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Spawn preparation and cultivation of *Volvariella volvacea* (Bull. ex Fr.) Singer on paddy straw substrate

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ABSTRACT

Volvariella volvacea (paddy straw mushroom) is an important edible mushroom cultivated widely in tropical and subtropical regions due to its rapid growth, nutritional value, and medicinal properties. The present study investigated spawn preparation and cultivation of *V. volvacea* using paddy straw as the primary substrate. Pure cultures were established under controlled laboratory conditions, followed by spawn production and indoor cultivation. Growth characteristics, fruiting behavior, yield, and biological efficiency were evaluated. The results showed that pinhead formation occurred within 15 days, and a yield of 2.05 kg per 10 kg of substrate with a biological efficiency of 20.5% was obtained. The findings indicate that appropriate substrate preparation, environmental conditions, and spawn quality are important factors associated with successful cultivation. Despite its commercial importance, production of *V. volvacea* remains limited by suboptimal practices. This study provides practical insights into spawn preparation and cultivation techniques that may support improved and sustainable mushroom production.

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INTRODUCTION

Mushrooms are classified as macro fungi, characterized by their fleshy and distinct spore-bearing fruiting bodies. They belong to the Pluteaceae family (Kotl. and Pouz) within the class Basidiomycetes (Singer, 1961) and are typically found growing above ground, in soil, or on other food substrates. Among the 12,000 known species of mushrooms, over 2,000 have been identified as edible. However, only about 35 species are widely accepted for consumption, with a limited number being commercially cultivated. Additionally, nearly 200 wild species are utilized for medicinal purposes (Chen *et al.,* 2019). Mushrooms are regarded as a delicacy, offering high nutritional and functional value, and are acknowledged as nutraceutical products. Their appeal has increased due to various advantages, including organoleptic qualities, medicinal properties, and economic importance. Furthermore, mushrooms are being explored as a potential alternative to muscle protein, owing to their high digestibility (Vinay *et al.,* 2021).

Mushroom sporocarps are rich in minerals such as potassium, iron, copper, zinc, and manganese. Additionally, mushrooms serve as a significant source of vitamin D, which is absent in other dietary supplements, alongside these proteins and minerals. (Pehrsson *et al.,* 2003). The unique bioactive compounds found in mushrooms possess immune-modulating effects and enhance human immune function, thereby lowering the risk of cancer and tumor development. Nonetheless, mushroom cultivation in Asian nations commenced over 1000 years ago, with scientific cultivation beginning only in the early 20th century when pure cultures of mushrooms were developed from spores and tissues. *Volvariella volvacea* is the most widely cultivated edible mushroom species (Walde *et al.,* 2006) and due to its delightful flavor, it ranks third among essential mushrooms (Ramkumar *et al.,* 2012; Thiribhuvanamala *et al.,* 2012) also noted for its rapid growth rate compared to other species (Rajapakse, 2011). This mushroom is also commonly referred to as paddy straw mushroom, straw mushroom, and Chinese mushroom. The first recorded cultivation occurred in China in 1822 (Chang, 1969).

The sporocarp of *V. volvacea* is characterized by a grayish to black, egg-shaped vulva in its juvenile stage, which ruptures to allow the pileus to expand to a nearly flat form. The straw mushroom is considered a nutritious food source (Feeney *et al.,* 2014). It is rich in protein, phosphorus, and potassium (Ahlawat and Tewari, 2007), while being low in alkalinity, cholesterol, and fat, and is free of salt. This mushroom contains bioactive metabolites that contribute to its rich taste, flavor, and pleasant aroma, as well as notable biological properties such as antioxidant (Hung and Nhi, 2012), antimicrobial (Chandra and Chaubey, 2017), anti-inflammatory, anti-coagulant, anti-hypersensitive, and anti-cancer effects.

