

## Isolation, purification of *Scopulariopsisfimicola* and effect of cropping period on *Calocybeindica*

### Abstract

The *Calocybeindica* (Milky Mushroom) affected by large number of biotic and abiotic factors during cultivation. Among them, white plaster mould caused by *Scopulariopsisfimicola* encounter the compost and casing soil during cultivation and adversely affect the spawn run, growth and yield of *C. indica*. The present research work on various aspects of milky mushroom cultivation was conducted during 2019-21. June and August months (29.2<sup>o</sup>- 35.8<sup>o</sup>C temperature and 73.00-85% per cent relative humidity) are most suitable for milky mushroom cultivation. The associated mycoflora was isolated from infected casing soil, collected from farmers cultivating milky mushroom house/field. Eleven associated mycoflora viz., *Aspergillus niger*, *A. flavus*, *A. flavusparasiticus*, *Penicilliumcitreonigrum*, *Acremonium sp.*, *Alternariaalternata*, *Curvularialunata*, *Fusarium oxysporum*, *F. solani*, *Rhizopus stolonifera* and *Scopulariopsisfimicola* were isolated and identified. The cropping period during April 2020 to October 2020 the moisture content, temperature and relative humidity varied from 74 to 75 per cent, 26.9 to 36.6 °C and 73 to 88 per cent and the spawn run during the rest of the month varied from 19 to 21 days. The maximum time of spawn run was recorded at 21 days in the month of October 2020 and minimum time of spawn run was recorded at 20 days in the month of July and August 2020.

**Keywords:** Mushroom, Mould, *Calocybeindica*, *Scopulariopsisfimicola*, Neem, Tulsi.

### 1. Introduction

Mushroom are fleshy macro fungi with large fruiting bodies usually growing on dead and decaying material in the form of thread-like structures called hyphae that form a network (mycelium), after the mycelium has grown profusely and absorbed sufficient food material, it forms the reproductive structures, which generally come out of the substrate and form large fruiting bodies, commonly referred as mushroom. The history of mushroom is very old as the origin of man himself. These have existed for millions of years and mankind has used their large fruiting bodies, both for flavour and nutrition. Theophrastus (372-287 BC), the great Greek Philosopher wrote about the food value of mushrooms during Middle Ages, when they comprised royal dishes for the Greek and Roman Emperors. However, their commercial cultivation has become established as an industry only during the turn of the present century and few other mushrooms like *Flammulina velutipes* (enokitake), *Pholiotanameko* (nameko), *Auricularia* spp. (black ear), *Tremella fucifera*

**Comment [WU1]:** No paragraphing in abstract and maintain 1.0 spacing

**Comment [WU2]:** Rephrase. The statement is not connecting

**Comment [WU3]:** This present study tries to check the cultivation of milky mushroom across seasons between 2019 to 2021....

**Comment [WU4]:** Remove

**Comment [WU5]:** June and August (...) gave the highest yield of milky mushroom under the conditions treated

**Comment [WU6]:** Eleven microbes were isolated and identified from the casing soil, there include .....

**Comment [WU7]:** Try and rephrase

**Comment [WU8]:** Try and conclude well

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**Comment [WU10]:** Rephrase

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*rmis*(jellyfungus),*Strophariarugoso-annulata*(giant fungus) and *Tuber melonosporum*(truffle) have been exploited only in the present century (Sharma, 1990).

According to a conservative estimate, 1.5 million species of fungi actually exist and of these the number of known fungal species is about 69,000 (Hawksworth,1991). Among these known and described fungal species, about 14,000 are fleshy **macrofungi** and of these approximately half are edible (Chang and Miles, 1993) about 3000 species belonging to more than 30 genera are edible fungi. Among all reported edible fungionly 10 are commercial grown and 4-5 are produced on an industrial scale (Tewari, 2005).

**Mushrooms** are good source of high-quality protein, fat and other important vitamins. In addition, they contain vitamin B1, B2, 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, antitumor, antioxidant, antiviral, hypocholesterolemic and hypoglycemic effects (Cheung, 2010). In India, wide range of agro-climatic conditions exist, which favour cultivation of a large variety of agricultural and horticultural crops. Therefore, a major output of most agricultural and food processing operations is the unusable lignocellulosic waste material, whose predominant components are cellulose (35-50%), hemicelluloses (20- 30%), and lignin (10-25%). ~~In addition to these, some minor components, such as, proteins, oils, and ash make up the remaining fraction (Rashad *et al.*, 2009).~~ Lignocellulosic waste materials are one of the most abundant naturally available and renewable complex organic carbons. The annual production of lignocellulosic waste is 150 billion tonnes worldwide (Saber *et al.*, 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. India produces about billions of tonnes of agricultural residues and waste annually even if 1% of it is utilized to produce mushrooms, India can emerge as the major mushroom producer of the world in future (Chadha, 1994). However, the mushroom production can be developed into a big industry in India. One such promising mushroom of the tropical region is the milky mushroom (*Calocybe indica* P&C), whose wild forms were reported first time by Purkayastha and Chandra in 1974 from Calcutta markets, and thereafter, tried to make it domesticate.

