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RESEARCH ARTICLE OPEN ACCESS

Comparison of Colony Morphology, Sporophore Characters and Yield Performance of Wild and Cultivated Milky Mushroom Isolates

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Abstract

The present investigation was carried out to identify morphology and yield performance of milky mushroom isolates for both the wild and cultivars. In this study, 17 wild isolates (CBE-TNAU-1513 to 1526, CBE- TNAU-1603, 1604 and CBE-TNAU-1701), seven cultivated strains (CI-13-02, 04, 06 and CI-14-02, 03, 04 and CI-14-06) and Tamil Nadu Agricultural University APK2 (*C. indica*) variety were compared. Colony characters of all the 25 isolates of milky mushroom grown on PDA medium were recorded. Among the isolates, CBE- TNAU-1523, CBE-TNAU-1603 and APK2 were found to be fast growing covering the maximum radial growth of 90 mm in Petri dish within 7 days. In order to find out the best performing wild isolate, the observations have been recorded with morphometric characters *viz*., days for spawn run (DFSR), days for pin head formation (DFPF), pileus and stipe measurements including the pileus: stipe ratio for all the strains were recorded. The complete spawn run was faster in APK2 (10.3 d). However, the mycelial impregnation in the casing soil was comparatively quick with the isolate CBE-TNAU-1515, which also reflected in early pinning with this isolate (8.4d). The strains *viz*., CBE-TNAU-1517, 1521, 1522, 1523 and APK2 possessed milky white, robust and companulate sporodomes having thick cylindrical stipe, which was found to be moderately bulged at the base. When selected based on yield attributes, significantly increased yield was obtained with the strain CBE-TNAU-1523 (972 g per bed with 194.5 per cent bio-efficiency).

Keywords: Colony morphology; wild isolate; milky mushroom; yield attributes; pileus: stipe ratio.

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INTRODUCTION

Edible mushroom species have been found in association with 13,000-year-old archaeological sites in Chile. The magnitude of fungal diversity was estimated to be at least 1.5 million species of which, 140,000 species produce fruiting bodies of sufficient size and suitable structure to be considered as macro fungi (Chang and Miles 1992). The man who lived between 3400 and 3100 BC in Europe was found with two types of wild edible mushrooms *viz., Boletus edulis* and *Tricholoma sp.* The first reliable evidence of mushroom consumption dates to several hundred years BC in China (Boa, 2004), they have found not only for nutrition and taste but also for their healing properties

Calocybe indica (P&C), commonly known as milky mushroom, introduced from India is highly suitable for cultivation in the warm humid tropical regions (Purkayastha and Chandra, 1976; Thakur and Singh, 2014). Sustainable yield, robust nature, alluring white colour, long shelf-life and potential market expansion of milky mushroom have attracted considerable attention by growers and consumers in India (Krishnamoorthy *et al.,* 1998; Chakraborty and Sikdar, 2010, Krishnamoorthy and Balan, 2015). The mushroom is exceedingly rich in protein, lipids, fibre, minerals, carbohydrates and contains essential amino acids (Mallavadhani *et al.,* 2006 and Alam *et al.,* 2008). Milky mushroom is also an admirable source of thiamine, riboflavin, nicotinic acid, pyridoxine and ascorbic acid. It grows well at a temperature range of 25-35°C with relative humidity more than 85 per cent (Krishnamoorthy, 1995; Pani, 2012). Therefore, it can be cultivated throughout the year in the tropical plains of India. Total commercial production techniques and the first milky mushroom variety *Calocybe indica* var. APK2 was introduced for the first time in the world by Krishnamoorthy *et al.* (1998).

Some of the tropical mushroom species belonging to the proposed new genus, have had a complex and confusing identification between the species, owing to the presence of same morphological characters (Pegler *et al.,* 1998). *Calocybe indica* has been placed under *Calocybe* – Sect. I. *Calocybe* (Guttatae) (Fr.) Sing. and belongs to the family Tricholomataceae of the order Agaricales (Singer, 1961). The hymenophoral trama, which is regular, but for the slight divergence below the hymenia. The pileopellis is filamentous and the basidia are carminophillic. Spores are thin walled, hyaline, which lack highly differentiated cystidia, although several cylindrical units with granular contents and clamp connections are present. This species is very close to *C. gambosa* (Fr.) Sing. differing in the slightly larger and broadly ellipsoid basidiophore. *C. eborina* reported by Pegler (1983) is also a pure white species whereas, *C. cyanocephala* (Pat.) Pegler and *C. cyanea* Singer ex Redhead & Singer contain violet or yellow pigments in the pileus, stipe and lamellae. Using DNA sequences, *C. cyanea* has been closely allied to *C. carnea*. *C. cyanea* appears to be more closely related to the temperate species, *C. onychina* (Fr.) Donk, typically found in coniferons forests in Europe and North America. Even though they are clearly different species, these two taxa share several similarities of macroscopic and microscopic features.

Calocybe bipigmentata (Singer) has dark reddish brown pileus; *C. alneti* (Singer) has convex-depressed pileus with a pale greyish brown disc and the stipe appears fuscous yellow; *C. coniceps* (Singer) has pale cream pileus with a greyish brown disc; *C. atropapillata* (Singer) has bluish grey pileus; *C. cyanocephala* (Pat.) Pegler has lamellae with violaceous or lilac hues (Singer, 1948). Appearance of *C. cyanea* from a xerophytic habitat in Mexico indicates the wide adaptability of the genus. Kost (1984) identified that the genus *Tricholoma colossum* (Fr.) Quɪl. forms large basidiomata, but differs from *Tricholoma staude* (Fr.) and its hemiangiocarpic development resulting in an annulate stipe, together with differences in the subhymenial layer, basidia and by the presence of true cheilocystidia and pleurocystidia. Furthermore, it lacks in clamp connections.

Inyod *et al.* (2017) indicated that differentiation of morphological characteristics of five isolates of *Macrocybe crassa* had been very difficult (DOA, DOA1, 4, 7 and 10) with that of *M. gigantea* or *Tricholoma giganteum.* However, they can be partially differentiated by the shape of stipes, which are cylindrical and swollen at the base. Presence of clavate basidia and basidiomata are almost similar in *M. crassa* (Berk.) Pegler & Lodge or *T. crassum* (Berk.), which completely

differed from *M. gigantean* (Massee). On the other hand, molecular analyses of all the five isolates revealed 57-99 percent similarity with *T. giganteum,* may be due to the limited ITS rDNA sequences of *M. crassa* available in the biological sequence data bases.

Macrocybe has been treated in *Tricholoma* until Pegler *et al.* (1998), who segregated *Macrocybe* from *Tricholoma* and ranked it to a genus status using morphological and molecular characteristics. Species of *Macrocybe* are saprophytic, large with clamped hyphae, while those of *Tricholoma* possess clampless hyphae and are obligatory ectomycorrhizal. Molecular analysis based on the larger sub-unit of rDNA could separate *Macrocybe* from *Tricholoma* which shows a close relationship with *C. indica* and *C. gambosa* (Pegler *et al.,* 1998; Razaq *et al.,* 2016). In this study, the objective was framed to identify the morphology and yield performance of milky mushroom isolates for both the wild and cultivars.

