### Cultivation of *Calocybe indica* (P & C) in Konkan Region of Maharashtra, India

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**Abstract** Considering suitability of climatic conditions and economic aspects in Konkan region of Maharashtra, cultivation of *Calocybe indica* was undertaken with locally available substrates viz. paddy straw, horse gram waste, wild grass (*Themeda quadrivolvis*), bamboo leaves and different casing materials such as vermicompost, sand + soil (1:1 v/v), dried biogas spent slurry and combination of sand +soil +dried biogas spent slurry (1:1:1 v/v). Among the four substrates evaluated for cultivation of milky mushroom, paddy straw was the best with 81.05 per cent biological efficiency followed by horse gram waste (BE 50 %) and bamboo leaves (BE 40.62 %), but wild grass (*Themeda quadrivolvis*) was the poorest substrate. The biological efficiency of *Calocybe indica* was doubled by using a combination of sand +soil +dried biogas spent slurry (BE 180.32%) or vermicompost (BE 176.28 %) as casing material. Use of dried biogas spent slurry alone also recorded 130 per cent biological efficiency but combination of sand + soil (BE 79.94 %) was inferior. From the present study it was concluded that the maximum biological efficiency of *Calocybe indica* (P & C) in Konkan conditions can be obtained by using paddy straw as a substrate encased with sand +soil +dried biogas spent slurry (1:1:1 v/v) or Vermicompost during summer season.

Keywords: Calocybe indica, Konkan, biological efficiency, substrates, casing

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### **1. Introduction**

Mushroom consumption is getting popular day by day due to their high nutritional and medicinal values. Directorate of Mushroom Research Solan, different AICRP Mushroom centers, State agricultural universities and NGOs in different parts of the country have taken tremendous efforts to disseminate mushroom cultivation technology to the grass root level. As a result, the rural masses in remote areas have realized its importance as a source of lively hood.

Mild tropical humid climate with the temperature in a range of 25-35°C and relative humidity around 70-90 per cent persists throughout the year in Konkan region of Maharashtra. Small and marginal farmers in Konkan are not in a position to take any summer season crop due to unavailability of sufficient irrigation water. Huge quantity of ligno-cellulosic crop residues such as paddy straw, horse gram waste, cowpea waste, weeds etc. is generated through cropping practices throughout the year, which can be efficiently utilized for the cultivation of mushrooms.

First attempt for cultivation of *Calocybe indica* was made by Purkayastha and Nayak in 1981. But till today it is cultivated only in few parts of the country [13]. There is need to introduce the cultivation technology of this mushroom into new agro climatic zones of the country as the biological efficiency of this mushroom is much greater than that of oyster mushroom [17].

Milky white mushroom is basically a summer mushroom as it requires higher temperature  $(30-35^{\circ}C)$  and relative humidity in a range of 85-90 per cent [17]. Therefore, cultivation of *Calocybe indica* (P & C) stands to be the best option during summer season.

Considering suitability of climatic conditions and economic aspects, cultivation of *Calocybe indica* was undertaken to assess suitability of locally available substrates and the effect of different casing materials on biological efficiency this mushroom under Konkan conditions.

### 2. Materials and Methods

The pure culture of summer mushroom *C. indica* was procured from Directorate of Mushroom Research (DMR) Solan, and augmented on PDA by frequent sub culturing. Culture was maintained at  $28 \pm 2^{\circ}$ C in a BOD incubator.

Five locally available substrates viz. paddy straw, horse gram waste, bamboo leaves, wild grass (*Themeda quadrivolvis*) and vermicompost were used to assess the biological efficiency of *C. indica*. Paddy straw and wild grasses (*Themeda quadrivolvis*) were chopped in to 5-7 cm pieces, while horse gram waste and bamboo leaves were used as such. Half kg quantity of each substrate was weighed separately on an electronic balance (10 kg capacity). All the substrates were soaked overnight in 0.1 percent carbendazim solution. Excess water in the

substrates was drained off by placing the substrates on a clean, cemented platform with desired slope. The substrates were pasteurized by dipping in hot water (80-90°C) for 2 hrs. After 2 hrs the excess water was drained off and the substrates were allowed to cool at room temperature. Moisture in substrates was adjusted to 60 per cent by drying them in sunlight. All the substrates were then spawned @ 3 per cent on wet weight basis and filled in polypropylene bags (16 x 21 cm) so as to make cylindrical mushroom beds. Each bag was plugged with cotton plug. Mushroom beds thus prepared were then transferred to iron shelves in spawn run room. Complete darkness and 30±2°C temperature was maintained in this room till completion of mycelial run. Beds with full white mycellial growth were opened and transferred to the cropping room on a cemented floor. Each bed was encased with 2 cm thick casing material i. e. sand + soil (1:1 v/v). Casing layer was kept moist by spraying water., relative humidity 80- 90 per cent and  $30 \pm 2^{\circ}$ C temperature was maintained in the cropping room. Five replications were maintained for each treatment.

