



## Domestication potential, acute toxicity, and nutritional properties of a white strain of *Pleurotus djamor* Rumph. ex Fr. (Agaricales, Basidiomycota) from Sri Lanka

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### Abstract

The industrial mushroom diversity in Sri Lanka lies within a narrow range; therefore, it is necessary to introduce novel mushroom species to the industry. *Pleurotus djamor* Rumph. ex Fr. strain PL01-White is a white local mushroom in Sri Lanka with a relatively larger pileus than the commercial strain and is considered a delicacy by locals. In the present study, we domesticated and optimized the cultivation conditions for this mushroom. This mushroom can be successfully cultivated in rubber sawdust with 65.5% moisture, 10% rice bran (w/w), 2.5% CaCO<sub>3</sub> (w/w) and 0.2% MgSO<sub>4</sub> (w/w). These mushrooms are nutritionally important, having a crude protein content of 26.038±0.183% (of dry weight) and 37.5±2.0% crude fibre content (of dry weight). The mineral content of the mushroom was analysed via Inductively Coupled Plasma-Mass Spectrometry. Potassium (K) is the most abundant macroelement (18950 ± 7.0 mg/kg), while Zn is the most abundant microelement (85.0 ± 0.85 mg/kg). The heavy metals, As, Pb, and Hg contents were identified as lower than those recommended by Codex Alimentarius. The *in vitro* cytotoxicity of the boiled mushroom extract (LC<sub>50</sub> – 0.0460 g/mL) determined by the *Artemia* toxicity assay was lower than that of the raw mushroom extract (LC<sub>50</sub> – 0.0003 g/mL).

**Keywords** – Cytotoxicity – Mushroom domestication – Nutritional content – Optimization – Oyster mushroom

### Introduction

In Sri Lanka, there is a large diversity of mushrooms, however only a few are used for culinary purposes and are rarely used for medicinal purposes. There is a high demand for the few wild edible species already consumed by locals due to the characteristic taste, texture, and

fragrance of those mushrooms. Although there is good demand for mushrooms as nonanimal origin protein sources in Sri Lanka, the market supply of cultivated or wild-picked mushrooms is inadequate to satisfy this demand. Only a few mushroom species, such as *Volvariella volvacea* (Pluteaceae), *Agaricus bisporus* (Agaricaceae), *Pleurotus ostreatus* (Pleurotaceae), *P. cystidiosus*, *P. sajor-caju*, *P. djamor*, and *Calocybe indica* (Lyophyllaceae) are cultivated commercially, novel species need to introduce into to the market. Therefore, there is substantial potential for the domestication of wild edible mushrooms in the country and for cultivation under artificial conditions to meet demand.

The United Nations Development Programme (UNDP) introduced commercial mushroom cultivation to Sri Lanka in 1985 (Rajapakse 2014), during which only *Volvariella volvacea* (straw mushroom) was cultivated from paddy straw. The Sri Lanka Export Development Board pioneered the establishment of facilities for mushroom spawn production in Sri Lanka (Rajapakse 2014, Karunarathna et al. 2017). *Pleurotus ostreatus* is the first introduced mushroom species (Rajapakse 2014) and currently, *P. cystidiosus* and *P. eous* have become popular among mushroom farmers (Karunarathna et al. 2017), while *P. ostreatus* remains the leading species. Subsequently several mushroom species such as *P. sajor-caju* and *P. djamor* were introduced to the mushroom industry in Sri Lanka, and several attempts to introduce novel species have been recorded.

