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The effect of substrates on the production of tropical mushroom *Macrocybe crassa*

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Abstract

The wild Thai isolate strain, the Department of Agriculture (DOA-10), of the mushroom, *Macrocybe crassa*, was grown on seven individual substrate formulas in order to measure mycelium development, productivity, morphological characteristics, and the nutritive value of the fruit-body. The most suitable substrate for cultivating this mushroom was spent mushroom, substrate-based substrate (T6), which consisted of spent mushroom substrate from *Pleurotus eryngii* cultivation, fine rice bran, $MgSO_4H_2O$, CaO, and fine corn seeds in a ratio of 100:3:0.2:1:0.5 (w/w). Mycelium growth rate and the number of fruit-bodies were higher when grown on this substrate formula compared to others. Moreover, this strain showed the fewest days to complete spawn running and primordial initiation. Additionally, its yield was highest at 237.21 g/0.6 kg of substrate, and the biological efficiency was highest at 65.89%. Mushroom morphology showed that the largest pileus and stipe diameters were found to be 117.00 and 37.20 mm, respectively, on spent mushroom substrate-based substrate (T6). Dry *M. crassa* strain DOA-10 grown on this substrate contained carbohydrates and crude proteins at 74.75% and 30.89%, respectively, which were higher than the other formulas. These results confirmed that spent mushroom substrate-based substrate (T6) is of high quality and could be a promising substrate for commercial cultivation of *M. crassa* native strain DOA-10.

Keywords: Fungi, Basidiomycetes, Biological efficiency, Substrate formula, *Tricholoma crassum*

1. Introduction

Mushrooms have a high nutritional value, and their fruit-bodies are an excellent food source for human. Mushroom cultivation could decrease environmental waste and agricultural residues and provide alternative choices of high-quality food production and animal feed. An effective method of mushroom cultivation is to make use of, and manage, agricultural residues, including environmental waste. Mushroom substrate mainly made from any organic material that contains cellulose, hemicellulose, or lignin [1]. In Asian countries, rice is a major crop and rice straw can be used as a basic substrate for mushroom cultivation at a reasonable cost; and, in general, rice straw is a good substrate for mushroom production. Corn leaves are agricultural waste from corn production that can be used for *Volvariella volvacea* cultivation. Sawdust is a common basal ingredient in synthetic substrate formulations for mushroom production [2]. Sawdust has also been recommended as the best substrate for oyster mushroom cultivation, which agrees with the findings of Hami [3], who studied oyster mushroom production on sawdust from various woods and discovered that *Pleurotus ostreatus* produced the highest yield, consistence with the findings of Shah et al. [4], who found that a maximum average yield of *P. ostreatus*, at 646.9 g, could be estimated from sawdust. Spent mushroom substrate (SMS) is residual by-product of several mushroom cultivation cycles. Some authors have attempted to evaluate mushroom cultivation on spent mushroom substrate, which has shown that this substrate could be used in order to recultivate other edible mushrooms, such as *Pleurotus sajor-caju*, after cultivation of shiitake, and *Coprinus comatus* or *Agaricus bisporus* after cultivation of *Ganoderma lucidum* or *Flammulina velutipes* [5].

Wild edible mushrooms are expensive because they only grow during wet seasons in specialized habitats. These wild mushrooms include *Macrocybe crassa* or *Tricholoma crassum* (Berk.) Sacc. [6]. *M. crassa* is known in Thailand by a variety of names, including Hed-Taen-Rad (northeast), Hed-Jan (north), Hed-Hua-Sum (south), and Hed Yai or Hed-Tub-Tao-Khao (central) [7]. Similar to other mushrooms, *M. crassa* has a high nutritional value and has a great potential for commercial cultivation. Sumpao et al. [8] found that different substrates, such as peanut shells, sawdust mixed with horse manure, and sawdust mixed with corn seed meal, could be used for *T. crassum* cultivation. Chingdaung et al. [9] reported that substrates containing lotus seed, mixed with horse manure, or sawdust with fresh manure were better than a substrate consisting of sawdust mixed with cow manure; however, with the substrate of only sawdust, mycelium growth was very slow. A mixture of rice straw, corn cob meal, and cotton seed meal in a ratio of 2:2:1 could be used for *T. crassum* cultivation; moreover, the combination of corncob meal, sawdust, and cornmeal at a ratio of 15:4:1 (v/v) was the best option and supported higher yields of basidiocarps [10]. Hed Taen Rad was grown easily and effectively on rubber tree sawdust [7]. However, there is insufficient knowledge regarding the best cultivation technique to improve quality and productive yields, which is necessary in order to identify the most suitable substrates for commercial cultivation.