MATERIALS AND METHODS

Collection of cultures

The wild strains of milky mushroom *viz.,* CBE-TNAU-1513 to 1525, 1603, 1604 and 1701were collected from different habitats at various geographical locations in Tamil Nadu during 2014-2017. The collected isolates were pure cultured and the passport data for individual isolate was prepared based on their habit, habitat and morphological features. Along with these strains, the cultivated milky mushroom *Calocybe indica* var. APK2 (released from TNAU) and 8 different cultures obtained through AICRP on mushroom from the ICAR-Directorate of Mushroom Research, Solan, Himachal Pradesh were used in the experiments conducted as per objectives.

In vitro testing of growth

In vitro studies were carried out to study the growth characters of the strains. To 100 mL of sterilized PDA medium, 0.3 mL of 500 ppm streptomycin sulphate was added and thoroughly mixed. The medium was cooled to 45°C and poured into sterile Petri plates. After solidification 9 mm mycelial discs taken from an actively growing 7 d old culture were aseptically placed at the centre of the Petri dishes. The dishes were incubated at 30 ±2°C. The growth parameters like colony diameter, colony colour and morphology were recorded at regular intervals. The changes observed in the Petri dishes were recorded and photographed.

Cultural and agronomic traits

The cultures of 25 isolates including *C. indica* var. APK2 were inoculated on sorghum spawn. After complete colonization, they were used for bed preparation. In order to verify the yield and yield attributing characters of milky mushroom isolates comparison with the ruling var. APK2, poly house trails were conducted at Mushroom Research Laboratory, Tamil Nadu Agricultural University, Coimbatore. The mycelial cultures of isolates *viz.,* CBE-TNAU-1513, 1517, 1521, 1522, 1523 were inoculated on sorghum grain spawn. After complete colonization, mushroom beds were prepared following "polybag method" described by Baskaran *et al.* (1978) and Krishnamoorthy (2003). Polythene bags of 60 x 30 cm size and 100 G thickness were used for bed preparation and 500 g of paddy straw on dry weight basis was used. Layer spawning was followed using two per cent spawn rate. The beds were incubated at room temperature (30± 2°C) for spawn running and cropping. In the cropping rooms 80-90 per cent relative humidity was maintained. After 15 days, when the beds were fully colonized, they were cut into two equal halves and steamed casing soil (black loam soil, pH 8.3) was applied uniformly, up to 2cm over the spawn run beds. The moisture level at the casing surface was maintained by regular water spray. The bags were further incubated in a partially sunken poly house roofed with blue coloured high density polythene sheet. The observations on days for spawn run (DFSR), days for pinhead formation (DFPF), number of buttons formed per bed (NBF), number of buttons harvested per bed (NBH) after 30, 37 and 45 d and morphological characters like stipe length and breadth; pileus diameter and thickness of test strains were recorded and compared with the ruling variety APK2. Total yield per 180 g of paddy straw substrate, average weight of the individual sporophore and bio-efficiency were also recorded as given below:

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushrooms harvested / bed}}{\text{Dry used of the subtrate used / bed}} \times 100$$

Microscopic observations of morphometric characters

Microscopic observations on morphometric characters for selected milky mushroom isolates *viz*., CBE-TNAU-1519, 1523, 1701 were made and compared with the existing var. APK2 by placing a drop of the KOH or Congo red staining solution (one g in 100mL distilled water) directly, on the tissue surface of gills and hymenial layer. Observation on immediate and subsequent (3-5 min.) colour changes (Largent *et al.,* 1977) were recorded. The observation on the diameter of hyphae, basidiophore, basidiospore and cystidia were also made with the help of image analyzer (N-400T, Optika, Italy).

Table 1. *In vitro* assessment of colony morphology

Isolates	Radial growth (mm)			DTCP	Colony morphology on PDA
	3d	5d	7d		
APK2	45.5^{a}	86.0^{a}	90.0^{a}	7.3^{a}	Pure white, thick cottony growth, smooth margin
CI-13-02	32.6^{f}	79.5^{b}	89.3^{a}	8.6^{a}	White thick cottony, aerial, smooth margin
CI-13-04	28.6^{g}	65.1^{d}	85.4^{b}	9.3^{b}	Thick cottony with smooth margin
CI-13-06	29.8^{g}	61.3^{f}	84.3^{b}	9.6^{b}	Creamy white, cottony aerial growth
CI-14-02	18.6^{i}	61.2^{e}	78.1^{d}	10.6^{bc}	White radiating colony with wavy margin
CI-14-03	8.6^{k}	41.6^{g}	67.3^{e}	11.0^{bc}	Creamy white turns to dull white
CI-14-04	22.3^{i}	36.1^{h}	63.1^{f}	13.1^{c}	Pure white sectoring upward growth
CI-14-06	28.6^{g}	64.2^{de}	86.5^{ab}	9.0^{b}	Thick cottony, smooth margins
CBE-TNAU-1513	36.6^{d}	79.1^{b}	89.0^{a}	8.6^{a}	Pure white with wavy margins
CBE-TNAU-1514	26.3^{gh}	70.0^{cd}	88.4^{a}	9.6^{b}	White, aerial, thick cottony
CBE-TNAU-1515	39.3^{c}	74.3^{c}	84.1^{b}	9.1^{b}	Milky white, thick cottony
CBE-TNAU-1516	38.3^{c}	72.1^{c}	85.1^{b}	8.1^{a}	Thin cottony with smooth margin
CBE-TNAU-1517	37.6^{c}	74.4^{c}	86.1^{ab}	8.3^{a}	Thin cottony, aerial mycelium
CBE-TNAU-1518	35.3^{e}	68.1^{d}	79.4^{cd}	8.0^{a}	Creamy white, aerial mycelium
CBE-TNAU-1519	42.6^{b}	78.3^{b}	88.5^{a}	8.6^{a}	Slightly yellowish with aggregated mycelium
CBE-TNAU-1520	25.3^{h}	68.5^{d}	83.8^{b}	10.5^{bc}	Creamy white, aerial mycelium, radiating growth
CBE-TNAU-1521	33.3^{ef}	67.4^{d}	79.0^{d}	9.0^{ab}	White, aerial growth, sparsely aggregated
CBE-TNAU-1522	29.6^{g}	67.2^{d}	79.4^{cd}	9.6^{ab}	Thin cottony, aerial mycelium
CBE-TNAU-1523	46.6^{a}	83.1^{a}	90.0^{a}	7.3^{a}	Pure white, silk thread like, quickly aggregating
CBE-TNAU-1524	27.3^{gh}	81.2^{ab}	89.5^{a}	8.6^{a}	Thick cottony with smooth margin
CBE-TNAU-1525	15.0^{j}	59.0^{f}	83.1^{b}	10.4^{bc}	Thick cottony, upwards, smooth margin
CBE-TNAU-1526	14.6^{j}	61.3^{e}	79.6^{c}	9.0^{b}	White cottony, thin mycelium
CBE-TNAU-1603	45.2^{a}	82.2^{ab}	90.0^{a}	7.3^{a}	Thin cottony growth with smooth margin
CBE-TNAU-1604	28.3^{g}	59.5^{f}	81.2^{c}	10.1^{b}	Radiating colony with wavy margin
CBE-TNAU-1701	37.6^{c}	63.2^{e}	82.5^{c}	9.3^{b}	Dull white , sparsely aggregated mycelium
C.D(P=0.05)	1.42	3.10	3.40	1.58	

DTCP - Days taken to cover 90 mm Petri dish; DAI-Days after inoculation
Mean of 3 replications,
Means followed by a common letter are not significantly different at P = 0.05 by one way ANOVA.

