Chiang Mai J. Sci. 2016; 43(5) : 959-971
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

First Successful Cultivation of the Edible Mushroom *Macrolepiota dolichaula* in Thailand

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Received: 3 February 2015
Accepted: 1 September 2015

ABSTRACT

The wild edible mushroom *Macrolepiota dolichaula* is widely consumed in China, India and northern Thailand as seasonal delicacies. Optimal cultural conditions, spawn production, cultivation parameters in compost and an outdoor method for inoculating spawn of *M. dolichaula* in orchard soils were investigated. Among the nine media tested, the optimum mycelial growth was observed in malt extract agar (MEA). This mushrrom can grow at a wide range of temperatures and pH values, although the optimum temperature and pH for the mycelial growth were 30°C and pH 7.0 respectively. Among the seven substrates tested, red sorghum was the best substrates for spawn production. Red sorghum spawn was fully colonized within 14 days following inoculation. The mushroom grew well in compost made of a pasteurized mixture of rice straw, rice bran, gypsum, calcium carbonate, urea and diammonium phosphate that was composted, using the outdoor method. After application of casing, basidiomes were obtained at 25°C and 92% relative humidity. Fructification took place at 41 days from spawning and yields from four flushes from four compost baskets were recorded. Basidiomes were also observed from the spawn inoculated sites in the orchard during the rainy season, ten months following spawn inoculation into the soil. This report provides valuable information concerning the possibility to cultivation *M. dolichaula* in compost and gardens or orchards soils in Thailand.

Keywords: domestication, basidiome production, mycelial condition, spawn inoculation

1. INTRODUCTION

Macrolepiota is a genus of white spored, gilled mushrooms of the family Agaricaceae (Agaricales, Basidiomycota) introduced by Singer [1] and has subsequently been studied by many researchers [2-5]. *Macrolepiota* species differ from other genera of Agaricaceae in three prominent features; a large and fleshy pileus, squamules scattered on the pileus giving various patterns, and a prominent annulus, which is often movable [3]. *Chlorophyllum molybdites* (G. Mey.) Massee. is a somewhat morphologically similar, but poisonous mushroom and is often mistaken for edible species within the genus *Macrolepiota* [6]. One

of the best ways to avoid confusing *C. molybdites* with *Macrolepiota* species is by making spore print. *Chlorophyllum molybdites* gives a green spore print, whereas *Macrolepiota* species produces a white spore print [7]. *Macrolepiota dolichaul*a (Berk. & Broome) Pegler & R.W. Rayner, *M. detersa* Z.W. Ge, Zhu L. Yang & Vellinga, *M. mastoidea* (Fr.) Singer, *M. procera* (Scop.) Singer, and *M. rachodes* (Vittad.) Singer are edible species of *Macrolepiota* which are highly appreciated for their nutritional and culinary values [5]. These species are consumed as wild delicacies in some African countries, China, Europe, India, North America and northern Thailand [3, 5, 8 -10].

Macrolepiota dolichaula contains biologically active molecules, such as water soluble polysaccharides (PS-I and PS-II) which were isolated from basidiocarps extracts of this mushroom. The branched glucan obtained from PS-1, showed significant splenocyte, thymocyte and macrophage activation [11]. *Macrolepiota dolichaula* also contains appreciable quantities of carotenoids, flavonoids, phenolic and the vitamins A, B_1, B_2 and C [12]. The presence of alkaloids in the extract, relatively high protein and carbohydrate contents in *M. dolichaula* is also an indication that this mushroom is of pharmacological importance and is good for human consumption [5].

Macrolepiota dolichaula is frequently collected from grasslands and disturbed areas such as roadsides in India [5, 12] and in northern Thailand during rainy season [N. Ratchaisin, personal. communication]. Unsustainable practices such as burning forests and over harvesting may result in over exploitation and loss of wild mushrooms [13]. The best way to preserve these high demand edible macrofungi is through understanding the ecology of mushroom, identifying cultivation parameters, domesticating and exploring the importance of each species through research.

