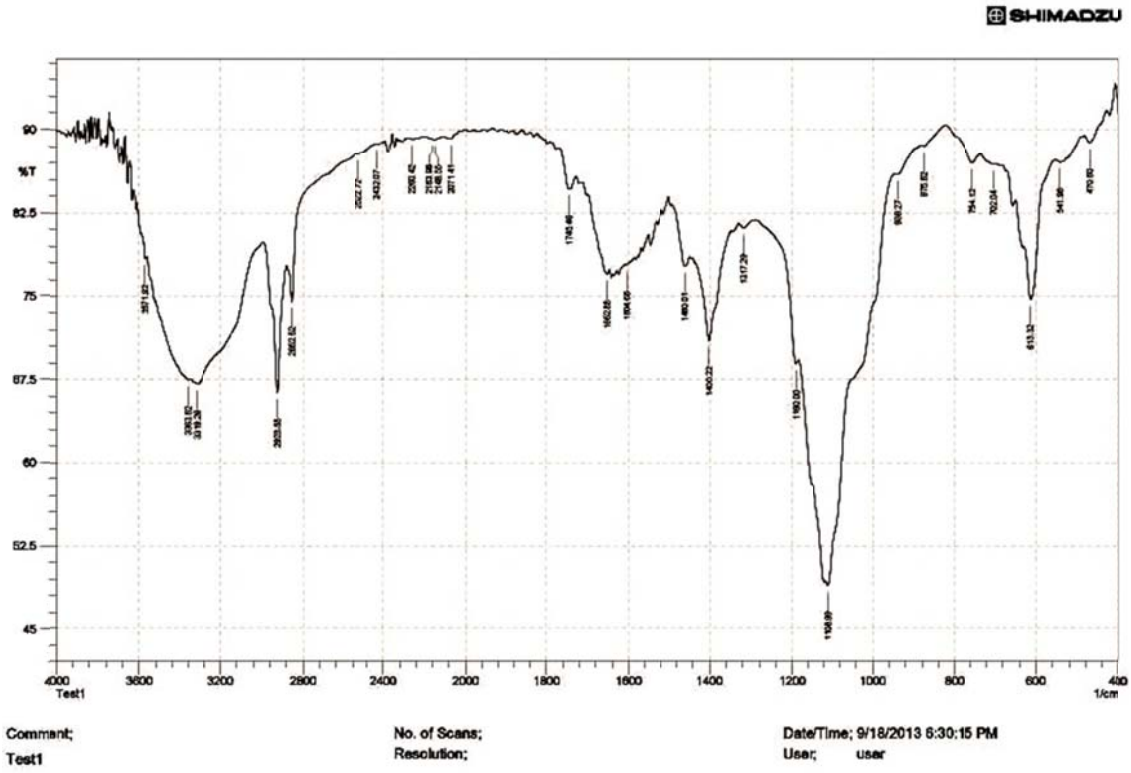

Figure 3. FT-IR Spectrum of *Calocybe indica.*

Conflict of Interests

The author(s) have not declared any conflict of interests.

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