International Journal of Agricultural Technology 2022Vol. 18(4):1809-1824
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

The cultivation of Milky Mushroom (*Calocybe indica* P&C) in the Plastic Bag in Thailand

Sornprasert, R.[1*], Kasipar, K.[1], Katekunlaphan, T.[1], Tongchure, S.[2] and Sukkapan, P.[1]

[1]Faculty of Science, Chandrakasem Rajabhat University, Bangkok, 10900, Thailand; [2]Faculty of Agriculture and Life Sciences, Chandrakasem Rajabhat University, Bangkok, 10900, Thailand.

Sornprasert, R., Kasipar, K., Katekunlaphan, T. Tongchure, S. and Sukkapan, P. (2022). The cultivation of Milky Mushroom (*Calocybe indica* P&C) in Thailand. International Journal of Agricultural Technology 18(4):1809-1824.

Abstract The cultivation of milky mushroom (*C. indica* P&C) in the plastic bag had full colonized on the substrate was found to be fastest on the formula 14 (rice straw). The fastest primordia to fruiting body development period was found with formula 11 (bamboo leaves) with the minimum and maximum of average ambient temperature of 30.68°C and 31.86°C. Formula 9 (corn cob) resulted in the highest fruiting body weight, while the formula 11 gave the highest amount of fruiting bodies. For the duration of full colonized, mycelia to primordia and primordia to fruiting bodies, formula 10 (cat tail) resulted in the fewest total days. Meanwhile, formula 15 (banana pseudostem) gave highest the Biological efficientcy. The crude extract of fruiting bodies with ethanol were analyzed by the thin layer chromatography and 4 bands were detected with R_f 0.16, 0.26, 0.32 and 0.54. After bioactive compounds analyzing by chemical reaction, flavonoid, saponin, tannin, phenolic and terpenoid were found, which agrees with the spectroscopy tests. For the nutritional value analysis, proteins, fats, carbohydrates, ash, moisture and minerals were found in different quantities in the fruiting bodies. The consideration on an annual basis, formula 9 showed the lowest total annual cost. There were three cases of fresh mushrooms price/kg including 250, 400 and 700 baht/kg, considered on an annual basis, formula 10 resulted in the highest return. If considering profits, formula 9 brought the greatest profit per bag, while if considered annually, formula 10 generated the highest profit. Moreover, if the price was 250 baht/kg, formula 10, formula 11, formula 14 and formula 15 would result in the shortest payback period within 1 year, while formula 1 (rice husk), formula 3 (rain tree sawdust), formula 9, formula 2 (khae na sawdust) and formula 5 (para rubber sawdust), had 2-year payback periods. Moreover, formula 10 is presented to be the best net present value when compared to the other formulas.

Keywords: Milky mushroom, Bioactive, Nutritional value, Costs and returns

Introduction

Milky mushroom (*Calocybe indica* P&C) grows on a wide range of seeding materials including rice straw, wood sawdust, seeds, or agricultural wastes. In India, production of this mushroom resulted in very high production at the same rate as straw

[*]**Corresponding Author:** Sornprasert, R.; **Email:** sornprasert_r@hotmail.com

mushrooms and *Pleurotus* sp. because of its good taste, nutritiousness and pharmaceutical properties (Miller, 1994). In Thailand, the preliminary surveys have found that farmers and government agencies are increasingly interested in the cultivation of milky mushrooms, there is a lack of supporting academic information. Additionally, Thailand has a wide range of biomaterials suitable for mushroom cultivation. Therefore, we are interested in the cultivation of milky mushrooms with suitable substrates for mushrooms growth. The milky mushroom grows well at 30-35^{o}C, while other mushrooms such as *Pleurotus* sp. gave lower numbers of fruiting bodies. Furthermore, milky mushroom produced large and high weight fruiting bodies that had a good taste, well textured, and price about 250 - 700 baht/kg of fresh weight (Yoelao, 2017; Government Public Relations Department, 2018) which is a higher price than other mushrooms. Moreover, the bioactive compounds which the antidiabetic substances namely flavonoid, saponin, tannin, phenolic and nutrients substances were found in these mushroom (Prabu and Kumuthakalavalli, 2014). Therefore, we are interested in studying milky mushroom cultivation. The objectives of this research were to study the suitable cultivation substrates for the growth of milky mushroom, bioactive compounds of the milky mushroom and the costs, returns, and financial analysis of milky mushroom cultivation.

Materials and methods

The suitable cultivation substrates for the growth of the milky mushroom

Pure culture preparation: Fresh fruiting bodies were obtained from @Home Farm in Nakhon Ratchasima Province, Thailand. The tissues of the fruiting bodies were cut and transferred on PDA Potato Dextrose Agar) (Sornprasert *et al*., 2019; Soytong *et al*., 2019) on the culture plate, and incubated at room temperature for 5 days. Then the mycelia were transferred to the PDA in test tubes to obtain the pure culture. The pure cultures were then transferred to the PDA culture plates and then incubated at room temperature for 10 days. Agar blocks with the mycelia on the surface were cut by cork borer with a 0.5 cm diameter. Preparation of spawn was done by using soaked sorghum seeds for 12 hours, filtered and boiled in clean water for 30 minutes. They were then filtered and packed into two-thirds of the 250 ml cultivation bottles. The cultivation bottles were then sterilized by autoclave at 121^{o}C and 15 psi for 30 minutes. After the sorghum seeds cooled, a piece of agar block was transferred to each cultivation bottle and incubated at room temperature until the mushrooms were fully grown on the sorghum seeds. Preparation of substrates were done with 15 formulas included formula 1 rice (*Oryza sativa*) husk, formula 2 khae na (*Dolichandrone serrulata*) sawdust, formula 3 rain tree (*Albizia saman*) sawdust, formula 4 mango tree (*Mangifera indica*) sawdust, formula 5 para rubber (*Hevea brasiliensis*) sawdust, formula 6 cinnamon tree (*Cinnamomum verum*) sawdust, formula 7 coconut (*Cocos nucifera*) coir dust, formula 8 bagasse (*Saccharum* sp.), formula 9 corn (*Zea mays*) cob, formula 10 cat tail (*Typha angustifolia*), formula 11 bamboo (*Bambusa* sp.) leaves, formula 12 brab palm (*Borassus flabellifer*)pericarp, formula 13 water hyacinth (*Eichhornia crassipes*), formula 14 rice (*O. sativa*) straw and formula 15 banana (*Musa* sp.) pseudostem. A total of 100 kg of each substrate was mixed with rice bran (5 kg), calcium carbonate (1 kg), gypsum (0.5 kg), and magnesium sulphate (0.2 kg) and then the moisture was adjusted to 70%. Next, 700 g of mixed substrates were packed in each plastic bag, followed by steaming at 100^{o}C for 3 hours. 15spawns of sorghum seeds from spawn preparation were inoculated in substrates and incubated at room temperature.