RESULTS AND DISCUSSION

In vitro cultural and morphological characters

Colony characters of all the 25 isolates of milky mushroom grown on PDA medium were recorded. Among the isolates, CBE- TNAU-1523, APK2 and 1603 were found to be fast growing covering the maximum radial growth of 90 mm in Petri dish within 7 days (Figure 1). However, the linear growth of the isolates CI-13-02, CI-14-06, CBE-TNAU-1513, 1514, 1517 and 1519 were also found to be statistically on par covering approximately 86 mm of growth during the same

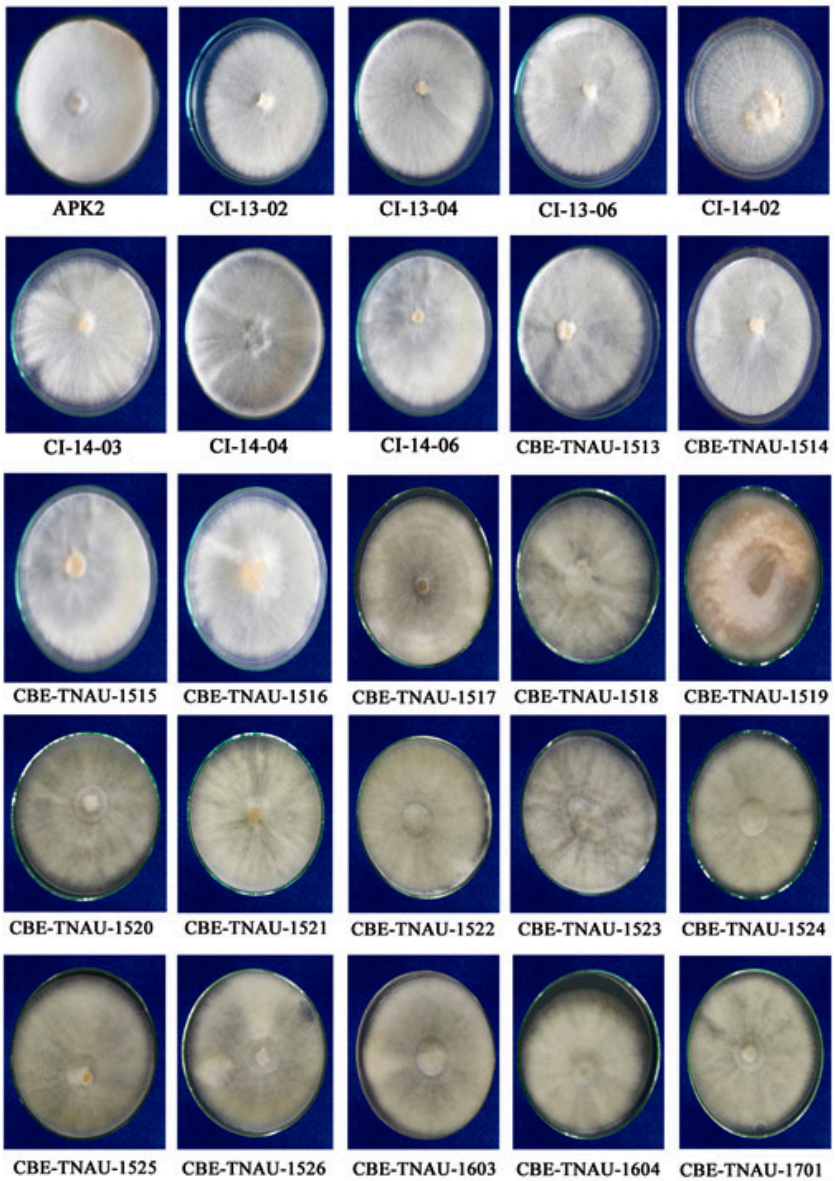

Fig. 1. Cultural characters of milky mushroom isolates on PDA medium. White thick cottony growth with more or less smooth margin was observed in APK2, CI-13-02, C1-13-04, CI-14-06, CBE-TNAU-1514, 1515 and 1517. Thick cottony slightly yellowish colony growth was prominent in cases of CBE-TNAU-1522 and 1524. Silk thread like meandering mycelium was observed with respect to CI-14-04, CBE-TNAU-1519, 1521 and 1523. The isolates *viz.,* CBE- TNAU-1523, CBE-TNAU-1603 and APK2 isolates were covering the maximum radial growth of 90 mm in Petri dish within 7 days.

Table 2a. Comparison of spawn run, pinhead formation and morphology of fruiting bodies

