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Cultivation technology and spawn production of *Volvariella volvacea*: Paddy straw mushroom

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Abstract
Volvariella volvacea is majorly cultivated edible species that includes in the family Pluteaceae of the Basidiomycetes. This mushroom has some fallowing common names such as paddy straw mushroom, straw mushroom, Chinese mushroom, and warm mushroom. Firstly, it was grown in china in 1822. In India, systemic cultivation was started in1940. This mushroom usually grows on variety of lignocellulosic waste materials like cotton wastes, rice straw, oil palm pericarp, banana leaves. This straw mushroom is enriched with terpenes, amino acids, minerals, polypeptides, phenolic compounds and sugars. It also helps in preventing chronic hepatitis, arteriosclerosis, hyperlipidemia, anticancer, antiallergic, anti-inflammatory. Straw mushroom is been cultivated in different methods and problems in cultivating by the diseases and pests.

Keywords: Basidiomycetes, cultivation methods, paddy straw mushroom, spawn production, *Volvariella volvacea*

Introduction
Mushroom classified as a macro fungus and has a fleshy and distinct spore bearing fruiting body of fungus is a family member of Pluteaceae (Kotl. and Pouz) of class basidiomycetes (Singer, 1961) [50] typically grown above land, or soil or other food substrate. More than 2,000 mushroom species have been observed as edible among 12000 species, but nearly 35 are mostly accepted for consumption and limited species are commercially cultivated and almost 200 wild species are purposed for medical use (Beulah, 2013). Mushrooms are considered a delicacy of high nutritional and functional value and are recognized as a nutraceutical product; they are gone into interest due to their merits such as organoleptic, medicinal properties and economical significance Mushrooms are being considered as a possible muscle protein replacement due to their high digestibility (Pavel, 2009).

Mushroom sporocarp are enriched with minerals like potassium, iron, copper, zinc and manganese. Furthermore, mushrooms provide a good source of vitamin D, which is not found in other food supplements, in addition to these proteins and minerals. (Pehrsson *et al.*, 2003) [40]. Specialized bioactive compounds in mushrooms have immune-modulating properties and improve human immune function to reduce risk of cancer and tumor growth. However, production of mushroom in Asian countries started before 1000 years ago, start of scientific cultivation only at the beginning of 20th century when pure mushrooms cultures were prepared from spore and tissue. *Volvariella volvacea* is the most popular edible mushroom species cultivated (Walde *et al.* 2006) [58] and due to its pleasurable taste, it ranks third among essential mushrooms (Ramkumar *et al.* 2012; Thiribhuvanamala *et al.* 2012) [44, 54], also its rapid growth rate in comparison to other species (Rajapakse 2011) [43]. Other common names for this include paddy straw mushroom, straw mushroom and Chinese mushroom. Cultivation was recorded for the first time in china in 1822 (Chang, 1969) [17].

In global market, currently paddy straw mushroom ranks sixth, contributing for around 5-6% (Ahlawat *et al.* 2011) [4]. Tropical and subtropical regions are best suited (Bao *et al.* 2013) [8] and grows best at high temperatures (Obodai and Odamtten 2012) [38]. In India, it contributes around 7% of total mushroom production (Sharma, 2017) [49]. In India paddy straw mushroom was firstly cultivated in 1940; although its systemic cultivation was first trialed by Thomas and his colleagues in 1943. Paddy straw mushroom raise on atypical lignocellulosic waste materials such as rice straw, cereal straws, cotton wastes, oil palm pericarp, banana leaves and sugarcane bagasse, etc. (Chang, 1974) [18].

