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Effect of Vermicompost–Soil–Cocopeat Casing Mixture on Growth, Morphology, and Biological Efficiency of Milky Mushroom (*Calocybe Indica)*

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ABSTRACT

The study on the effect of casing materials on *Calocybe indica* was carried out from April 30 to August 1 at the Science Business and Innovation Laboratory of HICAST. The experiment was conducted under ambient environmental conditions, where the temperature ranged between 21–27°C and relative humidity remained within 65–80%. A Completely Randomized Design (CRD) was employed with four treatments Soil + Farmyard Manure (1:1), Soil + Spent Mushroom Substrate (1:1), Cocopeat + Vermicompost (2:1), and Vermicompost + Soil + Cocopeat (2:1:1) each replicated four times to evaluate their influence on yield, number of fruiting bodies, and morphological traits of Calocybe indica. The Vermicompost + Soil + Cocopeat mixture significantly outperformed other treatments, producing the highest number of fruiting bodies (8.5 and 3.25 in first and second harvests), greatest total yield (487.5 g), and highest biological efficiency (97.5%). Soil + FYM followed with 435 g yield and 87% efficiency, while Cocopeat + Vermicompost gave moderate performance (383.75 g, 76.75% efficiency). Soil + SMS consistently resulted in the lowest yield (358.75 g, 71.75% efficiency) and poorest morphological characteristics. In terms of morphology, the Vermicompost based casing produced the longest and thickest stipes and one of the widest pileus diameters. Based on these findings, the Vermicompost + Soil + Cocopeat casing mixture is suggested for improved growth and yield of Milky mushrooms under similar environmental conditions.

Keywords: Biological efficiency, *Calocybe indica*, casing materials, vermicompost, morphological traits

INTRODUCTION

Mushrooms are valued worldwide as a nutritious food source and a reservoir of bioactive compounds with medicinal importance. They are increasingly recognized as functional foods owing to their anti-inflammatory, antioxidant, antibacterial, and immunomodulatory properties (Singh *et al*., 2018; Rijal *et al*., 2021). With low fat content, high-quality protein, essential vitamins (B_1, B_2, B_{12}), and minerals such as potassium, calcium, and iron, mushrooms serve as a healthy dietary component that bridges the nutritional gap between vegetables and meat (Mattila *et al*., 2001; Patel *et al*., 2012). Among the edible species, milky mushroom (*Calocybe indica*) has drawn attention for its ability to grow on a variety of agricultural and industrial wastes, making it an ideal candidate for sustainable farming systems (Chang, 2007). *Calocybe indica*, first identified in 1974 in West Bengal by Purkayastha and Chandra, is now considered one of the most promising tropical mushroom species. After its successful domestication and introduction of the APK2 variety in Tamil Nadu (Krishnamoorthy *et al*., 1998), it has become the third most commercially cultivated mushroom in India after button and oyster mushrooms (Kumar *et al*., 2018). Its robust milky white sporophores, long shelf life, and ability to fruit at higher temperatures (30 ± 2 °C) make it particularly suitable for warm regions where low-temperature mushroom cultivation is difficult (Pani, 2010; Kumar *et al*., 2012). Although *C. indica* is well adapted to tropical climates, commercial production in South Asia including Nepal remains limited. The absence of standardized cultivation techniques, especially concerning casing practices, constrains yield and consistency (Panda and Biswas, 2021). Casing, the application of a surface layer over the colonized substrate, is critical for fruit body initiation and development. It regulates the microenvironment by maintaining moisture, aeration, and microbial activity, while providing mechanical support to developing sporophores (Elangovan *et al*., 2024; Yadav, 2006). The physical and chemical properties of the casing texture, porosity, pH, and water-holding capacity greatly influence the shift from vegetative to reproductive growth, thereby determining yield and biological efficiency (Farsi *et al*., 2011; Tewari, 2005). Traditionally, peat moss has been the preferred casing material in temperate regions, but its cost, limited availability, and non-renewable nature restrict its use in South Asia (Sassine *et al*., 2007). Consequently, sustainable and locally available alternatives such as soil, farmyard manure, cocopeat, vermicompost, and spent mushroom substrate (SMS) warrant evaluation for tropical conditions.

