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### Evaluation of different spices and medicinal plant leaf powder on production potential of milky mushroom (*Calocybe indica* P&C)

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#### Abstract

Milky mushroom (*Calocybe indica* P&C) also known as "*Dudh Chhatta*" and "white summer mushroom" because it has milky white appearance and tropical in nature respectively. Milky mushroom is being used by mushrooms growers for round the year production of mushrooms with button and oyster mushrooms. The present study has been conducted to evaluate the effect of different spices and medicinal plant leaf powder on production potential of CI-20-03 strain of milky mushroom (*Calocybe indica P&C*). Result has been revealed that the maximum Yield (796.08 g/kg DS with 79.60% BE) was observed in Wheat Straw + 2.5% ginger leaf powder. Whereas, minimum yield (600.50 g/kg DS with 60.05% BE) was observed in Wheat Straw + 5.0% turmeric leaf powder in case of spices plants leaf powder. Similarly maximum Yield (767.08 g/kg DS with 76.70% BE) was observed in Wheat Straw + 2.5% tulsi leaf powder). While, minimum yield (600.50 g/kg DS with 60.05% BE) was observed in Wheat Straw + 5.0% lemon grass leaf powder in case of medicinal plants leaf powder.

Keywords: Milky mushroom, onion, garlic, turmeric, zinger, neem, citrus lemon, lemon grass, tulsi, leaf powder

#### 1. Introduction

Milky mushroom (Calocybe indica P&C) also known as "Dudh Chhatta" and "white summer mushroom" because it has milky white appearance and tropical in nature respectively. Milky mushroom is robust, fleshy and milky white in colour and large sized sporophores. Calocybe indica belong to Phylum Basidiomycota, class Basidiomycetes, order Agaricales and family Tricholomataceae (Kirk et al., 2008) <sup>[6]</sup>. Now a days milky mushroom attracted greater attention of consumers as well as mushrooms growers because it has much potential for market demand. The dried sporophores of this mushroom contain 21.4% protein, 4.95% fat, 12.9% fibre, 48.5% carbohydrates, 4.00% soluble sugar, 2.95% starch and 13.1% ash. In addition to this it has most of the mineral salts such as Potassium, Sodium, Iron and Calcium (Alam et al., 2008)<sup>[2]</sup>. It has longer shelf life of 3-4 days which give sufficient time to the grower to sell out own produce in the remote areas market where the demand and price is much more to local market. As it grows in hot humid climate, thus it has highly suitable for cultivation in the most of the plains of India. It can be easily grown temperature range between 25-35 °C. In India milky mushroom has become the third commercially grown after button and oyster mushrooms. Therefore, milky mushroom is considered as a better alternative option to the mushrooms growers for round the year production of mushrooms to earn good profit from mushrooms cultivation throughout the year particularly in tropical region where button and oyster mushrooms cant not grown without creating artificial environment.

#### 2. Material and Method

Under the present study, experiments were conducted for the evaluation of spices and medicinal plant leaf power on the production potential of milky mushrooms. Experiments on effect of different spices and medicinal plant leaf power. Before conducted experiments basic preparation and technique include selection of substrate, size/weight and height of bags, method of substrate sterilization, spawn rate, method of spawning, ingredients of casing, pH of casing, method of casing sterilization and thickness of casing etc.

#### 2.1 Experimental site

These present experiments had been done on Mushrooms Research and Training Centre collaborated with department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut 250110 (Uttar Pradesh) India. This university is situated on the western side of the Delhi-Dehradun highway at a distance of 10.0 km in the north of Meerut City. The Meerut district is situated between 29<sup>0</sup> 01'N latitude and 77<sup>0</sup> 45'E longitude at an altitude of 237 meters above the mean sea level.

