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Effect of casing material on the yield of milky mushroom (*Calocybe indica*)

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ABSTRACT

This research aimed to study the effect of casing material on the yield of milky mushroom (*Calocybe indica*). The experiment was assigned in a completely randomized design with 6 treatments and 4 replications each. The treatments were casing materials as follows: 1) loam soil:manure, 2) loam soil:manure:rubber sawdust, 3) loam soil:manure:rubber sawdust:sand, 4) loam soil:vermicompost, 5) loam soil:vermicompost:rubber sawdust, and 6) loam soil:vermicompost:rubber sawdust:sand. In each treatment, the ratios of casing materials were 1:1 by volume. The pinhead initiation, the number, fresh weight, size of the fruiting body, and biological efficiency were recorded. The results found that different casing materials showed significant differences in pinhead initiation, number of fruiting bodies, and the length of the stipe, but different casing materials did not show any significant difference in the diameter of the pileus, the fresh weight of milky mushroom, and percentage of biological efficiency. The diameter of the pileus was 15.13–19.65 cm. The fresh weight was from 938.3–1,238.3 g/5 bags, and the percentage of biological efficiency was from 40.60–46.29%. The loam soil, manure, and rubber saw dust tended to give the best fresh weight at 1,238.3 g/5 bags.

Keywords: milky mushroom, *Calocybe indica*, casing material

INTRODUCTION

Milky mushroom or milky white mushroom (*Calocybe indica*) was first reported from India by Purkayastha and Chandra in 1974 (Kerketta et al., 2018a; Kumar et al., 2011; Kerketta et al., 2018b), and its first artificial cultivation in 1976 on a mixture of soil, sand and maize meal (Subbiah and Balan, 2015). It becomes the third commercially grown mushroom in India, after button and oyster mushrooms (Kerketta et al., 2018a). This mushroom is now distributed in the tropical parts of the world. Various workers developed cultivation techniques (Subbiah and Balan, 2015). This mushroom is a small genus of about 40 species of mushroom, which is edible and is cultivated in India (Kerketta et al., 2018a). It is grown on humus-rich soil (Subbiah and Balan, 2015), a temperature range of 25–35°C with 75–90% relative humidity (Subbiah and Balan, 2015; Navathe et al., 2014; Kerketta et al., 2018b). Milky mushroom is basically a summer mushroom because it requires higher temperature and relative humidity (Navathe et al., 2014). It has a long shelf life without any refrigeration and can be stored for up to 7 days at room temperature. The market value of this mushroom is very high. The cultivation technology of this mushroom is easy and cheap. It has a robust size, attractive color, high nutritive value, good taste, and unique texture (Subbiah and Balan, 2015; Kerketta et al., 2018a; Kumar et al., 2011). These reasons have attracted the attention of growers. Milky mushroom is rich in protein, fiber, carbohydrates, and vitamins and contains abundant essential amino acids, but the liquid content is low (Alam et al., 2019; Kerketta et al., 2018b). It is reported as a therapeutic food as well as for preventing hypertension and hypercholesterolemia. It is a good source of antioxidants that help to reduce oxidative damage in the human body (Alam et al., 2019; Prabu and Kumuthakalavalli, 2016). In Thailand, government agencies and farmers are interested in growing milky mushrooms because of the high price of 250–700 THB per kilogram, and there are many kinds of cultivation substrates (Sornprasert et al., 2022). The milky mushroom cultivation process consists of spawn production, substrate preparation, mushroom bed preparation, room maintenance during spawn run and mushroom production, harvesting and packaging, and management of spent mushroom substrate. For the spawn production, the mycelium growth in potato dextrose agar or malt extract is 8–10 days with pH between 5.5 and 8.5 and optimum temperature around 30 to 35°C. The temperatures below 25°C or above 38°C did not support the growth of milky mushroom. The best substrate for milky mushroom spawn production is sorghum or wheat grains (Subbiah and Balan, 2015). The cultural substrates such as rice straw, wheat straw, mustard straw, maize straw, waste cotton, pigeon pea, sugarcane bagasse, and rubber sawdust are used for mushroom production (Subbiah and Balan, 2015; Kerketta et al., 2018b). In this step, casing is required to change from the vegetative to the reproductive stage of humicolous mushrooms that grow on soil, especially milky mushroom (Subbiah and Balan, 2015). The casing material or casing soil should have high water holding capacity, good air space ratio, and contain neutral to alkaline conditions (Subbiah and Balan, 2015). The clay loam, sand, soil, farm yard manure, vermicompost, dried spent biogas slurry, or spent oyster mushroom substrate can be used as casing material (Amin et al., 2010; Kumar and Chandra, 2013; Navathe et al., 2014). However, there are many types of research that focus on the cultural substrates, but not casing materials. This research aimed to evaluate the effect of different casing materials on the growth and yield of milky mushroom (*Calocybe indica*).

