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RESEARCH ARTICLE

CULTIVATION, NUTRITION, BIOCHEMICALS AND ENZYME ANALYSIS OF PADDY STRAW MUSHROOM (*Volvariella volvacea*)

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ABSTRACT

The edible Paddy straw mushroom is the most extensively cultivated mushrooms in tropical and sub-tropical regions. Studies were conducted on the cultivation, nutritional analysis, biochemical and enzyme production of this mushroom. Among the various method of cultivation, circular bed method was the best method and it showed the good growth. The nutritive values of the mushroom were rich in proteins and fibres and observed a lower amount of lipid. The screening and biochemical determination showed presence of saponin, alkaloids, terpenoids, sugar, flavonoids and sterols. Amylase, cellulase and laccase are important enzymes that can be used for various biological activities. Laccase exhibit good enzyme production than other enzyme like amylase and cellulase. This investigation may provide a basic knowledge about the *Volvariella volvacea,* and give valuable information for further study.

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INTRODUCTION

Volvariellavolvacea is the fifth most important edible mushroom in the world according to yield (Chang 1993). It is tropical and subtropical saprophytic fungus belonging to the family Pluteaceae of Basidiomycetes. It is commonly known as paddy straw mushroom, Chinese mushroom and tributary mushroom. *Volvariella volvacea* is also known for its unique aroma and texture. The nutritional value of these mushrooms depends on the type of the agricultural waste used for its production (Roy *et al*., 2014).Enzymes occur in all living organisms includingmushroom and it is used for hydrolysis; oxidation, reduction, or metabolism (Quimio, 1989; Wang, 1989). It plays a vital role in the mushroom development, nutritive value and flavour (Jonathan, 2002). The enzyme like amylase and cellulase from various fungal sources has largely been screened for commercial utility (Wang, 1989; Diez and Alvarez, 2001). Celluloytic enzymes play a significant role in natural biodegradation process (Jonathan and Adeoyo, 2011).In the developing countries like India, most of the people do not met the balanced diet. This may be mainly because of uncontrolled population, poverty and inadequate food for humans. To overcome this problem, low cost with highly nutritive food is needed. Today mushrooms like *Volvariellavolvacea* fulfil all this need. Generally, it is rich in protein, vitamins, fibre and minerals, which is easily digested and it has no cholesterol content (Isikhuemhen and Okhuoya, 1999). Artificial cultivation of *Volvariella volvacea* do not required much cost and it can be easily cultivated by the farmers. The substrate used for cultivation of mushrooms is mainly agro-industrial wastes (Bisaria and Ghose, 1981; Jonathan *et al*., 2010). The present studyfocused to evaluate cultivation, biochemical and enzyme production of paddy straw mushroom of *Volvariella volvacea.*

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MATERIALS AND METHODS

Collection of Mushroom

The paddy straw mushroom *Volvariella volvacea*(MTCC No:957) was purchased from Microbial Type Culture Collection centre, Chandigarh (HP).

Medium

The specific medium of fungi Malt extract Glucose Yeast Peptone (MGYP) broth and agar were used and the culture was

stored in Deep freezer at 4ºC. MGYP+Starch, MGYP with 1% CMC (Carboxyl Methyl Cellulose), MGYP medium were used for enzyme production.

Chemicals

Mercuric chloride, Iodine, Chloroform, H_2SO_4, Ferric chloride, HCl, Sodium hydroxide, Nitric acid, Soluble starch, Dinitrosalicyclic acid, Sodium potassium tartarate, Carboxymethyl cellulose, Sodium acetate buffer were used in the Centre for Bioscience and Nanoscience Research Institute, Echanari, Coimbatore.

Cultivation of Mushroom

Substrate for mushroom cultivation

The spawn substrate of sorghum grains was purchased from locally available Thanjavur market. The Agrowaste of paddy straw was collected from CM farm house, Sankaranatharkudikkadu village in Thanjavur (Dt).

Production of Spawn

Spawn of *V. volvacea*was produced using Sorghum grains and the substrate was half boiled after which air dried for 1h. Calcium carbonate ($CaCo_3$) 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 121ºC for 20min. After which the bags were cooled at room temperature atleast for 4hr. Bags were inoculated individually using the culture of *V. volvacea*. From a single mother spawn, 25 sub spawn bags were prepared.

Preparation of Bed

Paddy straw was collected selectively and soaked in water for 4-6hrs and that straw was autoclaved at 121ºC 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. After authentication, the fresh, healthy mushrooms of *Volvariella volvacea* (Bul. ex. Fr.) Singerwere properly dried in shade for 2-3 weeks. It was pulverized in a blender, sieved and used for further studies.

