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Physical, Chemical and Biological Properties of Casing Soil Used for Milky Mushroom (*Calocybe indica* P&C) Production

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Experiments were conducted to find out the effect of different casing soils *viz.,* peat, clay loam soil, sandy soil, biogas slurry, FYM, cocopeat on the yield and yield attributing parameters of milky mushroom (*Calocybe indica* var.APK2). Among the different casing media, clay loam soil has given the maximum yield (390 g / bed containing 250 g of paddy straw substrate on dry weight basis recording 156% bio-efficiency) and more number of buttons (8.1 /bed) than other casing media. Analysis of physical, chemical and biological properties of clay loam soil revealed that it contained high pH 8.40. Cation Exchange Capacity (CEC) was high in clay loam (28.20 milliequivalents / 100 g of soil). Bulk density and particle density were comparatively higher in sand (1.67 g/cc) and clay loam soils (1.33 g/cc). The maximum pore space was recorded in clay loam soil (55.01 per cent) followed by peat (39.57 per cent) and sand (20.46 per cent). The WHC revelaed high per cent in peat soil (46.27) followed by clay loam soil (39.04). Hence, peat and clay loam soils required less water to maintain surface moisture and a delay in spraying did not lead to the total drying of bed surface. In addition, the population of *Pseudomonas* spp were found to be high in clay loam soil (34.0 cfu/ g of soil).

Key words: Milky mushroom, *Calocybe indica*, Casing soil properties

Calocybe indica P&C, a tropical edible mushroom popular in India loves warm humid climate. It is mainly a grassland species, saprophytic in nature and sometimes ectomycorrhizal with *Cocos nucifera, Borossus flabellifer, Tamarindus indicus* and *Peltaphorum ferruginum.* Casing is an important agronomic practice followed in the cultivation of any humicolous mushroom (Krishnamoorthy and Balan, 2015) and milky mushroom is not an exception. Casing triggers off the change from vegetative to generative phase. Compact casing interfaces impede the diffusion of harmful metabolic gases on mushroom bed surface (Mac Canna, 1983). The casing layer must be sufficiently loose to facilitate primordia emergence (Sassine *et al.,* 2005). Casing soil also protects the compost against desiccation and support the growth of *Agaricus blazei* and provide anchorage for the developing sporophores (Colauto *et al.,* 2011). Purkayastha (1984-1985) used loam soil and sand (1:1), mixed with calcium carbonate at 12 per cent level (pH 7.0) for casing *C.indica* beds. Purkayastha (1984-1985) used loam soil and sand (1:1), mixed with calcium carbonate at 12 per cent level (pH 7.0) for casing *C.indica* beds. However, Krishnamoorthy *et al*., (1998) reported increased yield and bio-efficiency when clay loam soil was used for the cultivation of milky mushroom. In addition to physical and chemical properties, the biological properties of the casing soil are known to influence the induction of fruiting bodies in case of *Agaricus bisporus* (Grewal and Rainey, 1991). The required physical and chemical properties of a good casing should be high porosity and water holding capacity (WHC), 7.2–8.2 pH, low content of soluble inorganic and organic nutrients, and free of disease and pests (Taherzadeh and Jafarpour, 2013). Trehalose was found to have growth-promoting effects on the mycorrhization-helper bacteria (MHB), *Pseudomonas monteilii,* when inoculated with the ECM fungus *Pisolithus albus* (Duponnois and Kisa, 2006). Trehalose released by the mycelium of *Laccaria bicolor* in soil was shown to attract *Pseudomonads* (Frey-Klett *et al.,* 2007). With this background, the current investigation was made to screen the best casing medium for growing milky mushroom and to determine its physical, chemical and biological properties.

