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Nutritional analysis of cultivated *Pleurotus giganteus* in agricultural waste as possible alternative substrates

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Abstract

Pleurotus giganteus is a wild edible mushroom that was successfully domesticated in Thailand. In this research, a *P. giganteus* strain (MFLUCC23–0016) was isolated into PDA agar medium with the best growing spawn detected in millet and sawdust at 11.10 ± 0.34 mm/day, 9.02 ± 0.40 mm/day respectively. Fruiting bodies occurred 14 days after unsealing the substrate bag, which produced large basidiomata and obtained a higher total yield of fresh weight from the sawdust and sawdust + corn cob substrate at 180.77 ± 44.41 g, 176.60 ± 17.65 g, respectively, compared to sawdust + rice straw and sawdust + sugarcane bagasse (35.30 ± 14.15 g, 34.52 ± 11.29 g, respectively) in 60 days with developed fruiting bodies in a 7-day cycle. Three soil casing formulas could be used for the casing of the soil for the *P. giganteus* fruiting trial, in which T1 obtained a higher total fresh weight at 278.54 ± 89.04 g followed by T2 at 218.35 ± 92.53 g and T3 obtained the lowest yield at 137.28 ± 67.42 g. Nutritional analysis of 100 g of dried *P. giganteus* showed 61.32 ± 0.0% of carbohydrates content, 20.318 ± 0.8 (g/100 g) of protein content, 15.6 ± 0.5% of moisture content, 14.28 ± 0.3% of fiber content, 5.61 ± 0.9% of ash content, and 2.98 ± 1.58 (g/100 g) of fat content.

Keywords – cultivation **–** edible mushroom – fiber content **–** nutritional analysis – *Pleurotus* – soil casing

Introduction

Pleurotus (Fr.) P. Kumm. belongs to Pleurotaceae Kühner (Agaricales). 769 taxon names for *Pleurotus* are listed in Index Fungorm (http://www.indexfungorum.org/, 2022), with a total of 25 identified species with *P. ostreatus* (Jacq.) P. Kumm. as is the type species (He et al. 2019). The main morphological characteristics of *Pleurotus* are defined by pleurotoid basidiomata, decurrent lamellulae, smooth and elongated to cylindrical basidiospores, a dimitic hyphal system with skeletal hyphae and generative hyphae, and clamp connections (Corner 1981). This genus is distributed in both tropical and temperate areas (Chang & Miles 2004). *Pleurotus* is an edible mushroom that may be known as the oyster, abalone, or tree mushroom, and has been cultivated worldwide (Mahari et al. 2020).

Pleurotus giganteus (Berk.) Karun. & K.D. Hyde is an edible cultivated mushroom that can be found in the soil often on buried wood. It is known as the giant oyster mushroom and was identified for the first time as *Lentinus giganteus* in Sri Lanka by (Berkeley 1847). Later,

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Karunarathna et al. (2012) transferred this species to *Pleurotus* based on morphological and phylogenetic analysis. This species has been found and reported in many countries, such as Malaysia (Corner 1981), Thailand (Karunarathna et al. 2012), and Laos (Phonemany et al. 2021, Łuczaj et al. (2021). Bioactivity and medicinal properties of *P. giganteus* have been investigated in vitro and in vivo, such as antioxidant, antifungal, anti-inflammatory, anti-cancer, anti-diabetic, anti-dengue, hepatoprotective activity, Neurite outgrowth stimulating activity, and Genoprotection (Phan et al. 2018). Furthermore, many compounds have been isolated from extracts of *P. giganteus* e.g. alkaloids, fatty acids, organic acids, phenolics, sterols and triterpenoids (Moroney 2012, Phan et al. 2018, Yadav et al. 2020). In previous studies of *P. giganteus*, many report various biological activities and medicinal properties, optimal medium, PH, temperature, and optimal cultivation (Klomklung et al. 2012, Kumla et al. 2013, Klomklung et al. 2014, Soytong & Asue 2014, Phan et al. 2018, Yadav et al. 2020). Wild *P. giganteus* has been reported to be successfully domesticated using sawdust as a substrate with a temperature for mycelia growth on soybean agar (*Glycine max* L.) at 25–30 °C, and a of pH 5.0 to 6.5 (Klomklung et al. 2014). The best spawn was reported on soybean at 30 °C (Klomklung et al. 2012). The soil casing has been shown to be important to maintain moisture and trigger the use of fruiting bodies to obtain a higher yield for *P. giganteus*, such as honeycomb cinder soil (Qin et al. 2016), and garden soil supplemented with 1% nano-KS1 (Soytong & Asue 2014). However, this mushroom has only been investigated in terms of certain aspects of its cultivation, with its cultivation needs in terms of finding cost-effective and higher yielding substrates, as well as the factors needed to stimulate growth-fruiting body formation of mushrooms requiring further study. Therefore, this study aimed to provide agricultural waste that could be used as an alternative substrate to grow *P. giganteus* and to test the effect of soil casing on fruiting bodies production. Additionally, the nutritional content of the fruiting of cultivated mushrooms was recorded.