Strains	DFSR	DFPF	Elongation stage (3d)					Harvesting stage (5d)					Maturation stage (7d)				
			Pileus		Stipe		P:S ratio	Pileus		Stipe		P:S ratio	Pileus		Stipe		P:S ratio
			Dia. (cm)	T (cm)	L (cm)	B (cm)		Dia. (cm)	T (cm)	L (cm)	B (cm)		Dia. (cm)	T (cm)	L (cm)	B (cm)	
APK2	10.3^{a}	9.5^{ab}	3.0^{c}	1.3^{b}	4.1^{a}	2.3^{c}	1:1.4	4.1^{a}	2.3^{bc}	5.2^{a}	2.7^{b}	1:1.4	5.5^{a}	2.3^{a}	6.8^{ab}	3.4^{a}	1:1.4
CI-13-02	13.1^{d}	10.2^{b}	3.6^{e}	1.1^{a}	5.4^{cd}	3.1^{ef}	1:1.5	4.3^{b}	2.4^{c}	6.3^{c}	3.1^{c}	1:1.5	6.0^{a}	2.4^{ab}	7.5^{c}	4.5^{bc}	1:1.3
CI-13-04	12.1^{c}	11.3^{bc}	3.1^{c}	1.0^{a}	4.0^{a}	2.0^{a}	1:1.3	5.6^{f}	3.1^{e}	6.7^{d}	3.3^{c}	1:1.2	7.5^{f}	3.9^{d}	7.8^{cd}	4.2^{b}	1:1.0
CI-13-06	14.2^{ef}	10.2^{b}	3.5^{e}	1.5^{bc}	4.1^{a}	2.3^{c}	1:1.8	5.7^{f}	3.3^{f}	7.2^{e}	4.3^{e}	1:1.3	7.1^{ef}	3.5^{c}	8.1^{d}	5.1^{d}	1:1.1
CI-14-02	12.3^{c}	9.6^{ab}	3.3^{d}	1.2^{ab}	4.0^{a}	2.0^{a}	1:1.2	4.9^{d}	2.8^{d}	7.3^{e}	4.7^{f}	1:1.5	6.9^{e}	3.0^{b}	8.1^{d}	5.0^{cd}	1:1.1
CI-14-03	14.0^{e}	10.3^{b}	3.0^{c}	1.4^{b}	5.0^{b}	2.5^{d}	1:1.6	5.0^{d}	3.2^{ef}	6.8^{d}	3.8^{d}	1:1.4	7.0^{e}	3.4^{bc}	7.5^{c}	3.9^{b}	1:1.1
CI-14-04	15.3^{fg}	11.5^{c}	4.5^{i}	2.1^{d}	5.6^{d}	2.3^{c}	1:1.0	6.2^{h}	4.3^{i}	6.9^{de}	3.7^{d}	1:1.1	8.0^{h}	4.5^{ef}	8.2^{d}	4.2^{b}	1:1.1
CI-14-06	14.2^{ef}	10.7^{b}	3.2^{cd}	1.7^{cd}	4.3^{ab}	2.1^{ab}	1:1.3	4.7^{c}	2.8^{d}	6.7^{d}	4.3^{e}	1:1.4	7.7^{fg}	3.1^{b}	7.5^{c}	4.7^{c}	1.1:1
CBE-TNAU-1513	15.2^{f}	12.1^{cd}	3.2^{cd}	2.2^{de}	5.2^{c}	2.6^{de}	1:1.6	6.3^{h}	3.7^{g}	5.8^{b}	3.8^{d}	1.1:1	7.9^{h}	3.8^{d}	6.4^{a}	5.2^{d}	1.2:1
CBE-TNAU-1514	13.1^{d}	10.3^{b}	3.1^{c}	1.0^{a}	5.1^{c}	2.5^{d}	1:1.6	5.9^{fg}	2.8^{d}	8.0^{g}	6.3^{ij}	1:1.4	7.5^{f}	3.0^{b}	9.1^{f}	5.1^{d}	1:1.2
CBE-TNAU-1515	13.2^{d}	8.4^{a}	2.1^{a}	1.3^{b}	4.3^{a}	2.1^{ab}	1:2.0	4.7^{c}	4.2^{j}	7.6^{ef}	5.8^{hi}	1:1.6	6.7^{de}	4.4^{e}	8.2^{d}	5.8^{e}	1:1.2
CBE-TNAU-1516	14.6^{f}	10.7^{b}	3.9^{f}	1.4^{b}	5.8^{d}	2.6^{de}	1:1.5	4.8^{cd}	2.3^{b}	7.8^{fg}	5.7^{h}	1:1.6	7.5^{f}	2.5^{ab}	8.6^{e}	5.9^{e}	1:1.1
CBE-TNAU-1517	13.4^{de}	10.6^{b}	3.6^{e}	1.5^{bc}	4.7^{b}	2.4^{cd}	1:1.3	5.3^{e}	3.0^{e}	5.7^{ab}	2.3^{a}	1:1.0	6.3^{b}	3.2^{b}	6.8^{ab}	4.9^{cd}	1:1.0
CBE-TNAU-1518	12.2^{c}	11.1^{bc}	2.4^{b}	1.0^{a}	4.6^{b}	2.3^{c}	1:2.0	6.2^{h}	3.2^{ef}	8.3^{h}	6.8^{j}	1:1.3	7.5^{f}	3.5^{c}	9.3^{f}	5.2^{d}	1:1.2
CBE-TNAU-1519	14.1^{e}	10.3^{b}	3.6^{e}	2.0^{d}	6.2^{e}	2.6^{de}	1:1.8	5.8^{f}	2.7^{c}	5.3^{a}	5.2^{g}	1:1.1	8.5^{i}	2.9^{b}	7.4^{bc}	5.1^{d}	1.1:1
CBE-TNAU-1520	13.6^{d}	11.5^{c}	4.3^{gh}	1.2^{ab}	5.7^{d}	2.8^{e}	1:1.3	4.9^{d}	2.5^{c}	6.6^{d}	4.7^{f}	1:1.3	6.7^{de}	2.7^{ab}	7.5^{c}	4.7^{c}	1:1.1
CBE-TNAU-1521	14.1^{e}	10.3^{b}	2.1^{a}	1.3^{b}	4.5^{ab}	2.4^{cd}	1:2.1	4.1^{a}	2.1^{b}	6.8^{d}	4.9^{fg}	1:1.7	6.3^{b}	2.5^{ab}	7.8^{cd}	4.5^{bc}	1:1.2
CBE-TNAU-1522	13.6^{de}	9.8^{ab}	2.2^{a}	1.6^{c}	5.1^{c}	3.1^{ef}	1:2.3	3.9^{a}	1.7^{a}	6.2^{c}	5.3^{g}	1:1.6	5.7^{a}	2.3^{a}	7.2^{b}	4.5^{bc}	1:1.2
CBE-TNAU-1523	11.3^{b}	9.7^{ab}	3.2^{cd}	1.4^{b}	4.3^{ab}	2.4^{cd}	1:1.3	4.2^{ab}	1.5^{a}	5.4^{a}	3.9^{de}	1:1.3	5.9^{a}	2.0^{a}	6.6^{a}	5.3^{de}	1:1.3
CBE-TNAU-1524	14.6^{f}	10.3^{b}	3.4^{de}	1.0^{a}	5.9^{de}	3.3^{f}	1:1.7	4.8^{cd}	2.3^{b}	6.7^{d}	4.2^{e}	1:1.4	6.3^{b}	2.5^{ab}	7.1^{b}	5.1^{c}	1:1.1
CBE-TNAU-1525	14.0^{e}	10.8^{b}	4.2^{g}	2.3^{e}	4.6^{b}	2.3^{c}	1:1.0	5.0^{d}	2.7^{c}	6.8^{d}	4.8^{f}	1:1.4	6.4^{bc}	3.1^{b}	7.6^{c}	5.3^{de}	1:1.2
CBE-TNAU-1526	15.6^{g}	14.2^{d}	3.1^{c}	1.5^{bc}	5.2^{c}	3.0^{e}	1:1.7	4.6^{c}	2.3^{b}	7.7^{f}	5.5^{h}	1:1.7	6.2^{b}	3.0^{b}	8.5^{e}	5.9^{e}	1:1.4
CBE-TNAU-1603	16.4^{h}	12.7^{cd}	3.3^{d}	1.3^{b}	4.3^{ab}	2.2^{bc}	1:1.3	5.3^{e}	3.4^{f}	7.2^{e}	5.3^{g}	1:1.4	7.1^{ef}	3.5^{c}	8.4^{de}	4.6^{bc}	1:1.2
CBE-TNAU-1604	14.1^{e}	10.3^{b}	3.4d^{e}	1.6^{c}	5.6^{d}	2.3^{c}	1:1.6	5.0^{d}	2.9^{de}	7.3^{e}	6.1^{i}	1:1.5	6.4^{bc}	3.2^{b}	9.4^{f}	4.8^{c}	1:1.5
CBE-TNAU-1701	16.3^{h}	11.3^{bc}	4.1^{g}	1.3^{b}	5.1^{c}	2.0^{a}	1:1.2	6.3^{h}	4.0^{h}	7.9^{g}	5.8^{hi}	1:1.3	7.2^{ef}	4.1^{de}	8.7^{e}	5.1^{d}	1:1.2
CD=(P=0.05)	0.61	1.63	0.12	0.13	0.51	0.08		0.21	0.15	0.27	0.19		0.56	0.38	0.22	0.40	

DFSR-Days for spawn run; Dia.-diameter; T-thickness; L-length; B-breadth; P:S-pileus:stipe ratio

DFPF-Days for pinhead formation

Mean of 5replication

Means in a column followed by the same letter are not significantly different at P=0.05

