

Original Research Article

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Assessment of Mycelial Growth and Yield Attribute of *Calocybe indica* P and C

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ABSTRACT

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The milky mushroom (*Calocybe indica*) is a robust, fleshy, milky white, umbrella like mushroom, which resembles button mushroom. It grows well at a temperature range of 25-35°C and relative humidity more than 80%. Present experiments were conducted to see the effect of different media, temperature range on mycelial growth yield performance of *Calocybe indica*. The result revealed that the average mycelial growth of all strain (CI-1, CI-4, CI-522, CI-524 and CI-530) was maximum in PSA (Potato sucrose agar) medium followed by PDA (Potato dextrose agar) medium. Similarly 30°C temperature found to be suitable for maximum mycelial growth of different strains. The mycelial growth of each strain was differed significantly on all tested media and temperatures. Sorghum and wheat grains raised spawn gave maximum yield (718.33g and 685g/kg of dry wheat substrate) with highest biological efficiency.

Introduction

Milky mushroom (*Calocybe indica*) is a promising mushroom which was introduced to the world of edible mushrooms from India and commercialized from the Tamil Nadu Agricultural University, Coimbatore. It is also known as “Dudh Chhatta/Milky Mushroom” because of its milky white appearance and large sized sporophores or as “white summer mushroom” because of its tropical nature. It is more attractive with excellent shelf-life, grows on several agricultural wastes and on wide range of temperatures (Singh *et al.*, 2015).

This mushroom was first reported from West Bengal, India (Purkayastha, 1974).

First attempts on the induction of fruit bodies of *Calocybe indica* in culture was made by Purkayastha and Chandha in 1976 (Purkayastha and Nayak, 1981); (Chakravarty *et al.*, 1981). For the first time milky mushroom was cultivated successfully in North Bengal in summer time (June-August) when the temperature and humidity is high which is suitable for milky mushroom cultivation. The cultivation process resembles that of oyster mushrooms but for the additional process of casing. The mushroom can be harvested from 30-34 days after spawning. The advantages of this mushroom over other mushrooms are easy method of cultivation, less investment, very attractive

fruiting body, pleasing milk white color, long shelf life, more nutritious and less time to grow (Bokaria *et al.*, 2014).

Detailed studies on preferential physiological and cultural requirements for growing *C. indica* were reported. Since this mushroom is morphologically similar to *Agaricus bisporus* (button mushroom), it has been quite popular in southern Indian states and slowly getting popular in other countries (China, Malaysia, and Singapore). Methods have been developed to cultivate this mushroom on commercial scale since late nineties. Chhattisgarh has been bestowed with huge diversity of *Calocybe indica* and Mushroom Research Laboratory, IGKV Raipur has a huge collection of *C. indica* from different areas of Chhattisgarh (AICMIP Annual Report 2015-16). However a systematic study of this species is still lacking in context to this particular state or Central India *per se*. Therefore the present experiment was conducted to explore the media and temperature requirement for mycelial growth and assess the biological efficiency of milky mushroom.

Materials and Methods

Cultures and their maintenance

The culture of different strains of *Calocybe indica* namely CI-1, CI-4, CI-522, CI-524 and CI-530 were taken from the All India Co-ordinate Mushroom Improvement Project, Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya Raipur on and the cultures were revived on fresh PDA (Potato Dextrose Agar) medium.

Effect of media and temperature on radial growth

Five different media *i.e.* Potato dextrose agar, potato sucrose agar, wheat extract, malt

extract and Asthana and Hawker's, medium were used to know the effect of media on mycelial growth of five strains of *C. indica*. 20 ml of each medium was poured in sterilized Petri dishes. At the time of pouring a little amount of streptomycin was added in each medium to inhibit the bacterial growth. After solidification of medium the plates were centrally inoculated with a 7 days old culture of respective strain of *C. indica*. The inoculated plates were incubated at, 30°C and 4 replications were maintained for each treatment. The observations were recorded for mycelial growth in till the mycelial growth in any treatment reached at the periphery of the plates. The plates containing medium (PDA) were inoculated with actively growing pure culture of each strains of *C. indica*. These inoculated plates were kept at different temperature *i.e.*, 22°C, 25°C, 30°C, and 33°C for incubation. The observations were recorded for mycelial growth. Four replications were kept in each treatment.