Macrolepiota species are saprobic and hence have the potential to be cultivated [14, 15, 16]. They can be easily isolated in agar media and can decompose agricultural wastes such as rice straw, rice bran, sawdust, and rice hulls. *Macrolepiota procera* [17, 28] *M. zeyheri* (Fr.) Heinem [18] *M. bonaerensis* (Speg.) Singer [19] *M. gracilenta* (Krombh.) Wasser [14] and *M. detersa* [16] have been shown to be cultivable. Domestication of wild edible species such as *M. dolichaula* and other *Macrolepiota* species using agricultural waste residues or by inoculating spawn in soils of fruits orchards has a huge potential. This process will not only help in decreasing the burning of waste which causes significant environmental problems, but also can supply additional nutritional mushrooms in fresh markets and supermarkets. Although the cultivation of *M. gracilenta* has been achieved in composted substrates in Thailand, the yield is presently low and needs to be improved [14]. Like *M. gracilenta*, *M. dolichaula* also has a great potential for domestication, but its cultivation has never been attempted in India [5], nor in Thailand.

Most cultivable mushrooms have specific requirements for the mycelial growth as well as for the successful fructification. The production of mushrooms is effected by various factors such as types of compost, nutrients present in the growth medium, pH of the growth medium, and temperature, humidity, aeration and various biotic factors [20]. Hence there is always a need to study and characterize the optimal conditions of these parameters/factors for mushroom production.

Inoculating spawn of *M. dolichaula* in orchard soils for producing edible basidiomes and the stimuli triggering fructification also needs investigating. Since this mushroom is

saprobic and grows in grasslands on litter layers, creating ecological conditions suitable for the production of the mushroom in fruit orchards by utilizing agricultural and industrial wastes should be explored. The purpose of this study is to identify the optimal mycelial growth conditions, cultivation methods in compost and spawn inoculation in orchard soils for domestication of *M. dolichaula*.

2. MATERIALS AND METHODS

2.1 Strain Selection

Basidiomes of *M. dolichaula* were collected from two different grassland sites (near a pond and under a tree) during the rainy season, in Bandu, Chiang Rai Province, Thailand. Two strains were identified as *M. dolichaula*, based on the macroscopic and microscopic characteristics [3]. Molecular methods were used to confirm the identity of the strains. Herbarium specimens of the mushroom strains were made by drying the specimens in silica gel and deposited in Mae Fah Luang University herbarium (Herb. MFLU). Stock cultures were maintained on PDA slants at 4°C in Mae Fah Luang University culture collection (MFLUCC). The axenic cultures were deposited in Mae Fah Luang University culture collection (MFLUCC) as MFLUCC 13-0579 and MFLUCC14 0742.

Fresh tissues from two mature mushroom pilei resulted in two strains, MFLUCC 13-0579 and MFLUCC 14-0742 which were aseptically transferred to MEA with adjusted pH to 5.5 in Petri-dishes to serve as starter cultures for subsequent experiments. The cultures were incubated at 16, 18, 20, 25 and 30°C for eight days after which colony diameters were measured to determine the growth rates. Strain MFLUCC-13-0579 was found to grow faster than MFLUCC-14-0742 and was chosen as a representative strain for further studies on mycelial growth and cultivation methods. Molecular sequence analysis was performed to confirm the identity of *M. dolichaula* strain MFLUCC-13-0579. ITS sequence data was selected for this purpose as this region is often used as a DNA barcode for fungal identification [21]. For this analysis, the mushroom samples (both dried and mycelium) of MFLUCC 13-0579 was sent for the extraction of genomic DNA, amplification and sequencing to Biodiversity and Climate Research Centre (BiK-F) Laboratory, Main Frankfurt, Germany. The ITS sequence data was used to identify the similarity with other taxa using the NCBI nucleotide blast program and the accession number is submitted to GenBank.

