Original Research Article
Unleashing the Potential of Paddy Straw Substrate Preparation Methods for Enhanced Yield and Biological Efficiency in milky mushroom (*Calocybe indica* P&C)

ABSTRACT

Mushrooms, particularly *Calocybe indica*, are highly regarded for their distinctive flavour and numerous health benefits. This study investigates various substrate preparation methods: rolled paddy straw, intact paddy straw, and chopped paddy strawto optimize the cultivation of *C. indica*. Employing a Completely Randomized Design (CRD), we analysed growth parameters, yield, and biological efficiency of mushrooms cultivated on these substrates.The results indicated that chopped paddy straw was the most effective substrate, significantly reducing the time for spawn run to 14.20 days, pinhead formation to 11.80 days, and sporophore maturation to 10.20 days. This method yielded the highest overall production at 664.50 g, along with a biological efficiency of 66.45%. In contrast, intact paddy straw exhibited moderate growth performance, while rolled paddy straw resulted in the lowest yield at 451.30 g and took the longest time for all growth stages, which may be attributed to less compactness in the substrate beds.The number of sporophores produced also varied, with chopped paddy straw yielding an average of 10.6 sporophores, compared to 8.6 from intact paddy straw and 7.2 from rolled paddy straw. Although stipe length and pileus diameter showed non-significant differences among treatments, they did not contribute meaningfully to yield or biological efficiency.Overall, the findings highlight that chopped paddy straw is the most suitable substrate preparation method for cultivating *C. indica*, enhancing both yield and efficiency in production. This research contributes valuable insights for mushroom cultivation practices, particularly in tropical regions where high temperatures prevail.

Keywords: Calocybe indica, substrate preparation, paddy straw, yield, biological efficiency.

1. INTRODUCTION

Mushrooms, are renowned from ancient periodfor their unique aroma and taste but also for their numerous health benefits[1]. They have two distinct morphological phases such as vegetative phase as mycelia and reproductive phaseas fruiting body[2]. They contain bioactive compounds like polyphenols, polysaccharides, and antioxidants, which offer therapeutic potential and can be used in functional foods[3,4,5]. Their medicinal properties include anticarcinogenic, antimicrobial, and anti-inflammatory activities, making them valuable for both nutrition and health applications. Exploring edible mushrooms further can enhance their use in promoting human well-being[6].Mushroom production in India dates back over 1,000 years, but scientific cultivation only began in the early 20th century. The 21st century has seen a significant surge in the cultivation of various edible and medicinal mushrooms. Currently, India commercially grows five main mushroom species: white button

(*Agaricus bisporus*), oyster (*Pleurotus* spp.), paddy straw (*Volvariella volvacea*), milky (*Calocybe indica*), and shiitake (*Lentinula edodes*)[7]. Among these, the commercial cultivation of the milky white mushroom (*C. indica*) remains an area with considerable potential for further exploration.

Milky mushroom (*Calocybe indica*) is gaining popularity globally due to its large size, appealing milky color, delicious flavor, impressive shelf life, and unique texture, along with its sustainable yield [8,9].It is also known by several names, including "summer mushroom" for its ability to grow in high temperatures and "Dutt Chatta" because of its striking pure white colour. The initial documentation of *C. indica* occurred in India by Purkayastha and Chandra in 1974[10]. Different strains of this species show significant diversity in adapting to various temperatures, pH levels, and yield potential based on the substrates used [11,12]. One noteworthy strain, discovered by Krishnamoorthy [13] in a sugarcane field near Coimbatore, was subsequently released as the APK-2 variety by Tamil Nadu Agricultural University and is now grown under controlled conditions [14].*C. indica* is a promising addition to the domestic mushroom repertoire, especially suited for tropical regions, and has gained popularity in Tamil Nadu, Andhra Pradesh, and Karnataka [15].

