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Research Paper

Yield Improvement of Calocybe Indica Fruiting Bodies (Milky Mushroom) From Locally Available Unexplored Lignocellulosic Substrates

Microbiology

KEYWORDS : Calocybe indica cultivation, substrate, reeds, casing materials, shade, Spent Mushroom Substrate.

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ABSTRACT

This research concentrated on yield improvement of C. indica on unexplored locally available lignocellulosic materials such as paddy straw, reeds, banana stem, sugar cane leaves, sugar cane bagasse, coir pith and sorghum husk. The maximum bioefficiency of C. indica was obtained from paddy straw (134.16%) followed by reeds (104.30%). Different casing materials (garden soil, coir pith compost, vermicompost), different shade (blue, yellow and dark) were influenced for yield enhancement of C. indica and maximum bioefficiency was recorded in vermicompost (140.27%) and blue shade (134.16%) respectively. Seven spent mushroom substrate were analyzed for highest reduction of lignin, acid detergent lignin, cellulose and hemicellulose. Coir pith recorded for the highest reduction of lignin (25.29%) and acid detergent fiber (18.27%). Sugar cane bagasse recorded for highest reduction of cellulose (31.62%) and banana stem and for hemicelluloses (22.96%). The reeds can be utilized for successful cultivation of C. indica at commercial level than the paddy straw.

1. Introduction

The majority of the agricultural wastes primarily were used as cattle feed and remaining million tons of agricultural wastes are discarded, burned and neglected. The edible mushrooms can be successfully cultivated by exploiting varied locally available agricultural wastes in different regions and can be widely used as human food or as supplementary food and also generate additional income by utilizing the resources available.

Calocybe indica is a tropical edible mushroom of Indian origin and can be cultivated indoor in high temperature and humidity areas (Purkayastha & Chandra, 1974). *C. indica* commonly known as the 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 (Krishnamoorty et al*.,* 1998). The 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 (Pani, 2010). It contains 32.3% protein on dry weight basis and possesses 41% crude fibers, different types of vitamins, minerals (Chandravadana et al*.,* 2005 & Dandi et al., 2009). Due to their high content of vitamin, protein and minerals, mushrooms are considered as "Poor man's Proteins". Mushrooms can be used for the food to solve the malnutrition problem. Mushrooms have good nutritional value particularly as a source of protein that can enrich human diets, especially in some developing countries where animal protein may not be available and are expensive (Pandey, 2004 & Manandhar, 2003). *C. indica* can be cultivated in all types of lignocellulosic agricultural residues such as paddy, wheat, barley, maize, groundnut haulms, grasses, cotton and leaf fall of trees and sugarcane leaves banana stem and different grass. Presently all the commercial growers are utilizing paddy straw and wheat straw for *C. indica* cultivation. Whereas paddy cultivation in many parts of India have been dwindling mainly due to the cost of cultivation and labour shortages. Furthermore, paddy and wheat straw are widely used as cattle feed and are thus costly (Chandravanshi et al*.,* 2012). In other hand reeds are naturally growing in river delta sides mainly used as mat weaving purpose remaining materials unutilized. The present study was focused on cultivation of *C. indica* by utilizing different locally available agricultural wastes, also exploiting different casing materials and roofing materials to improve the bioefficiency. Reeds as an alternative substrate for cultivation of *C. indica* were explored in this study. The SMS of *C. indica* was analyzed for the lignin, hemicelluloses, cellulose and fiber contents.

2. Materials and Methods

2.1. Mushroom strain

The pure mycelial culture of *C. indica* was obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India and maintained at 25°C. Pure culture was stored at 4°C.

2.2. Production of *C. indica* spawn

Sorghum grains were half boiled for 30 min in boiling water until they become soft. Cooled grains were mixed with calcium carbonate (2 %, W/W). Half boiled grains were individually filled (250 g/bag) in a poly propylene bags (13 × 26 cm) and plugged with non absorbent cotton. The grain filled bags were sterilized in autoclave at 15 lb pressure (121°C) for 90 min and allowed to cool at room temperature. Seven days old mycelial discs (5 mm) of *C. indica* was aseptically inoculated in the spawn bags and incubated at 28 ± 2°C and dark chamber for 15 - 25 days.