Ambient temperature was recorded at 06:00-09:00 a.m., 09:00 a.m.-12:00 p.m., 12:00-03:00 p.m. and 03:00-06:00 p.m. until the full colonized on the substrates. The substrates fully covered with mycelia were placed in a fruiting body growing house. Water was applied to the substrates twice a day at 08:00 a.m. and 04:00 p.m. while ambient temperature was measured at 06:00-09:00 a.m., 09:00 a.m.-12:00 p.m., 12:00-03:00 p.m. and 03:00-06:00 p.m. The relative humidity in the fruiting body growing house was maintained about 75-80% with clean water during the 90 days period. The data collected included the mushroom mycelia growth; the development of primordia; the development of fruiting bodies and biological efficiency (B.E.).

The experimental design was performed using a completely randomized design (CRD) with 15 treatments, 5 replicates, 20 bags for each replicate and the mean values were analyzed by DMRT (Duncan's New Multiple Test).

The bioactive compounds of the milky mushroom

The crude extracts were characterized with thin layer chromatography (TLC) according to Nandhasri and Parawet (2006). The well-growth fruiting body in the suitable substrates were dried at 60^{o}C for 72 hours and 100 g of dried mushroom was ground, soaked in 95% ethanol for 5 days at room temperature, and then the solvent was evaporated with a rotating vacuum evaporator. The crude extracted was dissolved in 8.0:2.0 v/v of dichloromethane : methanol solvent and 2.0:3.0:0.1 v/v of hexane : dichloromethane : methanol solvent as mobile phases on the TLC plate (silica gel 60 F_{254}) as a stationary phase. Then the bands on TLC were investigated under UV 254 and 366 nm and R_f was calculated.

Alkaloids were analyzed by Dragendorff's test. Flavonoids were analyzed by Shinoda test. Saponins were analyzed by the frothing test. Tannins and phenolics were analyzed by the ferric chloride test. Terpenoids were analyzed by Salkowski test. Cardiac glycosides were analyzed by Keller-Keliani's Test according to Luanratana (1993). The functional groups of biochemical compositions of crude extracts were analyzed by Infrared Spectroscopy (Perkin Elmer EX FT-IR), in which the spectra were measured in the range between 500 and 3500 cm^{-1}. The Ultraviolet (UV) absorption spectra were used to investigate the chemical compositions of crude extract, which was performed in methanol on a JASCO V-530 spectrometer and the principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε. (Nanna, 2006).

The protein was analyzed by AOAC (2016d), fat was analyzed by AOAC (2016b), carbohydrate was analyzed according to Sullivan and Carpenter (1993), ash was analyzed by AOAC (2016a), moisture was analyzed by AOAC (2016c), Ca, Na, K, Mg were analyzed by AOAC (2016e), Se was analyzed by AOAC (2016f), P was analyzed by AOAC (2016g), Cu, Mn, Zn, Fe was analyzed by AOAC (2016h), and energy was analyzed according to Heleno *et al*., (2009).

Costs, returns and financial analysis of milky mushroom cultivation

The analyzed costs included variable costs (implicit and explicit costs), fix cost and total costs. Revenue analysis included total revenue (TR), average revenue (AR) and profit. The financial analysis included payback period (PB) and net present value (NPV).

Results

The suitable cultivation substrates for the growth of the milky mushroom

At 50 days, it was found that the mycelia were white with an abundant density with formulas 1-3, 5, 9-11, 14 and 15. Moderate densities were found with formulas 6 and 8, while poor densities were observed with formulas 4, 7, 12 and 13. Formulas 14 gave the fastest full colonized period, followed by formulas 10, 1, 2, 15, 5, 11, 3, 7, 8, 6, 9, 12, 4 and 13. When compare the average among these formulas, it was found that formula 14 with a full mycelia growth period differed to a statistically significant degree from the other formulas at a 95% confidence level (Table 1). The average ambient temperature between 06:00-09:00 a.m., 09:00 a.m.-12:00 p.m., 12:00-03:00 p.m. and 03:00-06:00 p.m. were 31.58°C, 32.41°C, 32.50 °C and 31.91°C, respectively.

The fastest period of primordia development was found in formula 10 followed by formulas 11, 15, 14, 1, 5, 8, 3, 2, 9 and 6. However, formulas 4, 7, 12 and 13 were not developed to primordia. Formulas 10 and 11 differed from the other formulas to a statistically significant degree at a 95% confidence level

(Table 1, Figure 1). The average ambient temperature during 06:00-09:00 a.m., 09:00 a.m.-12:00 p.m., 12:00-03:00 p.m. and 03:00-06:00 p.m. were 30.75^{o}C, 31.69^{o}C, 31.79^{o}C and 30.71^{o}C, respectively.

Figure 1. The development of mycelia to primordia of milky mushroom of formula 1, average age at 24.26 days (A); formula 2, average age at 29.06 days (B); formula 3, average age at 26.08 days (C); formula 5, average age at 25.57 days (D); formula 6, average age at 34.78 days (E); formula 8, average age at 25.98 days (F); formula 9, average age at 32.82 days (G); formula 10, average age at 9.70 days (H); formula 11, average age at 9.79 days (I); formula 14, average age at 18.74 days (J) and formula 15, average age at 15.10 days (K)

Formula 11 had the fastest the period of fruiting body development from primordia, followed by formulas 10, 5, 15, 9, 3, 1, 14 and 2. However, no fruiting body was developed with formulas 4, 6-8, 12 and 13. It was found that the period of fruiting body development differed with formula 11 than the formulas to a statistically significant degree at a 95% confidence level (Table 1, Figure 2). The average ambient temperature during 06:00-09:00 a.m., 09:00 a.m.-12:00 p.m., 12:00-03:00 p.m. and 03:00-06:00 p.m. were 30.73^{o}C, 31.60^{o}C, 31.86^{o}C and 30.68^{o}C, respectively.

The fresh fruiting body weight of formula 9 was the highest, followed by formulas 3, 14, 10, 1, 11, 15, 2 and 5, while the average weight was 73.94 g, 64.11 g, 56.97 g, 56.68 g, 55.71 g, 54.74 g, 54.09 g, 50.03 g and 47.70 g, respectively. It was found that the mushroom weight of formula 9 differed from other formulas to a statistically significant degree at a 95% confidence level. The amount of fruiting body of formula 11 was the highest, followed by formulas 10, 14, 15, 1, 9, 5, 2 and 3. It was found that formula 11 had a statistically significantly different amount of fruiting body than the other formulas at a 95% confidence level (Table 1).