As a relatively recent addition to edible mushrooms, milky mushroom stands out because most traditional varieties like oyster and button mushrooms thrive in cooler temperatures (below 25°C).In tropical regions, mushroom cultivation can be costly due to the necessary infrastructure [16]. In the absence of low-temperature control facilities during the summer months, it becomes essential to choose mushroom species that can thrive in high temperatures.The identification and cultivation of edible mushroom varieties that thrive in temperatures between 30 and 38°C have posed significant scientific challenges. However, milky mushroom, which prefers higher temperatures of 30 to 35°C, can be successfully cultivated in hot, humid climates throughout the summer [17,11].The milky mushroom is one of the most suitable edible mushrooms for cultivation in such conditions [18].Generally chopped paddy straw method or well chopped any other substrates only used for cultivation of mushroom. However, the appropriate substrates preparation methods for milky mushroom cultivation are crucial. Thus, the present study investigates that different method of preparation of paddy straw substrate suitable for milky mushroom cultivation.

2. MATERIALS AND METHOD

2.1 Source of Culture

A pure culture of milky mushroom (*C. indica* P&C) was obtained from the Tropical Mushroom Research Station at OUAT, Bhubaneswar. This culture was maintained on sterilized potato dextrose agar (PDA) medium and kept at room temperature throughout the investigation. Subsequent culturing occurred every three months. *C. indica* was cultivated using paddy straw, an agro-waste product from paddy crops collected from an agricultural farm at Visva Bharati.

2.2 Isolation and Maintenance of Pure Culture

Pure cultures of C. indica were prepared from fresh fruiting bodies using tissue culture methods [19]. The freshly harvested fruiting body of C. indica was cut in half with a sterile blade under aseptic conditions in a laminar flow chamber. To sterilize the blade and forceps, they were swabbed with cotton soaked in 50% alcohol, then heated over a flame. The surface of the fruiting body was also sterilized using an alcohol-soaked cotton swab. A sterile needle was then used to take fresh tissue from the centre of the pileus, which was immediately transferred to Petri plates and culture slants containing PDA medium. After inoculation, the plates and slants were incubated in a BOD incubator set to a temperature of 27°C ± 2°C to create an optimal growing environment. After 7 to 10 days, the fully grown plates and slants were stored in a refrigerator for preservation and future studies, as well as for further multiplication.

2.3Preparation of Spawn

A mother culture was prepared using healthy wheat grains as the substrate, which were boiled for 30 minutes and shade-dried for 2 to 3 hours. Grains with 50-55% moisture were then mixed with 2% calcium carbonate ($CaCO_3$) and 2% gypsum ($CaSO_4$) on a dry weight basis. The mixture was placed in 250 ml conical flasks, plugged with non-absorbent cotton, and autoclaved at 121°C and 15 psi for 2 hours. After cooling overnight, the flasks or bottles were aseptically inoculated with pure culture and incubated at 27°C ± 2°C for 10 to 15 days. For bed spawn, healthy wheat grains were prepared in the same manner as the mother culture. These grains were filled into clear polythene bags (12×18 cm) at 150 g per bag, with the mouths plugged with non-absorbent cotton and secured with rubber bands. The bags were then autoclaved for 2 hours at 15 psi and 121°C. After autoclaving, they were aseptically inoculated with mother spawn and kept in a dark environment at 28-30°C for 15 to 20 days. Fresh spawns were prepared separately for each experiment.

2.4 Preparation of substrate

Different method of substrate preparation includes Rolled paddy straw, Intact paddy straw without chopping and chopped paddy straw substrate was prepared for milky mushroom cultivation. (i) **Rolled paddy straw**: Chemically treated paddy straw bundles were rolled and kept inside the polypropylene bags in the layer by layer. (ii) **Simply filled paddy straw:** Chemically treated without chopped paddy straws were filled in the polypropylene bags as like as chopped paddy straw. (iii) **Chopped paddy straw**: Mushroom beds prepared by as usual commercial cultivation method like 10-15 cm chopped paddy straw filled in polypropylene bags. Each treatment beds prepared with five replications. The substrate, adjusted to 60% moisture, was filled into clear polypropylene bags measuring 16" × 18" with a thickness of 150 gauges. The substrate was added using a layering method, with the bottom and top layers being thinner than the other layers. The beds were prepared with 4% spawning, involving 4 to 5 layers of spawn. The openings of the spawned beds were sealed with rubber bands, and 15 to 20 holes were made in the bed surface. The beds were then hung on nylon ropes in a dark room, maintaining an appropriate temperature of 25-35°C and relative humidity of 80-90% for the spawn run. Once the mushroom mycelium had fully colonized the substrate, casing was applied.

