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Single spore isolation and interspecific hybridization of *Calocybe* spp.

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ABSTRACT

For the development of improved strain(s) of *Calocybe* spp. with desirable traits, interspecific hybridization of Milky white mushroom (*C. indica*) and St. George's mushroom (*C. gambosa*) was done by intermating their single spore cultures. *In vitro* evaluation of both the species for mycelial characteristics, growth rate, colony colour, days taken for completion of full growth were recorded. Single spore isolates of the two species were developed through serial dilution technique. Hybridisation of single spores was done by dual culture technique using hyphal anastomosis. Of the 121 crosses done 42 were compatible and 79 crosses incompatible.

Keywords: *Calocybe indica, C. gambosa,* Interspecific hybridization, mycelial growth rate

Mushrooms play an important role in social empowerment of women and alleviate rural unemployment (Gogoi *et al*., 2019). *Calocybe indica* is the fourth most popular and commercially grown mushroom in India after button, oyster and paddy straw mushroom. This mushroom is also known as "Milky mushroom or Dudh Chatta". It has robust milky white sporophore with high fruiting body weight. This mushroom was first reported in India from West Bengal (Purkayastha and Chandra, 1974). Major part of India is endowed with tropical and subtropical climate suitable for the cultivation of milky mushroom for most of the year. The approximate annual production of this mushroom in India is estimated to be over 3000 tonnes (Sharma *et al*., 2017). The cultivation of milky mushroom has gained momentum in tropical areas of India *viz*., Andhra Pradesh, Goa, Karnataka, Kerala, Maharashtra, Tamil Nadu and Uttar Pradesh. Mature fruit body of *C. indica* contains approximately 17.2% protein, 4.1% fat, 3.4% crude fibre and 64.26% carbohydrate on dry weight basis. Due to alkaline ash and high fibre content, it is highly suitable for people with hyperacidity and constipation (Gupta, *et al*., 2011).

Initially mushroom cultivation was mostly oriented towards the cultivation aspects rather than breeding, but now greater attention has been given to develop new strains/ hybrids by breeding programmes. The character, behaviour and overall performance of a strain are outcome of the genotype of the mushroom strain and the environmental conditions where it is grown**.** The need of the hour is not only to explore other new species but also to improve the existing species through various breeding techniques for higher yield, better quality, texture, colour and taste to meet the rising demands of the increasing population. The breeding programme for mushrooms in India has been mainly limited to introduction, but the procurement and introduction of the existing commercial strains from abroad is a short-term strategy of mushroom breeding. Besides introduction, selection has also been reported in many cases using tissue culture, multi-spore culture and single spore cultures. Existence of variability in

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morphological traits and growth rate of mycelium of homokaryotic single basidiospores can be exploited for the development of inter-strainal hybrids. Strategies with special reference to strain improvement using hyphal anastomosis as a potential tool has been reported for improving breeding potentials of edible mushrooms (Jandaik, 1997)**.** Promising hybrids have been developed in button mushroom (Singh *et al*., 2016). Strain improvement in *Pleurotus* utilizing hybridization technique was attempted by Anitha (1998). An interspecific hybrid was developed by pairing compatible monospore cultures of two different species of *Pleurotus* namely *P. platypus* (Pts-1) MK-3 and *P. citrinopileatus* (Pc-1) MK-2, which had several desirable traits. A potential isolate of *C. indica* collected at Coimbatore, Tamil Nadu out yielded hither to known cultivated mushrooms around the globe (with an average bio-efficiency of 142 per cent in paddy straw) and it was designated as APK-2 (Subbiah, 2014)**.** Hybrid development was attempted by crossing two strains of *Calocybe* with desirable characters (Heera, 2006). The hybrid out yielded the parentswith respect to yield and nutrient status. Molecular characterisation of hybrid and their parents revealed that hybrid had 44.40 per cent similarity with one parent and 28.57 per cent similarity with other parent. Strain improvement was also done in milky mushroom (*Calocybe indica*) using UV rays and gamma irradiation. The improvement in Ci-3, a high yielding strain of *C. indica*, was attempted using mutagenic treatment on protoplasts by irradiation with UV light and chemicals like NTG, Ethidum bromide, 5'-Bromouracil. The mutants had enhanced endoglucanase and xylanase activity. Four mutants (CMN-3, CMN-9, CMN-11 and CMB-4) of *C. indica* developed by Kaur *et al*., 2011 gave significantly higher yield, better sporophore characters than the parent. In an another report on production of inter-generic somatic hybrid by PEG mediated protoplast fusion between *P. florida* and *C. indica* by differential tolerance of NaCl level, the hybrid had *Calocybe* as the dominant parent with increase in bio-efficiency and γ-linoleic acid content (Chakraborty and Sikdar, 2009).

The present study is an attempt for improvement of *Calocybe indica* (milky mushroom) by interspecific hybridization for yield and quality.