#### 2.2 Preparation of herbal powder

For the preparation of plant leaf powder eight spices and medicinal plants were selected. Among eight plants, four plants were spices plant and four medicinal plant namely Onion (Allium cepa), Garlic (Allium sativum), Turmeric (Curcuma longa), Zinger (Zingeber officinale) Neem (Azadirechta indica), citrus lemon (Citrus limon) lemon grass (Cymbopogon citratus) and Tulsi (Ocimum tenuiflorum). The collection work had been done season wise from the modipuram, Meerut, Uttar Pradesh. Fresh healthy leaves were harvested from the plants at maturity stage and bring in the laboratory. Thereafter, cutting of leaves has been done with help of scissors or sharp knife to removed damaged and dead part of the leaf. The leaves had been properly washed in the tape water in a plastic tub. The sunshine utilized to drying entire amount of plant leaves until the completely ready for making powder. The leaves before making power dried in a ventilated oven at 40 °C for 24 hours. Thereafter well dried leaves of plants ground in mixie jar and ground material passed through 2 mm pore size sieve. The moisture content of powder was maintained 6.34%. The powder was stored in air tight plastic containers at 4 °C for further experimental use (Kumar et al 2022a)<sup>[8]</sup>.

These experiments were conducted to examine the effect of spices and medicinal plant leaf powder on production potential of CI-20-03 strains of milky mushroom. In these experiments nine treatments including control had been taken i.e.  $T_1$  (Wheat Straw + 2.5% garlic leaf powder),  $T_2$  (Wheat Straw + 5.0% garlic leaf powder), T<sub>3</sub> (Wheat Straw + 2.5% onion leaf powder), T<sub>4</sub> (Wheat Straw + 5.0% onion leaf powder) T<sub>5</sub> (Wheat Straw + 2.5% turmeric leaf powder), T<sub>6</sub> (Wheat Straw + 5.0% turmeric leaf powder), T<sub>7</sub> (Wheat Straw + 2.5% ginger leaf powder), T<sub>8</sub> (Wheat Straw + 5.0% ginger leaf powder) and T<sub>9</sub> Control (Wheat Straw only) in case of spices plant leaf powder. Similarly in case of medicinal plant leaf powder equal number of treatments including control had been taken i.e.  $T_1$  (Wheat Straw + 2.5% neem leaf powder),  $T_2$ (Wheat Straw + 5.0% neem leaf powder), T<sub>3</sub> (Wheat Straw + 2.5% citrus lemon leaf powder),  $T_4$  (Wheat Straw + 5.0%) citrus lemon leaf powder) T<sub>5</sub> (Wheat Straw + 2.5% lemon grass leaf powder),  $T_6$  (Wheat Straw + 5.0% lemon grass leaf powder),  $T_7$  (Wheat Straw + 2.5% tulsi leaf powder),  $T_8$ (Wheat Straw + 5.0% tulsi leaf powder) and T<sub>9</sub> Control (Wheat Straw only). The method of substrate preparation was same as described by (Srivastava et al., 2014; Kumar et al., 2012 and Kumar et al., 2011) <sup>[17, 7, 10]</sup>. Before bagging of the substrate all the treatments were mixed with the substrate. The bagging of the substrates had been done and maintained three replications of all the treatments. Following observations such as Days For Spawn Run (DFSR), Days For First Harvesting (DFFH), Days For Cropping Period (DFCP) Number Of Fruiting Bodies (NOFB), Length Of Stipe cm (LOS cm),

Width Of Pileus cm (WOP cm), Average Weight g/Fruiting Body (AW g/FB), Yield g/kg Dry Substrate (Yield g/kg DS), and Biological Efficiency (BE)% were observed.

#### 2.3 Statistical analysis

The data collected from present study were analyzed with the help of analysis of variance table whenever required. The F calculated value of each treatment were tested with F tabulated value of ANOVA table and critical difference (CD) was calculated at 1 per cent of significance under the programme of OPSTAT for comparing treatment means (Gomez and Gomez, 1996; Chandel, 1993)<sup>[5, 4]</sup> as followed by (Kumar *et al.*, 2022a; Kumar *et al.*, 2022b; Saini *et al.*, 2022 and Singh *et al.*, 2022)<sup>[8, 11, 15, 16, 8, 11]</sup>.