V. volvacea sporocarp is grayish to black egg-shaped vulva at young and rupture to expand the pileus up to nearly flat. Straw mushroom regarded as healthy food (Belewu 2005; Feeney *et al*., 2014; USITC, 2010) [49, 24]. It has high amounts of protein, phosphorus and potassium (Ahlawat and Tewari, 2007) [1] as well as low in alkalinity, cholesterol, fat and it is a salt free. Bioactive metabolites that provide surplus taste, flavor and pleasant aroma and prominent biological properties just like antioxidant (Hung and Nhi, 2012) [28, 29], antimicrobial (Chandra and Chaubey, 2017) [15], anti-inflammatory, anti-coagulant, anti-hypersensitive, anti-cancer (Hobbs 1995) [26]. This mushroom can grow on a variety of cellulosic materials and has a C:N ratio of 30:40, which is very high in comparison to other cultivated mushrooms, it can also grow rapidly and easily on uncomposted substrates. Only three main species of straw mushroom i.e., *V. volvacea, V. diplasia, V. esculanta* are artificially cultivated, among several species of *volvariella* have been reportedly grown for food (Ahlawat, 2011) [4].

Nutrient composition and sensory properties

Nutrient composition and sensory properties are described by their various proximate constituents. Paddy straw mushroom consists of high moisture content (90%), carbohydrates, fats, protein, fiber (chitin), essential organic acids (arginine, glycine, alanine, serine etc.), vitamins (biotin, thiamine, riboflavin and great amounts of vitamin C) and other vital minerals (potassium, sodium and phosphorus), unsaturated-fatty acids (Table 1) and also with reduced calorific value (Chang and Buswell, 1996; Jiskani, 2001; Buigut, 2002; Ouzouni *et al*., 2009). Based on the presence of carbonyl compounds and octavalent carbonate alcohols the aroma of straw mushroom is confined. The aroma of straw mushroom also relies on the contents of various elements like nitrogen, phosphorus, sulfur, zinc, potassium, iron, amino acids, nucleotides and as well as the auto oxidation of unsaturated fatty acids (Grzybowski, 1978).

Table 1: Health benefits of *Volvariella volvacea*

Content	Composition (qty/100gm of fresh mushroom)
Moisture content	90.40 gm
Fat	0.25 gm
Protein	3.90 gm
Fiber	1.87 gm
Riboflavin	1.63-2.98 mg
Thiamine	0.14 mg
Niacin	2.40 mg
Vitamin C	18.00 mg
Iron	1.70 gm
Potassium	0.32 gm
Phosphorus	0.10 gm
Calcium	5.60 gm

Metabolites of paddy straw mushroom and their actions

Volvariella volvacea is an adequate source of steroids, terpenes and polypeptides (Shwetha and Sudha, 2012) and various phenolic compounds which cause to increase in antioxidant activity like tannins, phenolic acids and flavonoids. The high free phenolic compounds which account for the major contributor for the antioxidant property. Dried straw mushroom and sporocarp of mushroom that carry great amounts of antioxidant enzymes; peroxidase, catalase, glutathione peroxidase, superoxide dismutase, glutathione-S-transferase, glutathione reductase. Methanol and water extracts of mushroom regarded as good antioxidant activity reduce risk against some chronic angiogenic diseases like arthritis, cancers, cardiovascular disease (Cheung *et al*., 2003), neurodegenerative diseases (Joseph *et al*.,1999) and chronic inflammation, (Ames *et al.*, 1993) [41]. Amounts of cardio toxic proteins in the protein extracts of straw mushroom knowns as volvatoxin and flammutoxin which limits the respiration rate in tumor cells (Cochran, 1978). It also carries proteins and polysaccharides that possess anti-tumor properties (Zhang *et al.*, 1994) [61].

Spawn Production

Spawn is the propagating material of mushroom, by growing mycelium in its substrate. This we commonly called as Seed of Mushroom has it is used as ready to mix in the beds. We can raise it by single spore culture technique, multi-spore technique or tissue culture technique.

