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First successful cultivation and nutritional composition of *Macrocybe gigantea* in Sri Lanka

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Abstract
The wild edible mushroom *Macrocybe gigantea* is widely consumed as one of the prime seasonal delicacies in the tropical and subtropical regions of the world. In this study, *M. gigantea* was identified through morphological and phylogenetic analyses. Spawn production and cultivation parameters of *M. gigantea* were investigated for the first time in Sri Lanka. The mycelial growth was observed in potato dextrose agar medium, while paddy grains were used for spawn production. A mixture containing rubber sawdust (100 kg), rice bran (18 kg), $CaCO_3$ (2.5 kg), gypsum (1 kg), and $MgSO_4$ (0.35 kg) was tested as a substrate for colonization bags. In addition, gene sequence-data, proximate analysis, energy value, and mineral elements of cultivated *M. gigantea* were studied. Mycelia in mushroom growth bags were cultivated under the temperature range of 27–30 °C and relative humidity of 60 %. Three weeks after inoculation, the primordia appeared and it took four additional days until the occurrence of young fruit bodies. A second flush was harvested 3 weeks after the first. Proximate analysis, energy value and mineral element analysis were recorded as 85.3 % moisture, 0.8 % ash, 1.6 % fat, 2.3 % protein, 10.0 % carbohydrate, 0.28 % potassium (K), 0.00064 % iron (Fe), 0.0024 % sodium (Na), and energy 63.6 kcal/100g. This study provides valuable information concerning the cultivation and nutritional composition of *M. gigantea* in Sri Lanka.

Keywords: domestication, fruit body production, mineral element analysis, mycelial condition, proximate analysis, spawn inoculation

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Introduction
Macrocybe gigantea, commonly known as *Tricholoma giganteum*, was reported for the first time in West Bengal, India (Pegler et al. 1998). This species belongs to the family *Tricholomataceae*, which contains a large number of fairly fleshy gilled mushrooms with white spores (Kui et al. 2021). According to the outline of fungi, it is also listed under the family *Biannulariaceae* (Wijayawardene et al. 2020). Species belonging to *Macrocybe* are characterized by white, cream to greyish, or ochraceous, and convex, umbonate to depressed pileus (Pegler et al. 1998). *Macrocybe* has been treated in *Tricholoma* until Pegler et al. (1998) segregated it from *Tricholoma* and ranked it as a genus using distinct morphological and molecular characteristics (Razaq et al. 2016). *Macrocybe* species are widely distributed in tropical regions, worldwide (Pegler et al. 1998). The genus is similar to another edible mushroom genus, *Calocybe*, both having conspicuous large basidiomata. But *Macrocybe* species differ from *Calocybe* in lacking siderophilous granulation in the basidia and in molecular characteristics (Devi and Sumbali 2021).

Macrocybe gigantea is an edible species with many varieties recognized and is cultivated in wild tropical/ subtropical regions of the world. It has a sweet taste and is rich in nutritive components such as proteins, polysaccharides, fat, amino acids, and many mineral elements (Wang et al. 2004). Nutritionally, *M. gigantea* contains 24.1 % protein, 10.2 % carbohydrate, and 5.6 % glycogen in dry weight (Pamitha and Latha 2014). According to Liu et al. (2012), *M. gigantea* contains high levels of mineral elements such as Ca, Mg, and Zn. Besides, it is important primarily due to its flavor but is also considered a healthy supplement to the diet. According to Giri et al. (2013), its nutritive value compares favourably to that of most vegetables. In addition to edibility, *M. gigantea* possesses a number of medicinal properties. It shows excellent antioxidant activity (Gaur and Rao 2016) through the inhibition of lipid peroxidation and superoxide radical scavenging activity (Banerjee et al. 2007). In addition, it shows antibacterial and antitumour activities (Mau et al. 2002, Dai et al. 2009, Giri et al. 2012). Laccase with a novel N-terminal sequence extracted from fresh fruiting bodies of *M. gigantea* inhibited HIV-1 reverse transcriptase with an IC50 of 2.2 l μM (Wang and Ng 2004).

