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Cultivation and determination of nutrient contents of an edible wild Thai *Hymenopellis*

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Abstract

Hymenopellis species are taxonomically well-studied and widely distributed in tropical and temperate regions. It is well-documented in eastern and north America and can also be found in several Asian countries, including Thailand. In this study, we collected a wild *Hymenopellis* specimen in northern Thailand. The morphological and phylogenetic analyses inferred from a combination of the internal transcribed spacers (ITS) and the large subunit (nrLSU) of ribosomal DNA confirmed the taxonomic placement of the collected specimen to the genus *Hymenopellis*. Limited studies have documented the cultivation of *Hymenopellis* species. So far, only China commercially cultivates this mushroom. Pure mycelium was isolated from the internal tissues on malt extract agar (MEA). Sorghum grain was used in spawn preparation. Spawns were inoculated in bags of four different substrate formulas, and sawdust was used as the main substrate and other additives. The cultivation process was conducted for four months. *Hymenopellis* sp. (MFLU 19-1658) has an average yield ranging from 10.8±17.9 to 115.5±73.5 grams of basidiomata per substrate formula with an average biological efficiency ranging from 3.0±5.0 to 32.1±20.4% with the highest values exhibited by (A) sawdust (98%) + CaO (1%) + gypsum (1%), followed by (B) sawdust (30%) + corncobs (60%) + rice bran (8%) + CaO (1%) + gypsum (1%). The nutrient contents of the cultivated basidiomata were also determined in this study. The results indicated that the obtained basidiomata contained carbohydrate, crude fat, crude fiber, and protein at 49.2, 8.0, 16.5, and 17.8%, respectively. This study is the first to document the nutrient contents of the genus *Hymenopellis*.

Keywords – basidiomata production – biological efficiency – nutrition – phylogeny – yield

Introduction

Wild edible mushrooms are traditionally used by many countries worldwide as food and medicine (Li et al. 2021, Mortimer et al. 2021). They are appreciated not only for their flavour but also for their nutritional and medicinal properties (Niego et al. 2021a). *Hymenopellis* is one of the largest genera in the oudemasielloid/xeruloid taxa of the family Physalacriaceae (Petersen & Hughes 2010), with *H. radicata* R.H. Petersen as the type species. *Hymenopellis radicata* was first described in 1786 as *Agaricus radicatu*s (Relhan 1786). Currently, *Hymenopellis* comprises 58

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records belonging to 41 species in the Index Fungorum (http://www.indexfungorum.org/). Relevant characteristics for *Hymenopellis* species delimitation include moist to glutinous pileus with pseudorrhiza and basidiomata ranging from small to large (Petersen & Hughes 2010). This genus is widely distributed in tropical and temperate regions (He et al. 2019). This genus has a well-documented distribution in eastern and north America, with 13 species recorded in Asia (Petersen & Hughes 2010, Niego et al. 2021b), of which only *H. raphanipes* (as *H. chiangmaiae*) (Petersen & Nagasawa 2006, Yang et al. 2009, Petersen & Hughes 2010) and *H. radicata* have been found in Thailand (Chandrasrikul et al. 2011). *Hymenopellis* species, especially *H. raphanipes*, are edible mushrooms (Hao et al. 2016). *Hymenopellis* can produce large basidiomata, which could be an ideal food source, but the nutrient content of this mushroom is not yet established. *Hymenopellis* has also been recorded as a source of bioactive compounds, such as mucidin (Gao et al. 2017), oudenone (Liu et al. 2013), oudemansin (Anke et al. 1990), and polysaccharides (Rosa et al. 2003, 2005, Liu et al. 2021). Subsequently, bioactive compounds extracted from this mushroom demonstrated different bioactivities, such as antifungal (Anke et al. 1990, Weber et al. 1990), antioxidative, anti-inflammatory, and lung-protective effects (Gao et al. 2017). Although *Hymenopellis* has been recognised for its bioactivities, no further application has been pursued for this genus.

There are limited studies on the cultivation of *Hymenopellis* species, but some successful attempts are documented for *H. radicata* (as *Oudemansiella radicata*) growing on sawdust substrate (Gao 2000, Kim et al. 2005). In China, *H. raphanipes* is the sole commercially cultivated species (Hao et al. 2016), but this mushroom has not been reported for cultivation in European or American countries. In Thailand, no strain of *Hymenopellis* has been reported to be cultivated. In this study, we cultivated the wild Thai strain of *Hymenopellis* (MFLU19-1658) by using different substrates formula. The combinations of sawdust were determined as the main substrate and additives to get the maximum yield and highest biological efficiency. The nutrient contents of this strain were also determined. We hope to grow this strain; therefore, it can be introduced into Thailand's agricultural sector as a new native mushroom source.

Materials & Methods

Mushroom collection

The fresh basidiomata of *Hymenopellis* were collected from Mae Fah Luang District, Chiang Rai Province, Thailand. The fresh basidiomata were photographed in the field, while details of habitat, habit, macro-morphological features (e.g., the colour of the basidiomata, lamellae, and stipe), and location were recorded. Colour, name, and codes were given according to the methods employed by Kornerup & Wanscher (1978). Then, basidiomata were carefully collected and kept in aluminium foil, labeled, and brought to the laboratory for micro-morphological and further studies. The specimens were photographed, measured, and described in the laboratory. The strain was isolated using the tissue culture technique on malt extract agar (MEA) and incubated at 25°C for seven days. The mycelial culture was subcultured on MEA for later use. Specimens were dried at 45 to 50°C until they were completely dried. The dried specimen was deposited at the Mae Fah Luang University Herbarium (MFLU).