a. Single spore culture technique

Choose an unopened mushroom fruiting body, clean the mushroom with cotton and 70% alcohol to remove the dirt from it and then cut the stripe lower portion. Fruiting body is placed in sterile petri-plate having a spiral wire with pointed end and covered properly with beaker. It should be kept undisturbedly for a night at room temperature. Remove the beaker and fruiting body along with spiral wire stand and petridish is covered aseptically. Spore collection up to 10^{-7} or 10^{-8} till 10-20 spore/ml count prepared by serial dilution technique. The plates should be incubated in BOD at 30±2 °C till small colonies are observed and these colonies are picked and transferred to PDA media fallowed by incubation to grow it as a single spore culture (Kaur and Sodhi, 2015) [33]. Observe growth under microscope to select the single spore isolates. Further it is multiplied by picking spores and sub-culturing Potato Dextrose Agar media by incubating at 34±2°C for 7 to 10 days in BOD incubator.

b. Multi-spore technique

Cultures of mycelia are uplifted from the spore print with 5mm disc and the spores are placed in sterile petri-plate containing Potato Dextrose Agar media and incubated at 30±2 °C for 8 days (Akinyele and Adetuyi, 2005) [5]. For vigor maintenance, fresh isolations were done from the sporocarps every time after 2-3subcultures (Nannapaneni, 2017)

c. Tissue culture technique

Tissue culture technique is used to get pure culture of V. volvacea from fresh fruiting body (Jonathan *et al*., 2009). Mushroom is cut into two equal parts with sterile, cool knife without touching fruiting body inner surface. Make small tissue pieces from stipe, pileus connecting point and placed it in a Potato Dextrose Agar (PDA) media containing petri-plate and subculturing of fungus was done at an interval of one month and stored at 25±°C (Biswas and Layak, 2014) [12]. The mycelial cultures can use directly in spawn substrate (Nie, 2016).

Preparation of spawn substrate

For spawn substrate various materials are used in alone or combination which favors the mycelium. Maximum used substrates are grains like wheat, sorghum and rye, rice straw cuttings, cotton wastes etc. The methods used for spawn substrate preparation as fallows.

Grain spawn

Spawn of mushroom are produced by using grains like wheat,

sorghum and rye. Full mycelial growth in short time observed in the combinations of 50% wheat grain and 50% rice bran (Tripathy, 2010) [55]. Healthy grains were boiled for 10-15 min. and air dried for 1 hr. Gypsum and lime are mixed with grains in the ratio 2:1 (Jandaik *et al*., 1976) [31]. The grains are mixed with 20gm of calcium carbonate to 1 kg grains in a container (Sanchez *et al*., 2002). The grains were filled in poly propylene cover closed by non-absorbent cotton and autoclaved the grains at 121°C for 20 minutes fallowed by cooling for 4 hr in a room temperature (Fig. 1). Inoculate the mycelial cultures and incubate at 34±2°C for 10-15 days (Karnan *et al*., 2016).

Fig 1: Commercial spawn prepared with wheat grains (Biswas, 2014)

Straw spawn

Rice straw of good quality should be soaked in water for 3-4 hours and 1% lime was added (Nie, 2016), then dried and substrate was supplemented with steamed horse gram powder at 2% on dry weight basis fallowed by filling in polypropylene bags and it should be closed by the non-absorbent cotton (Thiribhuvanamala *et al*. 2012) [54]. Before inoculation with spawn the substrate is pasteurizedfor 3 hrs. and cooled (Fig. 2). Then incubate this at 34±2 °C for 10-15 days (Okere *et al.,* 2015).

Fig 2: Commercial spawn prepared with paddy straw (Ahlawat and Tewari, 2007) [1]

The optimal temperature is 30-35 °C for mycelial growth and 28-30 °C for fruiting body of paddy straw mushroom (Le-Duy-Thang, 2006), if the temperature goes beyond 45 °C or falls below 15°C mycelium does not grow. After the mycelia is completely colonized in spawn substrate, it is regarded as it is ready for using as a seed. But, if it is unused, it should be removed from the incubator and kept in lower temperatures to avoid further mycelial growth, aging and spoiling. For the storage of spawn substrates, the temperature should range from 15-20°C at this point the mycelial growth is limited without losing viability for longer duration (Ahlawat, 2003). Mushroom grows best with the relative humidity around 70-90% (Biswas and Layak 2014) [12] and pH is 6.5; anything higher inhibits mycelial growth (Akinyele and Adetuyi, 2005) [5]. In favorable growing conditions, crop cycle completes in 4-5 days (Biswas 2014) [12].