Sri Lanka is a tropical island nation and due to its isolation as an island and its tropical climate, the biodiversity of the country is remarkable. Along with all other living organisms, fungal diversity in Sri Lanka is comprehensive, with a prediction of inhabiting more than 25000 species (Karunarathna et al. 2017). Among those, only about 2000 were taxonomically recorded, while some have been identified recently (Li et al. 2016). The most popularly consumed wild edible mushrooms in Sri Lanka are *Termitomyces* species which include *Termitomyces microcarpus*, *Termitomyces eurhizus*, *Termitomyces heimii*, *Termitomyces cartilaginous* and *Termitomyces srilankensis* (Pegler & Vanhaecke 1994, Ediriweera et al. 2023). However, these mushrooms can be collected only in the wild, and commercial cultivation is ineffective or unsuccessful due to the commensalistic relationship between termites and the fungus (Rahmad et al. 2014). A few cultivation trials have been reported with successful results (Rouland-Lefèvre & Bignell 2002, Gong & Guan 2020, Ahmad et al. 2021). In addition, several other wild mushroom species, such as *Auricularia* sp., *Tremella* sp., *Pleurotus* spp., *Volvariella volvacea*, *Schizophyllum commune*, *Coprinus* sp., *P. giganteus*, *Macrocybe gigantea*, *Lentinus squarrosulus*, *Lentinus sajor-caju*, *Flammulaster fulvoalbus*, *Laetiporus sulphureus*, and *Pleurotus tuber-regium* are consumed.

Although many wild edible mushrooms occur in Sri Lanka, only a few have been domesticated. Domestication of the wild mushroom species in Sri Lanka was initiated several decades ago by Udugama & Wickramaratna (1991). In this study, they successfully domesticated milky mushrooms (*Calocybe indica*) and black ear mushrooms (*Auricularia* sp.). *Pleurotus giganteus* is one of the delicacies of Sri Lankan cuisine and is a comparatively large mushroom (Karunarathna et al. 2012). With these characteristics, this mushroom has a high potential for domestication, and several studies have been conducted to domesticate this mushroom (Udugama & Wickramaratna 1995, Namalee et al. 2016). Bandaranayake et al. (2011) attempted to domesticate a medicinal mushroom, *Ganoderma lucidum*, for the first time in Sri Lanka. Miriyagalla et al. (2022) investigated the cultivation potential of three Sri Lankan wild edible mushroom species, *Lentinus sajor-caju*, *Lentinus squarrosulus*, and *P. tuber-regium*.

Although there are more than a thousand species of edible mushrooms worldwide, only approximately 130 species have been domesticated (Thawthong et al. 2014). Specifically, saprophytic mushrooms were domesticated, but some symbiotic mycorrhizal mushrooms were also cultivated semi-industrially (Lakhanpal 1995, Halling 2006, Morte & Andrino 2014, Reyna & Garcia-Barreda 2014, Morte et al. 2021). In addition, there is much evidence for the cultivation of some parasitic (Liu et al. 2019) and other obligate symbiotic mushroom species (Gyu 2015, Ahmad et al. 2021).

Furthermore, mushrooms are an affordable and sustainable source of protein that provides a strong substitute for animal proteins. In addition to having a high nutrient content, mushrooms are

produced at a significantly cheaper cost than typical animal products. The introduction and cultivation of new mushroom species are particularly favourable from an economic perspective.

The *P. djamor* mushroom is a white strain found in Sri Lanka and is considered a culinary delight. In the present study, we identified the domestication possibility of this mushroom strain while optimizing laboratory culture, spawn preparation, and cultivation. The nutritional quality and mineral content of the mushroom were also assessed to validate its nutritional aspects. The cytotoxicity of the mushroom was also identified to confirm its edibility.

## Materials & Methods

### Isolation of the pure culture

*Pleurotus djamor* strain PL01-White was collected from Weddagala, Kalawana, Sri Lanka (6.475418 °N, 80.415525 °E). Mycelia were isolated on Benomyl containing Agar (BA) added with 50 µg/mL gentamycin, which was prepared based on the methods described by Carey & Hull (1989) and Russell (1956). The surface of the mushroom fruiting body was wiped with 70% ethanol, and a piece of internal tissue was subsequently inoculated onto the medium and incubated at 25°C until mycelial growth was observed. The pure cultures of the mushrooms were obtained by continuous sub-culturing and hyphal tipping, following the method of Brown (1924). The cultures were deposited at the Department of Botany, University of Sri Jayewardenepura, Nugegoda, Sri Lanka.