Species confirmation based on morphology and phylogeny

Macro-morphological characteristics of the *Hymenopellis* specimen (i.e., pileus, lamellae, and stipe) were described based on the collected specimen and cultivated basidiomata. The preparation of the *Hymenopellis* sample to describe micro-morphological characteristics was based on the laboratory techniques described by Clémençon (2009). Important features were examined using a Motic SMZ-171 dissecting microscope, and specific features were noted based on Vellinga & Noordeloos (2001). Microscopic characteristics were observed using a Nikon Eclipse Ni, DS-Ri2 compound microscope with dried samples rehydrated and mounted in 5% KOH to retain the original colour. Specific characters, i.e., basidiospores, basidia, cystidia, and pellis, were described

following the glossary of Vellinga & Noordeloos (2001). The dimension of 60 basidiospores and 30 for each other characters were measured. Measurements are provided as (a)b-c-d(e), where 'a' and 'e' are the extreme values, 'b-d' are the 5th and 95th percentiles, the range containing 90% of all values, and 'c' is the average. Q denotes the length/width ratio, and Q* is the average value (Niego et al. 2021c).

DNA was isolated from the dried internal tissue of the specimen using the Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology, Hangzhou, China), following the manufacturer's protocol. The ITS and nrLSU region were amplified by polymerase chain reaction (PCR) using ITS1/ITS4 and LR0R, LR5 primers, respectively (Vilgalys & Hester 1990, White et al. 1990). Agarose Gel Electrophoresis was used to check for the quality of DNA. PCR products were purified and sequenced in both directions, using the PCR primers, by Sangon Biological Engineering Technology and Services (Shanghai, China). The quality of each generated sequence was checked using Bioedit Sequence Alignment Editor v. 7.0.9.0 (Hall 1999) and assembled using SEQMan Pro software (DNA Star, Madison, USA).

The sequences were aligned using MAFFT v. 7.450 (Katoh et al. 2019) on the server accessed at http://mafft.cbrc.jp/alignment/server/. TrimAl was used to eliminate ambiguously aligned positions from the alignments in strict mode (Capella-Gutierrez et al. 2009). Reconstruction of the phylogenetic tree was performed with maximum likelihood (ML) and MrBayes with ITS and nrLSU lengths of 672 and 864 bp, respectively. ML analysis was performed through RAxML-HPC2 v. 8.2.10 (Stamatakis 2014) on the web server CIPRES Science Gateway V. 3.3 (Miller et al. 2010) with the GTRGAMMA model. The branch support was estimated with 1,000 rapid bootstrap replicates. Before running MrBayes, the best-fit nucleotide substitution model for ITS and nrLSU was selected using jModeltest v. 2.1.10 (Darriba et al. 2012) based on the Akaike Information Criterion (AIC). For the two gene regions, the HKY+G model was selected as the best model. For BI analysis, Markov Chain Monte Carlo (MCMC) sampling was performed using MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Two runs of five simultaneous MCMC chains were run for 5,000,000 generations, with trees and parameters sampled every 1,000th generation, for a total of 10,000 samples. The first 25% of samples were discarded as the burn-in phase. The remaining samples were used to calculate posterior probabilities (PP) and the majority rule consensus tree. The trees were viewed using FigTree v1.4.2 (Rambaut 2012).

Spawn production

Hymenopellis spawn was prepared using sorghum grain (*Sorghum bicolor*) (Thongklang & Luangharn 2016). Sorghum grains were soaked overnight in water and then boiled for 15 minutes. The excess water was drained, and the cooked grains were air-dried for 1 hour. Two hundred grams of grains were then transferred to each 300 ml bottle container and autoclaved at 121°C for 15 minutes. All bottles were left to cool down at room temperature. Each bottle was inoculated with three mycelial plugs (0.5 cm in diam.) of *Hymenopellis* sp. grown on MEA. The inoculated bottles were incubated in the dark at 25ºC for 21 days until the mycelia fully colonized on the surface of the grains.

Substrate preparation

Cultivation substrates were prepared in four different formulas, of which sawdust was used as the main substrate (Table 1). The moisture content in all substrate formulas was manually adjusted at 70% relative humidity by adding water. Ten bags were prepared for each substrate formula. Each bag was filled with 600 g of mixed substrate. All substrate bags were autoclaved for 30 minutes at 121°C and cooled at room temperature. Each bag was then inoculated with spawn (20 g) and incubated in the darkroom at 25ºC for 30 days until the mycelia thoroughly colonized the bags.

Cultivation

The cultivation experiment was conducted at the fully covered mushroom house at Mae Fah Luang University, Chiang Rai, Thailand. Two fully colonized bags from each substrate combination were arranged in a tray (30 × 20 × 15 cm), giving equal spacing between them. Each tray was covered with dryland soil at 60–100 cm depth. Five replicates were prepared for each sawdust combination. Each tray was randomly arranged and labeled. Automatic sprinklers were set to water the trays for 15 minutes daily to maintain the moisture level at 70%. Environmental temperatures and humidity were monitored using thermometers and hygrometers, respectively. The temperature ranged from 24ºC to 40ºC during the day and 14ºC to 26ºC at night during cultivation.