MATERIALS AND METHODS

Culturing and morphological characterization of sporocarp of *Calocybe* spp.

The experiment was conducted at AICRP on Mushrooms, Department of Plant Pathology, College of Agriculture, Vellayani. Sporocarp of *C. indica* and *C. gambosa* were obtained from AICRP on Mushrooms and Instructional Farm, College of Agriculture Vellayani, respectively. The cultures of the mushrooms were prepared following the standard technique of tissue isolation on potato dextrose agar (PDA) medium. The Petri dishes of tissue isolation were incubated at room temperature (26±3°C). The mycelial initials from the tissues were sub-cultured on to PDA slants and maintained for further studies. The nature of mycelial growth, rate of mycelial growth, colony characters and time taken for growth completion in Petri dish were recorded.

Spawn preparation and cultivation

Mother spawn of *Calocybe* spp. were prepared by inoculating the paddy grains with the respective culture (*C. indica* and *C. gambosa*). The colour of the mycelium and days required for full spawn growth were recorded. The beds were prepared in cocopeat amended with vermicompost, wheat bran, $CaCO_3$ substrate at 4.5: 4: 2: 0.5 proportions. Casing was done 21 days after complete spawn run. The yield parameters *viz*., number of sporophores, total yield and average weight of the sporophores were noted. Morphological characteristics of sporocarp including the stipe characters (length, diameter), pileus characters (diameter, thickness, colour) gill characters and basidial characters were studied.

Isolation and development of monosporus cultures

Isolation of single spores to develop monosporus culture (homokaryons) is one of the key steps for developing hybrids through dikaryotization by intermating of compatible homokaryons.

1. *Spore print:* The spore print of *C.indica* and *C.gambosa* was obtained using the method proposed by Petersen and Ridly (1996). The freshly opened and healthy mushrooms were selected to prepare a spore print. The pileus of the mushroom was laid flat with gills down on inside a sterilized Petri dish. The Petri dish was sealed properly with paraffin and the entire unit was placed without any disturbance for 4 hours. The spores were collected for serial dilution.

2. *Serial dilution:* Serial dilution was done by the method proposed by Bahukandi and Sharma (2012). A small loop of spore from the spore print was taken with the help of sterile needle and suspended in sterile distilled water. From this suspension 10^{-4} dilution was made by serially diluting the suspension, the spore concentration was as low as up to 4-5 spores under low power microscopic field (10x). From this concentration a loop of 0.1 ml of spore suspension were carefully plated on plain potato dextrose peptone agar medium in Petri plates and incubated at 25±1°C for germination. After 9 days of incubation, small isolated colonies appeared which were transferred to the PDA slants and incubated for 25±1 ° C for 7-10 days. These cultures were examined for the clamp connections. The cultures with no clamp connections were confirmed as monokaryons under 40 X and cultures showing presence of clamp connections (dikaryons) were discarded.

3. *Morphological characterization of basidiospores and single spore isolates:* Cultural characters of single basidiospores from *C. indica* and *C. gambosa* were observed on PDA media. Single spore isolates were grown on sterilized Petri-plates with PDA. A mycelial disc of 5 mm diameter was transferred aseptically to the centre of each Petridish and incubated at room temperature (27 ± 1ºC). Observations on radial growth, colour of the colony, growth rate and appearance of *C.indica* and *C. gambosa* and their single spore isolates were recorded at regular intervals.

4. *Inter-specific hybridization:* Eleven single spore cultures of each species *C. indica* (A) and *C. gambosa* (B) were selected for crossing. Inter specific hybridization of monokaryotic cultures were performed by dual culture technique. One centimetre mycelial disc of seven days old monosporous/ single spore cultures of the above two species were cut with the help of sterile cork borer and placed 2.5cm apart in the two opposite sides of the Petri plate containing PDA medium, permitting the monokaryons to grow towards each other. The plates were incubated at 27±1°C for 10 days. Depending on their compatibility, the positive dikaryons were picked, where there was barrage formation at the zone of confrontation. The crosses were considered positive when cottony fluffy growth from the confrontation zone showed clamp connections indicating dikaryotic nature. One twenty-one crosses were made using 11 single spores of each *Calocybe* spp. The macro-morphology of thick barrages, line transects, fluffiness of growth and lytic area by means of pigments were observed. The bits from the barrage formation were subcultured on to PDA media. Similarly, the monosporous cultures used as parents for making hybrids were also maintained on PDA medium. The nature of mycelial growth and growth rate of parents and their crosses were recorded regularly.

The two parents (*C. indica* and *C. globosa*) with seven replications were utilised for the cultural and sporophore characters. The data were analysed using

CRD. The data obtained from the experiments was subjected to analysis of variance (ANOVA). Critical difference (CD) calculated at 5 per cent level of significance was used for comparison of different treatment means. Standard error mean and standard deviation of observations were also calculated. Statistical analysis of the data was done using GraPES software, KAU.

$$\sigma M = \sigma/\sqrt{N}$$

σM stands for the standard error of the mean, σ stands for the standard deviation and N is the size of sample.