2.2 Experiment 1. Effect of Different Media on Mycelial Growth

Nine different agar based media (Table 1) were tested in this study. They included potato dextrose agar (PDA), malt extract agar (MEA), corn meal agar (CMA), oat meal agar (OMA), sabouraud dextrose agar (SDA), rice bran extract agar (RBEA), rice straw extract agar (RSEA), compost extract agar (CEA) and coconut extract agar (COEA). All these media were adjusted to pH 5.5 using 1 N HCl or 1 N NaOH. All equipment and media used in this study were sterilized at 15 psi for 15 minutes at 121°C. All sterilized media were supplemented with amoxicillin (250mg/L) to prevent bacterial contaminations. A 6 mm diameter mycelial plug was cut from the edge of the eight day old colonies grown on MEA and aseptically transferred to the center of each medium plate. For each medium, there were three replicate cultures. The inoculated plates were then incubated at 30°C for eight days. Colony diameters were measured and recorded every two days during incubation until fungal colonies reached the edge of 9 cm Petri-dish. Subsequently, plates

with growing mycelium were melted in the microwave oven. In addition, the medium was melted and washed away with approximately 100°C water, to extract mushroom mycelia, which was dried at 45°C for 24 hours and weighed [22]. This was performed every two days after inoculation for up to eight days.

Table 1. Composition of various culture media.

Nutritional reagents	PDA	MEA	CMA	OMA	SDA	RBEA	RSEA	CEA	COEA
Agar	15	20	15	12.5	20	20	20	20	20
Corn meal			2						
Glucose						10	10	10	10
Peptone		5			10				
Malt extract		25							
Dextrose	20				40				
Oat meal				60					
Rice bran						100			
Rice straw							100		
Compost								100	
Coconut water									1000*
Potato	100								

Potato dextrose agar (PDA). Malt extract agar (MEA). Corn meal agar (CMA). Oat meal agar (OMA). Sabouraud dextrose agar (SDA). Rice bran extract agar (RBEA). Rice straw extract agar (RSEA). Compost extract agar (CEA). Coconut extract agar (COEA). *1000 ml of coconut water was used.

2.3 Experiment 2. Effect of Temperature on Mycelial Growth

Because the mycelium grew best in MEA, this medium was used for the evaluating the effect of temperature on mycelial. The fungal colonies were incubated on this medium for eight days at 16, 18, 25, 30 or 35°C in three replicates. The mycelial growth was assessed by measuring the colony diameter of the mycelium. The dry mass of mycelium was recorded by weighing the dried mycelium as described for Experiment 1.

2.4 Experiment 3. Effect of pH on Mycelial Growth

The most suitable medium (observed in Experiment 1) and the optimal temperature (observed in Experiment 2) were used in this experiment (Experiment 3) determining the optimal pH for mycelial growth. pH values were adjusted to 4.0, 5.5, 6.0, 7.0, 8.0 or 9.0 with 1N HCl or 1N NaOH using a digital pH meter before autoclaving. Plates containing these pH treatments were aseptically inoculated in three replicates as described for Experiment 1. All inoculated plates were incubated at 30°C. The mycelial growth was evaluated by colony diameter and mycelial dry mass.

2.5 Experiment 4. Suitability of Agricultural Waste and Cereal Grains for Spawn Production

Rice straw (RS), rice bran (RB), saw dust (SD), rice hull (RH) (agricultural substrates), and soybean (SB) and sorghum (S) (grains)

were tested for their suitability for spawn production. Rice straw was chopped into 2-3 cm pieces, soaked overnight in water and boiled for 5-10 minutes. All the substrates did not need to be chopped, but were also soaked overnight in water and boiled for 5-10 minutes. The excess water was removed by drying the substrate, until the moisture content was around 60 %. Each substrate was transferred into glass tubes (200×30 mm), in which each substrate was uniformly filled to 100 ml volume before autoclaving as described by Philippoussis *et al.* [23]. Two mycelial plugs, approximately 6 mm in diameter were aseptically placed on the surface of each cooled substrate and incubated at 30°C for 14 days in three replicates. Uninoculated tubes with each medium were used as a control. Linear growth and colonization rate in glass tubes was identified by measuring the visible progression of mycelia into the substrate every 2 days for 14 days.