Materials & Methodology

Mushroom strains, and identification

Mushroom samples were collected from the Chiang Mai Province, Mae Taeng District during the rainy season. The strain of *P. giganteus* (MFULCC23-0016) was isolated from pileus tissues using sterile forceps and put into Petri-dishes with Potato dextrose agar (PDA). The mycelium culture was subcultured with PDA for the extension of mycelium and incubated at 30 °C for 2 weeks. The dried samples and the culture collection were deposited in the Mae Fah Luang University herbarium and the Mae Fah Luang University culture collection.

The fresh basidiomata were described by following Largent (1986) and color coded by following Kornerup & Wanscher (1987). The dried samples were examined by following the procedures of Vellinga (2001). Micromorphology characteristics were observed by rehydrating with water, 5% KOH and Congo red was used for dyeing. Fifty basidiospores were measured. Other important characteristics such as basidia, cheilocystidia, and hyphae were measured with n = 25 for each characterization. The dimensions of microscopic structures are given as follows: (a–)b–c–d(−e), in which c presents the average, b the 5th percentile, d the 95th percentile and minimum and maximum values a and e are shown in parentheses. Q, the length/width ratio of the spores in the side view was provided in the same format.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from dry specimens using the Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou), by following the manufacturer's instructions. The ITS region, and nrLSU regions were amplified by polymerase chain reaction (PCR). For amplification of ITS, the primers ITS1-F and ITS4 (Gardes & Bruns 1993, White et al. 1990), the nrLSU with primers LR0R and LR5 (Vilgalys & Hester1990, White et al. 1990). The PCR amplification, purification, and sequencing of ITS and LSU were performed using PCR primers by Shanghai sangon Biological Engineering Technology & Services Co., Ltd. The PCR

cycle for ITS and LSU was set up: 3 mins at 94 °C; 35 cycles of 30 s at 94 °C, 40-50 s at 50-55 °C, 1 min at 72 °C; 10mins at 72 °C.

Effect of Spawn production:

Various materials including corncobs (*Zea mays* L.), coconut residue (*Cocos nucifera* (L.)), sugarcane bagasse (*Saccharum officinarum* L.), millet (*Panicum miliaceum L.*), wheat (*Triticum aestivum* L.), paddy rice (*Oryza sativa* L. var. *glutinosa*), rice berry (*Oryza sativa* L.), and sorghum (*Sorghum bicolor*) were used for optimal spawning. The grains were placed in vitro and sterilized at 121 °C for 15 minutes. The tubes containing the grains were inoculated with a mycelium plug (approximately 0.5 cm diam), and the culture was incubated at 30 °C in the dark. The experiment was carried out in five replicates. The length of the liner mycelium was measured and recorded every other day for 14 days.