RESULTS AND DISCUSSION

Observations on nature and growth rate of the *C.indica* and *C. gambosa* obtained by the standard tissue culturing technique from their respective sporocarp on potato dextrose agar medium are presented in Table 1. The isolates of *C.indica* had white fluffy mycelial growth while *C. gambosa* had cottony growth. *C. gambosa* had a significantly faster growth rate than *C. indica* as indicated by the radial growth at different days after inoculation (5, 7, 9, 11 and 13) (Fig. 1). The rate of mycelial growth of *C. gambosa* was 0.73cm day^{-1} as compared to 0.65cm day^{-1} in *C. indica*. The mycelium of *C. gambosa* completed full growth in Petridish in 13 days after inoculation while *C. indica* took 15 days. Similar observations have been made by other workers. Phutela and Phutela (2012) reported that *C. indica* produced thick mat of fluffy mycelium with maximum colony area of 63.6 cm^2 on potato dextrose agar and it was thick strandy mat with area of 60.8 cm^2 on Malt agar in 10 days of incubation. Krishnamoorthy *et al*. (2015) reported the time taken for full growth in mycelium of *Calocybe* in PDA and malt extract medium was 8-10 days.

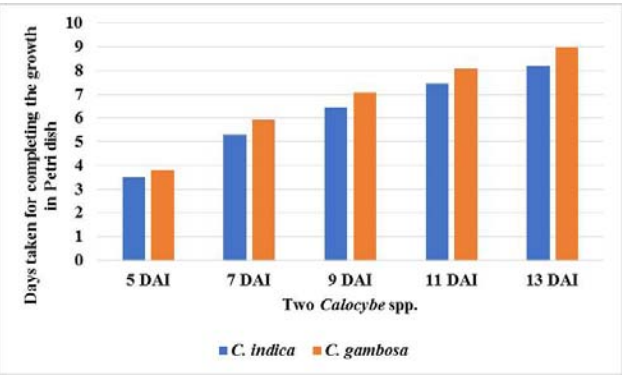

Fig. 1. Radial growth of the mycelia of *Calocybe* spp. at different DAI in PDA media

The microscopic observation on the mycelial characteristics indicated a higher mycelial width in *C. gambosa* (26.55 µm) while in *C. indica* it was only 17.95 µm. The size of basidia was 39.41 x 6.71 µm in *C. indica* when compared to 30.46 x 4.97 µm in *C. gambosa*. The basidiospores of *C. gambosa* were 11.61 x 9.03 µm while in *C. indica* it was comparatively small (9.74 x 8.39 µm). The cheilocystidia was larger in *C. indica* measuring 43.91 x 9 µm compared to *C. gambosa* (21 x 5.14 µm) (Table 2).

The growth of *C. gambosa* in mother spawn in paddy grains took about 25 days for complete

Table 1. Mycelial characters of *C.indica* and *C. gambosa* in PDA medium

***Calocybe* spp.**	**Nature of mycelial growth**	**Colour of mycelia**	**Mycelial growth rate (cm day^{-1})***
C. indica	Thick fluffy with smooth margin	Pure white	0.65 (0.81)^b
C. gambosa	Cottony with regular margin	Off white	0.73 (0.86)^a
SEm±			0.008
CD (0.05)			0.014
CV (%)			2.98

*Mean of seven replication; Value in parenthesis are square root transformed value

Table 2. Microscopical observations of mycelia, basidia and basidiospores of *Calocybe* spp.

Calocybe spp.	Mycelia*	Basidia*		Basidiospores*		Cheilocystidia*	
	Width (µm)	Length (µm)	Breadth (µm)	Length (µm)	Breadth (µm)	Length (µm)	Breadth (µm)
C. indica	17.95 (4.23)[b]	39.41 (6.28)[a]	6.71 (2.59)[a]	9.74 (3.12)[b]	8.39 (2.90)[b]	43.91 (6.63)[a]	9.00 (3.00)[a]
C. gambosa	26.55 (5.15)[a]	30.46 (5.52)[b]	4.97 (2.23)[b]	11.61 (3.41)[a]	9.03 (3.00)[a]	21.00 (4.58)[b]	5.14 (2.26)[b]
SEm±	0.76	0.61	0.16	0.14	0.08	0.40	0.24
CD (0.05)	2.346	1.881	0.479	0.433	0.262	1.250	0.740
CV (%)	9.05	4.62	3.55	3.48	2.58	3.31	8.99

*Mean of seven replication; Values in parenthesis represent square root transformed values; CD values mentioned are for transformed values; SEm± stands for standard error of difference of two means

substrate colonization while it was 30 days in *C. indica*. In the commercial spawn, *C. indica* took about 25 days and *C. gambosa* 20 days for complete colonization of the substrate (Table 3).