Table 1 Formula of cultivation substrate used for growing *Hymenopellis* sp. MFLU19-1658.

Substrate formula	Percentage of substrate components (%)					
	Sawdust	Sorghum	Rice bran	Corncobs	CaO	Gypsum
A	98	-	-	-	1	1
B	79	19	-	-	1	1
C	79	-	19	-	1	1
D	30	-	8	60	1	1

Yield data, biological efficiency, and statistical analysis

Monitoring was done from the first appearance of the primordium up to the last flush. Mature basidiomata were harvested from each flush and weighed (fresh weight) in the laboratory. Yield data and biological efficiency were calculated. The total weight of fresh mushrooms per spent substrate is used to calculate yield, while biological efficiency is calculated using the formula: weight of harvest/weight of a dry substrate) x 100% (Liang et al. 2019; Thongklang et al. 2020). Statistical analysis was conducted using one-way analysis of variance (ANOVA) at a significant level of $p<0.05$ to check the difference between the substrate combinations. The mean and standard deviation were also determined.

Nutritional analysis

The cultivated basidiomata of *Hymenopellis* sp. (MFLU 19-1658) were dried for 24 hours at 45ºC and powdered using a blender. Protocols for each analysis performed in this study are listed below.

Moisture content analysis

Moisture content analysis was done by the oven drying method (Nielsen 2010). Six disposable aluminium pans were pre-dried at 100ºC for 24 hours. Three grams of powdered mushroom sample was placed in each pan and weighed accurately. Samples were put in an oven and dried at 103±2ºC for 18 hours. After drying, the samples were put in the desiccator to lower the temperature and weighed. Percentages of moisture and dry matter were calculated as follows.

$$\%\text{Moisture} = \frac{\text{wt of } H_2O \text{ in sample}}{\text{wt of wet sample}} \times 100$$

$$\%\text{Moisture} = \frac{(\text{wt of wet sample + pan}) - (\text{wt of dried sample + pan})}{(\text{wt of wet sample + pan}) - (\text{wt of crucible})} \times 100$$

$$\%\text{Dry matter} = 100 - \%\text{ moisture}$$

Ash content analysis

Ash content analysis was done by the dry ashing method following Jame (1995). Three crucibles were pre-heated at 525ºC for 24 hours. Three grams of the powdered mushroom sample

were placed in each crucible and weighed accurately. Crucibles were put in a muffle furnace, and the samples were dried at 525ºC for 4 hours. After drying, samples were stored in a desiccator and weighed accurately. The percentage of ash in a wet weight basis (wwb) and dry weight basis (dwb) were calculated as follows.

$$\%\text{Ash (wwb)} = \frac{\text{wt of ash}}{\text{wt of sample}} \times 100$$

$$\%\text{Ash (wwb)} = \frac{(\text{wt of ashed sample + wt of crucible} - (\text{wt of crucible})}{(\text{wt of wet sample + crucible}) - (\text{wt of crucible})} \times 100$$

$$\%\text{Ash (dwb)} = \frac{\%\text{ ash (wwb)}}{(100 - \%\text{ moisture})} \times 100$$

Fat content analysis

Fat content was determined by the continuous Soxhlet methods using organic solvent following Nielsen & Carpenter (2017). Three extraction thimbles were prepared. Around 3 g of sample was weighed and placed in each thimble. Pre-dried extraction cups were weighed, labeled, and placed in the Soxhlet extractor. Exactly 70 ml of petroleum ether was put in the set-up by using a dispenser. The program was set following the manual guidelines for the equipment. After finishing the process, the extraction cups were put into the oven to dry at 105ºC for 2 hours. The extraction cups were then placed in a desiccator to cool before being weighed. The percentage of fat (wwb and dwb) was calculated as follows.

$$\%\text{Fat (wwb)} = \frac{(\text{wt of cup + fat}) - (\text{wt of cup})}{(\text{wt of wet sample})} \times 100$$

$$\%\text{Fat (dwb)} = \frac{\%\text{ fat (wwb)}}{(100 - \%\text{ moisture})} \times 100$$

Protein content analysis

The protein content of mushroom samples was determined using the Kjeldahl method, following Nielsen (2017a). Digestion was started by turning on the digestion block and setting the temperature to 420ºC. Three digestion tubes were prepared. Approximately 1g of the sample was weighed and recorded, then placed in each digestion tube. Exactly 5 g of catalyst and 12 ml of concentrated sulfuric acid were placed in each tube with the sample. After being arranged in the rack, the digestion tubes were placed in the digestion block. The exhaust system was then turned on. The digestion process was completed in about 45 minutes or until the samples became clear. The samples were taken from the digestion block and cooled. The samples were diluted with 20 ml of distilled water.

The distillation process was done following the manual of the distillation equipment. An appropriate volume of boric acid (25 ml) was dispensed into the receiving flask. The receiving flask was placed on the distillation system and submerged in the boric acid solution. NaOH solution (50 ml) was delivered to the tube during the distillation process. The steam generator was set for 4 minutes to distill the sample. The colour of boric acid was then changed from red to green. The same procedures were applied to all three tube samples.

The titration was done using standardized HCl solution in three sample replicates in tubes and one blank. The normality of HCl was recorded. Methyl red indicator (five drops) was added to each tube and then titrated with the standardized HCl solution. The colour changed from green to pink.

