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A new casing formulation for enhancing yield of milky mushroom, *Calocybe indica*

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ABSTRACT: The global popularity of milky mushrooms, *Calocybe indica*, is increasing due to their impressive size, appealing milky appearance, delicious taste, attractive colour, excellent shelf life, and unique texture. The casing layer is crucial for inducing fructification in milky mushrooms, providing moisture, physical support, and facilitating gas exchange. The present study evaluated various casing material combinations to find suitable options for commercially cultivation of milky mushroom *C. indica*. Different raw materials, *viz*., raw coir pith, Arka Fermented Cocopeat (AFC), MnSO4, milky mushroom grown substrate, and sandy, loamy soil, were evaluated individually and in different combinations. It was observed that the combination of AFC and sandy, loamy soil in a ratio of 60:40 resulted in a higher milky mushroom yield of 156.90g/kg wet substrate. Similarly, casing sandy loamy soil treated with MnSO4 (150 ppm) resulted in a higher yield of 198.83g/kg wet substrate. Additionally, the combination of casing soil and milky mushroom colonized substrate in a ratio of 90:10 led to a higher mushroom yield of 174g/kg wet substrate. The casing combination of the mushroom colonized substrate (10%), MnSO4 (100 ppm), AFC (600g), and sandy loam soil (400g) exhibited the highest mushroom yield of 241.2g/kg wet substrate.

Keywords: AFC, *Calocybe indica,* casing, milky mushroom, MnSO4, spawn, CAC-ing.

INTRODUCTION

The milky mushroom, *Calocybe indica*, also known as the white summer mushroom or *Dudh Chhatta*, is gaining global popularity due to its impressive size, appealing milky appearance, delicious taste, attractive color, excellent shelf life, and unique texture (Chadha and Sharma, 1995). It is now India's third most commercially grown mushroom, following the white button (*Agaricus bisporus*) and oyster (*Pleurotus* spp.) mushrooms. Initially collected in the wild from West Bengal, India, by Purkayastha and Chandra in 1974, the production technology for *Calocybe indica* was introduced by Purkayastha and Nayak in 1979 and further improved in 1981. The ICAR-IIHR has standardized its cultivation technology. Milky mushrooms are becoming increasingly prevalent worldwide due to their robust size, attractive milky appearance, delicious taste, appealing color, excellent shelf life, unique texture, and sustainable yield (Chang, 2007; Alam et al., 2010). Unlike most edible mushrooms, which require low temperatures (< 25°C) for commercial cultivation, the milky mushroom thrives in warmer climates (30–35°C), making it suitable for hot, humid regions throughout the summer (Pani, 2010; Kumar et al., 2012). This adaptability addresses the challenge of cultivating mushrooms in warm weather without the need for expensive infrastructure (Thakur and Singh, 2014).

In commercial cultivation, casing is a crucial agronomic practice for milky mushrooms. The casing layer, applied after the germination phase, stimulates the transition from vegetative to reproductive growth (Pardo et al., 2004). Effective casing materials must have high water-holding capacity, good air-space ratio, porosity, and bulk density (Yadav, 2006). Researchers have used various materials for casing, including peat moss, loam soil, spent mushroom substrate, coconut coir, biogas slurry, and farmyard manure (Krishnamoorthy et al., 2000). The CAC-ing technique (Compost Added at Casing) involves adding small amounts of fully colonized mushroom mycelium substrate to the casing soil, potentially increasing mushroom yield (Ratnoo and Anila Doshi, 2012). This promising technology, currently used in button mushroom cultivation, could be experimented with for milky mushrooms. The quality of the casing layer is influenced by factors such as texture, compactness, pH, structure, water holding capacity, porosity, and bulk density. Traditionally, growers have used locally available soils, well-decomposed spent compost, and farmyard manure as casing materials. However, exploring new alternative combinations can lead to consistent and improved biological efficiency. The present study focuses on developing new casing material formulations (different casing combinations, CAC-ing experiments, and micronutrients) to achieve consistent higher biological efficiency and improved cultivation methods for *C. indica* in tropical regions.