Paddy straw mushroom, also known as grass mushroom, derives its name from its cultivation on rice straw. This mushroom is a significant dietary component due to its rich flavor, aroma, and nutritional benefits. Scientifically classified as *Volvariella volvacea*, it is a Holobasidiomycete that belongs to the Plutaceae family (Mond *et al.,* 2021). This species accounts for 6% of the global mushroom production, predominantly utilized in the South Asian region. Over 100 species of *Volvariella volvacea* (Bull.ex.Fr) Singh have been documented worldwide (Kurtzman and Yang, 1982). The paddy straw mushroom thrives in high temperatures, making it primarily cultivated in the tropical and sub-tropical areas of Asia, including countries such as China, Taiwan, Thailand, Indonesia, India, and Madagascar. The life cycle of *Volvariella volvacea* consists of six maturity stages: pinhead, tiny, button, egg, elongation, and mature stages (Najmu *et al.,* 2022).

Depending on the geographical area and climatic conditions, *V. volvacea* is grown either in outdoor settings or within controlled indoor environments. The choice of substrates for cultivating *V. volvacea* in a specific nation is primarily determined by the quantity of accessible free resources (Amir *et al.,* 2023).

Mushroom cultivation is a significant and lucrative agribusiness that offers employment opportunities for rural women. The paddy straw mushroom grows rapidly allowing for harvest within two weeks of bed preparation. The demand for mushrooms is rising daily in Odisha. The agro-climatic conditions in Odisha are ideally suited for the cultivation of paddy straw mushrooms (Mijan, 2024). Nevertheless, most of the edible fungi that are presently cultivated belong to medium- and low-temperature varieties, while high-temperature varieties are quite scarce; this results in a limited availability of edible fungal varieties in the market during the high-temperature season (Ali *et al.,* 2024). These circumstances also contribute to the consistently high price of *V. volvacea* throughout the year, potentially enhancing the profits for mushroom farmers in comparison to those of other edible fungal varieties (Wang *et al.,* 2025). The present investigation was carried out to find out the spawn preparation, cultivation of *Volvariella volvacea* on paddy straw substrate and supplements for yield enhancement.

MATERIALS AND METHODS

Sample collection

Volvariella volvacea mushroom culture were purchased from India. The spawn further used to make a pure culture under controlled for further laboratory works (Fig. 1).

Fig. 1. Cultivation of spawn

Culture inoculation and identification

Volvariella volvacea mushroom cut placed on Petri plates containing potato dextrose agar medium (PDA). The Streptomycin sulphate (100mg/L) was added to prevent the growth of bacteria. Then it was monitored every day for growth of fungal colonies. Fungi growing out from the samples were subsequently transferred to fresh PDA plates. The identification of fungal species *V. volvacea* were done by microscopical characteristics and standard manual.

Spawn preparation

The initial culture for spawn production from any authorized agency (Tamil Nadu Agricultural University, Coimbatore). Additionally, it can be cultivated using tissue culture methods, single spore culture techniques, or multispore culture techniques as outlined in previous chapters. The culture of paddy straw mushrooms cannot be maintained at low temperatures. Therefore, cultures are typically kept at 17-20°C or higher. We can produce the spawn of this mushroom on various substrates such as grains, straw, etc. Many individuals prefer to utilize paddy grains or even paddy straw for spawn production. The procedure for creating the spawn remains the same as discussed in earlier chapters. However, for paddy straw mushrooms, after inoculation, the bags are incubated at temperatures ranging from 30-35°C. Complete colonization of the substrate occurs within 5-7 days, and the spawn is ready for use within a week. Spawn cannot be stored and should be utilized within 10-15 days.

Indoor cultivation method

We have acquired knowledge regarding the technique of outdoor cultivation which is notably straight forward and necessitates minimal facilities. Nevertheless, the yield remains quite low. By contrast, we can achieve significantly higher yields through indoor cultivation of this mushroom. In this environment, it is cultivated within specially designed insulated rooms equipped with steam pasteurization facilities. The substrate utilized for its growth can consist solely of a combination of this waste with paddy straw. We compost the substrate outdoors for a duration of four days, followed by pasteurization and conditioning for an additional four days within the cropping room itself. The specifics of the various stages involved in the indoor method are outlined below.

Fruiting and harvesting

Approximately 20 days post-spawning, pin-heads began to emerge. Typically, the straw mushroom is harvested before it reaches its full size, specifically at the button stage, just prior to the rupture of the volva that encases the cap and subsequently during the egg stage. The initial flush persisted for roughly 4 to 5 days, succeeded by a second flush approximately one week later. Nevertheless, the yield from the second flush was merely 10% of that from the first. The overall duration of the harvesting process extended up to 2 months.