Macrocybe gigantea is an edible mushroom with cultivation potential (Razaq et al. 2016, Devi and Sumbali 2021). Artificial cultivation of this mushroom is still at a minimum level in comparison to other cultivated mushroom species. According to previous studies, this species can easily and successfully be cultivated in wheat straw. Also, the study of Devi and Sumbali (2021) suggested the use of pearl millet (*Cenchrus americanus*) spawn for the quick and successful cultivation of *M. gigantea*. According to Kushwaha et al. (2016), lentil straw is a promising substrate for the cultivation of *M. gigantea. Macrocybe gigantea* is a new record for Sri Lanka. The objectives of this study are to identify Sri Lankan *M. gigantea* using morpho-molecular characteristics and analyze the nutritional composition and its cultivation on compost substrate.

Materials and methods

Sample collection

Fruit bodies of *M. gigantea* (Figure 1) were collected in March 2019 from Kananwila, Horana in Kalutara district of Sri Lanka (6.759505 N, 80.053864 E). The photographs and macromorphological notes were taken *in situ* before the mushrooms were collected and environmental conditions such as the current temperature in the area and relative humidity were recorded. After the macro-characteristics were recorded, the mushroom samples were used for pure culture isolation. Once the culturing work was completed, the mushroom samples were dried in an electric food drier at 40 ℃. The dried mushroom samples were deposited in the fungal herbarium at the Department of Biosystems Technology, Faculty of Technology, University of Ruhuna, Sri Lanka (FUOR).

Figure 1 *Macrocybe gigantea* fruit bodies in the field

Mushroom identification

Examination of morphological characteristics of *M. gigantea* was performed at the FUOR. During the process, the macro-characteristics such as size, shape, and structure of the pileus and stipe were recorded. Colour codes used in the macromorphological description were based on Kornerup and Wanscher (1978). For micromorphological characteristics examination of dried samples, sections of gills were squashed in 5 % KOH and stained with Congo Red for microscopic observation under a Nikon Eclipse Si upright microscope. For microscopic characteristic descriptions, 30 basidiospores and 30 basidia were measured, and the abbreviation Q refers to the length/width ratio of individual basidiospores. The photographs of micro-characteristics were taken with the digital camera (Nikon DS 1000) attached to the microscope.

DNA extraction, PCR and sequencing

The genomic DNA of the mushroom specimens (three specimens) was extracted from dried samples using a Bio-spin Fungus Genomic DNA Extraction Kit, following the manufacturer's instructions (Hangzhou Bioer Technology Co., LTD, Hangzhou, P.R. China). The nuclear ribosomal internal transcribed spacer (ITS) region was amplified using the primer pair ITS1/ITS4 (White et al. 1990). The amplification process was carried out for a total volume of 25 µL comprising 1.0 µL of template DNA, 9.5 µL of double-distilled water, 1.0 µL of each primer (2 µL in total), and 12.5 µL of 2× Power Taq PCR Master Mix solution. The latter consisted of a premixed, ready to use solution that included 0.1 Units/ µL Taq DNA polymerase, 500 µm of dNTP mixture each (dATP, dCTP, dGTP, and dTTP), 20 mM of Tris–HCl pH 8.3, 100 mM KCl, 3 mM of MgCl2, stabilizer, and enhancer. During the polymerase chain reaction (PCR), the sample underwent 35 cycles according to the following settings: denaturation (95 ℃, 30 s), annealing (52 ℃, 30 s), extension (72 ℃, 1 min), and final extension (72 ℃, 10 min). Amplified products were confirmed on a 1 % agarose gel electrophoresis stained with ethidium bromide. The amplified PCR fragments were sequenced at Genetech, Sri Lanka. The nucleotide sequence data obtained in this study were deposited in NCBI-GenBank.