MATERIALS AND METHODS

Studies were conducted during 2017-21 at ICAR-

Indian Institute of Horticultural Research, Bengaluru. Various raw materials were used during experimentation, including raw coir pith, Arka fermented cocopeats, MnSO4, mushroom-grown substrate, and soil. Standard mycological techniques were followed, such as pure culture production through tissue culture, maintaining pure cultures through sub-culture, and using low-temperature and sterile water storage methods. Cultivation experiments were conducted on sterile paddy straw, raw coir pith, commercial coco peat, and Arka fermented cocopeats (sterilized at 121°C, 15-18 psi pressure, for 25 minutes to 2 hours, depending on the substrate). Spawn was prepared using sterile sorghum grains (sterilized at 121°C, 15-18 psi pressure, for 3 hours). Polypropylene (PP) bags of the required sizes were used as containers for both spawn production and mushroom cultivation. Cultures were inoculated under laminar airflow conditions and incubated at 30±2^{0}C. The cultivation experiments were inoculated with 5% spawn in a spawning room and incubated in a spawn-running room under ambient conditions. After the complete spawn run, the bags were cased according to treatment requirements, and necessary humidity, light and ventilation were maintained using a high-pressure pump fogger.

Casing material preparation: Casing substrate mixing was prepared as required, and mixed cased material with 60-70% moisture was mixed with chalk powder (8%) to bring the pH to 7.5-8. It was then pasteurized at 80^{o}C for 2 hours.

RESULTS

The results indicated that the highest yields were achieved with soil (100%), raw coir pith + soil (60:40), and raw coir pith + soil (20:80), recording 139.67g, 133.97g and 128.40g per kg of wet substrate, respectively (Table 1). Raw coir pith contains lignin, which inhibits mushroom fungus growth, resulting in poor yields. Therefore, the highest yields were obtained from soil alone (Control), raw coir pith + soil (60:40), and raw coir pith + soil (20:80). Similar findings of lower yields with coir pith were reported by Chinara and Mahapatra (2022). Compared to other substrates, the poor growth of *C. indica* mycelium in coir pith likely influenced the mushroom production in the present study. It can be concluded from this study that fresh coir pith delays the growth of *C. indica*. Pani (2012) also observed a gradual reduction in fruiting bodies and productivity with delayed casing soil application.

Table 1. Study of different combinations of raw coconut coir pith along with soil for production of *C. indica* on paddy straw substrate

Treatment	2017-18		2018-19		Average	
	Yield (g)	BE (%)	Yield (g)	BE (%)	Yield (g)	BE (%)
Raw coir pith (100%)	102.7	29.34	83.8	23.94	92.10	26.36
Raw coir pith + Soil (80:20)	103.8	29.66	106.2	30.34	128.40	36.78
Raw coir pith + Soil (60:40)	69.7	19.91	114.2	32.63	133.97	38.39
Raw coir pith + Soil (40:60)	94.7	27.06	131	37.43	86.27	24.67
Raw coir pith + Soil (20:80)	140.7	40.20	130.2	37.20	121.60	34.79
Soil (100%)	161.3	46.09	129.9	37.11	139.67	39.97
CV	4.68		5.34			
CD(0.01)	4.24		3.93			

During 2017-18 and 2018-19, six different combinations of commercial cocopeat pith and soil were evaluated as casing materials for the growth and yield of *C. indica* on a paddy straw substrate. The results showed that casing with soil (100%) and commercial coco pith + soil (60:40) yielded higher results, with 138.10g and 126.05g per kg of wet substrate, respectively (Table 2). Applying casing materials provides physical support to the milky mushrooms in the bags and induces sporophore formation. Additionally, Kerketta et al. (2018) found that various casing materials positively affected the growth and yield of milky mushrooms. Casing soil protects and supports mushrooms against pests and diseases, aids in developing sporophores, and facilitates gaseous exchange necessary for mushroom growth and development (Krishnamoorthy, 2016). Similar results were also reported by Satish et al. (2022) and Amin et al. (2010).