The volume of the HCl titrant used was recorded. The percent nitrogen and percent protein were calculated using the formula below. The conversion factor used for nitrogen to protein was 6.25.

$$\%\ N\ (wwb) = \frac{\text{Normality HCl}}{1000} \times \frac{\text{Corrected acid vol. (ml)}}{\text{wt of sample (g)}} \times 14\left(\frac{\text{g of N}}{\text{mol}}\right) \times 100$$

$$\%\ \text{Protein (wwb)} = \%\ N \times \text{Protein Factor}$$

Remark:
Corrected acid vol. = (ml std. acid used for sample) – (ml std. acid used for blank)

Crude fiber content analysis

The crude fiber in mushroom samples was determined as follows (Nielsen 2017b). Crude fiber is composed of cellulose, hemicellulose, and lignin, the residue after chemical digestion with hot sulfuric acid (1.25% w/v) and hot sodium hydroxide (1.25% w/v). The crucibles to be used were pre-dried (W_1) and weighed. The powdered sample (1 g) was accurately weighed and placed in the crucible. The crucible was placed in the Fibertec cold extraction unit. Exactly 25ml of acetone was added to the crucible and left to filter for 10 minutes. This procedure was carried out three times before being washed with water. The crucible was placed in the Fibertec hot extraction unit, and 150 ml of hot, 1.25% w/v sodium hydroxide, followed by sulfuric acid, was added. Four drops of n-octanol were added to prevent foaming and heated till boiling for 30 minutes. The acid was filtered and washed three times with hot, distilled water. The crucible was again put in the Fibertec hot extraction unit, added 150 ml hot 1.25% w/v sodium hydroxide followed the previous process with sulfuric acid. The crucible was then placed in the Fibertec cold extraction unit, filled with 25 ml of acetone, filtered for 10 minutes, and repeated three times. The solvent was then evaporated, and the crucible was dried at 130ºC for 2 hours. The crucible was cooled in the desiccator and weighed accurately (W_2). The sample in the crucible was then ash dried at 525±25ºC for 4 hours. It was then cooled in the desiccator and weighed (W_3). The percentage of crude fiber (wwb) was calculated as follows.

$$\%\ \text{Crude fiber (wwb)} = \frac{(W2 - W3)}{W1} \times 100$$

Where:

W1 = Sample weight (g)
W2 = Weight of crucible + residue (g)
W3 = Weight of crucible + ash (g)

Carbohydrate content

Carbohydrate content is determined following Goyal et al. (2006).

$$\text{Available carbohydrate} = 100 - (\text{ash} + \text{crude fat} + \text{crude fiber} + \text{protein})$$

Determination of energy value

The Energy value was determined by multiplying the mean values of carbohydrate, crude fat, and crude protein with the Atwater factors as described by Onyeike et al. (1995) and expressing the result in kilocalories per 100 g sample with the following formula.

$$\text{Energy value}\left(\frac{\text{kcal}}{\text{100g sample}}\right) = 4 \times (\text{protein}) + 9 \times (\text{fat}) + 4 \times (\text{carbohydrate})$$

Statistical analysis

The statistical analysis of the data was performed by ANOVA at a 5% level of significance using the program IBM SPSS Statistics V21.0. Means were separated using Duncan's multiple range statistical test.

Results

Phylogenetic analyses of cultivated species

Sequences obtained from our specimen were compared with the closely related taxa in GenBank using the Basic Local Alignment Search Tool (BLAST). Fifty-three taxa, including twenty-seven related sequences and three outgroup taxa, *Paraxerula americana* (Dörfelt) R.H. Petersen, *Strobilurus conigenoides* (Ellis) Singer, and *Xerula pudens* (Pers.) Singer retrieved from GenBank were used to infer phylogenetic relationships with the newly generated sequence. Outgroup taxa were chosen based on the combined ITS and nrLSU phylogeny in Hao et al. (2016). Results showed that Thai *Hymenopellis* sp. MFLU19-1658 belongs in the clade consisting of *H. raphanipes* TENN59800, *H. furfuracea* JM98-155, and *Xerula* BCC56836 with 100% bootstrap support and 1.0 probability. With 85% bootstrap support and 0.96 posterior probability, *Hymenopellis* sp. MFLU19-1658 is closely related to *Xerula* BCC5683 with the ITS genetic distance of 1.92% (6/313) (Fig.1).

Taxonomic confirmation of the wild and cultivated species

Hymenopellis R.H. Petersen 2010 Fig 2–3

Description:— *Basidiomata* medium-sized to large size. *Pileus* 15–135 mm diam., seen from above is circular, side view is broadly convex with broad umbo to plane to depressed or indented when old, dark-brown (6E8) when in primordial stage, light brown (5D5) to dark brown (5E5–8) when mature, turning darkest brown (6F8) when old; non-hygrophanous, somewhat laccate surface especially when wet, glabrous to radially wrinkled especially towards the center; margin plane to uplifted, with edge minutely scalloped, dark brown to black; context cream (1A3), unchanging when cut, consistency soft to somewhat rubber-like. *Lamellae* 5–8 mm width, attachment adnate with decurrent line, white to cream (1A3), spacing greater than 1 mm apart; lamellar margin even to irregularly wavy at old age, dark brown to black; lamellulae present, 2-tier arrangement. *Stipe* 20–150 mm long to ground line, 3–6mm diam near apex, 5–10 mm diam near ground line, central, attenuate upward, thickening or swollen at the base, off-white, lighter (5A2) from the pileus becomes darker (5D4) towards the base, surface dry, minutely furfuraceous to distinctly scabrous especially when mature, with patches of dark brown caulocystidia, with canal when cut vertically; context white, unchanging when cut; pseudorrhiza present, tapering gradually. *Annulus* and *volva* absent. *Spore print* white. *Smell* indistinct. *Taste* mild.