Casing plays a vital role in milky mushroom cultivation, ensuring proper moisture retention and gas exchange, while an unsuitable casing can hinder pinhead formation and reduce yield (MacCanna, 1983; Subbiah and Balan, 2015). The thickness and composition of the casing have also been found to influence productivity, with 2–2.5 cm identified as optimal for *C. indica* (Pani, 2012; Subramanian and Shanmugasundaram, 2015). Vermicompost, in particular, is known to enhance microbial activity and moisture retention, supporting better fruiting body formation (Nagaratna and Mallesha, 2007). Despite this, systematic comparisons among various locally available mixtures remain limited in the Nepalese context. Therefore, the present study was undertaken to evaluate the effect of different casing mixtures Soil + FYM (1:1), Soil + SMS (1:1), Cocopeat + Vermicompost (2:1), and Vermicompost + Soil + Cocopeat (2:1:1) on the growth, yield, and biological efficiency of milky mushroom (*Calocybe indica*) cultivated under ambient Nepalese conditions using polyethylene-bag systems. The results are expected to contribute to the standardization of casing practices for sustainable milky mushroom production in tropical environments.

MATERIALS AND METHODS

Experimental site

The experiment was conducted from April 30 to August 1, 2025, at the Science Business and Innovation Laboratory of the Himalayan College of Agricultural Sciences and Technology (HICAST), Kirtipur, Kathmandu, Nepal (27.6°N, 85.3°E). The site represents a subtropical environment with an annual rainfall of approximately 1025 mm and maximum summer temperatures around 31 °C.

Experimental designs and treatments

A Completely Randomized Design (CRD) was employed with four treatments and four replications (16 units total). Casing mixtures were prepared using locally available materials Soil + FYM (1:1), Soil + Spent Mushroom Substrate (1:1), Cocopeat + Vermicompost (2:1), and Vermicompost + Soil + Cocopeat (2:1:1) selected to compare microbial activity and moisture-retention effects on yield and morphology of *Calocybe indica*. Details of the treatments are presented in Table 1.

Substrate preparation

Paddy straw was used as the substrate for milky mushroom production. It was soaked in water for 19 hours, drained, and pasteurized with hot water steam for

2 hours. The substrate was then cooled on a disinfected surface until it reached 25°C, after which 1% $CaCO_3$ and 2% $CaSO_4$ were mixed in. This prepared substrate was used for spawning.

Table 1. Treatments details

T1	Soil + FYM (SFYM) (1:1)
T2	Soil + SMS (SSMS) (1:1)
T3	Cocopeat + Vermicompost (CPVC) (2:1)
T4	Vermicompost + Soil + Cocopeat (VSCP) (2:1:1)

Inoculation and incubation

Fresh, fully colonized spawn of *Calocybe indica* was inoculated at a 5% rate (wet weight basis) into the substrate using 16" × 24" polypropylene bags. Inoculation was done by alternate layering of pasteurized straw and spawn to ensure uniform distribution. The bags were tightly secured, compressed to remove air pockets, and perforated for aeration. All operations were performed under hygienic conditions to prevent contamination. The inoculated bags were incubated in a dark room under ambient conditions (21–25°C temperature and 65–80% relative humidity) until the substrate was completely colonized by white mycelium, indicating healthy growth.

Casing

After full colonization, casing was applied to stimulate fruiting. Casing mixtures were prepared using garden soil, FYM, vermicompost, cocopeat, and spent mushroom substrate in different ratios to form four treatments. The mixtures were sterilized by autoclaving at 121°C and 15 psi for 2 hours. Once cooled, about 1 inch of casing material was spread evenly over the colonized substrate surface. The cased bags were kept in a well-ventilated area with indirect light and maintained moist through regular watering until pinhead formation and fruiting occurred.