Materials and Methods

Mushroom Bed Preparation

Cylindrical polythene bag mushroom beds (60 x 30 cm size and 100 gauge thickness) were prepared by following the methods suggested by Baskaran *et al.,* (1978) and Sivaprakasam, (1980) using paddy straw substrate. A total quantity of 0.5 kg of paddy straw (dry weight basis) was used for making each bed and layer spawning @ 5% to the wet weight of the substrate was followed (Sivaprakasam,1980) . Substrate moisture at the time of bed preparation was 65 %. After preparation, the mushroom beds were incubated at 30 ± 2°C for spawn running. Relative humidity ranged from 65 to 70 per cent in the spawn running rooms. After 15 days, when the beds were

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found fully colonized by the mushroom fungus, each bed was cut into two equal halves (each bed contained 250g of paddy straw substrate (dry weight basisi) colonized by the milky mushroom fungus) and applied with casing soil. After casing the beds were shifted to cropping room where, the temperature was maintained at 33±2°C and RH at 85-90 per cent. The roof of cropping room was lined with transparent HDP blue polythene sheet (120 G thick Silpaulin sheet).

Preparation of casing soils and casing

Peat soil (pH 6.0), clay loam soil (pH 8.4), sand (pH 6.3), biogass slurry (pH 7.3), well decomposed farm yard manure (pH 7.6) and cocopeat (pH 6.8) were used in this experiment. The casing medium was taken in mud pots and the moisture content was adjusted to 20 per cent following the method of Devadoss (1971). The pots filled with soil were steamed in an autoclave at 1.2 kg/cm^2 for 60 min and used for casing after 24 h. The casing soil was uniformly spread over the spawn run beds to a height of 2 cm. Regular spraying with water on the casing surface was done to maintain the required moisture level.

Determination of physical and chemical properties of casing soil

Physical characters of casing soil namely, electrical conductivity, cation exchange capacity (CEC), texture, bulk density, particle density, water holding capacity (WHC), pore space and percolation rate were estimated following the methods of De (1965) and the pH was estimated by digital pH meter. Chemical analysis of each sample was done for essential nutrients namely, nitrogen, phosphorus, potash and organic carbon following standard methods (AOAC., 1970).

Effect of calcium carbonate supplementation to casing soil

Steamed clay loam soil was mixed with calcium carbonate at the rate of 2.5, 5.0 and 10.0 per cent (W/W) level and used for casing. Soil, not mixed with calcium carbonate served as control.

Estimation of microbial population in casing soil

The population of fungi, bacteria and actinomycetes in the casing soil was estimated by serial dilution technique using Rose Bengal agar (Martin, 1950) for fungi; soil extract agar (Allen, 1957) for bacteria; King's B medium (King *et al.,* 1954) for pseudomonads and Kuster's agar (Kuster and Williams, 1964) for actinobacteria. Ten g sample of casing medium was transferred to 250 ml conical flask containing 100 ml of sterile distilled water. The flask was shaken on a rotary shaker for 10 min. Serial dilutions up to 10^6 were prepared with sterile pipette and dilutions of 10^3, 10^5 and 10^6 were used for actinomycetes, fungi and bacterial population count, respectively. One ml of the aliquot was aseptically transferred to sterile Petri dishes and agar medium after thawing and cooling was added. The plates were gently shacked to mix the medium and aliquot. Later,the Petri dishes were incubated at room temperature (30± 20°C). The colonies appearing on the plates were counted and expressed as number of colony farming units (cfu) per g of air-dried substrate or casing soil.

Result and Discussion

Effect of different casing soils on yield

Among the different soils used for casing, clay loam soil (pH 8.4) gave the maximum yield of 388.0 g of mushrooms per bed with more number of buttons (8.0). Peat soil (pH 6.0) was the second best medium for casing (Table 1). In sandy soil, the fungus took more number of days for the production of pinheads (10.2 d) and they attained harvesting maturity only after 10.6 days. Again, the average weight of individual mushroom, pileus weight and stipe weight were much less in mushrooms harvested from sandy soil (Table 1). Casing is generally done to provide anchorage and essential reserves for the developing sporophores over an uniform surface (Shandilya, 2002). Amin *et al.,* (2010) indicated that the cow dung and loamy soil (3 cm thick) was the best casing medium for growing *Calocybe indica*. But, compared to the results obtained in the present study, the bio-efficiency