Table 1. The growth of milky mushroom with 15 formulas

Formulas	Mycelia growth		(2)	Fruiting body development from primordia							
	Color/ Density at 50 days	(1) Fully colonized (days)	Primordia development (days)	(3) Age (days)	Fresh fruiting body (g)	Amount of fruiting body	Cap diameter (cm)	Stalk diameter (cm)	Stalk length (cm)	Total days (1)+(2)+(3)	B.E.
1. Rice husk	❂/ +++	41.08 ± 0.05^{b}	24.26 ± 0.30^{d}	17.52 ± 0.95^{de}	55.71 ± 1.24^{c}	5.48 ± 0.05^{d}	5.59 ± 0.11^{bc}	2.32 ± 0.08^{c}	12.14 ± 0.27^{cd}	82.86	11.45 ± 0.55^{e}
2. Khae na sawdust	❂/ +++	46.17 ± 0.13^{c}	29.06 ± 1.17^{f}	19.34 ± 0.77^{e}	50.03 ± 1.62^{de}	5.08 ± 0.03^{e}	5.86 ± 0.14^{ab}	1.88 ± 0.07^{d}	12.46 ± 0.30^{abc}	94.57	13.43 ± 0.35^{d}
3. Rain tree sawdust	❂/ +++	54.07 ± 0.57^{g}	26.08 ± 0.36^{de}	15.52 ± 0.50^{d}	64.11 ± 3.51^{b}	5.00 ± 0.00^{e}	6.14 ± 0.21^{a}	2.43 ± 0.13^{c}	11.24 ± 0.46^{d}	95.67	19.43 ± 0.80^{c}
4. Mango tree sawdust	❂/ +	97.87 ± 0.41^{m}	-	-	-	-	-	-	-	-	-
5. Para rubber sawdust	❂/ +++	53.15 ± 0.08^{e}	25.57 ± 0.67^{ef}	10.41 ± 1.14^{bc}	47.70 ± 1.30^{e}	5.15 ± 0.04^{e}	4.64 ± 0.12^{e}	2.25 ± 0.06^{c}	12.27 ± 0.29^{bcd}	89.13	9.94 ± 0.06^{f}
6. Cinnamon tree sawdust	❂/ ++	67.03 ± 0.55^{j}	34.78 ± 0.83^{g}	-	-	-	-	-	-	-	-
7. Coconut coir dust	❂/ +	61.19 ± 0.61^{h}	-	-	-	-	-	-	-	-	-
8. Bagasse	❂/ ++	62.88 ± 0.14^{i}	25.98 ± 0.11^{de}	-	-	-	-	-	-	-	-
9. Corn cob	❂/ +++	78.58 ± 0.97^{k}	32.82 ± 0.47^{g}	12.55 ± 0.35^{c}	73.94 ± 3.35^{a}	5.45 ± 0.80^{d}	5.03 ± 0.27^{de}	3.20 ± 0.08^{a}	12.52 ± 0.41^{abc}	123.95	20.14 ± 1.62^{b}
10. Cat tail	❂/ +++	40.91 ± 0.28^{b}	9.70 ± 4.65^{a}	9.70 ± 4.47^{b}	56.68 ± 12.28^{c}	6.58 ± 3.25^{b}	4.96 ± 0.83^{de}	2.82 ± 2.09^{b}	13.28 ± 2.67^{ab}	60.31	18.39 ± 0.28^{c}
11. Bamboo leaves	❂/ +++	53.68 ± 1.32^{f}	9.79 ± 5.64^{a}	7.03 ± 3.25^{a}	54.74 ± 15.36^{cd}	7.47 ± 1.28^{a}	5.37 ± 0.98^{bcd}	2.29 ± 0.61^{c}	11.38 ± 2.21^{d}	70.50	10.12 ± 0.12^{f}
12. Brab palm pericarp	❂/ +	86.06 ± 0.04^{l}	-	-	-	-	-	-	-	-	-
13. Water hyacinth	❂/ +	103.02 ± 0.54^{n}	-	-	-	-	-	-	-	-	-
14. Rice straw	❂/ +++	40.18 ± 0.07^{a}	18.74 ± 0.64^{c}	17.56 ± 0.61^{de}	56.97 ± 0.97^{c}	5.90 ± 0.06^{c}	5.55 ± 0.10^{bc}	2.41 ± 0.04^{c}	13.35 ± 0.22^{a}	76.48	21.13 ± 0.20^{b}
15. Banana pseudostem	❂/ +++	46.76 ± 0.20^{d}	15.10 ± 1.01^{b}	11.50 ± 0.58^{bc}	54.09 ± 1.07^{cd}	5.62 ± 0.08^{cd}	5.09 ± 0.19^{cde}	2.60 ± 0.05^{bc}	12.76 ± 0.36^{abc}	73.36	24.77 ± 0.07^{a}

Note: ❂ = white mycelia, + = poor density, ++ = moderate density, +++ = abundant density, The same alphabet in each column means not statistical significantly different at 0.05 level of probability

Table 2. Crude extraction of fruiting body of milky mushroom by

Bands	Distance of solute (cm)	Distance of solvent (cm)	R_f
A	0.80	5.00	0.16
B	1.30	5.00	0.26
C	1.60	5.00	0.32
D	2.70	5.00	0.54

Table 3. Bioactive compound of crude extraction of milky

Bioactive compound	Assays	Results
Alkaloids	Dragendorff's test	–
Flavonoids	Shinoda test	+
Saponins	Froth test	+
Tannins and phenolics	Ferric chloride test	+
Terpenoids	Salkowki's test	++
Cardiac glycosides	Keller-Keliani's test	–

Note: ++ : strong positive, + : positive, – : negative

Figure 2. The fruiting body of milky mushroom of formula 1, average age at 17.52 days (A); formula 2, average age at 19.34 days (B); formula 3, average age at 15.52 days (C); formula 5, average age at 10.41 days (D); formula 9, average age at 12.55 days (E); formula 10, average age at 9.70 days (F); formula 11, average age at 7.03 days (G); formula 14, average age at 17.56 days (H) and formula 15, average age at 11.50 days (I)

