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Original article

Selection of *Macrocybe crassa* mushroom for commercial production

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ABSTRACT

Macrocybe crassa or *Tricholoma crassum* (Berk.) Sacc. (synonym) is a wild, edible mushroom in Thailand. This mushroom has a large fruiting body with meaty texture and a delicious taste. However, this mushroom is not commercially cultivated at a large scale. In this study, five strains of *M. crassa*—DOA, DOA-1, DOA-4, DOA-7 and DOA-10—were cultivated on a ratio of rubber tree sawdust to fine rice bran to magnesium sulfate ($MgSO_4.7H_2O$) to calcium oxide (CaO) of 100:3:0.2:1 (weight per weight). Their growth, productivity and characteristics of the fruiting body were investigated. The results revealed that DOA-10 gave the highest yield (215.10 g per 0.6 kg of substrate) with 59.26% biological efficiency. Observation showed that its pileus diameter, stalk diameter and stalk length were 6.78 cm, 2.75 cm and 13.54 cm, respectively. The dried samples of DOA-10 contained protein, carbohydrates, ash and fat at 13.71%, 68.08%, 12.06% and 2.49% respectively. Moreover, they contained high contents of both macronutrients and micronutrients: potassium (43,100 mg/kg), calcium (492.00 mg/kg), magnesium (1046.66 mg/kg), sodium (936.33 mg/kg), iron (283.21 mg/kg), zinc (53.79 mg/kg) and manganese (17.55 mg/kg). These results confirmed that DOA-10 is a promising strain for commercial cultivation.

Introduction

Mushroom cultivation is a major agro-industry and it has become popular as a source of healthy and functional food, and its production is also increasing (Aida et al., 2009). *Macrocybe crassa* or *Tricholoma crassum* (Berk.) Sacc. (its synonym) is a wild, edible mushroom and non-toxic (Teaumroong et al., 2002; Pradhan et al., 2010). It is a fungus belonging to the genus *Macrocybe*, in the family of *Tricholomataceae*, and the order of Agaricales (Chang and Hayes, 1978). It has a high production yield with outstanding, enormous, fleshy basidiomata which may exceed 80 kg fresh weight and it shows high biological efficiency, which is more than for many edible mushrooms (Corner, 1993). The large size and flavorful fruiting bodies of some *Macrocybe* species make them a valuable source of food, such as lower cholesterol and immuno-modulating effects in humans (Hoshi et al., 2005). Species of *Macrocybe* were formerly placed within *Tricholoma*, but segregated in the section *Leucorigida* morphologically on the presence of clamp connections, and ecologically on the absence of ectomycorrhizal associations (Pegler et al., 1998).

They produce a fleshy convex pileus and can be found in every region of Thailand and in neighboring countries (Payapanon and Srijumpa, 2008). Several related species such as *Tricholoma albobrunneum*, *Tricholoma flavovirens*, *Tricholoma paneolum*, *Tricholoma equestre*, *Tricholoma terreum* and *Tricholoma georgii*, are found in Europe and America while only a few edible species of this family, such as *Tricholoma matsutake* and *Macrocybe crassum*, are found in the Asian region—in Japan, Thailand and Sri-Lanka—(Huffman et al., 1989). In Thailand, *M. crassum* has various common names among local people such as *hed-tin-rad* (northeast), *hed-jan* (north), *hed-hua-sum* (south) and *hed yai* or *hed-tub-tao-khao* (central) (Petcharat, 1996). Natural *M. crassa* is generally rather expensive and rare, because it is usually found only once a year, particularly in the rainy season (Teaumroong et al., 2002). Moreover, varying the substrate media for mushroom cultivation resulted in different yields due to biological and chemical differences between the substrate media and their quality which may been due to the genotype of the mushroom strains (Ragunathan and Swaminathan, 2003). However, it can easily and successfully be cultivated on rubber tree sawdust (Payapanon and Srijumpa, 2008). However, still no work has been done to determine the suitability of this locally available lignocellulosic waste for cultivation or to determine the most cost compatible strains under environmental

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conditions. The main objectives of this research were to evaluate the yield parameters of different strains of *M. crassa* through traditional cultivation methods on rubber tree sawdust substrates and to determine their growth and the productivity and characteristics of the fruiting body to identify the best strain of *M. crassa* mushroom most suitable for commercial cultivation.