For commercial mushroom cultivation, reducing growth times in order to save on costs and increase yield is of paramount importance. Hence, the main objective of this experiment was to evaluate the influence of locally available substrates on growth, yield performance, and nutrient content of the fruiting body of *M. crassa*. The experiments also looked into the suitability of locally available lignocellulosic wastes for increasing *M. crassa* fruit-body productivity and quality.

2. Materials and methods

2.1 Spawn preparation and mycelial growth

Macrocybe crassa strain the Department of Agriculture (DOA-10) was provided by Departement of Agriculture, Ministry of Agriculture and Co-operatives, Bangkok, Thailand (collected between 2005 and 2006 from Prachuap Khiri Khhan province, Thailand). This strain has a high production yield, as evidenced by cultivation on a substrate mixture of rubber tree sawdust, fine rice bran, magnesium sulfate ($MgSO_4H_2O$), and calcium oxide (CaO) at a ratio of 100:3:0.2:1 (w/w), as well as comparisons with other strains [11]. A pure culture of *M. crassa* strain DOA-10 was grown on PDA media for 7 d. Natural sorghum grains were soaked in water for 12 h in order to soften them before being utilized for spawn production. The grains were boiled and drained, and the moisture content of the grain was adjusted to around 50%. The sorghum grains were filled to a 2/3 capacity in 250-mL heat-resistant glass bottles and were autoclaved for 30 min at 121 °C. Five mycelium discs (Ø5 mm were cut from Potato Dextrose Agar (PDA) media and placed on top of the prepared grains. The bottles of sorghum inoculated were kept at room temperature in dark, sterile cabinets until they were completely colonized (14 d).

Individual substrates with different ingredients were prepared as demonstrated in the Table 1. These materials were analyzed for their carbon (C) and nitrogen (N) contents. Spent mushroom substrate from the cultivation of *P. eryngii* on para rubber sawdust was used.

Table 1 Various substrates and supplement combinations (weight/weight) used in the experiments.

Substrate formulations	Para rubber sawdust	Rice straw	Corn cob	Spent mushroom compost	Supplements				
					Fine rice ran	Zeolite	Fine seed corn	Cow dung	C/N ratio
T1	100	-	-	-	3	-	-	-	250.0
T2	100	-	-	-	3	3	-	-	250.0
T3	100	-	-	-	5	-	-	-	250.0
T4	95	5	-	-	3	-	-	-	175.0
T5	25	-	75	-	3	-	0.5	-	97.9
T6	-	-	-	100	3	-	0.5	-	490.0
T7	-	100	-	-	3	-	-	25	58.5

Approximately 600 g of each substrate mixture was placed in 1-kg autoclavable polypropylene bags, which were then sterilized in a broad cast-iron steamer for 3 h at 95 °C. After that, 10-15 grain spawn seeds were added to each autoclaved substrate bag. Samples for spawn running were incubated at 25 ± 3 °C in an incubating room. Fifty replicates of each treatment (substrate formula) were used for the experiment. The days required to complete spawn running (DCS) (i.e., the number of days it takes for the mycelium to completely colonize the substrate after being inoculated) and mycelium growth rate (MGR) were noted by daily measurements until the samples were completely covered by the mycelia.