2.6 Observations on Mycelium Growth and Fructification of *M. dolichaula* on Outdoor Turned Compost

The compost consisting of sterilized mixtures of composted rice straw, rice bran, gypsum, calcium carbonate, urea and diammonium phosphate (DAP) were mixed at a percentage of 86.20%, 8.62%, 1.72%, 1.72%, 1.72% respectively. It was manually prepared by the outdoor turned composting system, and used for obtaining basidiomes of *M. dolichaula*. After 24 days of fermentation of substrates, the first phase compost was obtained, which was subsequently pasteurized at 60°C for six hours. Ten baskets containing 5 kg of pasteurized compost were prepared for spawn inoculation. For each basket 100 g of spawn of *M. dolichaula* was manually transferred to the 5 kg of pasteurized compost pH 7.5 and 60 % moisture content on sterilized plastic sheet and thoroughly mixed. The homogenized mixture was wrapped with the clean transparent plastic sheet to maintain the moisture content and to avoid contamination. The inoculated and the non-inoculated substrate in the basket were then incubated at 25-30°C and 70-80 % relative humidity for 41 days in a controlled mushroom growing room.

At 41 days when ca. 80°90% of the compost in the inoculated baskets was covered by mycelia, a 2.5 cm casing consisting of soil and humus having a pH of 7.2 was applied evenly over the mycelia, followed by watering of the casing layer to saturation (avoiding over watering) with a sprayer. After the mycelia fully covered the casing layer, conditions for fructification were adjusted, the relative humidity of the growing room was increased manually to 85-90% to stimulate fruiting by spraying water over the mycelium and on floor once a day. The humidity and temperature inside the growing house were monitored by using a hygrometer and a thermometer. Ventilation was increased to provide more oxygen and gases were extracted with exhaust fans. Time was recorded in days for the completion of growth of mycelium on substrates, appearance of primordia and maturation of basidiomes. Basidiomes were harvested every three days, until two weeks. All flushes and all stages of fruiting bodies harvested were measured as a total yield in this study. The mushroom yield in each basket was evaluated by using the percentage of biological efficiency (BE) [24] as given in the equation Biological efficiency, % = FWM/DWC×100; where FWM and DWM corresponds to fresh weight of mushroom and dry weight of compost respectively. To check the postharvest quality of this fungus, semi-matured and matured basidiomes were stored at room temperature. Some

basidiomes of these two stages were wrapped in tissue papers, placed in plastic bag separately and stored in refrigerator.

2.7 Observations on Intentional Cultivation of *M. dolichaula* in Fruit Orchard Soils

The observational experiment was conducted to assess the potential of intentionally cultivating *M. dolichaula* in fruit orchards. The sites chosen for inoculation were under the fruit trees such as guava (*Psidium guajava*), lychee (*Litchi chinensis*) and banana (*Musa* sp.) plants. There were two inoculation times tested in order to identify the most favourable time for the spawn inoculation. The first involved 60 different sites that were inoculated during the end of the rainy season, after *M. dolichaula* had stopped fructifying in the natural habitats. A further 60 sites were inoculated during the dry winter season, after six month from the first inoculation. Inoculated sites were proximate to the previously inoculated sites. The spawn used for inoculation in all the sites was made on the same day for each of the inoculation times. The soil pH was measured using a digital pH meter and recorded on the day of inoculation by taking samples of soils from the inoculated sites. Soils under the fruit trees had an average pH of 7.5. Pasteurized compost obtained from mushroom compost and rice straw collected from surrounding rice growing areas was frequently placed over the inoculated areas. All inoculated sites were marked and photographed for easy recognition.

2.8 Inoculation Procedures

Basic protocols for inoculating spawn in different areas around an orchard were adapted from Paul [25]. Fifty grams of sorghum spawn were directly inoculated into a pit of approximately 45 cm wide and 15 cm deep. One meter was maintained between each inoculation. Inoculated sites were marked and checked after two months. To improve moisture contents and fertility of the soils for the mycelial growth, sites were further covered with soil and rice straw collected from the surrounding areas.

2.9 Data Analysis

Where possible the data were analyzed statistically in terms of variance and mean showing statistical significance using Duncan's multiple range tests by SPSS-16 program.

