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YIELD PERFORMANCE OF COLLECTED WILD MILKY MUSHROOM (*CALOCYBE* SP.)

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Abstract

Milky mushroom (*Calocybe indica*) is an indigenous tropical edible mushroom becoming popular among people these days during summer and rainy season. The morphological characters like pileus size, stipe size, gills, spore print colour and colonies characters of a wildly collected *Calocybe* sp. (DMRO-600) were found as 4.50- 6.16 cm, 7.5- 8.5cm, distinctly formed crowded with emarginated, white and circular with creamy white, respectively. Out of five grain substrates evaluated for spawn production, wheat grains proved as best substrate followed by sorghum grains, pearl millet grains and soybean product (Nutrella). For cultivation of this mushroom, five locally available substrates in pure form and in combinations with wheat straw were evaluated. Out of these, wheat straw substrate gave highest yield (1052.50 g), maximum number of fruiting bodies (40.75), early spawn run (21.50 days) along with early first harvest (33.25 days), followed by yield obtained from wheat straw + paddy straw (932.50 g), paddy straw (841.25 g), wheat straw + sugarcane bagasse (840.56 g), sugarcane bagasse (825.0 g), maize straw (703.75 g), wheat straw + maize straw (596.25 g), wheat straw + dehulled maize cobs (543.75 g) and dehulled maize cobs (503.75 g). Hence this study confirms the suitability of locally wild strain DMRO-600 for cultivation to mushroom growers by using wheat grains for spawn production and wheat straw as substrate for its cultivation for achieving higher yield.

Key words : *Calocybe* sp., spawn, substrates, yield.

Introduction

Milky mushroom (*Calocybe indica* P & C) was first reported in India by Purkayastha and Chandra in 1974. It belongs to the kingdom Fungi, phylum Basidiomycota, class Agaricomycetes, order Agaricales and family Lyophyllaceae. This is an indigenous tropical mushroom, suitable for cultivation during summer and rainy season. It naturally grows on the humus rich soil under road side tree, in garden and forest during rainy season. Wildly collected species of *Calocybe* were sold in Kolkata, Tamil Nadu and Rajasthan and their nutritional and medicinal quality were confirmed by Purkayastha and Chandra (1974), Doshi *et al*. (1989) and Krishnamoorthy (1995). In India, mushrooms vernacularly known as "*Khumbi*", "*Chhatra*", "*Kukurmutta*", "*Dhengri*", "*Dharti ka phool*", "*Doodh chatta*" etc.

A substrate is an important substance for making spawn and growing mushrooms. Various kinds of agricultural wastes including wheat straw, paddy straw, maize, bajra, cotton stalks and leaves, sugarcane bagasse, dehulled maize cobs, tea and coffee waste and coconut coir substrate can be utilized for cultivation of *Calocybe indica*. There is no need to compost the substrate for its cultivation as the mycelium can degrade the cellulose, hemicelluloses and lignin by secretion of various extracellular enzymes. This mushroom requires a temperature of 30-35ºC and relative humidity of 70-80% for cultivation which is conducive to environmental conditions of most part of India (Singh *et al*., 2009; Amin *et al*., 2010; Upadhyay, 2010 and Gitte *et al*., 2014). During last decade milky mushroom has become a major commercially cultivated species in south India particularly Tamil Nadu, Andhra Pradesh and Karnataka and during last 4-5 year its cultivation has become popular in north India as well. Its high biological efficiency (100%), better keeping quality, white attractive colour, suitable for pickle and chutney, can be grow on wide range of agricultural wastes and simple cultivation technique are major factors

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for its popularity among mushroom growers and consumers. Therefore, keeping in view the importance of milky mushroom in India, detailed studies of a collected wild *Calocybe* sp. (DMRO-600) were carried out on different aspects of cultivation.

Materials and Methods

Collection of culture

The fruiting bodies of this specimen were collected under the base of Peepal tree (*Ficus religiosa*), near the village of Mukund Pur, Distt. Sultanpur of Uttar Pradesh at longitude E.081^{0}50.310", latitude N. 26^{0}29.732" and Elev. 329 ft, when a survey was made for the collection and conservation of wild edible mushroom during rainy season of 2013-14. The collected specimen was brought to the laboratory and isolated. The culture of the specimen, passport data and their photograph of the naturally growing fruiting bodies was submitted to the Directorate of Mushroom Research, Chambaghat, Solan (H.P.), India. They identified, it as *Calocybe* sp. and given its accession number as DMRO-600.