Biological efficiency

The biological efficiency was determined by dividing the fresh weight of all the mushrooms by the weight of the dry substrate and then multiplying the result by 100, as illustrated below.

BE%= {(Fresh weight of mushroom)/(Dry weight of substrate)} ×100

Yield

The yield was determined by measuring the fresh weight of 2-3 flushes, which was expressed as weight (g) per unit of dry substrate.

Yeild (kg)= {Fresh weight of 2-3 flushes/Dry substrate (kg)}×100

RESULTS

Pure culture and identification

The *Volvariella volvacea mushroom* spawn that had been cultivated on the mushroom bed and subsequently harvested. The harvested mycelium was then sporulated on PDA plates and incubated at room temperature for 72 hours (Fig. 2). Finally, the *V. volvacea* that had been inoculated were subjected to microscopic and macroscopic characterization and their identification was carried out by the Indian Biotrack Research Institute, Thanjavur – 613 005, Tamil Nadu, India.

Fig. 2. Pure culture and slide identification (*Volvariella volvacea*)

Macroscopic and microscopic characters

The identification of paddy straw mushroom (*V. volvacea*) was performed by examining both their morphology. Morphological identification involved the growth of mycelia, analyzing the spore print and spore size, as well as measured the length and diameter of the (mycelium pileus and stipe). The cap usually measures between 4 and 12 cm in diameter, although it can expand to a maximum of 16 cm. Initially, it is egg-shaped, transitioning to a convex form, then to a bell shape, and ultimately flattening as it matures. The coloration is typically whitish to grayish-brown, frequently darker at the center, and is adorned with silky fibers. The edges are smooth and lack striations, the gills are detached from the stem (not connected). They are densely packed and undergo a color transformation as the mushroom develops: starting as white, transitioning to pink, and ultimately turning brownish-pink as a result of the spores' coloration.

The stem measures between 6 and 12 cm in length and has a thickness of 1 to 1.5 cm, being centrally connected to the cap. Its surface is smooth, exhibiting a whitish or brownish hue, and possesses a silky texture. A notable characteristic is the lack of an annulus (ring) on the upper section of the stem. A notable, robust, sack-shaped cup is located at the base of the stem, enveloping it. The volva generally appears brownish-gray to almost black externally and is whitish internally. This characteristic is essential for identification. The color of the spore print is a shade of brownish-pink. This stands in stark contrast to the white spore print of the poisonous *Amanita phalloides* (commonly known as the death cap), which is a similar-looking species. The flesh is white and remains unchanged in color when cut. The scent and flavor are not especially unique. Juvenile specimens might emit a faint aroma, occasionally likened to that of rose petals; however, this characteristic is not a dependable means of identification.

Spawn preparation

Spawn preparation entails the cleaning and sterilization of a substrate such as grains, followed by the inoculation of this substrate with a mushroom culture within a sterile environment. The inoculated substrate is then incubated until the mycelium has completely colonized it, thereby rendering it suitable for mushroom cultivation (Fig. 3). Thoroughly wash grains like sorghum to remove dirt and debris. Boil the grains for 15-30 minutes until slightly growth. This removes chaff and prepares the grain for mycelia growth. Drain the excess water and spread the grains on a cloth to dry in the shade until the surface moisture is gone. This is crucial to prevent clumping and bacterial growth. Mix in calcium carbonate ($CaCO_3$) at a rate of about 20 grams per kilogram of grain. This helps balance the pH and prevents clumping. Populate sterilized bags (such as polypropylene bags) or jars until they are approximately three-quarters full with the prepared grain. Ensure that a secure closure, conducive to gas exchange, is established (for instance, using a cotton plug and a paper cover).