2.5 Casing of The Bed

During the spawn run period, the casing mixture was prepared using a 1:1:1 ratio of garden soil, farmyard manure (FYM), and sand. This mixture was sterilized in an autoclave at 121°C and 15 psi for 2 hours, then allowed to cool to room temperature overnight. Completion of the spawn run was indicated by the milky white colour of the substrate bed, a result of the white mycelium of *C. indica*. Once the spawn run was fully established, the mouths of the beds were opened, and the casing mixture was spread over the surface to a thickness of 1 inch. The beds were then transferred to the cropping room for further development.

2.6 Estimation of Yield and Biological Efficiency

Mushrooms were hand-harvested by gently twisting and pulling mature fruiting bodies over a 50-60 day cropping period, with three harvests at 10-15days interval. Each fruiting body's weight was measured immediately using a 1 g sensitivity balance. Total yield was calculated by summing the fresh weights from all harvests, expressed as grams per unit of dry weight substrate. Yield and biological efficiency were determined using specific formulas:

$$\text{Yield (g)} = \frac{\text{Total weight of sporophores (g)}}{\text{Total number of sporophore}}$$

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

2.7 Statistical Analysis

The experimental data were analysed employing a Completely Randomized Design (CRD). To evaluate the significance of the results, the critical difference (CD) was determined at a 1% significance level.

3. RESULTS AND DISCUSSION

Various methods of substrate preparation such as rolled paddy straw, intact paddy straw and chopped paddy straw were used to evaluate theperformance on yield parameters of *C.indica*.

3.1 Time for Spawn Run and Pinhead Initiation

It was evident from the table 1 that, chopped paddy straw substrate has taken minimum time for completing the spawn run (14.20 days), pinhead formation (11.80 days), and sporophore maturation (10.20 days) followed by intact paddy straw substrate which showed moderate spawn running period (16.20 days) and pinhead formation (13.20 days). However, rolled paddy straw substrate took maximum

Table 1. Effect of different methods of substrate preparation on spawn run, pinhead formation and first harvest of *C. indica*

Treatments	Days for spawn running	Days for pinhead formation	Days for first harvest
Rolled Paddy straw	17.00	16.00	12.80
Intact paddy straw	16.20	13.20	11.80
Chopped Paddy straw	14.20	11.80	10.20
SEm (±)	0.48	0.40	0.37
CD @ 1%	1.47	1.23	1.15
CV %	6.74	6.54	7.21

time (17 days) for spawn run, pinhead formation (16 days) and sporophore maturation (2.80 days), Fig. 1. In intact and rolled paddy straw substrate the mycelia were not able to colonized properly. Due to poor colonization of mycelia in rolled and intact paddy straw substrate as compared to chopped paddy straw substrate may took long spawn run period, more days for pinhead formation and highest sporophore maturation period.

