International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Pilot Scale Cultivation of Calocybe Indica by Utilizing Reeds as the Substrate and Nutritional Analysis of its Harvested Fruit Bodies

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Abstract: ***The present study focused on pilot scale cultivation of Calocybe indica throughout the year (12 months) by utilizing reeds as the substrate. The efficacy of reeds as the alternate substrate and recorded the maximum bioefficiency (121.83%) at the month of April. Totally 71.23kg of fresh fruit bodies were harvested from 65 kg of dry substrate with bioefficiency of 115.74%. The nutritional parameters of harvested fruit bodies were analysed viz., carbohydrate (57.27g/100g), protein (28.73g/100g), fat (0.73g/100g), crude fiber (37.52g/100g), ash (8.80g/100g) and folic acid (57.0µg/100g). The mineral contents parameters of harvested fruit bodies were analysed viz., calcium (135mg/100g), iodine (227µg/100g), iron (45.91mg/100g), magnesium (177.3mg/100g), manganese (1.5mg/100g), phosphorus (0.88g/100g), potassium (2.92g/100g) and zinc (13.9mg/100g). Among seven vitamins tested vitamin B3 was recorded maximum (22.94mg/100g). Non detectable level of pesticides such as DDT, p.p.DDT, alpha BHC, beta BHC, gamma BHC and delta BHC. Heavy metal contents were recorded such as arsenic, cadmium, mercury at non detectable level whereas chromium (2.07mg/kg), copper (2.41mg/kg), lead (0.22mg/kg), selenium (4.60mg/kg) recorded. Hence the present shows a positive path that C. indica can be cultivated using alternative substrate and its efficacy was tested at pilot scale. As this mushroom known to several medicinal properties this study will be further focused towards pharmaceutical applications.***

Keywords: *Calocybe indica* cultivation, reeds, bioefficiency, nutritional parameters

1. Introduction

Mushrooms have existed for millions of years and the mankind has regarded them as a valuable food. Mushrooms with their flavour, texture, nutritional value and with very high productivity level per unit area rightly have been identified as an excellent food source to fight malnutrition in developing countries. Mushroom cultivation is the only current economically viable biotechnology process, where in waste materials or negative value, crop residues may be converted into valuable food [1]. Growing edible mushrooms is the most efficient method of bioremediation of the large quantity of lignocellulosic wastes generated annually through activities of agriculture and allied activities [2]. It can help in recycling the organic wastes into profitable products [3]. *Calocybe indica* (milky mushroom) is a potentially new species to mushroom growers. It is a robust, fleshy, milky white, umbrella like mushroom which resembles button mushroom. It grows well at a temperature of 25-35°C and relative humidity more than 80%. Milky mushrooms can be cultivated throughout the year in the entire plains of India [4-5]. This mushroom can be harvested from 24-28 days after spawning and the total crop cycle is 45-50 days. Most importantly the milky mushroom has an extended shelf life of 3-5 days compared to other cultivated species, making it more amenable to handling, transportation and storage. So, there is a growing interest among the farmers towards milky mushroom. *C. indica* mushroom is growing through year whereas the maximum yield was recorded in summer month (March to July) [6]. The mushroom is rich in protein, lipids, fibres, minerals, carbohydrate and contains an abundant amount of essential amino acids. It is an excellent source of thiamine, riboflavin, nicotinic acid, pyridoxine and ascorbic acid [7-8]

On a dry weight basis, they are considered to be good sources of digestible proteins (10–40%), carbohydrates (3–21%) and dietary fibre (3–35%). Mushrooms contain all the essential amino acids and are limiting in the sulphur containing amino acids, cysteine and methionine [9-10]. Although mushrooms contain all the main classes of lipids, including free fatty acids, mono-, di- and tri-glycerides, sterol esters and phospholipids, their levels are low at approximately 2– 8% (on dry weight basis) and the calorific value of most mushrooms is also low. Mushrooms are excellent sources of thiamine (vitamin B1), riboflavin (vitamin B2), nicotinic acid (vitamin B3), biotin and ascorbic acid (vitamin C). Edible mushrooms in cooked or other processed forms are nutritionally sound and good dietary component for vegetarians [11].

According to USD (United States Department of Agriculture) a National Nutrient Database, Mushrooms are high in protein, vitamins and essential elements including calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, copper, manganese and selenium[12]. Mushrooms are a group of fungi with good source of high quality proteins, rich in vitamins and minerals and its nutritional value equal to meats, eggs and milk with low calorie and cholesterol free. Mushrooms preserved by drying have a good flavor, prevents microbial deterioration and enhances the appearance.

Therefore our present research on cultivation of *C. indica* by utilizing reeds as an alternative substrate was carried out at pilot scale. The yield performance of *C. indica* was continuously monitored for a period of 12 months. The nutritional parameters were also estimated to harvested *C. indica* fruit bodies powder.