Media preparation

Potato Dextrose Agar (PDA) medium was used for isolation, purification and maintenance of the mushroom culture.

Isolation and purification of the culture

Freshly collected young fruiting bodies of the specimen brought to the laboratory for tissue culture, under aseptic condition. Young basidiocarp was cleaned with sterilized distilled water and dipped into 0.1% mercuric chloride solution for 1 min. The basidiocarp was air dried and split open longitudinally from centre and vegetative tissues were cut from the collar region (junction of pileus and stipe). These bits (2-3 mm) were then washed in sterilized water to remove mercuric chloride ($HgCl_2$) and placed in the Petri-plates having PDA media. Inoculated plates were incubated at 32^{0}C±1 in BOD incubator.

Purification of the culture was done within 5-6 days of isolation when the new mycelium growing over the media was observed. A bit of mycelium from advancing zone was taken with the help of sterilized inoculation needle and it carefully transferred on to the potato dextrose agar medium slant. The observations of colony characters in pure culture were noted.

Evaluation of different substrates for spawn preparation of *Calocybe* sp.

Spawn is the seed or vegetative mycelium of mushroom required for mushroom cultivation. Various kinds of grains were successfully used by different workers to prepare the spawn (Amle *et al*., 2007 and Senthilnambi *et al*., 2011). In this study, grains of wheat, maize, jowar, bajra and soybean product (Nutrella) were used due to their easy availability and low cost. All the selected grains were thoroughly washed in sufficient water two to three times to remove soil debris, straw and other undesired materials and boiled in double volume of water for 25-30 minutes. After boiling, the grains were placed on sieve to drain out the excess water and spread on clean floor to dry the surface area to minimize stickiness for a few hours. Now the grains were mixed with 20 g of calcium sulphate (gypsum) and 5gm of calcium carbonate @ per kg dry weight basis of grains. Mixed grains were filled in 20 cm long test tubes up to 2/3 volume and plugged with non-absorbent cotton. Mouth of the test tubes was wrapped with aluminum foil with the help of a rubber band. After that test tubes were sterilized in autoclave at 15 lb psi or 121^{0}C for 1.5 hours. The test tubes were then shifted to a room for cooling aseptically. A bit of mycelium of *Calocybe* sp. was aseptically transferred to these test tubes and incubated at temperature of 32±1^{0}C in BOD incubator. Linear mycelial growth was measured on each substrate after 12 days of incubation.

Evaluation of different substrates for yield of *Calocybe* sp.

Preparation of substrates

In order to find out best substrate for the cultivation of this mushroom, five locally available substrates, namely, paddy straw, wheat straw, maize straw, sugarcane bagasse and dehulled maize cobs in pure form and also in combination (1:1) with wheat straw were evaluated in RBD with four replications. Quantity of dry substrates per bag was kept 1.5 kg.

The substrates were chopped in to 3-5 cm pieces. All the substrates were soaked in water containing carbendazim (75ppm) and formalin (500ppm) for 14-18 hours. After wetting each of the substrate was taken out from the solution and excess water was drained out. To restrict the chance of contamination all the tested substrates were again sterilized in autoclave at 22 lb psi for 2 hours.

Spawning

A moisture content of about 65% was maintained prior to spawning. Spawning was done by layer method @ 4% of the wet weight basis of the prepared substrate in polypropylene bags of 60×40 cm size with 100 gauge thickness. After spawning bags were shifted to spawn room and kept in dark place where temperature between

Table 1 : Effect of different substrates on mycelia growth of *Calocybe* sp.

S. no.	Substrates	Linear growth of mycelium (cm)
1.	Wheat grain	11.875
2.	Bajra grain	10.750
3.	Jowar grain	10.875
4.	Maize grain	9.500
5.	Soybean cake (Nutrella)	8.750
C.D at 5%		**0.5**

Table 2 : Effect of different substrates on yield of *Calocybe* sp.