Materials and methods

Mycelial development of Macrocybe crassa mushroom

Mycelial discs (10 mm diameter) of five native strains (with substantially high production yield)—DOA, DOA-1 (collected from Bangkok province), DOA-4 (collected from Chiang Rai province), DOA-7 (collected from Chai Nat province) and DOA-10 (collected from Prachuap Khiri Khan province)—were obtained from the strain bank of the Department of Agriculture, Ministry of Agriculture and Cooperative, Bangkok, Thailand, and were carefully transferred to the upper surfaces of prepared sorghum grains. Inoculated grain bottles were tightly secured using moist cotton wool and covered with sterile aluminum foil. They were kept in dark, sterile cabinets at ambient room temperature for 14 d until they were fully colonized. The substrates employed in the production and in-depth mycelial development assessments of *Macrocybe* were prepared using rubber tree (*Hevea brasiliensis*) sawdust mixed with fine rice bran, magnesium sulfate ($MgSO_4.7H_2O$) and calcium oxide (CaO) in the ratio of 100:3:0.2:1 (weight per weight). Water was added to adjust the moisture content to 65%. Samples of the substrate mixture (each approximately 600 g) were used to fill 1 kg capacity autoclavable polypropylene bags to only three-quarters of their capacity and then steamed for 3 h in a large cast-iron steamer. When the temperature in the mushroom spawn cooled down to room temperature, 10–15 seeds of grains spawn were added to the bags and incubated at approximately 25 °C for spawn running until the bags were fully covered with mycelia. Data were collected on a daily basis for the mycelium running rate (MRR), days required to complete spawn running (DCS), primordial initiation (DFPI) and the number of fruiting bodies (NFB).

Production on sterile substrate

The upper end of the bags was cut off and the spawn mushrooms were neatly arranged in a basket and placed on shelves in the growing room. The surface of the compost was covered with a 2.5–5 cm thick layer of steamed casing soil, which was necessary to initiate fruiting. After casing, the relative humidity of the room was maintained between 85% and 90%. Each treatment was composed of 50 replicates of strains in each treatment. After fructification, one flush of mushrooms in each bag was harvested when the cap had started to fold. The cultivation substrates were maintained for 2 mth. The yields of mushrooms and their different quality parameters were recorded regularly. The biological efficiency percentage (BE) was calculated using the following formula: BE = (Fresh weight of fruit body/Dry weight of substrate) × 100 (Chang and Miles, 1989).

Morphological characterization procedures of the basidiomata

The macroscopic characteristics of the basidiomata collected from each isolate were recorded according to Largent (1977). Qualitative characters such as the color and shape of the cap, the color of the stipe and the color of the spore print of the mushroom were visually evaluated. For microscopic analysis, the dry material was rehydrated in 70% ethanol according to Largent et al. (1977). Free-hand transverse sections 0.1 mm thick were made from rehydrated basidiocarps using a sharp surgical blade. The sections were immersed in a diluted solution of methyl blue stain and covered with cover slips. The color and size of basidiospores were examined.

Nutritional analysis

The protein, fat, ash, crude fiber and total carbohydrate contents were determined using the procedures recommended by Association of Official Analytical Chemists (1995) and Manzi et al. (2001). The mineral contents of *M. crassa* mushrooms—potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), zinc (Zn), copper (Cu)—and the manganese (Mn), lead (Pb), cadmium (Cd) and arsenic (As) contents were determined using atomic absorption spectrophotometry (Willard et al., 1988). Total phosphorus was measured using the phosphorus-vanodomolybdate method (Munson and Nelson, 1990). The glucan concentration was measured using a Mushroom and Yeast Glucan Assay Kit (K-YBGL 09/2009; Megazyme International Ireland Ltd.; Bray, County Wicklow, Ireland).

