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RESEARCH ARTICLE

OPTIMIZATION OF CASING PROCESS FOR ENHANCED BIOEFFICIENCY OF *CALOCYBE INDICA*, AN INDIGENOUS TROPICAL EDIBLE MUSHROOM

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ABSTRACT

Casing is an important process in the cultivation of milky mushroom and it greatly determines the yield of the mushroom. The current investigation was undertaken to determine the effect of casing layer thickness in the growth and yield of *Calocybe indica*, an indigenous tropical edible mushroom. The pure culture was grown in potato dextrose agar medium and maintained. The spawn of *Calocybe indica* was prepared from the pure culture using white sorghum seeds as substrate. The spawn was inoculated in the paddy straw substrate filled bags and maintained in the mushroom cultivation chamber for the spawn running and growth of mushrooms. After the complete mycelium spreading in the bags, the casing process were done with varying thickness of casing layer viz. 1.5, 2.5 and 3.5 cm. The bags were monitored for the growth of the mushrooms and yield in each of the bags were recorded. The bags with casing layer thickness of 2.5 cm recorded the maximum yield and bioefficiency.

INTRODUCTION

Mushroom is a member of higher fungi seen with the naked eye and usually picked by hands, shaped fleshy fruiting body, widely used as food and food supplements for millennia. It is an important food item concerning human health, nutrition and disease prevention (Chang, 1996). Mushrooms are incredibly popular foods in most countries. Edible and medicinal mushrooms are regarded as the ideal health foods. They are well appreciated for their exquisite taste and flavor and are consumed both in the fresh and processed forms(Stamets, 2000). However, for a common man mushrooms are still considered as one of the curiosities of nature and many of them are widely consumed for their flavor and aroma. Their nutritive and medicinal values were known as early as 1500 BC based upon many ancient literatures (Sagakami *et al*., 1991; Wasser and Weis, 1999). Mushrooms represent one of world's greatest untapped resources of nutritious and palatable food. They possess extensive and efficient enzyme systems to degrade successfully a wide variety of inexpensive substrates such as lignin, cellulose, hemicelluloses, pectin and other industrial wastes resulting in the cheapest method of waste disposal as well as production of protein rich food. Paddy straw is reported as the most suitable substrate for cultivation of white summer mushroom, *Calocybe indica* (Krishnamoorthy and Muthusamy 1997; Pani, 2010). Cultivation of edible mushrooms might be the only current process that combines the production of protein-rich food with the reduction of environmental pollution (Sa´ nchez, 2010). It represents one of the most efficient biotechnological processes for lignocellulosic organic waste recycling (Mandeel *et al*., 2005).

Among the various edible mushroom *Calocybe* genus consists of about 20 species of mushroom, including *Calocybe indica*, which can be cultivated throughout the year in the entire of India even in hot humid climate (Kalha *et al.*, 2011). It is becoming more popular, due to its robust size, attractive color, sustainable yield, delicious taste, and unique texture. It has become the third commercially grown mushroom in India, after button and oyster mushrooms (Purkayastha and Nayak, 1979).

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. 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). Among the fungi, mushrooms have been used for untold centuries as food and medicine. Edible and medicinal mushrooms not only convert the huge lignocellulosic biomass waste into human food, but most remarkably can produce notable mycopharmaceuticals, myconutriceuticals and myocosmeceuticals for many years, mankind has benefited from green plants as a source of drugs

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and herbal remedies. Fungi, on other hand, have not been considered in any significant way. However this is changing rapidly. The prominence of fungi can now be seen increasingly as evidenced by their use as a major source of pharmaceuticals and medicinal foods (Law and Ng, 2001).

Since thousands of years, edible fungi have been revered for their immense health benefits and extensively used in folk medicine. Specific biochemical compounds in mushrooms are responsible for improving human health in many ways. These bioactive compounds include polysaccharides, tri-terpenoids, low molecular weight proteins, glycoproteins and immunomodulating compounds. Hence mushrooms have been shown to promote immune function; boost health; lower the risk of cancer; inhibit tumor growth; help balancing blood sugar; ward off viruses, bacteria, and fungi; reduce inflammation; and support the body's detoxification mechanisms. Increasing recognition of mushrooms in complementing conventional medicines is also well known for fighting many diseases (Singh *et al.*, 2011).

