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Effect of sugarcane leaves as substrate on production milky mushroom (CI.-16-02 and CI.-16-03)

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Abstract
Milky mushroom (*Calocybe indica*) is one of the most important mushrooms commonly called as "Kuduk".Milky mushroom was commercialized as a new variety *C. indica*, var. APK2 from the Tamil Nadu Agricultural University, Coimbatore, India and can be cultivated throughout the summer season. This mushroom is well appreciated due to its large-sized milky white sporophores, simple production technology and low capital investment. There is no need to compost the substrate for its cultivation as the mycelium can degrade the cellulose, hemicelluloses and lignin by secretion of various extracellular enzymes. Attempts on identification of potential strain and cultivation technology has been for the immurement inoculation technology. supplementation in casing and substrates, evaluation of cheap locally available substrates, Temperature and use of organic material etc.
Are needed the present investigation were carried out on Commercial production with different ratio of sugarcane leaf with wheat straw on milky mushroom of two strain of *Calocybe* spp. (CI.-16-02, CI.-16-03). The result is present investigation shows that different substrates on yield of milky mushroom such as result indicated that in CI-16-03 maximum yield was observed in sugarcane leaf + wheat straw (2:1). Minimum days for spawn run (19.50days) were observed in sugarcane leaf + wheat straw (2:1). The Minimum days for pin head formation (21.75 days) were observed in sugarcane leaf + wheat straw (2:1). The Minimum days for first harvesting (27.00 days) were observed in sugarcane leaf + wheat straw (2:1). The Maximum number of pinhead initiation (62.25) were observed in sugarcane leaf + wheat straw (2:1). The Maximum numbers of fruiting bodies (24.75) were observed in sugarcane leaf + wheat straw (2:1). The Maximum average weight of fruiting bodies (27.42 gm) was observed in sugarcane leaf + wheat straw (2:1).

Keywords: Mushroom, fruiting body, casing

Introduction
The mushroom cultivation is unique in the sense that it is the most efficient and economically viable biotechnological process for the conversion of lignocellulosic waste material into high quality protein rich palatable food. Nowadays, there is a need to promote the cultivation of mushroom to meet the challenges of increasing world population as well as energy crisis. World mushroom production has increased more than 25-fold during the last 35 years (from about 1 billion kg in 1978 to about 27 billion kg in 2012). It is a robust, fleshy, milky white, umbrella like mushroom, which resembles button mushroom. The species is suitable for hot humid climate. relative humidity more than 80%. Milky mushroom was commercialized as a new variety *C. indica*, var. APK2 from the Tamil Nadu Agricultural University, Coimbatore, India and can be cultivated throughout the summer season. This mushroom is well appreciated due to its large-sized milky white sporophores, simple production technology and low capital investment. Commercial cultivation of this species is still in its infancy in India. It is suitable for hot humid climate and can be cultivated almost throughout the year in India except few places. Milky mushroom (*Calocybe indica*) is one of the most important mushrooms commonly called as "Kuduk" but popularly known as "Dudhichhata". It was first reported from India by Purkayastha and Chandra in 1974. According to systemic position, *Calocybe indica* belongs to the sub division basidiomycotina, class basidiomycetes, order Agaricales and family Tricholomataceae. This mushroom requires a temperature of 30-35 ºC and relative humidity of 70-80% for cultivation which is conducive to environmental conditions of most part of India (Singh *et al*. 2009; Amin *et al*. 2010; and Gitte *et al*. 2014) [6, 1, 4]. Total mushroom production in India reached 1, 29, 782 metric tonnes during 2016, and different state–wise mushroom production in India is highest production in Punjab around 18thousand metric tonnes followed by Odisha and Haryana **(**DMR Solan data) in India. Directorate of mushroom research solan and AICRP center were carried out on different aspects of cultivation agricultural wastes including wheat straw, paddy straw, maize, bajra, cotton stalks

and leaves, sugarcane bagasse, dehulled maize cobs, tea and coffee waste and coconut coir substrate can be utilized for cultivation of *Calocybe indica*. There is no need to compost the substrate for its cultivation as the mycelium can degrade the cellulose, hemicelluloses and lignin by secretion of various extracellular enzymes. This mushroom requires a temperature of 30-35 ºC and relative humidity of 70-80% for cultivation which is conducive to environmental conditions of most part of India. In spite of sincere efforts made by various workers, limited success on cultivation of milky mushroom was achieved until 2010. As it is revealed from the literature, not much work has been done on *C. indica* as it is new introduction to mushroom world. Attempts on identification of potential strain and cultivation technology has been made till today but further research still needs to be carried out in the area such as, supplementation in casing and substrates, evaluation of cheap locally available substrates, Temperature and use of organic material etc.