2. Materials and Methods

2.1 Spawn preparation

Half cooked sorghum grains were mixed with calcium carbonate at the rate of 2 per cent (20 g/kg of seed) and filled in poly propylene bag (300g) and were autoclaved at 1.4kg/cm^2 pressure for 1.5 to 2.0 hour. The sterilized bags were aseptically inoculated with pure mycelium of *C. indica*, maintained on potato dextrose agar (PDA) slopes and incubated at room temperature (28±2 ℃). The spawn growth was completed in 12 to 14 days.

2.2mushroom Bed Preparation

Milky mushroom was cultivated by using reeds as substrate. Chaffed reeds bits of 3-5cm in length were soaked in cold water for 8 hour and after draining the excess water the reeds bits were boiled for 30 to 45 minutes in a separate drum with fresh water. The excess water was drained and the substrate was shade dried to remove excess moisture.

Mushroom beds were prepared by using 30X60cm size polythene bags of 100 gauge with two holes laterally. The reeds (sterilized) placed as one layer over that sandwiched with spawn of *C. indica* in alternative layer (6%) so that, entire bed contained 7 to 8 layers of reeds alternative with the spawn. The beds were incubated for complete the spawn run in the mushroom beds. After completion of spawn- run, the cylindrical beds were cut horizontally into two equal halves. Over the each half bed, casing soil (soil steamed for 1 hour, pH 8.0) was applied to a height of 1-2 cm. Beds after casing were incubated in cultivation chamber [13].

2.3 Cultivation Chamber

Beds after preparation are incubated in a room with a temperature range of 30±2℃. Rectangular pit was dug at east to west direction. Twelve nos of L angles were bended to curved shape and installed on corner side at every 5 feet with the help of cement concrete in both side of the mushroom shed. The hallow pipes were installed on L angle pipe bind with steel rope at 3 feet ones. A brick wall was constructed in the both corner side of pit. Door (3′ × 2′) and windows was installed in the east side wall. The shed roof was covered with blue colour tarpaulin (made of silpaulin and 90 GSM thicknesses sheet). Inside the chamber the temperature was kept around 30-35℃ and relative humidity more than 90 percent. The beds were uniformly and regularly sprayed with water. To assess the yield performance of *C. indica* in different months in a year, mushrooms beds were laid out from January, 2011 to December, 2011 at monthly intervals. Yield of mushroom especially bioefficiency along weather parameters such as maximum, minimum temperature, humidity and rain fall were also recorded.

2.4 Yield and Bioefficiency

Total weight of all the fresh 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 [14].

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

2.5 Analysis of Nutritional Parameters

The fruit bodies of harvested *C. indica* mushrooms were dried in an oven at 40°C for 48 hours. The dried *C. indica* powder was sent to Accreditation laboratory which SGS laboratory, Chennai for nutritional parameters estimation.

3. Results and Discussion

The bioefficiency of *C. indica* by utilizing reeds as substrate was evaluated continuously for a period of 12 months. The maximum bioefficiency (121.83 %) of *C. indica* was recorded during the month of April 2011, the temperature ranged between 34.8 ℃ to 26.9 ℃, 90 to 75 % of relative humidity and 30 mm rainfall (Table 1). The minimum bioefficiency (42.50 %) was recorded during the month of November, 2011, temperature ranged between 29.2 ℃ to 19.5 ℃, 85 to 95 % relative humidity and 330.1 mm rain fall.

Table 1: Bioefficiency of *C. indica* during different month of year 2011

Month (2011)	Temperature(℃)		Relative humidity		Total Rain fall	Bioefficiency (%)
	Maximum	Minimum	Maximum	Minimum		
January	33.1	24.2	95	80	0.75	116.66
February	33.5	25.8	90	75	0	119.16
March	34.4	25.7	90	75	0	120.00
April	34.8	26.9	90	75	30.0	121.83
May	36.7	27.2	85	75	11.5	118.33
June	35.4	25.1	90	70	87.5	120.00
July	35.8	24.3	90	75	47.5	108.33
August	34.1	24.5	95	75	23.0	106.66
September	34.6	23.6	95	75	124.0	104.16
October	29.2	23.7	95	85	105.0	50.00
November	29.2	19.5	95	85	330.1	42.50
December	30.1	23.6	95	80	109.5	51.66

Key: mm- milli meter

In pilot scale cultivation of *C. indica* fruit bodies, the complete colonization of mycelium in mushroom bed was recorded on day 10, followed by the casing soil was applied on day 16 and the pinhead primordial was appeared on day 27. The overall yield (75.05 kg/65 kg) and bioefficiency (115.46 %) of fresh fruit bodies of *C. indica* were recorded within 57 days utilizing reeds as alternative cellulosic substrate under pilot scale cultivation (Table 2).