In order to study the effect of casing material on the biological efficiency of *C. indica*, paddy straw substrate and four different casing materials such as sand + soil (1: 1 v/v), vermicompost, dried spent biogas slurry, and combination of sand + soil + dried spent biogas slurry (1:1:1 v/v) were used. All the casing materials were subjected to intermittent sterilization for three consecutive days in an autoclave. Five replications were maintained

per each treatment of casing material. Two centimeter thick layer of casing was given at the top the bed. Casing layer was kept moist by spraying fresh water as and when required. Observations on days required for spawn run, case run period, days required for pin head formation, yield data (number and weight of fruit bodies up to 4 weeks) were recorded.

Complete experiment was carried out in completely randomized design (CRD). The data recorded were statistically analyzed and the differences exhibited by the treatments were tested for their significance as per the methods suggested in reference no [5].

### **3. Results**

## **3.1.** Effect of Substrates on Biological Efficiency of *C. indica*

Five locally available substrates such as paddy straw, horse gram waste, bamboo leaves, wild grass and vermicompost were used to ascertain the best suitable substrate for cultivation of *C. indica* in Konkan region. It is revealed from the data presented in Table 1, that the minimum period was required to colonize horse gram waste (14 days) followed by vermicompost (16 days) and paddy straw (17 days) respectively. They were followed by wild grass and bamboo leaves. Maximum period (26 days) was required for complete colonization of bamboo leaves.

Table 1. Effect of Substrates on Biological Efficiency of C. Indica

Tr. No.	Substrates	Spawn run Period (days)	Pinhead Formation (DAC)	No of fruiting bodies	Average weight of fruit body (g)	Yield per Kg dry substrate (g)	BE (%)
T <sub>1</sub>	Paddy straw	17	16	12	67.5	810.5	81.05
<b>T</b> <sub>2</sub>	Horse gram waste	14	14	9	55.56	500.0	50.00
<b>T</b> <sub>3</sub>	Bamboo leaves	26	23	4	101.56	406.25	40.62
$T_4$	Vermicompost	16	18	3	49.75	149.25	14.92
<b>T</b> <sub>5</sub>	Wild grass	19	15	4	40.37	161.5	16.15
	$SEm \pm$					12.46	
CD at 5 %						37.55	
	CD at 1%					51.94	
	CV %					6.15	

Early emergence of pin heads occurred on horse gram waste (14 days) followed by wild grass (15 days). They were followed by paddy straw (16 days) and vermicompost (18 days) respectively. Delayed pin head formation was recorded only in bamboo leaves where pin heads were appeared 23 days after casing. The overall spawn run period ranged between14 –26 days and pinhead initiation period between 14-23 days.

Maximum number of fruiting bodies (12) was recorded on paddy straw. This treatment was followed by horse gram waste (9) bamboo leaves and wild grass (4) and vermicompost (3), respectively.

Maximum biological efficiency of *C. indica* was recorded on paddy straw (81.05%) which was subsequently followed by horse gram waste (50%), bamboo leaves (40.62%), wild grass (16.15%) and vermicompost (14.92%), respectively. The biological efficiency on wild grass was numerically higher than that on vermicompost but both these treatments were statistically at par.

The data in the table indicate that the biological efficiency on bamboo leaves was quite low as compared

to paddy straw and horse gram waste but the average weight of the fruiting body on this substrate was maximum (101.56 g) and it was 34.06 g more than that in paddy straw.

It is clear from the results of this experiment that in respect of time required from spawning to pinhead appearance, biological efficiency and average weight of fruit body, paddy straw was the best substrate for cultivation of *C. indica*.

# **3.2.** Effect of Casing Material on Biological Efficiency of *C. indica*

In this experiment, paddy straw was encased separately with four different casing materials viz, vermicompost, sand + soil, spent biogas slurry, sand +soil+ spent biogas slurry to assess their effect on the biological efficiency of *C. indica*.