Table 3. Mycelial growth of *Calocybe* spp. in spawn (paddy grains)

Calocybe spp.	Days taken for complete substrate colonization in	
	Mother spawn	Commercial spawn
C. indica	30.36 ±1.10	24.82± 0.62
C. gambosa	24.86 ±0.69	20.07± 0.45
SEm±	0.35	0.21
CD (0.05)	1.074	0.64
CV (%)	3.28	2.42

Values are mean ± SD of seven replications

The spawn of *Calocybe* spp. were used for bed preparation using cocopeat as substrate (Fig. 2). The days taken for spawn run, pin head initiation and first harvest was lesser in *C.indica* i.e. 25, 37 and 44 days, respectively while in *C. gambosa* it was 31, 45, 53 days, respectively (Table 4). The sporophore characters varied widely between *Calocybe* spp. The yield characters indicated that *C. indica* produced more number of small sporophores (16) in comparison to *C. gambosa* (8). The average sporophore weight was higher in *C. gambosa* (109.3 g) than *C. indica* (38.1 g). The biological efficiency of *C. gambosa* was higher (83.1%) when compared to *C. indica* (61.2%) (Table 5). *C. gambosa* produced large fruiting bodies with a maximum pileus diameter and thickness (13.2 and 1.9 cm) and a stipe length of (17.8 cm). *C. indica* had a pileus diameter and thickness

Fig. 2. Yielding beds of (a) *C. indica* and (b) *C. gambosa*

Table 4. Days for spawn run, pin head formation and first harvest of *Calocybe* spp.

Calocybe spp.	Days taken for complete spawn run	Days taken for pinhead formation	Days taken for first harvest	Crop period (days)
C. indica	25.21±0.64[b]	37.21±1.87[b]	44.29±1.63[b]	59.86±2.19[b]
C. gambosa	30.79±0.79[a]	45.14±3.24[a]	52.57±3.69[a]	69.57±2.51[a]
SE(m) ±	0.24	0.99	1.08	0.89
CD (0.05)	0.74	3.08	3.32	2.74
CV (%)	2.27	6.42	5.89	3.64

Total yield from three harvest. Values are mean ± SD of seven replications.
Values followed by similar superscripts are not significantly different at 5 % level.

Table 5. Yield characters of *Calocybe* spp.

Calocybe spp.	No. of sporophores/ bag	Sporophore weight (g)	Total yield* (g/ kg)	Biological efficiency (%)
C. indica	16.14±4.10[a]	38.07±5.79[b]	615.86±9.96[b]	61.59
C. gambosa	8.0±2.08[b]	109.29±17.60[a]	847.86±25.47[a]	84.79
SE(m) ±	1.23	4.95	7.31	
CD (0.05)	3.79	15.25	22.52	
CV (%)	26.93	17.78	2.64	

*Total yield from three harvest. Values are mean ± SD of seven replications
Values followed by similar superscripts are not significantly different at 5 % level

Table 6. Sporophore characteristics of *Calocybe* spp

Calocybe spp.	Colour	Pileus			Stipe			Gill/cm^2
		Shape	Diameter (cm)	Thickness (cm)	Length (cm)	Diameter (cm)	Shape	
C. indica	Milky white	Convex	9.0 ±0.99	1.6±0.08	10.83±0.68	7.6±0.40	Clavate	24±2.16
C. gambosa	Creamish white	Convex	13.20±0.51	1.9±0.25	17.64±0.87	8.9±0.23	Clavate	19±1.79
SEm±			0.30	0.07	0.29	0.13		0.75
CD (0.05)			0.92	0.21	0.91	0.39		2.32
CV (%)			7.32	10.11	5.60	3.99		9.53

Values are mean ± SD of 10 sporocarps

of 9.0 and 1.6 cm, respectively. The stipe length was smaller than *C. gambosa* (10.8 cm). The maximum gills per cm was observed in *C.indica* (24) followed by *C. gambosa* (19) (Table 6) (Fig. 3).

Single spore isolates of *C. indica* (parent A) were assigned prefix A and those of *C. gambosa* (parent B) were assigned prefix B. Accordingly, 11 single spore isolates from each species were labelled as A1 to A11 (Fig. 4) and B1 and B11 (Fig. 5). The nature of mycelial growth, rate of mycelial growth and days taken for complete growth in Petri dish was recorded. The single spore cultures showed a wide variation in nature of mycelial growth from fluffy, cottony to sparse growth. The rate of mycelial growth ranged from 0.54 to 0.77 cm day^{-1}. A2 and A5 were fast

Fig. 3. Morphological characters of *Calocybe indica* (top) and C. *gambosa* (bottom): a. Pileus, b. Stipe, c. Gills