2.2 Mushroom production and morphological characterization

The top end of the sample bags was removed. Colonized (by mycelium) substrates were arranged in baskets and then placed on shelves in a greenhouse (temperature, 25–30 °C; relative humidity, 85%). A 2.5-cm thick layer of sterilized casing soil was applied to the substrates' surfaces, which consisted of loamy soil, leaf debris, and charcoal, at a ratio of 10:2:1 (w/w). The culture was constantly wet in order to maintain the required relative humidity (85%). The cultures were irrigated by spraying with water, once or twice a day. After covering the substrates with casing soil, the time (days) for first primordial initiation day after application of casing (DFPI) and the number of fruit-bodies (NFB) were recorded. Mature mushrooms (noted as the point where the pileus started to fold) from each bag were harvested. The mushroom cultivation process lasted for two months. The yield and quality of the mushrooms (Pileus diameter, stipe diameter and stem length) were measured frequently; furthermore, the biological efficiency (BE) [1] was computed using the formula below:

$$BE = (\text{Fresh fruit-body weight} / \text{Dry substrate weight}) \times 100 \quad (1)$$

2.3 Nutritional analyses

Nutritional analyses were carried out on a dry matter basis at the Thailand Institute of Scientific and Technological Research (TISTR). Total carbohydrates, proteins, fats, crude fiber, and ash were determined using standard methods [12]. Mineral content, macronutrients, and micronutrients were analyzed using spectrophotometric atomic absorption [13], and total phosphorus was determined using the phosphorus-vanodomolybdate method [14]. A Mushroom and Yeast Glucan Assay Kit (K-YBGL 09/2009; Megazyme International Ireland Ltd.; Bray, County Wicklow, Ireland) was used to determine the glucan content.

2.4 Statistical analyses

The data from the study were statistically analyzed using a completely randomized design (CRD) with 50 replicates (n = 50). Analysis of Variance (ANOVA) at a 5% significance level was performed adopting SAS software (version 9.1) [15].

3. Results

3.1 Spawn running and mycelial development

The results revealed that the mycelial colonies of all *M. crassa* strains were white and cottony, with an abundance of aerial hyphae. Three to four days after spawn addition, mycelia could be seen running through the substrate, and the isolates gradually proceeded through the formulations, dividing at various rates. The day-to-day rates of mycelial development of various substrates are displayed in Table 2. *M. crassa* grew fastest on the rice straw-based substrate (T7) and DCS in 30.00 d, followed by spent mushroom substrate-based substrate (T6) (32.00 d). Moreover, the results showed that mycelial thickness and density were higher in the spawn of T6 than in those of T7.

The highest MGR for DOA-10 was observed for T7 at 4.80 mm per day, but it was not statistically different from the MGR of T6 (4.60 mm/d). The days to first primordial initiation, after the surface of the substrates (DFPI) of DOA-10 were cased with soil, were lowest for T6 (13.00), which is significantly different from the other treatments. The highest number of fruiting bodies (NFB) was observed for T6 (31.00), which was notably different ($p \leq 0.05$) from that of the other substrate formulas.

Table 2 Mean values of growth trend and yield of *M. crassa* strain DOA-10 cultured on various substrate formulations (mean ± SD; n = 10).

Substrate formulations	DCS (d)	MGR (mm/d)	DFPI (d)	NFB
T1	$35.00^{a}\pm0.73$	$4.30^{b}\pm0.04$	$14.05^{bc}\pm1.09$	$24.00^{e}\pm0.56$
T2	$35.00^{a}\pm0.85$	$4.30^{b}\pm0.01$	$14.00^{bc}\pm1.07$	$25.00^{c}\pm0.77$
T3	$35.00^{a}\pm0.79$	$4.00^{b}\pm0.03$	$14.25^{bc}\pm0.44$	$28.00^{b}\pm0.89$
T4	$33.00^{b}\pm0.56$	$3.30^{d}\pm0.06$	$16.00^{a}\pm0.32$	$15.00^{f}\pm0.63$
T5	$34.00^{ab}\pm0.79$	$3.80^{c}\pm0.02$	$15.00^{ab}\pm0.56$	$17.00^{e}\pm0.44$
T6	$30.00^{c}\pm1.12$	$4.60^{a}\pm0.05$	$13.00^{c}\pm0.76$	$31.00^{a}\pm0.64$
T7	$32.00^{b}\pm0.65$	$4.80^{a}\pm0.03$	$13.30^{c}\pm0.66$	$21.00^{d}\pm0.89$

Note: Values with identical letter in the same column do not differ significantly at the 5% probability level based on the Tukey test.