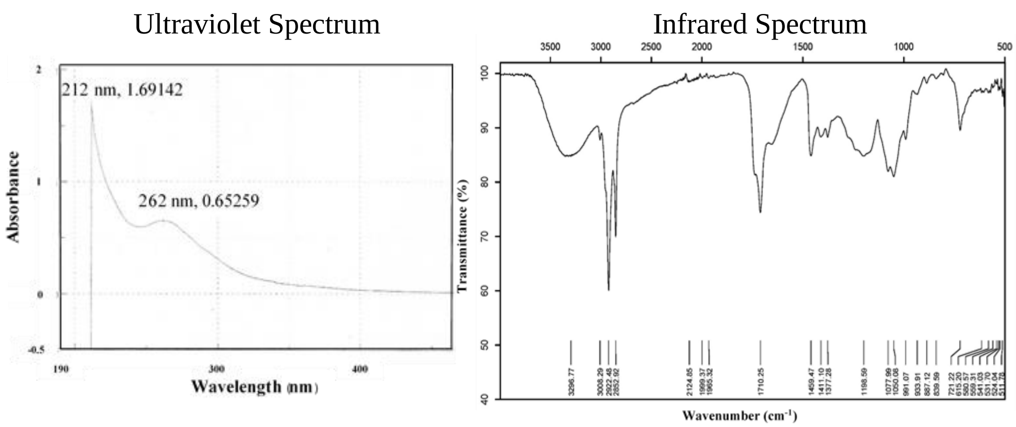

Figure 3. Chromatogram of crude extract of milky mushroom's fruiting

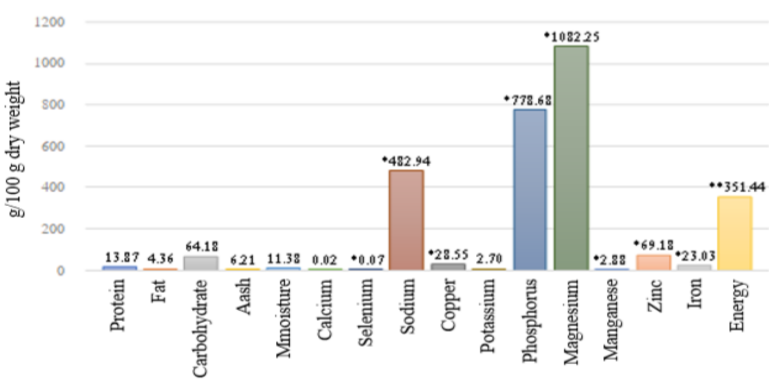

Figure 4. Amount of nutrients from fruiting body of milky mushroom

Note: * mg/100 g dry weight **Kcal/100 g dry weight

The average cap diameter was longest with formula 3, followed by formulas 2, 1, 14, 11, 15, 9, 10 and 5. It was found that formula 3 had a statistically significantly different cap diameter than the other formulas at a 95% confidence level. The average stalk diameter was highest in formula 9, followed by formulas 10, 15, 3, 14, 1, 11, 5 and 2. It was found that formula 9 had a statistically significant different stalk diameter than the other formulas at a 95% confidence level. For the length of stalk, formula 14 was longest, followed by formulas 10, 15, 9, 2, 5, 1, 11 and 3. Formula 14 had a statistically significant different average length of stalk than the other formulas at a 95% confidence level (Table 1).

The total days for mycelium to fully covered the substrates, the period for mycelia develop to the primordia and the period for the primordia develop to the fruiting body showed that formula 10 did so in the least amount of time, followed by formulas 11, 15, 14, 1, 5, 2, 3 and 9 at 60.31, 70.50, 73.36, 76.48, 82.86, 89.13, 94.57, 95.67 and 123.95 days, respectively. The B.E. was highest with formula 15, followed by formulas 14, 9, 3, 10, 2, 1, 11 and 5 at 24.77, 21.13, 20.14, 19.43, 18.39, 13.43, 11.45, 10.12 and 9.94, respectively (Table 1).

The bioactive compounds of the milky mushroom

Formula 15 was selected for bioactive compound analysis since it had the highest B.E. The results indicate that the crude extract was a brownish orange viscous liquid with a characteristic mushroom smell. The crude extract weight was 9.27 g (9.27%). TLC analysis showed 4 bands: A, B, C and D bands, with R_f 0.16, 0.26, 0.32 and 0.54, respectively (Table 2).

Bioactive component analysis showed that flavonoids, saponins, tannins, and phenolics were observed in the crude extract, giving positive results (+). Interestingly, terpenoid analysis showed a strong positive result (++). However, alkaloid and cardiac glycoside analysis showed negative results (-) (Table 3).

Ultraviolet spectroscopy analysis of the crude extract showed the highest absorbency at 212 and 262 nm, which are characteristics of aromatic and conjugate substances. Besides, infrared spectroscopy showed signals at 3296 cm^{-1} representing hydroxyl group (-OH) of alcohol capable for hydrogen bond formation, 3008 cm^{-1} representing benzene ring which are around 3000 – 3100 cm^{-1}, 2922 and 2852 cm^{-1} representing the C-H bond of methylene (CH_2)and methyl (CH_3) which are found between 2840 – 3000 cm^{-1}, 1710 cm^{-1} representing carbonyl compound (C=O) of ketone or aldehyde, 1459 cm^{-1} representing C=C in aromatic compounds, 1411 and 991 cm^{-1} representing double bond of $RCH=CH_2$, and 1198 cm^{-1} representing C-O of alcohol or phenol which are found between 1000-1260 cm^{-1} and 721 cm^{-1} representing C-X (X = halide group). Therefore, the functional groups found in the crude extract included alcohol, carbonyl, aromatic, as well as double-bond (Figure 3).

Nutrition analysis showed that the crude extract contained 13.87 g protein, 4.36 g fat, 64.18 g carbohydrate, 6.21 g ash and 11.38 g moisture per 100 g dry weight. Moreover, mineral analysis showed that the crude extract also contained 0.02 g Ca, 0.07 mg Se, 482.94 mg Na, 28.55 mg Cu, 2.70 g K, 778.68 mg P, 1,082.25 mg Mg, 2.88 mg Mn, 69.18 mg Zn and 23.03 mg Fe per 100 g dry weight of the crude extract. In addition, the crude extract yielded 351.44 Kcal energy per 100 g dry weight (Figure 4).

Cost, return benefit and investment of the milky mushroom

Considering the experimental resulted with culturing materials suitable for the development of milky mushrooms with 15 Medias, formulas were found to be the most promising, which are formulas 1-3, 5, 9-11, 14 and 15. Then, there was an analysis of different annual crop rounds which were 4.41, 3.86, 3.82, 4.10, 2.94, 6.05, 5.18, 4.77 and 4.98 crops. Moreover, different culturing materials produced different average weight per bag which were 55.71 g, 50.03 g, 64.11 g, 47.70 g, 73.94 g, 56.68 g, 54.74 g, 56.97 g and 54.09 g, respectively.

The lowest production costs were 6.68 baht/bag for formulas 2, 3, 9-11 and 15, followed by formula 5 (6.91 baht/bag), formula 1 (7.55 baht/bag) and formula 14 (8.12 baht/bag) however, the annual production cost of formula 9 was the lowest at 16,544.82 baht/year followed by formula 3 (20,705.63), formula 2 (20,917.75), formula 5 (22,808.19), formula 15 (26,251.70), formula 1 (26,608.50), formula 11 (27,216.50), formula 14 (30,779.39) and formula 10 (31,397.85) baht/year, respectively.

