Original Research Article

Assessing the Impact of Casing Material Thickness on Yield and Biological Efficiency of Milky Mushroom (*Calocybeindica* P&C)

ABSTRACT

Calocybeindica, known as the milky mushroom, has significant potential for agricultural expansion and economic development, particularly in West Bengal, India. This study investigates the impact of different casing material thicknesses (0.5", 0.75", 1.0", and 1.5") on the yield and biological efficiency of C. indica. Using a Completely Randomized Design (CRD), the effects on primordia initiation, sporophore maturation, yield, and biological efficiency were assessed. Results indicated that a casing thickness of 1.0" was optimal, leading to the quickest primordia initiation (10.4 days) and sporophore maturation (9 days), and yielding the highest total yield (717.5 g) and biological efficiency (71.75%). In contrast, thinner and thicker casings resulted in lower yields and efficiencies. The findings suggest that a 1.0" casing thickness significantly enhances C. indica cultivation, providing practical recommendations for commercial mushroom farming. This optimization can support the development of milky mushroom farming in West Bengal, offering new opportunities for local farmers and contributing to regional agricultural diversification and economic growth.

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Keywords: *Calocybeindica,* casing thickness, Yield, Biological efficiency, Commercial cultivation

1. INTRODUCTION

The milky mushroom, scientifically known as *Calocybeindica*, is characterized by its robust and fleshy nature, with a distinctive milky white colour. It is notable for its long shelf life, which extends from 4 to 5 days, making it a viable option for both fresh consumption and extended use in culinary purposes. The discovery of this mushroom dates back to 1974, when Purkayastha and Chandra identified it in the northeastern region of India, specifically in West Bengal [1]. This initial discovery was significant but did not immediately lead to widespread cultivation or commercialization.

The importance of *Calocybeindica* gained renewed attention in 1997 when Krishnamoorthy rediscovered the mushroom in Tamil Nadu, southern India. Despite its initial discovery and subsequent rediscovery, *Calocybeindica* did not enter commercial cultivation until 1998. This year marked a pivotal moment when Krishnamoorthy and colleagues successfully cultivated the mushroom at the Regional Research Station in Aruppukkottai. This effort resulted in the introduction of a new variety, *Calocybeindica* var. APK2, which represented the first commercial cultivation of this species globally [2].

The genus *Calocybe*, which originated in India, currently encompasses nearly 40 species. These species are predominantly cultivated in tropical and subtropical regions that offer the high temperatures and relative humidity essential for their growth and development [3]. The cultivation of *Calocybeindica*

has demonstrated significant potential due to its favourable nutritional profile, including high levels of protein, lipids, fiber, polysaccharides, and essential amino acids, while being low in fatty acids [4].

Calocybeindica primarily thrives in the southern part of the Indian subcontinent. Regions such as Tamil Nadu, Andhra Pradesh, Karnataka, and Kerala provide the optimal climatic conditionscharacterized by high and moderate temperatures throughout the yearthat are necessary for the successful cultivation of this mushroom [3]. The climatic conditions in Orissa are also suitable for the growth of *Calocybeindica* except during the cooler months from November to February, which may impede its cultivation.

However, despite the favourable conditions in the northeastern part of India, including West Bengal, *Calocybeindica* has not been commercially cultivated in this region. West Bengal is predominantly agricultural, with over 90% of its cultivators being small and marginal farmers [5]. This contrasts sharply with the successful commercialization of other mushroom varieties, such as button and oyster mushrooms, which are cultivated throughout the year in West Bengal. The absence of standardized cultivation practices for milky mushrooms in this region represents a significant gap in agricultural development.

The potential for *Calocybeindica* to contribute to local agriculture and improve the livelihoods of small and marginal farmers in West Bengal is substantial. For commercialization of this mushroom production needs to optimize the different cultivation process such as substrate preparation and its sterilization technique, and its growing conditions etc. Among these casing materials are crucial in mushroom cultivation as they influence the moisture retention, temperature regulation, and overall growth conditions for the fungi. The current study aims to exploring the effects of different casing material thicknesses on the yield and biological efficiency of *Calocybeindica* in the lateritic belt of West Bengal. By establishing optimal casing thicknesses, the study aims to provide practical recommendations for commercial cultivation, thereby enhancing the viability of milky mushroom farming in West Bengal.