3.2 Mushroom production and morphological characterization

M. crassa strain DOA-10, cultivated on substrate T6, had the highest yield (237.21 g) with significant differences ($p \leq 0.05$), as shown in Table 3 and Figure 1. BE varied from 38.67% to 65.59%. Fruit-body sizes showed a large pileus with a pale cream surface at 60.60–117.00 mm. Pileus shapes varied from convex to slightly concave. The stripes were 13.00–37.20 mm in diameter, and 69.00–136.70 mm in length, with cylindrical and swollen bases. The largest pileus diameter (DP) was on substrate T6, and the longest stipe (LS) of DOA-10 was on substrate T3. The largest stipe diameter (DS) was observed on substrate T6.

Table 3 Mean of yield and morphological characteristics of *M. crassa* strain DOA-10 cultivated on various substrate formulations.

Substrate formulations	Fruit-body weight/bag (g)	Pileus diameter (mm)	Stipe length (mm)	Stipe diameter (mm)	Dry weight of Substrate (g)	BE (%)
T1	204.37^{b}	67.80^{c}	135.40^{a}	27.50^{b}	370.00^{b}	55.24^{b}
T2	205.00^{b}	94.30^{b}	116.80^{d}	18.60^{e}	370.00^{b}	55.41^{b}
T3	206.25^{b}	73.30^{c}	136.70^{a}	27.00^{b}	370.00^{b}	55.74^{b}
T4	162.73^{c}	71.30^{c}	69.00^{f}	13.00^{f}	380.00^{a}	42.48^{c}
T5	139.20^{d}	60.60^{e}	73.80^{e}	20.00^{d}	360.00^{c}	38.67^{e}
T6	237.21^{a}	117.00^{a}	130.00^{b}	37.20^{a}	360.00^{c}	65.89^{a}
T7	118.59^{e}	67.20^{c}	122.40^{c}	24.80^{c}	300.00^{d}	39.53^{d}

Note: The identical superscripts in a column do not vary statistically at 5% probability level.

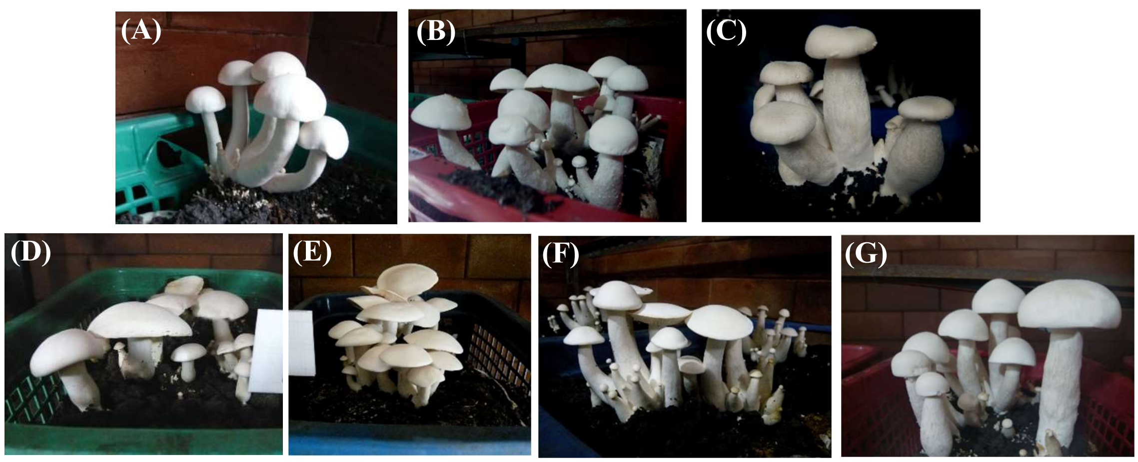

Figure 1 *M. crassa* strain DOA-10 cultivated on various substrate formulations after casing for 20 days (A) T1, (B) T2, (C) T3, (D) T4, (E) T5, (F) T6, and (G) T7.