### Morphological identification of the mushroom species

Tentative identification of the selected wild mushroom species was performed by analysing the morphological characteristics using keys described by Arora (1986) and Pegler (1986). The macromorphological characteristics were initially studied and the micromorphological characteristics of gills, spores and mycelia were observed through the microscope. The Q-values of the spores were calculated using the method described by Høiland (2012).

### Molecular identification of the mushroom species

DNA was extracted from fresh mushroom mycelia using the protocol by Guo et al. (2000) with slight modifications. First, approximately 500 milligrams of mushroom mycelia were ground with approximately 500 µL of 4X cetyltrimethylammonium bromide (CTAB) buffer, and the resulting extract was incubated at 65°C for approximately one hour with occasional shaking. Then approximately 500 µL phenol: chloroform: isoamyl alcohol at 25:24:1 was added to the extract, which was vigorously shaken for approximately 2 minutes until an emulsion formed, and the upper aqueous layer was obtained after centrifugation at 13,000 rpm for 15 min. Next, a volume of ice-cold iso propyl alcohol equivalent to two third the volume of the extract was added to the same tube, and the mixture was mixed by inverting a few times and placed in an ice bath for 15 min. The sample was centrifuged again at 13,000 rpm for 7 min, after which the pellet was obtained. After that, 200 µL of freshly prepared 70% ethanol was added to the sample, and the tubes were gently inverted and subjected to centrifugation at 13,000 rpm for 7 min to obtain the pellet.

For DNA barcoding, the universal fungal barcoding region nuclear ribosomal Internal Transcribed Spacer (ITS) region (nc ITS1-5.8S-ITS2 rDNA sequence) (Schoch et al. 2012, Badotti et al. 2017) was amplified using PCR. The protocol of Manamgoda et al. (2012) was followed for PCR, with slight modifications. The nuclear ribosomal ITS region was amplified using the PCR primers ITS1 and ITS4 (White et al. 1990). The amplification program included an initial denaturation step at 95°C for 3 min; 30 cycles of amplification (denaturation at 94°C for 30 s, primer annealing at 52°C for 30 s, and extension at 72°C for 1 min); and a final extension at 72°C for 10 min. The resulting PCR product was subsequently sequenced using the Sanger sequencing method.

The low-quality regions of the sequencing result data were trimmed using the software Unipro UGENE v43.0 (Okonechnikov et al. 2012), and other necessary adjustments

were made using Sequence Manipulation Suite version 2 (Stothard 2000). The contig assembly of the ITS sequences was performed using the CAP3 Contig Assembly programme (Huang & Madan 1999). The resulting sequences were subsequently used as queries for the BLAST® tool (Zhang et al. 2000, Okonechnikov et al. 2012) to identify the potential target species of the mushrooms. Type sequences were selected as the target; if they were unavailable, highly similar sequences were used as the target (MegaBLAST [https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE\_TYPE=BlastSearch&LINK\_LOC=blasthome]). The species of the mushroom was identified based on sequence similarity, as described by Raja et al. (2017).

Phylogenetic placement of the studied mushroom species was performed by phylogenetic analyses of the generated ITS sequences with selected ITS sequences of several closely related species from the GenBank database (Table 1) (Benson et al. 2012) based on studies by Barbosa et al. (2018) and Razaq et al. (2016). The necessary adjustments to the obtained sequences were made using the software Unipro UGENE v43.0 (Okonechnikov et al. 2012), and the sequences were aligned using ClustalW v2.0 (Larkin et al. 2007). Using the software MEGA v11 (Tamura et al. 2021), maximum likelihood phylogenetic analysis (Huelsenbeck & Crandall 1997) was performed using the bootstrap method test of phylogeny (Felsenstein 1985) and Kimura's 2-parameter model (Kimura 1980) with 1,000 bootstrap replicates.