The returns of production indicated that there were very different prices, so three prices were considered, 250, 400 and 700 baht/kg as a result, the three highest returns were formula 9 (18.49, 29.58 and 51.76 baht/bag); formula 3 (16.03, 25.64 and 44.88 baht/bag) and formula 14 (14.24, 22.79 and 39.88 baht/bag). The annual returns show that formula 10 made the highest returns of 68,606.20,109,769.92 and 192,097.36 baht/year, followed by formula 11 (56,681.13,90,689.82 and 158,707.18 baht/year) and formula 14 (54,377.75, 87,004.39 and 152,257.69 baht/year).

The highest profit of production per bag were formula 9 made 11.81, 22.90 and 45.08 baht/bag followed by formula 3 (9.35, 18.97 and 38.20 baht/bag) and formula 10 (7.49, 16.00 and 33.33 baht/bag). Meanwhile, annual profit showed that formula 10 could make the highest profits which were 37,208.35, 78,372.07 and 160,699.51 baht/year followed by formula 11 (29,464.63, 63,473.31 and 131,490.67 baht/year) and formula 3 (28,212.84, 57,563.92 and 116,266.08 baht/year).

The payback period (PB) showed that with a price of 250 baht/kg, the shortest PB was 1 year for formulas 10, 11, 14 and 15 on the other hand, formulas 1, 3, 9, 2 and 5 could pay back in 2 years.

Net present value (NPV) showed that if the investment costs were 50,000 baht with 5 years, and the discount rate was 13%, formula 10 would be the best case to invest with NPV=191,303.88, 336,086.20 and 625,650.85 baht followed by formula 11 (NPV=149,360.66, 268,977.05 and 508,209.85 baht) and formula 14 (NPV= 141,259.11, 256,014.57 and 485,525.50 baht) (Tables 4 and 5).

Table 4. Costs, returns and investment costs on milky mushroom production

Programs	Formula 1		Formula 2		Formula 3		Formula 5		Formula 9	
Crops/Year	4.41		3.86		3.82		4.10		2.94	
1. Fixed cost (baht)	50,000.00		50,000.00		50,000.00		50,000.00		50,000.00	
2.Variable cost	baht/ bag	baht/ year	baht/ bag	baht/ year	baht/ bag	baht/ year	baht/ bag	baht/ year	baht/ bag	baht/ year
Labor cost	0.70	2,468.99	0.70	2,468.99	0.70	2,468.99	0.70	2,468.99	0.70	2,468.99
Material cost	6.85	24,139.51	5.98	18,448.77	5.98	18,236.65	6.21	20,339.20	5.98	14,075.84
3. Total cost	7.55	26,608.50	6.68	20,917.75	6.68	20,705.63	6.91	22,808.19	6.68	16,544.82
4.Return										
Case 1	13.93	49,080.74	12.51	38,618.91	16.03	48,918.47	11.93	39,067.65	18.49	43,546.75
Case 2	22.28	78,529.18	20.01	61,790.25	25.64	78,269.55	19.08	62,508.25	29.58	69,674.80
Case 3	39.00	137,426.07	35.02	108,132.94	44.88	136,971.72	33.39	109,389.43	51.76	121,930.91
5. Profit										
Case 1	6.38	22,472.24	5.83	17,701.15	9.35	28,212.84	5.02	16,259.46	11.81	27,001.93
Case 2	14.73	51,920.68	13.34	40,872.50	18.97	57,563.92	12.17	39,700.05	22.90	53,129.98
Case 3	31.45	110,817.57	28.35	87,215.18	38.20	116,266.08	26.48	86,581.24	45.08	105,386.08

Programs	Formula 10		Formula 11		Formula 14		Formula 15	
Crops/Year	6.05		5.18		4.77		4.98	
1. Fixed cost (baht)	50,000.00		50,000.00		50,000.00		50,000.00	
2. Variable cost	baht/ bag	baht/ year	baht/ bag	baht/ year	baht/ bag	baht/ year	baht/ bag	bath/ year
Labor cost	0.70	2,468.99	0.70	2,468.99	0.70	2,468.99	0.70	2,468.99
Material cost	5.98	28,928.87	5.98	24,747.52	7.42	28,310.41	5.98	23,782.72
3. Total cost	6.68	31,397.85	6.68	27,216.50	8.12	30,779.39	6.68	26,251.70
4. Return								
Case 1	14.17	68,606.20	13.69	56,681.13	14.24	54,377.75	13.52	53,824.56
Case 2	22.67	109,769.92	21.90	90,689.82	22.79	87,004.39	21.64	86,119.30
Case 3	39.68	192,097.36	38.32	158,707.18	39.88	152,257.69	37.86	150,708.78
5. Profit								
Case 1	7.49	37,208.35	7.01	29,464.63	6.13	23,598.35	6.85	27,572.86
Case 2	16.00	78,372.07	15.22	63,473.31	14.67	56,225.00	14.96	59,867.60
Case 3	33.00	160,699.51	31.64	131,490.67	31.76	121,478.29	31.19	124,457.08

Note: case 1 =250 baht/kg, case 2 =400 baht/kg, case 3 =700 baht/kg (Yoelao,2017; Government Public Relations Department, 2018)

Table 5. Payback Period (PB) and Net Present Value (NPV) analysis on milky mushroom production