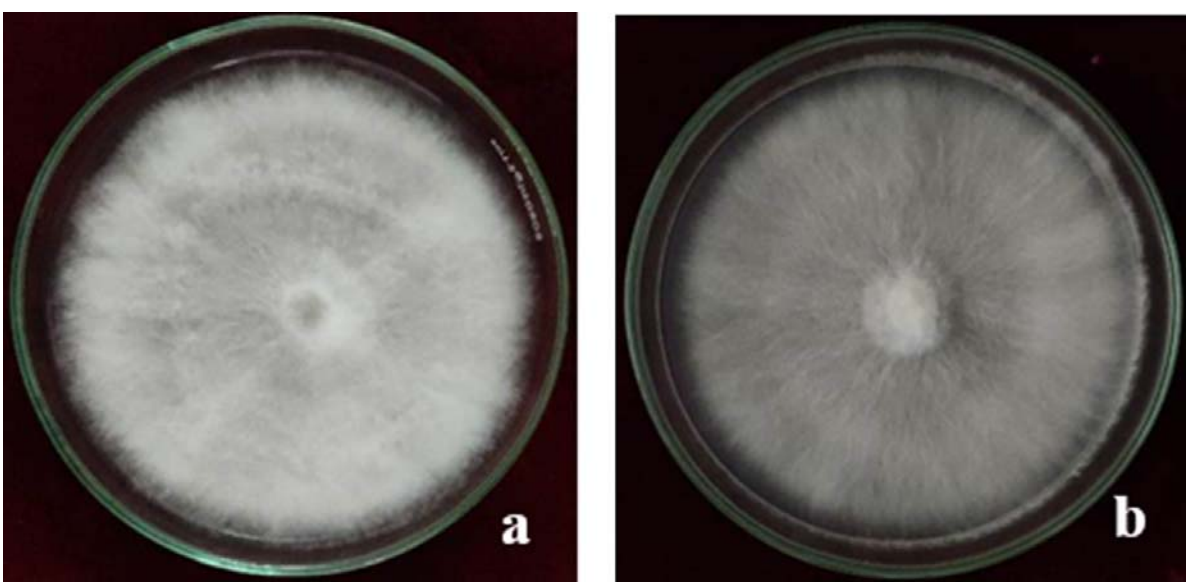

Fig. 4. Mycelial characteristics of *C. indica* and *C. gambosa* grown in PDA medium at 15 DAI (a) *C. indica* (b) *C. gambosa*