Statistical analysis

A completely randomized design was used in all experiments. Data collected were subjected to ANOVA at the 5% level of significance using the SAS version 9.1 software package (SAS Institute, 2005).

Results and discussion

Mycelial development of Macrocybe crassa mushroom

The findings showed that mycelial colonies of all strains of *M. crassa* were white and cottony, with abundant aerial hyphae. The mycelia running through the substrate could be observed 3–4 d after spawn addition, and the isolates slowly progressed down the formulations completely ramifying them at different rates. The daily mycelial growth rates of different strains are presented in Table 1. The mushroom strain DOA-10 had the fastest complete spawn running of 35 d on the sawdust substrate whereas DOA, DOA-4 and DOA-7 took about 37 d for full saturation. Yadav et al. (2013) reported that the spawn running of *Tricholoma* sp. in polythene bags was completed in 30–35 d. The highest MRR was observed for DOA-10 (0.42 cm/d) which was significantly different from the MRR of DOA-7 (0.39 cm/d), DOA-1 (0.39 cm/d), DOA-4 (0.37 cm/d) and DOA (0.31 cm/d). The rate of mycelial growth may have been influenced by the quality of the inoculants and by the strain of mushroom. Stamets (2000) recommended using dense and thick mycelia for inoculation of grains for spawn formation.

Table 1
Growth and yield pattern of five strains of *Macrocybe crassa* cultivated on sawdust.

Parameter	Mushroom strain[a]				
	DOA	DOA-1	DOA-4	DOA-7	DOA-10
MRR[b] (cm/d)	0.31b	0.39a	0.37a	0.39a	0.42a
DCS[c] (d)	37.00b	36.00a	37.00b	37.00b	35.00a
DFPI[d] (d)	12.00c	14.05b	15.25a	13.05b	15.30a
NFB[e]	4.35b	4.75b	5.05a	5.25a	5.75a

[a] Means followed by the same letter in the same column are not significantly different at the 5% probability level according to Tukey tests.
[b] Mycelial running rate.
[c] Days required to complete spawn running.
[d] Days to first primordia initiation.
[e] Number of fruiting bodies.

After casing, the DFPI of DOA took the fewest days (12.00 d) which was significantly different from DOA-7 (13.05 d), DOA-1 (14.05 d), DOA-10 (15.30 d) and DOA-4 (15.25 d). In *Pleurotus* spp. the primordial initiation days generally occurred at 20–30 d (Ragunathan and Swaminathan, 2003; Yildiz and Karakaplan, 2003), The NFB refers to the total number of fruiting bodies and it is directly proportional to the yield. The highest NFB was observed for DOA-10 (5.75) which was significantly different from the NFB of DOA-7 (5.25), DOA-4 (5.05), DOA-1 (4.75) and DOA (4.35). Moreover, the NFB values obtained from *Macrocybe* mushroom in this experiment were higher than those using cultivation in wheat straw mixed with different supplements—termitorium soil (5%), cotton linter (7.5%), cotton linter (5%), maize meal (5% and 7.5%), wheat bran (7.5%) and cotton seed cake (7.5%), which were 4.75, 4.75, 4.25, 4.00, 4.00 and 2.75, respectively (Yadav et al., 2013).