**Table 1** GenBank accessions of the nrITS regions used in the phylogenetic placement of the *Pleurotus djamor* strain PL01-White

Species	GenBank accession	Reference
<i>P. albidus</i>	KF280334.1	Menolli et al. (2014)
<i>P. albidus</i>	KF280333.1	Menolli et al. (2014)
<i>P. citrinopileatus</i>	KX688471.1	Kawai et al. (2008)
<i>P. citrinopileatus</i>	MN546044.1	Menolli et al. (2014)
<i>P. cornucopiae</i>	DQ342325.1	Hebin et al. (2006)
<i>P. cystidiosus</i>	MH862689.1	Vu et al. (2019)
<i>P. cystidiosus</i>	NR_103594.1	Schoch et al. (2014)
<i>P. djamor</i>	KX061789.1	Li et al. (2020)
<i>P. djamor</i>	MN546045.1	Li et al. (2020)
<i>P. djamor</i>	KF280326.1	Menolli et al. (2014)
<i>P. djamor</i>	KF280324.1	Menolli et al. (2014)
<i>P. djamor</i>	KJ754107.1	Otieno et al. (2015)
<i>P. djamor</i>	KJ754109.1	Otieno et al. (2015)
<i>P. djamor</i>	KX836373.1	Li et al. (2017)
<i>P. djamor</i>	KX061789.1	Li et al. (2017)
<i>P. djamor</i>	KJ754115.1	Li et al. (2017)
<i>P. eryngii</i>	KX836357.1	Li et al. (2020)
<i>P. ferulaginis</i>	NR_158885	Zervakis et al. (2014)
<i>P. floridanus</i>	KX836149.1	Li et al. (2017)
<i>P. giganteus</i>	KY951477.1	Avin et al. (2017)
<i>P. giganteus</i>	MH233962.1	Fui et al. (2018)
<i>P. giganteus</i>	MH233963.1	Fui et al. (2018)
<i>P. nebrodensis</i>	KU612943.1	Zhao et al. (2016)
<i>P. nebrodensis</i>	KU612942.1	Li et al. (2020)
<i>P. nebrodensis</i>	HM998835.1	Zervakis et al. (2014)
<i>P. ostreatus</i>	NR_163515.1	Unpublished type material
<i>P. ostreatus</i>	AY450345.1	Petersen & Krisai-Greilhuber (1996)
<i>P. sajor-caju</i>	AM110142.1	Smith et al. (2006)

**Table 1** Continued

Species	GenBank accession	Reference
<i>P. tuberregium</i>	AF109978.1	Li et al. (2017)
<i>P. tuoliensis</i>	KU612910.1	Zhao et al. (2016)
<i>P. tuoliensis</i>	KU612911.1	Zhao et al. (2016)

### Identification of the optimum culture conditions

The optimum growth temperature for mushroom mycelia was determined by culturing them in artificial culture media and mycelial growth was measured by incubating them at different temperatures ranging from 15°C to 35°C. The growth of the mycelia was then measured in eight directions from the agar block to the mycelial front at two-day intervals until the mycelia fully covered the culture plate. The mean mycelial growth rates were calculated, and the significant differences between the means were identified for selecting the optimum temperature using Tukey's pairwise comparison post-hoc test with one-way Analysis of Variance (ANOVA) using Minitab 17 Statistical Software.

Similarly, *P. djamor* strain PL01-White mushrooms were inoculated on PDA at various pH values ranging from 4.0 to 10.0. The growth of the mushrooms was measured, and the mean growth rates at each pH were compared to identify the optimum pH for growth. The *P. djamor* strain PL01-White mushroom was cultured on various culture media; ½ Potato Dextrose Agar (½ PDA), Benomyl containing Agar (BA), Corn Meal Agar (CMA), Malt Extract Agar (MEA), Oat Meal Agar (OMA), Potato Dextrose Agar (PDA), Peptone Dextrose Yeast extract Agar (PDYA), Potato Dextrose Sucrose Agar (PDSA) and Sabouraud Dextrose Agar (SDA).