S. no.	Substrates	Average days for spawn run	Average days for first harvest	Average no. of fruiting bodies/bag	Average yield (g/bag)	Biological efficiency (%)	Pileus size (cm)	Stipe size (cm)
1.	Wheat straw	21.50	33.25	40.75	1052.50	70.50	6.16	8.5
2.	Paddy straw	26.25	39.50	37.00	841.25	57.50	5.15	8.0
3.	Sugarcane bagasse	23.50	36.25	34.50	825.00	55.75	5.04	7.75
4.	Maize Straw	25.40	41.25	32.25	703.75	47.25	5.30	8.0
5.	Dehulled maize cobs	27.50	36.50	25.00	503.75	32.50	5.40	7.0
6.	Wheat straw + paddy straw (1:1)	22.50	33.50	34.50	932.50	64.00	4.75	7.50
7.	Wheat straw + sugarcane bagasse (1:1)	23.25	39.25	39.25	840.56	57.00	5.38	8.35
8.	Wheat straw + maize straw (1:1)	25.35	36.25	37.50	596.25	38.75	4.50	7.5
9.	Wheat straw + Dehulled maize cobs (1:1)	24.50	35.50	39.25	543.75	40.50	5.13	8.25
S.Em. ±		0.63	0.57	0.56	11.45	0.56	0.19	0.16
CD at 5%		1.84	1.67	1.65	33.40	1.63	0.56	0.47

25-30°C and relative humidity 80% were maintained till mycelium colonized the substrates and then the substrates were ready for casing.

Casing

Casing provides physical support, moisture and allows gases to escape from the substrates. Casing material was prepared by using Garden soil (50%), sand (25%) and FYM (25%). Casing material was chemically treated with formaldehyde solution (2%) about a week in advance of casing. Casing material was spread about 3-4 cm thickness on roughled uniform top surface of the bags and slightly pressed. Temperature 25-30^{0}C and RH 80-85% were maintained till case run.

Cropping

One week after casing when mycelium emerged on casing soil, the environmental conditions were changed in cropping room by providing fresh air through ventilation and light 120-150 lux for 6-8 hours and temperature 30-35^{0}C and RH 80-85% were maintained. Watering was done twice in a day by using the knapsack sprayer/ atomizer.

Protection during cultivation

During bag filling and spawning, floor areas was sprayed with formaldehyde (1%). Spawn running room was sprayed with formaldehyde (0.5%) and malathian (0.1%) once in a week. During casing and cropping a mixture of carbendazim 1 g + formaldehyde 5 ml. in a litre of water was sprayed before casing and spray was repeated again after a week. Malathian 0.1% was sprayed further on next day after casing to avoid the flies.

Harvesting

Mushrooms were harvested by holding the cap and twisting the fruiting bodies.

The observations with respect to days required for spawn run, first harvest, number of fruiting bodies and yield were recorded and results summarized in table 2.

Results and Discussion

In study, regarding cultural and the morphological characters of *Calocybe* sp., the size of the pileus of the fruiting body was between 4.50 and 6.16 cm in diameter,

Fig. 1 : Naturally grown fruiting.

Fig. 3 : L.S. & view of fruiting body.

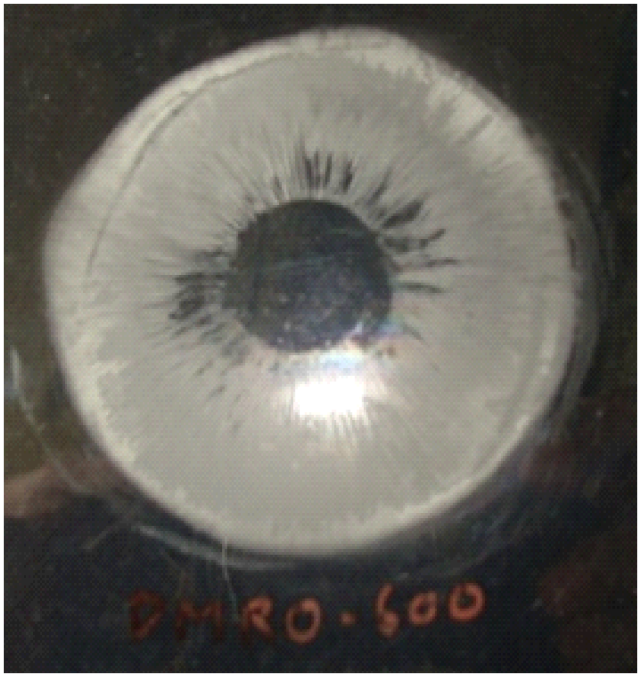

Fig. 2 : Spore print.