Nutritional analysis of paddy straw mushroom

The samples were analyzed for chemical composition (moisture, proteins, fat, carbohydrates and ash) using the AOAC procedures (AOAC, 1995). The crude protein content (N × 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 ºC. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal) = 4 × (g protein) + 3.75 × (g carbohydrate) + 9 × (g fat).

Biochemical analysis

Biochemical test were used to analysis the primary and secondary compounds. Proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds (Harborne, 1998), which are given below;

Test for Alkaloids

Mayor's test: Dissolved filtrate 1 ml treated with Mayor's reagent (Potassium mercuric iodide). Formation of a yellow coloured precipitate indicatedthe presence of alkaloids.

(Mercuric chloride + few drops of Iodine solution)

Test for Terpenoids

Crude extract 2 ml was dissolved in 2 ml of chloroform and evaporated to dryness. To this 2 ml of Con H_2SO_4 was added and heated for about 2min. A grayish colour indicated the presence of terpenoids.

Test for Phenol and Tannin

Crude extract was mixed with 2 ml of 2% solution of $FeCl_3$. A blue green (or) black colorization indicated the presence of phenol and tannin.

Test for sugar

The little amount of substance mixed with equal volume of Fehling's A and B solution heated in water bath. Formation of red colour indicated the presence of sugar.

Test for Saponins (Froth test)

To 3 ml of extract were diluted with 2 ml of distilled water and this test tube was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponin.

Test for Flavonoids

To 4 ml of crude extract was mixed with few fragment of magnesium ribbon and Con HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

Test for Quinines

To the 1% test substance 2% sodium hydroxide was added. Blue green (or) red colour indicated the presence of quinines.

Test for Protein

The 4% extract were treated with few drop of concentrated nitric acid. Formation of yellow colour indicated the presence of protein.

Test for Sterols

The 3 ml of crude extract was mixed with 2 ml of chloroform and con H_2SO_4 was added sidewise. A red colour is produced in the lower chloroform layer indicated the presence of steroids.

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, di nitrosalicyclicacid, 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 (Zhou *et al*., 2009). 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

Sorghum grains substrate was used for the preparation of spawn bottle to cultivate paddy straw mushroom. In sorghum grains substrate spawn developed in short period and produced high yield than other substrates (Figure 1). By using this spawn, *V. volvacea*is cultivated on agro waste material as a substratum called paddy straw. It was the oldest and commonly used technique. Among various methods used circular method was a good simple method and gave best yield than others in this study (Figure 2). Pin heads appeared after 4-5 days of spawning. The mycelia covered the paddy straw in about 12-15 days.

Table 1. Nutritional composition of the Paddy straw mushroom (*Volvariella volvacea*)

Nutrition parameter of the paddy straw mushroom	Nutrition content of paddy straw mushroom (g/100g)
Moisture (%)	82.20± 0.45
Proteins(%)	52.12± 0.02
Fibre(%)	10.07±0.13
Fat (%)	06.03± 0.03
Carbohydrates (%)	43.45 ± 0.57
Ash (%)	05.00±0.27
Energy (kcal)	383.21± 01.94

All parameters except moisture were presented for dried matter

Table 2. Biochemical compounds detected in the mushroom (*Volvariella volvacea*)

Biochemcial compounds	Alkaloids	Terpenoids	Phenol & Tannins	Sugar	Saponins	Flavonoids	Quinines	Protein	Steroids
V. volvacea	+	+	_	+	+	+	_	+	+