#### 3. Result and Discussion

# **3.1.** Effects of different spices plant leaf powder on production of CI-20-03 strain of milky mushroom.

The results of different spices leaf powder shown in Tableland Fig.1 that maximum Yield (796.08 g/kg DS with 79.60% BE) was observed in Wheat Straw + 2.5% ginger leaf powder which was significantly at par with Wheat Straw + 2.5% onion leaf powder with (783.83 g/kg DS with 78.38% BE) and Wheat Straw + 2.5% garlic leaf powder with (761.50 g/kg DS with 76.15% BE) and significantly higher than all others treatments. Whereas, minimum yield (600.50 g/kg DS with 60.05% BE) was observed in Wheat Straw + 5.0% turmeric leaf powder which was significantly at par with Wheat Straw + 5.0% garlic leaf powder with (610.83 g/kg DS with 61.08% BE) and Wheat Straw + 5.0% onion leaf powder with (638.50 g/kg DS with 63.85% BE) and significantly lower than all treatments.

Similarly, maximum LOS (16.17 cm with WOP 7.08 cm) was observed in Wheat Straw + 2.5% ginger leaf powder which was significantly at par with Wheat Straw + 2.5% onion leaf powder with (15.83 cm with WOP 6.91cm) and Wheat Straw + 2.5% garlic leaf powder with (15.25 cm with WOP 6.91 cm) and significantly higher than all others treatments. However, minimum LOS (12.92 cm with WOP 5.50 cm) was found in Wheat Straw + 5.0% turmeric leaf powder which was significantly at par with Wheat Straw + 5.0% garlic leaf powder with (13.33 cm with WOP 5.62 cm) and Wheat Straw + 5.0% onion leaf powder with (13.83 cm with WOP 5.75 cm) and significantly lower than all treatments.

The maximum NOFB (14.67 with AW 52.62 g/FB) was found in Wheat Straw + 2.5% ginger leaf powder which was significantly at par with Wheat Straw + 2.5% onion leaf powder with (14.00 with AW 50.35 g/FB), Wheat Straw + 2.5% garlic leaf powder with (13.67 with AW 50.04 g/FB) and Wheat Straw + 2.5% turmeric leaf powder with (13.33 with AW 49.25 g/FB) and significantly higher than all others treatments. While, minimum NOFB (11.33 with AW 43.37 g/FB) was found in Wheat Straw + 5.0% turmeric leaf powder which was significantly at par with Wheat Straw + 5.0% garlic leaf powder with (11.67 with AW 43.62 g/FB), Control, 11.67 NOFB and Wheat Straw + 5.0% onion leaf powder, with AW 43.67 g/FB) respectively as well as significantly lower than all treatments.

Although, the minimum DFSR (21.00 with 25.67 DFFH) was found in Control which was significantly at par with Wheat Straw + 2.5% ginger leaf powder with (23.00 with 26.33 DFFH) and Wheat Straw + 2.5% onion leaf powder with (23.67 with 27.67 DFFH) as well as significantly lower than all treatments. Whereas, maximum DFSR (28.33 with 32.33 DFFH) was reported in Wheat Straw + 5.0% turmeric leaf which was significantly at par with Wheat Straw + 5.0% garlic leaf powder with (27.67 with 31.67 DFFH) and Wheat Straw + 5.0% onion leaf powder with (26.33 with 30.33 DFFH).

However, the minimum DFCP (72.00) was found in Control which was significantly at par with Wheat Straw + 2.5% ginger leaf powder, 73.00 as well as significantly lower than all treatments. Whereas, maximum DFCP (85.00) was reported in Wheat Straw + 5.0% turmeric leaf which was significantly at par with Wheat Straw + 5.0% garlic leaf powder, 83.33 and Wheat Straw + 5.0% onion leaf powder, (83.00).

## **3.2.** Effects of different medicinal plant leaf powder on production of CI-20-03 strain of milky mushroom

The results of different medicinal plant leaf powder shown in Table-2 and Fig. 2 that among the applied treatments of different medicinal plant leaf powder the maximum Yield (767.08 g/kg DS with 76.70% BE) was observed in Wheat Straw + 2.5% tulsi leaf powder which was significantly at par with Wheat Straw + 2.5% neem leaf powder with (749.17 g/kg DS with 74.91% BE) and Wheat Straw + 2.5% citrus lemon leaf powder with (729.17 g/kg DS with 72.91% BE) as well as significantly higher than all others treatments. Wheat Straw + 2.5% lemon grass leaf powder with (695.17 g/kg DS with 69.51% BE). However, minimum yield (600.50 g/kg DS with 60.05% BE) was observed in Wheat Straw + 5.0% lemon grass leaf powder) which was significantly at par with all other treatments viz., Wheat Straw + 5.0% citrus lemon leaf powder with (603.17 g/kg DS with 60.31% BE), and Control with (643.17 g/kg DS with 74.91% BE) and Wheat Straw + 5.0% tulsi leaf powder with (650.17 g/kg DS with 65.01% (BE).

