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IMPACT OF DIFFERENT SUBSTRATES FOR SPAWN PRODUCTION AND PRODUCTION OF MILKY MUSHROOM (CALOCYBE INDICA)

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ABSTRACT

Milky mushroom (*Calocybe indica*) has become the third commercially grown mushroom in India after button and oyster mushrooms. This mushroom is gaining popularity due to its attractive robust, white sporocarps, long shelf life, sustainable yield, delicious taste, unique texture and cholesterol free foods with certain important medicinal properties including their antiviral effect. The varied production potential of different substrates is due to the variations in their physical properties and nutritional composition. *C.indica* is rich in protein, lipids, mineral, fibre, carbohydrate and is abundant with essential amino acids. In addition to this, it has most of the mineral salts required by human body such as potassium, sodium, phosphorus, iron and calcium. Due to its alkaline and higher fibre content, it is highly suitable for people with hyperacidity and constipation. Experiment was conducted in the Mahewa region, Prayagraj district of Uttar Pradesh. In this study, Locally available substrates wheat straw, paddy straw, pea straw, cotton waste, maize straw, sugarcane bagasse and wheat straw in combination with paddy straw were evaluated to find out the best suitable substrate for spawn production of *C.indica.* Amongst the various spawn substrates evaluated for spawn production, pearl millet grain was found to be the best spawn substrate for early spawn run. Six locally available substrates such as; wheat straw, paddy straw, pea straw, cotton waste, maize straw, sugarcane bagasse, wheat straw in combination with paddy straw were evaluated for growth and yield parameters for the cultivation of milky mushroom. The probable reason for this finding may be that paddy straw has high water holding capacity, porosity and bulk density. Paddy straw was found to be the best substrate for cultivation of milky mushroom.

KEYWORDS: ***Calocybe indica, mycelial growth, spawn production, different substrates***

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INTRODUCTION

In India, mushrooms vernacularly known as Khumbi, Chhatra, Kukurmutta, Dhingri, Dhartikaphool, etc. *C indica*is the third important commercially grown mushroom in India after button and oyster mushroom[1]. During last decade milky mushroom has become a major commercially cultivated species in south India particularly Tamil Nadu, Andhra Pradesh and Karnataka and during last 4-5 year its cultivation has become popular in north India as well. Ever increasing production in mushroom like *C. indica* pose challenge to the current supremacy of button mushroom in the world market[2]. Taxonomic position of milky mushroom falls in Phylum-Basidiomycota, Class-Agaricomycetes, Order-Agaricales, Family-Tricholomataceae[3,4]. This is an indigenous tropical mushroom, suitable for cultivation during summer and rainy season. *C. indica* is rich in protein, lipids, mineral, fibre, carbohydrate and essential amino acids[5,6]. It is also an excellent source of thiamine, riboflavin, nicotinic acid, pyridoxine, biotin and ascorbic acid[7]. In addition to this, it has most of the mineral salts required by human body such as potassium, sodium, phosphorus, iron and calcium. Due to its alkaline and higher fiber content it is highly suitable for people with hyperacidity and constipation[8]. A substrate is an important substance for growing mushrooms. Various kinds of grains were successfully used by different workers to prepare the spawn[9,10]. A wide range of diverse cellulosic substrates are used for cultivating mushrooms. Paddy straw, wheat straw, soybean straw, sugarcane baggase, cotton waste and coconut coir pith are the common lignocellulosic substrates used for cultivation of the mushroom[11,12]. 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. After complete mycelial formation casing is done to provide a reservoir of water for the developing fruiting body. Casing layer plays an important role in inducing fructification by changing vegetative phase to reproductive phase[13]. This mushroom requires a temperature of 30-35º C and relative humidity of 70-80% for cultivation which is conducive to environmental conditions of most part of India[4,14-16]. Keeping this in a view, the present study was conducted to find out the potentiality of different grains for spawn production and agricultural wastes for milky mushroom production (*C. indica*).

MATERIALS AND METHODS

The experiment was conducted in the Mahewa region, Allahabad district of Uttar Pradesh.

Effect of different substrates for spawn preparation of Calocybe indica.

Media preparation

Potato Dextrose Agar (PDA) medium was used for isolation, purification and maintenance of the mushroom culture.

