

BIODIVERSITY EXPLORATION OF MILKY MUSHROOM (*CALOCYBE INDICA* P&C) - CONCEPT TO COMMERCIALIZATION

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ABSTRACT

Indian mushroom industry is witnessing a fabulous change in recent years with respect to the types and strains cultivated. *Calocybe indica* P&C popularly known as milky mushroom is relatively new to world mushroom lovers. This species is highly suitable for commercial exploration in the warm humid tropical zones around the world. Purkayastha and Chandra in 1974 [1] identified the fungus for the first time from India. Limited attempts were made in cultivation of this species until 1990s. During 1992 an isolate of *Calocybe indica* was collected near a coconut tree (*Cocos nucifera*) at Coimbatore, Tamil Nadu, India. This isolate was found to out yield hither to known cultivated mushrooms around the globe (with an average bio-efficiency of 142 per cent in paddy straw). Further, this tropical edible mushroom variety possessed incomparable shelf life. During 1994-1998 the technology was severally field tested under University Adoptive Research Trials (ART) and Multi Location Trials (MLT) involving mushroom farmers in the entire state. Finally, the technology for commercial cultivation and the new variety APK-2 has been introduced for the first time in the world from Tamil Nadu Agricultural University, Coimbatore, India during 1998. From the concept to commercialization, the journey was very tough and challenging. Systematic studies were undertaken during 1992-94 to standardize the physical, physiological, biochemical and cultural requirements for the commercial cultivation of milky mushroom. Continuing with the efforts through a research project funded by ICAR to develop milky mushroom hybrids (2003-2006); and also through ICAR - All India Coordinated Research Project on Mushroom (2000-2014), we have made sustained efforts to collect 25 wild isolates from different habitats and locations in Tamil Nadu. Many of these isolates were found to have mycorrhizal association with the finer roots of *Cocos nucifera*, *Borokus flabellifer*, *Peltaphorum ferrugenum*, *Delonix regia* and *Tamarindus indicus*. Sometimes they have also been found exclusively humicolous. Cultures of at least five isolates have been deposited in the National Repository (NCBI Gen accession No. AY636067) at the Directorate of Mushroom Research (DMR), ICAR, Solan, Himachal Pradesh, India. At present, the annual milky mushroom production in Tamil Nadu state alone is approximately 530 tonnes worth of Rs. 8.0 crore. More than 120 milky mushroom growers are distributed throughout the state. Horizontal spread of the technology is done through ICAR-AICRP, KVKs and State extension functionaries.

Keywords: Milky mushroom, *Calocybe indica* P&C, biodiversity, variety APK-2, commercial prospects

INTRODUCTION

Mushroom production is the best biotechnology process for integrated agro-waste management in rural areas. As an integral part of secondary agriculture, mushroom growing helps to create sustainable rural employment, in addition to addressing protein malnutrition. Indian mushroom industry is witnessing a tremendous change in recent years with respect to the types and strains cultivated. *Calocybe indica* P&C popularly known as milky mushroom is relatively new to world mushroom lovers. This species is highly suitable for commercial exploration in the warm humid tropical zones around the world. Purkayastha and Chandra in 1974 [1] identified the fungus for the first time from India. Limited attempts were made in cultivation of this species until 1990s. Milky mushroom (*Calocybe indica*) is a tropical edible mushroom with incomparable shelf life and yield, relatively new to the world mushroom lovers. The technology for commercial cultivation and the new variety APK-2 has been introduced for the first time in the world from Tamil Nadu Agricultural University, Coimbatore by Krishnamoorthy *et al.* during 1998 [2]. As on date, button mushroom (*Agaricus bisporus*) available in the market has certain common features with that of milky mushroom. However, cultivation of button mushroom requires huge investment on infrastructure, which is not normally affordable to small farmers. Although some seasonal growers in north India cultivate button mushroom, availability of quality compost and spawn is a major constraint. Further, this mushroom prefers to grow

only under low temperature conditions (15-20 °C). Hence, year around cultivation in a small scale is much difficult. Moreover, button mushroom needs to be carefully transported in cold chain to reach distant markets. Many a times, before it reaches the consumers, lose its original shape, becomes brown, flattened and the market quality is totally lost. Milky mushroom var. APK-2 has several advantages as compared to button mushroom. The technology is much flexible for a small farmer to a big industry. Obviously, there is no doubt that this Indian origin mushroom will have greater stake holders in the world market very soon.