3.3 Nutritional analyses

The nutrient analyses for mushroom strain DOA-10, cultivated on various substrate formulas, are shown in Table 4. Results revealed that total carbohydrates and protein content ranged from 50.61% to 76.61%, and 13.71% to 30.89%, respectively.

The amount of crude fat of *M. crassa* varied from 1.22% to 2.76% on a dried basis. The maximum content of crude fat, ash, and crude fiber, at 2.76%, 12.39%, and 2.54%, respectively, were recorded from substrate T2. The beta glucan contents of DOA-10, cultivated on the seven substrate formulas, are reported in Table 4, and ranged from 33.97 g to 45.87 g, per 100 g, on a dry basis. Macronutrient and micronutrient values are presented in Tab. 5. The maximum phosphorus value in the fruit-bodies was recorded for substrate T6 (9100 mg/kg). The potassium, on the other hand, varied from 33,600 to 47,800 mg/kg (dry weight basis). *M. crassa* strain DOA-10 contained the maximum level of calcium and magnesium on substrate T1 (492.63 mg/kg) and T4 (1434.77 mg/kg), respectively. Sodium contents varied between 50.62 and 1466.02 mg/kg.

Table 4 Mean of nutritive values of *M. crassa* strain DOA-10 (g/100 g dwb) cultivated on various substrate formulations.

Substrate formulations	Moisture	Protein	Fat	Carbo hydrates	Ash	Crude fiber	β-glucans	Energy value (Kcal)
T1	7.54^{e}	18.71^{d}	2.49^{ab}	58.08^{cd}	12.26^{a}	2.38^{b}	43.14^{b}	348.86^{d}
T2	15.72^{a}	18.21^{d}	2.76^{a}	57.74^{cd}	12.39^{a}	2.54^{a}	40.65^{bc}	300.42^{f}
T3	12.01^{d}	20.89^{c}	1.99^{bc}	50.61^{d}	10.34^{b}	1.98^{c}	40.83^{bc}	283.53^{g}
T4	13.30^{c}	15.60^{e}	2.42^{ab}	63.42^{bc}	11.81^{a}	2.03^{c}	45.87^{a}	327.18^{e}
T5	14.11^{b}	18.27^{d}	1.49^{cd}	76.61^{a}	12.13^{a}	1.79^{d}	41.17^{bc}	381.74^{b}
T6	14.35^{b}	30.89^{a}	1.76^{cd}	74.75^{ab}	12.07^{a}	1.74^{d}	33.97^{e}	371.76^{c}
T7	13.51^{c}	28.69^{b}	1.22^{d}	62.88^{bc}	9.23^{c}	1.70^{d}	36.99^{d}	409.02^{a}

Note: Means sharing the same superscript in a column are not significantly different at a level of 5% probability.

Table 5 Mean of macronutrient and micronutrient values of *M. crassa* strain DOA-10 (mg/kg dry weight) cultivated on various substrate formulations.

Treatments	Macronutrients					Micronutrients		
	P	K	Ca	Mg	Na	Fe	Zn	Mn
T1	6200^{d}	43,100^{c}	492.63^{a}	1046.66^{d}	936.33^{b}	283.21^{ab}	53.79^{bc}	17.55^{a}
T2	6700^{c}	33,600^{e}	68.30^{c}	899.94^{e}	577.54^{c}	236.04^{ab}	96.72^{a}	7.36^{b}
T3	9000^{a}	42,400^{cd}	118.49^{c}	1115.81^{c}	914.05^{b}	263.68^{ab}	63.20^{bc}	20.24^{a}
T4	8000^{b}	45,800^{b}	265.19^{b}	1434.77^{a}	864.67^{b}	376.83^{a}	107.75^{a}	9.32^{b}
T5	6200^{d}	34,500^{e}	ND	923.50^{e}	50.62^{d}	262.95^{ab}	67.34^{b}	ND
T6	9100^{a}	47,800^{a}	499.09^{a}	1474.00^{a}	960.72^{b}	282.14^{ab}	58.72^{bc}	16.16^{a}
T7	8900^{a}	41,000^{d}	133.14^{c}	1277.47^{b}	1466.02^{a}	ND	44.60^{c}	ND

Note: Values with the same letter in the same column are not statistically different at a 5% probability level.
ND: Not detected.