Mushrooms have been a food supplement in various cultures and they are cultivated and eaten for their edibility and delicacy. They fall between the best vegetables and animal protein source. Mushrooms are considered as source of proteins, vitamins, fats, carbohydrates, amino acids and minerals (Jiskani, 2001). Due to its alkaline ash and high fiber content it is highly suitable for the people with hyper acidity and constipation. (Pokhar *et al*., 2014).

C. indica is rich in protein, mineral, fiber, carbohydrate, and is abundant with essential amino acids (Alam *et al*., 2008, Mallavadhani *et al*., 2006). It is an excellent source of thiamine, riboflavin, nicotinic acid, pyridoxine, biotin, and ascorbic acid (Breene *et al*., 1990).

Meeting the food demand for the increasing population from the limited land resource is a big challenge for our Indian democracy in this vulnerable climate change era. In addition to this, wide spread malnutrition and associated diseases are more common among the economically poor population. This compels us to search for cheap alternative quality nutritional sources for our huge population. Non green revolution otherwise referred as mushroom farming is one among the apt ways to meet this challenge because mushrooms grow on wastes without requiring additional land besides its exceptional nutritional and medicinal properties (Singh *et al*., 2011).

In view of the importance of mushroom cultivation, this study has been undertaken to study the effect of casing layer thickness in the growth and yield of *Calocybe indica*, a tropical Indian edible mushroom, cultivation of which can play an important role in people's progress.

MATERIALS AND METHODS

Mushroom culture

The mushroom culture of *Calocybe indica* was procured from Vijaya mushrooms, Coimbatore. The species was sub cultured and maintained in Potato dextrose agar medium (PDA) at room temperature as slants and in pertriplates (Shivaprakasam and Kandaswamy, 1983).

Mushroom spawn production

The mushroom spawn was prepared on white sorghum grain. The mature grain procured from local market was well cleaned and boiled in water for 30 min. The boiled grain was mixed with 2% calcium carbonate. 300g of calcium carbonate mixed grain was filled in polypropylene bags of size 11 inch x 5 inch and sterilized for 15 psi for one hour. The sterilized bags were cooled to room temperature and inoculated with the mushroom culture maintained in slants (Ram *et al*., 2013). The culture inoculated bags were kept undisturbed at room temperature for 20 - 25 days. Completely mycelium spread spawn bags were ready for preparation of mushroom beds.

Cultivation technology

The procedure of Krishnakumari *et al*., 2014 was followed for the cultivation of *Calocybe indica* mushroom. The well matured paddy straw were cut into 3- 5 cm in length and soaked in water overnight. For paddy straw substrate sterilization, the overnight soaked paddy straw was washed and sterilized for 45 min in steam and shade dried in a dust free place until the moisture content is around 50 - 60 %. The shade dried paddy straw was filled in polypropylene bags. The bags were perforated with 1cm diameter holes (6 Nos) before packing. The 35 day old matured mushroom spawn was dispersed gently and used for mushroom bed preparation. A layer of paddy straw is followed by sprinkling of one hand full of spawn over the first layer. Likewise, five layers of spawn and seven layers of paddy straw were filled and the bag was tied with a nylon thread and hanged in the mushroom cultivation chamber for spawn running and growth of mushrooms. The beds are left undisturbed till the day of complete mycelium spreading. The temperature of 25 - 30°C and humidity of 80% is maintained in the chamber.