Material and Methods

The study was carried out in Mushroom Laboratory, Department of plant pathology at S. V. P. University of Agriculture and technology, Meerut, U.P. India, during 2017. The experiments were laid out in the Complete Randomized Design (C.R.D.) with six treatments and replicated four.

Establishment of pure culture

The culture of two strains (CI-16-02 and CI-16-03) of *Calocybe indica* were purified and maintained by single hyphal tip method. For this purpose, the cultures were grown on Potato Dextrose Agar Medium (PDA) in sterilized Petri plate. Single branched hyphae from the periphery of the growing colony were marked under low power (10x) in the compound microscope and transferred to PDA slants. These culture tubes were incubated at 28 ± 1 ^{0}C for about a week, again subculture on PDA and then stored in a refrigerator at 10 ^{0}C and or room temperature for further use.

Spawn Production

For most studies, the spawn was prepared on wheat grains in 500 ml capacity wide mouthed glass bottles. To achieve the objective, the grains were cleaned to remove any broken, shriveled grains either by sieving or winnowing or by hand picking of undesired grains. After this, the grains were soaked overnight in clean water and then washed. Now, they were boiled in water for 15 minutes taking care that grains should not split but remain slightly hard after boiling. The partially boiled grains were spread in thin layer over a wire net to remove excessive water and enable them to cool about 25-30 ^{0}C. The cooled grains were then mixed with 1.2 percent commercial grade gypsum ($CaSO_4$) and 0.3 percent calcium carbonate ($CaCO_3$). Gypsum prevents the sticking of wheat grains together and calcium carbonate maintains the pH 5.5-7.5. The grains were then filled in clean glass bottle up to 2/3rd of its capacity. The bottles were plugged with non absorbent cotton and covered with butter paper. These bottles were then sterilized at 121 ^{0}C (1.1 kg/cm^2 pressure) for 60 minutes on two consecutive days. Sterilized bottles were taken out from the autoclave, while still warm and were shaken to avoid clumping of grains.

Wheat grains in sterilized bottles were inoculated with 9 mm disc of a vigorously grown 7 days old culture of different strains of *Calocybe indica*. Inoculated bottles were incubated at 28±1 ^{0}C and shaking was done after 7 days. Entire grains were covered with fine mycelial growth after 20 days. This spawn is known as mother spawn spawn. Which was prepared on sterilized grains in polypropylene bags inoculated with a spoon full of mother spawn.

Cultivation technology substrate for this experiments was bright, good quality wheat straw alone with dry sugarcane leaves in deffrent ratio which was soaked in a water tank chemically treated with Carbendazim 7.0 gm and formalin 100ml/100lit of water for overnight (18 hrs.), so as to make it soft. Thereafter, straw was taken out from the tank and spread on cemented floor treated with 2 per cent formalin for two hours to drain out excess of water. This is now ready to mix with spawn @ 6 per cent of the wet weight of substrate and after spawning this mixture was filled in polythene bags at the rate 1.0 kg/bag. The upper portion of the polythene bags was folded and stapled. Thereafter, 8-10 small holes were made on polythene bags with the help of nail, to permit proper aeration during spawn run. These bags were placed in the mushroom crop room at relative humidity of 80-85 per cent and temperature maintained 28±2 ^{0}C. After the full spawn run in the wheat straw, it becomes white compact mass covered with mycelial growth which was also sticking to the polythene bag after two to three weeks.

After 15-18 days of spawning casing was done. Casing mixture was prepared by mixing of Farm Yard Manure+ Garden Soil+ SAND (1:2:2w/w) each two years old and treated with formalin (4%) solution and kept covered for 48-72 hrs. The pH of casing was maintained 8.5 by using Calcium Carbonate and Calcium Sulphate. A 2.5 cm thick layer was applied uniformly over the surface of substrate. Relative humidity 80-90 per cent was maintained in the crop room by sprinkling water through sprayer. Pin head initiation started after 10-12 days of casing.