2.3. Mushroom bed preparation

The locally available lignocellulosic substrates such as paddy straw (PS), reeds(R), banana stem (BS), sugarcane bagasse and leaves (SCB and SCL), coir pith (CP) and sorghum husk (SH) were selected for mushroom bed preparation. Mushroom beds were prepared in polypropylene bags of size (30 × 60 cm). The substrates were sterilized at 121°C for 90 minutes and shadow dried up to 60% moisture. The cylindrical polypropylene bags were filled up to 7 - 8 cm layer height with the processed substrates and 10 g of bed spawn was inoculated on the substrate along the circumference of the bags. The substrate was again layered to 5 cm height and spawn was inoculated along the corners of the mushroom beds with gentle pressing of the substrate in each layer for tight packing. The process was repeated until eight layers of spawn

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and substrate (90 cm) were packed. The inoculated bag was perforated (12 no's) with sterilized teasing needles. The beds were incubated for 15 to 17 days to complete the spawn run and maintained temperature at 25 - 28°C with relative humidity of 85 %.

After the complete colonization of mycelium in the mushroom beds, the cylindrical beds were cut horizontally into two equal halves. Over the each half bed, casing materials (steamed for 1 hour) was applied 1-2 cm. Beds after casing were incubated in polythene chamber (12 × 6 × 4 feet size) having blue, yellow and thatches sheets as roofing materials for different shade. The different casing materials like alkaline (garden) soil, coir pith compost and vermi compost were applied to the mushroom beds and recorded its bioefficiency. Coir pith compost and spent mushroom substrates was converted into vermicompost (Nagarajan et al., 1985).

2.4. Construction of mushroom shed for *C. indica*

The partially sunken chamber lined with blue and yellow colored high density tarpaulin (Silpauline company) sheet (90 Gram per square meter), thatches as roof materials individually.

2.5. Yield and bioefficiency

Total weight of all the fruiting bodies harvested from all the four pickings were measured as total yield of mushroom. The bioefficiency (yield of mushroom per kg substrate on dry wt. basis) was calculated by the following formula (Chang *et al.,* 1981).

$$B.E.\ (\%) = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

2.6. Bio chemical analysis of substrates before and after harvest

Cellulose, hemicelluloses, lignin and fibre contents in the lignocellulosic substrates were determinated by following of Goering and Van Soest, 1975 and AOAC, 1975.

3. Results and Discussion

Mycelium colonization of *C. indica* in mushroom beds containing different substrates such as paddy straw and reeds was initiated on day 2 and fully colonization on day 10 (Table 1). The mushroom bags were cut into two half and alkaline garden soil as casing medium was applied on 15th day for paddy straw, followed by reeds on 17th day. The pin head primordia of *C. indica* was observed on day 28 in mushroom bed containing paddy straw whereas reeds took 29 days to initiate the pin head primordia. The pin head primordia was developed into matured fruit bodies on day 30 in mushroom bed containing paddy straw whereas reeds took 32 days.

Among seven lignocellulosic substrates, paddy straw recorded for the maximum production of fruit bodies (804.99 g) with the bioefficiency of *C. indica* (134.16 %) harvested in 4 intervals (300.00 g/29 - 30 days, 253.33 g/37 - 38 days, 185.00 g /45 - 46 days and 66.66 g/52 - 54 days respectively). Next to the paddy straw, reeds influenced for the production of fruit bodies (678.32 g) with the bioefficiency (104.30 %) harvested in 4 intervals (273.33 g/30 - 32 days, 215.00 g/38 - 40 days, 128.33 g /47 - 49 days and 61.66 g/55 - 57 days respectively) and (Table 2) (Plate 1).

Cultivation of *C. indica* on reeds recorded bioefficiency (104.30 %) which was slightly less than paddy straw but higher than when compared to banana stem, sugar cane leaves, sugar cane bagasse, coir pith and sorghum husk. (Saranya *et al.,* 2011) investigated on growth and yield performance cultivation of *C. indica* on different substrates. The yield of fresh mushroom obtained from paddy straw, teak leaves, sugarcane trash substrates were 1140, 745 and 570 g / kg respectively. Our results were also in agreement with earlier studies (Krishnamoorthy, 2003; Tewari, 2004 and Chaubey *et al.,* 2010).

