

Status of milky mushroom (*Calocybe indica*) in India-a review

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ABSTRACT

The ever increasing population particularly in third world countries is creating problems of food, nutritional security, health and environment. Increase in crop production is resulting in generation of huge amount of agricultural waste creating environmental pollution. These wastes can be used in mushroom production there by producing valuable food with better nutritional and medicinal values, employment generation and spent mushroom can be used to produce organic manure for agricultural and horticultural crops. Varied agro-climatic conditions and availability of agricultural and industrial wastes in India (>700 million tonnes) offer great opportunities for cultivation of different mushrooms on commercial scale. Introduction of new mushrooms is important to meet out the increasing public appetite for new and different mushrooms. In this context milky mushroom (*Calocybe indica*) has great scope in our country. Moreover, Indian population being vegetarian, consumption of mushrooms would certainly augment their diet which is deficient in proteins and minerals. Mushroom consumption can thus prove a boon to growing children as well as breast feeding mothers. Mushroom consumption has proved beneficial for the patients suffering from hypertension, high sugar and heart problems. Among the new mushrooms, milky mushroom is an important mushroom and is gaining popularity recently. It is fourth most largely growing mushroom in India and being tropical in nature, the mushroom is grown commercially in many parts of the country particularly in southern parts of India. This mushroom is most popular in Tamil Nadu.

Keywords Mushroom, *Calocybe indica*, production, Review

History

Calocybe indica P&C known as milky mushroom or dudh chatta or summer mushroom is a big sized delicious mushroom reported in India by Purkayastha and Chandra (1976). It is an attractive mushroom with large, milky white sporophores, belonging to the family Tricholomataceae of the order Agaricales. It grows in nature on humus soil under the road side trees in the forest. It is sold in city

and village markets collected from forests in West Bengal and liked because of its attractive robust white sporocarps, long shelf life and taste. It grows at a temperature range of 25-35 °C (Sharma *et. al.*, 2008). Natural occurrence of milky mushroom in plains of Tamil Nadu and Rajasthan has also been reported (Doshi *et. al.*, 1989; Krishnamoorthy, 1995). In spite of sincere efforts by different workers only limited success was achieved on the cultivation of this mushroom until 1998. Krishnamoorthy (1995) identified a

potential strain of milky mushroom occurring in a sugarcane field near Coimbatore and was later released as a new variety called APK 2. The best temperature optima for cultivating this mushroom are 30-35 °C. Some workers have reported that 32 °C was ideal for spawn run and fruit body initiation was more at 30 °C and 90% RH. Yield reduction was reported at temperature below 25 °C. Complete darkness less favoured fruit body formation and diffused light helped in the elongation of stipe. Strandy and vigorous mycelial growth of fungus was observed when it was incubated at 600-800 lux light intensity. Maximum yield were obtained when the cased beds were incubated at 1600 lux light. At higher light intensity stipe length was very much reduced whereas, pileus breadth increased substantially. During a survey conducted during 2009-2010 in south west monsoon season in Koliyoor area of Thiruvanthapuram district, Kerala, a new species of *Calocybe*, *C. gambosa* (Fr) Donk was collected and pure culture isolated. The sporophores have bigger sized pileus and club shaped stout and elongated stipe. Cultivation technology was standardized by polybag method on paddy straw and 1:1:1 sand-soil-vermicompost mixture as casing material. Average fruit body weight ranged from 250-620g. Farm trials conducted in ten locations of six districts of Kerala indicated the highest biological efficiency of 137.4% compared to 90.06% for *C. indica*. First flush appeared early (32day) in *C. gambosa* while it took 39.5 days in *C. indica*. A benefit cost ratio of about 3:1 was achieved owing to low cost substrates.

Milky mushroom was cultivated on four different agro-residues viz paddy straw, coirpith, wood shaving and banana trash.

The energy value of substrates worked out based on cellulose, hemicellulose and lignin content revealed highest energy value of 466 K Cal per 100 gm substrate in coirpith followed by banana trash (435 K Cal per 100 gm), paddy straw (391 K Cal per 100 gm) and wood shavings (380 K Cal per 10 gm values) (Usha, 2007).

Nutritive and medicinal value

Nutritive value of milky mushroom is comparable with other mushrooms. Mature fruit body of *C. indica* contains highest protein (17.2% on dry weight basis), while young pin heads contain the lowest proteins (15% on dry weight basis), 4.1% fat, 3.4% crude fibre and 64.26% carbohydrate on dry wt basis. Mature fruit bodies contain 4% soluble sugars, 2.9% starch and 7.43% ash. The fruit body contains 12 amino acids, namely, alanine, aspartic acid, glutamine, glutamic acid, glycine hydroxyl proline, histidine, lysine, threonine, tryrosine, valine, arginine, and proline. Out of all amino acids gluycine is predominant (10.8g/100g protein). In addition to this, it has all the mineral salts required by human body such as potassium, sodium, phosphorus, iron and calcium. Due to alkaline ash and high fibre content, it is highly suitable for people with hyperacidity and constipation (Doshi, *et.al.*, 1988). Mushrooms have been reported to have a therapeutic potential against several age related processes. Sivaprakasam *et al.*, (1986) and Doshi *et al.*, (1988), recorded 20.2% protein in milky white mushroom (on dry weight basis) while Krishnamoorthy *et al.*, (2000) reported 32.2% protein (dry weight basis) in milky mushroom. It is also reported that milky mushroom contains higher protein than button and oyster mushrooms (Krishnamoorthy *et al.*, 2000). This

