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Studies on combined effect of casing material and substrate on the productivity of milky mushroom (*Calocybe indica*, CI-3 strain)

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Abstract : Total proteins present in mushrooms is much more than that of the plants. In view of this, Food and Agriculture Organisation (FAO) has recommended mushroom as a food item that can contribute significantly to protein nutrition of people especially in the developing countries like India, where many of the people are vegetarian. The milky mushroom (*Calocybe indica*) has an attractive white sporophore, ability to grow at temperature above 30 ºC and high biological efficiency (80%). Maximum yield of *C. indica* (488.3g/500g of dry substrate) was obtained on paddy straw with farm yard manure as the casing material. Even if one percent of agro-residues produced in Indian peninsula are utilized to produce mushrooms, India will emerge as the major mushroom producing country of the world.

Key words: *Calocybe indica*; milky mushroom; productivity.

1. Introduction

Mushroom are the fleshy macrofungi with large fruiting bodies that belong either to Ascomycotina or Basidiomycotina. Mushroom are appreciated, not only for their flavor and texture but also for their chemical and nutritional characteristics. The protein content of mushroom is less than that in the animals but much more than that in the plants. They have low fat content, high fibre, all essential amino acids and important minerals (Sadler, 2003).

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On exposure to UV-light, mushrooms are known to produce large amount of vitamin D (Koyyalamudi *et al.,* 2009) In addition, they contain vitamin B_1, B_2, C, E, K, nicotinic acid, pantothenic acid and biotin. Apart from being recognized as nutritious foods, certain mushrooms are also important sources of biologically active compounds with potential pharmacological effects, such as, anti-tumor, antioxidant, antiviral, hypocholesterolemic and hypoglycemic effects (Cheung, 2010).

Food and Agriculture Organisation (FAO) has recommended mushroom as a food item that can contribute significantly to protein nutrition of people especially in the developing countries like India. The Indian peninsula with diverse agro-climatic zones, alone generates about 5000 million tonnes of agricultural-residues annually. Even if one percent of it is utilized to produce mushrooms, India will emerge as the major mushroom producing country of the world (Chadha, 1994). Although cultivation of edible mushrooms in India started way back in 1961, it emphasized only on the temperate mushrooms. However, our country can make rapid progress in mushroom industry by cultivating and commercializing some of the tropical and subtropical mushrooms. One such promising mushroom of the tropical region is the milky or summer mushroom (*Calocybe indica* P&C), whose wild forms were reported for the first time by Purkayastha and Chandra (1974) from Calcutta markets, and thereafter, attempts were made to domesticate it. This mushroom has an attractive white sporophore, robust size, excellent shelf life, ability to grow at temperature above 30^{o} C, high biological efficiency (80%) and easiness in post-harvest handling.

The first attempt towards induction of fruiting bodies in milky mushroom was made by Purkayastha and Chandra (1976a). However, till date only limited success has been achieved on its cultivation and productivity in the North Indian States. Therefore, a need was felt to standardize its cultivation methodology on locally available lignocellulosic wastes of Jammu division (Jammu and Kashmir State) and make special efforts to increase its yield potentials. Jammu division has tropical to sub-tropical type of climate with optimum temperature of 25-35^{o}C existing for most period of the year. In addition, a wide range of agro-wastes, garden-wastes and forest wastes are available in this region, many of which have not been utilized so far for the cultivation of *Calocybe indica* (CI-3 strain).

2. Materials and methods

2.1 Procurement of *C. indica*

Pure culture of tropical mushroom *C. indica* (CI-3 strain) was procured from Punjab Agricultural University, Ludhiana, Punjab (India). The strain used for fruiting ability was maintained on potato dextrose agar (PDA) and malt extract agar (MEA) medium at room temperature and subculturing was done at regular interval of three months to sustain their fruiting vigour.