Basidiospores (12.5)13.0–14.0–16.0(17.0) × (10)10.5–12.5–13.5(14) µm (Q = 1.0–1.2, Q* = 1.1), subglobose, broadly ellipsoid, obovoid thin-walled, smooth, hyaline in 5% KOH. *Basidia* (50.0) 52.0–60.0–68.0 (78.0) × (14.5) 15.0–16.0–17.5 (17.8) µm (Q = 2.7–4.5, Q* = 3.5), 4-spored, clavate, without clamp connection. *Cheilocystidia* (45.5) 60.0–72.0–80.0 (97) x (6.0) 7.0–10.0–12.0 (15.0) µm, crowded, clamped, pedunculate, narrowly clavate, narrowly cylindrical, narrowly lageniform, narrowly fusiform, smooth, thin-walled, hyaline in 5% KOH. *Pleurocystidia* - (90.0) 100.0–115.0–128.0 (141.0) × (16.0) 18.0–25.0–30.0 (32.0) µm, broadly fusiform with broadly rounded apex, smooth, lower portion thin-walled, semi-collapsed; content homogeneous, dull yellowish in 5% KOH. *Hymenophoral trama* irregular, made of thin-walled, hyaline hyphae. *Pileipellis* is an ixotrichoderm composed of a single element. *Pileocystidia* 21.0–43.0 × 10.0–20.0µm, pileal hairs not observed, with scattered intracellular light brown (6D8) pigment in 5% KOH. *Stipitipellis* a hymeniderm with apical *caulocystidia* 40–65–90 × 7–11–16 µm, narrowly fusiform to subcylindric, gregarious to caespitose; contents with intracellular light brown (6D8) pigment in 5% KOH. *Clamp connections* absent in all tissue types.

Fig. 1 – Phylogenetic tree generated from ML analysis of ITS and nrLSU data set for *Hymenopellis* with three outgroup species; *Paraxerula americana*, *Strobilurus conigenoides*, and *Xerula pudens*. Bootstrap support values (≥70%) and posterior probabilities (≥0.9) (BS/PP) are given above the branches. All terminals are labeled with the species name and voucher ID, and the newly generated sequences from this study are in bold.

Habitat and distribution:—Solitary or gregarious. The first time was recorded in Chiang Rai Province, Thailand with three basidiomata growing near each other in soil covered with litter in a mixed deciduous forest.

Specimen examined:—Thailand, Chiang Rai Province, Mae Fah Luang District, elev. 1,110 m, tropical mixed deciduous forest, 14 June 2019, A.G. Niego, MFLU 19-1658, GenBank OP630831 (ITS) and OP630832 (nrLSU).

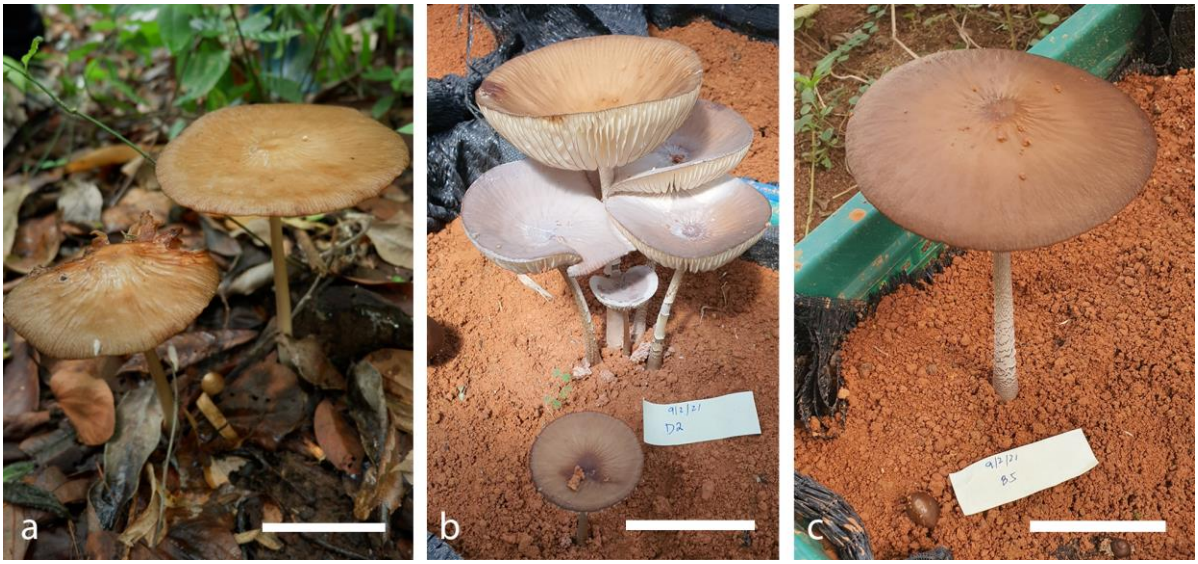

Fig. 2 – *Hymenopellis* sp. (MFLU 19-1658). a Mature basidiomata of the wild *Hymenopellis* sp. MFLU 19-1658 in the forest. b–c Mature basidiomata of cultivated *Hymenopellis* sp. MFLU 19-1658 in the mushroom house at Mae Fah Luang University, Thailand on 52nd day of cultivation (5th flush) with a daytime temperature of 24ºC. Scale bar = 5 cm.