Cultivation

Thomas *et al.* (1943) introduced *V. esculenta* cultivation for the first time. On the bundles of paddy straw of 10 kg soaked in water and placed on the raised wooden platform. For the cultivation of paddy straw mushroom different waste materials are used as a substrate. Before 1970, the only substrate using was rice straw in the cultivation of paddy straw. Later the rice straw was partially replaced by cotton waste from 1971, as it is acts as a heating material which favors the growth and provides stable yields of paddy straw mushroom. By using only paddy straw for composting is not sufficient as it carries low amounts of nutrients and has a slow decomposition rate (Anonymous, 1983) [6]. A variety substrate used for growing such as rice straw, cotton wastes, water hyacinth, banana leaves, oil palm pericarp wastes, sugarcane bagasse (Jandaik, 1976) [31]. This mushroom needs high cellulose, low lignin and produces a variety of cellulase enzymes.

Conventional method

Rice straw is commonly used as a substrate in the traditional method of bed preparation (Khan, 1991). Rice bundles are tied and then cut into appropriate pieces (Reyes *et al*., 1991). Hand-threshed paddy straw can be used to make 0.75-1.0 kg paddy straw bundles. Rice straw bundles collected should be sun-dried to maintain moisture. Place the prepared in cleaned water for nearly 12-18 hrs. in a water tank of cement floor as it is a important process which helps in the composting process (Reyes, 2000) [45] and then drain out excess water. Prepare a layer by placing the four bundles in line and another four bundles also placed in a similar manner combined forming a layer of eight bundles by spawning between the first and second, second and third, third and fourth layers, a total of four layers will be formed and then spread the red gram powder near the spawn region It is suggested that a bed of 30-40 kg paddy straw, 500 gm spawn, and 150 gm red-gram powder be used. Spawns are used in between layers by leaving some margins (Biswas and Layak, 2014) [12]. For maintaining required temperature (30-35°C) and humidity (80-85%) beds are top pressed and plastic sheets are covered (Rajapakse.2011) [43]. After 7-8 days polythene sheets are removed and control the temperature nearly 28-32°C. After 4-5 days removal of sheet mushroom will start appeared. It is continued growing for more 10-15 days and harvested at button and egg stage.

Improved cage method

Substrate using cotton gives notably higher yield compared to paddy straw (Makandura, 2011). Caged method evidenced greater yield and biological efficiency among bed, spiral and heap methods (Biswas, 2014). Take paddy straw of dried,

fresh and hand threshed free from competitive moulds (Fig. 3). Prepare bundles 25 cm×10 cm and those bundles are immersed in water for overnight with addition of calcium carbonate fallowed by steam sterilization records good yield (Sudha *et al*., 2017) [51]. Disinfected cage is taken and place 10 bundles in cage at the bottom evenly and spawning should be done. Similarly, make a total of six layers one above the other with intermittent spawning. Spray 0.2% Dithane Z-78 and 0.1% Malathion solutions and cover with polythene sheet followed by binding cage with jute sting (Biswas, 2014) [12]. Full growth of mycelia observed in 5 days at a temperature 30-35°C with relative humidity 80-90% (Zikriyani *et al*., 2018) [62]. The polythene sheet is removed after the spawn run is over, and humidity is controlled. Harvest the mushroom at button and egg stages which is most preferred by consumers (Chang and Miles, 2004; Ahlawat and Tewari, 2007; Jamjumroon *et al*., 2012) [1, 30] and apply water gently for 2nd harvest.