The sporophores of *Calocybe indica* were harvested after the maturity. The yield obtained from the strains in 7 weeks harvesting period were compared with each other). Adequate moisture humidity (80-90%) and ventilation in the crop room. The fruiting bodies must be protected from direct sunlight but some diffused light (2500-3000 Lux) was allowed to induce fruiting body formation. The crop room floor and walls were sprayed with 0.1 per cent Malathion or Sevin to protect it from insect infestation. To prevent the fungal infection, two sprays of Carbendazim 0.01 per cent were given. Biological efficiency was calculated using the following formula:

The experiment was conducted to find the beneficial Effect of different substrates on yield of milky mushroom such as different substrates sugarcane leaf + wheat straw (3:1), sugarcane leaf + wheat straw (2:1), sugarcane leaf + wheat straw (1:3), sugarcane leaf + wheat straw (1:2) and sugarcane leaf + wheat straw (1:1) on both strains (CI-16-03 and CI-16-02). Days for spawn run (DFSR), days for pin head formation(DFPF), days for first harvesting(DFFH), number of pin head initiation(NOPI), number of fruiting bodies(NOFB) and total yield were recorded.

Results and Discussion for the Present Expariment

The result indicated that in CI-16-03 maximum yield was observed in sugarcane leaf + wheat straw (2:1), 680g/kg of dry substrates with 68.00% Biological Efficiency (B.E.). which was followed by sugarcane leaf + wheat straw (1:1), 550g/kg of dry substrates with 55.00% B.E. while minimum yield was observed in control (460.00g/kg dry substrates with 46.00% B.E.) which was followed by sugarcane leaf + wheat straw (1:3), (470 g/kg of dry substrates with 47.00% B.E.) which was statistically lower then all other treatment. The Minimum days for spawn run (19.50days) were observed in sugarcane leaf + wheat straw (2:1). While maximum days for

spawn run (23.50 days) were observed in control. The Minimum days for pin head formation (21.75 days) were observed in sugarcane leaf + wheat straw (2:1). Maximum days for pin head formation (28.15 days) were observed in control which was statistically higher than all other treatment. The Minimum days for first harvesting (26.75 days) were observed in sugarcane leaf + wheat straw (2:1) which was statistically lower than all other treatment. While maximum days for first harvesting (31.00 days) were observed in control which was statistically higher than all other treatment. The Maximum number of pinhead initiation (62.25) were observed in sugarcane leaf + wheat straw (2:1), while minimum numbers of pinhead initiation (54.00) were observed in sugarcane leaf + wheat straw (3:1) which was statistically lower then all treatment. The Maximum numbers of fruiting bodies (24.75) were observed in sugarcane leaf + wheat straw (2:1). Which was significantly higher than all other treatment. While minimum number of fruiting bodies (19.00) were observed in control. Which was significantly lower than all other treatment. The Maximum average weight of fruiting bodies (27.47 gm) was observed in sugarcane leaf + wheat straw (2:1). Which was significantly higher than all treatment. While minimum average weight of fruiting bodies (21.86 gm) was observed sugarcane leaf + wheat straw (1:3). Which was significantly lower than all other treatment as shows table.

The result was indicated that in CI-16-02 maximum yield was observed in sugarcane leaf + wheat straw (2:1), (670 g/kg of dry substrates with 67.00% biological efficiency). Which was followed by sugarcane leaf + wheat straw (1:1), (540 g/kg of dry substrates with 54.00% biological efficiency) while minimum yield was observed in control (450.00 g/kg dry substrates with 45.00% biological efficiency) which was followed by sugarcane leaf + wheat straw (1:3), (460 g/kg of dry substrates with 46.00% biological efficiency) which was statistically lower than all other treatment. The Minimum days for spawn run (19.25 days) were observed in sugarcane leaf + wheat straw (2:1) which was statistically at par with sugarcane leaf + wheat straw (1:1), (20.25). While maximum days for spawn run (23.25 days) were observed in control at par with sugarcane leaf + wheat straw (1:3), (22.50) which was statistically. The Minimum days for pin head formation (21.50 days) were observed in sugarcane leaf + wheat straw (2:1). Maximum days for pin head formation (28.50 days) were observed in control which was statistically higher than all other treatment. The Minimum days for first harvesting (26.25 days) were observed in sugarcane leaf + wheat straw (2:1) which was statistically lower than all other treatment. While maximum days for first harvesting (33.00days) were observed in control which was statistically higher than all other treatment. The maximum number of pinhead initiation (63.00) were observed in sugarcane leaf + wheat straw (2:1) while minimum numbers of pinhead initiation (53.00) were observed in control which was statistically lower than all other treatment. The Maximum numbers of fruiting bodies (25.00) were observed in sugarcane leaf + wheat straw (2:1). Which was significantly higher than all other treatment. While minimum number of fruiting bodies (19.50) were observed in control. Which was significantly lower than all other treatment. The Maximum average weight of fruiting bodies (26.80) was observed in sugarcane leaf + wheat straw (2:1). Which was significantly higher than all treatment. While minimum average weight of fruiting bodies (21.30 mm) was observed sugarcane leaf + wheat straw (1:3). Which was significantly lower than all other treatment as shows.