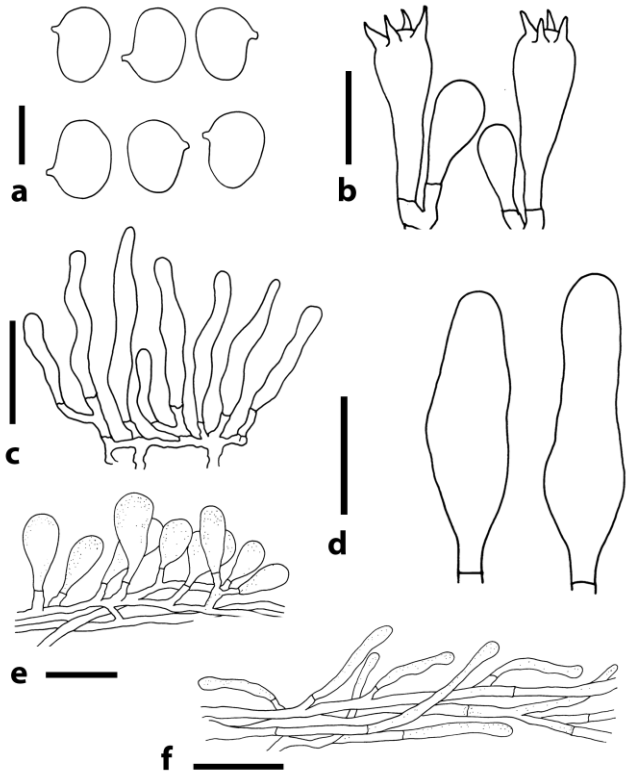

Fig. 3 – Micromorphological characteristics of *Hymenopellis* sp. MFLU 19-1658. a basidiospores. b basidia. c cheilocystidia. d pleurocystidia. e pileipellis. f Mid-stipe caulocystidia. Scale bars: a (10 µm), b (25 µm), c-d (50 µm), e-f (20 µm)

Cultivation yield and nutrient content

The strain *Hymenopellis* sp. MFLU 19-1658 fully colonized the sawdust bags 60 days after inoculation. The cultivation of *Hymenopellis* sp. MFLU 19-1658 resulted in ten flushes, with the first appearing 30 days after tray preparation covered with soil and lasting 86 days (Fig. 3, b–c). The size of fruiting bodies ranges from 3.0 cm to 13.5 cm pileus diameter and 3.0 cm to 17.0 cm stipe length. Growing *Hymenopellis* sp. MFLU 19-1658 in sawdust (A) has an average weight (g/tray) of 115.5±73.5 g, which is slightly higher than that in the combination of sawdust and corncobs at 84.2±67.6 g (D). Sawdust with sorghum (B) can also grow this mushroom with an average weight of 61.0±21.1 g, while adding rice bran to sawdust (C) has the lowest yield at 10.8±17.9 g. The biological efficiency ranges from 3.0±5.0% (C) to 32.1±20.4% (A) (Table 2). As for the flushes, the highest average weight (D) was 119.3 g, with BE at 33.1% from the fifth flush (Table 3). The result of the ANOVA showed that the f-ratio value was 2.83003 and *p*-value was 0.052034. The difference in average yield between treatments is not significant at $p < 0.05$.

Table 2 Data comparison between the different substrate combinations.

Content		***Hymenopellis* sp. MFLU 19-1658**
Primordia appearance after inoculation (days)		90
Number of flushes		10
Yield, the average weight of mushroom (g/tray) in substrate combination		
	A	115.5±73.5 **a/A**
	B	61.0±21.1 **ab/AB**
	C	10.8±17.9 **b/B**
	D	84.2±67.6 **ab/A**
Biological Efficiency (%)		
	A	32.1±20.4 **a/A**
	B	16.9±5.9 **ab/AB**
	C	3.0±5.0 **b/B**
	D	23.4±18.8 **ab/A**

Notes: The data was expressed as mean ± standard deviation; value with different letters are significantly different under Duncan's (capital letters) and Waller-Duncan's (lowercase letters) multiple range, followed Dunnett's T3 post hoc test at $p< 0.05$. A, B, C, and D represent the different substrate combinations.

Table 3 Comparison of mushroom yield in each flush in different substrate combinations (5 replication).

	A		B		C		D	
Flush	Ave wt. (g)	BE (%)	Ave wt. (g)	BE (%)	Ave wt. (g)	BE (%)	Ave wt. (g)	BE (%)
1	15.5	4.3	4.9	1.4	0.0	0.0	10.4	2.9
2	13.4	3.7	29.6	8.2	0.0	0.0	0.0	0.0
3	33.4	9.3	0.0	0.0	0.0	0.0	0.0	0.0
4	55.1	15.3	37.8	10.5	5.7	1.6	46.0	12.8
5	11.3	3.1	40.0	11.1	24.1	6.7	119.3	33.1
6	45.5	12.6	12.0	3.3	0.0	0.0	5.5	1.5
7	18.2	5.0	12.7	3.5	0.0	0.0	0.0	0.0
8	27.6	7.7	34.3	9.5	0.0	0.0	13.0	3.6
9	24.9	6.9	0.0	0.0	0.0	0.0	26.8	7.4
10	43.8	12.2	8.6	2.4	0.0	0.0	34.6	9.6

Notes: Ave wt, average weight; BE, biological efficiency. A, B, C and D represent the different substrate combinations.