Phylogenetic analyses

The phylogenetic analyses were conducted based on ITS sequence data. The ITS sequence obtained for our strain was subjected to NCBI-BLAST analysis to find out the closely related taxa. The reference nucleotide sequences (Table 1) were retrieved from GenBank based on the recently published data (Razaq et al. 2016, Karlsen-Ayala and Smith 2020). The sequences were initially aligned using MAFFT V.7.036 (http://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2018) and the necessary changes were done manually using Bioedit v.7.2 (Hall 1999). Maximum likelihood analysis (Figure 2) was performed using RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using GTR+I+G model of evolution. Evolutionary models for phylogenetic analyses and Bayesian information criterion were obtained using the jModelTest2 on XSEDE (2.1.6) and MrBayes on XSEDE (3.2.7a) respectively in the CIPRES Science Gateway platform.

Table 1. Names, voucher codes, countries, and corresponding GenBank* numbers of the taxa used in the phylogenetic analyses. (The newly generated sequence is indicated in black bold).

Name	Isolate/voucher	Country	Accession number
Calocybe carnea	CBS552.50	Switzerland	AF357028
C. fallax	5972	Italy	JF907774
C. gambosa	8064	Italy	JF907775
C. ionides	13284	Italy	JF907780
C. ionides	HC77/133	Switzerland	AF357029
C. naucoria	HC80/103	Switzerland	AF357030
Calocybe sp.	GG255_86	Netherlands	GU234094
Calocybe sp.	GG253_86	Netherlands	GU234085
Leucocalocybe mongolica	HMJAU:24942	China	KC413945
L. mongolica	HMJAU:24941	China	KC413944
L. mongolica	HMJAU:24946	China	KC413949
L. mongolica	HMJAU:24945	China	KC413948
L. mongolica	HMJAU:24944	China	KC413947

L. mongolica	HMJAU:24943	China	KC413946
Macrocybe gigantea	R 65	Pakistan	LK932288
M. gigantea	R 43	Pakistan	LK932289
M. gigantea	R 18	Pakistan	LK932290
M. gigantea	R 79	Pakistan	LK932291
M. gigantea	R 89	Pakistan	LK932292
M. gigantea	Lyo	Pakistan	LK932287
M. gigantea	**FUOR0008AGS**	**Sri Lanka**	**OM953523**
M. gigantea	-	India	JN006792
M. gigantea	-	China	EU051917
M. gigantea	SCAU 1	China	JX041888
M. gigantea	SCAU 3	China	JX193694
M. gigantea	Dongguanzhuang	China	JX068527
M. gigantea	SCAU 2	China	JX068526
M. sardoa	29083a	India	MN017542
M. sardoa	KUBOT-KRMK-2020-01	India	MT880333
M. titans	mt3	-	MZ519068
M. titans	-	Italy	LR992036
Tricholoma argyraceum	AF00.242	France	HQ184104
T. argyraceum	AB03.1	France	HQ184103
T. cingulatum	N95210	Netherlands	AF349697
T. myomyces	-	Ireland	AY082607
Tricholomopsis flammula	SR161	Pakistan	FR822742

*The above strains do not have type sequences. However, well-accepted strains from previous studies were used in the phylogenetic analyses.

Mushroom cultivation

Mushroom cultivation was done in the laboratory of Teclink International (Pvt) Ltd.

Pure culture isolation

The field-collected fresh fruit bodies were cleaned using a sterilized brush followed by surface disinfection using isopropyl alcohol (99 % v/v). A small inner part of the pileus tissue was aseptically removed and implanted on the petri dish containing PDA (Himedia MH096, India) inside a laminar flow hood. The composition of PDA was given as potato infusion 200, dextrose (glucose) 20, Agar 15 (Gms/Litre). PDA (40 g) was mixed in 1 liter of distilled water and heated until the PDA powder dissolved in full. After pouring 20 mL/PDA in Petri dishes, they were autoclaved at 140 ℃, 20 psi for 40 minutes. The inoculated dishes were incubated at room temperature of 27–30 ℃ in dark conditions.

Spawn production

Paddy grains were used for spawn production. The paddy grains were bought from a farmer and cleaned well with chlorinated (tap) water. Paddy grains were transferred into a stainless-steel utensil and gypsum was mixed in water well. Two full tablespoons of gypsum were added for 4 kg of paddy grain. Paddy grains were soaked overnight (12 hours). The utensil was heated until the water boiled and the paddy grains were kept inside for 10 minutes. Thereafter, water was drained and paddy grains were spread on a cleaned glass table to dry out until no more moisture was left.