Harvesting

Mushrooms were harvested when the caps were fully expanded by gently twisting and pulling them by hand. Harvested mushrooms were cleaned to remove casing material and dirt before recording yield data.

Observation and data collection

Growth and yield parameters were recorded at regular intervals to assess the effects of different casing mixtures on the performance of *Calocybe indica*. Observations were taken from all replications, and mean values were used for analysis.

The following parameters were measured:

1. **Days to pinhead initiation:** Number of days from casing application to the first visible appearance of pinheads.
2. **Days to first and second harvest:** Number of days from casing to the first and second flush harvests, respectively.
3. **Number of fruiting bodies per bag:** Count of mature mushrooms harvested per experimental unit during each flush.
4. **Stipe length (cm) and diameter (cm):** Measured from the base to the pileus and at the midpoint of the stipe using a digital caliper.
5. **Pileus diameter (cm) and thickness (cm):** Recorded at the widest part of the cap and its center using a vernier caliper.
6. **Fresh weight per fruiting body (g):** Measured immediately after harvest using a digital weighing balance.
7. **Total yield per bag (g):** Cumulative fresh weight of all fruiting bodies obtained per experimental unit from both flushes.
8. **Biological efficiency (BE %):** Calculated using the formula:
   (Total fresh weight of mushrooms / Dry weight of substrate) × 100

All observations were made on freshly harvested mushrooms. Morphological measurements were taken from randomly selected representative fruiting bodies in each replication.

Statistical analysis

Data were compiled in MS Excel (v16.0) and analyzed using ANOVA in R Studio (v4.4.1). Mean differences were compared using the Least Significant Difference (LSD) test at a 5% significance level. Figures and tables were prepared in Excel.

RESULTS AND DISCUSSION

The application of different casing materials significantly influenced all major growth, yield, and morphological parameters of *Calocybe indica* in this study. The Vermicompost + Soil + Cocopeat (VSCP; 2:1:1) casing mixture consistently demonstrated superior performance across nearly all metrics evaluated, establishing itself as the most effective treatment. Specifically, VSCP prompted the most rapid initiation of the fruiting cycle, resulted in the highest production of fruiting bodies, and yielded the greatest total biomass with the highest biological efficiency. Furthermore, mushrooms grown under the VSCP treatment exhibited superior morphological characteristics, indicative of higher quality. The following subsections detail the effects on each parameter, with the performance of the VSCP mixture consistently outperforming the other treatments, including the traditional Soil + Farmyard Manure (SFYM) blend.

Effect of different casing materials on days to pinhead formation and harvest.

The application of different casing materials significantly affected the time required for pinhead initiation and harvest of *Calocybe indica*. The VSCP mixture showed the most rapid fruiting response, initiating primordia within 11.5 days and reaching the first and second harvests at 11.7 and 8.5 days, respectively.

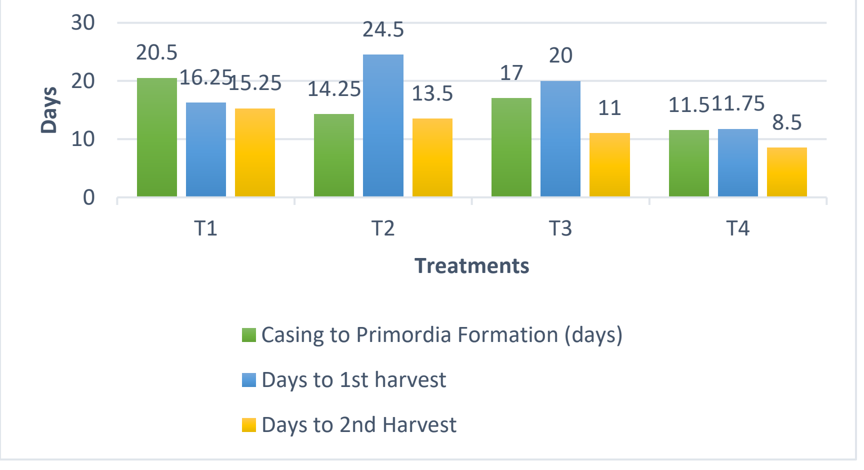

Figure 1. Effect of casing materials on days to primordia formation, days to 1st and 2nd harvest