mushroom has higher dry matter (14.4%) and fiber content (61.1%). Milky mushroom also contains higher sugars and fat (59.9% and 0.67%, respectively) compared to other edible mushrooms. Saranya *et al.*, (2011) reported that the type of substrates and supplements used for mushroom cultivation had greatly influenced the proximate composition including antioxidants. These authors also reported increased levels of calcium, phosphorus and iron in the milky white mushroom. Ragul (2013) reported that chitosan from *C. indica* sporophores ranged from 2.5% to 2.9% on dry weight basis. The beta-glycans present in dietary fibers of mushrooms are reported to have stimulatory effect on immune system with anti-mutagenic, anticancer and antitumor activities (Crisan and Sands, 1978). Good amount of minerals (Ca, K, Mg, Na, and P) and trace elements (Cu, Fe, Mn, and Zn) from milky white mushrooms (Mattila *et al.*, 2001; Zahid *et al.*, 2010).

Reactive oxygen species and free radicals causes a number of diseases such as rheumatoid arthritis, cirrhosis and life threatening diseases like cancer in the human body. There are vitamins, compounds and enzymes like vitamin E, C, polyphenols, carotenoids, glutathione, superoxide dismutase, catalase etc. help in neutralizing free radicals in the human system. Milky mushroom contains good amount of vitamin A (0.275mg per g) (Alam *et al.*, 2008), vitamin C (1.03mg /100 g) (Selvi *et al.*, 2007), vitamin E (tocopherol) (Mattila, 2000) (2.8 mg/g) and Glutathione (0.025 nmole/g). Vitamin C is a free radical scavenger and inhibitor of lipid peroxidation whereas Vitamin E is an antioxidant that protects membranes, lipids and lipoproteins. The most abundant non-protein thiol

(Phenolic compounds with sulfur in place of oxygen) in animal cells is glutathione, which exist is both reduced (GSH) and oxidized (GSSG) state. The GSH is essential for protein and DNA synthesis, regulation of enzyme activities and protection against free radicals (Selvi *et al.*, 2007). Mirunalini *et al.*, (2012) and Babu and Rao (2013), have reported in vitro antioxidant activities of *C. indica* extracts. The results showed higher DPPH scavenging activity, reducing power, chelation, and hydrogen peroxide scavenging activity in *C. indica* compared to *Agaricus bisporus*. Interestingly, the stipe of *C. indica* exhibited more chelation, hydrogen peroxide scavenging activity, flavonoid and total phenolic contents as compared to its cap.

Govindan *et al.*, (2014) evaluated the possible protective effects of milky mushroom fruiting body polysaccharides (CIFBP) against oxidative stress in streptozotocin (STZ) induced diabetic rats. Diabetes was induced in overnight fasted adult Wistar strain albino female rats weighing 150-180g by single intraperitoneal injection of freshly prepared streptozotocin (60mg/kg body weight) in 0.1M citrate buffer (pH 4.5) and NAD (110mg/kg body weight). Three days after STZ injection diabetic rats received CIFBP orally at the doses of 200 and 400 mg/kg daily for 30 days. The effect of CIFBP on glucose, glycosylated haemoglobin (HBA1C), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), glutathione reductase (GR), reduced glutathione (GSH), vitamin C, thiobarbituric acid reactive substances (TBARS) and hydroperoxide in serum, liver, kidney and pancreas were studied. The levels of glucose,

HBA1C, TBARS and hydroperoxide were increased significantly whereas; activities of enzymic and level of non-enzymic antioxidants were decreased in STZ induced diabetic rats. The antioxidant effect of CIFBP was compared with glibenclamide, a well known and anti-hyperglycemic drug. This study indicates that CIFBP possesses a significant favourable effect on anti-oxidant defense system in addition to its anti-diabetic effect.