Isolation and purification of the culture

Young basidiocarp was cleaned with sterilized distilled water and dipped in 0.1% mercuric chloride solution for 1 minute. The basidiocarp was air dried and split open longitudinally from centre and vegetative tissues were cut from the collar region (junction of pileus and stipe) into bits. These bits (2-3 mm) were dipped in mercuric chloride ($HgCl_2$) for few seconds and then washed in sterilized distilled water. Bits were then inoculated in the petri-plates containing potato dextrose agar (PDA) media and incubated at 32^0C±1 in BOD (Bio-Oxygen Demand) incubator. Transfer of the culture was done within 5-6 days of isolation. A bit of mycelium from advancing zone was taken with the help of sterilized inoculation needle and was carefully transferred to the PDAslant. Spawn is the seed or vegetative mycelium of mushroom. Various kinds of grains have been successfully used by different workers to prepare the spawn[9,10]. In this study, five substrates maize grain, pea, wheat grain, sorghum grain and pearl millet grain were evaluated due to their easy availability and low cost. The experiment was conducted in Completely Randomized Designs (CRD)with five replications. All the selected grains were thoroughly washed in sufficient water two to three times to remove soil debris, straw and other undesired materials. These were boiled in double volume of water for 25-30 minutes. After boiling, the grains were placed on sieve to drain out the excess water and was spread on clean floor to dry the surface area, minimize stickiness for a few hours. Now the grains were mixed with gypsum@ 2% and calcium carbonate @ 0.5% per kg dry weight basis of grains. Mixed grains were filled in glucose bottle up to 2/ 3 volumes and plugged with non-absorbent cotton. Mouth of the glucose bottle was wrapped with aluminium foil with the help of a rubber band. After that glucose bottle were sterilized in autoclave at 15 lb psi or 121^0C for 1.5 hours. The glucose bottles were then shifted to a room for cooling aseptically. Mycelium of *C.indica* was aseptically transferred to this glucose bottle and incubated at temperature of 32±1^0C in BOD incubator. Linear mycelial growth was measured on each substrate after 12 days of incubation.

Preparation of substrates

Six locally available substrates like wheat straw, paddy straw, pea straw (pod), cotton waste, maize straw and sugarcane bagasse in combination (1:1) with wheat straw were selected for the cultivation of this mushroom. All the selected substrates were chopped in to 3-5 cm pieces and soaked in water containing carbendazim (75 ppm), formalin (500 ppm) and nuvan (65 ppm) for 14-18 hours[17]. Each substrate was taken out from the solution and excess water was drained out for 2-3 hrs. The experiments were evaluated in (Completely randomized designs) CRD with three replications.

Spawning

A moisture content of about 65% was maintained in the wet substrate prior to spawning. Spawning was done @ 5% by wet weight basis of the prepared substrate in polypropylene bags of 60 × 40 cm size with 100 gauge thickness thickness.[18] After spawning bags were shifted to spawn room and kept in dark place where temperature (25-30 ^{0}C) and relative humidity (85-90%) were maintained till mycelium colonized the substrates and then the substrates were ready for casing.

Figure 1
Spawning

Casing
Casing material provides physical support, moisture and allows gases to escape from the substrates. Casing material was prepared by using garden loam soil (50%), sand (25%) and FYM (25%). The casing soils were prepared by thorough mixing of the selected casing soils in the proper ratio. Each casing soil was chemically sterilized with 4 per cent formalin solution @ 500ml/cubic feet and covered with plastic sheet for 72hours. Casing material was spread about 3-4 cm thickness on roughed uniform top surface of the bags and slightly pressed. Temperature was maintained at 25-30^{0}C and relative humidity at 85-90% till case run. The mycelium emerged on casing soil after 10 days, the environmental conditions were changed in cropping room by providing fresh air through ventilation and light for 6-8hrs, relative humidity 90-95% were maintained by spraying of the water thrice a day.

Figure 2
Casing

Harvesting
Mushroom was harvested by holding the cap and twisting the fruiting bodies. Days required for pinhead initiation, first harvest, number of fruiting bodies, yield and biological efficiency were recorded.

Figure 3
Harvesting

Biological efficiency
Biological efficiency of mushroom on fresh weight basis was calculated by formula given by [19].

$$\text{Biological efficiency (\%)} = \text{Total biological yield/total substrate used} \times 100$$

RESULT

Effect of different substrates on spawn production of C. indica.