3. RESULTS

3.1 Strain Selection

The mycelia of both *M. dolichaula* strain MFLUCC 13-0579 and MFLUCC 14-0742 grew well between 25°C-30°C. *Macrolepiota dolichaula* strain MFLUCC 13-0579 collected from grasslands near a pond grew faster than strain MFLUCC14 0742 which had been collected from under the shade of a large tree (Table 2). The sequenced data was obtained for the fastest growing strain MFLUCC 13-0579. The NCBI blast using nucleotide sequences showed *M. dolichaula* MFLUCC 13-0579 having 100% similarity scores with *M. dolichaula* and is a new record of this species for Thailand. The GenBank accession number is KP859148. This strain was used for further studies.

3.2 Effect of Culture Media on Mycelial Growth

Of nine different media tested the best colony diameter as well as the mycelial dry mass was observed on MEA (7.83 cm, 0.41g) after eight days of mycelial incubation at 30°C (Table 3). The medium MEA was followed by CEA (7.83 cm. 0.33g), PDA (9.00 cm, 0.08 g), OMA (6.77 cm, 0.29 g) and SDA (5.87cm, 0.43 g).

Table 2. Growth rate (cm/8 days) of *M. dolichaula* strains MFLUCC-13-0579 and MFLUCC-14-0742 in MEA at different temperature after eight days.

Temperature (°C)	Colony diameter		Growth rate	
	MFLUCC-13-0579	MFLUCC-14-0742	MFLUCC-13-0579	MFLUCC-14-0742
16	2.73±0.200^{c}	2.33±0.06^{e}	2.73	2.33
18	4.10±0.100^{bc}	3.63±0.15^{d}	4.10	3.63
20	5.83±2.25^{ab}	4.53±0.06^{c}	5.83	4.53
25	6.40±0.10^{a}	5.60±0.10^{b}	6.40	5.60
30	7.53±0.06^{a}	6.30±0.10^{a}	7.53	6.30

Within a column, values followed by the same letter are not significantly different at $P = 0.05$ by Duncan's test.

Table 2. Mycelial diameter and dry mass of *M. dolichaula* grown on media at 30°C after eight days.

Media	Colony diameter (cm)	Mycelial dry mass (g)
PDA	9.00 ± 0 00^{a}	0.08 ± 0.01^{c}
MEA	7.83 ± 0.47^{b}	0.41 ± 0.01^{a}
CEA	7.83 ± 0.47^{b}	0.33 ± 0.02^{b}
OMA	6.77 ± 0.76^{cd}	0.29 ± 0.07^{b}
SDA	5.87 ± 1.10^{cd}	0.43 ± 0.00^{a}
CMA	5.87 ± 0.31^{cd}	0.28 ± 0.06^{b}
SEA	5.73 ± 0.35^{d}	0.07 ± 0.01^{c}
COEA	5.57 ± 0.15^{d}	0.06 ± 0.01^{c}
RBEA	5.53 ± 0.25^{d}	0.06 ± 0.00^{c}

Within a column, values with the same letter are not significantly different ($p < 0.05$) by the Duncan's test.*The full form of media are mentioned in Table 1.

Table 3. Mycelial diameter and dry mass of *M. dolichaula* grown on MEA at different temperature after eight days.

Temperature (°C)	Colony diameter (cm)	Mycelial dry mass (g)
16	2.54 ± 0.02^{d}	0.21 ± 0.016^{b}
18	3.14 ± 0.12^{c}	0.23 ± 0.011^{b}
25	6.28 ± 0.03^{b}	0.28 ± 00.02^{a}
30	7.60 ± 0.10^{b}	0.31 ± 0.013^{a}
35	0.00 ± 0.00^{e}	0.00 ± 0.000^{c}

Within a column, values with the same letter are not significantly different ($p < 0.05$) by the Duncan's test.