Govidan *et al.*, (2014b) also investigated the protective effect of *C. indica* crude polysaccharides (CICP) against D-galactose induced cognitive dysfunction, oxidative damage and mitochondrial dysfunction in mice. Mice were subcutaneously injected with D-galactose (150mg/kg per day) for 6 weeks and were administered CICP simultaneously. Aged mice receiving vitamin E (100mg/kg) served as positive control. Chronic administration of D-galactose significantly impaired cognitive performance oxidative defence and mitochondrial enzyme activities as compared to control group. The results showed that CICP (200 and 400mg/kg) treatment significantly improved learning and memory ability in Morris water maze test. Biochemical examination revealed that CICP significantly increased the decreased activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), mitochondrial enzymes-NADH dehydrogenase, malate dehydrogenase (MDH), glutathione isocitrate dehydrogenase (ICDH), Na⁺, K⁺, Ca²⁺, Mg²⁺ ATPase activities elevated the lowered total anti oxidation capability (TAOC), glutathione (GSH), vitamin C and decreased

the raised acetyl choline esterase (AChE) activities, malondialdehyde (MDA), hydroperoxide (HPO), protein carbonyls (PCO), advanced oxidation protein products (AOPP) level in brain of aging mice induced by D-gal in a dose dependent manner.

In vitro and *in vivo* antidiabetic activity of milky mushroom exhibited significant results for its α -amylase (89.49 \pm 3.54% at 1.0mg/ml) and α -glucosidase activity (67.30 \pm 2.93% at 1.0mg/ml) in a dose dependent manner. The methanolic extract showed significant activity at tested dose level (200mg/kg b.w.), which was comparable to glibenclamide, a standard antidiabetic drug. The presence of phytochemicals namely phenols, flavonoides, saponins and tannins may be responsible for such antidiabetic activity. Results reveal that milky mushroom can be used as a potential antidiabetic agent (Prabu and Kumuthakalavalli, 2014). Chelladurai *et al.*, (2014) conducted studies to evaluate the *in vivo* cultivation technology, proximate composition, mineral content and spectrum analysis of edible milky mushroom of *Calocybe indica*. Moisture, crude protein, carbohydrate, dietary fibre, total lipids, ash, ether extract, pH, nitrogen and carbon content in mushrooms were analyzed. The results were found to be in 89, 14.9, 5.36, 8.02, 4.6, 7.05, 3.15, 5.4, 3.57 and 33.60% mg/100 g, respectively. The values of copper, manganese, zinc, iron, calcium, phosphorous, potassium and sodium content in mushrooms were found to be 0.44, 0.36, 0.05, 0.13, 0.51, 0.38, 1.35, 1.35 and 0.21 mg/100 g, respectively. Fourier transform infrared spectroscopy (FT-IR) spectrum of the mushroom indicated the presence of OH, COOH and NO₂ functional groups. The ultra-violet (UV) absorption

showed at 294 nm with a shoulder at 321 and 379 nm indicating the presence of aromatic nature of the compounds.

Investigation of the proximate composition and mineral content (Ca, P, Mg and Fe) was carried out on milky mushroom (*Calocybe indica*) cultivated on paddy straw supplement with 5, 10 and 20 per cent (on paddy straw dry weight basis) of ragi flour. Control was cultivated on paddy straw alone. Results showed that the proximate composition remained largely unchanged. However, only the five per cent treatment had mineral contents that were either significantly higher (Ca and P) or indifferent (Mg and Fe) from those of the control. The 10 and 20 per cent treatments had their content for phosphorus only being significantly higher than the control. Ca, Mg and Fe were significantly lower than the control in the 10 per cent treatment while Mg and Fe were significantly lower in the 20 per cent treatment. The study indicated that supplementation with ragi flour does not enhance the nutritional composition of milky mushroom significantly despite the richness of the supplement material used. But the findings reaffirmed the findings of others that mushroom in general and milky mushroom in particular is a high protein and low fat product (Kamugisha and Sharan, 2005).

Chandravadana *et al.*, (2005) analysed the volatile flavour composition of dry milky mushrooms (*Calocybe indica*) by capillary GC and compared with that of fresh mushrooms. A total of 20 components were identified. Drying significantly reduced the concentration of 1-octen-3-ol, n-octanol and 3-octanone and increased the concentration of n-hexanal; 2,4-decadienol; 2,4-nonadienol;

2-octen-1-ol; 1-hexanol; decanol and linalool oxide.

Volatile compounds

Mushrooms produce a number of volatile compounds varying with the species, variety and sometimes cultural conditions (Rapior *et al.*, 1996; 1997). The flavor profile also changes when mushrooms are dried, primarily due to the high level of oxidation (Morath *et al.*, 2012). These volatile compounds include alcohols, aldehydes, ketones and oxides (Beltran-Garcia *et al.*, 1997; Jennings and Shibamoto, 1980; Picardi and Issenberg, 1973). In *C. indica*, a total of 20 compounds have been identified (Chandravadana *et al.*, 2005). 1-octen-3-ol (58.3%) and n-octanol (17.9%) of the total volatile fractions are the two of the most abundant compounds present in fresh *C. indica* sporophores. In addition, eight carbon volatiles including 1-octen-3-one, 3-octanone and 3-octanol have been also reported.