In this study, five locally available substrates were evaluated, to find out the suitable substrate for spawn production of *C. indica* (Table 1).The days taken for spawn run on different substrates ranged from 12.40 days to 17.60 days. Pearl millet grains took minimum days for complete mycelial growth (12.40 days) followed by sorghum grains (13.60 days), wheat grains (15.00 days), pea grains (16.20 days) and maize grains (17.60 days). All kinds of grains used as substrates for spawn production show significant differences among each other. The findings were in close agreements with the present results [3,10]. They found that wheat grains and sorghum grains as good substrates for spawn production of *C. indica*.

Table 1
Effect of different substrates on mycelia growth of Calocybe indica

S.No.	Substrates	Average mycelial growth (Days)
1.	Maize grain	17.60
2.	Pea grain	16.20
3.	Wheat grain	15.00
4.	Sorghum grain	13.60
5.	Pearl millet	12.40
	SEd.	**0.88**
	C.D%	**1.86**

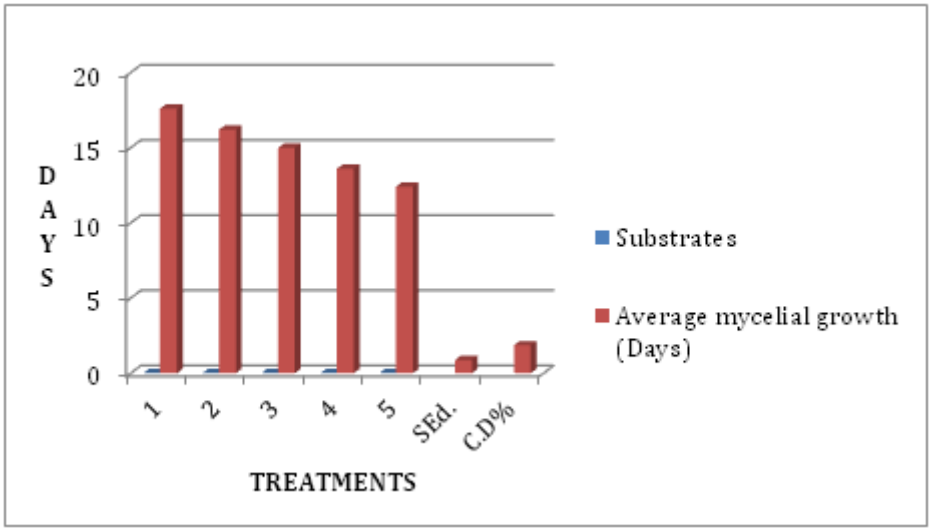

Figure 4
Effect of different substrate on the average mycelial growth of C. Indica

Effect of different substrates on C. indica.

In order to find out the best substrates for the cultivation of *Calocybe indica*, six locally available substrates were used alone or in combination. It is evident from the data that wheat straw substrate took minimum days for complete spawn run of *C.Indica* (18.16 days) followed by wheat straw + paddy straw (18.33 days), sugarcane bagasse (20.16 days), pea straw (21.17 days), paddy straw (22.16 days), maize straw (22.70 days).However, cotton waste took maximum days for complete spawn run of *C.Indica* (23.00 days) (Table 2).The days required for first harvest ranged from 25.00 to 31.10 days. Wheat straw took minimum days for the first harvest (25.00 days) followed by wheat straw + paddy straw (26.50 days), sugarcane bagasse (27.03 days), paddy straw (29.16 days), pea straw (30.86 days), maize straw (31.00 days).However, cotton waste took maximum days for first harvest (31.10 days) (Table 2).The average maximum number of fruiting bodies/bag ranged from 10.66 to 20.00. Maximum number of fruiting bodies/bag was obtained in paddy straw (20.00) followed by wheat + paddy straw (15.66), wheat straw (15.00), maize straw (14.00), sugarcane bagasse (13.00), pea straw (12.00). However, minimum number of fruiting bodies/bag (10.66) was recorded in cotton waste (Table 2). The total yield ranged from 483.33g to 842.00g/bag. Maximum yield was recorded in paddy straw (842 g) followed by wheat + paddy straw (645.00 g), wheat straw (628.00 g), maize straw (625.00 g), sugarcane bagasse (558.66 g), pea straw (525.00 g)/bag, respectively. However, significantly lowest yield (483.33g) was recorded in cotton waste substrate (Table 2).The total biological efficiency ranged from 38.26 to 21.96%. Maximum biological efficiency was recorded in paddy straw (38.26 %) followed by wheat + paddy straw (29.31 %), wheat straw (28.54 %), maize straw (28.40 %), sugarcane bagasse (25.34 %), pea straw (23.86 %), respectively. However, significantly lowest biological efficiency (21.96 %) was recorded in cotton waste substrate (Table 2).These results are in close agreements with the findings of [20,21]. They reported that the wheat straw + paddy straw substrate gave minimum