3.3 Effect of Temperature on the Mycelial Growth

The most unfavorable temperature was 35°C since no mycelium grew in this temperature treatment (Table 4). The mycelium grew best at 30°C, but the growth was not significantly different from that at 25°C. The growth of mycelium accelerated as the incubation temperature was increased from 18°C to 30°C (Figure1)

Table 4. Mycelial diameter and dry mass of *M. dolichaula* grown on MEA at different pH values at 30°C after eight days.

pH	Colony diameter (cm)	Mycelial dry mass (g)
4.0	5.62 ± 0.06[d]	0.10 ± 0.09[c]
5.5	6.37 ± 0.08[b]	0.20 ± 0.02[b]
6.0	6.43 ± 0.06[b]	0.23 ± 0.02[b]
7.0	8.52 ± 0.03[a]	0.30 ± 0.02[a]
8.0	6.06 ± 0.14[c]	0.25 ± 0.01[ab]
9.0	4.23 ± 0.23[e]	0.21 ± 0.02[b]

Within a column, values with the same letter are not significantly different ($p < 0.05$) by the Duncan's test.

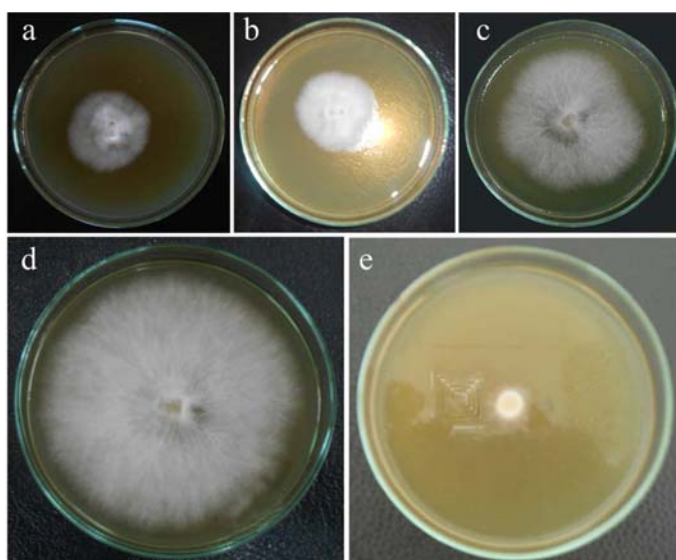

Figure 1. Colony diameter of *M. dolichaula* MFLUCC-13-0579 at different temperatures. a. 16°C. b. 18°C. c. 25°C. d. 30°C. e. 35°C.

3.4 Effect of pH on Mycelial Growth

Among the various pH values tested in this study (pH 4.0, 5.5, 6.0, 7.0, 8.0, 9.0) in MEA at 30°C, the optimum pH for the mycelial growth was observed at pH 7.0 (Table 4).

3.5 Effect of Different Agricultural Wastes and Cereal Grains for Spawn Production

The optimal mycelial growth of *M. dolichaula* was observed in sorghum at 30°C after two weeks following mycelial inoculation. Statistical analysis indicated no significant difference in mycelial linear growth between sorghum and soybean grains (Table 5).

Table 5. Mycelial linear growth of *M. dolichaula* MFLUCC-05-13-0579 in agricultural waste and cereal grains at 30°C.

Spawning substrate	Mycelial linear growth (cm)
Red sorghum	11.6±0.07[a]
Soybean	10.9±0.00[a]
Rice straw	5.6±0.06[bc]
Rice bran	5.4±0.00[c]
Rice hull	5.0±0.02[c]
Sawdust	4.2±0.00[d]
Groundnut hull	4.0±0.07[d]

Within a column, values with the same letter are not significantly different ($p < 0.05$) by the Duncan's test.

3.6 Fructification on Composted Substrate

After 21 days following spawn inoculation approximately 90% of compost was covered by mycelia. After ten days following casing, viable mycelium was observed only in four baskets, whilst six baskets were contaminated by fungicola and were discarded. Primordia were observed in the remaining four baskets at 25°C after 16 days of casing. Matured and semi-matured fruiting bodies in four baskets appeared after 3-4 days after primordia formation at 25°C and 92 % relative humidity (Figure 1). The average weight of mushrooms harvested in four flushes per basket was 239.75g and this represented a biological efficiency of 7.99 %. Some semi-matured fruiting bodies fell over due to their large cap and relatively long stipe. Basidiomes were harvested every three days, until two weeks. As time passed contamination set in. The contamination occurred first in the casing layer and then affected the fruiting bodies after the fourth flushes. Approximately 200 g of contaminated fruiting bodies from the four baskets was discarded. The causative organisms of the contamination were identified as *Trichothecium roseum* and *Cladobotryum protrusion*. Their identity was confirmed using molecular DNA analysis [J. Sun personal. Communication].