Table 2. Study of different combinations of commercial cocopeat along with soil for production of *C. indica on* paddy straw substrate

Treatment	2017-18		2018-19		Average	
	Yield (g)	BE (%)	Yield (g)	BE (%)	Yield (g)	BE (%)
Commercial coco pith (100%)	139.3	39.46	91.3	26.09	115.30	32.78
Commercial coco pith + Soil (80:20)	79.5	22.52	126.9	36.26	103.20	29.39
Commercial coco pith + Soil (60:40)	116.9	33.12	135.2	38.63	126.05	35.88
Commercial coco pith + Soil (40:60)	78.8	22.32	142.8	40.80	110.80	31.56
Commercial coco pith + Soil (20:80)	94.4	26.74	137	39.14	115.70	32.94
Soil (100%)	146.3	41.44	129.9	37.11	138.10	39.28
CV						
CD(0.01)						

During 2017-18 and 2018-19, six combinations of Arka fermented cocopeat (AFC) and soil were evaluated as casing materials for the growth and yield of *C. indica* on a paddy straw substrate. The results indicated that AFC + soil (60:40) and AFC + soil (80:20) yielded higher mushroom yield, with 156.90g and 146.25g per kg of wet paddy straw substrate, respectively, compared to the other treatments. The lowest yield was recorded with control soil (100%) at 113.45g per kg wet substrate (Figure 1). Pradeep Singh Shekhawat et al. (2023) also reported similar results: commercial cocopeat combination treatments obtained the highest mushroom yields.

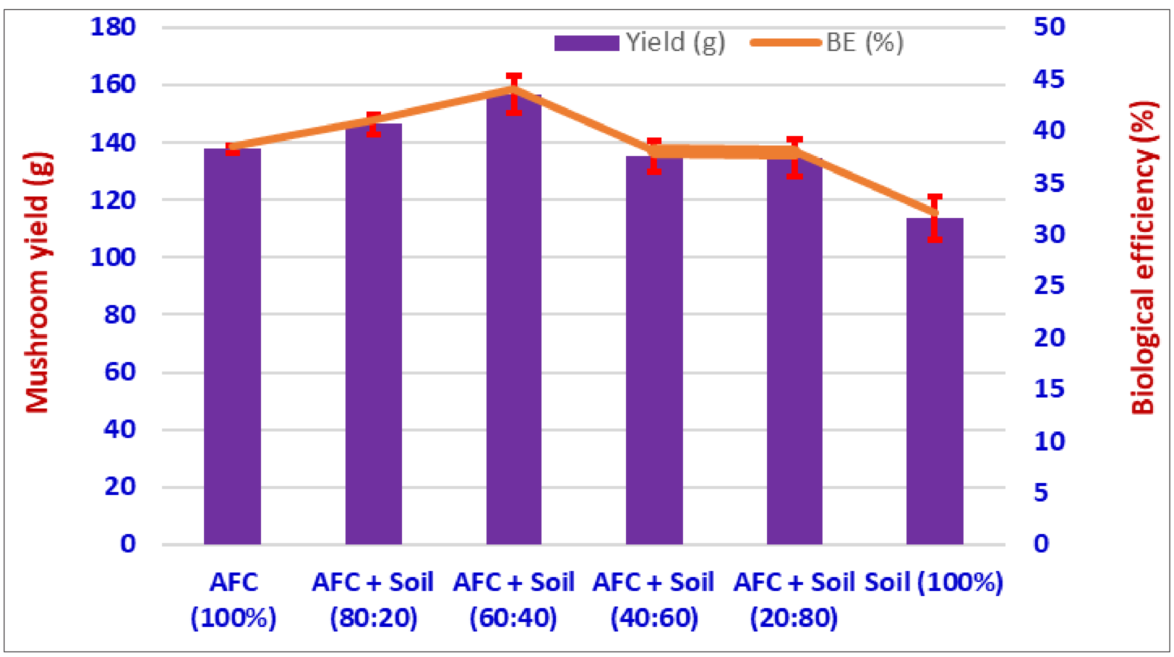

Fig. 1. Effect of different combinations of Arka fermented Cocopeat (AFC) along with soil on production of *C. indica* with paddy straw substrate