Orderly, maximum LOS (15.50 cm with WOP 6.75 cm) was observed in Wheat Straw + 2.5% tulsi leaf powder which was significantly at par with Wheat Straw + 2.5% neem leaf powder with (14.58 cm with WOP 6.29 cm), Wheat Straw + 2.5% citrus lemon leaf powder with (14.17 cm with WOP 6.25 cm), Wheat Straw + 2.5% lemon grass leaf powder with (14.33 cm with WOP 6.16 cm) and Wheat Straw + 5.0% tulsi leaf powder with (14.08 cm with WOP 6.00 cm) as well as significantly higher than all others treatments. However, minimum LOS (13.08 cm with WOP 5.50 cm) was found Wheat Straw + 5.0% lemon grass leaf powder which was significantly at par with all other treatments *viz.*, treatment number Control with (13.17 cm with WOP 5.50 cm) and Wheat Straw + 5.0% citrus lemon leaf powder with (13.33 cm with WOP 5.66 cm).

The maximum NOFB (13.33 with AW 50.95 g/FB) was observed in Wheat Straw + 2.5% tulsi leaf powder which was significantly at par with Wheat Straw + 2.5% neem leaf powder with (13.00 with AW 49.78 g/FB), Wheat Straw + 2.5% citrus lemon leaf powder with (12.67 with AW 49.60 g/FB) and Wheat Straw + 2.5% lemon grass leaf powder with (12.67 with AW 49.25 g/FB) as well as significantly higher than all others treatments. While, minimum NOFB (10.67 with AW 44.58 g/FB) was found in Wheat Straw + 5.0% lemon grass leaf powder which was significantly at par with all other treatments *viz.*, Control with (11.00 with AW 45.71 g/FB), Wheat Straw + 5.0% citrus lemon leaf powder with (11.33 with AW 44.66 g/FB) and Wheat Straw + 5.0% neem leaf powder with (11.67 with AW 45.63 g/FB). However, the minimum DFSR (21.67 with 26.67 DFFH) was found Control which was significantly at par with Wheat Straw + 2.5% tulsi leaf powder with (22.33 with 27.33 DFFH), Wheat Straw + 2.5% neem leaf powder with (23.00 with 27.33 DFFH) and Wheat Straw + 2.5% citrus lemon leaf powder with (23.67 with 28.67 DFFH) as well as significantly lower than all treatments. Whereas, maximum DFSR (28.33 with 31.33 DFFH) was reported in Wheat Straw + 5.0% lemon grass leaf powder which was significantly at par with Wheat Straw + 5.0% citrus lemon leaf powder with (26.33 with 30.67 DFFH) and statistically lower than all treatments. Although, the minimum DFCP (73.00) was found in Control which was significantly at par with Wheat Straw + 2.5% tulsi leaf powder (74.00), treatment number Wheat Straw + 2.5%neem leaf powder (75.00) and Wheat Straw + 2.5% citrus lemon leaf powder (76.33) as well as significantly lower than all treatments. Whereas, maximum DFCP (84.00) was reported in Wheat Straw + 5.0% lemon grass leaf powder which was significantly at par with Wheat Straw + 5.0% citrus lemon leaf powder (82.00) and Wheat Straw + 5.0% neem leaf powder (81.00) as well as statistically higher than all treatments.