Alam *et al.,* (2010) [1, 2] also investigated the most suitable supplements and their levels for the commercial cultivation of milky white mushroom. Rice bran, maize powder, and wheat bran with their different levels were used as supplements to evaluate the yield and yield contributing characteristics of *C. indica.* Primordia initiation was observed between 13.5 and 19.3 days. The results indicated that the 30% maize powder supplement was effective for producing viable fruiting bodies. The maximum diameters of the pileus and stalk were observed with 30% maize powder. The highest biological and economic yield and biological efficiency were also obtained with 30% maize powder as a supplement. The results indicate that increasing the supplement level resulted in less biological efficiency, and that 30% maize powder was the best supplement level for rice straw substrate to cultivate milky white mushrooms.

Kumar *et al.,* (2013) [5] also reported that on *Calocybe indica* is one of the best edible mushrooms which can be grown at high temperature or summer season. It replaces the non-vegetarian food. The present experiment was conducted to find out the efficacy of different substrates such as paddy straw, wheat straw, soybean straw, coconut coir pith, cotton waste and sugarcane baggase for the cultivation of milky mushroom. Among the six different substrates, wheat straw substrate was superior which recorded minimum days for spawn run 15.67 days, pinhead formation 28.67 days and for first harvest 33.67 days with highest no. of fruit bodies.

Table 1: Effect of Sugarcane leaves as substrate on production milky mushroom (CI.-16-02 and CI.-16-03).

S. No.	Different Pulse additives	DFSR		DFPF		DFFH		NOPI		NOFB		Yield (g/kg dry substrate)		Average Weight (g/FB)		Biological efficiency (%)	
		Strain CI		Strain CI		Strain CI		Strain CI		Strain CI		Strain CI		Strain CI		Strain CI	
		16-02	16-03	16-02	16-03	16-02	16-03	16-02	16-03	16-02	16-03	16-02	16-03	16-02	16-03	16-02	16-03
1.	sugarcane leaf + wheat straw(3:1)	22.00	21.00	26.00	25.75	29.25	29.75	57.75	57.25	22.00	22.00	480.00	490.0	21.81	22.27	48.00	49.00
2.	sugarcane leaf + wheat straw(2:1)	19.25	19.50	21.50	21.75	26.25	27.00	63.00	62.25	25.00	24.75	670.00	680.0	26.80	27.47	67.00	68.00
3.	sugarcane leaf + wheat straw(1:3)	22.50	22.75	26.50	26.50	31.50	31.00	56.50	56.50	21.00	21.50	460.00	470.0	21.90	21.86	46.00	47.00
4.	sugarcane leaf + wheat straw(1:2)	21.25	21.50	24.50	24.00	28.50	27.50	54.50	54.00	23.00	22.75	490.00	500.0	21.30	21.97	49.00	50.00
5.	sugarcane leaf + wheat straw(1:1)	20.25	19.75	23.00	23.50	27.25	26.75	59.75	59.50	24.00	24.00	540.00	550.0	22.50	22.91	54.00	55.00
6.	Control (wheat straw)	23.25	23.50	28.50	28.50	33.00	31.00	53.00	54.75	19.50	19.00	450.00	460.0	23.07	24.21	45.00	19.00
	CD@ 5%	1.93	2.30	0.99	2.01	1.23	1.51	1.63	1.58	1.17	1.43	12.22	12.22	-	-	-	-
	SE (M)	0.64	0.76	0.33	0.67	0.41	0.50	0.54	0.53	0.39	0.47	4.08	4.08	-	-	-	-

Average of four replications

DFSR= Days for spawn run, DFPF= Days for pin head formation, DFFH= Days for first harvesting, NOPI= Number of pin head initiation, NOFB= Number of fruiting body.

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