During 2019-20 and 2020-21, five different concentrations of MnSO4 and soil were tested as casing materials for the growth and yield of *C. indica* on a paddy straw substrate. The results showed that casing soil treated with 150 ppm MnSO4 recorded the highest yield at 198.83g per kg of wet substrate, followed by casing soil treated with 100 ppm MnSO4, which yielded 193.77g per kg. The lowest yield was recorded in the control group (soil), with 135.55g per kg wet substrate (Table 4). MnSO4 content was analyzed in all harvested mushroom samples, and the results indicated that the MnSO4 treated casings had significantly higher MnSO4 content than the untreated control (Figure 2). Similar findings were reported by David et al. (2006), who observed that adding manganese to casing soil increased button mushroom yield by 9.6% to 11.8% compared to the control. Saheb and Golnoosh (2023) also reported that applying manganese and iron to casing soil increased button mushroom yield by 11.2% compared to the control.

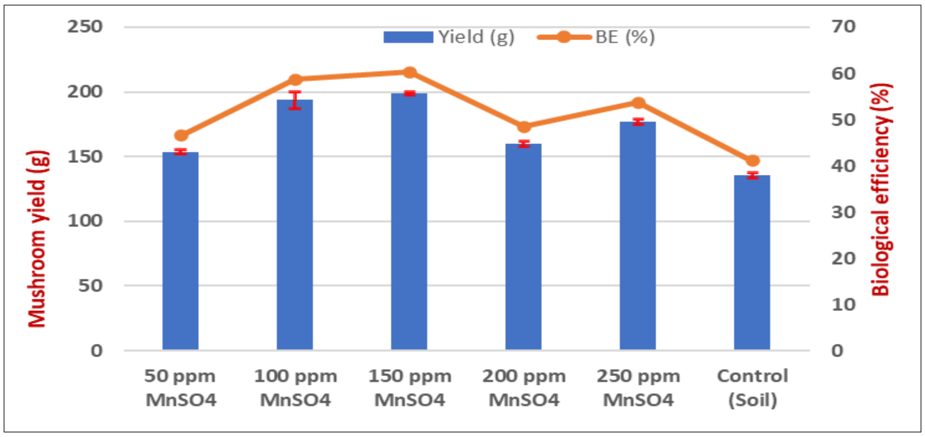

Fig. 2. Evaluation of different concentration of $MnSO_4$ along with soil as a casing for growth and yield of milky mushroom

Five combinations of casing soil and mushroom-colonized substrate were evaluated for their impact on the growth and yield of *C. indica* grown on a paddy straw substrate. The results showed that the combinations of casing soil + mushroom-colonized substrate at ratios of 90:10 and 92:8 recorded higher mushroom yields, producing 174 g and 169 g per kg of wet substrate, respectively. These yields were comparable and outperformed the other treatments and the control. The control group recorded the lowest yield, with 135.85 g per kg wet substrate (Fig 3). The CAC-ing technique was evaluated in this study and resulted in significant yield increases of 36.13% to 43.01% over a 32-day harvest phase. The increased yields from the CAC-ing technique may be attributed to the higher population of Pseudomonas and improved aeration due to the addition of crushed colonized compost in the casing layers (Nair and Hayes, 1975).