A tissue test was carried out to determine the wetness of the grains (three random samples of one teaspoon of grains were placed on tissue and checked whether the tissue does not get wet). Two hundred grams of paddy grains were then packed in polypropylene autoclavable bag and sterilized at 140 ℃, 20 psi for 3 hours. The grain bags were removed from the autoclave and kept in the laboratory for cooling. Cooled grains were inoculated with a small piece (2 cm × 2 cm) from the fully colonized petri dish and incubated at 27–30 ℃ under dark conditions. The mycelial growth was observed by direct observation and contaminated bottles were removed. The time taken for complete colonization was recorded as 28 days.

Substrate preparation and inoculation

The main substrate was rubber sawdust that was obtained from Horana, Sri Lanka and rice bran was the second ingredient for the substrate, which was purchased from rice mills in the same area. The two ingredients were mixed with $CaCO_3$, Gypsum ($CaSO_4.2H_2O$), and $MgSO_4$ mechanically adding water to the limit of 36 %. The ingredients were mixed according to the below formula; rubber sawdust 100 kg, rice bran 18 kg, $CaCO_3$ 2.5 kg, Gypsum 1 kg, and $MgSO_4$ 0.35 kg. Fifty-seven polypropylene bags were filled with 2.5 kg substrate in each bag, placed in a plastic tray, and loaded into the retort in retort racks. The substrate bags were sterilized in a bio-mass retort for 5 hours at 128 ℃. After 5 hours, the substrate bags were unloaded to the cooling area and kept for 24 hours at room temperature of 27–30 ℃. The bags were inoculated with two teaspoons of grain spawns and incubated at 27–30 ℃.

Fruit body production

After complete colonization, the bags containing the spawned substrates were moved to the growth house at Techlink International (Pvt) Ltd for fruit body production. The bags were monitored regularly and contaminated bags were removed from the incubation area. A dried sterilized casing layer prepared by mixing humus clay with compost bought from an agriculture material shop was laid on the top of the bag for one-inch thickness and saturated with clean water. The temperature of the growth house was ranging from 27 ℃ and 30 ℃ and the room was ventilated with natural air through insect-proof nets. The relative humidity (RH) was maintained between 60 % and 75 % and water was hand sprayed twice per day. The natural light was taken in through the nets. The mycelial development was daily monitored through direct observations until the complete colonization of the substrates. Frequent fungal contaminants such as *Rhizopus* and *Trichoderma* were also checked and the contaminated bags were immediately displaced to a distant shelf to avoid contamination of the neighbouring bags (chain contamination). The primordia were harvested sixteen times within two months when the fully-grown fruiting bodies appeared. The average number of primordia of each bag, the average number of mature fruiting bodies of each bag, and the average total weight of fresh fruiting bodies per bag were recorded. Biological efficiency was calculated using the bellow equation:

Biological Efficiency (BE) = (average total weight of fresh fruiting bodies per bag/weight of dry substrate) × 100 %

Nutritional component analysis

Fruiting bodies of *M. gigantea* (250 g × 3) were washed, cut into pieces and freeze-dried for 6 hr to prepare the sample for analysing the proximate composition and mineral elements. Then the dried samples were ground with a mortar and pestle. The powdered samples were stored in a sterile polythene bag until the nutritional analyses. Nutritional analysis was carried out at IDB-SGS food laboratory, Sri Lanka (Igile et al. 2020).

Moisture, protein, fat, carbohydrates, ash, and energy values were determined according to the Association of Official Analytical Chemists (AOAC 1984) and the Food and Agriculture Organization (FAO 1986) (Igile et al. 2020).

Atomic Absorption Spectrometry (AAS) was used to analyse the iron (Fe), potassium (K), and sodium (Na) composition. A 1 g of the powdered sample was placed in a crucible and ignited in a muffle furnace at 300 ℃ for 6 hours. Then the ashes were allowed to cool at room temperature (30±2.0 ℃). The cooled sample was digested with a mixture of 20 mL 4M HNO_3 and 60 % perchloric acid and diluted with 100 mL of de-ionized water. An aliquot of the diluted sample was analysed for Fe, K, and Na by the atomic absorption spectrophotometric method described by James (1995) and the AOAC (1990) (Igile et al. 2020).