Harvested fruiting bodies remained fresh for a day in normal conditions without any treatment. Fruiting bodies that were wrapped in tissue papers and sealed in plastic bag and stored in a refrigerator remained fresh up to three days. Thereafter, fully matured cap started to become brown after two days following picking.

3.7 Fructification of *M. dolichaula* in Orchards

The spawn inoculated soils produced mushrooms approximately one month earlier than natural populations at ten months after the first spawn inoculation. Fruiting was most abundant at the time when there was persistent rain over a prolonged time (e.g. three days), during which the relative humidity of air was in between 80% - 85%. During this period of high humidity and temperature of 27°C, many basidiomes were obtained under the lychee, guava and banana plants (Figure 2). However, the basidiomes were most abundant under the banana plants. A total of 3.32 kg of mushroom was harvested during the rainy season from 30 of the first 60 sites. Mushrooms were harvested from the same sites over a month during the day when there was heavy rainfall and the average air humidity was at a range of 80%-85% and temperature 25-30°C. After a month, rainfall decreased and there was a drop in relative humidity and fructification gradually stopped. The basidiomes were free of diseases. The average pH of soils under the fruit trees where fructification occurred was 7-7.5 and there was no difference in pH during the inoculation and fructification period. In addition the sixty sites, which were the second set of inoculations during the winter, did not result in any basidiomes.

4. DISCUSSION

This is the first attempt that we are aware of to cultivate the edible *M. dolichaula* in Thailand, both on compost and in orchard soils. The mycelium of the *M. dolichaula* grows in all agar based culture media. The most suitable medium for the mycelial growth was MEA followed by CEA. The mycelium grew best at 25°C-30°C. The growth sharply decreased at temperatures below 18 C and no growth occurred at 35°C. This finding is in agreement with Shim *et al.* [28], in which mycelial growth of *M. procera* gradually increased with the increase in temperature to 30°C, while growth was greatly suppressed at 35°C. The optimal

temperature indicated that *M. dolichaula* grows better in summer and autumn season in subtropical and tropical regions and is a potential mushroom to develop in poor and developing countries in Asia. Fungi grow differently at different pH levels. Some tropical mushrooms produce fruiting bodies in a neutral or slightly acidic pH of 7.0 or 6.0 [29]. Optimal growth of *M. dolichaula* in MEA occurred at pH 7.0, which concurs with the finding of Rizal *et al.* [16] for *M. detersa*. Kumla *et al.* [30] also confirmed pH 7.0 as suitable for the optimal growth of *Pleurotus giganteus*.

Figure 2. Basidiomes of *M. dolichaula* grown on compost. A. fruiting bodies at different stages of development. B. pileus with annulus. C. squamules on pileus. D. and E. bud stages of fruiting bodies. F. mature fruiting bodies. Scale bars: A, B = 5 cm. C, D, E, F = 5 cm.

The mycelia of *M. dolichaula* colonized all the agricultural wastes and cereal grains tested in spawn production. However the growth rate differed among the materials used for spawn production. The greatest mycelial production occurred in red sorghum followed by soybean grains. Sorghum and soybean have larger grains which may attribute to a greater food reservoir [31] and better surface aeration within the grains, as a result there may be improved respiration of the mycelium. Due to the low cost and readily availability of sorghum, it is generally used for spawn production in Thailand and other sub-tropical and temperate countries [32]. Senthilnambi *et al.* [33] observed that sorghum used as substitute for the bulk substrate was suitable for early spawn run and higher yield of *Calocybe indica*. Kumla *et al.* [34] identified that a mixture of sorghum grain with other bulk substrates, like sawdust infused with fungal host solution helped in producing the highest yield of *Phlebopus portentosus*.