Position the containers within an autoclave or pressure cooker and carry out sterilization at 15 psi (equivalent to around 121°C) for a minimum duration of 90 minutes to two hours. Following sterilization, permit the bags to cool entirely in a clean, sterile space. It may be beneficial to activate a UV light for 20 minutes prior to commencing. Within a sterile setting, such as a laminar flow hood, segment a pure mushroom culture into portions and insert a piece into each bag using sterilized cork borer. Secure the bags and gently combine the contents. Position the bags in a clean, dark area at a stable temperature (approximately 25°C) and incubate for a duration of 10-15 days. Over a period of 10 to 15 days, the mycelium will proliferate across the grains, resulting in a white and fluffy appearance. After complete colonization, the spawn becomes suitable for mushroom cultivation. It may be kept in a cool, dark environment for several days, or for extended storage, it should be refrigerated.

Initial spawn

Preparation of spawn Final spawn growth

Fig. 3. Preparation of spawn and spawn development

Mushroom bed preparation

A mushroom bed suitable for *Volvariella volvacea*, it is essential to soak and drain paddy straw layer it within a bag or cage and intersperse spawn along with a supplement such as gram powder between each layer. Ultimately, the bed should be covered with a plastic sheet, and the appropriate temperature and humidity must be maintained until the onset of fruiting. Immerse paddy straw bundles in water for a duration of several hours (for instance, 6–8 hours or overnight). Remove the surplus water on a sanitized surface. It is possible to disinfect the bundles during the soaking process using formalin and incorporate calcium carbonate to mitigate stickiness. Begin by placing a layer of straw bundles, approximately 5–10 cm in height at the base of your bag or cage. Disperse a handful of spawn across the straw layer, focusing on the periphery. Additionally, you may incorporate a supplement such as gram powder atop the spawn. Proceed to alternate layers of straw and spawn until you achieve the desired height or exhaust all available materials. For instance, you might construct five layers of straw and four layers of spawn. Place a clean plastic sheet over the bed's surface to preserve humidity and temperature. Ensure the bed is situated in a dark environment with a temperature range suitable for the spawn-running phase. After a duration of 7 to 8 days, remove the plastic covering and ensure that the temperature remains constant while sustaining a relative humidity of approximately. Mushrooms are expected to begin emerging within a few days.

Mushroom harvest

The rapid growth of this particular mushroom was facilitated by its preference for high temperatures and moisture. The entire process, from the initial spawning to the first crop harvest, typically takes around 20 to 25 days. The first flush, which lasts for approximately 15 days, accounts for a significant portion, ranging from 70 to 90% of the mushroom yield were harvested and calculated (Fig. 4; Table 1).

Fig. 4. Fruiting bodies of *Volvariella volvacea*

Table 1. Growth, yield and biological efficiency of *Volvariella volvacea* using paddy straw substrate

Strain	Spawn run (days)	Pin head formation (days)	No. of fruit bodies/1000g	Av. fruit body weight (g)	Yield (kg)/10kg substrate	Biological efficiency (%)
V. volvacea	8	15	10.21	30.12	2.05	20.5

Yield and harvesting

Once the plastic is removed, mushrooms will begin to emerge within approximately 4-5 days and can be harvested at either the button or egg stage. Under optimal conditions, yield of as much as 2.05kg of mushrooms per 10kg of substrate has been documented, resulting in a biological efficiency of 20.5% (Table 1). Various factors that may impact yield include the specific strain of mushroom, the type of substrate utilized (cotton waste may yield more than paddy straw) and the incorporation of supplements and boosters. The caged method has also been shown to produce higher yield and biological efficiency in comparison to other techniques such as the bed, spiral, or heap methods.

DISCUSSION

The impact of various strains on the yield and yield-related parameters of paddy straw mushroom (*Volvariella volvacea*) is detailed. Among the different grains utilized in the investigation of various strains of *V. volvacea*, jowar emerged as the most effective, demonstrating statistical equivalence to wheat. This study aligns with the findings of Zakhary *et al.,* 1984; Tripathy *et al.,* 2009) regarding *Volvariella diplasia*. The evaluation of paddy straw mushroom on wheat, jowar, oat and rice revealed that jowar facilitated accelerated growth, achieving full spawn run in 8 days for strain one 11 days and 10 days for strain two, respectively. These results were comparable to those observed for full spawn run in wheat across the evaluated strains. This observation corroborates the results reported by (Kumar *et al.,* 1975). Furthermore, the yield of *V. volvacea* and *V. dysplasia* increased by 5-10% when cultivated on wheat grain spawn compared to other substrates. The highest growth and productivity have been documented from spawn cultivated on wheat grains which was exhibited maximum growth but also enhanced biological efficiency (Purkayastha, 1980).