Morphology and molecular characterization

Calocybe is a small genus of about 40 species of mushroom (Kirk *et al.*, 2008), which is edible and is cultivated in India. The name is derived from the Ancient Greek terms kalos “pretty”, and cubos “head” (Nilson and Persson, 1977). Around nine species of *Calocybe* are found in neotropical regions. Sixty accessions of the specialty mushroom germplasm maintained in the ICAR-DMR, Solan repository were characterized using DNA fingerprinting techniques. Phylogenetic analyses based on Random Amplified Polymorphic DNA profiles and direct sequencing of 5.8S rRNA gene region revealed intergeneric, inter and intra specific variations in *Volvariella*,

Lentinula, *Ganoderma* and *Calocybe* groups of the accessions. Multiple sequence alignment of all the accessions within species exhibited polymorphism in ITS-1 and ITS-II but not in the conserved 5.8S rRNA gene regions. In all four types of *V. volvacea*, two types each of *Lentinula*, *Ganoderma*, *Calocybe* and one type of *Trametes versicolor* sequences were obtained (Singh, *et al.*, 2003). Shekar and Singh (2014) established phylogenetic relationship among the eleven commercially cultivated edible mushrooms, namely, *A. bisporus*, *A. bisporus* (Portobello), *P. eryngii*, *L. edodes*, *H. tessellates* (Brown shimeji), *H. tessellates* (white shimeji), *F. velutipes*, *P. ostreatus*, *P. djamor*, *C. indica* and *P. florida* using RAPD markers. Mushrooms varieties were also screened for phytochemicals such as cardiac glycosides, anthraquinones, terpenoids, proteins, flavonoides, saponins, tannins, lignins and phenol. All the samples showed positive results for terpenoids and proteins and showed negative result for anthraquinones, flavonoides, tannins, lignins and phenol. Most of the samples found positive for cardiac glycosides and saponins.

Chakraborty and Sikdar (2010) focused on the production of somatic hybrid sporophores through PEG-mediated protoplast fusion between *Calocybe indica* var. APK2 and *Pleurotus florida*. They used NaCl tolerance to screen the hybrid strains. Basidiocarps could be successfully generated from eight out of fourteen hybrid lines that were maintained in culture. Hybridity of the fusant lines was established on the basis of their colony morphology, mycelial growth rate and hyphal traits, while the fruit-body-generating lines were demarcated on the basis of nature of sporophores, isozyme and RAPD markers. On the basis of RAPD profiles of the fusant

lines were classified into the microgenome and macrogenome insertion types. Notably, *P. florida* was genetically distant from the hybrid lines, while *C. indica* was phylogenetically the dominant parent. Significant increase in bio-efficiency and α -linoleic acid content in these hybrid lines indicated quantitative as well as qualitative improvement of the newly developed somatic hybrids.

Physiology and biochemistry

Upadhyay and Tripathi (2014) estimated the influence of temperature on biomass production, change in pH, total protein production, laccase, tyrosinase, aryl alcohol oxidase (AAO), manganese peroxidase (MnP), lignin peroxidase (LiP) and versatile peroxidase (VP) activity on wheat straw medium, Saborauds medium and modified Czapek Dox medium (10g/l glucose) after 6,12,18 and 24 days. *C. indica* produced versatile peroxidase in all three culture medium and maximum quantity was recorded on wheat straw medium after 12 days at 35 °C. Maximum protein was recorded on straw medium and least in Czapek Dox medium. Wheat straw medium produced maximum laccase after 24 days at 30 °C (187.38u/ml). Maximum biomass was recorded on Saborauds medium at 30 and 35 °C after 24 days.

Raina *et al.*, (2014) determined the contents of vitamin D2 and sterols in some wild and cultivated mushrooms. Four mushrooms *C. indica*, *G. lucidum*, *P. florida* and *V. volvacea* were grown on two synthetic and three semi-synthetic media. The mycelial biomass of each mushroom was subjected to extraction of ergosterol and its identification using high performance liquid chromatography (HPLC). The ergosterol content ranged from 113 to 403mg/g with lowest retention peak was observed in *P.*

florida showing 113mg ergosterol per gram whereas, *C. indica* showed 243mg ergosterol per gram. Highest retention peak was observed in *G. lucidum* showing 403 mg ergosterol per gram of sample.

Mushrooms are considered to be natural nutraceuticals and are cultivated for both edible and medicinal purposes. Many edible mushrooms possess enriched proteins and some medicinal properties such as antibacterial, antifungal, antiviral, and anti-AIDs. Based on both nutritional and medicinal properties, Karuppaiya Periasamy (2005) focused on the antibacterial substances and their efficacy. Antibacterial substances were isolated from culture filtrates, fresh mycelia, and dried fruiting bodies (basidiomata) of an Indian milky mushroom, *Calocybe indica*. Chandry (Tricholomataceae) and an oyster mushroom, *Pleurotus ostreatus* (Jacq.:Fr.) P.Kumm. (Pleurotaceae) (Purkayastha and Chandra, 1974). The antibacterial activity against some human pathogenic bacteria, such as *Bacillus* spp. *Escherichia coli*, *Vibrio cholerae*, and *Salmonella thiphi*, was studied and the maximum activity was observed in the dried fruiting bodies of *Calocybe indica*, extracted with the solvent ethyl acetate, followed by *Pleurotus ostreatus*. Two different compounds of blue and green colour with Rf values of 0.86 and 0.95 could be separated by thin layer chromatography and are worth future analyses by mass spectrum and nuclear magnetic resonance (NMR).