***V. volvacea*Mother spawnSub spawn bottle**

Figure 1. Sorghum grains using prepared spawn bottle

Figure 2. Cultivation of *Volvariella volvacea* using circular bed method

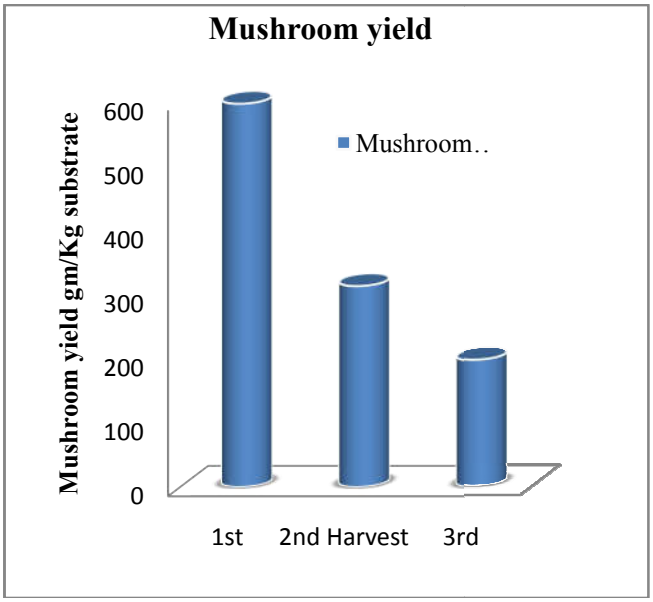

Figure 3. Yield rate of *Volvariella volvacea*

First harvest has been taken within 15- 20 days whereas the second harvest has been seen in 20-23 days and the third harvest has been carried out in 25-28 days, the yield rate of the mushroom shown in figure 3.The optimum temperature for the growth of *V.volvacea* was 30- 35°C.The nutritive value of *V. volvacea* on dry weight contains water, protein, fat, carbohydrates, crude fibre and ash were analysed and tabulated (Table 1). The results of biochemical screening indicates the presence of alkaloids, terpenoids, sugar, saponins, flavonoids, protein and steroids. Quinine, phenol and tannins were absent in the methanolic extract of *V. volvacea* (Table 2).

The enzymes of amylase, cellulase and laccase are the extra cellular enzymes. In this study the quantity of enzymes like cellulase, amylase and laccase were observed and the amount of enzymes were 0.024unit, 0.076unit and 0.355unit/mL respectively.

DISCUSSION

A variety of waste materials had been used other than paddy straw for cultivation of the *V. volvacea,* which included oil palm bunch (Naidu, 1971) oil palm pericarp waste, (Graham and Yong, 1974) banana leaves and saw dust, (Chua and Ho, 1973), cotton waste (Chang, 1974) and sugarcane bagasse (Hu *et al*., 1976). The common method employed for paddy straw mushroom cultivation are conventional method, improved cage cultivation, circular method (Thakur *et al*., 2003) outdoor method (Chang, 1982; Hu, 1985) indoor method (Quimio, 1993) indigenous chinese cultivation practice (Chang and Miles, 2004) The yield observed in this study agreed with the observation of Belewu, (2005) for similar mushrooms. The optimum temperature observed in the present study was similar to Chandra and Purkayastha, (1977) who observed 32°C as the optimum temperature for the vegetative growth of *V. volvacea.* Likewise, Jonathan *et al*., (2004) reported that *V. esculenta* grew fairly well in the temperature 35°C. There was a reduction in the weight of the substrate paddy straw and this showed that the mushroom had the ability to degrade lignocelluloses materials (Manson *et al*., 1989). It is the rising problem that straw alone was not sufficient as a compositing material as it did not contained sufficient nutrients and so it gave very low mushroom yield (10- 15% of dry substrate (Roy *et al*., 2014). To justify the above statement Manson *et al*., (1989) reported that the highest yield of *V. volvacea* was obtained from wheat grain with rice bran and Thiribhuvanamala *et al*., (2012) had studied the oil palm bunch waste with rice straw.*V. volvacea* was a good source of polypeptides, terpenes, steroids (Shwetha and Sudha, 2012) and phenolic compounds such as flavonoids, phenolic acids and tannins which contributed to high antioxidant capacity (Roy *et al*., 2014).The free phenolics were higher in this mushroom which is the major contributor to the antioxidant activity. Methanol and water extracts of *V. volvacea* were found to have a rich antioxidative activities which helped in the prevention of cardiovascular diseases, cancer (Cheung *et al*., 2003)neuro-degenerative diseases (Joseph *et al*., 1999) inflammation and problems caused by cell and cutaneous aging (Ames *et al*., 1993).

Some extra cellular enzymes (pectinolytic enzymes) like cellulase, hemicellulase and lignase played key role in the developmental stages of *Volvariella*sp(Roy *et al*., 2014). In *Agaricusblazei* and *Podoscyphabooleana*, very good amylase activities (0.60 and 0.24 unit/ml) were recorded at 25°C and 30°C, respectively. Conversely, little or no amylase and cellulase activity were observed at 35-40°C (Gbolagade *et al*., 2006). This is because enzyme activity is reduced at high temperatures (Griffin, 1994). In contrast to the above findings, the enzyme laccase givesgood yield at 35°C in *Schyzophilum commune*(Irshad *et al*., 2011) and laccase from *Trametesversicolor* had 45°C as an optimum temperature (Stoilova *et al*., 2010). Similarly in the present study, the enzyme laccase showed the good yield when compared to amylase and cellulase. That was mainly due to *V. volvacea* required high temperatures for (30-35°C) vegetative growth and fruiting (Zhao and Chang, 1997) and so it is called warm mushroom.In conclusion, based on our results it was concluded that, *V. volvacea* have been cultivated by simple traditional method and required low investment for cultivation. Likewise,

it possesses rich nutritional value and has been widely consumed as one of a delicious food in many countries. Moreover enzymes present in the mushroom have the ability to cure many diseases. Thus, it seems to be economically, nutritionally and pharmaceutically very important and useful species.

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