MATERIALS AND METHODS

Field surveys have been conducted since 1992 during South West monsoon (June to September) and North East monsoon (October to December) months in the tropical plains of Tamil Nadu, India. Field data related to the habit and habitat of occurrence of milky mushroom was recorded. Collected specimens have been identified both by morphological and molecular techniques. Cultures have been maintained in PDA slants and some of them have been deposited in the National Repository maintained at DMR, Solan, Himachal Pradesh, India. Sorghum grain spawn was prepared and commercial cultivation techniques have been perfected over years (1992-1998) following standard methods described by Krishnamoorthy [9].

RESULTS AND DISCUSSION

Calocybe indica is popularly known as Dhuthichatta in India. Approximately 20 species of *Calocybe* have been described worldwide by Pegler [3] and Singer [4]. This genus has wide distribution from temperate to tropical climate, although it loves to grow under warm humid weather. *Calocybe* is mainly a grassland species, saprophytic in nature and sometimes ectomycorrhizal. Milky mushroom normally grows on humus soil on road side and forest areas. Almost about four decades ago, *Calocybe indica* was a wild edible mushroom reported from India by Purkayastha and Chandra [1]. Although success was achieved in the induction of fruit bodies of *C. indica* in culture by Purkayastha [5], only limited attempts were made on its commercial cultivation until 1998 by Purkayastha and Nayak [6]; Doshi *et al.* [7] and Pandey and Tewari [8]. Meanwhile, Krishnamoorthy [9] collected an isolate of *Calocybe indica* near a coconut (*Cocos nucifera*) tree adjacent to a sugarcane field near Coimbatore, Tamil Nadu, India. Pure cultures of the specimen were made and preliminary attempts have been made to perfect the commercial production techniques.

From the concept to commercialization, the journey was very tough and challenging. In fact, the initial laboratory work was started in the Mushroom Research Laboratory, Department of Plant Pathology, TNAU, Coimbatore during 1992 as a part of Ph.D thesis work by the author. Systematic studies were undertaken during 1992-94 to standardize the physical, physiological, biochemical and cultural requirements for the commercial cultivation of milky mushroom. With the limited literature scan (only seven at that time, all Indian work and most of the work was reported by Purkayastha and Chandra [1,5,6] from West Bengal, India); and also with minimum experience in working with edible mushrooms, several attempts were made to induce the fruiting bodies of milky mushroom with paddy straw as the basal substrate. More specific attempts were made to design the cropping room for cultivation. However, no significant achievement was possible. It seemed to be very complex. At this crucial stage, the first author was transferred and posted to one of the Regional Research Stations of TNAU at Aruppukottai, during the hot summer month of May-1994. The ambient temperature at that time was around 42 °C. Minimum facilities were available to continue the research work. Luckily, a 15'x12' size room with glass windows all around in the first floor of the residential quarters was available to continue the research. The side walls of the room were partly soaked due to the seepage of water from the overhead tank. Enough sunlight passed through the glass window in to the room from morning to evening. With all hesitation and gloomy mind set, some of the mushroom beds (at case running stage) transported from the TNAU main campus at Coimbatore were dumped inside the new and the only facility available. For the next ten days, the room was completely closed. Surprisingly, and as a blessing in disguise, robust mushrooms each weighing 0.5 to 1.6 kg with milky white colour; and definite shape were found growing on the casing surface. This was the turning point in commercializing milky mushroom technology. Till this stage, the actual cultural requirements viz., high temperature (30-35 °C); high humidity (>85%) and increased levels light intensity were not known to the research team. Later, the blue light requirement of the milky mushroom fungus was established which paved the way to design simple

Table 2. Wild isolates of milky mushroom collected during the survey (1992-2014)