Agricultural wastes for cultivation

Four different formulas were used in this study, which is shown in (Table 1), corn cobs (*Zea mays* L.), rice straw (*Oryza sativa* L. var. *glutinosa*), and sugarcane bagasse (*Saccharum officinarum* L) were mixed with rubber sawdust (*Hevea brasiliensis* Müll.Arg.) with a ratio of 1:1 (Thongklang & Luangharn 2016) and rubber sawdust alone was used as a control. Rice bran, pumice sulfate, gypsum ($CaSO4.2H_2O$), yeast, calcium carbonate ($CaCO_3$), molasses, and EM (Effective Microorganism) were also added. The substrate bags (800 g per bag) were sterilized for 1 hour at temperatures of 121 °C. The bags were left to cool then approximately 50 g of the spawn was inoculated and incubated in dark conditions at 28 ± 1.09 °C until the mycelia had grown to completely fill the bags. Watering was carried out twice daily in the morning and evening using a tap water sprayer until the fruiting bodies had fully developed. The results were harvested manually and recorded daily. The experiment was performed with 15 repetitions for each substrate, and the experiment was carried out for 60 days.

Table 1 The ratio of experimental substrates (w/w) for the cultivation of *Pleurotus giganteus*.

Treatment	The composition of the bag (w/w)
Sawdust	
Sawdust + corn cobs	1:1
Sawdust + rice straw	1:1
Sawdust + sugarcane bagasse	1:1

(1:1), means the ratio of experimental substrates (w/w).

Casing and nutrient supplementation

The full mycelium in the bag substrate was opened after 30 days and transferred to the tray. The bag was placed in the center of the tray and then covered with soil approximately 3 cm deep. Humus soil was used as the main casing material for growth with three different soil casing formulas as shown in (Table 2). The first formula (T1) used 25% humus soil, 35% lime sand, 25% calcium carbonated, and 15% Peat. The second formula (T2) used 50% lime sand, 25% calcium carbonate, and 25% peat. The third formula (T3) used 100% humus soil and removed tough parts before use (Table 2). After the soil casing was applied, the trays were incubated in a mushroom house at 30 °C, 90% humidity, and the trays was watered every week. The moisture of the trays was maintained by spraying water outside the trays two to three times per day. The experiment was carried out on five replicated trays. The mushrooms product that developed were harvested when the cap started to curl. Data was collected each day for 60 days.

Statistics Analysis

Statistical significance for the mycelium growth rate of spawn production, the total wet weight of mature fruiting bodies harvested, the average of wet weight, and data analysis were determined by variance of means using Tukey's test with a significance of $P < 0.05$. The biological

efficiency (B.E.) was recorded. B.E means the total weight of fresh mushrooms/weight of dry substrate) x 100% (Liang et al. 2019).

Table 2 The ratio of the soil casing formula.

Treatment	The composition of the soil casing formula (%)			
	Humus soil	**Lime sand**	**Calcium carbonated**	**Peat**
T1	25	35	25	25
T2		50	25	15
T3	100			

Nutritional analysis

The basidiomata of *P. giganteus* were proximately analyzed. Fresh basidiomata were dried at 50 °C for 24 hours or until completely dried and powdered using a blender.

Determination of Ash Content

Ash refers to the inorganic matter remaining after the complete oxidation of organic matter in mushroom samples. Ash content analysis was performed by dry ashing (Jame 1995). Six crucibles were preheated at 525 °C for 24 hours. Three grams of powdered mushroom samples were placed in each crucible and accurately weighed. Crucibles were placed in a muffle furnace and samples were dried at 525 °C for 4 hours. After being dried, the samples were stored in a desiccator and accurately weighed. The percentages of ash on a wet weight basis (wwb) and dried weight basis (dwb) were calculated as follows:

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

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

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

Determination of Fat Content Analysis

Two grams of powder samples were placed on paper and on a thimble, to which the Soxhlet method was applied using organic solvent following (Nielsen & Carpenter 2017) as follows. Pre-dried weight in an extraction cup was recorded, and the cup was then placed in a Soxhlet extractor, 70 ml of petroleum ether was added using a dispenser, the program was setup following previously described instructions (Fat was continuously extracted, with an organic solvent. The solvent was heated and volatized and then condensed above the sample. Solvent continues to drip through the sample to extract fat). After finishing, the cup was heated in a hot air oven at 105 °C for 2 hours and let it cool in the desiccator and the fat content was measured by the weight loss of the sample or of the fat in solvent. The percentage of fat (wwb and dwb) was calculated as follows:

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

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

Determination of carbohydrates

The carbohydrate content of *P. giganteus* was determined by the following equation (Raghuramulu et al. 2003):

$$\text{Carbohydrate (g/100 g sample)} = 100 - \frac{(\text{moisture} + \text{fat} + \text{protein} + \text{ash} + \text{crude fiber})\ \text{g}}{100\ \text{g}}$$

Determination of Fiber Content

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 samples (1g) were accurately weighed and placed in the crucible. The crucibles were placed in the Fibertec cold extraction unit. Exactly 25 ml of acetone was added to the crucible and left for 10 minutes to filter. This process was repeated three times and then washed with water. The crucibles were placed in the Fibertec hot extraction unit and added with 150 ml of hot 1.25% w/v sulfuric acid, diatomaceous earth, and filter aid. Four drops of n-octanol were added to prevent foaming and heated to boiling for 30 minutes. The acid was filtered and washed 3 times with hot distilled water. The crucible was again placed in the Fibertec hot extraction unit and 150 ml of 1.25% w / v hot sodium hydroxide was added which was then treated with sulfuric acid. The crucibles were placed in the Fibertec cold extraction unit and filled with 25 ml of acetone, then filtered for 10 min, a process which was repeated 3 times. The solvent was evaporated, and the crucible was dried at 130 °C for 2 hours. The crucibles were cooled in the desiccator and accurately weighed (W_2). The samples in the crucible were ashed at 525 ± 25 ºC for 4 hours and then cooled in the desiccator and weighed (W_3). The calculation for the percentage of crude fiber (wwb) is as follows:

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

Note: W_1 = sample weight (g)
W_2 = weight of crucibles + residue (g)
W_3 = weight of crucibles + ash (g)

Determination of Moisture Content Analysis

Moisture content analysis was done after oven drying (Nielsen 2010). Six disposable aluminum pans were pre-dried at 100 °C for 24 hours. Approximately three grams of powdered mushroom samples were placed in each pan and accurately weighed. The samples were placed in an oven and dried at 103 ± 2 ºC for 18 hours. After drying, the samples were put in the desiccator to lower the temperature and weighed. The percentage of moisture and dry matter was calculated as follows:

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

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

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

Determination of protein content

The protein content of the mushroom samples was determined using the Kjeldahl method (Nielsen 2010). Digestion was started by pre-heating the digestion block to 420 °C. Six digestion tubes were prepared. Approximately one gram of sample was weighed and recorded, and then placed in each digestion tube. Exactly five grams of catalyst and 12 ml of concentrated sulfuric acid were placed in each tube with samples. The digestion tubes were placed on the rack and placed in the digestion block; then the exhaust system was activated. The digestion process was completed in about 45 minutes or until the samples became clear. Samples were taken from the digestion block and cooled. The samples were diluted with 20 ml of distilled water.

The distillation process was performed following the manual of the distillation equipment. An appropriate volume of boric acid (25 ml) was dispensed in the receiving flask. The receiving flask was placed in the distillation system and submerged in the boric acid solution. A NaOH solution (50 ml) was delivered to the tube during the distillation process. The steam generator was set to 4 minutes to distill the sample. The color of boric acid changed from red to green. The same

procedures were applied to all 6 tube samples. The titration process was carried out using standardized HCl solution in 6 sample replicates in tubes with 1 blank. The normality of HCl was recorded. The methyl red indicator (5 drops) was added to each tube and titrated with the standardized HCl solution. The color changed from green to pink. The volume of HCl titrant used was recorded. The conversion factor to be used for nitrogen to protein was 6.25. The percent nitrogen and the percent protein were calculated using the formula:

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

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

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

Results

Identification

The specimen was identified as *P. giganteus* characterized by large basidiomata, pileus was 40–50 mm diam., convex to plano-concave, then depressed in the center when at maturity, brownish orange to reddish gold (6C6–8), fibrillose, brown to dark brown in the center or depress zone becoming pale toward the margin; margin present small flattened scales, incurved at maturity. Lamellae deeply decurrent, white to pale yellow (4A2) in age, 2–4 mm wide. Stipe 50–100 × 6–12 mm, central, solid with tapering pseudorrhiza, squamules concolorous with pileus. Context 10–12 mm, white with soft when young and rather solid when mature. Spore print white to whitish. Basidiospores (6.1–)6–7–9(–9.5) × (3.5–)4–5–7(–7.5) µm, Q = (1.05–)1.2–1.49–2.15(–2.44), subglobose to ellipsoid, hyaline, inamyloid, smooth, and thin-walled. Basidia (24–)27–34.5–38(–40.2) × (6.5–)7–8.6–10(–10.5) µm, clavate, thin-walled. Cheilocystidia (15.9–)16–19.4–27(–27.5) × (6–)6–7.5–8(–8.5) µm, clavate with long appendage and slightly swollen at the apex, hyaline, thin-walled. Pleurocysidia absent. Hyphal system dimitic consist with generative hyphae and skeletal hyphae. Pileipellis a trichoderm composed hyphae, 3.5–5 µm wide. Clamp connections were present. Our strains were collected from the Ban Pha Deng temple site near Karunarathna et al. (2016) who first reported collection site at the Mushroom Research Centre in Ban Pha Deng, Mae Taeng District.

Table 3 showed that the BLAST results of the ITS1 + ITS2 sequences of *P. giganteus* (MFLUCC23–0016) were very similar to *P. giganteus* (MFLU08–1371 and CMU54–1) with 100% and 98.77%, respectively. The sequence was deposited in GenBank. The records are deposited in the Greater Mekong subregion database (Chaiwan et al 2021).

Effect of grain media on spawn production

The mycelium growth rate of *P. giganteus* was investigated in vitro in various grain media. After incubation at 25 °C for 12 days, the millet and sorghum grains did not show a significantly higher growth rate compared to other grain media by statistical analysis. In (Table 4), millet and sorghum grain had the highest growth rate with a compacted mycelial density (11.10 ± 0.34, 9.02 ± 0.40 mm/day, respectively); followed by rice paddy with a thin mycelial density (4.02 ± 3.14 mm/day); rice berry and wheat had the lowest growth rate with lightly compacted mycelium density (1.21 ± 1.2 mm/day, 1.86 ± 2.21 mm/day). The results showed that these cereal grain media could be used to promote growth of *P. giganteus* mycelia.

Effect of agricultural waste on fruiting production

The primordia of *P. giganteus* were formed after four weeks of incubation at 28 °C and at a humidity of 85% in darkness. Subsequently, the bags were opened and transferred to the mushroom house temperature at 30.6 ± 0.9 °C, humidity 90% and illuminated conditions. The fruiting bodies of *P. giganteus* matured in 7 days. The mushroom product from sawdust, sawdust +

corn cobs, sawdust + rice straw, and sawdust + sugarcane bagasse is shown in (Table 5). After 60 days we found that there was no significant difference in the total fresh weight of the fruiting bodies product of sawdust and sawdust + corn cobs at 180.77 ± 44.41g, 176.60 ± 17.65 g, with no significant difference in the average weight of the fruiting bodies at 51.13 ± 13.25 g, 44.13 ± 4.41 g, respectively. The number of the fruiting bodies were 35 and 29, respectively; followed by no significant total fresh weight of sawdust + rice straw and sawdust + sugarcane bagasse at 35.30 ± 14.15 g, 34.52 ± 11.29 g, respectively, such that the average weight and the number of the fruiting bodies were 21 showed no statistically significant difference between the conditions. The biological efficiency was measured on sawdust + corn cobs and sawdust with no significant difference at 45.45 ± 4.54%, 42.20 ± 10.36%, respectively; followed by sawdust + rice straw and sawdust + sugarcane bagasse with no significant at 11.51 ± 4.61%, 10.55 ± 3.45%, respectively.