2.1 Spawn preparation

A spawn is a pure fungal culture, which is grown on softened grains in a sterilized condition. In the present investigation, grains of wheat were used for making spawn following the standard procedure described by Munjal (1973). In accordance with this method, healthy wheat grains were taken, washed several times and soaked in water for 6-8 hours. Thereafter, the grains were boiled for 30 minutes, air dried and mixed with 4% calcium sulphate ($CaSO_4$) and 2% calcium carbonate ($CaCO_3$), which help to keep the grains separate from one another and also help in adjusting the pH of the substrate at 7.0. After thorough mixing, the grains were put in milk bottles and polypropylene bags up to 3/4th of their capacity, stoppered with cotton plug and autoclaved at 15lb/sq. inch for 80 minutes. After cooling, the soft and sterilized wheat grains were inoculated with 3- 5 uniform sized mycelium discs of *C. indica* and incubated at 30±2^{o} C. Within 3-4 days, downward linear growth of the mycelium started in each bottle / bag and covered the grains completely in about 15-20 days. In this way, both mother and commercial spawn was prepared by using soft wheat grains.

2.3 Preparation of substrates

Some of the locally available agricultural wastes like straw of paddy (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.), sugarcane bagasse (*Saccharum officinarum* L.), stalk and dehulled cobs of maize (*Zea mays* L.) were used for the present investigation. In addition to agricultural wastes, some garden wastes like fallen leaves of burflower tree (*Anthocephalous cadamba* (Roxb.) Miq.), elephant apple (*Dillenia indea* L.), queen's flower (*Lagerstroemia speciosa* (L.) Pers.), buddha's coconut (*Sterculia alata* Roxb.), mixed garden litter; and some forest wastes like fallen needles of chir pine (*Pinus* *roxburghii* Sarg.), fallen leaves of peepal tree (*Ficus religiosa* L.), banjh oak (*Quercus leucotrichophora* L.) and mixed forest litter were collected and chopped into 2-5 cm pieces. 500g dry weight of each substrate was used for filling each bag. However, before filling the bags, all the substrates were soaked overnight in water and the excess water was drained off by placing them on a clean, cemented platform with desired slope. Later, these substrates were sterilized by dipping in hot water at 95^{o} C for 40 to 60 minutes, the excess water was again drained off and the substrates were allowed to cool at room temperature. Moisture in the substrates was adjusted to 55-60 per cent. All the substrates were spawned, filled in polythene bags (12 × 24) cm so as to make cylindrical mushroom bags. Each bag was sealed with rubber band and two holes were made at the bottom for aeration and drainage of excess water. These spawn filled bags were then transferred to hanging nets kept in the mushroom house. A temperature of 30±2 ^{0}C was maintained in the mushroom house till completion of spawning, that is, mycelial run. Mushroom bags with full white mycelium growth were cut into two equal halves, and each bed of substrate was encased with sterilized casing material, which consisted of garden soil, farm yard manure and their mixture (1:1). Casing layer was kept moist by spraying water, whereas the cropping room was maintained at a relative humidity of 75-90 per cent and a temperature of 30 to 35 ^{o}C. Three replicates were maintained for each treatment.

3. Results and discussion

During the present investigation, casing materials viz., garden soil, farm yard manure and their mixture (1:1), and lignocellulosic substrates (agricultural, garden and forest wastes) were studied for their combined effect on sporophore yield and biological efficiency.

Statistical analysis of the data presented in table 1 shows that among the five different agricultural wastes tested and the three types of casing materials used, maximum yield of *C. indica* (488.3g/500g of dry substrate) was obtained on paddy straw with farm yard manure as the casing material. This was followed in decreasing order by dehulled maize cobs, wheat straw, sugarcane bagasse and maize stalk, which yielded 425.0g, 388.5g, 276.6g and 271.1g of sporophores respectively when encased with farm yard manure. Biological efficiency achieved on these agricultural wastes was in accordance with the yield, being maximum (97.6%) on paddy straw and minimum (54.2%) on maize stalks. Casing is necessary for cultivation of *C. indica* as it stimulates formation of pinheads, provides anchorage and essential reserves for developing sporophores and even provides physical support to the mature sporophores. Similar observations have been noted earlier by Chang and Miles (2004) and Jarial *et al*. (2005) for the cultivation of some other mushrooms also. In addition, various other factors like texture, structure, water holding capacity, electrical conductivity and C:N ratio of casing layer affect the growth of mycelium within it and later formation of pinheads also (Angarish *et al.,* 2003; Singh *et al.,* 2009). A number of other workers have also found farm yard manure to be the best for casing when used along with sand or soil (Tandon *et al.,* 2006; Singh *et al*., 2007). However, Amin *et al*. (2010) recorded cow dung to be more efficient than farm yard manure.