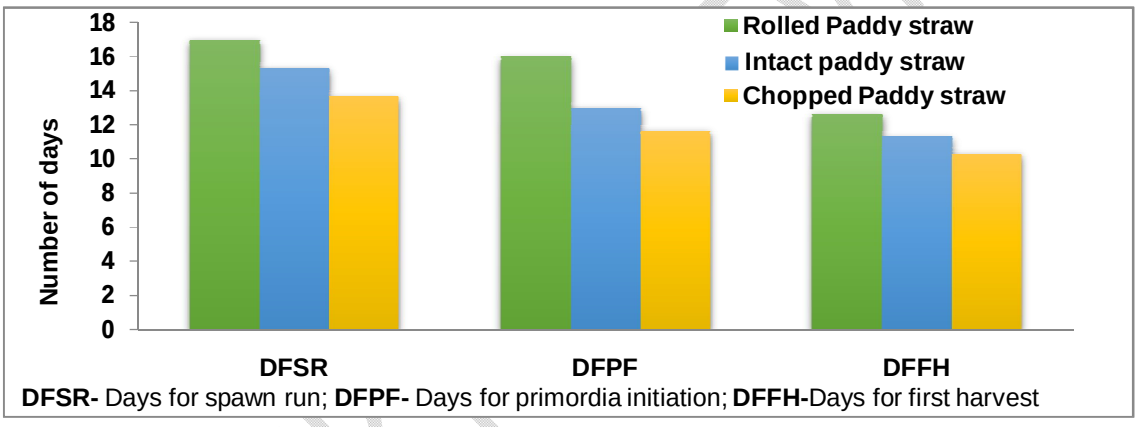


Fig. 1 Effects of various method of substrate preparation on spawn run, pinhead initiation and sporophore maturation of *C.indica*

3.2 Sporophore Production

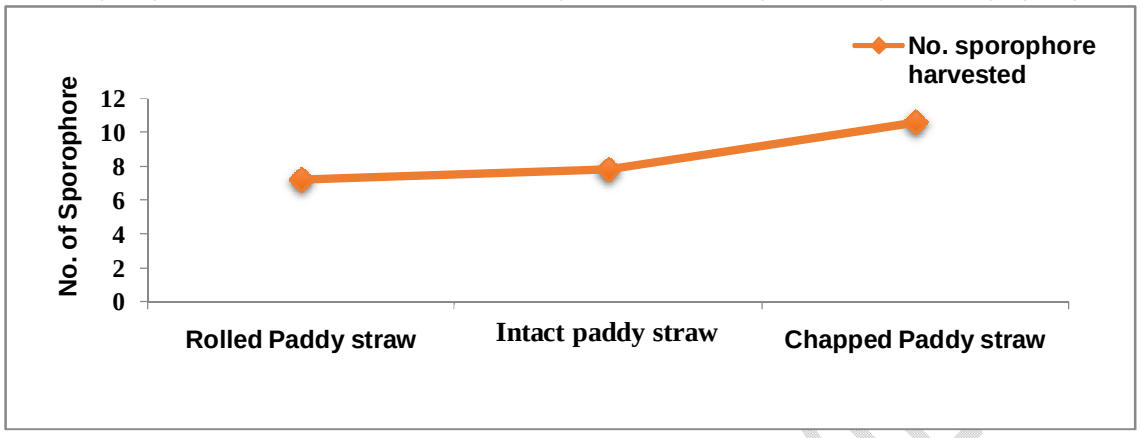
Maximum number of sporophores was recorded in chopped paddy straw bed (10.6) followed by intact paddy straw (8.60) and rolled paddy straw beds (7.20)(Table 2). Minimum number of sporophores was obtained from rolled paddy straw. Chopped paddy straw substrate showed a significant difference from both the methods of substrate preparation and this could clearly be understood from Fig. 2.The results

Table 2. Effect of different methods of substrate preparation on number of sporophore harvest of *C. indica*

Treatments	No. fruiting body harvest
Rolled Paddy straw	7.20
Intact paddy straw	8.60
Chopped Paddy straw	10.60
SEm (±)	0.47
CD @ 1%	1.45

CV %	11.92

of the current investigation indicate that inadequate colonization of mycelium on the substrate adversely impacts the colonization of the casing material, thereby ultimately affecting sporophore



production.