Table 2b. Comparison of sporophore characters

Isolates	Growth characters						Basidispore dia (µm)
	Pileus	Stipe	Colour	Gills	Spore print	Basidiospores	
APK2	Campanulate	Robust cylindrical	Pure white	White	White	Golden yellow	1.2
CI-13-02	Convex	Robust cylindrical	White	Yellowish white	White	Brown	1.5
CI-13-04	Flattened	Cylindrical	Dull white	Yellowish white	White	Brown	2.1
CI-13-06	Flattened	Cylindrical	Dirty white	Yellowish white	Creamy white	Brown	1.4
CI-14-02	Convex	Robust cylindrical	Dirty white	White	Creamy white	Brown	1.8
CI-14-03	Convex	Robust cylindrical	Pure white	Yellowish white	White	Golden yellow	1.0
CI-14-04	Flattened	Cylindrical	Pure white	White	White	Light yellow	1.2
CI-14-06	Convex	Cylindrical	Creamy	Yellowish white	Creamy white	Brown	1.6
CBE-TNAU-1513	Flattened	Bulged, cylindrical	Pure white	White	White	Brown	1.5
CBE-TNAU-1514	Flattened	Cylindrical	White	White	Dull white	Golden yellow	2.0
CBE-TNAU-1515	Flattened	Robust cylindrical	Dirty white	Brownish white	White	Golden yellow	1.4
CBE-TNAU-1516	Convex	Cylindrical	White	Yellowish white	White	yellow	1.8
CBE-TNAU-1517	Campanulate	Robust cylindrical	White	White	White	Golden yellow	2.3
CBE-TNAU-1518	Flattened	Cylindrical	White	White	Dull white	Brown	2.1
CBE-TNAU-1519	Flattened	Cylindrical	Dull white	White	White	Brown	1.6
CBE-TNAU-1520	Convex	Robust cylindrical	White	Yellowish white	White	Golden yellow	1.8
CBE-TNAU-1521	Campanulate	Robust cylindrical	Dull white	White	Dull white	Yellow	1.4
CBE-TNAU-1522	Campanulate	Robust cylindrical	Dull white	White	White	Yellow	1.3
CBE-TNAU-1523	Campanulate	Robust cylindrical	Pure white	White	White	Golden yellow	1.7
CBE-TNAU-1524	Flattened	Cylindrical	Creamy	Yellowish white	Dull white	Brown	1.5
CBE-TNAU-1525	Flattened	cylindrical	White	Yellowish white	Dull white	Brown	2.4
CBE-TNAU-1526	Convex	Robust cylindrical	Creamy	White	White	Brown	1.7
CBE-TNAU-1603	Convex	Robust cylindrical	Pure white	White	Dull white	Golden yellow	1.5
CBE-TNAU-1604	Flattened	cylindrical	White	Yellowish white	Dull white	Golden yellow	1.3
CBE-TNAU-1701	Flattened	cylindrical	Creamy	Yellowish white	White	Brown	2.1

Table 3. Comparison of yield performance

Isolates	DFSR	DFPF	NBF	NBH	Average weight (g/button)	Yield (g/ 500g) of paddy straw	Bio-efficiency (%)	Pileus dia. (cm)	Pileus thickness (cm)	Stipe length (cm)	Stipe breadth (cm)	P:S ratio
APK2	15.1^{ab}	8.4^{ab}	35.2^{ab}	16.1^{a}	57.3^{h}	922.5^{b}	184.5^{bc}	6.6^{a}	1.8^{e}	8.2^{jk}	2.2^{h}	1:1.2
CI-13-02	18.0^{c}	9.2^{b}	22.1^{f}	10.2^{e}	75.1^{f}	776.0^{i}	155.2^{i}	7.7^{ef}	1.9^{ef}	7.8^{hi}	2.1^{g}	1:1.0
CI-13-04	23.6^{e}	10.1^{c}	36.3^{a}	10.3^{e}	85.1^{d}	876.5^{cd}	175.3^{cd}	8.3^{g}	2.1^{g}	8.4^{k}	2.2^{h}	1:1.0
CI-13-06	24.5^{e}	12.1^{e}	27.8^{d}	9.1^{f}	76.4^{f}	695.2^{jl}	139.0^{l}	7.0^{b}	1.8^{e}	7.4^{ef}	2.0^{f}	1:1.1
CI-14-02	15.6^{ab}	8.8^{b}	26.4^{e}	11.3^{d}	67.1^{g}	758.2^{i}	151.6^{ij}	7.0^{b}	1.6^{cd}	8.4^{k}	2.2^{h}	1:1.3
CI-14-03	16.1^{b}	9.6^{bc}	24.2^{ef}	10.5^{e}	83.1^{de}	872.5^{cde}	174.5^{cde}	7.6^{cde}	2.0^{f}	7.5^{efg}	1.8^{d}	1:1.0
CI-14-04	23.2^{e}	10.5^{cd}	15.5^{g}	7.1^{j}	74.1^{f}	526.1^{n}	105.0^{n}	7.0^{b}	1.6^{c}	7.9^{hi}	2.0^{f}	1:1.2
CI-14-06	25.5^{ef}	12.8^{ef}	22.4^{f}	8.3^{hi}	100.3^{a}	832.4^{fg}	166.4^{fg}	7.1^{bc}	1.5^{bc}	7.5^{efg}	1.8^{d}	1:1.2
CBE-TNAU-1513	26.2^{f}	13.5^{f}	13.1^{g}	12.1^{c}	59.1^{h}	715.1^{kl}	143.0^{kl}	7.7^{e}	1.9^{ef}	8.2^{jk}	2.1^{g}	1:1.2
CBE-TNAU-1514	24.6^{e}	12.3^{e}	26.0^{e}	9.0^{fg}	71.1^{g}	639.9^{m}	127.9^{m}	6.7^{ab}	1.5^{b}	6.9^{cd}	1.7^{c}	1:1.1
CBE-TNAU-1515	25.4^{ef}	11.9^{e}	34.8^{b}	10.1^{e}	83.1^{de}	839.4^{fg}	167.8^{fg}	7.7^{ef}	1.8^{e}	8.5^{k}	2.1^{gh}	1:1.1
CBE-TNAU-1516	13.2^{a}	7.6^{a}	22.5^{f}	10.3^{e}	87.1^{d}	897.1^{bc}	179.4^{bc}	6.8^{ab}	1.5^{bc}	6.7^{bcg}	1.4^{a}	1.1:1
CBE-TNAU-1517	14.4^{a}	8.0^{a}	35.4^{ab}	8.4^{hi}	101.4^{a}	851.7^{defl}	170.3^{def}	7.2^{c}	1.8^{de}	7.8^{hi}	2.0^{f}	1:1.1
CBE-TNAU-1518	23.2^{e}	11.2^{d}	31.7^{c}	13.3^{b}	54.4^{hi}	723.5^{k}	144.7^{kl}	7.7^{e}	1.7^{de}	7.6^{fgh}	1.9^{e}	1:1.1
CBE-TNAU-1519	15.6^{ab}	8.6^{ab}	37.1^{a}	15.9^{a}	53.1^{hi}	844.2^{dfg}	168.8^{efg}	6.3^{a}	1.7^{d}	6.6^{b}	1.9^{e}	1:1.0
CBE-TNAU-1520	15.5^{ab}	8.6^{ab}	25.1^{ef}	9.1^{f}	59.0^{h}	912.7^{b}	182.5^{b}	7.0^{b}	1.4^{ab}	7.7^{h}	1.7^{c}	1:1.2
CBE-TNAU-1521	14.4^{a}	7.8^{a}	25.5^{e}	10.4^{e}	81.1^{e}	843.4^{efg}	168.6^{efg}	8.4^{g}	1.9^{ef}	8.8^{l}	2.0^{f}	1:1.0
CBE-TNAU-1522	27.6^{f}	13.4^{f}	13.4^{g}	11.4^{d}	71.4^{g}	813.9^{gh}	162.7^{gh}	7.3^{c}	1.6^{c}	7.2^{dei}	1.4^{a}	1.1:1
CBE-TNAU-1523	15.1^{ab}	14.1^{g}	30.2^{cd}	16.8^{a}	57.9^{h}	972.7^{a}	194.5^{a}	7.7^{e}	1.7^{de}	8.0^{j}	2.0^{fg}	1:1.1
CBE-TNAU-1524	18.7^{d}	9.8^{c}	28.2^{d}	9.4^{f}	83.5^{de}	784.9^{hi}	156.9^{hi}	7.5^{cd}	1.5^{b}	7.2^{dei}	1.5^{b}	1.1:0
CBE-TNAU-1525	26.1^{f}	13.5^{f}	29.0^{d}	8.1^{i}	97.1^{ab}	786.5^{hi}	157.3^{hi}	7.1^{bc}	2.0^{f}	9.6^{n}	2.3^{i}	1:1.1
CBE-TNAU-1526	25.2^{ef}	12.4^{e}	31.4^{c}	8.5^{hi}	100.4^{a}	850.0^{def}	170.0^{def}	6.5^{a}	1.6^{c}	6.6^{b}	1.9^{e}	1:1.1
CBE-TNAU-1603	16.2^{b}	9.8^{c}	32.4^{c}	8.6^{gh}	91.5^{c}	786.9^{hi}	157.3^{hi}	6.3^{a}	1.3^{a}	6.2^{a}	1.5^{b}	1:1.1
CBE-TNAU-1604	17.3^{c}	10.4^{cd}	26.5^{e}	8.3^{hi}	78.1^{f}	830.8^{fg}	166.1^{fg}	7.3^{c}	2.0^{f}	6.6^{b}	1.9^{e}	1:1.0
CBE-TNAU-1701	24.5^{e}	12.6^{ef}	31.4^{c}	7.2^{j}	104.1^{a}	739.1^{jk}	147.8^{jk}	6.8^{ab}	1.4^{ab}	9.1^{m}	2.2^{hi}	1:1.5
CD(P=0.05)	1.30	0.52	1.10	0.47	3.27	32.6	6.40	0.28	0.08	0.27	0.08	