### Identification of the optimum conditions for spawn preparation

To determine the optimum moisture content of spawn media for the growth of *P. djamor* strain PL01-White mushrooms, they were cultured on grain media with varying moisture contents based on the dry weight of the grains. The protocol outlined by Chang & Miles (2004) was utilized with some modifications to spawn production.

Paddy seeds (*Oryza sativa*) were washed to remove any dust, debris, and floating seeds and were subsequently sun-dried for two days. The dried seeds were then oven-dried at 105°C for 6 hours. Different masses of dry seeds were mixed with varying amounts of tap water to obtain moisture contents of 30%, 40%, 50%, 60%, and 70%, and left to soak overnight. The next day, the seeds were boiled in a closed container using a water bath until the seeds were split and cooked. Next, the seeds were mixed with CaSO<sub>4</sub> and CaCO<sub>3</sub> in proportions of 2% and 0.5%, respectively, of the initial dry weight of the seeds (Gupta & Sharma 1994). The bottles were filled with grains up to the neck, and the mouths were covered with cotton plugs and aluminium foil and sterilized by autoclaving. Next, each bottle was inoculated under aseptic conditions with a 1 cm<sup>2</sup> piece of mycelial advancing margin from the *P. djamor* strain PL01-White culture. The bottles were then incubated at 25°C.

The mycelial growth of *P. djamor* strain PL01-White was measured in grain media with different moisture contents, from the point of inoculation to the mycelial front on four sides of the bottle, at three-day intervals. Growth rates were calculated, and the means of the growth rates were compared using Tukey's pairwise comparison of one-way ANOVA with Minitab 17 Statistical Software to determine the moisture content leading to the fastest mycelial growth rate.

Similarly, spawn media were prepared using different types of grains to determine the most suitable grain type for mycelial growth. The mushroom was inoculated in triplicate on spawn bottles prepared as previously described; the moisture content was 30%. Sorghum (*Sorghum bicolor*), Finger millet (*Eleusine coracana*), Cowpea (*Vigna unguiculata*), Chickpea (*Cicer arietinum*), Paddy (*Oryza sativa*), Maize (*Zea mays*), Wheat (*Triticum* sp.) and Mung beans (*Vigna radiata*) were used. The growth rates of mycelia on grain media were calculated, and the means were compared using a post-hoc test, Tukey's pairwise comparison of one-way ANOVA, with the

aid of Minitab 17 Statistical Software to identify the most suitable grain type that led to the highest mycelial growth rate.

To determine the optimum  $\text{CaCO}_3$  and  $\text{CaSO}_4$  contents for the maximum growth rate of the mushroom spawn production, the grain spawns were supplemented with varying amounts of these compounds and the same procedure was followed as above.

### Determination of the shelf life of spawns

The shelf life of the spawn produced using the optimized media was determined by evaluating the initiation and growth rate of the mushroom mycelia. The spawns were stored at two temperatures, 25°C and 4°C, and a small amount of spawn seeds were cultured in the selected optimum laboratory culture media at one-month intervals until the viability of the spawn was lost, which was indicated by the lack of growth of the mycelia in culture media. The growth characteristics and rate of the re-cultured mushroom on laboratory culture media were determined in triplicate.

### Identification of optimum cultivation conditions

To determine their cultivability, the mushrooms were initially cultivated on Rubber (*Hevea brasiliensis*) substrate media with 60% moisture, which was prepared according to the composition shown in Table 2.

**Table 2** The ratio of components used to prepare the sawdust based substrate medium

Component	Amount mixed
Saw dust (dry)	100 kg
Rice bran	10 kg
$\text{CaCO}_3$	2.5 kg
$\text{CaSO}_4$	1 kg
$\text{MgSO}_4$	200 g

The ingredients were mixed well and then filled into polypropylene bags with a thickness of 230  $\mu\text{m}$  and dimensions of 35 × 18 cm. A neck ring was then fixed to the mouth of the bag, and a filter cap with an air-transferable sponge filter was applied. The bag was sterilized by steaming at 100°C in a 200 L steel drum for approximately 3 h (Tonomura 1978, Nguyen 2004, Kortei et al. 2018). The substrate bags were then inoculated with 5–10 g of grain spawn inoculum under aseptic conditions, covered with a filter cap, and incubated in an incubation chamber at 25±2°C until the mycelia fully colonized the substrate medium. Sufficient ventilation was provided to maintain a constant atmospheric  $\text{CO}_2$  concentration (Stamets 2000) of 410–460 ppm and daytime illuminance was maintained at 0–10 lx inside the incubation chamber to suppress fruiting initiation during incubation (Eger-Hummel 1980).