Fig 3: Improved cage method of cultivation (Ahlawat and Tewari, 2007) [1]

Outdoor cultivation

An outdoor cultivation was practiced in mid-1980's from the department of agriculture. Farmers, on other hand, are hesitant to grow mushroom using this method due to unpredictability of production and yield. Owing to inability to monitor environment factor like temperature, relative humidity and insect and issues (Fig. 4). Unlike with the oyster mushroom, the straw mushroom is extremely susceptible to the changes in the weather. Conventional outdoor method employs abed type solution and a variety of agricultural wastes such as dried paddy straw, rice stubbles, water lily, stalks, leaves and banana peels (Ryes and Abella, 1997) [46]. Immersed the bundles in running water or in 2% $CaCO_3$ solution. Calcium carbonate reduced the stickiness of compost and raised the pH by preventing anaerobic conditions (Hota S and Pani B.K., 2019) [27]. Prepared bundles are placed on raised platform and make four layers with each layer contain four bundles. Intermittent spawning is done followed by spreading red gram powder and then covered with plastic sheets until pin heads emerge (Hota S and Pani B.K., 2019) [27]. Polythene sheets are removed after 8 days of spawning. During rainy season sprinkling water is avoided and the first flush of marketable fruiting bodies normally emerges from edge of the mushroom beds after 10-14 days. Thakur *et al*., 2003 [53] and Godara 2002 conducted trials at Raipur in AICRP obtained biological efficiency from 2-5%. Button stage of V. *volvaceae* should be harvested by pulling cluster out of the bed (Reyes, 2000) [45].

Fig 4: Outdoor method of mushroom cultivation (L. V. Thuc *et al.,* 2020)

Spiral method

Thakur *et al*., (2003) [53] found some steps which involved in this method of cultivation at Raipur in Chhattisgarh. Cultivation period was increased for nearly 45 days and comparably yield also increased. Bundles of weight 1 kg are prepared and bundles are immersed in 2% $CaCO_3$ for 12 hrs. Water-soaked bundles are wrapped around a wooden pillar for height of 6 ft. and spawning along the wrapped bundles and gram flour is spread along the spawned area. Beds are covering with polythene sheet (Ahlawat and Tewari, 2007) [1]. Maintain the temperature around 32-34°C and humidity 85% with sufficient ventilation for 5-6 days. Polythene sheets are removed and temperature is lowered 28-32°C, relative humidity to 80% and water is applied gently. Mushroom are removed by twisting the fruiting bodies at egg stage (Maurya *et al*., 2020). More exposed surface area, more light penetration and more aeration credited higher yields in spiral method compared to cage and bed method (Thakur *et al*., 2003) [53].

Fig 5: Spiral method of mushroom cultivation (Thakur and Singh, 2014)

Indoor method

In the early 1970's the indoor method of warm mushroom cultivation was started all around the year with various agricultural wastes as substrate. Indoor cultivation of paddy straw mushroom substrate like paddy straw used preferably to improve productivity (Zinkriyani, 1951) [62]. Among different agricultural wastes cotton waste is used more preferably.

Introduction of cotton waste in replace of paddy straw in the cultivation enhanced and set a considerable yield in Hong Kong (Chang, 1979). Use of cotton wastes helps to retain water for longer period and it consists of cellulose and hemicellulose, thus contributes more yield (Fig. 5). By using cotton waste biological efficiency observed about 25-50% (Quimio *et al.*, 1990). The agricultural wastes used in the cultivation medium for *V. volvacea* are crucial because they influence the nutrient content of the mushrooms (Roy *et al.*, 2014).

Substrate is immersed in water with 1.5% $CaCO_3$ for 16 hrs. and after drying bundles are arranged in form of bed, 5 bundles in 4 layers + 2 i.e., 22 bundles per bed (Kaur and Sodhi, 2015) [33]. Substrate was left composting for 4 days and 20 kg wet bundles of are placed on the shelves by maintaining the room temperature for 32±2 °C fallowed by partial ventilation (Kumar *et al.*, 2019). These beds were kept in growing rooms for mycelial growth (Khanna and Kapoor, 2007). Favored mycelium growth is observed at the temperature 35 °C and humidity 85% in 5-6 days with little ventilation (Zikriyani *et al.*, 2018) [62]. Sheets are removed and temperature is lowered to 30°-25 °C. Allow little ventilation, supply of fluorescent light and supply ample water at primordia stage and at button stage harvesting practice is done (Ahlawat and Tewari, 2007) [1].