Table 3 GenBank accession numbers and BLAST search results of ITS1 + ITS2 sequences of *Pleurotus* species from this study against the GenBank database, I = Identity and QC = Query cover.

Species	Voucher, GenBank accession no.	The most similar sequence in GenBank	ITS1 identical sites	ITS2 identical sites	ITS1+ ITS2 identical sites	Species	Voucher no.	Locality	References
P. giganteus	MFLUCC 23-0016	KP120919, I = 100%*, QC = 99%.	234/234	243/243	477/477	*P. giganteus*	MFLU08-1371	Sri Lanka	Karunarathna et al. (2012)
P. giganteus	MFLUCC 23-0016	JQ724360, I = 98.77%*, QC = 97%.	233/234	235/241	468/475	*P. giganteus*	CMU54-1	Thailand	Kumla et al. (2013)

*Identity obtained from Blast search on the entire sequence of the ITS region amplicon.

Table 4 Effect of different types of spawn media on mycelia growth rates (mm/day) of *P. giganteus*.

Spawn media	Mycelial growth rate	Mycelial density
Millet	11.10 ± 0.34^{a}	Compacted
Rice paddy	4.02 ± 3.14^{b}	Thin
Rice berry	1.21 ± 1.26^{cb}	Lightly compacted
Sorghum	9.02 ± 0.4079^{a}	Compacted
Wheat	1.86 ± 2.21^{cb}	Lightly compacted

Effect of soil casing

Primordia appeared after the soil casing was applied for 14 days with a 7-day cycle at 30.8 ± 1.17 °C, 95% of humidity and under illuminated conditions. The effect of soil casing with three different formulas which add various degrees of nutrient supplementation on total fresh weights and the number of fruiting bodies of *P. giganteus* is shown in (Table 6). Each formula did not result in significant differences for the average weight of the fruiting bodies. However, the first formula (T1) yielded the highest fresh fruiting bodies weights at 218.35 ± 92.53 g and the number of fruiting bodies at 44; followed by the third formula (T3) which resulted in fresh fruiting bodies weights of 278.54 ± 89.04 g with 48 fruiting bodies; and the second formula (T2) with the lowest number of fruiting bodies at 137.28 ± 67.42 g with 25 fruiting bodies.

Nutritional analysis

The nutritional composition of *P. giganteus* was determined including the content of ash, carbohydrates, fat, fiber, moisture and protein of dried mushrooms grown on a sawdust substrate. (Table 7) shows the nutritional values in 100 g of dried *P. giganteus* which included the following: ash content was 5.61 ± 0.9%, carbohydrates content 61.32 ± 0.0 (%/100 g), fat content was 2.98 ±

1.5 8 (g/100 g), fiber content was 14.28 ± 0.3 (%/100 g), moisture content was 15.6 ± 0.5 (g/100 g), and protein content was 20.318 ± 0.8 (g/100 g).

Table 5 Effect of different types of substrates on fruiting body growth rates of *P. giganteus.*

Treatment (w/w)	Total number of fruiting bodies	Total fresh weight (g)	Average fruit weight (g)	Biological efficiency (%)
Sawdust	35	180.77 ± 44.41[a]	51.13 ± 13.25[a]	42.20 ± 10.36[a]
Sawdust + corn cobs	29	176.60 ± 17.65[a]	44.13 ± 4.41[a]	45.45 ± 4.54[a]
Sawdust + rice straw	21	35.30 ± 14.15[b]	10.12 ± 2.14[b]	11.51 ± 4.61[b]
Sawdust + bagasse	21	34.52 ± 11.29[b]	14.14 ± 3.33[b]	10.55 ± 3.45[b]

Table 6 Effect of soil casing with nutrient supplementation on the production of fruiting bodies of *P. giganteus*.

Treatment	Total number of fruiting bodies	Total fresh weight (g)	Average fruit weight (g)
T1	44	278.54 ± 89.04[a]	78.13 ± 28.1
T2	25	137.28 ± 67.42 [b]	76.11 ± 16.18
T3	48	218.35 ± 92.53[ab]	49.75 ± 7.16

Table 7 Comparing the proximate nutritional values of some *Pleurotus* species.