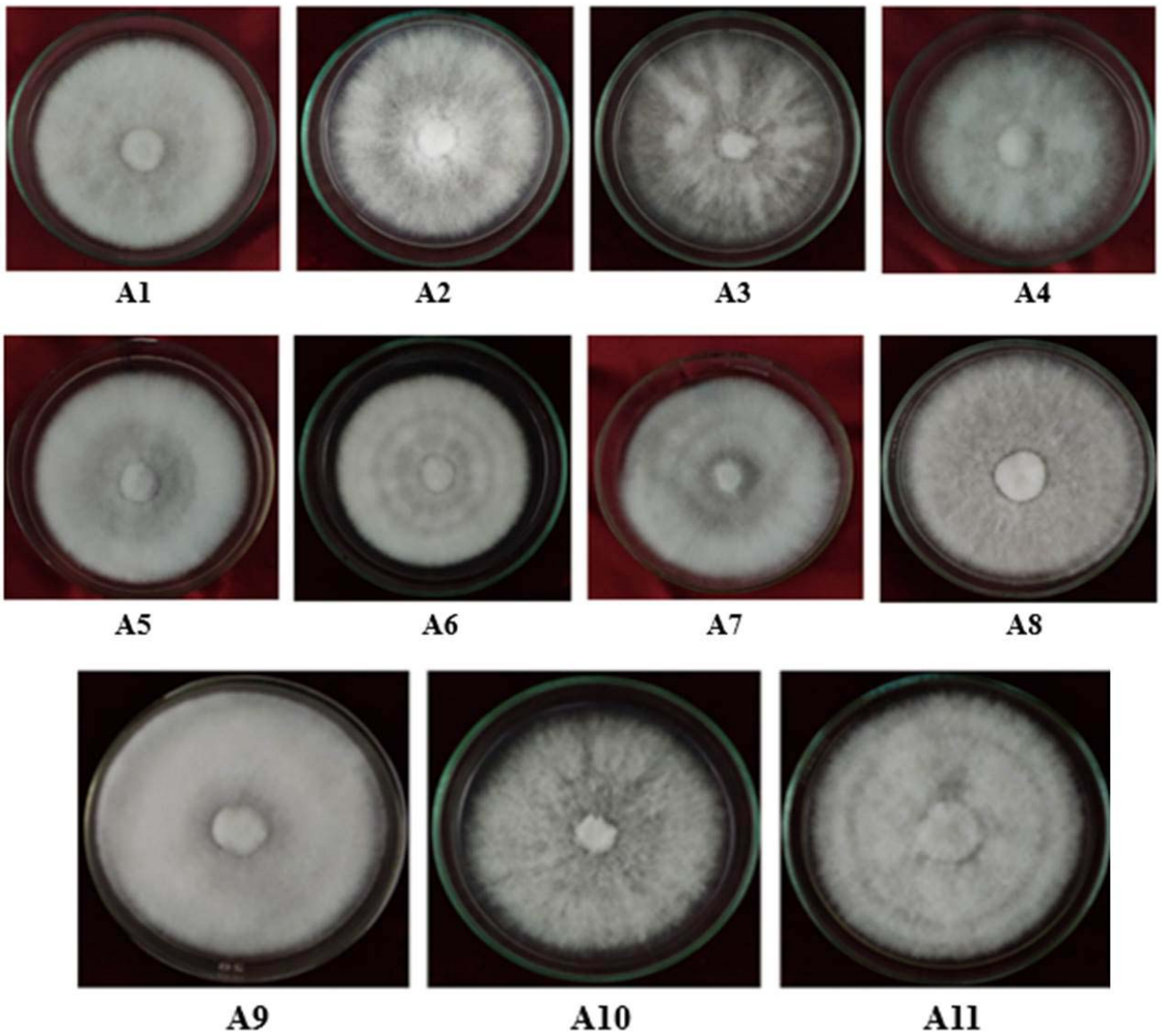

Fig. 5. Pure culture of single spores of *C. indica* (A1 to A11) in PDA medium 12 DAI

growers while A7 was a slow grower. The days taken for complete growth in Petri dish ranged from 12-17 days. Out of eleven monospores A2 and A5 took minimum of 12 days while A7 with 17 days for complete growth in Petri dish (Table 7). The monospores of parent (B) had fluffy, cottony or sparse mycelial growth. These monospores showed a mycelial growth rate ranging from 0.46 to 0.75 cm day^{-1}. The monospores B1 and B2 were fast growers with 0.75 and 0.69 mycelial growth rate, respectively. They took only 12 days to complete full growth in Petri dish when compared to B6 (19 days) (Table 8).

Out of 121 crosses done by dual culture technique between A1 to A11 with B1 to B11 by hyphal anastomosis, 42 were compatible as indicated by barrage formation (Table 9) (Fig. 6) and confirmed by presence of clamp connections while 79 crosses showed inhibition (Fig. 7 and 8). Twenty two selections were made based on the extent of barrage

Table 7. Mycelial growth characteristics of single spores of *C. indica* (Parent A)

Single spores	Nature of mycelial growth	Colour of mycelia	Rate of mycelial growth (cm day^{-1})	DTCP*
A1	Thick cottony with regular margin	Pure white	0.71 ± 0.008^{d}	13.00 ± 0.70^{d}
A2	Fluffy with irregular margin	White	0.75 ± 0.008^{b}	12.20 ± 0.45^{e}
A3	Sparse with radiating margin	Dull white	0.58 ± 0.008^{g}	15.90 ± 0.22^{b}
A4	Fluffy with regular margin	Pure white	0.63 ± 0.008^{f}	14.10 ± 0.74^{c}
A5	Cottony with regular margin	Pure white	0.77± 0.008^{a}	12.40 ± 0.42^{e}
A6	Thick cottony with concentric rings pattern	White	0.58 ± 0.008^{g}	16.10 ± 0.62^{b}
A7	Fluffy with irregular margin	White	0.54 ± 0.008^{h}	17.10 ± 0.32^{a}
A8	Thick fluffy with regular margin	Greyish white	0.73 ± 0.011^{c}	12.40 ± 0.29^{e}
A9	Fluffy with regular margin	Pure white	0.62 ± 0.008^{f}	14.28 ± 0.19^{c}
A10	Sparse with regular margin	Dull white	0.62 ± 0.008^{f}	14.18 ± 0.22^{c}
A11	Thick cottony with concentric pattern	White	0.68 ± 0.008^{e}	14.14 ± 0.49^{c}
SEm±			0.004	0.200
CD (0.05)			0.011	0.586
CV (%)			0.71 ± 0.008^{d}	13.00 ± 0.70^{d}

*Days taken for completion of growth in Petri plate

Table 8. Mycelial growth characteristics of single spores of *C. gambosa* (Parent B)