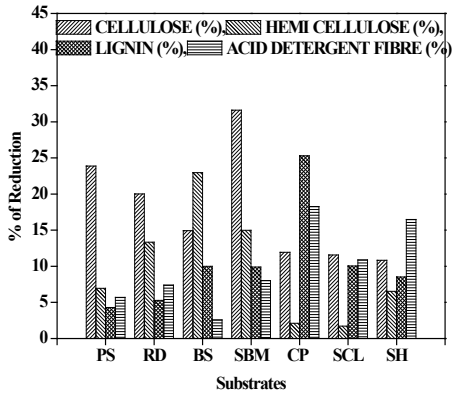

Fig. 1. Biochemical properties of lignocellulosic substrates before and after cultivation (SMS) of CI 01

PS- paddy straw, RD-reeds, BS- banana stem, SBM- sugarcane bagasse milled, CP- coir pith, SCL –sugarcane leaves, SH- sorghum husk, % of R-percentage of reduction.

Plate 1. Cultivation of *C. indica* using locally available lignocellulosic substrates

A. Paddy straw, B. Reeds, C. Banana stem, D. Sugar cane bagasse milled, E. Coir pith, F. Sugar cane leaves, G. Sorghum husk.

1. Cultivation of *C. indica* using locally available lignocellulosic substrates crop cycle days details

Substrates	DSR	DCSA	DPHF	DFH	DSH	DTH	DFH
PS	10	15	26-28	29-30	37-38	45-46	52-54
RD	10	17	28-29	30-32	38-40	47-49	55-57
BS	10	17	28-29	31-32	39-40	48-49	57-58
SBM	11	17	28-29	30-32	39-40	47-48	56-57
CP	12	18	30-31	39-40	49-50	58-59	-
SCL	11	17	28-29	31-32	38-39	46-47	-
SH	11	17	28-29	30-31	38-39	47-48	56-57

Key: DSR – Days for spawn run, DCSA – Days for Casing soil application, DPHF – Days for Pin head formation, DFH - Days for First harvest, DSH - Days for Second harvest, DTH - Days for Third harvest, DFH - Days for Fourth harvest.

Table 2. Cultivation of *C. indica* using locally available lignocellulosic substrates harvest details

Subst.	SW (g)	FH (g)	SH (g)	TH (g)	FH (g)	TMH (g)	BE (%)
PS	600	300.00± 57	253.33 ± 6.66	185.00±10.40	66.66±6.00	804.99	134.16± 0.48
RD	650	273.33±6.66	215.55±7.63	128.33 ±6.00	61.66±4.40	678.32	104.30±0.04
BS	650	208.33±8.33	158.33±8.33	78.33±4.40	55.00±2.88	499.99	76.92±0.88
SBM	650	200.00±14.43	180.00±5.00	95.00±10.40	51.66±1.66	526.66	81.02±0.67
CP	600	151.16±13.64	93.33±3.33	66.66±4.40	-	311.15	51.94±1.20
SCL	550	205.00±2.88	98.33±1.66	71.66±3.33	-	374.99	68.18±1.05
SH	600	200.00±5.77	118.33±4.40	83.33±4.40	48.33±4.40	374.99	74.99±0.96

Key: Subst. – Substrate, SW - Substrate weight in grams, FH - First harvest, SH - Second harvest, TH - Third harvest, FH - Fourth harvest, TMH- Total mushroom harvested, BE – Bioefficiency

Table 3. Influence of different casing media on bioefficiency of *C. indica*

DC	SW (g)	FH	SH	TH	FH	TH (g)	Bioefficiency (%)
PSGS	600	300.00±57	253.33 ± 6.66	185.00±10.40	66.66±6.00	804.99±4.40	134.16± 0.48
RDGS	650	273.33±6.66	215.55±7.63	128.33 ±6.00	61.66±4.40	678.32±3.12	104.30±0.04
PSVC	600	336.66±18.55	271.66±8.81	161.66±10.13	71.66±1.66	841.66±4.40	140.27±0.73
RDVC	650	325±14.43	211.66±7.24	136.66±11.66	51.66±4.40	725±2.88	111.53±0.44
PSCP	600	236.66±18.55	228.33±10.92	88.33±1.66	55±10.40	608.33±4.40	100.13±0.07
RDCP	650	240±5.77	193.33±8.81	103.33±13.33	65±7.63	601.66±4.40	92.55±0.67