days for spawn run and maturation of fruiting bodies and higher biological efficiency. Similarly, minimum days required for spawn run and for pinhead formation were seen on wheat straw for *C.indica*[21-23].However,[17]they obtained the best yield in paddy straw and sorghum stalks substrate (356 and 354.3g/bed).[24-26]Also reported that paddy straw was the best substrate for cultivation of *C.indica* which also supported the results of present finding. The probable reason for this finding may be that paddy strawhave high water holding capacity, porosity and bulk density. These factors would have directly affected the microbial build up and enhanced the initiation of pin heads of *C.indica.*

Table 2
Effect of different substrates on the growth and yield parameters of Calocybe indica.

S.No.	Substrates	Days required for pinhead formation (Days)	First harvest (Days)	No. of fruiting body/ bag	Average yield of (g/ bag)	Biological efficiency (%)
1	Wheat straw	18.16	25.00	20.00	842.00	28.54
2	Paddy straw	22.16	29.16	15.00	628.00	38.26
3	Pea straw	21.17	30.86	12.00	525.00	23.86
4	Cotton waste	23.00	31.00	10.66	483.33	21.96
5	Maize straw	22.70	31.10	14.00	625.00	28.40
6	Sugarcane bagasse	20.16	27.03	13.00	558.66	25.34
7	Wheat straw + paddy straw (1:1)	18.33	26.50	15.66	645.00	29.31
	SEd.	**0.40**	**0.40**	**0.68**	**1.71**	**0.08**
	C.D %	**1.53**	**1.09**	**2.26**	**5.70**	**0.26**

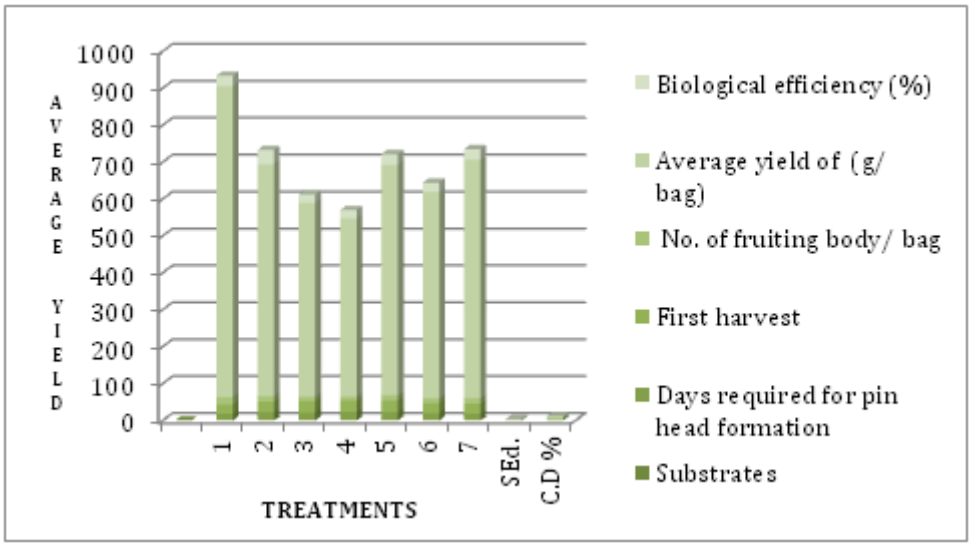

Figure 5
Effect of different substrates on the growth and yield parameters of C. indica.

CONCLUSIONS

The production of milky mushroom mainly depends on quality of spawn and substrate used. In the present investigation amongst the various spawn substrates used, pearl millet grain took minimum days for complete mycelial growth and was found to be the best spawn substrate. Paddy straw was found to be the best substrate for cultivation of milky mushroom which is in agreement with earlier reports of several scientists. The varied production potential of various substrates is due to the variations in their nutritional composition and physical properties.

AUTHORS CONTRIBUTION STATEMENT

Amit Kumar Maurya- Handled the whole experimental part and data collection. Vinny John- Assisted the experiment, performed the numerical calculations for the suggested experiment. Rakhi Murmu- carried out the research study, evaluated the results and drafted the manuscript. Dr. Sobita Simon- conceived the idea and guided me in conducting this research study and also reviewed the manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

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