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2. MATERIALS AND METHODS

2.1 Source of Culture

The pure culture of *Calocybeindica* used in this study was obtained from the Mushroom Research Laboratory, Department of Plant Pathology, Orissa University of Agriculture and Technology, Bhubaneswar, Orissa. This culture was transferred to sterilized Potato Dextrose Agar (PDA) slants and maintained at room temperature throughout the research period.

2.2 Preparation of Mother Spawn

Healthy wheat grains were used as the substrate for the preparation of mother spawn. The grains were boiled for 30 minutes and then shade-dried for 2-3 hours. Subsequently, the grains were mixed with 2% calcium carbonate (CaCO3) and 2% gypsum (CaSO4) on a dry weight basis to achieve a moisture content of 30-35%. The prepared grains were placed in 250 ml conical flasks or alcohol bottles, which were then plugged with non-absorbent cotton and autoclaved at 121°C and 20 lbs pressure for 2 hours. After autoclaving, the flasks or bottles were allowed to cool overnight before being inoculated

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aseptically with the pure culture. The inoculated flasks were then incubated at 27°C ± 2°C for 10-15 days.

2.3 Preparation of Bed Spawn

For the preparation of bed spawn, the procedure was similar to that of the mother spawn. Healthy wheat grains were prepared and mixed with calcium carbonate and gypsum, then filled into 12×18 cm clear transparent polythene bags at a rate of 150 g per bag. The bags were plugged with non-absorbent cotton and secured with rubber bands. The filled bags were autoclaved for 2 hours at 15 psi and 121°C. After autoclaving, the bags were inoculated aseptically with mother spawn and incubated in a dark place at 28-30°C for 15-20 days.

2.4 Preparation of Mushroom Beds

Substrate with 60% moisture content was filled into clear transparent polypropylene bags of size 16"×18" and 150-gauge thickness. The substrate was layered with the bottom and top layers being thinner compared to the intermediate layers. The beds were prepared with 4% spawning, employing a layering method with 4-5 layers of spawn. The mouths of the spawned beds were closed with rubber bands, and 15-20 holes were made on the surface of each bed. The beds were then suspended on nylon ropes in a dark room with an appropriate temperature range of 25-35°C and relative humidity of 80-90% to facilitate spawn run. After complete colonization of the substrate by the mushroom mycelium, casing was applied.

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2.5 Preparation of Casing Material and Casing of Beds

Casing material was prepared by mixing garden soil, farmyard manure (FYM), and sand in a 1:1:1 ratio. The mixture was sterilized in an autoclave at 121°C and 20 psi for 2 hours and allowed to cool to room temperature overnight. Once the milky white mycelium of *C. indica* had fully colonized the substrate, indicating the completion of spawn run, the mouths of the mushroom beds were opened. A casing mixture with varying thicknesses of 0.5", 0.75", 1.0", and 1.5" was spread over the surface of the beds. After casing, the beds were transferred to the cropping room for primordial initiation and sporophore production. To maintain surface wetness, the beds were watered twice or thrice daily. Additionally, the cropping room's relative humidity was regulated by covering the ventilated cemented floor with sand and watering it as needed.

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2.6 Harvesting of Mushrooms

Mushrooms were harvested by hand-picking, involving slight twisting and pulling of the matured fruiting bodies. During the cropping period of 50-60 days, three harvests were conducted at 10-15 days intervals. The weight of each individual fruiting body was recorded immediately using a single-pan weighing balance with 1 g sensitivity. The total yield of mushrooms from each replication was calculated by summing the fresh weights from all harvests, expressed as weight (g) per unit of dry weight substrate. Yield and biological efficiency were calculated using the following formulas:

$$\text{Yield (g)} = \frac{\text{Sum of weight of individual sporophores (g)}}{\text{Number of sporophore}}$$

$$\text{Biological ef iciency (\%)} = \frac{\text{Weight of fresh mushroom (g)}}{\text{Weight of dry substrate (g)}} \times 100$$

2.7 Statistical Analysis

Data collected from the experiments were analysed using a Completely Randomized Design (CRD) methodology. The critical difference (CD) was computed at a 1% significance level to determine the significance of the results.