Results

Phylogenetic results

The initial alignment consisted of 839 base pairs. The manual alignment was performed where necessary and final alignment consisted of 786 base pairs with gaps. Phylogenetic tree consists of 36 taxa including the

isolate obtained in this study. The RAxML analysis of the combined dataset yielded a best scoring tree (Figure 2) with a final ML optimization likelihood value of -4663.474955. The matrix had 468 distinct alignment patterns, with 19.10 % of undetermined characters or gaps. Parameters for the GTR + I + G model of the ITS were as follows: Estimated base frequencies; A = 0.250098, C = 0.198260, G = 0.216417, T = 0.335225; substitution rates AC = 1.515320, AG = 2.309057, AT = 1.109127, CG = 0.501654, CT = 3.089857, GT = 1.000000; proportion of invariable sites I = 0.000100; gamma distribution shape parameter α = 0.501320.

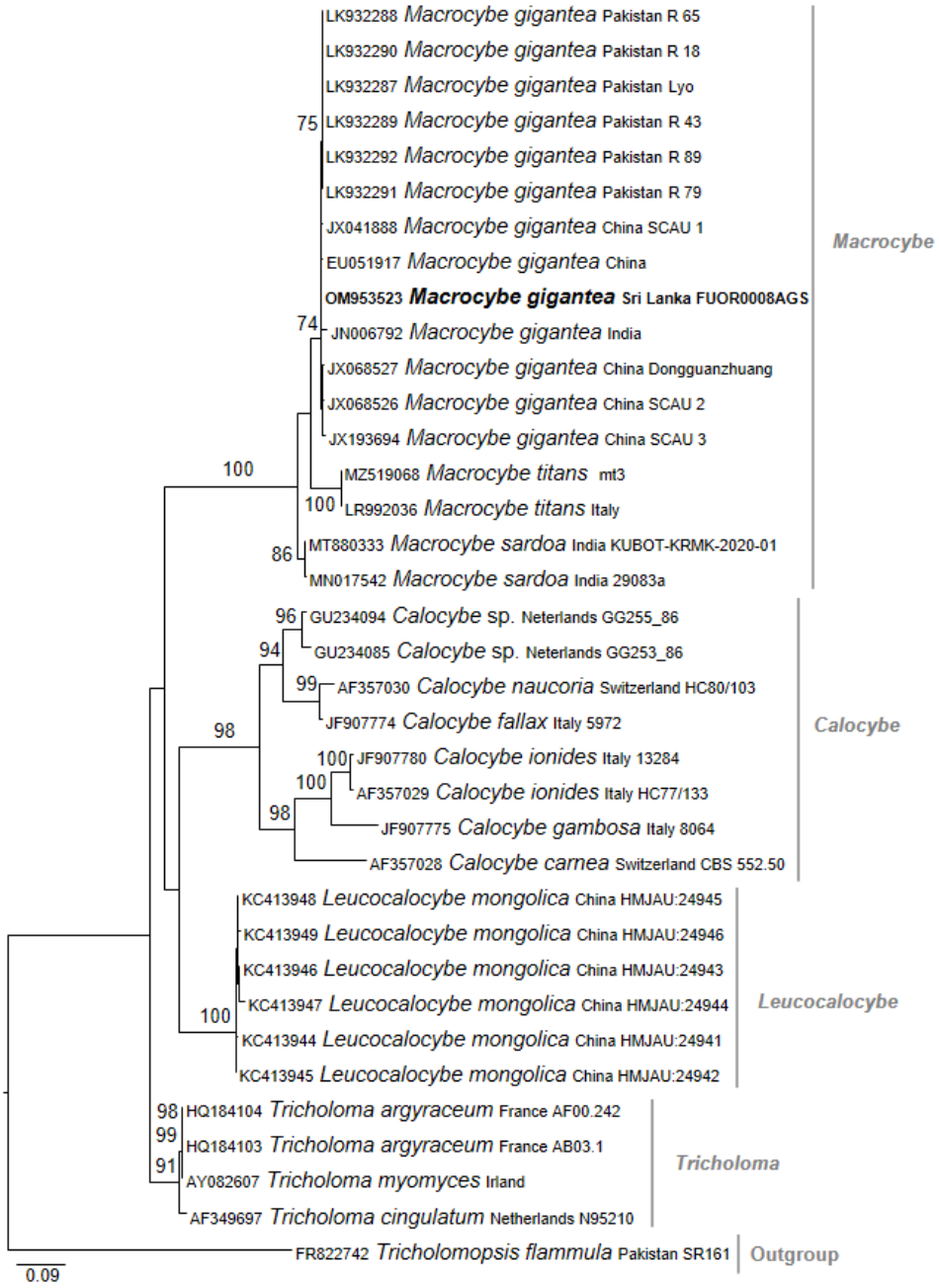

Figure 2 RAxML tree based on ITS sequence data. Bootstrap support values for maximum likelihood equal to or higher than 70 % are displayed on the nodes. The sequence of *M. gigantea* obtained in this study is indicated in black bold. The tree is rooted with *Tricholomopsis flammula* (SR161).

Taxonomy

Macrocybe gigantea (Massee) Pegler & Lodge, in Pegler, Lodge & Nakasone, Mycologia 90(3): 497 (1998) (Figures 1 and 3).

Index Fungorum number: 443590

Macro-morphological characteristics