Production on sterile substrate

Sawdust was considered as the most effective substrate for the production of the five different strains of *M. crassa*. The DFPI appeared at about 12–16 d after casing on the surface of spawn. The primordial morphology of the five strains of *M. crassa* is shown in Fig. 1. Mushroom yield and quality are summarized in Table 2. The biological yield was estimated on the basis of 600 g substrate. The highest yield was observed in DOA-10 (215.10 g) followed by DOA-7 (214.37 g), DOA-4 (211.46 g), DOA (189.00 g), and DOA-1 (124 g). Moreover, the fruiting bodies of DOA-10 had a large pileus diameter (6.78 cm) an average stalk diameter (2.75 cm) and stalk length (13.54 cm). The BE values of most strains were non significantly different, except for DOA-1. The BE from the five strains was higher than that reported by Yadav et al. (2013) (27.81%). The current results are in accordance with Peng et al. (2000) on the different biological efficiency values for different strains of king oyster mushroom on sawdust. These variations were mainly related to the spawn rate and fungal species used (Mane et al., 2007). The quality of *M. crassa* strains can be based on the length of stalk of DOA, DOA-1, DOA-4, DOA-7 and DOA-10 which on average were 11.40 cm, 12.10 cm, 8.45 cm, 12.75 and 13.54 cm, respectively. The stalk diameters on this substrate were significantly different ($p \leq 0.05$), ranging from 2.00 cm (DOA-4) to 2.80 cm (DOA). The pileus diameter of DOA (6.60 cm), DOA-1 (6.65 cm), DOA-7 (6.85 cm) and DOA-10 (6.78 cm) were not significantly different, except for DOA-4 (5.85).

Table 2
Evaluation of production and quality of five strains of *Macrocybe crassa* cultivated on sawdust.

Strain[a]	Fruiting bodies/bag (g)	DP[b] (cm)	LS[c] (cm)	DS[d] (cm)	BE[e] (%)
DOA	189.00a	6.60a	11.40d	2.80a	52.07a
DOA-1	124.00b	6.65a	12.10c	2.70ab	34.16b
DOA-4	211.46a	5.85b	8.45e	2.00c	58.25a
DOA-7	214.37a	6.85a	12.75b	2.83b	59.06a
DOA-10	215.10a	6.78a	13.54a	2.75ab	59.26a

[a] Means followed by the same letter in the same column are not significantly different at the 5% probability level according to Tukey tests.
[b] Diameter of pileus.
[c] Length of stalk.
[d] Diameter of stalk.
[e] Biological efficiency.

Morphological characterization procedures of the basidiomata

Five mushroom strains with *Macrocybe*-related morphology from cultivation in greenhouses were examined under a compound microscope for the shape, size and color of the mushroom cap, stipe color, color of spore prints and the color and size of spores. Two main cap shapes were observed—convex becoming slightly concave in shape (strains DOA, DOA-1, DOA-7 and DOA-10) and convex with a depressed center (DOA-4). Caps of the first group were between 6.15 cm and 11.70 cm in diameter with a pale cream surface and crowded lamellae. The stems were 9.01–13.67 cm in length with a cylindrical and swollen base, solid texture and an off-white surface