Code No.	Habit and Habitat	Soil type	Weight (g)	Texture	Colour	Pileus		Stipe	
						Diameter (cm)	Thickness (cm)	Length (cm)	Bredth (cm)
WC1	Humicolous	Black loam	250	rough	brownish white	9.3	1.7	3.1	3.0
WC2	Mycorrhizal - <i>Cocos nucifera</i>	Black loam	75	smooth	milky white	4.6	1.8	2.5	2.5
WC3	Mycorrhizal - <i>Cocos nucifera</i>	Red loam	900	rough	pale to white	13.5	3.0	10.0	2.7
WC4	Humicolous	Clay	78	smooth	white	5.2	2.4	2.8	2.6
WC5	Humicolous	Clay	800	scaly	white	15.8	2.7	8.7	4.0
WC6	Mycorrhizal - <i>Cocos nucifera</i>	Black loam	85	smooth	dull white	5.4	2.5	3.0	2.8
WC7	Mycorrhizal - <i>Cocos nucifera</i>	Black loam	1100	scaly	white	19.7	2.0	6.5	6.7
WC8	Mycorrhizal - <i>Cocos nucifera</i>	Red loam	2010	scaly	white	37.0	3.4	15.0	6.5
WC9	Mycorrhizal - <i>Cocos nucifera</i>	Red sandy	1080	smooth	white	17.5	2.5	8.0	6.0
WC10	Mycorrhizal - <i>Borossus flabellifer</i>	Black loam	700	rough	white	11.4	2.5	9.7	2.4
WC11	Mycorrhizal - <i>Delonix regia</i>	Black loam	120	smooth	milky white	8.0	2.2	4.2	2.4
WC12	Mycorrhizal - <i>Cocos nucifera</i>	Red sandy	150	rough	white	5.4	3.3	3.8	3.0
WC13	Humicolous	Black loam	55	smooth	dull white	6.2	2.4	2.8	2.4
WC14	Mycorrhizal - <i>Tamarindus indicus</i>	Black loam	200	rough	white	5.5	3.0	3.0	2.5
WC15	Humicolous	Black loam	660	smooth	white	5.0	2.6	10.4	3.0
WC16	Humicolous	Red loam	510	scaly	white	5.2	3.0	3.6	2.5
WC17	Mycorrhizal - <i>Cocos nucifera</i>	Red loam	140	smooth	white	4.8	2.8	3.0	2.2
WC18	Mycorrhizal - <i>Cocos nucifera</i>	Red sandy	620	rough	dull white	10.0	3.2	12.1	5.2
WC19	Mycorrhizal - <i>Cocos nucifera</i>	Red loam	65	smooth	milky white	6.5	3.0	2.8	4.6
WC20	Humicolous	Black loam	720	smooth	white	7.0	3.2	8.4	3.5
WC21	Humicolous	Black loam	540	smooth	white	8.0	3.6	7.4	3.2
WC22	Mycorrhizal - <i>Peltaforum</i> sp	Black loam	85	smooth	white	3.5	2.0	3.0	2.2
WC23	Mycorrhizal - <i>Cocos nucifera</i>	Red loam	400	scaly	pale to white	7.5	3.2	6.5	2.9
WC24	Humicolous	Black loam	7500	smooth	dull white	47.0	5.4	42.0	4.4
WC25	Mycorrhizal - <i>Cocos nucifera</i>	Red loam	820	smooth	pale to white	12.5	3.0	7.0	3.2

commercial mushroom growing sheds with natural ventilation. The period during 1995 -1998 was very much crucial during which the technology was severally field tested under University Adoptive Research Trials (ART) and Multi Location Trials (MLT) involving several mushroom farmers in the state over years (1994-1998). Finally, the first ever variety of milky mushroom *C. indica* var. APK-2 was released for commercial cultivation during 1998 by Tamil Nadu Agricultural University, Coimbatore, India by Krishnamoorthy *et al.* [2, 10, 11].

Milky mushroom var. APK-2 was found to out yield hither to known cultivated mushrooms around the globe (with an average bio-efficiency of 142 per cent (Table 1) in paddy straw). Its milky white color and robust nature are appealing to all. Hence, it is known as “Milky mushroom”. Continuing the efforts through a research project funded by ICAR to develop milky mushroom hybrids (2003-2006); and also through ICAR - All India Coordinated Research Project on Mushroom (since 1982), we have made sustained efforts to collect more than 25 wild isolates from different locations in Tamil Nadu (Table 2). Many of these isolates were found to have association with the finer roots of *Cocos nucifera*, *Borusus flabellifer* and *Tamarindus indicus*. Pure cultures of at least five isolates have been deposited in the National repository (NCBI Gen accession No. AY636067) at National Research Centre for Mushroom (NRCM), ICAR, Solan, Himachal Pradesh, India (presently ICAR-Directorate of Mushroom Research). Some of these isolates were found to out yield APK-2 milky mushroom.