It is revealed from data illustrated in Table 2 that among the four treatments maximum biological efficiency was obtained on paddy straw encased with the combination of sand + soil + spent dried biogas slurry (180.32%). This was followed by paddy straw encased with vermicompost (176.8%), spent dried biogas slurry (130%) and sand + soil (79.94%), respectively. Though the biological efficiency was numerically higher on paddy straw encased with combination of sand + soil + spent dried biogas slurry than paddy straw encased with vermicompost, yield on both the treatments was statistically at par. These two treatments were statistically significant with the remaining two treatments.

Table 2. Effect of Casing	Materials on Biologica	al Efficiency of C. Indica
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Tr. No.	Casing materials	Spawn run Period (days)	Pinhead Formation (DAC)	No of fruiting bodies	Average weight of fruit body (g)	Yield per Kg dry substrate (g)	BE (%)	
T <sub>1</sub>	vermicompost	17	12	15	117.52	1762.8	176.8	
<b>T</b> <sub>2</sub>	sand +soil	17	16	12	66.61	799.4	79.94	
<b>T</b> <sub>3</sub>	spent dried biogas slurry	17	26	6	216.67	1300.0	130	
<b>T</b> 4	sand + soil + spent dried biogas slurry	17	18	12	150.26	1803.2	180.32	
	$SEm \pm$					46.97		
	CD at 5 %				140.81			
CD at 1%					194.02			
CV %			7.42					
SEm: Mean Standard Error, CD: Critical Difference, CV: Critical Variance								

Early emergence of pinheads was recorded on paddy straw encased with vermicompost (12 days) followed by sand + soil (16 days) and combination of sand + soil + spent dried biogas slurry (18 days). Delayed pin head formation was observed on paddy straw encased with spent biogas slurry where pinheads appeared 26 days after casing. Maximum numbers of fruiting bodies were recorded on vermicompost (15) each weighing around 110 -120 g. This was followed by combination of sand + soil + biogas spent slurry and sand + soil where 12 fruiting bodies were harvested. Minimum numbers of fruiting

bodies were observed on spent biogas slurry (6). Even though the delayed primordial formation and only 6 fruiting bodies were obtained from casing with spent dried biogas slurry; the biological efficiency was 130 per cent and average weight of an individual fruiting body was 216.67 g.

It is evident from these results that the combination of sand + soil + spent dried biogas slurry as well as vermicompost are ideal casing materials for getting maximum biological efficiency of C. indica on paddy straw substrate.



Figure 1. Effect of substrate on biological efficiency of Calocybe indica, a: Paddy Straw, b: Horse gram waste



Figure 2. Effect of casing materials on biological efficiency of Calocybe indica, a: Vermicompost, b: Sand + soil + spent biogas slurry (1:1:1 v/v)

### 4. Discussion

Locally available substrates such as paddy straw, horse gram waste, bamboo leaves, wild grass and vermicompost were investigated for their suitability. Minimum period for substrate colonization (14 days) was recorded on horse gram waste while it was maximum (26 days) in bamboo leaves. In remaining three substrates the colonization occurred within 20 days. Bamboo leaves contain high amounts of phenolic substances and complex lignin compounds as compared to other substrates [15]. This might be the probable reason for delayed colonization in this substrate. Pin head formation period was also minimum (14 days) in horse gram waste and maximum in bamboo leaves (23 days).

Even though shortest period for colonization and pin head formation was observed on horse gram waste it failed to record higher biological efficiency. The reason for early colonization and pin head formation may be attributed to the higher protein content of horse gram waste but the reason for decreased biological efficiency could not be ascertained.

Comparison of the biological efficiency of *C. indica* on all the substrates revealed that maximum biological efficiency was on paddy straw (81.05%) which was followed by horse gram waste (50%), bamboo leaves (40.62%), wild grass (16.15%) and vermicompost (14.92%).

The reason for low biological efficiency on wild grass and vermicompost may be attributed to unavailability of necessary ligno-cellulosic compounds in required amounts for fruit body formation. Paddy straw was the best suitable substrate in respect of, spawn run period, pinhead formation time, average weight of fruit body and biological efficiency. Proportionate amounts of lignin, cellulose and hemicellulose in paddy straw might have played the important role in performance of the mushroom under study. Requirement of cellulose, hemicellulose, lignin, carbon as well as dry matter for colonization and their positive effect on sporophore production and nutrient content was discussed in reference no [7] on the study on bio-degradation and bioremediation capacity of C. indica. Superiority of paddy straw as compared to many other substrates such as maize stalk, sugarcane bagasse, ground nut haulms, soybean hay, black gram hay, sunflower stalk, cotton waste, sesamum stalk, coir pith and wheat straw for cultivation of C. indica has been reported by many workers [1,9,11,12,16,20,22].

