Isolation, purification of *Scopulariopsisfimicola* and effect of cropping period on *Calocybe indica*

Abstract

The *Calocybe indica* (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 (29.2^0-35.8^0C temperature and 73.00-85% per cent relative humidity) aremost suitable for milkymushroom 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. flavus parasiticus, Penicillium citreonigrum, Acremonium sp., Alternaria alternata, CurvulariaIunata, Fusarium oxysporum, F. solani, Rhizopus stolonifera and Scopulariopsisfimicola*were isolated and identified.The cropping period during April 2020 to October2020the moisture content, temperature andrelative humidity varied from 74to 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 21days 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, *Calocybeinidca, Scopulariopsisfimicola,* Neem, Tulsi.

1. Introduction

Mushroom are fleshy macro fungi with large fruiting bodies usually growing on dead and decaying material in theform of thread-likestructures called hyphae that forma 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 *Flammulinavelutipes*(enokitake),*Pholiotanameko*(nameko),*Auricularia*spp.(blackear),*Tremellafucifo*

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 allreported edible fungionly 10 are commercial grown and 4-5 are produced on an industrialscale (Tewari, 2005).

Mushroom 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 afood 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 producemushrooms,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 milkymushroom (*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 methodologyon 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^{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, manyof which have not been utilized so far for the cultivation of *Calocybe indica*.

2. Materials & Methods

The present investigation wasconducted at the Plant Pathology Research LaboratoryofAcharyaNarendraDevaUniversityofAgriculture & Technology, Narendra Nagar, Kumarganj, Ayodhya (U.P.).

2.1.Collectionofinfestedcasingmixturefromdifferentsamplingsite:

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 fromsuccessive 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 casingmixtureswere collected fromthe farmersalready cultivating milky mushroom in eastern U.P viz. Gorakhpur, Azamgarh, Gonda, Basti andAyodhya. The samples (25 g) were collected aseptically from each compost bag and was utilized for the isolation of fungi.

Nameofthe 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.IsolationoffungusfromCasing:

Casing sample (10 g) was suspended in 90 ml of 0.85% normal salinewater(pH7.0)andshakenvigorouslyat150rpmand18°Cfor1hour.The

resultingslurry was serially diluted (100 p1) to 900 p1 of 0.85% normal saline in each appropriate dilution (10-4) of this suspension (l00 p1) was spread plated in triplicate, on MEA medium cultures were incubated at 24±2°C for 2 days.

2.3. IsolationandPurificationofAssociatedMycoflora:

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 subsequently10 ml ofthis suspension was added into 990 ml of sterilized distilled water. Petri dishes containing theMalt Extract Agar (MEA) medium plus chlorophenicol (100 mg U") and Bengal Rose (50 mg L'1) were inoculated with 1 ml of the 1:1000 diluted soil suspensions. The plates were kept at roomtemperature (24 + 2°C) and the growth of the colonies were accompanied up to 96 hrs. The edge of the individualcolonieswere transferred seperatelyto the same mediumcontaining50 mg L'1 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 (l988),1-lammill(1970),Raper andFennell(1975),Rifai(1969),Schipper(1984).

andSutton(1980).

2.4.CultivationofMilkyMushroom

2.4.1. CollectionofCulture:

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

Media: Before preparation ofculture, the preparation ofmedium isessential where thevegetativegrowthofmushroomisbeingoccurredinvitroatPotato DextroseAgar(PDA)& Malt Extract Agar Medium.

2.4.1.1. MaltExtractAgarMedium:

Malt Extract : 20g

Agar-agar powder : 20g

Water	:	l000ml

PreparationofPotatoDextroseAgar(PDA)medium:

Peeledandsliced Potato	:	200g
Dextrose	:	20g
Agar(powder)	:	20g
Water	:	1000ml

Potatoeswere peeled, washed and sliced into small piecesand 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 500m1water in a conical flask with continuous stirring with a glass rod for 2-3 minutes now,both agarsolution and potatodecoction weremixed together. Thepreviously measured was also added to the mixed solution with stirring and made them homogenous. The volume was raised up to l000ml. The prepared media was then sterilized in autoclave at l5lbs pressure (120°C) 20 minutes. The medium during hot condition was poured in culture tubes up to l/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 sterilizedalong with medium in autoclave.

3. Result & Discussion

*3.1.*Isolation and purification of associated mycoflora (*Scopulariopsisfimicola*) 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 primordialstage (PS),and harvestingstage (HS)casing. The infested casingmixtures 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)

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

Plate1:Plastermould*(Scopulariopsisfimicola)*

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, CurvulariaIunata, Fusarium oxysporum, F. solani, Rhizopus stolonifera and Scopulariopsisfimicola*were isolated and identified.

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

3.2.Effectofcroppingperiodof*Calocybeindica*:

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 ^{0}C to 36.6 ^{0}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 attemperatureranges between 26.9 ^{0}C to 30.9^{0}C,relativehumidity

79.00to86.00 per cent and minimum 20 days in two months *i.e.* July and August 2020 at temperature ranges between 27.3 ^{0}C to 34.5 ^{0}C and relative humidity 81.00 to 85.00percent and time taken for initiation of pinhead varied between 28 to 32 days. Minimum being28daysinthethreemonths*i.e.*April,JulyandAugustattemperaturerangesbetween27.1 ^{0}C to 36.6 ^{0}C and relative humidity73.00 to 87.00 per cent being 31 daysin the month of October 2020 at temperature range between 29.9 ^{0}C to 30.9 ^{0}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 ofcropping period ofmilkymushroom (April 2020 toOctober 2020)

Cropping Period	Mean					Monthly average temperature during cropping period(^{0}C)		Monthly averageR. H. during cropping period(%)	
	Moisture content of substrate at the time of spawning (%)	Days taken for Spawn run	Days taken in pining	Days taken forfirst 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 October2020the moisture content, temperature andrelative humidity varied from 74to 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 21days 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 andAugust 2020. The maximum yield (491.07 gm bag^{-1}) 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 *Calocybe indica* was obtained at temperature range from 25to 38°C and relative humidity 75 to 85%. **Sarmah *et al.* (2006)** also reported that *Calocybe indica* was successfullygrown from May to August.The highestyield was recorded in themonthof Juneand mean temperature, relative humidity during this period 26.1 to 32.5 °C and 79.5 to 85 per cent.

Conclusion

Theeffect ofenvironmenton milky mushroom (*Calocybeindica*)cultivation,maximum yield (491.07gm bag-1)was recorded in the month ofJune and August 2020 at average temperature of 29.2^0 C and relative humidity 73.00 per cent in month of June. Whereas in August temperature was 35.8^0C and relative humidity 85.00 per cent was recorded.The associated mycoflora was isolated from infected casingsoil,collected from farmers cultivating milky mushrrom house/field. The eleven associated mycoflora viz., *Aspergillus niger, A. flavus, A. flavus parasiticus, Penicillium citreonigrum, Acremonium sp., Alternaria alternata, CurvulariaIunata, Fusarium oxysporum, F. solani, Rhizopus stolonifera and Scopulariopsisfimicola*were isolated and identified.

Declarations

Ethical approval: Not applicable.

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