Species name	Ash (%)	Carbohydrates (%)	Fat (g/100 g DW)	Fiber (%)	Moisture (g/100 g FW or DW)	Protein (g/100 g DW)	References
P. cornucopiae (Paulet) Quél.	6.58 ± 0.0	71.05 ± 0.0	1.30 ± 0.0	N/ A	9.07 ± 0.0	11.08 ± 0.0	Landingin et al. (2021)
P. cystidiosus O.K. Mill.	6.30 ± 0.00	N/ A	2.05 ± 0.0	20.05 ± 0.0	91.13 ± 0.0	15.68 ± 0.0	Hoa et al. (2015)
P. djamor (Rumph. ex Fr.) Boedijn	0.87 ± 0.22	N/ A	0.17 ± 0.01	3.10 ± 0.24	90.15 ± 0.27	0.12 ± 0.0	Zurbano et al. (2017)
P. eryngii (DC.) Quél.	5.99 ± 0.08	N/ A	3.4 ± 0.1	N/ A	9.47 ± 0.08	16.2 ± 0.3	Rodrigues et al. (2015)
P. florida Singer	8.69 ± 0.09	42.83 ± 2.54	2.4 ± 0.40	12.25 ± 0.30	89.23 ± 0.10	27.89 ± 0.23	Alam at al. (2008), Prasad et al. (2018)
P. giganteus	**5.61 ± 0.9**	**61.32 ± 0.0**	**2.98 ± 1.5**	**14.28 ± 0.3**	**15.6 ± 0.5**	**20.31 ± 0.8**	**This study**
P. giganteus	N/ A	64.7 ± 0.0	3.10 ± 0.0	32.45 ± 0.07	N/ A	19.2 ± 0.0	Phan et al. (2018)
P. ostreatus	10.91 ± 1.22	37.8 ± 2.5	2.18 ± 0.21	10.41 ± 1.84	8.45 ± 1.65	33.5 ± 0.22	Cohen et al. (2014), Tolera & Abera (2017)

FW, fresh weight; DW, dry weight; N/A, no data available; This study is designed in bold.

Discussion

Comparing the morphology of *P. giganteus* strains MFLUCC23-0016 with other *P. giganteus* strains reported on from different countries, such as the strains MFLU08–1371 from Sri Lanka described by Karunarathna et al. (2012), the strains of *P. giganteus* CMU54–1 from Thailand by Kumla et al. (2013), and the strains of *P. giganteus* HNL501302 from Laos by Phonemany et al. (2021), the characterization of MFLUCC23-0016 fit well with these reports which was further confirmed by molecular analysis.