Formula*	Cash flow (baht)					PB	NPV
	Year 1	Year 2	Year 3	Year 4	Year 5	(year)	(r=13%)**
1							
Case 1	49,080.74	49,080.74	49,080.74	49,080.74	49,080.74	2	122,628.31
Case 2	78,529.18	78,529.18	78,529.18	78,529.18	78,529.18	1	226,205.29
Case 3	137,426.07	137,426.07	137,426.07	137,426.07	137,426.07	1	433,359.26
2							
Case 1	38,618.91	38,618.91	38,618.91	38,618.91	38,618.91	2	85,831.63
Case 2	61,790.25	61,790.25	61,790.25	61,790.25	61,790.25	1	167,330.60
Case 3	108,132.94	108,132.94	108,132.94	108,132.94	108,132.94	1	330,328.55
3							
Case 1	48,918.47	48,918.47	48,918.47	48,918.47	48,918.47	2	122,057.57
Case 2	78,269.55	78,269.55	78,269.55	78,269.55	78,269.55	1	225,292.11
Case 3	136,971.72	136,971.72	136,971.72	136,971.72	136,971.72	1	431,761.20
5							
Case 1	39,067.65	39,067.65	39,067.65	39,067.65	39,067.65	2	87,409.97
Case 2	62,508.25	62,508.25	62,508.25	62,508.25	62,508.25	1	169,855.96
Case 3	109,389.43	109,389.43	109,389.43	109,389.43	109,389.43	1	334,747.93
9							
Case 1	43,546.75	43,546.75	43,546.75	43,546.75	43,546.75	2	103,164.00
Case 2	69,674.80	69,674.80	69,674.80	69,674.80	69,674.80	1	195,062.40
Case 3	121,930.91	121,930.91	121,930.91	121,930.91	121,930.91	1	378,859.20
10							
Case 1	68,606.20	68,606.20	68,606.20	68,606.20	68,606.20	1	191,303.88
Case 2	109,769.92	109,769.92	109,769.92	109,769.92	109,769.92	1	336,086.20
Case 3	192,097.36	192,097.36	192,097.36	192,097.36	192,097.36	1	625,650.85
11							
Case 1	56,681.13	56,681.13	56,681.13	56,681.13	56,681.13	1	149,360.66
Case 2	90,689.82	90,689.82	90,689.82	90,689.82	90,689.82	1	268,977.05
Case 3	158,707.18	158,707.18	158,707.18	158,707.18	158,707.18	1	508,209.85
14							
Case 1	54,377.75	54,377.75	54,377.75	54,377.75	54,377.75	1	141,259.11
Case 2	87,004.39	87,004.39	87,004.39	87,004.39	87,004.39	1	256,014.57
Case 3	152,257.69	152,257.69	152,257.69	152,257.69	152,257.69	1	485,525.50
15							
Case 1	53,824.56	53,824.56	53,824.56	53,824.56	53,824.56	1	139,313.44
Case 2	86,119.30	86,119.30	86,119.30	86,119.30	86,119.30	1	252,901.50
Case 3	150,708.78	150,708.78	150,708.78	150,708.78	150,708.78	1	480,077.63

Note: * = investment cost = 50,000 baht, ** = annual interest rate 13% of Bank for Agriculture and Agricultural Cooperative in 2018, case 1 = 250 baht/kg, case 2 = 400 baht/kg, case 3 = 700 baht/kg (Yoelao, 2017; Government Public Relations Department, 2018)

Discussion

At 50 days, white mycelia were found in all substrates. The mycelia density was characterized into three groups, namely abundant, moderate and poor. The minimum and maximum of average ambient temperature at 06:00 a.m.-06:00 p.m. were 31.58^{o}C and 32.50^{o}C. Development of primordia was fastest in formula 10, followed by formulas 11, 15, 14, 1, 5, 8, 3, 2, 9 and 6 with the minimum and maximum of average ambient temperature were 30.71^{o}C and 31.79^{o}C. However, formula 11 took the least amount of time to develop from primordia to fruiting body with the minimum and maximum of average ambient temperature were

30.68^{o}C and 31.86^{o}C. The results show that mycelia growth well on rice husk, khae na sawdust, rain tree sawdust, mango tree sawdust, para rubber sawdust, cinnamon tree sawdust, coconut coir dust, bagasse, corn cob, cat tail, bamboo leaves, brab palm pericarp, water hyacinth, rice straw and banana pseudostem. These substrates had different growth rates in accordance with Buakhom (1996) which reported that the growth of mushroom mycelia on different seeding materials can affect growth, including rice husk (Navathe *et al*., 2014), bagasse (Amin *et al*., 2010), corn cob (Ukoima *et al*., 2009) and rice straw (Thakur and Singh, 2014).

Moreover, the minimum and maximum of ambient temperature also affected the growth of mushrooms, since the mycelia growth was affected by 31.58^{o}C and 32.50^{o}C, the primordia development was influenced by 30.71^{o}C and 31.79^{o}C and the fruiting body development was affected by 30.68^{o}C and 31.86^{o}C. This is evident since the growth phase of mycelia requires a relatively higher ambient temperature than in the growth phase of the primordia and the fruiting body, which is in agreement with Thakur and Singh (2014) which reported that milky mushrooms grow well between 30-35^{o}C. Meanwhile, this temperature range resulted in *Pleurotus* sp. giving fewer mushrooms and also complies with Sornprasert *et al*. (2017) which reported that the term of mycelia growth *Macrocybe crassa* required temperatures that exceed the growth phase of the primordia to develop into fruiting body. Meanwhile, Hanmoungjai (2014) reported the growth phase of *Astraeus hygrometricus* mycelia grew well at 30^{o}C and resulted in a medium mycelium density. In terms of growth, the fruiting body prospered well at 27^{o}C.

For the total number of days during the period of full colonized on substrates, the period of development of mycelia to primordia and the period of development of fruiting body, formula 10 grew in the shortest period of time. However, formula 9 had the highest weight of fresh fruiting body.

Moreover, formula 11 provided the largest number of fruiting bodies. formula 3 gave the highest cap diameter. Formula 9 showed the largest stalk diameter. Besides, the longest mushroom stalk was found in formula 14. Nevertheless, there was no correlation between fruiting body weight, cap diameter, stalk diameter and stalk length, which is inconsistent with Pongthornpruek and Kraivuttinun (2013) which reported that hte number of fruiting bodies, cap diameter and stalk length are related.

Formula 15 (banana pseudostem) had the highest B.E. followed by formula 14 (rice straw), formula 9 (corn cob), formula 3 (rain tree sawdust), formula 10 (cat tail), formula 2 (khae na sawdust), formula 1 (rice husk), formula 11 (bamboo leaves) and formula 5 (para rubber sawdust).

Our results suggest that mycelia can be developed into fruiting body in plastic bags by pulling out the bottleneck and the bottle lid without casing, with processed with loam, loam mixed with manure, or loam mixed with coconut coir dust and manure as with the approach of Bhupathi *et al*. (2017); Srioon (2018). Therefore, these substrates are suitable for fruiting body development since they contain appropriate chemical compositions. Banana pseudostem contains 2.5% protein, 26.1% fiber, 1.0% Ca, 3.0% K, 0.1% P, 0.42 % Mg, 2.87 mg Mn, 0.05 mg Cu, 6.37 mg Fe and 1.41 mg Zn per 100 grams of dried weight (Limmatvapirat *et al*., 2007). Rice straw contains 32-47% cellulose, 19-27% hemicellulose and 5-24% lignin (Garrote *et al*., 2002; Saha, 2003). Corn cob contains 1.90% protein and 33.03% fiber (Snitwong, 1997). Rain tree sawdust contains 3.69% protein, 61.51% fiber, 0.33% ash, 29.85% carbohydrate, 0.13% P, 0.58% K, 0.47 % Ca and 0.04% Mg (Charoenkid *et al*., 2013). Cat tails contains 63.0% cellulose, 8.7% hemicellulose, 9.6% lignin, 1.4% fat and 2.0% ash (Duke and Wain, 1981). Khae na sawdust contains 2.29% protein, 0.08% fat, 36.17% fiber, 40.66% carbohydrate and 9.55% ash (Khaing *et al*., 2018). Rice husk contains 1.9-3.0% protein, 0.3-0.8% fat, 34.5-45.9% fiber, 26.5-29.8% carbohydrate, 13.2-21.0% ash, 9.0-20.0% lignin, 28.0-36.0% cellulose and 12.0% hemicellulose (Kongseree, 2003). The highest chemical composition in bamboo leaves is K, followed by Ca, N, Mg and P (Korntong, 2015). Para rubber sawdust contains 57.99% fiber, 41.24% lignin, 0.25% N, 0.04% P, 0.21% K, 0.02% Na, 0.10% Mg, 1.60% protein, 16.0% carbohydrate and 0.35% fat (Hiranpradit *et al*., 1989; Maneesri, 2004).