These results were accordance with findings of Mohit et al., (2019) <sup>[13]</sup> on different organic additives, they evaluated two doses 1 and 2% of three different pulse additives viz., black gram, cowpea and pea and reported maximum yield 690 g/kg dry substrate and average weight of fruiting bodies (26.22 gm) in sugarcane leaf + wheat straw (2:1) with pea flour @ 2% with CI-16-03 strain of milky mushroom. Kumar, (2019) <sup>[12]</sup> studied and reported yield 610.00 g/kg dry substrate with 61.00% biological efficiency and yield 590.00 g/kg dry substrate with 59.00% biological efficiency with pigeon pea powder @ 1% and followed by yield 570.00 g/kg dry substrate with 57.00% biological efficiency and yield 550.00 g/kg dry substrate with 55.00% biological efficiency with black gram powder @ 1% in case of two strain (CI- 17-04 & CI-17-08) of C. indica respectively. A similar study has been done by Alam, et al., (2010)<sup>[1]</sup> on different organic additives to evaluate the yield and yield contributing characteristics of C. indica. They reported 30% maize powder supplement was effective for producing fruiting bodies, as well as maximum pileus diameter and stalk length biological and economic yield with 30% maize powder. Navathe (2013)<sup>[14]</sup> studied and reported maximum biological efficiency (119.10%) on paddy straw supplemented with 10% wheat bran (93.76%) and biological efficiency with supplementation wheat bran 5%.

Kumar, (2009)<sup>[9]</sup> also studied and reported minimum time for spawn run (17.33 and 17.67 days), average weight per fruit body (35.49 g and 31.29 g) as well as numbers of pinhead initiation (110 and 108/bag) in supplemented with mustard cake in two strains (Cl-6 and CI-4) of C. indica respectively. Whereas maximum numbers of fruiting bodies (24.00 and 24.33/bag) were harvested with lentil powder supplemented treatment from cake in two strains (Cl-6 and CI-4) of C. indica respectively. While, maximum yield was harvested (648.33 and 599.67 g/kg of dry substrate with 64.83% and 59.97% BE) in soybean flour treatment from both strains Cl-6 and CI-4 respectively. A similarly Amle et al., (2007)<sup>[3]</sup> also studied on various organic supplement additives on yield parameters of *C. indica*. They reported highest number of pin heads per bag (28.33), number of sporophores per bag (12.33), average sporophore height (128.17 mm), average pileus diameter (63.17 mm), and yield (444.94) g/kg of dry substrate with maize grains.

S. No.	Treatment	DFSR	DFFH	DFCP	NOFB	LOS (cm)	WOP (cm)	AW g/FB	Yield g/kg DS	<b>BE</b> (%)
1.	$T_1$	24.33	28.33	78.33	13.67	15.25	6.91	50.04	761.50	76.15
2.	$T_2$	27.67	31.67	83.33	11.67	13.33	5.62	43.62	610.83	61.08
3.	T3	23.67	27.67	77.00	14.00	15.83	6.91	50.35	783.83	78.38
4.	$T_4$	26.33	30.33	83.00	12.33	13.83	5.75	43.67	638.50	63.85
5.	T5	25.00	28.33	79.33	13.33	15.00	6.50	49.25	687.83	68.78
6.	T <sub>6</sub>	28.33	32.33	85.00	11.33	12.92	5.50	43.37	600.50	60.05
7.	<b>T</b> <sub>7</sub>	23.00	26.33	73.00	14.67	16.17	7.08	52.62	796.08	79.60
8.	$T_8$	25.67	30.33	82.00	12.33	14.17	6.17	45.04	657.50	65.75
9.	T9	21.00	25.67	72.00	11.67	13.92	5.91	46.83	680.50	68.05
	CD at 1 %	2.08	1.82	1.99	1.41	0.87	0.49	2.39	39.49	-
	SE(m)	0.69	0.61	0.66	0.47	0.29	0.16	0.80	13.17	-
	C.V	4.8	3.6	1.4	6.4	3.5	4.5	2.9	3.3	-

Table 1: Effects of different spices plant leaf powder on production of CI-20-03 strain of milky mushroom.

Average of three replication

DFSR = Days for spawn run, DFFH = Days for first harvesting, DFCP = Days for cropping period, NOFB = Number of fruiting bodies, LOS = Length of stipe cm, BOS = Breadth of Pileus cm, AW g/FB = Average weight g/fruiting body, Yield gm/kg DS = Yield g/kg Dry Substrate, and BE % = Biological efficiency %.