Key: DC – Different composting, PSGS – Paddy straw Garden soil, RDGS– Reeds Garden soil, PSVC – Paddy Straw Vermi Compost, RDVC– Reeds Vermi Compost, PSCP – Paddy Straw Coir pith Compost, RDCP – Reeds Coir pith Compost, TH (g) – Total Harvest in grams

Table 4. Influence of different roofing materials on the bio efficiency of *C. indica*

DL	SW (g)	FH (g)	SH (g)	TH (g)	FH (g)	TMH (g)	BE (%)
PSB	600	300.00±57	253.33 ± 6.66	185.00±10.40	66.66±6.00	804.99±4.41	134.16± 0.48
RDB	650	273.33±6.66	215.55±7.63	128.33 ±6.00	61.66±4.40	678.32±3.12	104.30±0.04
PSY	600	300± 5.77	238.33±19.64	198.33±1.66	61.66±10.13	798.33±4.40	133.05±0.73
RDY	650	253.33±8.81	238.33±13.64	125±12.58	56.66±6.00	675±5.77	100.31±0.15
PSD	600	125±2.88	86.66±14.52	56.66±4.40	35±7.63	320±27.53	53.33±4.59
RDD	650	128.33±1.66	103.33±6.66	51.66±3.33	25±14.43	308.33±7.26	47.43±1.11

Key: DL – Different lighting, , PSB – Paddy straw blue, RDB – Reeds blue, PSY – Paddy Straw Yellow, RDY – Reeds Yellow, PSD – Paddy Straw Dark, RDD – Reeds Dark.

3.1. Influence of different casing media on bioefficiency of *C. indica*

In order to improve the bioefficiency of *C. indica* three different casing medium were applied individually and the mushroom beds were incubated under blue light. Among the three casing media, paddy straw applied with vermicompost recorded for the maximum bioefficiency (140.27 % and 111.53 % of *C. indica* in paddy straw and reeds respectively. (Kale, 1998: Nagaratna and Mallesha, 2007) reported that the vermicompost SMS as the casing medium recorded for the high bioefficiency (111.98 %) of *C. indica* (Table 3).

3.2. Influence of different roofing materials on the bioefficiency of *C. indica*

In order to further improve the bioefficiency of *C. indica,* three types of roofing materials were studied along with paddy straw and reeds. Among the three roofing materials, blue polythene sheet influenced for maximum bioefficiency (134.16 % and 104.30 %) of *C. indica* in paddy straw and reeds respectively) whereas the shed laid with thatches as roofing material significantly reduced the bioefficiency (53.33 % and 47.43%) of *C. indica* in paddy straw and reeds respectively (Table 4). The bioefficiency of *C. indica* was correlated with research findings (Theradimani *et al.,* 2001; Krishnamoorthy, 2003 and Pani, 2011) where, reported that blue coloured shade influenced for maximum bioefficiency of *C. indica.*

3.3. Biochemical analysis of spent mushroom substrates

Among the seven *C. indica* spent mushroom substrates, CP spent substrate recorded for the highest reduction of lignin (25.29%). SBM recorded highest reduction of cellulose (31.62%) where as BS recorded highest reduction of hemicelluloses (22.96 %) and CP recorded highest reduction of acid detergent fiber (18.27%) (Fig. 1) content was reduced with maximum rate in fructification stage and minimum in spawn run stage (Zadrazil, 1975). Similar findings were reported by (Kausar, 1988) in which the crude fibre, cellulose and lignin contents dropped significantly in the rice straw biodegraded with *Pleurotus ostreatus* and *Pleurotus sajor-caju.* The results from the present investigation are in consonance with (Moorthy, 1981: Singh *et al*., 1989) observed that cellulose, hemicellulose and lignin are degraded up to an extent of 75 % during the growth period.

The present study concluded that *C. indica* can be cultivated by different type of lignocellulosic substrate and among those reeds was a best alternative and replacement of traditional substrates. The spent mushroom substrates as casing materials was enhancing yield of *C. indica*. The present study further leads us to cultivate and commercialize the *C. indica* production using reeds for the benefit of society.

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