Fruit bodies medium to large, Pileus; 4–7 cm in diam., convex or hemispheric when young, broadly convex to plano-convex when mature, Surface; smooth, glabrous, pastel yellow (2A4), canary yellow (2B7) to chrome yellow (2A8) when young, topaz brown (5C5) to yellow ochre (5C7) or banana yellow (4B7) at maturity, margin; tightly incurved when young and incurved when mature, lined, concolourous with the surface, smooth and equal, Lamellae; adnexed, yellowish white (2A2) or pale yellow (2A3) when young, light yellow (2A5) or pale yellow (3A3) at maturity, crowded with intermingled lamellulae of 4 different lengths (0.8, 1.2, 2 and 2.3 cm) in a mature fruit body, lamellulae; thick, smooth and moist, context; thick, fleshy, yellowish white (3A2) to pale yellow (3A3), stipe; solid, fleshy, 14–17 × 2.5–4 cm, cylindrical, elongated, clavate with slightly swollen base, stipe surface; smooth, often canary yellow (2A4) to pale yellow (1A3), densely squarrulose closer to the apex and base, Odour; pleasant when young, strong at maturity, taste; mild.

Micro-morphological characteristics

Basidiospores; ellipsoid or slightly ovate, (5.7) 5.7–6.3 (6.22) × (4.6) 4.5–5.3 (5.32) µm, Qaverage=1.18 (n=30) smooth, hyaline or light brown, thin-walled, prominent with oil drops or granules, Basidia; 22.5–18.1 × 4.1–4.9 µm, 4-or occasionally 2-spored, clavate, pinkish brown to hyaline, thin-walled, dense with oil droplets, basal clamp connections present, Cystidia; absent, but hyphal filament projections visible, Metuloids; present, Lamellar edge; fertile, Hymenophoral trama; regular, hyaline, composed of thin-walled, parallel hyphae; Pileipellis; a cutis of narrow hyphae, clamp connections present.