The first attempt towards induction of fruiting bodies in milky mushroom was made by Purkayastha and Chandra (1976a). However, there is limitation in cultivation and productivity. Therefore, a need was felt to standardize its cultivation methodology on locally available lignocellulosic wastes in North India and make special efforts to increase its yield. North India has tropical to sub-tropical type of climate with optimum temperature of 25-35°C existing for most period of the year. In addition, a wide range of agro-wastes, garden-wastes and forest wastes are

**Comment [WU12]:** Macro-fungi

**Comment [WU13]:** Mushroom is a good source of high quality protein

**Comment [WU14]:** And other important vitamins which includes vitamin B1,.....

**Comment [WU15]:** Start as a new sentence

**Comment [WU16]:** Add a reference

**Comment [WU17]:** Remove

**Comment [WU18]:** To patients that are in need of protein especially in India

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available in this region, many of which have not been utilized so far for the cultivation of *Calocybe indica*.

## 2. Materials & Methods

The present investigation was conducted at the Plant Pathology Research Laboratory of Acharya Narendra Deva University of Agriculture & Technology, Narendra Nagar, Kumarganj, Ayodhya (U.P.).

### 2.1. Collection of infested casing mixture from different sampling sites:

In the present study casing material were amended with farm yard manure (FYM) in the ratio of 3:1. These casing materials were collected from the commercial farm of Mushroom growers. The samples were drawn from successive stages of each casing, namely, 0-day casing (when casing was applied over the mycelium impregnated compost, i.e. at the time of casing), mycelium impregnated stage (MIS) of casing at primordial stage (PS), and harvesting stage (HS) casing. The infested casing mixtures were collected from the farmers already cultivating milky mushroom in eastern U.P viz. Gorakhpur, Azamgarh, Gonda, Basti and Ayodhya. The samples (25 g) were collected aseptically from each compost bag and was utilized for the isolation of fungi.

Name of the farmer	Place
1. Farmer no.1	Gorakhpur
2. Farmer no.2	Azamgarh
3. Farmer no.3	Gonda
4. Farmer no.4	Ayodhya
5. Farmer no.5	Haidergarh

### 2.2. Isolation of fungus from Casing:

Casing sample (10 g) was suspended in 90 ml of 0.85% normal saline water (pH 7.0) and shaken vigorously at 150 rpm and 18°C for 1 hour. The

resulting slurry was serially diluted (100 p1) to 900 p1 of 0.85% normal saline in each appropriate dilution (10<sup>-4</sup>) of this suspension (100 p1) was spread plated in triplicate, on MEA medium cultures were incubated at 24±2°C for 2 days.

**Comment [WU21]:** Explain why normal saline was used in place of serial water? What is MEA media. From literature fungi grows within 72h not 48h.

### 2.3. Isolation and Purification of Associated Mycoflora:

The mycoflora associated with casing soil were isolated according to Warcup (1950). The casing soil samples (25 g) were suspended in 225 ml of sterilized distilled water (1:10 dilution) and subsequently 10 ml of this suspension was added into 990 ml of sterilized distilled water. Petri dishes containing the Malt Extract Agar (MEA) medium plus chlorophenicol (100 mg U<sup>-1</sup>) and Bengal Rose (50 mg L<sup>-1</sup>) were inoculated with 1 ml of the 1:1000 diluted soil suspensions. The plates were kept at room temperature (24 + 2°C) and the growth of the colonies were accompanied up to 96 hrs. The edge of the individual colonies were transferred separately to the same medium containing 50 mg L<sup>-1</sup> of chlorophenicol and the growth was accompanied for 96 h. The isolates were identified after growth on Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) medium, by observing its macroscopic characteristics (colour, texture appearance and diameter of the colonies) and microscopic (microstructures), according to Baijal and Mehrotra (1985), Bissett (1991), Domschet (1993), Pitt (1988), I-lammill (1970), Raper and Fennell (1975), Rifai (1969), Schipper (1984) and Sutton (1980).