Fig. 4 : Lab grown fruiting bodies.

its shape was convex at first later expanded and flattened, cuticle is easily peeled, mat polished, some-time appressed scales present at or around the centre, margin regular in curved, smooth, non- striate. Stipe surface was dry and attached centrally, some-times eccentric, cylindrical with sub-bulbous base, up to 8.5 cm long, white, eartilaginous, fibrillose, base solid, without annuals and volva. Gills were distinctly formed crowded, emarginated, separable, white, non intervened, unequal, pliable, thick, attenuated towards margin. Spore print colour of the fungus was white and colonies were creamy white. The cultural and morphological characters of the fruiting bodies of *Calocybe* sp. observed in the present study are similar to those described by Varshney (2007) and Purkayastha and Chandra (1985) for *Calocybe indica*.

In order to find out the best mycelium growth substrate of *Calocybe* spp., for spawn production revealed (table 1) that wheat grains substrate supported maximum mycelial growth (11.875 cm), which was significantly superior to the other substrates tested. The next best substrates for its growth were sorghum (10.875 cm) and bajra grains (10.75 cm), both were significantly at par to each other. Maize grains supported good mycelial growth, whereas, poor mycelium growth was recorded on soybean product (Nutrella). The findings of Amle *et al*. (2007) and Senthilnambi *et al*. (2011) are in close agreements with the present results. They found maize grains and sorghum grains as good substrates for spawn production of *C. indica*.

In order to find out best substrate for the cultivation of this mushroom, five locally available substrates in pure form and also in combinations with wheat straw in 1:1 proportion were evaluated and the obtained results (table 2) are discussed as-

The data show that wheat straw substrate taken minimum days (21.5) for spawn followed by wheat straw + paddy straw, wheat straw + sugarcane bagasse (WS + SB), which were significantly at par with each other. Among all the tested substrates, dehulled maize cobs took maximum days (27.50) for completing the spawn run.

A perusal of data (table 2) further revealed that overall 33.25 to 41.25 days were taken for first harvest among different tested substrates. Wheat straw (33.25 days) and wheat straw + paddy straw (33.50 days) were taken minimum days for first harvest, which were at par with each other. However, maize straw was taken maximum days (41.25) for first harvest.

The data presented in table 2 revealed that wheat straw (40.75) produced maximum number of fruiting bodies followed by wheat straw + sugarcane baggasse (39.25), wheat straw + dehulled maize cobs (39.25), wheat straw + maize straw (37.50) and paddy straw (37.00). Non significant differences were recorded regarding the fruiting bodies produced on wheat straw, wheat straw + sugarcane baggasse, and wheat straw + dehulled maize cobs. Among all the tested substrates, dehulled maize cobs produced significantly minimum number (25.00) of fruiting bodies.

Perusal of data (table 2) further revealed that yield obtained from different substrates ranged from 503.75 to 1052.50 g/bag. Among tested substrates wheat straw substrate gave significantly highest yield (1052.50 g) followed by wheat straw + paddy straw (932.50 g). The next higher yield were obtained on paddy straw, wheat straw + sugarcane bagasse and sugarcane bagasse, 841.25 g, 840.56 g and 825.0 g/bag, respectively but the differences between the three were non-significant. However, significantly lowest yield (503.75 g) was recorded from dehulled maize cobs substrate.

The perusal of data in table 2 indicated that diameter of pileus was significantly highest (6.16 cm) on wheat straw. The next pileus sizes were developed on dehulled maize cobs (5.40 cm) and maize straw (5.30 cm), which were at par with each other.

The size of stipe (table 2) was highest on the wheat straw substrate followed by wheat straw + sugarcane bagasse and wheat straw + dehulled maize cobs.

These results are similar with finding of Tondan and Sharma (2006) and Bhatt *et al*. (2007). They reported that wheat straw and paddy straw substrate gave minimum days for spawn run and maturation of fruiting bodies and higher biological efficiency. Likewise, Krishnamoorthy and Muthusamy (1997) obtained the best yield in paddy straw and sorghum stalks substrate (356 and 354.3g/bed), which also supported the results of present finding.

The locally collected wild *Calocybe* sp. (DMRO-600) used in this study showed good quality fruiting bodies, high biological efficiency (70.50%) on wheat straw substrate and wheat grains found best substrate for spawn production.

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