Cultivation Technology

Spawn preparation

Spawn for milky mushroom can be prepared following standard technology on

wheat grains (Joshi and Sagar, 2016). Cultivation technology was illustrated by Theradimani *et al.*, (2001). Milky mushroom, *Calocybe indica*, with its ability to grow fairly at high temperatures with excellent shelf life seems to be the best alternative to such mushrooms. Due to this promising ability, an experiment was conducted to find the suitability of different grains as spawn substrates and their effect on yield parameters of *C. indica* (Senthilnambi, *et al.*, 2011). The results revealed the supremacy of sorghum grains as the most suitable substrate for early spawn run, yield and number of buttons harvested followed by ragi grain spawn.

Substrate, supplementation and processing

A variety of substrates were tested for the cultivation of *C. indica* (Purkayastha, and Chandra, 1976; Purkayastha and Nayak, 1979). They tried to induce fruit bodies in a number of growth media, including soil-sand, soil-sand-maize meal and soil-sand-pulse powder. Attempts were made to develop suitable substrate for higher production of *C. indica*. Purkayastha and Nayak (1981) grew *C. indica* on unsterilized, paddy straw-maize or wheat bran substrate. Purkayastha (1984) used chopped rice straw, pre-soaked for 18 to 24 hr in water and put in hot water for 2-3 hr. Doshi *et al.*, (1993) evaluated wheat straw, maize stalks, sorghum stalks, maize meal, rice meal, sorghum meal, and wheat bran as basal substrates for the production of *C. indica*. The results indicated that wheat straw was the best substrate for fruit body production. Krishnamoorthy and Muthusamy (1997) used different substrates viz., paddy straw, sorghum stalks, sugarcane bagasse, palmrosa grass, vetiver grass, groundnut

haulms, soybean hay, and paddy straw compost for cultivation of milky mushroom. Paddy straw and maize stalks gave significantly higher yield followed by sorghum stalks and vetiver grass. Palmarosa grass, soybean hay and groundnut haulms gave good yield (94-99% BE). Paddy straw compost was not found suitable for the cultivation of milky mushroom. Krishnamoorthy *et al.*, (2000) concluded that substrates like paddy straw and sorghum stalks were colonized more quickly by the milky white mushroom fungus compared to black gram hay, soybean hay, maize stalks and finger millet straw. The study also indicated that substrates like coconut coir pith compost, paddy straw compost and saw dust did not favor the growth of *C. indica*. Salam *et al.*, (2004) evaluated nine different combinations using retted, non-retted, composted coir pith in combination with paddy straw, spent mushroom substrate and neek cake in different combinations. They found non-retted coir pith in combination with 75% paddy straw. Tandon and Sharma (2006) found wheat straw as the best substrate amongst four different substrate tested for the sporophore yield of milky mushroom while saw dust was least preferred substrate. Supplementation with wheat bran resulted in the higher yield whereas, soybean meal gave the lowest yield. Geetha *et al.*, (2010) collected a new strain of milky mushroom from Koliyoor area of Thiruvananthapuram, Kerala. The new strain took more time for spawn run and primordial formation but out yielded the existing strain. Paddy straw and sugarcane bagasse were found to be best substrates with BE of 140 and 138%, respectively.. According to Jadhav *et al.*, (2014) stipe length, pileus diameter and average fruit body weight were high in mixture of wheat

and soybean straw (1:1). The highest yield/kg substrate and biological efficiency were recorded in mixture of straw, followed by soybean and wheat straw. Gitte, *et al.*, (2014) also evaluated the efficacy of different substrates such as paddy straw, wheat straw, soybean straw, coconut coir pith, cotton waste and sugarcane bagasse for the cultivation of milky mushroom. Among the six different substrates, wheat straw substrate was found superior with highest biological efficiency 146.3% followed by paddy straw.

Organic supplements like maize meal, wheat bran, rice husk and lucern at 5-7.5% to substrate at the time of spawning as well as at the time of casing is reported to enhance the yield in *C. indica* (Anon., 1989,1990,1991,1992). Salam *et al.*, (2004) retted, non-retted, composted coir pith and found highest yield in retted coir pith. Singh *et al.*, (2010) studied the effect of different substrate preparation methods, type and rate of supplementation, casing soil and cultural practices for *Calocybe indica* were investigated. Kumar *et al.*, (2012) evaluated 11 different supplements viz., wheat bran, soybean flour, pigeon pea powder, green gram powder, cotton cake, mustard cake, neem cake and lentil powder. Alam *et al.*, (2010) have used 30% maize powder to supplement paddy straw substrate in order to increase mushroom yields. More promisingly, supplements like soybean and cotton seed cake gave the highest absolute mushroom yields (64.8% and 59.2% increased biological efficiency over control).