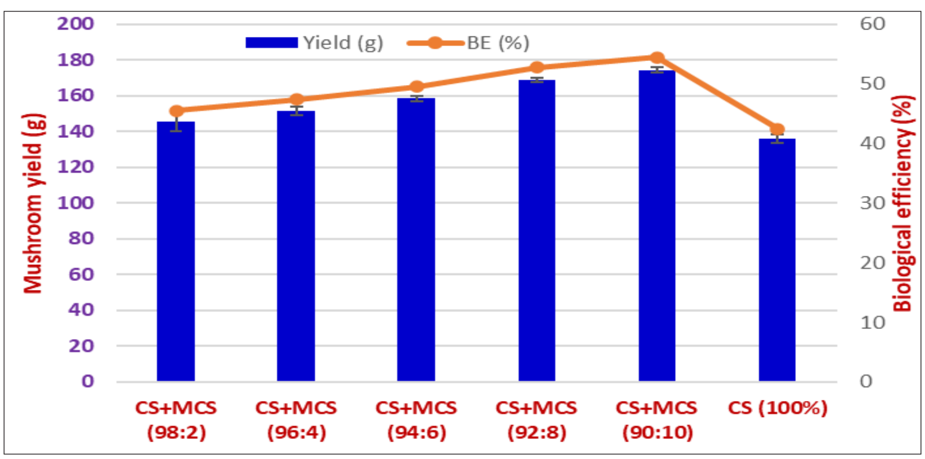

Fig. 3. Evaluation of Mushroom colonized substrate (CAC-ing) for production of milky mushroom *C. indica*

Six combinations of mushroom-colonized substrate, MnSO4, AFC+ soil, and control (soil) were evaluated as casing materials for the growth and yield of milky mushroom *C. indica*. Results showed that the casing combination of mushroom-colonized substrate (10%) + MnSO4 (100 ppm) + AFC (600 g) + soil (400 g) recorded the highest mushroom yield of 241.2 g/kg wet substrate. The combination of the mushroom colonized substrate (8%) + MnSO4 (150 ppm) + AFC (600 g) + soil (400 g), which yielded 208.6 g/kg wet substrate. The control treatment recorded the lowest yield of 174.4 g/kg wet substrate (Table 3). Similar results were obtained in previous studies that examined the effects of these treatments individually. However, this is the first time the combined effect of mushroom-colonized substrate, MnSO4, AFC, and soil as a casing formulation has been evaluated, and the combination significantly increased the milky mushroom yield. The varied production potential of different casing materials might be attributed to their distinct physical properties and nutritional compositions. Additionally, Kerketta et al. (2018) discovered that using various casing materials positively impacted the growth and yield of milky mushrooms. Casing soil protects mushrooms from pests and diseases, supports developing sporophores, and facilitates gaseous exchange, essential for growth and development (AS Krishnamoorthy, 2016).

Table 3. Combination of Arka fermented cocopeat, Cacing and micronutrients along with casing soil on the growth and yield of milky mushroom *C. indica*

Treatments	Yield (g/kg wet substrate)	Biological efficiency
Mushroom colonized substrate (8%) + 100 ppm $MnSO_4$ + AFC + Soil (60:40)	185.6	58.00
Mushroom colonized substrate (10%) + 100 ppm $MnSO_4$ + (AFC + Soil (60:40))	241.2	75.38
Mushroom colonized substrate (8%) + 150 ppm $MnSO_4$ + AFC + Soil (60:40)	208.6	65.17
Mushroom colonized substrate (10%) + 150 ppm $MnSO_4$ + AFC + Soil (60:40)	200.3	62.59
Mushroom colonized substrate (8%) + 100 ppm $MnSO_4$ + AFC + Soil (40:60)	206.3	64.48
Mushroom colonized substrate (10%) + 150 ppm $MnSO_4$ + AFC + Soil (40:60)	199	62.19
Casing soil (100%)	174.4	54.51
CV	5.89	
CD(0.01)	4.62	

*AFC- Arka fermented cocopeat

CONCLUSION

The casing layer plays a crucial role in milky mushroom yield by containing microorganisms that induce environmental changes, aiding the transition from the vegetative to the reproductive stage. Additionally, this layer provides necessary moisture and physical support and facilitates gas exchange during fruiting body formation. Meeting all these parameters with soil or coco peat alone is challenging. Based on the present investigation, the tested casing materials, combined with the mushroom-colonized substrate, MnSO4, AFC, and soil, have potential commercial applications for achieving improved quality and higher yields of milky mushrooms in India.

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