Pleurotus giganteus has been used as a medicinal substance and source of food and income for local people (Mortimer et al. 2012, Mortimer et al. 2014, Valverde et al. 2015). Therefore, studying and developing approaches to optimal cultivation is critical. Our results show that the mycelium growth rate was highest with the compacted mycelium density of *P. giganteus* in millet and sorghum. For other types of media grain, such as rice paddy, growth occurred but the mycelium density was thin. For rice berry and wheat grain the results did not suggest optimal use based on the mycelium growth of *P. giganteus*. Our results suggest that with rice berry and wheat grain the mycelium growth rate was very low with lightly compacted mycelium density, which agreed with Jayachandran et al. (2017) who reported that wheat grain was comparatively less effective for the production of *P. florida*. The effect of grain media for spawn production was similar to that of Klomklung et al. (2012) and Kumlar et al. (2013) who similarly reported the best substrate for spawn production of *P. giganteus*. Thulasi et al. (2010) reported that sorghum grain was the optimal substrate for spawn production of *P. eous* and *P. florida*. Agriculture wastes for fruiting bodies production, such as corn cob, rice straw, sugarcane bagasse, could be used as an alternative to mixing with sawdust for cultivated *P. giganteus.* The fruiting bodies product being dependent on the type of substrates that contained different percentages of lignin, cellulose and hemicellulose used for its cultivation, in agreement with Thongklang & Luangharn (2016) who reported using agricultural wastes for the production of *P. ostreatus*. Furthermore, according to previous studies *P. giganteus* was first grown on sawdust in northern Thailand at the laboratory scale, by Klomklung et al. (2012, 2014), Kumla et al. (2013), and Soytong & Asue (2014). The soil casing is important for the cultivation of *P. giganteus* to obtain a higher mushroom product by applying soil on top of the substrates and our study reported that three soil casing formulas could be used for the fruiting products. However, 25% humus soil mixed with 25% calcium carbonate, 35% lime sand, 15% peat obtained a higher product compared to the total wet weight of the fruiting product from 100% humus soil, and without humus soil mixed with 50% lime sand, 25% calcium carbonate, 25% peat. Based on our results on the effect of the soil casing, we suggest that 25% humus soil + 25% calcium carbonate + 35% lime sand + 15% peat be used as the soil casing. Furthermore, based on the result of this study, it was found that 100% humus soil could be an alternative to the soil casing and 50% lime sand + 25% calcium carbonate + 25% peat could also be used due to its good air permeability, but could negatively impact the ability to retain much moisture. This study agrees with Soytong & Asue (2014) who used ordinary garden soil as the main casing material and reported garden soil supplemented with 1% nano-KS1. Additionally, reported by Qin et al. (2016) who tested seven types of soil casing used for cultivated *P. giganteus* (named as *Panus giganteus*) such as sugarcane filter mud, mushroom fermentation residue, paddy soil, yellow clay soil, honeycomb and cinder soil, spent straw mushroom substrate and water-soaked rotten straw.

The results of the proximate of *P. giganteus* in sawdust substrates were found to have a very low amount of fat but were rich in carbohydrates, protein, moisture, and ash content with a large amount of fiber. Our study had similar results to previous studies by Landingin et al. (2021) who reported the approximate mass of *P. cornucopiae,* which had a large amount of carbohydrates, protein, ash, and moisture content, with a lower fat content. The comparison of the proximate nutritional values of some species of *Pleurotus* is shown in (Table 7). Phan et al. (2018) who reported a review of the proximate of *P. giganteus* found that the amount of fiber and fat was higher (32.45 ± 0.07%, 3.10 ± 0.0%, respectively) than our strains (14.28 ± 0.3%, 2.98 ± 1.5%); the protein contents of our strains were determined to be higher (20.31 ± 0.8 g/100 g) than previously reported (19.2 ± 0.0 g/100 g); the carbohydrates in this study (61.32 ± 0.0%) can be compared with Phan et al. (2018) at (64.7 ± 0.0%). The ash content of our study was 5.61%, which is relatively low compared to other *Pleurotus* species in (Table 7), e.g., *P. ostreatus* (10.91 ± 1.22%) (Cohen et al. 2014, Tolera & Abera 2017); *P. florida* (8.69 ± 0.09%) (Alam et al. 2008, Prasad et al. 2018); and *Agaricus* sp. (10.1–10.90%) (Zakhary et al. 1983). However, nutritional status is affected by several factors, such as the type of mushrooms, the stage of development, the part sampled, the level of nitrogen available, and the location (Flegg & Maw 1977, Kurkela 1972, Motskus 1973).

Mushrooms have been consumed by humans for decades, and nutritional analyses of mushroom species reveal that they are rich in protein, carbohydrates, and dietary fiber with low fat (Cheung 2010, Valverde et al. 2015). The results of the nutritional analyses of *P. giganteus* cultivated found in this study confirmed that this mushroom can be compared with other species of *Pleurotus* (see Table 7) and *P. giganteus* can be an alternative food that provides health benefits. Consequently, the use of agricultural waste has a high potential for mushroom cultivation. Therefore, more study is necessary on how to increase fruiting production by changing the ratio and supplementing for sawdust + corn cob substrates. In addition, the formula for the soil casing changes the percentage of the nutrient supplement, as well as the types of soil.

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