DFCR –Days for spawn run; DFPF-Days for pinhead formation;
NBF- Number of button formed;NBH- Number of button harvested; P:S-Pileus:stipe ratio
Mean of 5 replications,
Means in a column followed by the same letter are not significantly different at P = 0.05

Table 4. Comparison of microscopic and morphometric differences of milky mushroom isolates

Strain	Identified organisms	Siderophilous granulation	Cheilo cystidia	Clamp connections	Hymenial trama	Basidiophore (40x)		Basidiospore diameter (40X) in µm
						Dia. (µm)	Length (µm)	
APK2	*C. indica*	+	-	+	Regular	4.8	7.2	1.2-2.3
CBETNAU 1523	*C. indica*	+	-	+	Regular	5.4	8.4	1.3-1.8
CBE TNAU 1519	*T. giganteum*	-	+	-	Irregular	2.0	5.2	1.5-1.7
CBE TNAU 1701	*M. gigantea*	-	-	+	Regular or irregular	2.3	6.5	1.0-1.5

'+'presence or '-' absence

period of observation. The isolate CI-14-04 was found to be slow growing, which covered only 63.1 mm over a period of 7d (Table 1). White thick cottony growth with more or less smooth margin was characteristically observed with respect to the isolates APK2, CI-13-02, C1-13-04, CI-14-06, CBE-TNAU-1514, 1515 and 1517. Thick cottony slightly yellowish colony growth was prominent in cases of CBE-TNAU-1522 and 1524. Silk thread like meandering mycelium was found to be quickly aggregated either at the centre or in the margin of Petri dishes during the mycelial morphogenesis of CI-14-04, CBE-TNAU-1519, 1521 and 1523. Clamp connections in the mycelium were also frequently observed in the isolates *viz*., APK2, CBE-TNAU-1513, 1517, 1521, 1522 and 1523; whereas, in all other isolates clamp connections were observed occasionally. Such kind of varied growth in the culture for *Calocybe, Macrocybe* and *Tricholoma* has been indicated by Purkaysatha and Chandra (1974), Kalpana *et al*. (2005), Thiribhuvanamala (2011), Arun Kumar and Acharya (2014). Pegler (1983) also indicated colony colour variation of *Calocybe* spp. Kost (1984) indicated that clamp connections were absent in *Tricholoma* spp. Krishnamoorthy *et al.* (1998) reported that the time required for maximum mycelial growth of *C. indica* in culture media like potato dextrose agar or malt extract agar was 8 to10 days. Suman *et al.* (2018) reported that *Macrocybe gigantea* and *Calocybe indica* had produced fluffy and extensive aerial growth, which gave the appearance of cotton wool on PDA and MEA media.

Screening of isolates based on spawn run and fruiting body morphology

In order to find out the best performing wild isolate with respect to spawn run and fruiting body size, a preliminary trial was conducted using 1800 mL polypropylene bottles. The observations recorded with respect to spawn run and morphometric characters *viz*., days for spawn run (DFSR), days for pin head formation (DFPF), pileus and stipe measurements including the pileus: stipe ratio for all the strains tested are presented in Tables 3a, b and Figure 2a, b. Observations on DFSR showed that, complete spawn run was faster in APK2 (10.3 d). However, mycelial impregnation in the casing soil was comparatively quick with the isolate CBE-TNAU-1515, which was reflected

in early pinning with this isolate (8.4d). The results also showed variations with respect to DFSR (ranged from 10.3 to 16.4) and DFPF (ranged from 8.4 to 12.7) (Table 2a and Figure 2a, b). The strains *viz*., CBE-TNAU-1517, 1521, 1522, 1523 and APK2 possessed companulate robust sporophores, having cylindrical stipe, moderately bulged at the base. Tin these cases the pileus margin was found to be incurved. The pileus:stipe ratio at harvesting maturity (5d after pinhead formation) was found to be narrow in CI-14-04, CBE-TNAU-1513, CBE-TNAU-1519 (1:1.1), while it was found to be 1:1.4 in the cultivar. APK2 and 1:1.3 in case of CBE-TNAU-1523. In case of all other isolates, much variations existed with respect to pileus: stipe ratio (Table 2a). Although P:S ratio was observed to be narrower in some of the isolates, the pileus showed convex or expanded pileus with flattened margin (Table 2b). In such cases, the pileus thickness was more at the centre, which decreased towards the margin. Compact fruiting bodies with incurved pileus margin were conspicuously noticed in five of the *C. indica* isolates *viz*., CBE-TNAU-1517, 1521, 1522, 1523 and APK2. The colour of gills and spore print showed wide variations of white shade including creamy white, dull white and white with yellow tinge. The basidiospores were mostly brown or golden yellow and the diameter of the basidiospores ranged from 1.0 to 2.4 µm (Table 2b and Figure 2a, b). Pandey and Tewari (2003) also made comparison studies on the morphological characters and fruiting