3. RESULTS AND DISCUSSION

The study assessed the impact of different casing layer thicknesses (0.5", 0.75", 1.0", and 1.5") on several factors, including the initiation of primordia, the maturation period of sporophores, yield, and biological efficiency of *Calocybeindica*. The results of these evaluations are detailed in Tables 1 and 2.

3.1 Time for primordia initiation and sporophore maturation

The results revealed that a casing thickness of 1.0" was optimal for both early primordia initiation (10.4 days) and sporophore maturation (9 days). This was followed by the 0.75" casing thickness, which required 14.20 days for primordia initiation and 12.20 days for sporophore maturation. The 1.5" casing thickness took 16.40 days for primordia initiation and 12.40 days for maturation. The casing thickness of 0.5" resulted in the longest periods, with 17.4 days for primordia initiation and 14.40 days for fruiting body maturation (Fig. 1).

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3.2 Performance on pinheads and sporophore production

The effect of casing thickness on *Calocybeindica* production was also evaluated by measuring the number of pinheads and sporophores. The 1.0" casing thickness yielded the highest number of pinheads (28.60) and sporophores (12.6). In contrast, the 0.5" casing thickness resulted in the lowest counts, with 11.8 pinheads and 7.6 sporophores. The 0.75" and 1.5" casing thicknesses produced intermediate results, with 14.8 and 22.4 pinheads and 9.2 and 11.6 sporophores, respectively (Fig. 2).

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3.3 Performance on yield and biological efficiency

The yield and biological efficiency of *Calocybeindica* varied significantly with different casing layer thicknesses, as detailed in Table 2. The 1.0" casing thickness produced the highest yield (717.5 g) and biological efficiency (71.75%), followed by the 1.5" casing thickness, which resulted in a yield of 674.1 g and a biological efficiency of 67.41%. The 0.75" casing thickness yielded 591.5 g and a biological efficiency of 59.15%. Conversely, the 0.5" casing thickness resulted in the lowest yield (447.9 g) and biological efficiency (44.79%) (Fig. 3). Statistical analysis revealed no significant relationship between stipe length and pileus diameter in relation to yield and biological efficiency (Table 2).

Casing is a critical step in milky mushroom production as it triggers the reproductive phase of the mushroom and facilitates substrate aeration. Among the various casing thicknesses tested, 1.0” proved to be the most effective, promoting early pinhead initiation, a shorter sporophore maturation period, higher yield, and greater biological efficiency under the red and lateritic belt conditions of West Bengal. This thickness also significantly outperformed other casing layers. These findings are consistent with those of Chiwan [6], who identified a casing thickness of 10-25 mm as ideal for prompt primordial initiation and sporophore maturation.

Although the 0.75” casing thickness also led to early pinhead initiation compared to the 1.5” thickness, it resulted in fewer pinheads and sporophores. The superior performance of the 1.0” thickness is attributed to its ability to provide adequate substrate aeration and facilitate the easy upward growth of pinheads. Similar conclusions were reached by Sharma et al. [7], Prasuna [8], and Shukla [9], all of whom observed that a 1.0” casing thickness was optimal for yield and biological efficiency in *Calocybeindica*. However, this result partially contrasts with Krishnamoorthy's [10] findings, which suggested that a casing thickness less than 1.0” was preferable for achieving better yields of *C. indica*.

3.4 Effect of Casing Material

Casing, which involves covering the mycelial substrate with a layer of soil, is essential for supporting the mushroom canopy and ensuring proper fruiting. It signals the end of the vegetative growth phase and the commencement of mushroom reproduction. According to Phutela et al. [11], ideal casing material should be free from pests, diseases, competitor organisms, undecomposed vegetable matter, and should be neutral in pH.