Biological efficiency on bamboo leaves was quite low as compared to paddy straw but average weight of fruiting body was maximum (101.66 g) which was 34.06 g more than that in paddy straw. Reason behind the heavy fruiting bodies in this substrate was not understood. In the reviewed literature, no author has discussed regarding the average weight of fruiting body. Concurrence of the results of present study and literature suggests that paddy straw is the best substrate for cultivation of milky mushroom.

Casing is necessary for certain mushrooms such as *Agaricus, Lentinula, C. indica* etc. as it stimulates and promotes formation of fruiting bodies and provides physical support to the mature fruit bodies [3]. Casing acts as a platform for initiation of uniform fructification and also provides anchorage and essential reserves for developing sporophores of mushrooms [7].

In the present study, the effect of four different casing materials viz, sand + soil (1:1v/v), vermicompost, spent dried biogas slurry and combination of sand + soil + spent dried biogas slurry (1:1:1v/v) on sporophore development and biological efficiency was studied. Among the all treatments maximum biological efficiency was recorded on paddy straw encased with the combination of sand + soil + spent dried biogas slurry (180.32%) which followed by paddy straw encased with vermicompost (176.8%), spent dried biogas slurry (130%) and sand + soil (79.94%).

Various factors like texture, structure, water holding capacity, pore spaces, electrical conductivity, C: N ratio affect the mycellial growth in casing layer and pin head formation [2,20].

Use of municipal waste based vermicompost and its suitability as casing substrate for Agaricus bisporus was suggested by reference [6]. In the experiment conducted in reference no [18] to determine the best casing material for C. indica it was revealed that, the two year old cow dung (BE 100%) was better casing material than biogas spent slurry (BE 98.7%). Macro-elements such as P, K and microelements such as Mn, Mg, Fe and Cu are present in vermicompost and spent dried biogas slurry in available form [4]. Positive role of micronutrients viz., Mn, Mg, Fe and Cu in casing material comprising spent biogas slurry in development of sporophore of A. bisporus was proved in reference [2]. According to reference no [8] higher bio efficiency of C. indica by casing with vermicompost (BE 135.83 %) and biogas spent slurry (BE128.61%) in combination with garden soil. Use of vermi-products (vermicompost and vermi-wash) in cultivation of milky mushroom for improvement of yield and quality was suggested in reference no [22]. These findings are in concurrence with the results of present study where maximum biological efficiency was recorded in the casing material comprising spent biogas slurry in combination with sand + soil and vermicompost alone. These results suggest that the micro elements such as Mn and Cu in spent biogas slurry as well as in vermicompost not only stimulated fruit induction but also played a major role in further development of fruit bodies.

The least biological efficiency was recorded in the beds encased with combination of sand + soil. Physical, chemical and nutritional properties of casing materials affect the sporophore initiation [6,7,10,19]. The lateritic soil with acidic pH was used in combination with sand as casing material, in the present study. The low pH, less water holding capacity and unavailability of the essential nutrients such as Mn, Mg, Cu, and Fe required for fruiting and further development of fruit bodies might have resulted in low yield.

The results of present study are in agreement with those reported earlier and confirm that the biological efficiency of *C. indica* can be increased to 170-180 per cent by using a combination of spent dried biogas slurry + soil + sand or vermicompost as casing material. It is evident from these results that casing material plays a decisive role in increasing biological efficiency when it provides micro nutrients required for fruiting initiation and fruit body development.

### 5. Conclusion

On the basis of the results of present study it can be concluded that, *C. indica* can be successfully cultivated in Konkan region during summer season by using paddy straw supplemented with 10 per cent wheat bran. Casing with a combination of sand + soil + spent dried biogas slurry (1:1:1 v/v) or vermicompost alone results in increased biological efficiency of *C. indica*. In rice based cropping system, there is vast scope for cultivation of *C. indica* and therefore its cultivation technology needs to be popularized in the farming community.

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### Abbreviations

AICRP: All India Coordinated Research Project PDA: Potato Dextrose Agar DMR: Directorate of Mushroom Research

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