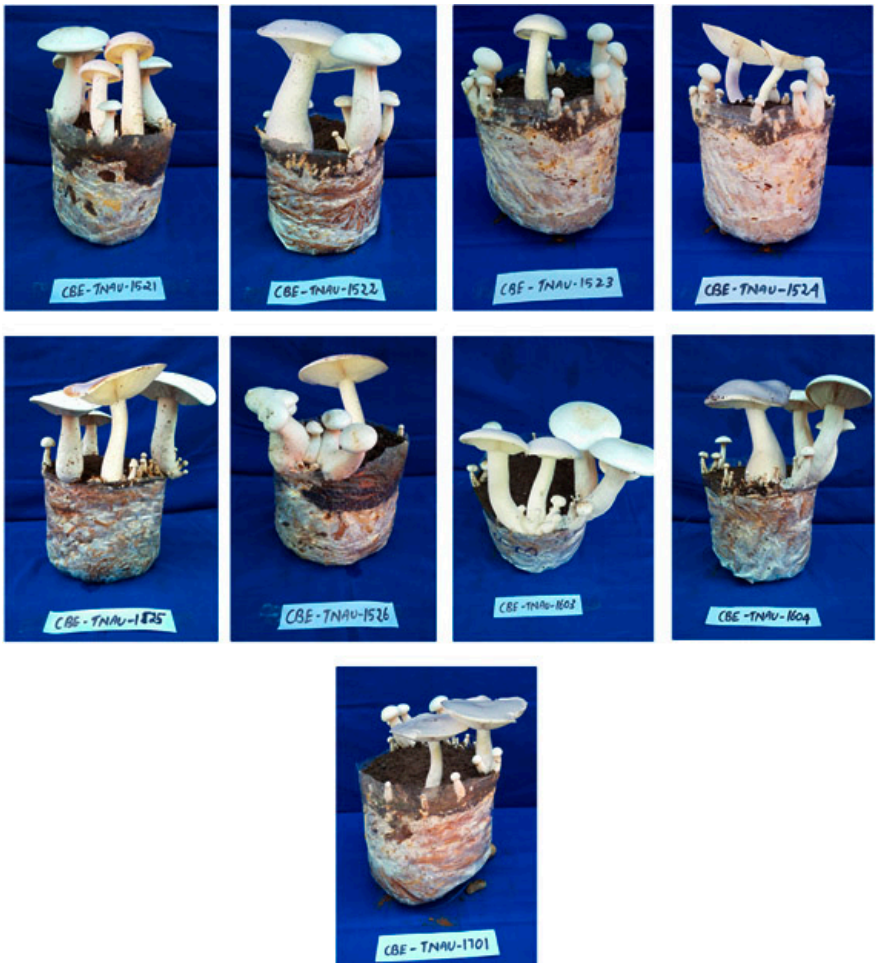

Fig. 2a, b. Morphological evaluation of milky mushroom isolates

body production of *Tricholoma giganteum* and *C. indica* and concluded that, both the species had a striking resemblance. The only difference noticed could be a very shallow stipe at the base and solitary appearance in *T. giganteum*, whereas sporophores of *C. indica* were growing in clusters with a slightly larger pileus. Contrarily, in the present stud, size wise observation in ascending order indicated *Calocybe, Tricholoma* and *Macrocybe*, respectively. Razaq *et al.* (2016) also indicated that milky mushroom (*Macrocybe gigantea*) was found to be fairly fleshy with large cap (7-8cm) fibrous stipe. In a similar study Singh *et al.* (2017) described about the morphological characters of *Calocybe* sp. They have reported that pileus size was found to be between 4.5 and 6.16 cm in diameter and produced convex to flattened fruiting bodies with appressed scales. Further they have observed that the surface of the stipe found to be dry and attached centrally, cylindrical at the apex with sub- bulbous base.

Screening the isolates based on yield performance

Gyurko (1982) indicated that the wild strains are usually vigorous, since nature is selecting only the vigorous to survive. It is not certain in all cases that, the wild strains are the best suited for our needs, because the aim of nature is different from that of human being. It is the matter of survival in nature and plenty of time is available for the organism to fit its level best to thrive. However, man wants to grow in a short time as much quantity of mushrooms

as possible. Kalpana *et al.* (2005) studied and compared the yield and flushing patterns of milky mushroom wild isolates with the cultivar APK2 and recorded the maximum yield and more number of buttons in WC2 wild isolate followed by WC6. During the present investigation, experiments were conducted to evaluate the performance of different strains, significantly increased yield was obtained with the strain CBE-TNAU-1523 (972 g per bed with 194.5 per cent bio efficiency). The number of buttons formed and harvested was 30.2 and 16.8, respectively. The average weight of individual mushroom in case of CBE-TNAU-1523 was 57.9g. The second best isolate was found to be APK2, which recorded 922.5 g of yield per bed (184.5 per cent bio-efficiency) with average weight of 57.3 g per mushroom. The var. APK2 produced a total number of 35.2 buttons, of which, only 16.1 attained the harvesting maturity. The performance of CBE-TNAU-1523 was also found to be statistically on par with that of APK2 (Table 3). Less number of buttons (10.2, 9.1, 7.1 and 7.2, respectively) and comparatively poor yields (776, 695, 526 and 739 g per bed, respectively) were noticed with the strains CI-13-02, CI-13-06, CI-14-04 and CBE-TNAU-1701. Gyurko (1982) indicated that the wild strains are usually vigorous, since nature is selecting only the vigorous to survive. It is not certain in all cases that, the wild strains are the best suited for our needs, because the aim of nature is different from that of human being. It is the matter of survival in nature and plenty of time is available for the organism to fit its level best to thrive. However, man wants to grow in a short time as much quantity of mushrooms as possible. Kalpana *et al.* (2005) studied and compared the yield and flushing patterns of milky mushroom wild isolates with the cultivar APK2 and recorded the maximum yield and more number of buttons in WC2 wild isolate followed by WC6.

Krishnamoorthy *et al*. (2000) and Krishnamoorthy and Muthusamy (1997) reported that the *C. indica* var. APK2 had a short crop cycle (7~8 wk) and produced good yield (140 kg fresh mushroom/100 kg dry paddy straw as substrate) with a biological efficiency of 140 per cent. Likewise, Tandon and Sharma (2006) and Bhatt *et al.* (2007) reported that wheat straw and paddy straw showed minimum days for spawn run and recorded good yield with higher biological efficiency. Sharma and Kumar (2008) evaluated the yield potential on different strains of *Calocybe indica viz.,* APK-2, CI-1, CI-3, CI-6 and CI-7 and

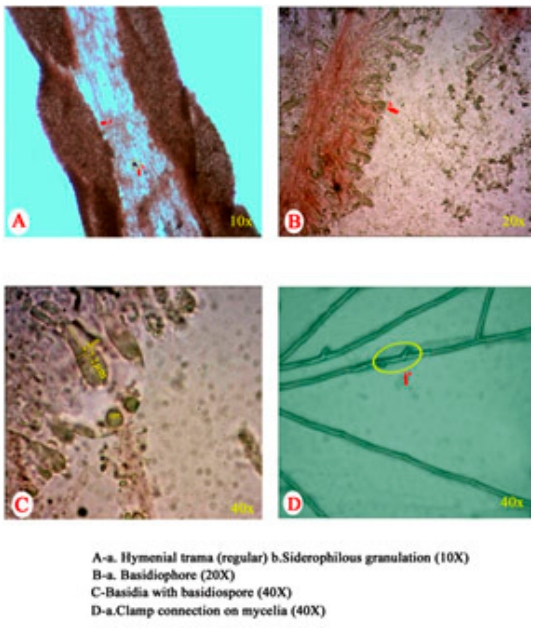

Fig. 3a. Microscopic observation of *Calocybe indica* (var.APK2)