Cultivation technology

Wheat straw was chemically treated. It was then spawned @ 4 % on wet weight basis. The mixture was filled in polythene bags at the rate 1.0 kg/bag. These bags were placed in the mushroom crop room at relative humidity of 80-85% and temperature of $28 \pm 2^{\circ}$ C. After 15-17 d of spawning, casing was done. Casing mixture was prepared by mixing of farm yard manure + vermi-compost + garden soil (1:1:1 w/w) and treated with formalin (4%) solution and kept covered for 48-72 h. The pH of casing was maintained at 8.5 using calcium carbonate and calcium sulphate. A 3 cm thick layer was applied uniformly on substrate. Pin head initiation started after 10-12 d of case-run. The sporophores were harvested after the maturity. The yield obtained from the strains 524, spawn prepared in different cereal grains during 7 weeks harvesting period were compared with each other. Observations on

growth parameters and sporophore yields were recorded. Biological efficiency was calculated as:

BE (%) = fresh wt of fruit body/dry weight of substrate.

Statistical analysis

Factorial with CRD (Completely randomized design) was used to statistical analysis. The analysis was performed online in OPSTAT (Online statistical analysis tool) analysis. The critical difference (C.D.) was calculated at 5% levels.

Results and Discussion

Colony characteristics

All the five strains showed cottony white mycelial growth with even margin on all tested media while strain CI-4 and CI-530 showed concentric ring also on potato sucrose agar except all medium. At different temperatures, all the strains showed absolute white mycelial growth with even margin but only difference was in strain CI-524, in which concentric ring was observed on PDA at 33 °C.

Effect of different media

The result revealed that the highest mycelial growth of *C. indica* was noticed in potato sucrose agar medium followed by potato dextrose agar medium and it was lowest in wheat extract agar medium under study. The mycelial growth of each strain varied significantly in all the media tested. In potato dextrose agar medium maximum radial growth of mycelium (9.00 cm) was recorded in strain CI-522. The other strains showed significant variation with each other ranging from 4.37 to 8.72 cm. In potato sucrose agar medium, the maximum average mycelial

growth was in strain CI-4 which was significantly superior to other strain.

The present findings are almost similar to the results obtained by Singh *et al.*, (2009) reported that *C. indica* grew well on all the tested media but maximum linear growth (7 cm) was recorded on wheat extract agar medium followed by PDA. Krishnamoorthy *et al.*, (2015) reported the majority of the time required for more radial growth in culture media like PDA or malt extract medium is 8 to 10 days (Table 1).

Effect of different temperatures

Among the tested temperatures, all strains showed maximum mycelial growth at 30°C followed by 33°C and minimum at 22°C on 7 d of incubation. The mycelial growth of each strain varied significantly at all the temperature tested. At 30°C temperature, strain CI-522 showed maximum radial growth (9.00 cm). However, other strains showed variation to each other giving diametric growth from 6-8 cm. At 33°C, maximum average mycelial growth 5.80 cm was recorded in strain CI-522 which was significantly superior to other strains. The least growth in strains was recorded at 22°C in CI-1 (2.93 cm).

These results are in accordance with the findings of Varshney (2007) reported temperature requirement from 25-35°C for mycelial growth of *Calocybe indica*. All strains of *Calocybe indica* showed maximum mycelial growth at 28°C followed by 32°C and minimum at 20°C. At 28°C temperature on 8th day's strain CI-6 was at par showing maximum diametric growth of mycelium (9.0 cm) in observations. Shukla *et al.*, (2013) studied on effect of temperature on mycelia growth of the strains of milky mushroom (*Calocybe indica*) viz. CI-4, CI-6, CI-7, CI- 8, CI-9 & CI-10 (Table 2).