After the complete mycelium spreading, the beds were taken from the chamber and cut into the two equal halves. The mycelium spread substrate is pressed on its top for the liberation of gases. The sterilized casing soil prepared was applied on the beds for thickness of 1.5 cm, 2.5 cm and 3.5 cm. Spraying of water was done on the casing soil and the beds were placed in the underground chamber for the growth of mushrooms. The temperature in the underground chamber was maintained at a range of 30 - 35 °C and a humidity of 80 - 85 %. water is sprayed on the beds at periodic intervals. Days of pin headed appearance, first, second and third harvest were recorded. The mushroom yield during first, second and third harvest were also recorded for calculating the bioefficiency of the mushroom. These observations were done for all the beds with varying levels of casing layer thickness. The total yield of the mushroom was recorded by calculating the weight of each mushroom harvested in all the three harvests. The bioefficiency of the mushrooms was calculated using the formula,

Bioefficiency (%) = Yield of fresh mushroom (g) / Total weight of dry substrate used (g) x 100

Statistical analysis

Statistical comparison was done at significance level, $P<0.05$ using SPSS package version 20. One way ANOVA followed by post hoc analysis of DMRT was performed.

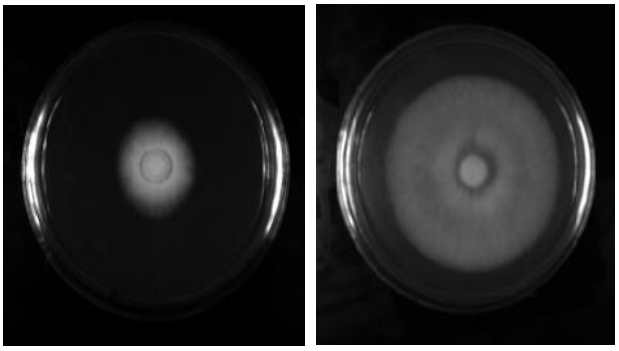
RESULTS AND DISCUSSION

The various stages of culture growth of *Calocybe indica* is shown in fig. 1 and the various stages of cultivation technology of *Calocybe indica* is shown in fig. 2. The well grown and matured spawn aging 35 days were used for the bed preparation. The beds hanged in the mushroom unit showed the mycelium spreading from the third day of hanging. The

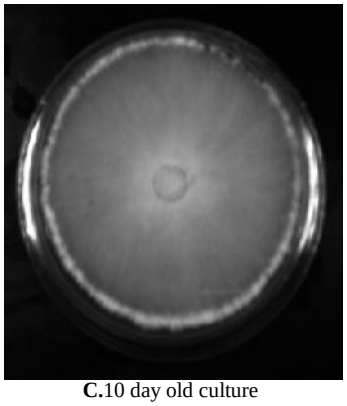
mycelium spreaded completely in all the beds of various treatments around 25-30 days. The beds applied with casing layer of 2.5 cm (Group 2) recorded with maximum yield of mushrooms followed by 1.5 cm (Group 1) and 3.5 cm (Group 3) casing layered beds. The primordial initiation was found to appear first in the group 2 beds subsequently followed by sooner growth of mushrooms and better yield. The group 3 beds gave minimum yield of mushrooms. The group 1 beds showed comparatively low yield than group 2 beds. This may be due to the fact that the casing layer is not sufficient enough for the growth of mushrooms. The day of pin headed appearance was 31.17 ± 0.76 days for Group 2 bed followed by 34.67 ± 1.04 days and 36.83 ± 1.26 days for Group 1 and Group 3 beds respectively (table.I). The days of first, second and third harvest in the Group 2 beds was on 35.67 ± 0.29 days, 42.17 ± 0.76 days and 47.5 ± 2.29 days respectively. Similarly for Group 1 and Group 3 beds, the first, second and third harvest ranged from 38.17 ± 1.26 days to 50.67 ± 2.52 days and 39.33 ± 0.76 days to 52.33 ± 2.08 days respectively. The yield of mushroom in Group 1, Group 2 and Group 3 beds were 1364 g, 1403 g and 1242 g respectively (table.I). Group 2 beds gave higher bioefficiency of 140.3% followed by 136.4% for Group 1 beds and 124.2 % for Group 3 beds. The Group 2 beds gave more yield in all the three harvests with 590.67±32.65 g , 669.67±13.57 g and 143.33±16.80 g in the first, second and third yield respectively.