Table 1. Effect of different casing soils on the yield of *C. indica*

Casing soil	pH	DFPF	DFFH	Yield (g/bed)	Bio-efficiency (%)	No. of buttons harvested	Average weight (g/button)	Size of mushroom			
								Pileus		Stipe	
								Breadth (cm)	Weight (g)	Length (cm)	Weight
Peat soil	6.0	8.0	8.0	341.0	136.4	7.0	49.1	5.5	26.7	7.5	21.8
Clay loam soil	8.4	8.2	8.6	390.0	156.0	8.1	51.1	5.8	26.2	6.9	23.2
Sandy soil	6.3	10.2	10.6	114.0	45.6	3.1	36.5	6.0	16.9	8.0	19.6
Bio-gass slurry	7.3	8.8	9.0	145.0	58.0	2.9	49.7	4.8	25.7	7.6	24.0
Farm yard manure	7.6	10.0	9.2	181.0	72.4	4.4	40.8	5.2	20.6	6.6	20.2
Coco peat	6.8	8.4	8.6	138.4	55.4	3.2	43.8	6.6	23.6	8.0	20.2
CD (P=0.05)		1.20	1.41	34.86		0.88	7.62	NS	8.35	1.08	NS

DFPF - Days for pinhead formation DFFH - Days for first harvest reported was much less. Krishnamoorthy *et al.,* (1998) and Pani (2012) concluded that application of casing soil up to 2 cm depth could be ideal for quick primordial induction, increased number of buttons and maximum mushroom yield in case of *Calocybe indica.*

Physical and chemical properties of casing soils and their influence on yield

The different casing media namely, clay loam soil, peat soil, sand, farm yard manure, biogass slurry and cocopeat were subjected to different physical and chemical analyses and the results are presented in Table 2. Among the casing media, clay loam soil exhibited alkaline pH (8.4) and the pH in peat soil was 6.0. Electrical conductivity was almost equal in both clay loam and peat soils (0.43 and 0.42 mmhos/ cm). Higher levels of nitrogen and phosphrous were noticed in clay loam than in peat. However, Potash content was much high in peat. Sandy soil exhibited less of N and P as compared to clay loam and peat

Table 2. Physical and chemical properties of casing soils

pH	Clay loam soil	Peat soil	Sandy soil	Biogass slurry	FYM	Coco peat
PH	8.40	5.40	6.30	7.30	7.60	6.80
EC (mmhos/cm)	0.43	0.42	0.34	-	-	0.80
N (per cent)	0.69	0.35	0.22	0.43	0.52	0.44
P (per cent)	0.30	0.21	0.25	1.36	1.52	1.52
K (per cent)	1.55	3.15	2.50	2.34	2.53	2.59
C (per cent)	0.37	0.84	0.12	9.80	10.05	14.10
CEC (*)	28.20	12.20	0.80	-	-	-
Ca++	16.00	7.50	0.80	-	-	-
Mg++	5.00	2.50	7.00	-	-	-
Na++	0.87	0.54	T	-	-	-
K+	0.77	0.03	0.06	-	-	-
Clay (per cent)	38.26	-	0.12	-	-	-
Silt (per cent)	25.83	-	0.30	-	-	-
Coarse sand (per cent)	33.87	-	82.17	-	-	-
Fine sand (per cent)	15.60	-	17.31	-	-	-
Bulk density (g/cc)	1.33	0.99	1.67	-	-	-
Particle density (g/cc)	2.37	1.63	2.21	-	-	-
WHC (per cent)	39.04	46.27	19.91	27.0	37.0	70.0
Pore space (per cent)	55.01	39.56	20.46	-	-	-
Water percolation (ml/h)	1240	377	1960	1118	1260	235