**Comment [WU22]:** While the used of chlorophenicol and Bengal rose  
2. You used two media PDA and MEA are you specific about any organism

**Comment [WU23]:** Check

### 2.4. Cultivation of Milky Mushroom

#### 2.4.1. Collection of Culture:

The pure culture of *Calocybe indica* was collected from Mushroom laboratory, Department of Plant Pathology, multiplied and maintained on freshly prepared medium for further use.

**Comment [WU24]:** *Calocybe indica*

**Media:** Before preparation of culture, the preparation of medium is essential where the vegetative growth of mushroom is being occurred in vitro at Potato Dextrose Agar (PDA) & Malt Extract Agar Medium.

**Comment [WU25]:** And

#### 2.4.1.1. Malt Extract Agar Medium:

Malt Extract : 20g

Agar-agar powder : 20g

Water : 1000ml

#### Preparation of Potato Dextrose Agar (PDA) medium:

Peeled and sliced Potato : 200g  
Dextrose : 20g  
Agar (powder) : 20g  
Water : 1000ml

Potatoes were peeled, washed and sliced into small pieces and boiled in tap water (500ml) in a conical flask 20-25 minutes then, it was strained by a strainer and the decoction was kept into a beaker. Agar powder is boiled with 500ml water in a conical flask with continuous stirring with a glass rod for 2-3 minutes now, both agar solution and potato decoction were mixed together. The previously measured was also added to the mixed solution with stirring and made them homogenous. The volume was raised up to 1000ml. The prepared media was then sterilized in autoclave at 15lbs pressure (120°C) 20 minutes. The medium during hot condition was poured in culture tubes up to 1/3 height, and kept the culture tubes in culture tube's rack. The culture tubes were then plugged with non-absorbent cotton plug and wrapped 5-6 tubes by brown paper/two folds of used news-paper and rubber band then wrapped tubes were kept into a wire net basket few clean Petri plate pairs were also wrapped with brown paper and sterilized along with medium in autoclave.

**Comment [WU26]:** 15 minutes  
I advise you check what you did. Or you can give us the reference to your methodology

### 3. Result & Discussion

#### 3.1. Isolation and purification of associated mycoflora (*Scopulariopsis fimicola*) from casing soil *in vitro*.

In the present study casing materials were amended with farm yard manure (FYM) in the ratio of 3:1 and these casing materials were collected from the commercial farm of mushroom growers. The samples were drawn from successive stages of each casing, namely, 0-day casing (when casing was applied over the mycelium impregnated compost, that is, at the time of casing), mycelium impregnated stage (MIS) of casing, casing at primordial stage (PS), and harvesting stage (HS) casing. The infested casing mixtures were collected from the farmers already cultivation milky mushroom in eastern U.P i.e. Gorakhpur, Azamgarh, Gonda, Basti and Ayodhya. The samples (25 gm)

**Comment [WU27]:** Your results were not properly discussed. Please try and do justice to it. Breakdown your points bit by bit

were collected aseptically from each compost bag was utilized for the isolation of associated mycoflora (Plate 1).



**Plate 1: Plaster mould (*Scopulariopsis fomicola*)**

The associated mycoflora was isolated from infected casing soil, collected from farmers cultivating milky mushroom house/field. The mycoflora were isolated on MEA (Malt Extract Agar) medium and then it was sub cultured on MEA medium slant. The purification was done by serial dilution method. The eleven associated mycoflora viz., *Aspergillus niger*, *A. flavus*, *A. flavus parasiticus*, *Penicillium citreonigrum*, *Acremonium sp.*, *Alternaria alternata*, *Curvularia lunata*, *Fusarium oxysporum*, *F. solani*, *Rhizopus stolonifera* and *Scopulariopsis fomicola* were isolated and identified.

**Vijay et al. (1988, 1996)** also studied in-vivo condition by inoculating with *S. maheshwarlarum*, *T. viride*, *F. acunjinatum*, *S. atra*, *Cladobotryum dendroides*, *C. indicum* and *V. fungicola*, these moulds in the compost at different stages of cultivation. Similar results were also reported by **Sharma and Satish (2005)**. **Fergus (1978)** isolated 50 different fungi from the compost after spawn run stage and similar study was carried out by **Shandilya (1989)**

### **3.2. Effect of cropping period of *Calocybe indica*:**

The data pertaining in Table-1 indicated that moisture content of the substrate at the time of spawning varied from 74 to 75 per cent.