Nutrient contents of *Hymenopellis* sp. MFLU 19-1658 from fresh and dried basidiomata are measured through different analyses. Results showed a high carbohydrate content of 49.2±0.9 g/100g and a high crude fat of 8.0±0.6 g/100g. Nutrient contents of the studied mushroom are listed in Table 4.

Table 4 Nutrient content of *Hymenopellis* sp. MFLU 19-1658

Content (g/100g)	Percentage (%)
Moisture (FW)	83.3±1.4
Ash (DW)	8.1±0.9
Crude fat (DW)	8.0±0.6
Crude fiber (DW)	16.5±0.5
Protein (DW)	17.8±0.6
Carbohydrate (DW)	49.2±0.9
Energy (Kcal/100g DW)	340.0

Notes: FW, fresh weight; DW, dry weight.

Discussion

The strain *Hymenopellis* sp. MFLU 19-1658 was confirmed morphologically to belong to *Hymenopellis*. The morphological characteristics of *Hymenopellis* sp. collected from this study are very similar to *Hymenopellis neurodermis* (Pat.) R.H. Petersen (Petersen & Hughes 2010), with the exception that the latter was a small basidiome with a pileus diameter of only 10 mm. *Hymenopellis neurodermis* was only described from the type specimen with limited macroscopic descriptions (Petersen & Hughes 2010) and no sequence data available. The diameter of the cultivated specimens ranges from 15 to 135 mm. The colour of the type specimen was dark brown (Petersen & Hughes 2010), while the cultivated specimens are dark brown when young, become lighter when mature, and become darker with age. The distinct resemblance between the type and the cultivated specimens is the darker margins of the lamellae due to the crowded cheilocystidia. The cheilocystidia are clamped and subcylindrical to narrowly fusiform. Pileal hairs are also not observed in the cultivated specimens. Other microscopic characteristics resemble the 4-spored basidia and are broadly fusiform with broadly rounded apical pleurocystidia. The type specimen of *H. neurodermis* was collected from Vietnam and is expected to be found in Southeast Asia, including Thailand (Petersen & Hughes 2010). Also, there are no sequences available for the type specimen of *H. neurodermis*, thus, the cultivated specimens cannot be confirmed phylogenetically and will be named *Hymenopellis* sp. The ITS sequence (with primers ITS1 & ITS4) of the specimen also exhibits polymorphism, as multi-peaks at the beginning and end of the ITS region and should be further resolved by using other gene regions to confirm its correct identity. Moreover, the cultivated specimen came from only one wild collection, thus, an additional collection is required to establish the correct taxonomic placement and if it is a novel species. With almost 50 species of *Hymenopellis* identified taxonomically (He et al. 2019), only 19 species have available sequences deposited in the GenBank, thus more taxonomic studies should be conducted to resolve the species identification of this genus.

Hymenopellis sp. MFLU 19-1658 was also identified as *Hymenopellis* based on phylogenetic analysis of the combined ITS and nrLSU sequences. The result showed that the specimen clustered with *Xerula* sp. BCC56836 was a previously found strain in Thailand (Sadorn et al. 2016). *Xerula* sp. BCC56836, however, was misidentified and fell in the group of *Hymenopellis* in the phylogenetic analysis. The morphology of *Hymenopellis* sp. and *Xerula* BCC56836 cannot be compared since the latter was sequenced through the mycelial culture only, with no access to the herbarium specimen. *Hymenopellis* sp. MFLU 19-1658 also clustered with *Hymenopellis raphanipes* TENN59800 and *Hymenopellis furfuracea* JM98-155, which are also misidentified since they both placed away from their clades. *Hymenopellis raphanipes* (Berk.) R.H. Petersen is closely related to *Hymenopellis* sp. MFLU19-1658 phylogenetically, however, they are distinctly