(*) ECE expressed as milliequivalents per 100 g of soil

and increased level of K when compared to clay loam soil. Among all the media, cocopeat contained more of organic carbon (14.10 per cent). When the three soils alone were considered, C content was higher in peat soil. Cation Exchange Capacity (CEC) was exceptionally high in clay loam (28.20 milliequivalents / 100 g of soil). All the cations namely, Ca++, Mg++, Na+ and K+ were also found to be high in clay loam soil. When the texture of the casing soils was examined, the percentage of clay

Table 3. Effect of mixing calcium carbonate to casing soil on yield

Level of $CaCo_3$ in casing soil (%)	DFPF	DFFH	Yield (g/ bed)	Bio-efficiency (%)	No. of buttons harvested	Average weight (g/ button)	Size of mushroom			
							Pileus		Stipe	
							Breadth (cm)	Weight (g)	Length (cm)	Weight
2.5	8.4	9.0	334.0	133.6	7.8	42.9	10.3	26.39	13.0	16.0
5.0	7.2	7.8	328.0	131.2	6.4	50.9	7.9	24.5	12.7	26.4
10.0	7.6	8.0	320.0	128.0	7.0	45.8	8.8	24.1	12.2	21.7
Control	8.8	9.0	318.5	127.5	7.3	43.6	9.0	22.9	10.4	20.7
CD (P=0.05)	0.78	0.79	NS		NS	NS	NS	NS	NS	NS

DFPF - Days for pinhead formation DFFH - Days for first harvest and silt were maximum in clay loam soil (38.26 and 25.83). Coarse and fine sand per cent was more in sandy soil. Bulk density and particle density were comparatively higher in sand and clay loam soils. The maximum pore space was recorded in clay loam soil (55.01 per cent) followed by peat (39.57 per cent) and sand (20.46 per cent). Among all the media water holding capacity (WHC) was exceptionally high in cocopeat followed by peat soil. WHC in clay loam soil and farm yard manure was almost equal.

Noble (1999) studied the properties of peat based casing soils and their influence on the water relations and growth of button mushroom.

The vermicompost was also because of fine texture and compact nature; high viscosity and electrical conductivity (EC) not suitable for use as a casing soil for *Agaricus blazei* (Taherzadeh and Jafarpour, 2013) and there is the possibility of mixing it with other substrates having low EC to increase the re-use potential. Besides, alkaline pH, electrical conductivity and cation exchange capacity were also considered important in the sporophore formation of *A.bisporus*. A good casing soil should have low availability of soluble inorganic ions and low electrical conductivity (Singh *et al.,* 1992). In the present study, clay loam soil alone possessed all these desirable characters. Visscher (1980) reported that soils containing higher levels of magnesium delayed sporophore production and reduced the yield upto 50 per cent in *A. bisporus*. But in the present study, clay loam soil contained more of magnesium (Table 2) than any other media tested. Yet, this soil gave the highest yield. Zied *et al .,* (2012) indicated that high amount of organic matter in casing medium could reduce the yield (1.67%) and biological efficiency (4.77%) during the cultivation of *Agaricus subrufescens* due to the continuous proliferation of the vegetative mycelium and a nutrient stress is always needed to induce fruiting. Water percolation was poor in cocopeat and peat soil, while it was much higher in sand. Farm yard manure and clay loam soil were almost on par showing moderate levels of water percolation (Table 2). Lambert and Humfeld (1939) and Flegg (1991) indicated that heavy clay soils yielded better than others. This could be correlated to the slow release of water to the growing sporocarps from such casing soils.