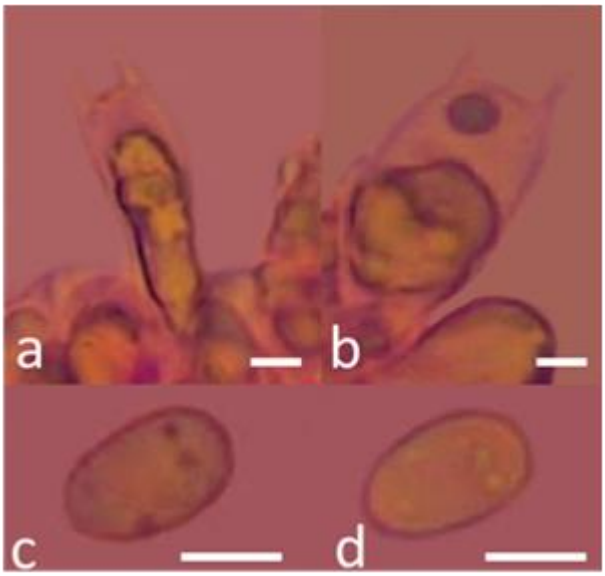

Figure 3 *Macrocybe gigantea* (FUOR0008AGS): a–b basidia, c–d basidiospores (Scale bars: a–b = 0.5 µm, c–d = 5 µm).

Material examined

Sri Lanka, Kananwila, Horana, Kalutara, gregarious, on moist humus soil mixed with rotting wood and decaying leaves, but the soil was heavily covered with fine sawdust**,** March 2019, M. Gamage (FUOR0008AGS).

Ecology, habit, and habitat

Basidiomata of collection used in the current study were found fruiting gregarious, on moist humus soil mixed with rotting wood and decaying leaves, but the soil was heavily covered with fine sawdust. The temperature of the substrate or soil was above 35 ℃ due to artificial covers. The sample was collected at a carpentry workshop in Kananwila. The area was cleaned to build the carpentry shed and the humus soil was covered with sawdust.

Nutrition analysis

Proximate analysis, energy value, and mineral element analysis of *M. gigantea* were recorded as moisture (85.3 %), ash (0.8 %), fat (1.6 %), protein (2.3 %), carbohydrate (10 %), potassium (0.28 %), iron (0.00064), sodium (0.0024 %) and energy (63.6 kcal/100g).

Spawn preparation, inoculation, and fruit body production

During the spawn production process, the grains were fully colonized in 28 days while the inoculated substrate bags were fully colonized in 55 days. Three weeks after casing, the primordia appeared and it took four days for the primordia to become young fruit bodies (Figure 4). A second flush was harvested three weeks after the first harvest. The average number of primordia of each bag was nineteen. The average number of mature fruiting bodies was twelve. The average total weight of fresh fruit bodies Per Bag in the first harvest was 337 g. The average total weight of fresh fruit bodies per bag after harvesting sixteen times was 1078 g. The biological efficiency was reported as 43 %.

Figure 4 Fruit bodies of cultivated *Macrocybe gigantea*

Discussion

Macrocybe gigantea has been recorded from Asian countries such as China, India, Nepal, and Pakistan and there is no other report of this species from the western hemisphere (Razaq et al. 2016). The mushroom specimens collected were confirmed as *Macrocybe gigantea* by both morphological characteristics and phylogenetic analysis. The morphological features of the specimens of this study closely resemble the morphological features of specimens studied by Razaq et al. (2016) in Pakistan. But the pileus of the Pakistani specimens (10–30 cm) (Razaq et al. 2016) and Udaipur, Northern Indian specimen (15–25 cm) (Roy Das et al. 2017) were comparatively larger than the specimen found in Sri Lanka (5–7 cm). The humidity, fresh air, temperature, and compact material are the major environmental factors that affect cap size, stalk diameter, and

stalk height (Schmidt 1983, Stamets 1993, AMGA 2004, Sher 2010). *Macrocybe gigantea* is a saprotrophic mushroom well adapted to occurring under higher temperatures (up to 40–50 ℃) (Razaq et al. 2016). Even the specimens were found at a carpentry workshop on humus soil covered with sawdust, this land was earlier covered with grass, rotting wood, and decaying leaves. Perhaps, mycelia colonized the rotting wood and leaves before placing the carpentry workshop.

The field specimen studied here was found in a well-ventilated area. The differences between the above physical factors may be the cause for the different sizes of the pileus in Pakistan and Sri Lanka. But the average basidiospore size (5.5 × 3.8 μm) and basidia size (27.5 × 7 μm) of the Pakistani specimens closely resemble basidiospore size (5.4 × 3.7 μm) and basidia size (28 × 7.2 μm) of the specimens found in this study. According to Devi and Sumbali (2021), *M. gigantea* requires a relative humidity of 70–80 %, duration of lights 8–10 hours, and temperature between 25–35 ℃ for its optimal growth. The specimens studied here were also found in an area with good sunlight but the relative humidity was lower (60 %) than the relative humidity of 70–80 % mentioned in Devi and Sumbali (2021).