4. Discussion

Agricultural residues, either as main substrates or in combination with supplements, can be used for growing *M. crassa* mushrooms. For the cultivation of this mushroom, we classified substrate tests into three groups: Group 1 included a para rubber sawdust-based substrate (T1) and used general substrate formulations for the cultivation of this mushroom. Group 2 included a para rubber sawdust-based substrate with different supplement volumes added (T2, T3). Finally, Group 3 included various substrates in order to substitute para rubber sawdust as the main substrate material with other agricultural wastes (T4, T5, T6, and T7). The supplements (fine rice bran, $MgSO_4 \cdot 7H_2O$, CaO, and zeolite) showed no significant differences in the number of days required to complete spawn running, mycelium growth rate, and yield. However, for comparison between the types of substrates, the time of mycelium growth of *M. crassa* strain DOA-10 was determined at 30–35 days, depending on the type of material used. The shortest days required completing spawn running and the primordium formation period was determined to be 30.00 and 13.00 days for spent mushroom substrate-based substrate (T6), this information is further elaborated on in Table 2. Previously, the days required for completion of spawn run and the primordial formation days of *M. crassa*, were observed to be 35.00–37.00 and 13.00–15.30 days, respectively [11]. The results were different due to the amount of carbon and nitrogen sources in the material. The growing periods and yields of mushroom were found to vary according to nitrogen source, variety, and dose [16]. Spent mushroom substrate-based substrate (T6), consisting of spent mushroom substrate from *P. eryngii* cultivation, fine rice bran, $MgSO_4 \cdot 7H_2O$, CaO, and fine corn seeds, at a ratio of 100:3:0.2:1:0.5 had a high mycelium growth rate and number of fruit-bodies of *M. crassa* strain DOA-10, whereas the number of days required to complete spawn running, and days to first primordial initiation, were the lowest. According to Yadav et al. [17], the spawning of *Tricholoma* sp. in polythene bags took 30.00-35.00 days to complete. Results obtained during mycelia growth showed that spent mushroom substrate-based substrate (T6) had the fastest rate of mycelia development and a higher fresh weight than the other substrates, similar to a report on Kenyan native wood ear mushrooms, which recorded faster growth on composted waste than on fresh waste [18,19]. Narain et al. [19] pointed out that lignocellulosic biomass, particularly the C/N proportion, influenced mycelial and primordial mushroom growth. The significant differences in growth and yield may be due to the chemical composition and the C/N ratio of the growth media [20]. Different growth responses could also be attributed to variations in the nutritional and porosity levels (O_2 availability) of the various formulas tested [21]. Spent mushroom substrate-based substrate (T6) contained 441% carbon, 0.9% nitrogen, and the C/N ratio was 490:1 (Table 1), which is similar to the chemical analysis results of spent mushroom substrate used for commercial corn [22].

The yield (g/0.6 kg of substrate), morphological characteristics, and BE (%) of *M. crassa* strain DOA-10, cultivated under controlled conditions, are shown in Table 3. Mushroom cultivation continued for three months, during which time one crop was harvested. The highest yield and BE were 237.21 g and 65.89%, respectively, on spent mushroom substrate-based substrate (T6). Moreover, a high quality of fruiting bodies was found in this substrate formula, which may have arisen from the biological structure of the substrate used for the culture. This result is comparable to other findings on the quantity and quality of mushrooms on various substrates [17,20]. Olivier [23] found that a substrate containing 0.7-0.9% N dry weight and a C/N ratio of 50:1 or more produced the highest output. The substrates utilized to cultivate oyster mushrooms provided all the nutrients required for growth, as well as a high carbon to nitrogen ratio and a high C/N proportion [24]. Yildiz and Karakaplan [16] stated that various N and C/N ratios for the substrates employed for the production of *Pleurotus* spp. impacted the mushroom yielding results. Therefore, C/N ratio of a substrate material should be considered in substrate preparation. The spent mushroom substrate contained a high amount of organic matter, and this stimulated fungus growth, resulting in a faster colonization. Differences in the biological and chemical compositions between the substrate medium and the genotype of the cultured mushroom affected growth. P. sajor-caju cultivated on spent substrate *Volvariala volvacea* had a significantly shorter incubation time, a lower number of days from total colonization of mycelial to primordial development, and improved yield and biological effectiveness [25]. Pantawee [26] reported that full spawning was achieved within three days using fermented manure, made-up of sawdust and horse or pig excreta, as a substrate for cultivation. Moreover, different yields resulted from varied substrate media for mushroom cultivation. The effects of the physical and chemical properties of substrates on yield and BE were investigated using P. sajor-caju and P. ostreatus [27]. BE was found to be directly related to the nitrogen, lignin, and ash content, as well as indirectly related to the amount of hemicellulose and carbohydrates of the substrates. These results suggested that BE was affected by different substrate formulas. Mushroom quality, stalk length, stalk diameter, and cap size were also affected by substrate material.