Table 4. Casing soil microflora (cfu / g of soil)

casing soil	before steaming				after steaming				at the time of pinning			
	F	B	A	P	F	B	A	P	F	B	A	P
Peat soil	18.3	41.3	33.7	23.3	2.3	18.7	31.3	16.0	7.3	24.3	33.0	26.3
Clay loam soil	19.0	48.0	38.3	29.3	1.3	25.7	21.0	21.0	3.0	32.7	28.3	34.0
Sandy soil	8.7	21.0	28.7	6.7	1.3	12.7	19.3	1.7	2.0	18.3	19.0	4.3
Biogass slurry	12.4	28.0	17.7	8.3	3.7	19.0	10.3	3.0	8.0	25.7	12.0	8.3
Farm Yard Manure	20.7	64.7	63.7	15.7	5.7	18.0	49.7	6.0	9.7	25.0	56.3	13.0
Cocopeat	15.3	51.7	27.0	4.3	8.7	14.7	17.3	0.6	10.0	18.0	22.3	2.3
CD (P = 0.05)	2.85	6.62	7.56	5.44	2.18	4.43	9.35	3.96	1.54	5.64	10.30	6.80

F - Total fungi (x 10^5 cfu); B - Bacteria (x 10^6 cfu); A - Actinobacteria (x 10^4 cfu); P - Pseudomonads (x10^6 cfu)

Effect of addition of calcium carbonate to casing soil

In a separate experiment clay loam soil (pH 8.4) used for casing was mixed with 2.5, 5.0 and 10.0 per cent level of calcium carbonate, in order to find out its effect on the yield of *C. indica.* The maximum yield of 334.0 g per beds was harvested when the casing soil was mixed with calcium carbonate at 2.5 per cent level. However, this observation was not statistically different from the yield obtained in the control or other treatments (Table 3). When days for pin head formation (DFPF) and days for first harvest (DFFH) were compared, pinheads appeared earlier and also attained the harvesting maturity well in advance in the treatments that involved mixing of calcium carbonate at the rate of 5.0 and 10.0 per cent level. Oei (2003) reported that calcium carbonate ($CaCO_3$) could be used to correct casing soil acidity, as it could elevate the pH and keep low Mg values in casing medium during button mushroom cultivation.

Influence of casing soil microflora

The results obtained in the experiment are given in Table 4. Before steaming, farm yard manure, clay loam soil and peat soil contained large population of total fungi. Bacterial and actinobacterial colonies were significantly higher in farm yard manure followed by clay loam and peat soil. Cocopeat also contained more population of bacteria as comparable to other casing media.. When the pseduomonads alone were counted, it was observed that clay loam soil contained more number of cfu followed by peat soil and farm yard manure (Table 4).

After steaming, the cfu of total fungi in cocopeat was found to be more followed by steamed farm yard manure and biogass slurry. Total bacterial count was maximum in clay loam soil followed by farm yard manure and others. The actinobacterial colonies were found to be higher in farm yard manure followed by peat soil and clay loam soils. However, the cfu of pseudomonads were continued to be higher in clay loam soil even after steaming followed by peat soil (Table 4). Compost or casing soil borne *Pseudomonas putida* is known to have a positive relationship in the primordial induction and fruiting body formation of button mushroom (Choudhary *et al.,* 2009).

When the casing soil samples were analyzed at the time of pinhead formation, it was noted that the cfu of total fungi were significantly more in cocopeat and farm yard manure (Table 4). Clay loam soil and sand contained lesser number of total fungal population. Saprophytic bacterial count was much higher in biogass slurry, farm yard manure and peat soil. However, when pseudomonads alone were counted in King's B medium, it was observed that clay loam soil contained more cfu than others. Counts of actinobacteria continued to be higher in farm yard.

From the results of the study, it is concluded that clay loam soil with pH 8.4; CEC 28.2 milli equivalents / 100 g of soil; bulk density and particle density at 1.33 g/cc; pore space 55.0 % and WHC 39.04% could be the best casing soil medium for growing milky mushroom. Further, clay loam soil required less water to maintain the required moisture level on bed surface. Because of good water retention and slow release, even a delay in spraying over the casing surface did not lead to the total drying of bed moisture. In addition, increased populations of soil pseudomonads (34.0 cfu/ g of soil) recorded in clay loam casing soil favoured the appearance of more number of fruiting bodies of milky mushroom.

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