After fully colonizing the bags with mycelia, they were transferred to the fruiting chamber. The bags were arranged according to a randomized block design (Dodge 2008). The relative humidity inside the fruiting chamber was maintained at 95–99% by using an ultrasonic mist and a water spraying system through a pressure pump. The  $\text{CO}_2$  concentration inside the fruiting chamber was maintained at approximately 400–470 ppm by providing sufficient ventilation, and the daytime illuminance was maintained at 50–100 lx.

The mushrooms were monitored from the initiation of primordia to the degradation, to determine the optimum harvesting stage. The stage that exhibited the maximum growth and sporulation initiation was identified as the optimum stage for harvesting.

The mushrooms were harvested, and their weight was recorded for each bag. Harvesting continued for 3–5 months, and the mean yields were compared for significant differences using Tukey's pairwise comparison of one-way ANOVA, with a significance level of 0.05, utilizing

Minitab 17 Statistical Software. When the ANOVA conditions were not satisfied, non-parametric pairwise comparisons were conducted using SPSS Statistics software version 21.0.

The biological efficiency of the mushrooms was calculated using Equation 1 (Chang, 1993).

### Equation 1

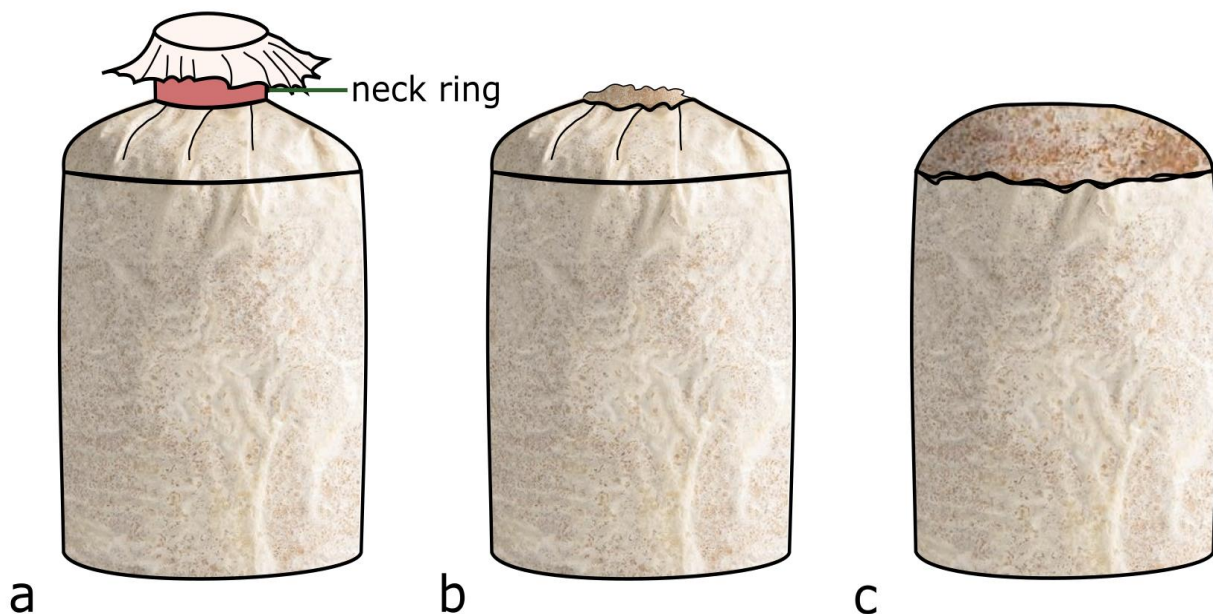
$$\text{Biological efficiency} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of the substrate}} \times 100\%$$

To identify the optimum biological efficiency, substrate media bags with different moisture contents of 50%, 56.5%, 61.5%, 65.5%, and 69.7% were prepared, and they were 900 g, 1,000 g, 1,100 g, 1,200 g, and 2,300 g, respectively. In all the samples, the amount of water was varied while keeping the amount of sawdust constant. Five replicates were prepared for each moisture content.