Single spores	Nature of mycelial growth	Colour of mycelia	Rate of mycelial growth (cm day^{-1})	DTCP*
B1	Cottony with regular margin	Dull white	0.74± 0.013^{a}	12.50 ± 0.64^{g}
B2	Fluffy with irregular margin	White	0.69 ± 0.016^{b}	12.40 ± 0.52^{g}
B3	Fluffy with regular margin	Milky white	0.64 ± 0.024^{c}	13.95 ± 0.37^{f}
B4	Cottony with regular margin	Creamish white	0.64± 0.024^{c}	14.25 ± 0.39^{ef}
B5	Cottony with regular margin	White	0.66 ± 0.019^{c}	14.00 ± 0.47^{f}
B6	Sparse with wavy margin	Greyish white	0.46 ± 0.016^{g}	18.80 ± 0.57^{a}
B7	Sparse with radiating margin	Dull white	0.61 ± 0.013^{d}	15.95 ± 0.72^{c}
B8	Fluffy with irregular margin	Pure white	0.59 ± 0.012^{e}	16.00 ± 0.79^{c}
B9	Cottony with regular margin	Dull white	0.54 ± 0.016^{f}	17.70 ± 1.20^{b}
B10	Thick fluffy with regular margin	Pure white	0.61 ± 0.016^{de}	14.90 ± 0.65^{de}
B11	Fluffy with concentric pattern	Pure white	0.61 ± 0.012^{de}	15.70 ± 0.48^{cd}
SEm±			0.008	0.294
CD (0.05)			0.022	0.838
CV (%)			0.74± 0.013^{a}	12.50 ± 0.64^{g}

*Days taken for completion of growth in Petri plate

Table 9. Hybridization of single spores of *Calocybe* spp.

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11
A1	-	+	-	+	++++	-	-	-	-	-	-
A2	+	+	-	+++++	-	+	+	-	-	+	+
A3	++	-	-	+	-	+++	-	-	-	+	+
A4	-	+++++	-	-	-	++++	-	-	+	++++	-
A5	++	-	+	-	++	-	-	-	-	-	-
A6	-	++++	+	++++	-	-	-	++++	-	-	-
A7	-	+	++++	-	-	-	-	-	-	-	-
A8	+	+	++++	-	++++	-	-	-	-	-	-
A9	+	-	-	-	-	-	++++	+++	-	+	-
A10	-	++++	-	++++	-	-	-	-	-	-	
A11	+++	-	-	++	-	-	-	+	-	++++	

+++++ Very thick barrage; ++++ Thick barrage; +++ Moderate barrage; ++ Thin barrage; + Very thin barrage; - No barrage
Combinations with very thin barrage were not selected for further studies

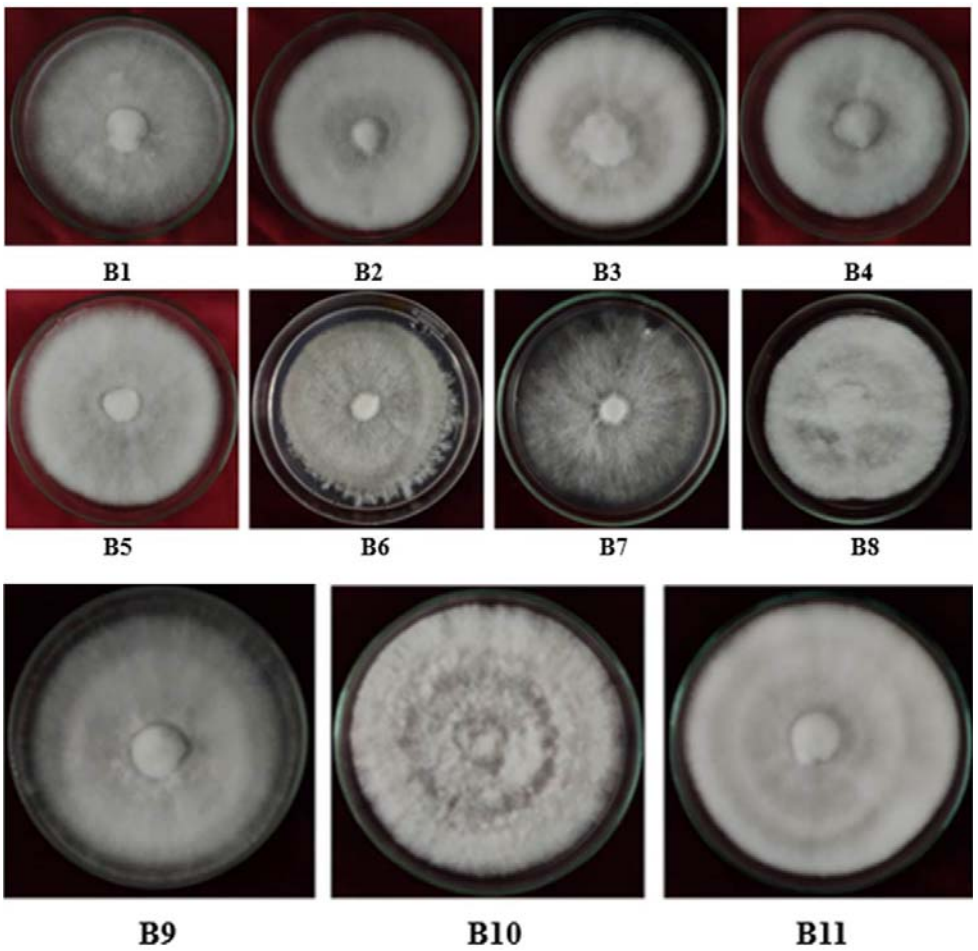

Fig. 6. Pure culture of single spores of *C. gambosa* (B1 to B11) in PDA medium 9 DAI