Some substrates were found to be unsuitable for milky mushroom cultivation since primordia was not found in some substrates, including mango tree sawdust (formula 4), coconut coir dust (formula 7), brab palm pericarp (formula 12) and water hyacinth (formula 13). This could be due to some chemical components that may affect the development of mycelia to primordia, or some essential chemical elements that are in inappropriate quantities. This underlying mechanism could also affect cinnamon tree sawdust (formula 6) and bagasse (formula 8) since primordia development was found with it, but no fruiting body was observed. Hence, further chemical analysis of these substrates is required to clarify this result. In addition, the application of formulas 1-3, 5, 9-11, 14 or 15 for the cultivation of milky mushroom requires consideration of other factors including quantity, availability, preparation processes and the cost of the substrates which reflect the production costs for farmers.

Analysis of the bioactive compounds in the crude extract of milky mushroom showed the consistent result between TLC and spectroscopy, indicating that it contained flavonoids, saponins, tannins, phenolics and terpenoids but it did not contain alkaloids or cardiac glycosides. This result in similar to Prabu and

Kumuthakalavalli (2014), though Sumathy *et al*. (2015) reported that alkaloids were observed in the crude extract of milky mushroom. Nutrition analysis indicated that the fruiting body of milky mushroom in this study contained protein, fat, carbohydrate, ash, moisture, Ca, Se, Na, Cu, K, P, Mg, Mn, Zn and Fe, which is in agreement with the findings of Pongsamart *et al*. (1985); Cuptapun *et al*. (2010) which reported that many types of mushrooms contain protein, fat, carbohydrate, ash, moisture, minerals and amino acids.

Total cost of milky mushroom production showed that formulas 2, 3, 9-11 and 15 had the lowest costs (6.68 baht/bag) followed by formulas 5, 1 and 14, respectively. This finding shows that khae na sawdust, rain tree sawdust, corn cob, cat tail, bamboo leaves, and banana pseudostem could be used as substates for mushroom production instead of, or mixed with para rubber sawdust which could decrease production costs and result greater profits. For three pricings (250, 400, and 700 baht/kg), formula 9 made the highest revenue (18.49, 29.58 and 51.76 baht/bag), followed by formulas 3, 14, 10, 1, 11, 15, 2 and 5, respectively.

However, formula 9 made the highest profits which were 11.81, 22.90 and 45.08 baht/bag followed by formulas 3, 10, 11, 15, 1, 14, 2 and 5, respectively. Furthermore, the payback period for formulas 10, 11, 14, and 15 were within 1 year which were shortest periods and formula 10 had the best NPV were 191,303.88, 336,086.20 and 625,650.85 baht at mushroom prices of 250, 400 and 700 baht/kg, respectively, followed by formulas 11, 14, 15, 1, 3, 9, 5 and 2, respectively. As a result, cat tail could be the substrate of milky mushroom instead or mixed with para rubber sawdust since it has the shortest of growth period at 60.31 days, indicating that this could result in 6.05 harvests and produce 56.68 g/bag which Pengpis *et al*., (2011) reported about straw mushroom cultivation by a short stack. Cat tail as a substrate then could make 3.75 kg of straw mushroom, had the best NPV with 6.96 6.96 baht/bag of costs and produced 57.11 g/bag.Thus if farmers want to get 30% profit from production cost, they could sell at 158.44 baht/kg or 182.81 baht/kg with a 50% profit.

Acknowledgements

This research is financial supported by Research and Development Institute Chandrakasem Rajabhat University. We also would like to thank Faculty of Science, Chandrakasem Rajabhat University for supporting facilities.

References

Amin, R., Khair, A., Alam, N. and Lee, T. S. (2010). Effect of different substrates and casing materials on the growth and yield of *Calocybe indica*. Mycobiology, 38:97-101.

AOAC (2016a). AOAC Official Method 920.153 ash of meat. *In* Official Method of Analysis of AOAC International. AOAC International, United States of America.

AOAC (2016b). AOAC Official Method 922.06 fat in flour. *In* Official Method of Analysis of AOAC International. AOAC International, United States of America.

AOAC (2016c). AOAC Official Method 925.45 loss on drying (moisture) in sugars. *In* Official Method of Analysis of AOAC International. AOAC International, United States of America.

AOAC (2016d). AOAC Official Method 981.10 crude protein in meat. *In* Official Method of Analysis of AOAC International. AOAC International, United States of America.

AOAC (2016e). AOAC Official Method 984.27 calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zine in infant formula. *In* Official Method of Analysis of AOAC International. AOAC International, United States of America.

AOAC (2016f). AOAC Official Method 986.15 arsenic, cadmium, lead, selenium and zine in in human and pet foods. *In* Official Method of Analysis of AOAC International. AOAC International, United States of America.

AOAC (2016g). AOAC Official Method 986.24 phosphorus infant formula and enteral products. *In* Official Method of Analysis of AOAC International. AOAC International, United States of America.

AOAC (2016h). AOAC Official Method 999.10 lead, cadmium, zine, copper and iron in foods. *In* Official Method of Analysis of AOAC International. AOAC International, United States of America.

Bhupathi, P., Subbiah, K. A. and Uthandi, S. (2017). Profiling of morphogenesis related enzymes of milky mushroom *Calocybe indica* P&C. Journal of Pharmacognosy and Phytochemistry, 6:2537-2543.

Buakhom, C. (1996). A Study on the Growth of *Pleurotus sajor-caju* in Fermented Straw Mixed with Pararubber Sawdust. (Master Thesis). Mahasarakham University, Thailand.