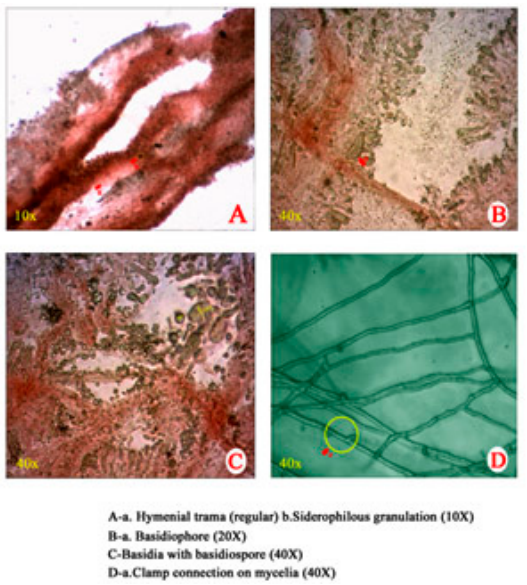

Fig. 3b. Microscopic observation of *Calocybe indica* (CBE-TNAU-1523)

recorded maximum biological efficiency with CI-6. They also reported that the highest average weight of fruiting bodies was obtained with APK2, which was found to be significantly higher than CI-6, CI-7 and CI-1. A similar study was also conducted by Dhakad *et al.* (2015), who evaluated the yield potential of different strains of *Calocybe indica viz.,* CI-4, CI-13, CI-14, CI-15 and CI-18 with wheat straw finally suggested that the strain CI-14 performed better with a yield potential of 811.3g kg^{-1} of dry wheat straw. When compared to this reports, it can be strongly concluded that, CBE-TNAU-1523 and APK2 could be the best strains for cultivation with paddy straw as substrate.

Microscopic observation

Calocybe indica has been placed under *Calocybe* – Sect. I. *Calocybe* (Guttatae) (Fr.) Sing. (Pegler, 1983). The hymenophoral trama was regular, but slight diversence was seen in the hymenium. The siderophileus granulation was highly present in basidia. Spores were thin walled, hyaline and creamy yellow to brown. Cystidia were not highly differentiated, although several cylindrical units with granular contents were observed. Clamp connections were also present in the hypha. The sporocarps were highly campanulate and robust. The genus has been classified under, *Tribus lyophylleae*, Fa: Tricholomataceae and Or: Agaricales (Singer, 1961). *Macrocybe* has been treated in *Tricholoma,* until it was segregated and ranked it to a separate genus using morphological and molecular characterization (Pegler *et al.,* 1998 and Razaq *et al.,* 2016). *Macrocybe* is also known as a tricholomatoid species with fleshy, cream to grayish, convex, umbonate to depressed basidiomata. However, Siderophilous granulation and cheilocystidia were absent in this species; whereas in *Tricholoma,* the cheilocystidia in basidia were highly prominent. This species possessed clamp connections in the hyphae.

Microscopic observations of mycelial characters and basidiospores of *C. indica*, *T. giganteum* and *M. gigantea* are presented in Table 4. The clamp connections and siderophilous granulation were noticed in *C. indica* (APK2 variety and CBE-TNAU-1523) and whereas, clamp connections and siderophilous granulation were absent in *T. giganteum* (CBE-TNAU-1519). *M.*

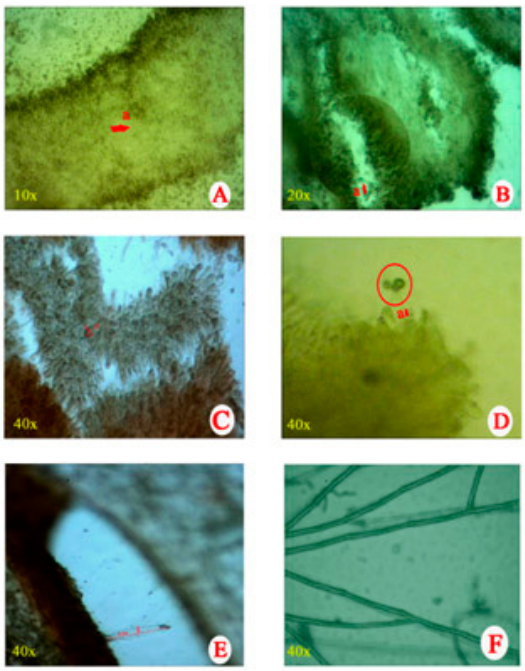

Fig. 3c. Microscopic observation of *Tricholoma giganteum* (Massee) (CBE-TNAU-1519)

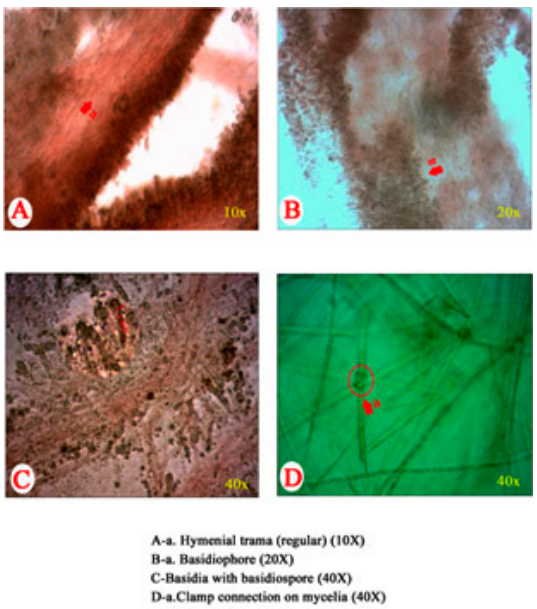

Fig. 3d. Microscopic observation of *Macrocybe gigantea* (Massee) (CBE-TNAU-1701)

gigantea (CBE-TNAU-1704) lacks siderophilous granulation in the basidia and cheilocystidia. The matured cheilocystidia were identified in *T. giganteum* (CBE-TNAU-1519) while, they were not seen in C*. indica* and *M. gigantea* isolates (Fig. 3a-3d) (Table 4).

CONCLUSION

The results clearly indicated that *C.indica* isolates always possessed companulate to highly companulate pileus with incurved margin; whereas, *T. giganteum* and *M. gigantea* could be differentiated by flattened to highly flattened cap. The stipe portion of *C.indica* isolates was moderately bulbous at the base and the stipe diameter was found to be gradually reduced towards the tip; whereas, in case of *T. giganteum* the pileus was highly bulbous at the base and reduced sharply, in width towards the apex. In case of *M. gigantea*, the stipe was stout and more or less cylindrical from base apex. Although P: S ratio was observed to be narrower in some of the isolates, the pileus showed convex to expanded margin with flattened margin. This kind of fruiting bodies with bigger size may face packaging and marketing problem. In general consumer preference is always for medium sized mushrooms weighing 40-45 g per button, having a narrow pileus and stipe ratio**.** Production of uniform fruiting bodies having defined pileus: stipe ratio is another challenge in milky mushroom cultivation. Further research will lead to identification of the gene activity and gene alteration through genome editing techniques to reduce the mushroom size and to improve the pileus and stipe ratio of milky mushroom.

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None.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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