different morphologically. *Hymenopellis raphanipes* differ from *Hymenopellis* sp. MFLU19-1658 by having mostly dark-coloured basidiomata and 2– and 4-spored basidia (Petersen & Hughes 2010), while the latter is strictly 4-spored. The basidiospores of *H. raphanipes* are also slightly larger (14–20 × 10–18 µm). The cheilocystidia are also distinctly large for *H. raphanipes* (42–150(–235) × 7–37 µm. *Hymenopellis raphanipes* is widespread from Australia to Japan and is expected to be found throughout Southeast Asia (Petersen & Hughes 2010). Phylogenetically, the clade of *Hymenopellis furfuracea* is distinct from *Hymenopellis* sp. MFLU19-1658. *Hymenopellis furfuracea* is also morphologically different from *Hymenopellis* sp. MFLU19-1658 by exhibiting more diverse colour variation with a paler pileal margin but sometimes deeply striatulate (Petersen & Hughes 2010). The pleurocystidia of *H. furfuracea* are more variable, (80–)90–175 × 27–47 µm, utriform-pedicellate to narrowly ten pin-shaped. The cheilocystidia of *H. furfuracea* are also much larger (32–160 × 8–36 µm) than *Hymenopellis* sp. MFLU19-1658. *Hymenopellis furfuracea* is strictly distributed in Eastern North America (Petersen & Hughes 2010). Although only two species of *Hymenopellis* are recorded in Thailand, we believe that there are more to be discovered. *Hymenopellis* species such as *H. furfuracea*, *H. radicata*, and *H. raphanipes* are edible mushrooms, though cultivation is limited (Gao 2000, Shim et al. 2006, Hao et al. 2016). Only China commercially cultivates *Hymenopellis raphanipes*, with an annual yield exceeding 20,000 tons. In Thailand, *Hymenopellis* has not yet been introduced as a food source. This study is the first attempt at cultivating *Hymenopellis* in this country using sawdust as the main substrate with different additives. The range of biological efficiency from this study (3.0±5.0–32.1±20.4%) is lower compared with the cultivation of *H. radicata* using oak sawdust with a biological efficiency of 100% (Gao 2000). Sawdust is the main substrate in mushroom cultivation; however, it is quite expensive since it has to be transported from the southern area of Thailand. The average price is 20 baht/Kg (0.60 USD/Kg) in Chiang Rai Province, and the higher freight fee contributed to the increase in price. Finding cheaper additives, such as corncobs, as an alternative to sawdust can help decrease the total cost of mushroom cultivation. *Hymenopellis* sp. MFLU 19-1658 could grow in the substrate with corncobs and sorghum as additives, thus, its cultivation can be less expensive. Also, it can grow well in the tropical climate in northern Thailand, therefore, there is no need for a high-maintenance mushroom house. Moreover, *Hymenopellis* sp. MFLU 19-1658 can tolerate a higher range of temperatures from 24–40ºC. Since this mushroom can grow well in environmental conditions with only moisture to be maintained in Thailand, the local people can have a better opportunity to grow this mushroom.

Mushrooms have become popular not only for their pleasant and unique flavours but also for their nutritional content, contributing to their increasing health benefits (Barros et al. 2008). The nutrient contents of *Hymenopellis* sp. MFLU 19-1658 are relative to those of other economically important mushroom species (Table 5). The ash content of *Hymenopellis* sp. MFLU 19-1658 is similar to that of *Lentinus sajor-caju* (Ao & Deb 2019), while the fat content is notably higher than all other listed species. *Hymenopellis* sp. MFLU 19-1658 has glossy and smooth pileus, which could be a fatty surface, thus contributing to higher fat content. However, this assumption needs further validation. It is also essential to identify the kind of fatty acids present in the mushroom. Fat is one of the components of energy value; therefore, higher fats contributed to a greater energy value of 340 Kcal/100g for *Hymenopellis* sp. MFLU 19-1658. Moreover, this cultivable species has relative protein and carbohydrate content as other species. This study showed that *Hymenopellis* can be a good source of nutrients like other economically important species. Since there is no pre-existing nutrient content for *Hymenopellis*, this study served as the baseline for this genus, guiding other future nutritional studies.

Table 5 Nutrient contents of some economically important mushroom species.

Species	Moisture (g/100g FW or DW)	Ash (g/100g DW)	Fat (g/100g DW)	Proteins (g/100g DW)	Carbohydrates (g/100g DW)	References
Hymenopellis sp.	83.3±1.4 (FW)	8.1±0.9	8.0±0.6	17.8±0.6	49.2±0.9	This study
Agaricus bisporus	90.09 + 0.07 (FW)	9.17 + 0.52	3.06 + 0.03	24.43 + 0.10	53.10 + 0.56	Goyal et al. (2006)
Grifola frondosa	4.8 ± 0.08 (DW)	4.7 ± 0.07	5.3 ± 0.09	18.3 ± 0.34	66.9 ± 8.4	Cohen et al. (2014)
Lentinula edodes	7.3 ± 0.10 (DW)	5.1 ± 0.05	0.8 ± 0.01	18.5 ± 0.16	68.3 ± 4.7	Cohen et al. (2014)
	82.8 ± 0.01 (FW)	5.59 ± 0.3	-	43.81 ± 0.02	38.44 ± 0.01	Ao & Deb (2019)
Lentinus sajor-caju	85.1 ± 0.02 (FW)	8.41 ± 0.2	-	62.27 ± 0.02	6.81 ± 0.01	Ao & Deb (2019)
	89.58 + 0.19 (FW)	7.46 + 0.30	1.96 + 0.12	25.65 + 0.05	52.46 + 0.43	Goyal et al. (2006)
L. squarrosulus	87.3 ±0 .02 (FW)	10.66 ± 0.4	-	27.86 ± 0.01	9.32 ± 0.01	Ao & Deb (2019)
	86.2 ± 0.01 (FW)	3.12 ± 0.2	-	18.77 ± 0.02	19.14 ± 0.01	Ao & Deb (2019)
Pleurotus ostreatus	8.2 ± 0.07 (DW)	7.1 ± 0.06	2.3 ± 0.07	33.5 ± 0.22	48.9 ± 2.7	Cohen et al. (2014)

Note: FW= fresh weight; DW= dry weight

Conclusion

Hymenopellis sp. (MFLU 19-1658), with a rich nutritional value, can be grown in sawdust and is suitable for the tropical regions of northern Thailand. This mushroom can be a promising food source, and its cultivation can be a potential alternative livelihood for people in Thailand. However, further confirmation in the laboratory is required to fully establish its edibility since some generally edible mushrooms can be toxic at certain doses.

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