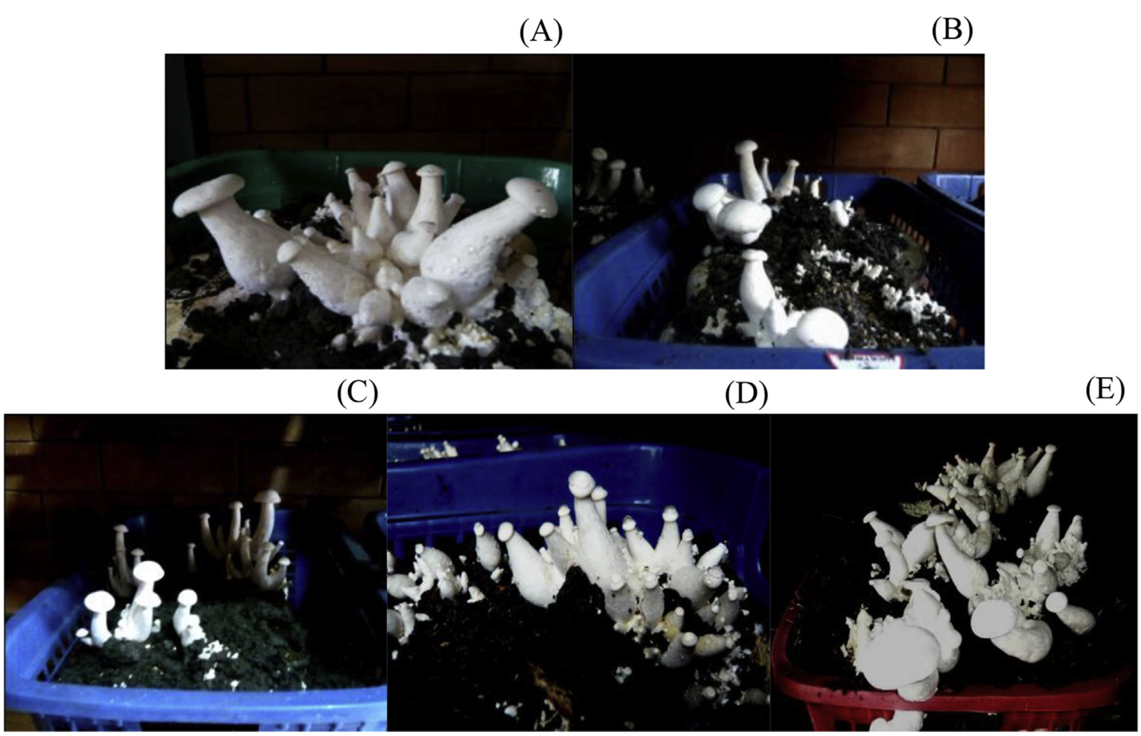

Fig. 1. Primordial morphology of five strains of *M. crassa* after casing for 16 d: (A) DOA; (B) DOA-1; (C) DOA-4; (D) DOA-7; (E) DOA-10.

similar to those reported by Payapanon and Srijumpa (2008). On the other hand, the pileus of the strain DOA-4 was between 5.81 cm and 8.95 cm in diameter with a pale cream surface, and a darker center. Lamellae were adnexed and crowded. Stipes were cylindrical, short with a length of 6.15–11.70 cm, solid texture and concolorous with the pileus surface. However, from the morphological characteristic studied, all strains were identified as *M. crassa*.

Nutritional analysis

The nutrient data analyzed for different strains of mushroom are summarized in Table 3. The nutritive values of dried *M. crassa* cultivated on sawdust substrate were evaluated. The result revealed that the moisture content of the dried mature fruiting bodies were 12.76%, 12.09%, 12.51%, 6.98% and 7.54% for DOA, DOA-1, DOA-4, DOA-7 and DOA-10, respectively. The total carbohydrates and protein contents estimated in dry powder of all *M. crassa* strains were between 53.79% and 68.08% and 11.85% and 16.10%, respectively. In this study, the maximum amount of protein was found in DOA-4 (26.10%) and the minimum in DOA-7 (11.85%). The strains of *M. crassa* contained crude protein levels of approximately 11.85–26.10% which are similar to *Lentinus edodes* and *Pleurotus ostreatus* (24.68% and 19.59%, respectively) according to Cuptapun et al. (2010). Yildiz et al. (2005) reported higher amounts of protein found in some *Tricholoma* species (29.7% for *Tricholoma auratum*, 31.1% for *Tricholoma nudum*, 46.5% for *T. terreum* and 50.5% for *Tricholoma ustale*). The crude protein contents in the *M. crassa* strains in the current study were higher than those in many other edible mushrooms such as *Astraeus hygrometricus* (8.48%), *Lentinus polychrous* Berk. (6.12%) and *Auricularia auricula* (7.94%). Therefore, *M. crassa* will be valuable for human consumption. Moreover, it contained a carbohydrates content of approximately 53.35–67.57% which is higher than in various mushrooms such as *A. auricula*, *Thaeogyroporus porentosus* (berk. ET. Broome), and *L. polychrous* Berk. The high levels of carbohydrate in *M. crassa* indicate that this mushroom is a good energy-food resource. The carbohydrate contents of *T. terreum*, *Tricholoma portentosum* (Diez and Alvarez, 2001), and *T. portentosum* (Barros et al., 2007) were reported as 31.1%, 34.6%, and 52.3% on a dry basis, respectively. On the other hand, *Tricholoma giganteum* contained a greater amount of carbohydrate (70.1%) than *T. terreum* and *T. portentosum* (Giri et al., 2013). The fat content on a dry weight basis of the *M. crassa* strains ranged from 1.30% to 2.49%. The highest crude fat was found in the strain DOA-10 while the strain DOA-1 had the lowest percentage of fat (1.30%). This variation could be attributed to the type of mushroom and interactions with the environment. The crude fiber and ash contents of this mushroom were higher than in other edible mushrooms. The highest ash content was obtained from DOA-10 and the lowest content from DOA-4 grown on sawdust ($p \leq 0.05$) as shown in Table 3. These values are close to the range for ash content in *Pleurotus* sp. (11.65–12.26%) according to Kurtzman (2005) and in *T. portentosum* (11.7% on a dry basis) according to Barros et al. (2007). The differences in ash content could have resulted from different mushroom species and substrates and could also be partly due to the water used in the cultivation (Kurtzman, 2005; Gregori et al., 2007; Ahmed et al., 2009).