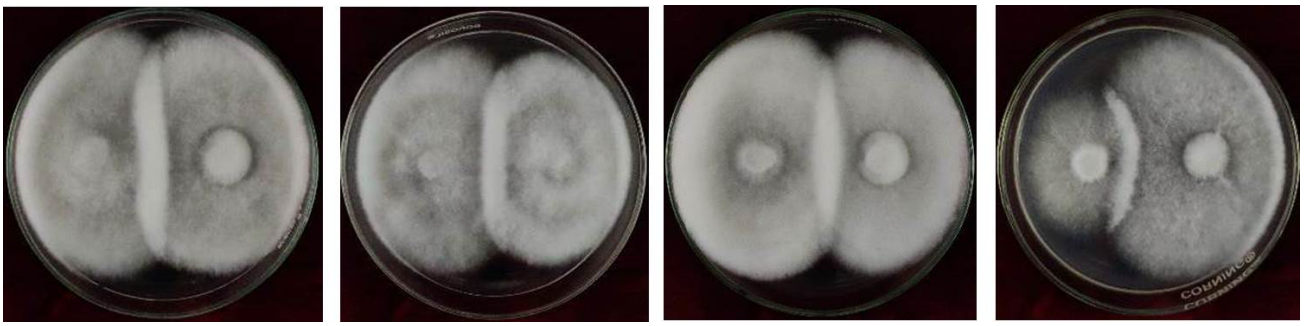

Fig. 7. Compatible crosses of *C. indica* and *C.gambosa*

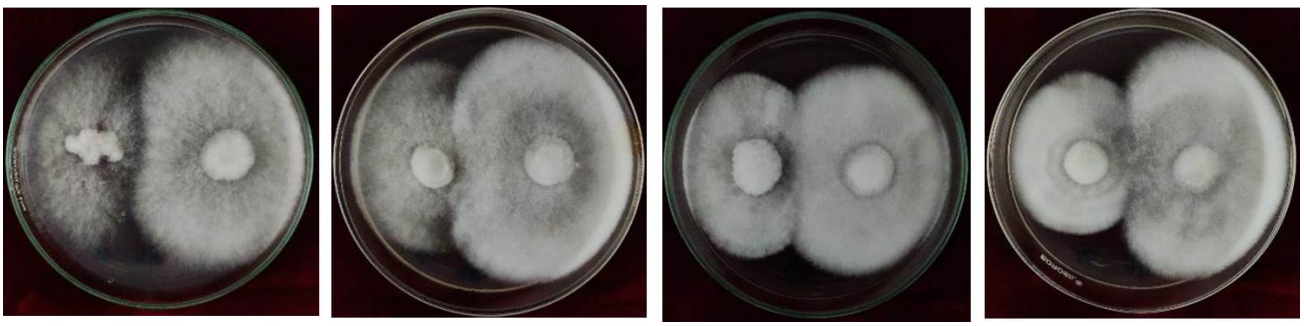

Fig. 8. Incompatible crosses of *C. indica* and *C. gambosa*

formation and the cross combinations showing very thin barrage were rejected. There are number of other reports on interspecific hyridization in other mushrooms. Gupta *et al.* (2011) intermated single spore isolates of *Pleurotus sajor-caju, P. florida, P. eous* and *Hypsizygus ulmaris* and developed inter-strainal hybrids of *Pleurotus* with desirable characters including less time for spawn run and higherbiological efficiency.

CONCLUSION

The pure culturing and morphological studies of cultures of the two *Calocybe* spp. revealed that the mycelia were septate with clamp connections. The mycelial growth was white fluffy with slow growth in *C. indica* whereas it was off white cottony mycelia with faster growth in *C. gambosa*. The mycelial width was larger in *C. gambosa*. Basidia and cheilocystidia were comparatively larger in *C. indica*. The size of the basidiospores were comparatively larger in *C. gambosa*. Mycelial run was faster in *C. gambosa* than *C. indica* in paddy grains when used as spawn substrate. During cultivation *C. indica* took less number of days for complete spawn run, pinhead formation, first harvest, and total crop period with comparatively more number of sporophores than that of C.*gambosa*. *C. gambosa* took more period for spawn run, days for pin head formation, days for first harvest with larger sporophores characterized by long stipe and greater sporophore weight that resulted in higher biological efficiency.

Of the 121 interspecific crosses, 42 showed dikaryotization as confirmed by presence of clamp connections. Isolate B9 was compatible with only one A isolate whereas isolate B2 showed highest compatibility and inter-mated with seven of the 11 isolates of *C. indica*. Variation in the type of barrage between different isolates indicates that the compatibility may not be complete and needs to be confirmed by growing these isolates.

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