Substrate media bags with different amounts of rice bran were prepared to identify the optimum rice bran content for cultivation. Five replicates of substrate bags were prepared for each rice bran, with amounts ranging from 5 to 25 kg per 100 kg of dry saw-dust and one set was prepared without adding rice bran.

Substrate media bags were prepared with varying amounts of  $\text{CaCO}_3$  to determine the optimum content for cultivating *P. djamor*. The tested ratios were 0.0 kg, 1.5 kg, 2.5 kg, 3.5 kg, 4.5 kg and 5.5 kg of  $\text{CaCO}_3$  per 100 kg of dry saw dust, with five replicates each. Additionally, one set was prepared without adding  $\text{CaCO}_3$ .

Different opening techniques were tested to determine the most effective bag opening method for maximizing mushroom yields. *Pleurotus djamor* strain PL01-White bags were opened in the fructification chamber using three different methods (Fig. 1). The first method involved removing only the filter cap while leaving the plastic neck ring intact. The second method involved cutting the polypropylene bag around the base of the plastic neck ring, and the third method involved removing the entire circular face of the substrate bag.



**Fig. 1** – Different bag opening methods for fructification of the *Pleurotus djamor* strain PL01-White. a Obtaining mushrooms through the plastic neck ring after removing the filter cap. b Removing the polypropylene bag at the neck of the substrate bag. c Removing the whole top circular face of the substrate polypropylene bag.

Substrate media bags of different sizes were prepared described previously in Table 3, to determine the optimum size for cultivating *P. djamor* strain PL01-White.

**Table 3** Different substrate bag sizes tested to identify the optimum size for the cultivation of *Pleurotus djamor* strain PL01-White.

Code	Bag weight (g)	Bag height (cm)	Bag diameter (cm)
BS/A	1300	20.0	10.0
BS/B	3250	22.0	20.0
BS/C	650	10.0	10.0
BS/D	500	14.0	8.0
BS/E	150	9.5	6.0

Different light intensities were applied to the mushroom bags during fruitification to determine the optimum illuminance for mushroom yield and quality. The light sources were adjusted to illuminances of  $0\pm 0.1$  lx,  $100\pm 0.1$  lx,  $500\pm 0.1$  lx,  $1,000\pm 1.0$  lx,  $5,000\pm 1.0$  lx, and  $10,000\pm 1.0$  lx. Mushroom yields were measured, and the colours of the mushrooms under different illuminance levels were recorded according to the colour guides of Kornerup & Wanscher (1978).

### Determination of the proximate composition

The moisture content was determined based on the AOAC 925.40 method and Nielsen (2017) with minor modifications. First, the weight of a pre-dried porcelain crucible with a lid ( $W_0$ ) was measured, followed adding approximately 15.00 g of fresh mushrooms, after which the crucible was reweighed ( $W_1$ ). The crucible was then covered with aluminium foil and heated in a forced draft oven at  $100^\circ\text{C}$  until a constant weight was reached. After cooling in a desiccator, the crucible and dried mushroom sample were reweighed ( $W_2$ ). The percentage moisture of the mushrooms was calculated using Equation 2.