This accelerated development can be attributed to the optimal physicochemical and biological properties of vermicompost, which harbors beneficial microbes producing growth regulators and volatile compounds that stimulate the transition from vegetative mycelium to reproductive fruiting bodies (Eger, 1961; Nagaratna & Mallesha, 2007). Moreover, the inclusion of cocopeat and soil created a porous, well-aerated structure that maintained ideal surface moisture while facilitating gas exchange, particularly CO_2 dissipation factors critical for early pinhead initiation (MacCanna, 1983; Subramanian & Shanmugasundaram, 2015). In contrast, the delayed pinning (14.25 days) and harvest (24.5 days) observed in the SSMS treatment suggest nutrient depletion and compaction, leading to poor aeration (Choudhary, 2011). The moderate performance of CPVC (17 days to pinhead formation) and the relatively delayed pinning in SFYM (20.5 days) reaffirm that a balanced combination of physical and microbial properties, as seen in VSCP, enhances mycelial fructification and reduces crop duration (Lambert & Humfeld, 1939; Pandey & Tewari, 1994; Bhatt *et al.,* 2007).

Table 2. Effect of casing materials on number of fruiting bodies

Treatments	Number of fruiting bodies	
	1st harvest	2nd harvest
SFYM (1:1)	6.5±0.288^{b}	2.75±0.478^{a}
SSMS (1:1)	5±0.408^{c}	2.50±0.288^{a}
CPVC (2:1)	6.5±0.288^{b}	2.25±0.250^{a}
VSCP (2:1:1)	8.5±0.288^{a}	3.25±0.478^{a}
LSD	0.994	1.197
F-value	***	NS
C.V (%)	9.743	28.922
Grand Mean	6.625	2.687

Signif. Codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.' 0.1 'NS' 1
Non-significant (NS), Significant (*), Highly Significant (**), Very Highly Significant (***) at 95 % level of confidence.

Effect of different casing materials on no. of fruiting bodies

Casing materials markedly influenced the number of fruiting bodies per flush. The VSCP mixture yielded the highest counts (8.5 in the first and 3.25 in the second flush), followed by SFYM and CPVC (6.5 each), while SSMS recorded the lowest (5.0). The superior performance of VSCP reflects its rich microbial population and sustained nutrient availability, both of which stimulate consistent fruiting across flushes (Nagaratna & Mallesha, 2007; Bhatt *et al.,* 2007).

The contrasting performance of CPVC, which produced a strong first flush but declined sharply in the second, highlights the difference between casings that promote initial fruiting and those that sustain productivity. The high water-holding capacity of cocopeat favored initial fruit formation (MacCanna, 1983), but its lower nutrient and microbial content limited subsequent flushes. In contrast, the biologically active and porous VSCP mixture maintained consistent fruiting through improved aeration and gradual nutrient release (Tandon *et al.,* 2006). These results demonstrate that integrating nutrient-rich vermicompost with soil and cocopeat supports both the initiation and continuity of the fruiting process.

Effect of different casing materials on yield of mushroom

The yield of *Calocybe indica* was significantly influenced by the type of casing material used (Table 3). The VSCP treatment produced the highest yield (325.25 g in the first flush and 162.25 g in the second), indicating its superior ability to support both initiation and sustainability of fruiting. This can be attributed to the biologically active and nutrient-rich nature of vermicompost, which enhances microbial activity and provides sustained nutrient release. Beneficial microbes such as *Trichoderma* and *Azotobacter* in vermicompost secrete growth regulators and enzymes that promote mycelial vigor and continuous fruiting (Nagaratna and Mallesha, 2007; Tandon *et al*., 2006). The combination of soil and cocopeat further improves aeration and moisture balance, providing ideal conditions for sustained yield (Pandey and Tewari, 1994).