Charoenkid, S., Sang, V., Manusom, K., Meesuk, S. and Majiew, S. (2013). Cultivation of potential economic mushrooms in the area with local grass. *In* Research and Development of Technology in Using Materials and Supplements in the Cultivation of Economic Mushrooms. Ministry of Agriculture and Cooperatives, Thailand.

Cuptapun, Y., Hengsawadi, D., Mesomya, W. and Yaieiam, S. (2010). Quality and quantity of protein in certain kinds of edible mushroom in Thailand, 44:664-670.

Duke, J. A. and Wain, K. K. (1981). Medicinal Plants of the World Volume 3. Plants Genetics and Germplasm Institute, Maryland.

Garrote, G., Dominguez, H. and Parajo, J. C. (2002). Auto hydrolysis of corncob: study of nonisothermal operation for xylooligosaccharide production. Journal of Food Engineering, 52:211-218.

Government Public Relations Department (2018). Trang province holds a new alternative to mickey mushrooms, earning 400 baht/kg better than para rubber tapping. Trang Provincial Public Relations Office, Thailand.

Hanmoungjai, W. (2014). Factors affecting on the growth of mycelium Hed-Pho (*Astreaus hygrometricus* Morgan) on agar medium and cereal grain medium. Naresuan University Journal: Science and Technology, 22:93-101.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A. and Ferreira, C. F. R. (2009). Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and hight performance liquid chromatography. Microchemical Journal, 93:195-199.

Hiranpradit, S., Sittibut, Y., Kancharoen, Y. and Tantayaporn, S. (1989). Effect of pH and carbon on nitrogen on the growth of fungi on sawdust from mushroom culture. Agricultural Science Journal, 22:375-395.

Khaing, K., Naing, A. P., Lae, K. Z. W., Nyo, K. T. and Ngwe, D. H. (2018). Investigation of chemical properties and biological activities of stem of *Coccinia cordifolia* Cogn. (Kin-Pon) and *Dolichandrone serrulata* Seem. (Tha-Khut). Journal of the Myanmar Academy of Arts and Science, 16:153-178.

Kongseree, N. (2003). Rice and Rice Products. Department of Livestock Development. Ministry of Agriculture and Cooperatives, Thailand.

Korntong, A. (2015). Research and Development on Mushroom. Department of Agriculture. Ministry of Agriculture and Cooperatives, Thailand.

Limmatvapirat, C., Charoenteeraboon, J. and Phaechamud, T. (2007). The chemical composition of bananas. Pharmatime, 5:51-60.

Luanratana, O. (1993). Extraction and Detection of Important Substances from Natural Products. Faculty of Pharmacy. Mahidol University, Thailand.

Maneesri, J. (2004). Utilization of para rubber sawdust as substrate for cellulase production. Proceedings of 42nd Kasetsart University Annual Conference: Fisheries, Agro-Industry, 347-353 p.

Miller, F. C. (1994). World trade in mushroom. *In* Souvenir National Symposium on Mushroom. Solan, India.

Nandhasri, P. and Parawet, W. (2006). TLC Atlas of 24 Thai Medicinal Plants Phytochemistry. Thammasat University Printing house, Thailand.

Nanna, Y. (2006). Spectroscopy for Organic Chemistry. Chulalongkorn University Press, Thailand.

Navathe, S., Borkar, P. G. and Kadam, J. J. (2014). Cultivation of *Calocybe indica* (P & C) in Konkan Region of Maharashtra, India. World Journal of Agricultural Research, 2:187-191.

Pengpis, U., La-ongpant, N. and Boondao, S. (2011). Application of Some Aquatic Weeds for Agricultural Media. Bureau of Research and Development. Rayal Irrigation Department, Thailand.

Pongsamart, S., Boonvisut, S. and Vooticumpree, P. (1985). Protein quality of wild edible mushrooms in Thailand. Thai Journal of Pharmaceutical Sciences, 10:149-163.

Pongthornpruek, S. and Kraivuttinun, P. (2013). The used of bamboo residues from chopstick production for mushroom cultivation material. RMUTP Research Journal, (Special Issue):20-26.

Prabu, M. and Kumuthakalavalli, R. (2014). Nutritional and phytochemical studies on *Pleurotus florida* (Mont.) Singer and *Calocybe indica* P&C. World Journal of Pharmaceutical Research, 3:4907-4913.

Saha, BC. (2003). Hemicelluloses bioconversion. Journal of Industrial Microbiology and Biotechnology, 30: 279-291.

Snitwong, C. (1997). The Use of Agricultural Waste in Cattle - Buffalo Farming. Department of Livestock Development. Ministry of Agriculture and Cooperatives, Thailand.

Sornprasert, R., Aroonsrimorakot, S., Hambananda, A., Kasipar, K., Sukkapan, P. and Saenkamol, P. (2019). The growth of *Antrodia cinnamomea* mycelia on different kinds of substrates in Thailand. International Journal of Agricultural Technology, 15:113-126.

Sornprasert, R., Hambananda, A., Kasipar, K. and Saenkamol, P. (2017). Comparison of Tin Raed mushroom (*Macrocybe crassa* (Berk.) Pegler & Lodge) cultivated with six substrate formulas. Journal of Agricultural Research & Extension, 34:1-12.

Soytong, K., Suthammarit, S. and Kanokmedhakul, S. (2019). Evaluation of bio-formulation from *Chaetomium elatum* ChE01 to control banana anthracnose caused by *Colletotrichum musae*. International Journal of Agricultural Technology, 15:665-674.

Srioon, K. (2018). Effects of amount of rice bran and number of sawdust spawn on the growth and yield of milky mushroom (*Calocybe indica*). Agricultural Science Journal, 49:193-206.

Sullivan, D. and Carpenter, D. (1993). Method of Analysis for Nutrition Labeling. AOAC International, Arlington.

Sumathy, R., Kumuthakalavalli, R., Krishnamoorthy, A. S. and Balan, V. (2015). Phytochemical investigation and total antioxidant potential of the milky mushroom, *Calocybe indica* Var.APK2 Cultivated in tamilnadu, India. International Journal of Pharma and Bio Sciences, 6:485-497.

Thakur, M. P. and Singh, H. K. (2014). Advances in the cultivation technology of tropical mushrooms in India. JNKVV Research Journal, 48:120-135.

Ukoima, H. N., Ogbonnaya, L. O., Arikpo, G. E. and Ikpe, F. N. (2009). Cultivation of mushroom (*Volvariella volvacea*) on various farm wastes in obubra local government of Cross River State, Nigeria. Pakistan Journal of Nutrition, 8:1059-1061.

Yoelao, C. (2017). Charee boonyawinit, a farmer who builds a city farm. Thailand Restaurant News, 18:23-24.

(Received: 1 September 2021, accepted: 30 May 2022)