While investigating the effect of different casing materials, farm yard manure (FYM) was also mixed with garden soil (GS) in the ratio of 1:1 and used for casing the bags with agricultural wastes. Perusal of data presented in table 1 shows that significant differences were obtained with respect to yield on bags encased with FYM and that on bags encased with mixture of FYM and GS. The yield was less on GS+FYM and more on farm yard manure (Table 1). Few other workers have also used farm yard manure mixed with other materials like garden soil (Shukla, 2008, Kumari and Kudada, 2018), sand (Bhatt *et al.*, 2007), moss (Sarmah *et al.*, 2006), spent dried biogass slurry (Navathe, *et al.*, 2014) and found their suitability as casing substrate for *C. indica*. It was observed through the present investigation that garden soil proved to be the least efficient casing material when used on different agricultural wastes (table 1).

Table 1: Effect of different combinations of casing materials and agricultural wastes on the productivity (g) and biological efficiency (BE) of *C. indica* (CI-3 strain).

Agricultural wastes	Casing materials: Garden soil (GS)		Farm yard manure (FYM)		Mixture (GS + FYM)		F-value	P-value
	Yield/500g of dry substrate	BE (%)	Yield/500g of dry substrate	BE (%)	Yield/500g of dry substrate	BE (%)		
Triticum aestivum (wheat straw)	271.0^{c} ± 28.5	54.2	388.5^{a} ± 9.87	77.7	365.7^{b} ± 11.9	73.1	8.96	<0.001
Oryza sativa (paddy straw)	349.3^{c} ± 23.8	69.8	488.3^{a} ± 9.45	97.6	412.0^{b} ± 29.8	82.4	7.75	<0.001
Zea mays (maize stalk)	216.0^{c} ± 11.6	43.2	271.1^{a} ± 16.02	54.2	255.7^{b} ± 8.83	51.1	4.91	0.016
Zea mays (dehulled maize cobs)	347.1^{c} ± 15.2	69.4	425.0^{a} ± 42.3	85.0	418.7^{b} ± 48.5	83.7	7.96	<0.001
Saccharum officinarum (sugarcane bagasse)	222.8^{c} ± 10.3	44.5	276.6^{a} ± 21.2	55.3	271.5^{b} ± 4.10	54.3	4.03	0.03

The values given are mean ± standard error. Fisher's LSD was applied when ANOVA detected significant difference (*P<0.001*) between casing materials and productivity. Values within a row followed by the same letter do not differ significantly.

Perusal of data presented in table 2 shows that statistically significant differences in yield were obtained in bags containing garden wastes and encased with GS/ FYM/ GS+FYM. All the garden wastes with a casing of farm yard manure gave better yield than that obtained on garden soil and a mixture of both. The yield in bags with farm yard manure varied from 376.5 to 481.8g, which is quite high and comparable to that obtained on agricultural wastes (271.1 to 488.3g) as depicted in table 1.

Table 2: Effect of different combinations of casing materials and garden wastes on the productivity (g) and biological efficiency of *C. indica* (CI-3 strain).