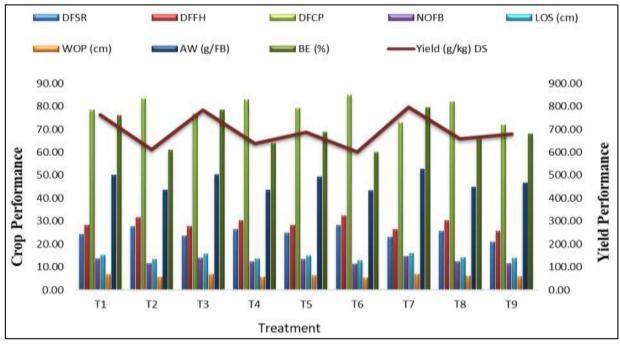


Fig 1: Effects of different spices plant leaf powder on production of CI-20-03 strain of milky mushroom.

S. No.	Treatment	DFSR	DFFH	DFCP	NOFB	LOS (cm)	WOP (cm)	AW g/FB	Yield g/kg DS	<b>BE</b> (%)
1.	T1	23.00	27.33	75.00	13.00	14.58	6.29	49.78	749.17	74.91
2.	T <sub>2</sub>	25.67	29.33	81.00	11.67	13.33	5.75	45.63	604.50	60.45
3.	T3	23.67	28.67	76.33	12.67	14.17	6.25	49.60	729.17	72.91
4.	T4	26.33	30.67	82.00	11.33	13.33	5.66	44.66	603.17	60.31
5.	T5	24.33	29.33	77.67	12.67	14.33	6.16	49.25	695.17	69.51
6.	T <sub>6</sub>	28.33	31.33	84.00	10.67	13.08	5.50	44.58	600.50	60.05
7.	T <sub>7</sub>	22.33	27.33	74.00	13.33	15.50	6.75	50.95	767.08	76.70
8.	T <sub>8</sub>	25.00	29.33	79.00	11.67	14.08	6.00	45.87	650.17	65.01
9.	T9	21.67	26.67	73.00	11.00	13.17	5.50	45.71	643.17	64.31
	CD at 1 %	1.81	2.08	2.80	1.27	0.99	0.39	1.61	49.44	-
	SE(m)	0.60	0.69	0.93	0.42	0.33	0.13	0.54	16.49	-
	C.V	4.3	4.2	2.1	6.0	4.1	3.8	2.0	4.3	-

Table 2: Effects of different medicinal plant leaf powder on production of CI-20-03 strain of milky mushro	om.
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Average of three replication

DFSR = Days for spawn run, DFFH = Days for first harvesting, DFCP = Days for cropping period, NOFB = Number of fruiting bodies, LOS = Length of stipe cm, BOS = Breadth of Pileus cm, AW g/FB = Average weight g/fruiting body, Yield gm/kg DS = Yield g/kg Dry Substrate, and BE % = Biological efficiency %.

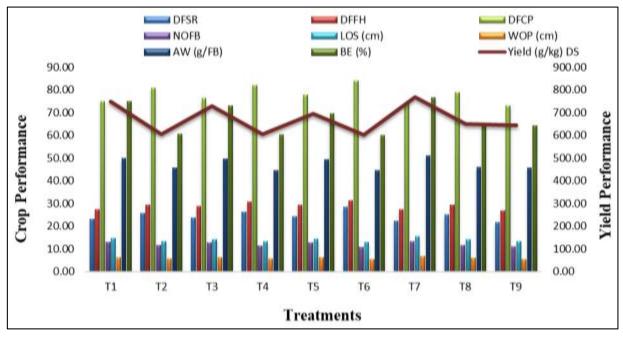


Fig 2: Effects of different medicinal plant leaf powder on production of CI-20-03 strain of milky mushroom

#### 4. Conclusion

On the behalf of this investigations the maximum Yield g/kg DS with BE, NOFB with AW g/FB and LOS cm with WOP cm, which was significantly more in case of both spices and medicinal plants leaf power at 2.5% concentration. Hence, we concluded that these spices and medicinal plant leaf power at 2.5% concentration are best supplement to increase the production of milky mushroom (CI-20-03).

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