MATERIALS AND METHODS

Preparation of substrates and culturing

The main cultural substrate in this study was 100% rubber sawdust mixed with 20% rice bran, 0.5% $MgSO_4$, 0.2% Na_2SO_4, 1% $CaCO_3$, and 1% glutinous rice flour (modified from Srioon, 2018). Water was added to adjust the humidity of the mixed substrate to about 60–70%. The 1,000 grams of substrate was put in a plastic bag (6.5 × 12.5 inches), and the opening of the bags was plugged with a cotton plug and covered with a plastic ring. The bags were sterile at 100°C for 4 h, and after that, left at room temperature for cooling and added around 20 seeds (2 teaspoons) of sorghum spawn. The bags were kept in a room for incubation at 26–32°C until the mycelium colonized the substrates. The number of days mycelium colonized all the substrates in the bags was recorded.

Experimental design and treatments

The experiment was conducted from October 2023 to April 2024, and the temperature of the cultural room ranged from 28–30°C. The spawn that grew on sorghum grain at 20 days old was used in this experiment and obtained from the plant protection laboratory, Faculty of Agriculture, Princess of Naradhiwas University. The experiment was carried out in a completely randomized design (CRD). There were six treatments with four replications each. The treatments were different casing materials being used in this experiment as follows: T1) loam soil:manure, T2) loam soil: manure:rubber sawdust, T3) loam soil:manure: rubber sawdust:sand, T4) loam soil:vermicompost, T5) loam soil:vermicompost:rubber sawdust, and T6) loam soil:vermicompost:rubber sawdust:sand. The ratios of all casing materials were approximately 1:1 by volume. Then, they were spread on the 5 mycelium running bags, which were contained in a plastic basket. The thick layer of the casing material on the substrate was 2.0 cm after that slightly moistened and kept in the cropping room for fruiting. A hand sprayer watering two times a day; the temperature was recorded during this time.

Yield and biological efficiency

For yield, the following parameters were recorded, including pinhead initiation, the number, fresh weight, and size (the length of stipe and diameter of pileus) of the fruiting body, and biological efficiency. The percentage of biological efficiency was calculated by using the following formula:

$$\%\text{Biological efficiency (\%B.E.)} = \frac{\text{Fresh weight of fruiting bodies}}{\text{Dry weight of cultural substrate}} \times 100$$

The production cost per treatment was also calculated in this study. The data were analyzed using a statistical program, and significant differences between treatments were declared at $P \leq 0.05$, and a mean comparison was conducted using Duncan's multiple range test.