Chemical treatment of wheat straw using carbendazim (35ppm) + formaldehyde (1000ppm) gave the highest yield followed by hot water treatment. Substrate

supplementation with 6% pulse mixture gave maximum production followed by gram husk (4%). Casing mixture consisting of FYM +RBH+Coir Pith (1:1:1) was found to be the best followed by burnt rice husk + farm yard manure (1:1).

Sohliya *et al.*, (2011) conducted an experiment on the effect of period of steaming for pasteurization of straw and gibberellic acid on the growth of milky mushroom (*Calocybe indica*). Direct steaming of straw for 1 hr and also for 30 min was practiced with application of gibberellic acid (GA3) at three different concentrations before spawning was done. It revealed that steaming and GA3 application increased the duration of mycelial run with reduction in yield of milky mushroom but it increased the protein content of fruit bodies as compared to control. The yield in the normal practice of boiling of straw for 1hr and sun drying was found better than steaming.

Casing

Casing means covering the top surface of bags after spawn run is over, with pasteurized casing material in thickness of about 2-3cm. Casing provides physical support, moisture and allows gases to escape from the substrate. Casing material is spread in uniform layer of 2-3 cm thickness and sprayed with solution of carbendazim and formaldehyde to saturation level (Doshi *et al.*, 1993). Use of various casing materials during the production of milky mushroom is well documented in literature with variable results (Sharma *et al.*, 1997, Singh *et al.*, 2007). Tandon *et al.* (2006) evaluated four casing material for the *C. indica* cultivation trials and found FYM + Loam soil (3:1) as the best suited casing soil. Tandon *et al.*

(2006) evaluated four casing material for the *C. indica* cultivation trials and found FYM +Loam soil (3:1) as the best suited casing soil. Nagarartna and Mallesha (2007) used vermicompost prepared casing using coir pith, crop residues, horse manure and analyzed for microbial population, pH, nitrogen and organic carbon. Vermi-compost as such and in combination with sand and soil tested as casing material for milky mushroom. Sand+ soil+ coir pith vermicompost casing mixture initiated maximum number of fruiting bodies with highest yield and bio-efficiency followed by sand+ soil+ crop residues vermicompost casing mixture. Sharma *et al.*, (1997) tried various casing materials and their combinations. They found that spent biogas slurry (100% BE) and 2 years old cow dung (98.7% BE) as highly suitable casing material for milky mushroom. Use of vermicompost (Geetha *et al.*, 2010) as casing material recorded maximum yield and BE (145%) followed by 1:1:1 mixture of sand, soil and vermicompost (140%). Kumar *et al.*, (2012) assessed the effects of eleven organic supplements in substrate and casing mixtures on yield of two strains (CI-6 & CI-4) of milky mushroom. Minimum time for spawn run, pinhead formation and first harvest was recorded in the mustard cake supplemented substrate. Maximum yield was obtained from soybean flour supplemented substrate whereas maximum yield was harvested in neem cake supplemented casing from strains CI-6 and CI-4, respectively. Among the tested casing thickness, the 0.5 cm/200 gm took less period for pinhead formation and first harvesting, while maximum yield was recorded in casing thickness 2.5 cm/1000gm from strains CI-6 and CI-4, respectively. Gitte *et al.*, (2014)

evaluated six different casing materials and found soil + sand as the best casing material in both paddy as well as wheat straw based substrate. Wheat straw as a substrate with soil + sand as casing material recorded minimum days for spawn run (15.67 days), pinhead formation (28.67 days) and first harvest (33.67 days) with highest no. of fruit bodies (24.33), diameter of pileus (7.66 cm), length of stalk (7.86 cm) and biological efficiency (146.3 per cent). Jadhav *et al.*, (2014) assessed the effect of nitrogen fixing and phosphate solubilizing biofertilizers and different substrate for improvement of casing quality and yield in milky mushroom. Out of eight treatments of biofertilizers viz. *Azotobactor* and PSB i.e. phosphate solubilizing bacteria (*Bacillus megaterium* + *Pseudomonas striata*) and their combinations, highest fresh weight (20.52g) as well as dry weight (0.65g) of mycelium was obtained in consortium of *Azotobactor* and PSB (*Bacillus megaterium* + *Pseudomonas striata*) and also the nitrogen content of the casing found to be increased. The yield of mushroom increased from 12.89% to 79.81% due to inoculation of bio-fertilizers. These results indicate that nitrogen fixing and phosphatase solubilizing bacteria could increase the quality of casing material.