Table 2: Cultivation of fruit bodies of *C. indica* on reeds as an alternative cellulosic Substrate at Pilot scale (100 kg of fresh mushroom / shed / crop cycle)

Growth parameters	*Crop cycle*
Spawn run (days)	10
Pin head formation (Days)	27
First harvest (kg/days)	30.52/30-32
Second harvest (kg/days)	19.22/38-40
Third harvest (kg/days)	15.25/47-49
Fourth harvest (kg/days)	10.06/55-57
Total fresh mushroom produced (kg/days)	75.05/55-57
Total fresh substrates utilized (kg/days)	65
Bioefficiency (%)	115.46 ± 1.52

In present study the biological efficiency of *C. indica* was continuously monitored for a period of 12 months at laboratory scale by utilizing reeds as the substrate and maximum bioefficiency (121.83 %) was recorded during the month of April, 2011. Further the production efficiency was scaled up to pilot scale level and also throughout the year cultivation (Plate1). Earlier report of [15] reported that *C. indica* was cultivated in paddy straw during different months of 2004 to 2005 and summer month (March to July) recorded the maximum biological efficiency (168.0 % to 180.2 %). *C. indica* required a temperature range of 30 to 32°C and more than 90% RH for its better yield and several report supports us [16-18].

Plate 1: Pilot scale cultivation of *C. indica* on reeds as substrates

4. Nutritional Properties of C. indica

4.1 Nutritional Properties

The fruit bodies of *C. indica* (/100g) contains carbohydrate (57.27 g), protein (28.73 g), fat (0.73 g), crude fiber (37.52 g), folic acid (57.0 µg), total ash (8.80 mg), moisture (14.47g) and energy values (350.57 kcal). Among seven vitamins (/100g) tested, vitamin B3 (22.94 mg) was recorded the highest amount whereas vitamin E content (DL: 0.2 mg) was the least (Table 3).

Mineral contents (/100g dried powder) contains calcium (135mg), iodine (227µg), iron (45.91mg), magnesium (177.3mg), manganese (1.5mg), phosphorus (0.88g), potassium (2.92g) and zinc (13.9mg) were recorded. Pesticides content such as DDT, p.p.DDT, alpha BHC, beta BHC, gamma BHC and delta BHC were recorded to be non detectable level.

The heavy metals contents (/kg) such as arsenic; cadmium and mercury were non detectable level. But the heavy metals such as selenium (4.60 mg), copper (2.41mg), chromium (2.07 mg) and lead (0.22 mg) was recorded in *C. indica*. Similarly several reports [19-20] recorded the dried *C. indicia* in nutrient contents (g/100g) such as carbohydrate (48.5), protein (21.4), fat (12.9), dietary fiber (12.9), ash (13.1) and energy (1566.23 kcal). Mushrooms are an important source of vitamins. The vitamins of group B are rich amount, particularly thiamine, riboflavin, pridoxine, pantotene acid, nicotinic acid, nicotinamide, folicacid and cobalamin as well as other vitamins such as ergosterol, biotin tocopherols [21]. Earlier reported [22-24] that Vitamin (mg/100g), A (0.215), C (0.4), E (0.80) and the moisture content of dried mushrooms is in the range of 10-15 % and has superior quality of protein content varying from 25-35 % on dry weight basis. Similarly reported [23-24] mineral contents in dried *C. indica* dried powder (mg/100g) calcium (20.65), iron (56.25), magnesium (12.82), manganese (1.64) phosphorus (67.99) arsenic (0.54), selenium (0.1) and zinc (12.86) were recorded.

Table 3: Analysis of nutrient components, vitamins and enumeration of microorganisms from *C. indica* mushroom powder

Parameters	*C. indica*
Nutrient composition	
Carbohydrate (g/100g)	57.27
Protein (g/100g)	28.73
Fat (g/100g)	0.73
Crude fiber (g/100g)	37.52
Total Ash (g/100g)	8.80
Folic acid (µg/100g)	57.0
Moisture (g/100g)	14.47
Energy (kcal/100g)	350.57
Vitamins	
Vitamin A (HPLC) (IU/100g)	0.50
Vitamin B1 (mg/100g)	0.25
Vitamin B2 (mg/100g)	2.25
Vitamin B3 (mg/100g)	22.94
Vitamin B6 (mg/100g)	0.61
Vitamin D3 (ug/100g)	4.0
Vitamin E (mg/100g)	0.2

Table 4: Analysis of minerals, heavy metals and from *C. indica* mushroom powder

Minerals	
Calcium (mg/100g)	135
Iodine (µg/100g)	227
Iron (mg/100g)	45.91
Magnesium (mg/100g)	177.3
Manganese (mg/100g)	1.5
Phosphorus (g/100g)	0.88
Potassium (g/100g)	2.92
Zinc (mg/100g)	13.9
Heavy metals	
Arsenic (mg/kg)	NDL(0.09)
Cadmium (mg/kg)	ND L(0.01)
Chromium (mg/kg)	2.07
Copper (mg/kg)	2.41
Mercury (mg/kg)	ND L(0.01)
Lead (mg/kg)	0.22
Selenium (mg/kg)	4.60

Key: NDL* - Not detectable limit,

5. Conclusion

Hence the present shows a positive path that *C. indica* can be cultivated using alternative substrate and its efficacy was tested at pilot scale. The present studies *C. indica* was recorded high nutritional and minerals contends and as this mushroom known to several medicinal properties this study will be further focused towards pharmaceutical applications.

6. Acknowledgements

The authors thank to the Department of Bio technology, India for their financial support and Shri. AMM Murugappa Chettiar Research Centre, Chennai and Inba seva sangam Sevapur for provided the laboratory facilities.

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