In addition, as a result of the great attention now given to biologically active substances such as polysaccharides in the field of functional foods, the result showed that the strains of *M. crassa* contained high β-glucans contents which adds to their high value adding potential because β-glucans from several mushrooms has been demonstrated to have great immunomodulation, antioxidant, antiinflammatory and analgesic properties (Bobek and Galbavy, 2001; Smiderle et al., 2008). This mushroom also contains mineral levels (mainly potassium, phosphorous, calcium, magnesium and sodium) which are higher than those reported from several mushrooms varieties such as *L. edodes*, *Pleurotus abalonus*, *P. ostreatus*, *T. porentosus* (Sunanta et al., 1983), *Agaricus bisporus*, *Auricularia polytricha*, *Lentinus sajor* (Afiukwa et al., 2013) and various cowpea varieties (Aletor and Aladetimi, 1989). Minerals are required for metabolic reactions, sensory stimulation, rigid bone formation and regulation of water and salt balance among others, while in children, minerals play an important role by helping to promote growth and better development and assist with developing immunity to normal day-to-day substances (Rush, 2000; Wintergerst et al., 2007). According to the data obtained from this research, *M. crassa* possesses a high level of phosphorous and potassium which are associated with the formation of bones and teeth, and play a key role in skeletal and smooth muscle contraction (Hays and Swenson, 1985; Soetan et al., 2010). They could also be very useful for those who suffer from hypophosphatemia and hypokalemia, which is a condition of low levels of soluble phosphate and potassium levels in the blood (He and MacGregor, 2008). As shown in Table 4, the highest phosphorus content of the fruiting bodies (7000 mg/kg) was in DOA-4 and the lowest content (5300 mg/kg) was in DOA grown on sawdust substrate. The

Table 3
Nutritive values of five strains of *Macrocybe crassa* cultivated on sawdust (SD) (g/100 g dry weight basis).

Strains	Moisture[a]	Protein[a]	Fat[a]	Carbohydrates[a]	Ash[a]	Crude fiber[a]	β-glucan[a]
DOA	12.76a	12.79d	1.86b	62.99a	12.04a	2.36d	44.91b
DOA-1	12.09c	14.94b	1.30c	63.92a	11.98ab	2.56b	46.56a
DOA-4	12.51b	26.10a	2.04ab	53.79b	11.65b	2.69a	40.86c
DOA-7	6.98e	11.85e	2.16ab	58.26ab	12.13a	2.45c	43.62b
DOA-10	7.54d	13.71c	2.49a	68.08a	12.26a	2.38d	43.14b

[a] Means followed by the same letter in the same column are not significantly different at the 5% probability level according to Tukey tests.

Table 4
Macronutrients and micronutrients values of five strains of *Macrocybe crassa* cultivated on sawdust (SD) (mg/kg dry weight).