Fig. 2 Effects of various methods of substrate preparation on sporophore production of *C.indica*

3.3 Performance on yield and biological efficiency

The effect of different methods of substrate preparation was evaluated for yield and biological efficiency. Maximum yield (664.5g) and biological efficiency (66.45%) were obtained from chopped paddy straw substrate beds, followed by intact paddy straw substrate beds yield of 531.5gand biological efficiency

Table 3. Effect of different methods of substrate preparation on yield and biological efficiency of *C. indica*

Treatments	Stipe (l) (cm)	Pileus (d) (cm)	Yield (g)	Biological Efficiency (%)
Rolled Paddy straw	10.44	2.66	451.30	45.13
Intact paddy straw	9.02	2.44	531.50	53.15
Chopped Paddy straw	10.84	3.10	664.50	66.45
SEm (±)	0.61	0.17	9.19	0.92
CD @ 1%	NS	NS	48.83	2.83
CV %	13.52	14.19	3.93	3.70

of 53.15% respectively (Table 3). The lowest yield (451.3g) and biological efficiency (45.13%) were recorded from rolled paddy straw substrate and these treatments were differed significantly from chopped paddy straw substrate beds(Fig. 3). Stipe length and pileus diameter were showed non-significant affiliation among the treatments, therefore, the contribution of stipe length and pileus diameter was reported to be very negligible towards yield and biological efficiency of *C.indica*.The findings of this study did not align with those of other researchers, as this specific aspect has not been previously explored. The use of rolled paddy straw resulted in the longest durations for spawn run,

primordia initiation, and sporophore maturation, attributed to the decreased compactness of the substrate beds. Consequently, this led to an extension of the overall cropping period.

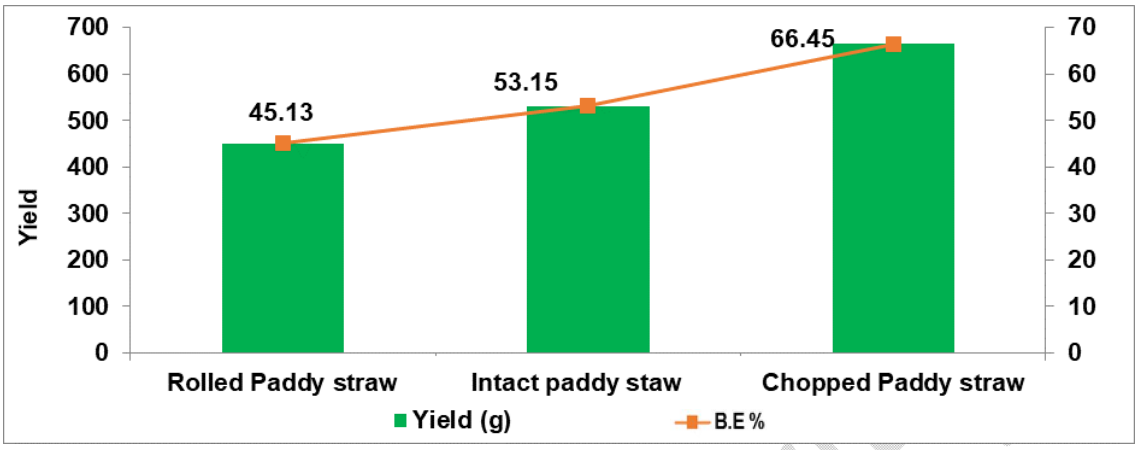


Fig. 3 Effect of different methods of substrate preparation on yield and biological efficiency of *C.indica*

4. CONCLUSION

The current investigation assessed the effects of different substrate preparation methods on the cultivation of milky mushroom (*Calocybe indica*). Three substrate preparation methods were compared: rolled, intact, and chopped paddy straw substrate. The results demonstrated that chopped paddy straw significantly enhanced the growth and yield of *C. indica*, with the shortest spawn run, pinhead formation, and sporophore maturation. In contrast, the rolled paddy straw substrate exhibited the longest time for these phases, indicating inadequate mycelial colonization due to its less compact structure. The chopped paddy straw method produced the highest yield and biological efficiency, whereas rolled paddy straw yielded the least. These findings suggest that the preparation of chopped paddy straw provides a more suitable substrate for the efficient cultivation of *C. indica*, leading to improved productivity to further enhance potential economic benefits for mushroom farmers in tropical climates.

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