Table.1 Effect of different media on radial growth of different strains of *C. indica* on 7 days of incubation

MEDIA	Mycelial Growth of Strains (cm)				
	CI-1	CI-4	CI-522	CI-524	CI-530
PDA	6.22	8.72	9.00	6.61	7.46
PSA	6.00	8.72	7.87	6.97	7.60
WE	4.98	5.45	5.62	3.83	4.95
ME	4.37	5.03	5.56	6.46	4.63
A&H	4.66	5.45	5.22	5.47	4.97
	SEm±			CD (5%)	
	Strains		0.078	0.220	
	Media		0.078	0.220	
	Strains × Media		0.174	0.491	

Table.2 Effect of different temperatures on radial growth of different strains of *C. indica* on 7 days of incubation

TEMPERATURE	Mycelial Growth of Strains (cm)				
	CI-1	CI-4	CI-522	CI-524	CI-530
22°C	2.93	3.57	3.31	3.36	3.45
25°C	5.15	5.68	5.68	4.37	5.33
30°C	6.22	8.72	9.00	6.61	7.46
33°C	5.36	5.53	5.80	4.01	5.07
	SEm±			CD (5%)	
	Strains		0.61	1.75	
	Temperatures		0.55	1.56	
	Strains × Temperatures		1.23	3.50	

Table.3 Yield performance of *C. indica* on different grains substrates raised spawn in wheat straw substrate

Yield g/ kg dry wheat substrates									
S No	Grains	Spawn Run (Days)*	Pin head Initiation (Days)*	Pileus Diameter (cm)**	Stipe Length (cm)**	Stipe Diameter (cm)**	Average Weight (g)**	Yield (g)*	BE (%)
1.	WHEAT	12	7	10.36	17.80	4.44	85	685.00	68.50
2.	SORGHUM	11	6	9.24	17.60	3.24	66	718.33	71.83
3.	BAJRA	11	6	5.68	11.40	3.34	62	310.00	31.00
4.	RAGI	12	6	6.93	12.00	3.50	60	283.00	28.30
5.	MAIZE	13	8	5.66	11.50	3.16	54	486.67	48.66
	SEm±	0.29	0.29	1.50	1.50	0.31	8.78	13.22	
	CD (5%)	0.93	0.93	NS	4.44	NS	NS	41.68	

(*) – Average of three replication

(**) – Average of five sporophore

(NS) – Non significant

They reported that the most suitable temperature for fast and full mycelial impregnation and growth was 30°C.

Yield performance of *C. indica* on different grains raised spawn on wheat straw substrate

The number of days required for spawn run by *C. indica* differed significantly with respect to different grains. Spawn prepared on sorghum and bajra grains required significantly less time (11 days) for spawn run whereas, took significantly more time by maize (13 days), wheat and ragi grain based spawn (12 days) for spawn run by *C. indica*. Similarly pin head initiation was quite earlier (6 days) in sorghum, bajra and ragi grain followed by wheat grain (7 days). However, pin head initiation took significantly more time when spawn was prepared using maize (8 days) (Table 3).

The other parameters like stalk length differed significantly. The stipe length of *C. indica* was highest in the fruit body produced from bed inoculated with wheat grain based spawn (17.80 cm) followed by sorghum (17.60 cm), ragi (12.00 cm) and maize (11.50 cm). However, the minimum length was showed by bajra (11.40 cm) grain. The average weight of sporophores, pileus diameter and stalk diameter did not show any significant difference with the type of grains used. However, the average weight of sporophores varied from 54-85g, pileus diameter from 5.66-10.36 cm, stalk diameter from 3.16-4.44 cm.

The fresh yield of *C. indica* differed significantly with type of grains used. The highest yield recorded on sorghum grains (718.33g) with BE (71.83%) followed by wheat grains (685g) with BE (68.50%) and minimum yield was recorded in ragi (283.00g) with BE (28%) followed by bajra

grains (310g) with BE (31 %) and maize grains (486.67g) with BE (48.66%).

Senthilnambi *et al.*, (2011) found the supremacy of sorghum grains as the most suitable substrate for early spawn run, which took only 13.7 days for hundred percent mycelial growths. The yield and number of buttons harvested were found maximum in the sorghum grain spawn followed by ragi grain spawn. The maize grain substrates took 19 days for complete spawn run and recorded low yield when compared to other spawn substrates. The days for pin head formation and first harvest of the crop were earlier in case of sorghum grain spawn followed by ragi spawn.

According to present experiment we can conclude that all strains (*i.e.* CI-1, CI-4, CI-522, CI-524, and CI-530) showed maximum mycelial growth on PSA followed by PDA and ME. Similarly all strains showed maximum mycelial growth at 30°C followed by 33°C and minimum at 22°C. The mycelial growth of each strain was varied significantly on all the media and temperature tested.

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