Table 1. Comparative yield performance of *Calocybe indica* var. APK2 in selected mushroom farms (MLT)

Name of the Mushroom Farm	Days for spawn run		Days for pinhead formation	Number of pinheads formed	Number of buttons harvested	Yield (g/ 500g of substrate)	Average weight (g/button)	Bio-efficiency (%)
	50%	100%						
A Prince 5 Mushroom Farm, Sooriampalayam,	7.51 ^a	14.54 ^a	23.67 ^{ab}	92.46 ^b	13.96 ^a	701.91 ^c	50.28 ^b	140.38
Sakthi Mushroom farm, Nasianur	7.86 ^{ab}	15.07 ^{ab}	24.12 ^b	82.59 ^c	12.78 ^{bc}	663.27 ^d	51.90 ^b	132.65
Maga Mushroom Farm, Coimbatore	9.52 ^d	14.54 ^a	23.17 ^{ab}	90.00 ^b	12.67 ^c	601.60 ^e	47.48 ^c	120.32
Annai Mushroom Farm Gopichettipalayam	8.43 ^c	15.39 ^b	22.94 ^a	93.47 ^b	12.85 ^{bc}	774.51 ^b	60.27 ^a	154.90
Sujji Mushroom Farm Kumarapalayam	7.94 ^b	15.22 ^{ab}	22.84 ^a	101.49 ^a	13.37 ^{ab}	817.50 ^a	63.28 ^a	163.50

Means followed by a common letter are not significantly different at five per cent level according to DMRT.

Mean of four replications

Panoramic view of a Milky mushroom farm variety with an average bio-efficiency ranging 167-187 % (Table 3). Milky mushroom is a close relative of the highly praised and excellent edible fungus known as St. George’s Mushroom (*C. gambosa* (Fr.) Sing.) in Europe. However, the sporophores of *C. indica* are attractive milky white in colour with incomparable shelf life (even up to 10 days without refrigerated storage). Commercial production of milky mushroom is much flexible from home growing to big industry.



Milky mushroom var. APK-2 released from TNAU, Coimbatore (1998)



A wild isolate (WC11) found to have mycorrhizal relationship with *Delonix regia*



Wild isolate (WC9) collected near *Cocos nucifera*



A wild isolate (WC24) weighing 7.5 kg



Small mushroom ! Big hope !!



Panoramic view of a Milky mushroom farm

Table3. Comparative yield performance of selected wild isolates of *C. indica* (2013-14)

Culture code	Days for spawn run		Days for pinhead formation	Number of pin-heads formed	Number of buttons harvested	Yield (g/ 500g of substrate)	Average weight (g/button)	Bio-efficiency (%)
	50 %	100 %						
WC 2	8.5 ^c	15.0 ^b	23.3 ^c	105.7 ^c	15.4 ^a	835.9 ^b	54.4 ^b	167.0
WC 6	8.0 ^b	15.0 ^b	22.6 ^b	106.5 ^c	15.7 ^a	909.8 ^c	58.1 ^c	182.0
WC 19	8.0 ^b	14.0 ^a	22.1 ^b	115.4 ^b	16.9 ^b	934.4 ^d	62.7 ^d	186.9
APK 2	7.5 ^a	14.5 ^a	21.5 ^a	145.1 ^a	15.3 ^a	738.5 ^a	48.3 ^a	147.7

Means followed by a common letter are not significantly different at five per cent level according to DMRT.

Mean values recorded in five different mushroom farms.

CONCLUSION

At present, the annual milky mushroom production in Tamil Nadu is approximately 530 t worth of INR 8.0 crore, More than 120 milky mushroom growers are distributed throughout the state of Tamil Nadu, India. Due to the horizontal spread of the technology through ICAR – All India Coordinated Research Project on Mushroom (functioning in 14 different centres in India including TNAU, Coimbatore with the headquarters at ICAR-DMR, Solan, HP) since 1998, the technology is spreading fast throughout the country for the past few years, especially in Kerala, Karnataka, Andhra Pradesh, Chhattisgarh, Orissa, Bihar and West Bengal. The average production capacities of individual units vary from 15 kg to 200 kg per day. In addition to regular growers, hundreds of home growers are also engaged in the production of milky mushroom throughout India. Regular training, hands on support and Agribusiness Incubation opportunities are provided by TNAU to the milky mushroom growers.

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