Table 3. Effect of different casing materials on yield of mushroom

Treatments	Average yield of mushroom (g)	
	1st harvest	2nd harvest
SFYM (1:1)	285.00±11.669^{c}	150.00±10.700^{a}
SSMS (1:1)	255.75±5.677^{d}	103.00±4.546^{b}
CPVC (2:1)	291.00±3.391^{b}	92.75±3.250^{b}
VSCP (2:1:1)	325.25±23.192^{a}	162.25±22.443^{a}
LSD	0.239	39.264
F-value	*	**
C.V (%)	0.053	20.067
Grand Mean	289.25	127

Signif. Codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.' 0.1 'NS' 1
Non-significant (NS), Significant (*), Highly Significant (**), Very Highly Significant (***) at 95 % level of confidence.

The SFYM treatment followed with a slightly lower yield (285.00 g and 150.00 g), suggesting that farmyard manure mixed with soil offers sufficient nutrients and good structure for fruiting but lacks the microbial richness of vermicompost (Krishnamoorthy, 2003). CPVC produced a strong first flush (291.00 g) but a sharp decline in the second (92.75 g), possibly due to compaction and limited microbial support in cocopeat-dominant mixtures (MacCanna, 1983). The SSMS treatment yielded the least (255.75 g and 103.00 g), likely due to nutrient depletion and the presence of inhibitory compounds in spent substrate (Choudhary, 2011). These results highlight that yield performance depends on the synergistic effects of nutrient content, porosity, and microbial activity, with VSCP proving most effective (Bhatt *et al*., 2007).

Effect of different casing materials on morphological traits of *Calocybe indica*

Casing composition markedly affected the morphological characteristics of *C. indica* (Table 4). The VSCP treatment produced mushrooms with the longest (8.06 cm) and thickest stipes (4.29 cm), largest pileus (9.98 cm), and greatest pileus thickness (3.05 cm), indicating robust and compact fruit bodies. This superior morphology can be linked to improved nutrient supply and microbial activity from vermicompost and soil, which promote efficient water uptake and assimilate partitioning, resulting in firm, fleshy structures (Nagaratna and Mallesha, 2007; Tandon *et al*., 2006). Cocopeat's moisture-holding capacity further maintains optimal turgor pressure, supporting healthy tissue formation and dense fruiting bodies. In contrast, the SFYM treatment produced moderately thick and firm mushrooms due to adequate nutrients but less microbial stimulation (Krishnamoorthy, 2003).

Table 4. Effect of different casing materials on morphological traits

Treatments	Avg. Stipe Length	Avg. Stipe Thickness	Avg. Pileus Diameter	Avg. Pileus Thickness
SFYM (1:1)	6.025 ± 0.345^{b}	3.462 ± 0.100^{b}	9.100 ± 0.453^{b}	2.637 ± 0.210^{ab}
SSMS (1:1)	5.300 ± 0.478^{b}	2.737 ± 0.143^{c}	6.562 ± 0.688^{c}	1.125 ± 0.208^{c}
CPVC (2:1)	5.775 ± 0.242^{b}	3.025 ± 0.066^{c}	12.100 ± 0.324^{a}	2.225 ± 0.277^{bc}
VSCP (2:1:1)	8.062 ± 0.408^{a}	4.287 ± 0.154^{a}	9.975 ± 1.007^{b}	3.050 ± 0.219^{a}
LSD	1.167	0.374	2.066	0.711
F-value	**	***	***	*
C.V (%)	12.051	7.190	14.220	18.768
Grand Mean	6.290	3.378	9.434	2.459

Signif. Codes: 0 '***' 0.001 '**' 0.01'*' 0.05 '.' 0.1 'NS' 1

Non-significant (NS), Significant (*), Highly Significant (**), Very Highly Significant (***) at 95 % level of confidence.