RESULTS AND DISCUSSION

The main cultural substrate used in this study was rubber sawdust, and the average time mycelium colonized all of the substrates in the bags was 67.2 (53−78) days. The different casing materials were studied on pinhead initiation, the number of fruiting bodies, the length of the stipe, the diameter of the pileus, and the fresh weight of the milky mushroom. The results obtained are shown in Table 1. Different casing materials show significant differences in pinhead initiation, number of fruiting bodies, and stipe length. The pinhead initiation was from 22–34 days. The loam soil:vermicompost showed the shortage period of pinhead initiation at 22.8 days, and loam soil: manure:rubber saw dust:sand showed the most extended period of pinhead initiation at 34.0 days. The loam soil:manure showed the best number of fruiting bodies at 11.5 fruiting bodies/5 bags. The loam soil: vermicompost showed the length of stipe at 17.6 cm but made no significant difference with loam soil: manure:rubber sawdust, loam soil:vermicompost: rubber sawdust, and loam soil:vermicompost:rubber sawdust:sand. Different casing materials showed no significant difference in pileus diameter, milky mushroom's fresh weight, and biological efficiency percentage. The diameter of the pileus was from 15.13−19.65 cm. The fresh weight was from 938.3–1,238.3 g/5 bags, and the percentage of biological efficiency was from 40.60–46.29%. The loam soil: manure:rubber sawdust trended to give the best fresh weight at 1,238.3 g/5 bags. The temperature during the reproductive time was between 26–32°C. The character of the fruiting body received from all treatments is shown in Figure 1.

The production cost per treatment is shown in Table 2. All treatments had the same cost in cultivation substrates and boxes but different in casing materials. The cost of vermicompost was relatively high, and the production cost of treatment 4, 5, and 6 was also high at 516.0, 415, and 310.5 THB, respectively. All treatments showed no difference in yields when using different casing materials. The price of milky mushroom in Thailand varies from 250–700 THB per kilogram (Sornprasert et al., 2022).

The cultural substrates used in this study were 100% rubber sawdust, 20% rice bran, 0.5% $MgSO_4$, 0.2% Na_2SO_4, 1% $CaCO_3$, and 1% glutinous rice flour. The average time mycelium colonized all of the substrates in the bags was 67.2 (53–78) days. The cultural substrate was modified from the study of Srioon (2018), which used rubber sawdust as the main substrate supplement with 20% rice bran and showed the best results in 51 days that mycelium colonized all of the substrates in the bags. However, this study took longer than the study of Srioon (2018). These may be due to differences in supplement substrate, incubation room conditions, or environmental conditions such as weather temperature, or humidity. The agricultural residues such as rice straw, wheat straw, mustard straw, pigeon pea straw, maize, sugarcane, sugarcane bagasse, sorghum leaf, banana leaf, or wide grass were used as the primary cultural substrates for milky mushroom production (Kumar and Chandra, 2013, Navathe et al., 2014; Nirupa and Kudada, 2018; Yadav et al., 2021; Kumar et al., 2012; Kerketta et al., 2018b, Selvaraju et al., 2015). Moreover, wood shaving or sawdust can be a cultural substrate (Maheswari et al., 2018; Yenjit et al., 2021; Srioon, 2018). In the past, the main cultural substrates were soil, sand, and maize meal; later, the milky mushroom was adapted to grow on straw, maize, or wheat bran substrate (Subbiah and Balan, 2015). However, paddy or wheat straws are favorable substrates for milky mushroom cultivation (Selvaraju et al., 2015; Kumar et al., 2012; Kerketta et al., 2018b).

The casing is essential in cultivating a mushroom, especially one that grows on the soil. The casing changes the vegetative to the reproductive stage. The casing materials should have a high water holding capacity, a space ratio for air exchange, and a suitable pH or neutral to alkaline (Subbiah and Balan, 2015). Several researchers investigated casing materials for the production of milky mushroom. For example, Yenjit et al. (2021) used soil mixed with 3.5% $CaCO_3$ as a casing material on top of a cultivation bag with a thickness of 2 cm. Srioon (2018) studied the numbers of sawdust spawn per pot on growth and yield of milky mushrooms by using loam soil + manure + coconut coir + rice husk charcoal 1:1:1:1 and found that sawdust spawn 5 bags/pot promoted the highest yields with the pinhead initiation period of 10 days. Many materials can be used as casing, for example, garden soil, sand, farm yard manure, coco dust, loamy soil, peat soil, biogas slurry, coir pith compost, vermin-compost (Singh et al., 2017; Sarker et al., 2020). These materials can be used alone or mixed before topping on the cultivation bags. The casing material may be treated with a 2% formaldehyde solution (Singh et al., 2017) or 12% $CaCO_3$ before use. The using vermicompost as casing material gave limited success. However, the composition, pH, EC, water holding capacity, porosity, and bulk density of casing materials are essential to selecting the casing substrates (Subbiah and Balan, 2015). Nowadays, the cultivation in plastic bags and exposure on the surface of the substrate by no casing material is applied.