The thickness of the casing material significantly influences primordial initiation, number of primordia, and overall yield. Krishnamoorthy [10] recommended a casing thickness of 1-2 cm for optimal yield. Conversely, Sharma et al. [7] found that a 1.0” casing thickness was superior for the yield of *C. indica*. Prasuna [8] also supported the use of a 1.0” casing thickness for achieving the highest yield, compared to 1.5” and 2.0” thicknesses. Shukla [9] tested various casing thicknesses (0.5, 1.0, 1.5, 2.0, and 2.5 inches) and identified 1.0-1.5” as the most effective for maximum yield.

Pani[12] investigated different casing depths and timings and found that a thickness of 10-25 mm was ideal for primordia initiation and sporophore production. Chiwan[6] also reported that a casing thickness of 15-25 mm was optimal for rapid primordial initiation and sporophore maturation.

4. CONCLUSION

The introduction of *Calocybeindica* cultivation in West Bengal, offers a promising new avenue for agricultural expansion and farmer prosperity. This study revealed that a casing thickness of 1.0” is ideal for cultivating milky mushrooms, significantly enhancing primordia initiation, sporophore maturation, yield, and biological efficiency. By adopting this optimal casing thickness, local farmers can achieve higher yields and improved crop quality, potentially transforming their agricultural practices and boosting

their incomes. These findings not only provide valuable guidance for commercial mushroom growers but also support the broader goal of diversifying agriculture in West Bengal, fostering economic growth, and improving the livelihoods of small and marginal farmers. The successful commercialization of milky mushrooms could thus play a crucial role in advancing rural development and sustainability in the region.

Table 1.Effect of casing material thickness on pinheads and fruiting body formation of *C.indica*

Casing Thickness	Days for pinhead formation	No. of pinheads	Days for fruiting body harvest	No. of fruiting body harvested
0.5”	17.40	11.80	14.40	7.60
0.75”	14.20	14.80	12.20	9.20
1.0”	10.40	28.60	9.00	12.60
1.5”	16.40	22.40	12.40	11.60
SEm (±)	0.48	1.18	0.64	0.48
CD @ 1%	1.44	3.55	1.93	1.44
CV %	7.35	13.64	12.00	10.46

Table 2. Effect of casing material thickness on stipe length, pileus, yield and biological efficiency of *C.indica*

Casing Thickness	Stipe length (cm)	Pileus diameter (cm)	Yield (g)	Biological efficiency (%)
0.5”	10.16	2.66	447.90	44.79
0.75”	9.76	2.90	591.50	59.15
1.0”	9.70	3.48	717.50	71.75
1.5”	8.92	3.22	674.10	67.41
SEm (±)	0.60	0.20	13.03	1.30
CD @ 1%	NS	NS	39.07	3.91
CV %	13.84	14.88	4.79	4.79

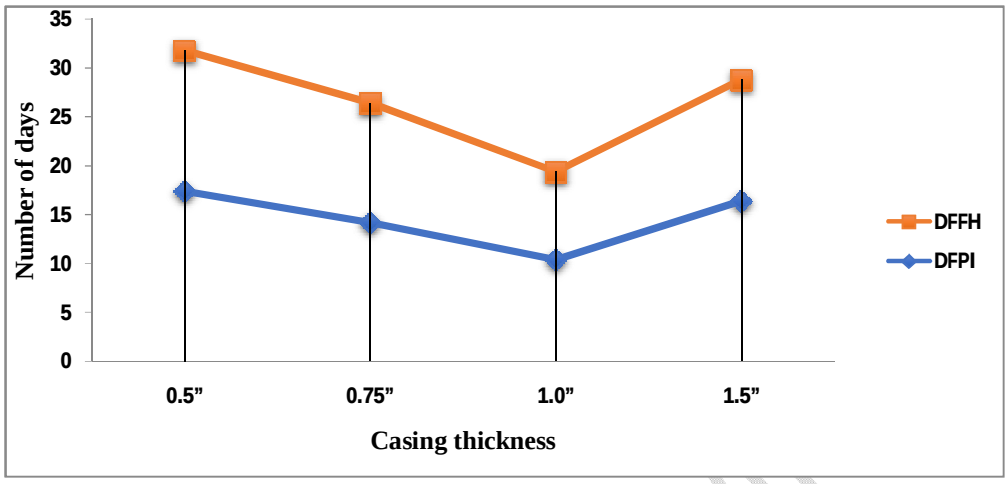

Fig. 1. Effects of various levels of casing thickness on pin head initiation and sporophore maturation of *C.indica*