The CPVC mixture developed mushrooms with wide caps (12.10 cm) but thinner stipes and pileus an outcome of excess moisture and limited nutrient content favoring cap expansion over compactness (MacCanna, 1983). The SSMS treatment resulted in small, thin fruit bodies, reflecting nutrient exhaustion and poor aeration (Choudhary, 2011). Overall, these morphological responses confirm that the VSCP mixture's aeration, microbial richness, and nutrient availability create optimal conditions for superior fruit quality and marketable traits (Bhatt *et al.*, 2007).

Effect of different casing materials on total yield and biological efficiency

The ultimate measures of cultivation success total yield and biological efficiency (BE) were strongly influenced by the choice of casing material (Table 5). The VSCP (2:1:1) mixture achieved the highest BE (97.5%) and total yield (1950 g), confirming its superior performance in converting substrate nutrients into fruiting biomass. Vermicompost's sustained nutrient release and microbial enzymatic activity enhance substrate degradation and nutrient mineralization, leading to high substrate-to-mushroom conversion efficiency (Tandon *et al.*, 2006). The soil and cocopeat components stabilize structure and maintain optimal aeration–moisture balance, supporting prolonged fruiting cycles (Pandey and Tewari, 1994). SFYM recorded a BE of 87.0%, showing good but slightly lower efficiency due to limited microbial diversity (Krishnamoorthy, 2003). CPVC achieved 76.75% BE, indicating good initial performance but rapid decline from nutrient depletion and compaction (MacCanna, 1983). SSMS showed the lowest BE (71.75%), confirming its unsuitability as a primary casing due to nutrient exhaustion and toxic residue effects (Choudhary, 2011).

Table 5. Effect of different casing materials on total yield and biological efficiency

Treatments	Total yield (g)	Biological Efficiency (%)
SFYM (1:1)	1740	87.00
SSMS (1:1)	1435	71.75
CPVC (2:1)	1535	76.75
VSCP (2:1:1)	1950	97.50

These findings establish VSCP as the most productive and biologically efficient casing, providing a balanced environment for consistent yield and economic sustainability in *C. indica* cultivation (Bhatt *et al*., 2007).

CONCLUSION

This study conclusively demonstrates that the composition of the casing layer is a decisive factor in the successful cultivation of milky mushroom (*Calocybe indica*). Among the tested formulations, the mixture of Vermicompost + Soil + Cocopeat (2:1:1) proved to be superior, consistently enhancing every critical aspect of production. It significantly accelerated the fruiting cycle, maximized the number and yield of fruiting bodies, and improved key morphological traits, resulting in the highest biological efficiency (97.5%). The success of this mixture is attributed to its synergistic combination of biological activation from vermicompost and optimal physical structure from soil and cocopeat, which together create an ideal microenvironment for pinhead initiation and fruit body development. Therefore, the VSCP mixture is highly recommended as an effective, sustainable, and readily available casing material for commercial and small-scale production of *Calocybe indica* under similar ambient conditions. Replacing traditional materials like FYM alone with this specific formulation can lead to more predictable cropping, higher profitability, and better-quality mushrooms. Future research could focus on fine-tuning the ratios of these components and evaluating the specific microbial consortia within the vermicompost that contribute to its efficacy.

AUTHOR'S CONTRIBUTIONS

Zenita Nepal: designed and conducted the experiment, performed mushroom cultivation, monitored growth parameters, recorded and interpreted the data, and drafted the manuscript.
Sundar Timalsina: performed statistical analysis using R Studio, generated graphs and tables, and validated data interpretation.
Alize Kumar Shah: assisted with substrate preparation, casing application, and watering.
Bidur P. Chaulagain: supervised the study, contributed to conceptualization, provided guidance on cultivation techniques, facilitated access to laboratory and cultivation facilities, and critically reviewed the manuscript.

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