The fructification of this mushroom occurred within 41 days after inoculation of spawn in compost at 25°C at 92 % relative humidity. Among the ten baskets used, only four baskets contained viable mycelia and produced fruiting bodies with a yield of 230-250g per basket, with the biological efficiency ranging from 7.66-8.33%. The yield and biological efficiency were relatively low. This was a result of contamination of mycelia by *Trichothecium roseum* and *Cladobotryum protrusion* and diseases on basidiomes after the fourth flush. Two organisms have previously been implicated by Poldmaa [35], Dong and Bian [36] as casual agents of diseases on cultivable such as *Coprinus comatus* and other mushrooms.

The inoculation of spawn of *M. dolichaula* in an orchard soils led to successful production of mushroom during the rainy season under the banana plants, guava and lychee trees. A total yield of 3.32 kg was obtained from the sites inoculated during the previous rainy season. The fructification in spawn inoculated sites occurred earlier than in the natural habitat. The mixture of compost and soil covered with rice straws under the occasionally

watered fruit trees provided suitable condition for mycelial growth. The provision of litter might have provided the fungal mycelium with the nutritional resources that promoted earlier fructifications. Fruiting bodies obtained under the fruit trees were healthy and contamination was not observed. Mycelium growth and fructification in the natural settings are naturally controlled by the weather patterns and the competitors are not concentrated in a tight space to cause a major contamination problem [37]. The sites which were inoculated during the dry winter season did not result in any basidiomes. This might be because the mushroom mycelia may need damp soil and warm conditions.

Maximum basidiomes were produced under banana trees. Humidity (85%), temperature (25-30°C) and various biotic and abiotic factors which were naturally present in the soil may have played important roles to trigger the fructification, as there was no significant difference found in the pH of the soil, during inoculation and fructification. The observation from the present study indicates that *M. dolichaula* is indeed a saprobe which does not require host plants for survival and fruictification. Cultivation in orchard soils is economical, and requires little effort. Thus the cultivation of this mushroom can supplement diets of farmers.

5. Conclusions

The optimal conditions for mycelial growth of *M. dolichaula* was found on (MEA), with pH 7 at 30°C. Mycelia utilized all the agricultural wastes used in this study, however the optimal mycelial growth occurred in red sorghum.

In our experiment most fruiting bodies fell over due to their long stipe supporting a heavy cap and disease was seen after applying casing. The growing strategies need adjusting to reduce these problems and an increase in aeration resulting in reduced carbon dioxide might affect the stipe length. Pasteurized compost, a sterilized casing layer and maintaining hygiene throughout the cultivation period may solve contamination and improve yield. Inoculating spawns during rainy season in fruits orchard, applying litter (e.g. rice straw) and watering the inoculated sites during dry days may enhance mycelial growth and faster establishment thereby, shorten the cropping time in soils.

Our studies show that *M. dolichaula* is cultivable indoor, outdoor and provides basic information to maintain the species, explore optimal cultivation conditions and make production possible throughout the year. In further studies, we will attempt to improve the mycelium growth and spawn preparation in non-sterile culture substrates. Initiatives will be taken to compare the yield with other cultivated *Macrolepiota* species. We will also identify innovative strategies to prevent contamination problems, and attempt to solve contamination using plant extracts. Aeration, humidity, nutrients and light plays vital roles in triggering fructifications in controlled cultivation methods, studies related with these factors will be carried out in order to improve yield and biological efficiency for commercial production of this mushroom in Thailand. For growing this mushroom in soil, innovative methods such as growing *M. dolichaula* along with other vegetables in fertile garden soils will be explored. Its commercial production not only promises a strong food alternative for mushroom lovers, but also promises to provide equal potential culinary options with edible species of *Agaricus*, *Lentinus*, *Pleurotus*, *Volvariella*, and *Letinula*.

Acknowledgements

We are grateful to Nonglak Ratchaisin, Anan Thawthong and Dhanushka Nadheeshan for their valuable help in collecting and

cultivation. This study was financially supported by Mae Fah Luang University, Royal Civil Service Commission of Bhutan and Thailand Research Fund grant - Taxonomy, Phylogeny and biochemistry of Thai Basidiomycetes (BRG 5580009).

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