Production

It takes about 10 days for mycelium to reach on top of casing layer when fresh air is introduced while maintaining temperature and R.H.. Light should be provided in long time. Watering is very important to get good and healthy crop. During rainy season controlled watering is required and watering once may be enough. During winter twice may be sufficient. However, during summer it is very important as loss of water is more

and it becomes very difficult to maintain required RH and moisture of the substrate. The changes thus made in environment, result in the initiation of fruiting bodies within 3-5 days in the form of needle shape which mature in about a week. Temperature is considered to be most important factor affecting growth and fructification of mushrooms. Evaluation of different temperatures for the mycelia growth of milky mushroom revealed that 35 °C is the optimum temperature under in vitro conditions. Experiments regarding light requirements during cropping revealed that it needs a photoperiod in excess of one hour per day (Tandon *et al.*, 2003, 2006). The effect of plant growth regulators on the sporophore production was studied by Theradimani *et al.*, (2001). A uniform size of sporophore with reduced stipe length, uniform pileus diameter and increased yield was obtained by spraying with kinetin 100ppm. The results of the experiment conducted on the effect of different light sources and different coloured highly density polythene chambers revealed that growth chamber with blue coloured high density polythene sheet roofing material with blue incandescent light significantly increased the sporophore yield of milky mushroom.

Several cases of less organized cultivation in thatched houses or under tarpaulin roofs surrounded by brick walls have also been reported with limited success (Raja and Ganesh, 2012). These sheds are not properly insulated and the growth requirements, including temperature and relative humidity, could fluctuate depending on the external environment. A one-year study on the yield of milky white mushrooms in such sheds, using rice straw as substrate, showed the best performance

during the months of May and June (peak summer season in South India) (Kumar *et al.*, 2012).

Strain evaluation

Productivity and quality of widely cultivated mushrooms mainly depend on strain make up, therefore many strains have constantly been produced with an aim of achieving higher yields and improved quality attributes such as accumulation of nutrients of interest, disease resistance, adaptability to wide temperature range and sporelessness. Sharma and Kumar (2008) evaluated different strains of milky mushroom viz. APK-1, CI-1, CI-3 and CI-6 on paddy straw based substrate. The strain CI-6 resulted in maximum biological efficiency (111.9%) which was significantly different from other strains whereas, strains APK-2 (89.2%), CI-7 (86.7%) and CI-1 (72.9%) were at par. The highest average weight of fruit body was 78.8g in APK-2. Dhakad *et al.*, (2014) tested five strains of milky mushroom viz. CI-4, CI-13, CI-14, CI-15 and CI-18 for growing behavior and yield potential. Minimum spawn run period (15-66 days) was observed in CI-14 while strain CI-15 took maximum time (34days). Highest average yield in first and second flushes was recorded in CI-14 (441.67g and 285g) strain but yield of third flush was higher in strain CI-13. Overall total yield was better in CI_14 (811.67g).

Genetic manipulation of mushrooms can be achieved in number of ways which include cross-breeding, protoplast fusion or protoplast mutagenesis. Mutagenesis has been attempted using a number of approaches. Chemical mutagenesis may be performed using agents such as NTG

(Nmethyl-N'-nitro-N-nitrosoguanidine), sodium nitrite, BU (5'-bromouracil), etc. and this usually results in a single nucleotide substitution in the target genome (Kaur *et al.*, 2011). They studied the yield performance of nine strains of *C. indica*, out of which Ci-3 strain out-yielded the other eight strains. Seven putative mutants of Ci-3 were selected on basis of their growth and enzyme activities used for evaluation of putative mutants. Four mutants viz., CMN-3, CMN-9, CMN-11 and CMB-4 out-yielded the parent when grown on wheat straw.

Shelf life and dehydration of milky mushroom

Dehydration studies were conducted on milky mushroom under room temperature, in oven (55 °C) and in sun. Polypropylene cover (80-100gauze) were best suited for the storage of sporophores at room temperature (4-5 days in 0% vent area) and under refrigerated conditions. The sporophores dehydrated at room temperature had better colour and morphology. The rehydration of oven dried samples was better and browning of sporophores was absent (Pandey *et al.*, 2002). Kasthuri *et al.*, (2007) assessed the physico-chemical qualities and shelf life of mushroom canned with three different concentrations of sodium chloride viz. 1%, 2% and 3% and tomato pulp made from both hybrid and local varieties as canning medium. Canned mushroom samples were analyzed periodically at monthly intervals for physico-chemical parameters such as protein, vitamin C, moisture content and drained weight. Analytical results of biochemical qualities' of canned mushrooms in different canning media showed a gradual decrease during storage period of one year. Same trend was