Strains	Macronutrients[a]					Micronutrients[a]				Heavy metals[a]		
	P	K	Ca	Mg	Na	Fe	Zn	Cu	Mn	Pb	Cd	As
DOA	5300d	35,300c	433.04a	651.15c	828.97c	213.44b	37.65ab	ND	13.07a	ND	ND	ND
DOA-1	6600a	43,000a	483.69a	959.70b	1152.24a	318.03a	57.23a	ND	12.01a	ND	ND	ND
DOA-4	7000a	43,100a	487.38a	922.89b	993.11b	321.01a	50.20ab	ND	18.87a	ND	ND	ND
DOA-7	5700c	40,900b	422.29a	717.75c	899.02bc	248.83ab	32.04b	ND	12.47a	ND	ND	ND
DOA-10	6200b	43,100a	492.63a	1046.66a	936.33b	283.21ab	53.79a	ND	17.55a	ND	ND	ND

[a] Means followed by the same letter in the same column are not significantly different at the 5% probability level according to Tukey tests.

differences in phosphorus content could be attributed to species and growing substrates (Kurtzman, 2005; Ahmed et al., 2009). The potassium content on a dry weight basis of the five strains of *M. crassa* was in the range 35,300–43,100 mg/kg. The highest iron content was found in DOA-10 and DOA-4 (43,100 mg/kg). In general, edible, wild mushroom species have an average potassium content of 34,350 mg/kg on a dry basis, making them an important and valuable potassium source for the human diet (Vetter, 1994). The highest zinc content of the fruiting bodies (321.01 mg/kg) was for DOA-4 and the lowest content (213.44 mg/kg) was for DOA grown on sawdust. According to Nikkarinen and Mertanen (2004), element concentration in the fruiting bodies of mushrooms is generally species-dependent and great differences exist in the uptake of individual trace elements. Therefore, the differences in the iron content of the mushrooms could be in part attributed to the *Macrocybe* strains. The dried samples of *Macrocybe* strains contained maximum levels of calcium and magnesium in DOA-10 at 492.63 mg/kg and 1046.66 mg/kg, respectively. On the other hand, the dried fruiting bodies of *T. terreum* contained 4526 mg/kg of calcium on a dry basis (Dursun et al., 2006). Moreover, no heavy metals (Pb, Cd, As) were detected in any of the mature fruiting bodies studied. The interaction effects of growing substrates and species were significantly different for zinc (Table 4), and the highest zinc content was recorded in DOA-1 (57.23 mg/kg) and the lowest was in DOA-7 (32.04 mg/kg). The results were in the range 46.70–76.70 mg/kg reported by Strmiskova et al. (1992) for oyster mushrooms.

M. crassa is an edible mushroom that has potential for commercial cultivation in Thailand. This study showed that the sawdust compound substrate developed can be used effectively for the cultivation of *M. crassa*. In addition, all the strains of *M. crassa*, (DOA, DOA-1, DOA-4, DOA-7 and DOA-10) produced high yields when cultivated on the sawdust. However, the *M. crassa* strain DOA-10 produced the highest yield (215.10 g per .0.6 kg of substrate) with 59.26% biological efficiency compared to other strains, though the mean values for DP and DS were lower than for strain DOA-7. The strain DOA-10 had a mean pileus diameter, diameter of stalk and stalk length of 6.78 cm, 2.75 cm and 13.54 cm, respectively. The total carbohydrates and protein contents estimated in dry powder of all *M. crassa* strains were between 53.79% and 68.08% and 11.85–16.10%, respectively. Moreover, the mushroom contained higher contents of both macronutrients and micronutrients. It contained high levels of protein, ash, fiber, β-glucans and minerals, making it a valuable source of food for new and alternative foods in conjunction with very low lipid contents. These results suggested that this mushroom could serve as a highly nutritious source including minerals for human consumption. Based on its mycelial development, production yield and the quality of the fruiting bodies, *M. crassa* strain DOA-10 is a suitable strain for commercial production.

Conflict of interest

The authors declare that there are no conflicts of interest.

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