It has been well established that physical and chemical state of substrate largely decides their suitability for mushroom growing (Zadrazil, 1978). The casing layer of 3.5 cm thickness in the beds reduced the number of sporophores and quantity of the mushroom. The reason for this may be mycelia cannot able to penetrate through the 3.5 cm thickness layer casing material. The day of pin headed appearance, first harvest, second harvest and third harvest in the group 2 beds were significantly different with that of group 1 and group 3 beds. The first, second and third yield of *Calocybe indica* obtained in the group 2 beds were significantly different with that obtained in group 1 and also with the yield obtained in the group 3 except for first yield.

This study revealed that the casing layer thickness of 2.5 cm was ideal for better yield of milky mushroom as it provides sufficient ventilation and necessary substrate for growth. Pani 2012 also reported that the casing layer of 2 cm thickness gave maximum yield of milky mushroom, which similar result is obtained in this study.



A. 3 day old culture **B.** 7 day old culture



C.10 day old culture

Fig.1 Various stages of culture growth of *Calocybe indica*

Table II Effect of casing layer thickness in the yield and bioefficiency of *Calocybe indica*

Groups	First yield (g)	Second yield(g)	Third yield (g)	Total yield (g)/bed	Bioefficiency (%)
Group 1	555.33±11.15[a]	542±13.076[b]	266.67±8.50[c]	1364	136.4
Group 2	590.67±32.65[b]	669.67±13.57[c]	143.33±16.80[a]	1403	140.3
Group 3	578.33±9.61[b]	466.67±9.07[a]	143.33±7.64[b]	1242	124.2

All the values are expressed as mean ± SD; n=6
Group 1 – 1.5 cm casing layer thickness beds
Group 2 – 2.5 cm casing layer thickness beds
Group 3 – 3.5 cm casing layer thickness beds
Mean values in the same column followed by different alphabets in the superscripts are significantly different (P<0.05, ANOVA, DMRT).

Table I Effect of casing layer thickness in the growth of *Calocybe indica*

Casing layer thickness (cm)	Pin headed appearance (days)	First harvest (days)	Second harvest (days)	Third harvest (days)
Group 1	34.67 ± 1.04[b]	38.17 ± 1.26[b]	43.5 ± 0.5[b]	50.67 ± 2.52[b]
Group 2	31.17 ± 0.76[a]	35.67 ± 0.29[a]	42.17 ± 0.76[a]	47.5 ± 2.29[a]
Group 3	36.83 ± 1.26[c]	39.33 ± 0.76[c]	45.33 ± 1.527[c]	52.33 ± 2.08[b]

All the values are expressed as mean ± SD; n=6
Group 1 – 1.5 cm casing layer thickness beds
Group 2 – 2.5 cm casing layer thickness beds
Group 3 – 3.5 cm casing layer thickness beds
Mean values in the same column followed by different alphabets in the superscripts are significantly different (P<0.05, ANOVA, DMRT).

CONCLUSION

Milky mushroom is one of the best edible mushroom that can be cultivated throughout the year in the tropical climate of India. Simple cultivation technology, low capital investment and long shelf life are some of the attributes which makes it a better choice for mushroom growers and also consumers.

Fig. 2 Various steps in the cultivation technology of *Calocybe indica*

(a) spawn running; (b) cutting of bed into two halves; (c) applying casing soil; (d) appearance of pin headed structures; (e) growth of mushrooms; (f) fully grown mushrooms

The efficiency of casing process is an important aspect in success of milky mushroom cultivation. So the present study aimed at finding out the suitable thickness of casing layer for obtaining maximum yield. The results obtained in the study showed that the 2.5 cm thickness of casing layer is appropriate for maximum yield of mushrooms. With the improved cultivation technique, mushrooms can be made available to the common public in a wide and cheap manner as other vegetables which can benefit them a lot.

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