According to Gaur et al. (2016), moisture content is the lowest in *M. gigantea* (82.6 %) when compared with *Agaricus bisporus* (90.9 %), *Calocybe indica* (89.4 %), *Lentinula edodes* (85.7 %), and *Lentinus sajor-caju* (88.7 %). The results of our study also show 85.3 % moisture content in fresh *M. gigantea* which is in accordance with the study of Gaur et al. (2016). Roy Das et al. (2017) also reported 71.13 % moisture percentage from *M. gigantea* found in Udaipur, Northern India. Also, Gaur et al. (2016) reported 16.4 g/100 g of protein in dry weight, but protein content in *M. gigantea* studied here is 2.3 %. According to Gaur et al. (2016), iron (Fe) percentage in *M. gigantea* is 0.0176 % and it is comparatively high in the present specimen (0.00064 %). The energy value in the specimen found in Northeast India was higher (336.81 kcal/100 g) (Roy Das et al. 2017) than measured in *M. gigantea* studied here. Also, according to Roy Das et al. (2017), proximate analysis and mineral element analysis were reported as follows, ash (4.53 g/100g), fat (1.27 g/100g), protein (31.04 g/100g), carbohydrate (52.01 g/100g), and iron (Fe) (0.27 mg/kg) whereas results of proximate analysis, energy value, and mineral element analysis of *M. gigantea* of this study are shown in Table 2.

Mushroom cultivation is an eco-friendly activity but very few studies have been done to find out the suitability of different cereal grains on spawn development and fruit body development of *M. gigantea*. Devi and Sumbali (2021) have tested the time period (in days) required for the spawn production and cultivation of *M. gigantea* on different agro wastes inoculated by different grain spawns. In their study, they prepared the spawn on bajra grain, wheat grain, and maize grain. Thereafter they tested the time period required for spawn run, pinhead formation, and production of the first flush on substrates such as wheat straw and paddy straw.

The substrate used in this study (paddy grains used for spawn development and rubber sawdust 100 kg, rice bran 18 kg, $CaCO_3$ 2.5 kg, gypsum 1 kg, and $MgSO_4$ 0.35 kg mixture used for fruit body production) takes more time for the spawn development and fruit body production respectively in comparison to the substrates used in Devi and Sumbali (2021). According to Inyod et al. (2016), *Macrocybe crassa* was cultivated on a substrate prepared by rubber tree sawdust, fine rice bran, magnesium sulfate ($MgSO_4.7H_2O$) and calcium oxide (CaO) in the ratio of 100:3:0.2:1 (weight per weight) and the proximate analysis of dried samples was reported as protein (13.71 %), carbohydrates (68.08 %), ash (12.06) and fat (2.49 %). Comparatively, *Macrocybe gigantea* in the present study shows a higher protein percentage than Inyod et al. (2016).

When considering the biological efficiency of *M. gigantea*, it is high when paddy straw is used as the main substrate (68.7 %) than wheat straw as the main substrate (66.26 %) (Devi and Sumbali 2021). According to Kushwaha et al. (2016), biological efficiencies (BE) of *M. gigantea* are mentioned as wheat straw - 42.18 %; chickpea straw – 70.12 %; lentil straw – 80.94 %; and lawn grass dry – 51.56 % and the highest BE was recorded from the mixture of wheat straw and lentil straw (81.41 %). According to Devi and Sumbali (2021), fruit body yield depends on both materials used in spawn preparation and substrate preparation.

Macrocybe gigantea is a good source of nutrients (Table 2). It is rich in protein and low-calorie fiber and contains necessary minerals for humans. The commercial cultivation of this mushroom species will be helpful as an alternative vegetarian food for Sri Lankans. The findings of this study are useful in developing an efficient method to cultivate the Sri Lankan strains of *M. gigantea.*

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