It was also evident from data given in Table-1 that from April 2020 to October 2020, temperature ranges between 26.9 °C to 36.6 °C and relative humidity 73.00 to 88.00 per cent and during course of investigation of *Calocybe indica* days taken to spawn run during rest of the months varied from 19 to 21 days. Maximum being 21 days in month of October, 2020 temperature ranges between 26.9 °C to 30.9 °C, relative humidity

**Comment [WU28]:** Remove this is a repetition of your methodology. Go straight to your results

**Comment [WU29]:** The full meaning should come first before the abbreviation

**Comment [WU30]:** Stop repetition of methods

**Comment [WU31]:** Remove from bold. Did the findings agree or disagree with your results?

**Comment [WU32]:** Type set. Explain the work in more details

**Comment [WU33]:** Remove

79.00 to 86.00 per cent and minimum 20 days in two months *i.e.* July and August 2020 at temperature ranges between 27.3 °C to 34.5 °C and relative humidity 81.00 to 85.00 per cent and time taken for initiation of pinhead varied between 28 to 32 days.

Minimum

being 28 days in the three months *i.e.* April, July and August at temperature ranges between 27.1 °C to 36.6 °C and relative humidity 73.00 to 87.00 per cent being 31 days in the month of October 2020 at temperature range between 29.9 °C to 30.9 °C and relative humidity 79.00 to 86.00 per cent. In the month of July mushroom was taken minimum 38 days for first harvest. While in month of October it took maximum time 40 days for first harvest.

**Table-1: Parameters of cropping period of milk mushroom (April 2020 to October 2020)**

Cropping Period	Mean					Monthly average temperature during cropping period (°C)		Monthly average R. H. during cropping period (%)	
	Moisture content of substrate at the time of spawning (%)	Days taken for spawn run	Days taken in pinning	Days taken for first fruiting	No. of fruiting harvested	Max.	Min.	Max.	Min.
April, 20	74	20	29	38	28	31.2	27.1	83.0	74.0
May, 20	75	21	30	37	26	36.6	30.5	85.0	75.0
June, 20	75	19	28	36	27	35.8	29.2	82.0	73.0
July, 20	74	21	28	38	29	33.4	28.9	87.0	78.0
August, 20	75	20	32	35	27	34.5	27.3	85.0	81.0
September, 20	74	19	29	40	20	31.7	28.4	88.0	80.0
October, 20	75	20	31	41	22	30.9	26.9	86.0	79.0

The experimental findings showed that cropping period during April 2020 to October 2020 the moisture content, temperature and relative humidity varied from 74 to 75 per cent, 26.9 to 36.6 °C and 73 to 88 per cent and the spawn run during the rest of the month varied from 19 to 21 days. The maximum time of spawn run was recorded at 21 days in the month of October 2020 and minimum time of spawn run was recorded at 20 days in the month of July and August 2020.

The time taken in initiation of pinhead varied from 28 to 32 days, the minimum

being 28 days and maximum 32 days in the month of April, July and August 2020. The maximum yield (491.07 gm bag<sup>-1</sup>) was recorded in the month of June and August 2020, similar findings were also reported by **Trivedi et al. (1991)** that the optimum yield of *Calocybeindica* was obtained at temperature range from 25 to 38°C and relative humidity 75 to 85%. **Sarmah et al. (2006)** also reported that *Calocybeindica* was successfully grown from May to August. The highest yield was recorded in the month of June and mean temperature, relative humidity during this period 26.1 to 32.5 °C and 79.5 to 85 per cent.

### Conclusion

The effect of environment on milky mushroom (*Calocybeindica*) cultivation, maximum yield (491.07 gm bag<sup>-1</sup>) was recorded in the month of June and August 2020 at average temperature of 29.2<sup>o</sup> C and relative humidity 73.00 per cent in month of June. Whereas in August temperature was 35.8<sup>o</sup>C and relative humidity 85.00 per cent was recorded. The associated mycoflora was isolated from infected casing soil, collected from farmers cultivating milky mushroom house/field. The eleven associated mycoflora viz., *Aspergillus niger*, *A. flavus*, *A. flavus parasiticus*, *Penicillium citreonigrum*, *Acremonium sp.*, *Alternaria alternata*, *Curvularia lunata*, *Fusarium oxysporum*, *F. solani*, *Rhizopus stolonifera* and *Scopulariopsis fimicola* were isolated and identified.

Comment [WU34]: Maintain the same front

### Declarations

**Ethical approval:** Not applicable.

### References

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Comment [WU35]: Use journals guidelines for referencing

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**Comment [WU36]:** Use updated journals like from 2015 and above