This study showed that the nutritional composition of the fruit-bodies differed significantly, depending on the substrates used. The variation of nutrient content was due to the quality and quantity of nutrients available in the substrates. Sturion and Oetterer [28] noted that the nutritional value of mushrooms can be greatly affected by cultivation substrates. Among the cultivation parameters, the carbon/nitrogen ratio is one of the most important factors in balancing biomass and biocomposite productions. Substrates with high amounts nutrients produced higher quality mushrooms. This suggested that the substrates affected the nutritional composition of the fruit-bodies [29]. The carbohydrate contents of *Macrocybe* mushroom strain DOA-10 were reported as being higher than those of *Tricholoma terreum*, *Tricholoma portentosum* and *Tricholoma portentosum* [30,31]. In addition, heavy metals such as lead (Pb), cadmium (Cd) and arsenic (As) were not found in all the matured mushroom fruit-bodies investigated. The carbohydrates present in different lignocellulosic wastes were more effectively utilized by the mycelium for vegetative growth [32]. This is in agreement with the fact that composting the substrates caused a breakdown of complex lignocellulosic compounds into simple carbohydrates by microorganisms, providing energy rich conditions for faster growth of mycelia [33]. Moreover, a high nitrogen content from the spent mushroom substrate was slowly released, and the mycelium then absorbed the nutrients and developed fruit-bodies. Moreover, the time taken by mycelia to start primordial formation depended on the type of substrate and supplement levels [34]. Moreover, the spent mushroom substrate contained many microorganisms from the breakdown of larger molecules, including cellulose and lignin, into simpler compounds [35]. These results suggested that a spent mushroom substrate was a suitable choice as a material for commercial cultivation of a wild Thai isolate of a tropical mushroom (*M. crassa*) to produce mushrooms of a high quality and yield.

5. Conclusion

This study showed that substrate combinations for *M. crassa* strain DOA-10 cultivation resulted in significantly different mushroom growth, yields, and nutritive values. *M. crassa* was fully grown on rice straw-based substrate (T7) in 30.00 d; however, there was no statistical difference in the mycelium growth rates between rice straw-based substrate (T7) and spent mushroom substrate-based substrate (T6). The study on DFPI showed the shortest time but the highest yield, and the BE for cultivated mushrooms on substrate T6 was significantly different compared to other substrates. Similarly, *M. crassa* strain DOA-10 cultivated on spent mushroom substrate-based substrate (T6) showed the highest amounts of crude proteins and carbohydrate content at 30.89% and 74.75%, respectively, with large pileus (stipes, 37.20 mm in diameter, and 130.00 mm in length). Moreover, mushrooms cultivated on this substrate had higher macronutrient and micronutrient contents compared to other substrates, particularly phosphorus at 9100 mg per kg. Substrate formula T6, consisting of spent mushroom substrate from *P. eryngii* cultivation, fine rice bran, $MgSO_4 \cdot 7H_2O$, CaO, and fine corn seeds, at a proportion of 100:3:0.2:1:0.5 (w/w) and a C/N ratio of 490:1 was, therefore, the most suitable and has a high potential for *M. crassa* strain DOA-10 cultivation.

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