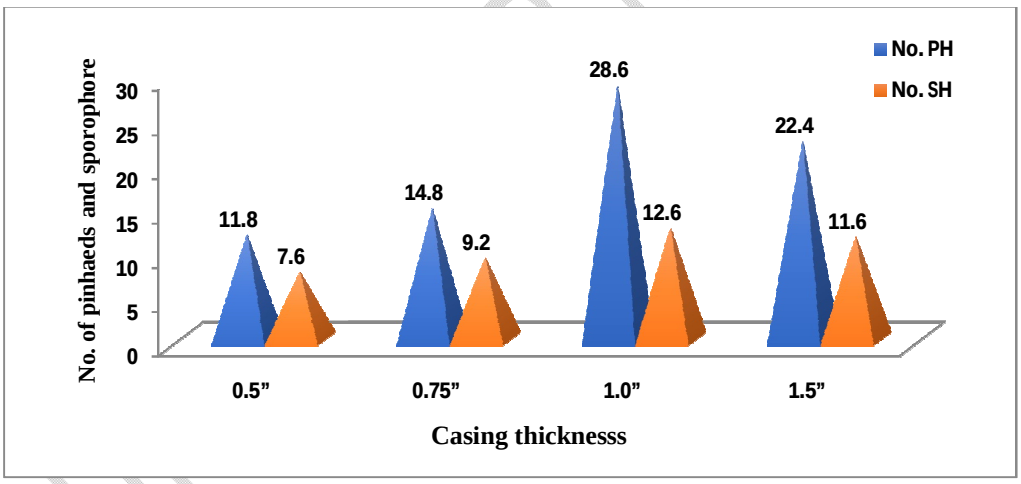

Fig. 2. Effects of different levels of casing thickness on pinhead initiation and sporophore harvest of *C.indica*

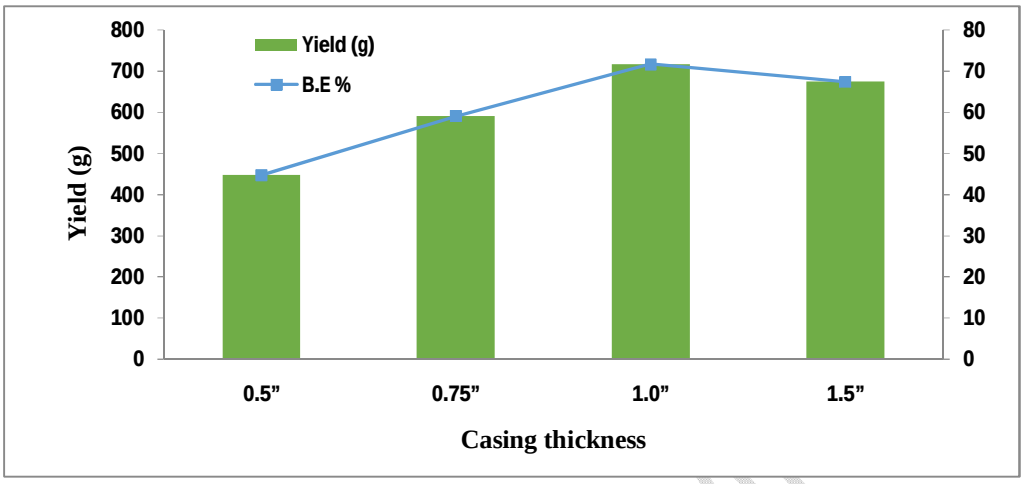

Fig. 3 Effects of various levels of casing material thickness on the yield and biological efficiency of *C.indica*

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