Table 1. Casing material on the growth and yield of milky mushroom (*Calocybe indica*)

Treatment	Pinhead initiation (day)	Number of fruiting body	Length of stipe (cm)	Diameter of pileus (cm)	Fresh weight (g/5 bags)	%B.E.
T1	28.0^{b}	11.5^{a}	13.3^{c}	15.1	1,201.3	43.1
T2	33.3^{a}	7.5^{b}	16.8^{ab}	19.6	1,238.3	46.3
T3	34.0^{a}	6.8^{b}	13.8^{bc}	18.2	1,095.6	41.9
T4	22.8^{c}	5.5^{b}	17.6^{a}	19.7	938.3	40.6
T5	30.5^{ab}	5.8^{b}	15.6^{abc}	18.7	1,215.2	45.4
T6	29.8^{ab}	7.5^{b}	15.0^{abc}	16.3	1,208.0	45.2
F-test	*	*	*	ns	ns	ns
% CV	10.07	31.10	12.78	15.24	21.10	13.63

abcDifferent superscripts in the same column indicate a significant difference (P < 0.05); *P < 0.05; ns = not significant. T1 = loam soil:manure; T2 = loam soil:manure:rubber sawdust; T3 = loam soil manure:rubber sawdust:sand; T4 = loam soil:vermicompost; T5 = loam soil: vermicompost:rubber sawdust; T6 = loam soil:vermicompost:rubber sawdust:sand.

Table 2. Production cost per treatment

Treatment	**Substrate** Bag(THB)	**Loam soil** kg(THB)	**Manure** kg(THB)	**Rubber sawdust** kg(THB)	**Sand** kg(THB)	**Vermicom-post** kg(THB)	**Box** Box(THB)	**Total** (THB)
T1	20(8)	13(2)	9(2)	-	-	-	4(7.5)	234.0
T2	20(8)	10(2)	7(2)	5(1)	-	-	4(7.5)	216.0
T3	20(8)	7(2)	4(2)	3(1)	7(0.5)	-	4(7.5)	218.5
T4	20(8)	13(2)	-	-	-	15(20)	4(7.5)	516.0
T5	20(8)	10(2)	-	5(1)	-	10(20)	4(7.5)	415.0
T6	20(8)	7(2)	-	3(1)	7(0.5)	5(20)	4(7.5)	310.5

T1 = loam soil:manure; T2 = loam soil:manure:rubber sawdust; T3 = loam soil manure:rubber sawdust:sand; T4 = loam soil:vermicompost; T5 = loam soil:vermicompost:rubber sawdust; T6 = loam soil:vermicompost:rubber sawdust:sand; kg = kilogram; THB = Thai Baht.

Loam soil:manure

Loam soil:manure:rubber sawdust

Loam soil:manure:rubber sawdust:sand

Loam soil:vermicompost

Loam soil:vermicompost:rubber sawdust

Loam soil:vermicompost:rubber sawdust:sand

Figure 1. The character of the milky mushroom (*Calocybe indica*) uses different casing substrates.

CONCLUSIONS

The different casing materials gave different significances for the cultivation of milky mushrooms, especially the pinhead initiation, number of fruiting bodies, and length of the stipe. Still, there were no differences in the yields. The loam soil plus vermicompost gave the shortest periods for pinhead initiation at 22.8 days, and loam soil plus manure gave the highest number of fruiting at 11.5.

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