Fig 6: Indoor method of mushroom cultivation (L. V. Thuc *et al.*, 2020) [35]

Fruiting and harvesting of paddy straw mushrooms

Paddy straw mushrooms are harvested at egg and button stage when diameter is up to 2 inches (Maurya *et al*., 2020). For higher protein content, increased palatability, and a longer shelf life, harvest during the button to egg-shaped stages. Mushrooms at button stage have good texture and flavour (Jamjumroon *et al.*, 2012, He *et al*., 2018) [30]. When mushrooms are harvested at the bottom stage of the growth cycle, there is a chance of higher profits (Tripathy and Sahoo, 2010) [55]. Fruiting bodies are detached from the substrate carefully by uplifting slightly and twisted gently. Straw mushroom is only that can be harvested in short period as compared to other mushrooms (Thiribhuvanamala *et al.*, 2012; Thakur and Singh, 2014) [54]. First harvest is generally done after 9-10 days of spawning and it usually lasts for 3 day, which add up to 70-90% of the yield. Optimum environment and addition of water is required for second flush and it constitutes only 10-30% of total yield.

Diseases and Pest Problems

Rice straw mushroom is extremely sensitive to environment including sunlight, temperature, water, oxygen and carbon dioxide. Sudden temperature changes can restrict or limit growth of straw mushroom. Sunlight is required for different growth stages from sphere to egg stages. Significant reduction of vitamin E and unavailable of vitamin D is observed with the absence of sunlight, and melanin pigment may not form in the mushroom. In India, straw mushroom is subject to competitor moulds. *Chaetomium* sp., *Alternaria* sp., *Sordaria* sp., are generally observed as contaminants on straw bundles of wheat, rice, Kans, Jowar, barley and maize (Gupta *et al.*, 1970) [25]. Other competitor mushrooms namely *Coprinus* sp., *Trichoderma* sp., *Psathyrella* sp., *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp., *Trichoderma* sp. and *Sclerotium* sp., are reported on the substrate (Munjal, 1975; Rangaswami, 1978; Bahl, 1984; Purkayasta and Das, 1991). Destructive diseases subjected to straw mushroom are orange mold (*Neurospora* sp.), plaster mold (*Scopulariopsis funicola*), green mold (*Triichoderma* sp.), acne mushroom (*Sclerotium rolfsii*, Muthukrishnan 1971) and bacterial rot (Kannayan, 1978).

Lime water with a conc. 0.5-1% can be used to cure these diseases. In straw mushroom common infested pests are phorids (*Megaselia* sp.), sciarids (*Bradysia tritici*, *Lycoriella auripilla*), spring tails (Lepidocrytus sp., *Seira iricolor*), and mites (*Tyrophagus* sp., *Rhizoglyphus echinopus*, *Histiostoma heinemanni*, *Hypoaspis miles*). Among these rapacious pests mites contribute more damage to mycelium and button. Use of combination of insecticide, fungicide and antibiotics (Malathion @0.025%, Dithane Z-78 or Benomyl @0.025%, tetracycline @0.025%) are suggested for the management of pests and diseases (Kannaiyan and Prasad, 1978).

Conclusion

Producing straw mushroom is not only a sustainable option but also helps in efficient utilization of agro-waste. Mushroom production and consumption have increased dramatically in many countries due to its numerous benefits and advantages. (Vizhanyo and Jozsef 2000; Bernas *et al.*, 2006) [57]. In india, Volvariella mushrooms contribute 7% of total mushroom production (Sharma *et al.*, 2017) [49]. This mushroom pertains good health benefits, due to the presence of bioactive compounds and the aroma relies on the composition. As the spawns are prepared for the germination of mushroom and used as seed for the cultivation. Outdoor cultivation is a conventional practice with low investment cost but generates less yield due to risk of environment changes and high incidence of diseases and pest. While, indoor method requires higher investment for mushroom rooms construction which helps to maintain environment and helps to enhance productivity.

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