observed with physical parameters such as moisture content and drained weight. Among the treatments, mushroom canned with tomato pulp from hybrid tomatoes performed better at all the quality aspects. Amuthan *et al.*, (1999) conducted the studies on osmo air drying of milky mushroom. Samples of milky mushroom were osmosed at different concentrations of common salt viz. 10, 15, 20 and 25% and dehydrated in a fluidized bed drier at 55C and 60%RH. The moisture removal was higher by osmosis at 25% concentration of salt in 6 hours duration. The osmosed samples took about 170 minutes to dry the samples compared with 195 minutes for control (air drying) and the complete rehydration of the mushroom osmosed with 25% salt concentration was obtained in a shorter period (50 minutes). The colour was bright for the samples dried after osmosis. Arora (2014) assessed effect of various cooking methods on the antioxidant potential by DPPH inhibition, thiobarbituric acid (TBA) reactive compounds and total phenols on common edible mushrooms of India viz. *A. bisporus*, *C. indica*, *V. volvacea*, *L. edodes* and *P. ostreatus*. It was found that antioxidant potential as DPPH inhibition in fresh mushrooms was found to be in the decreasing order as *A. bisporus*, *V. volvacea*, *C. indica*, *P. ostreatus* and *L. edodes*. Total phenol was found to be in decreasing order as *P. ostreatus*, *C. indica*, *V. volvacea*, *A. bisporus* and *L. edodes*. These mushrooms were also analysed after cooking by various methods as microwaving for 2min, boiling in water for 5 min and sautéing in sunflower oil for 2 min. Antioxidant potential as DPPH inhibition in case of all the mushrooms decreased in microwave treatment as well as in boiling which can be attributed to leaching of biomolecules whereas, increased slightly in sautéing of mushrooms that can

be attributed to concentration of biomolecules due to frying. TBA reactives decreased in all the mushrooms by all forms of cooking that indicates less of carbonyl compounds.

Diseases and pests

Pandey *et al.*, (2003) investigated the occurrence of competitor moulds and pathogens during the cultivation of milky mushroom. *Trichoderma harzianum* was the most problematic weed mould observed during spawn running. Cobweb disease caused by *Dactylium dendroides* caused complete loss of crop. Carbendazim at 0.01% could effectively control the weed mould and the pathogen without inhibiting the mushroom mycelium. Prochloraz Mn complex controlled the pathogen but inhibited *C. indica* mycelium by 46.69%.

Studies on sources of nematodes inoculum showed that the most potential sources of nematode dissemination in *Agaricus bisporus* were wheat straw, chicken manure and spent compost often used as casing material. Since chicken manure is not used in *Calocybe indica* nematodes were mainly disseminated in this mushroom through other two means. Besides, FYM, loam and platform soil also acted as source of nematode inoculum. Dipteran flies hovering in the farms also disseminated nematodes from one bed/room to other in both the cases. Interestingly, sporophores of *Calocybe indica* were found to harbour more nematodes as compared to that of *Agaricus bisporus* (Khanna *et al.*, 2006).

Kumar *et al.*, (2010) estimated the damage potential of the nematodes *Aphelenchoides swarupi* and *Aphelenchus avenae*, at 1000

individuals per 10 kg of compost, in milky mushroom (*Calocybe indica*) by inoculation of the nematodes at the times of spawning and casing. Nematodes inoculated at spawning did not cause significant mycelial depletion in *C. indica*. Feeding by nematodes occurred only after casing and, thus, significant mycelial depletion was observed at pinhead formation stage. Total production showed no significant differences among the nematode inocula irrespective of inoculation stage and nematode species involved. The flush pattern in *C. indica* inoculated with the nematodes showed no disturbance in weekly yield until the fourth week but production of fruiting bodies declined continuously thereafter up to the seventh week.

Conclusion

Mushroom industry has been grown @>7 % during the last few decades. Mushroom production in India for the year 2013 is roughly 1,29,000 tons contributing < 1% of total world production (Sharma *et al.*, 2017). Milky white mushrooms are highly suitable for commercial production in humid tropical and subtropical regions of the world where, the average temperature falls between 25! and 35! throughout the year (Navathe *et al.*, 2014). Due to its morphological appearance, higher shelf life, higher productivity, white color and low production cost, milky mushroom will have greater acceptability in the world market. The mushroom has a resemblance to button mushroom, which may again help in picking up the demand for this mushroom in the world.

Milky mushroom (*Calocybe indica*) can be grown on wide range of substrates such as straw of paddy, wheat, ragi, maize/bajra/

cotton stalks and leaves, sugarcane bagasse, cotton and jute wastes, dehulled maize cobs, tea/coffee waste etc. It has a simpler pasteurization process to kill harmful microbes, which can be done by steam or hot water treatment. Covering the top surface of bags after spawn run with pasteurized casing material, provides physical support, moisture and allows gases to escape from the substrate. With a temperature 30-35°C and R.H. 80-90%, it takes about 10 days for mycelium to reach on top of casing layer when fresh air is introduced. Light should be provided for long time. The changes thus made in environment, result in the initiation of fruiting bodies with in 3-5 days in the form of needle shape which mature in about a week.

With the simpler technology and less requirement of airconditioning, this mushroom may prove to be an alternative to button mushroom without incurring high infrastructural costs.

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