Garden wastes	Casing materials: Garden soil (GS)		Farm yard manure (FYM)		Mixture (GS+FYM)		F-value	P- value
	Yield/500g of dry substrate	BE (%)	Yield/500g of dry substrate	BE (%)	Yield/500g of dry substrate	BE (%)		
Anthocephalous cadamba (burflower tree leaves)	343.7^{c} ± 16.3	68.7	393.0^{a} ± 33.2	78.7	379.6^{b} ± 28.8	75.9	0.78	0.46
Dillenia indica (elephant apple leaves)	271.7^{c} ± 28.9	54.3	397.0^{a} ± 14.4	79.4	341.8^{b} ± 15.1	68.3	7.62	<0.001
Lagerstroemia speciosa (queen's flower leaves)	328.3^{c} ± 15.2	65.6	481.8^{a} ± 10.2	96.3	381.8^{b} ± 51.1	76.3	3.44	0.048
Sterculia alata (buddha's coconut leaves)	331.8^{c} ± 8.02	66.3	401.7^{a} ± 19.1	80.0	385.1^{b} ± 22.2	77.0	4.14	0.02
Mixed garden litter	254.7^{c} ± 26.1	50.0	376.5^{a} ± 9.63	75.3	357.8^{b} ± 10.4	71.5	11.68	<0.001

The values given are mean ± standard error. Fisher's LSD was applied when ANOVA detected significant difference (P <0.001) between casing materials and productivity. Values within a row followed by the same letter do not differ significantly.

Highest biological efficiency of *C. indica* was obtained on fallen leaves of *Lagerstroemia speciosa* (96.3%), followed in decreasing order by *Sterculia alata* (80.0%), *Dillenia indica* (79.4%), *Anthocephalous cadamba* (78.7%) and mixed garden litter (75.3%). Therefore, in respect of yield and biological efficiency, out of the five garden wastes that were used, *Lagerstroemia speciosa* proved to be the most superior substrate with casing of farm yard manure, whereas mixed garden litter with garden soil casing proved to be the least efficient combination. This shows that supplementing the substrate with casing enhances the yield and biological efficiency by maximizing the utilization of the substrate.

In addition to the agricultural and garden wastes, forest wastes in combination with different casing materials were also evaluated with respect to yield potential and biological efficiency. As depicted in table 3, among the various combinations of forest wastes and casing materials investigated, fallen leaves of *Ficus religiosa* encased with farm yard manure gave significantly highest yield of *C. indica* (425.0g), followed in decreasing order by fallen needles of *Pinus roxburghii* (372.6g), fallen leaves of *Quercus leucotrichophora* (368.7g) and mixed forest litter (335.6g) encased with FYM. As depicted in table 3, biological efficiency achieved by using forest wastes encased with FYM was also in accordance with the yield, being maximum on *Ficus religiosa* (85.0%), followed in descending order by *Pinus roxburghii* (74.5%), *Quercus leucotrichophora* (73.7%) and mixed forest litter (67.1%). The yield and biochemical contents of mushroom are reported earlier also to be affected by the nature of casing materials like bulk density, porosity, water holding capacity and pH value (Choudhary *et al*., 2009).

Table 3: Effect of different combinations of casing materials and forest wastes on the productivity (g) and biological efficiency of *C. indica* (CI-3 strain).

Forest wastes	**Casing materials**						**F- value**	**P- value**
	Garden soil (GS)		**Farm yard manure (FYM)**		**Mixture (GS+FYM)**			
	Yield/500g of dry substrate	**BE (%)**	**Yield/500g of dry substrate**	**BE (%)**	**Yield/500g of dry substrate**	**BE (%)**		
Ficus religiosa **(peepal tree leaves)**	311.1^{c} ± 13.9	62.2	425.0^{a} ± 7.67	85.0	319.6^{b} ± 35.5	63.9	5.18	0.013
Pinus roxburghii **(chir pine needles)**	252.1^{c} ± 27.2	50.0	372.6^{a} ± 11.7	74.5	311.8^{b} ± 13.8	62.3	7.85	<0.001
Quercus leucotrichophora **(banjh oak leaves)**	301.2^{c} ± 12.1	60.0	368.7^{a} ± 13.8	73.7	349.5^{b} ± 15.9	69.9	6.18	<0.001
Mixed forest litter	228.3^{c} ± 24.4	45.6	335.6^{a} ± 10.3	67.1	330.8^{b} ± 8.7	66.1	12.4	<0.001

The values given are mean ± standard error. Fisher's LSD was applied when ANOVA detected significant difference (P<0.001) between casing materials and productivity. Values within a row followed by the same letter do not differ significantly.

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