The formation of pin heads was observed to occur more rapidly in strain one specifically within a period of 15 days, with an average yield of fruiting bodies per 1000 g amounting to 10.81 g and a weight of the fruit bodies measuring 30.20 g. This was significantly higher than the yields observed in the other two strains indicating that strain one is the most productive strain in terms of yield (2.08 kg per 10 kg of substrate) and biological efficiency (20.8%) (Najmu *et al.,* 2022). Previous research has indicated that cultivating mushrooms beds is more effective than using bottles or plastic bags (Luu *et al.,* 2022). The primary methods for cultivating *Volvariella volvacea* include greenhouse cultivation and indoor cultivation following either primary or secondary fermentation treatments, with cultivation duration of 20 to 23 days (Vieira *et al.,* 2022). The microorganisms present in the compost and the surrounding culture environments coexist and interact with the mushrooms and this ecological relationship has been identified as a crucial factor influencing mycelium growth and the development of fruiting bodies (Zhu *et al.,* 2024).

Volvariella spp. thrive in tropical climates characterized by temperatures ranging from 30 to 35°C and relative humidity levels between 75 and 85% (Quimio, 1993). Nonetheless, the conventional cultivation techniques for paddy straw mushrooms differ across various countries, states, and even regions. In the current investigation, significant disparities in the bio-efficiency of *V. volvacea* were recorded when utilizing different types of beds. Additionally, the early emergence of pinheads and buttons was recorded on the 6th and 7th days, respectively. The enhanced weight and increased number of buttons, which contributed to the maximum yield observed in the circular compact bed, may be attributed to the uniformity of the growing conditions present in such beds (Gurudevan *et al.,* 2012). The subsequent most effective treatment was the square compact bed, which produced a yield of 711.2 g/bed, equating to a biological efficiency of 17.5%. The variations in bioefficiency can be linked to specific physical factors including temperature, aeration, moisture levels and the compactness of the beds. The circular compact bed likely maintained a consistent moisture level and temperature throughout its layers, thereby promoting better mycelial proliferation, leading to the production of a greater number of pinheads and buttons, ultimately resulting in increased yield. In contrast, the square compact bed experienced loosening of the straw at the corners which disrupted the mycelium. Similar findings were reported by (Sangeetha, 2002). Conversely, hollow beds were observed to produce higher yields as indicated by (Garcha *et al.,* 1989). However, in our study, the hollow beds failed to maintain uniform temperature and moisture levels between the layers due to unrestricted air movement, which ultimately led to a decrease in yield.

In this study, it was found that significantly greater yields were achieved when the beds were prepared during the months of July to September, during which the temperature ranged from 24 to 36 °C and the relative humidity was between 80% and 90%, resulting in a biological efficiency ranging from 14.9% to 20.80%.

CONCLUSION

In this research, high-throughput sequencing technology was employed to examine the characteristics of changes in substrate and culture environment biological communities following the cultivation of high- and low-yielding *Volvariella volvacea*. The study aimed to compare and analyze the differences in their composition and to investigate the variations in the metabolism and functionality of their microbial communities. The results indicated an increase in the proportion of microbial actinomycetes groups, a rise in microbial diversity, and an intensification of the oxidation-reduction processes in the culture environment over extended cultivation periods. Furthermore, alterations in the microbial community structure of the substrate were observed during cultivation, which may account for the reduced yield of *Volvariella volvacea*. This research has established a foundation for assessing the impact of the growing environment on mushroom yield. The cultivation of *V. volvacea* utilizing these enhanced agricultural methods, in conjunction with advanced storage techniques, can undoubtedly enhance both the yield and quality of mushrooms, thereby creating opportunities for large-scale commercial cultivation. *Volvariella volvacea*, while being a widely sought-after and commercially significant mushroom, yields less in comparison to other species. This paper compiles various strategies aimed at enhancing the yield and biological efficiency of *V. volvacea*, including advancements in spawn development and contemporary cultivation techniques.

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