#### Equation 2

$$\text{Percentage moisture} = \frac{W_1 - W_2}{W_1 - W_0} \times 100\%$$

$W_1$  - weight of the crucible and the fresh mushroom sample

$W_2$  - weight of the crucible and dry mushroom sample

$W_0$  - weight of the crucible with lid

The ash content was determined based on the methods of AOAC 923.03 and 967.04 and Harris & Marshall (2017) with slight modifications. The porcelain crucibles were dried in a laboratory oven at  $105^\circ\text{C}$  for 24 h and then transferred to a desiccator. After cooling to about  $60^\circ\text{C}$ , the weight of the crucible was measured ( $W_0$ ), followed by the addition of approximately 0.5 g of dried mushroom sample, and the weight of the crucible with the sample was measured ( $W_1$ ). The crucibles were then heated in a muffle furnace at  $550^\circ\text{C}$  for six hours with the lids open and were subsequently cooled in a desiccator with the lids closed. The weight of the crucibles with ash ( $W_2$ ) was measured immediately after removal from the desiccator, and the ash content was calculated using Equation 3. The samples were tested in triplicate for each mushroom.

#### Equation 3

$$\text{Percentage ash} = \frac{\text{Weight of ash (g)}}{\text{Weight of dry mushroom (g)}} \times 100\%$$

$$\text{Percentage ash} = \frac{W_2 - W_0}{W_1 - W_0} \times 100\%$$

$W_1$  - weight of the crucible and the dry mushroom sample (g)

$W_2$  - weight of the crucible and ash (g)

$W_0$  - weight of the crucible (g)

The fat contents of the mushroom samples were determined based on AOAC 963.15 and Carpenter (2010) with slight modifications. *Pleurotus djamor* strain PL01-White mushrooms were dried at 70°C for two days in a vacuum oven and ground into powder with a laboratory grinder. The weight of a Soxhlet extraction thimble was first measured ( $W_1$ ) and then re-weighed after adding approximately 3.5 g of mushroom powder ( $W_2$ ). A round bottom flask was then weighed ( $W_0$ ). Next, approximately 200 mL of petroleum ether with a boiling point of 30°C to 60°C was added to the thimble, and the flask was heated using a heating mantle and refluxed at a condensation rate of 5–6 drops per second for 16 hours. The solvent remaining in the extractor and flask was then evaporated at 100°C for approximately 2 hours until the weight of the flask became constant. The flask was then cooled in a desiccator and weighed ( $W_3$ ). This procedure was performed in triplicate for each mushroom species. The mushroom fat content was calculated using Equation 4.

#### Equation 4

$$\text{Percentage total fat} = \frac{\text{Weight of extracted fat (g)}}{\text{Weight of dry mushroom sample (g)}} \times 100\%$$

$$\text{Percentage total fat} = \frac{W_3 - W_0}{W_2 - W_1} \times 100\%$$

$W_1$  – weight of the extraction thimble (g)

$W_2$  – weight of the extraction thimble + mushroom sample (g)

$W_3$  – weight of the flask + fat extract (g)

$W_0$  – weight of the flask (g)

To determine the fatty acid profile, the mushroom fats were extracted into ether and converted into Fatty Acid Methyl Esters (FAME) following the protocol by Ostermann et al. (2014), using  $\text{BF}_3$  in methanol. The FAME composition was analysed using gas chromatography-mass spectrometry (GCMS) according to the protocol by Raman et al. (2020).

The total protein content was determined by quantifying the total nitrogen content based on the AOAC 955.04 method and Nielsen (2017b). Approximately 1 g of pre-dried mushroom powder was placed in a Kjeldahl digestion tube and digested using 25 mL of concentrated  $\text{H}_2\text{SO}_4$  and Kjeldahl tablets as catalysts for 2 hours in a DK 6/48-Velp Scientifica® digestion unit until the mushroom sample was fully solubilized. After cooling to room temperature, approximately 200 mL of distilled water was added to the flask, and the solution was neutralized by adding approximately 37 g of NaOH. The flask was then connected to a condenser and heated until ammonia was completely released, after which the released ammonia was trapped in 25.0 mL of 4% boric acid solution. The resulting dihydrogen borate anions were titrated with 0.1 M HCl in a mixture of methylene blue and methylene red indicator using a UDK 129-Velp Scientifica® Kjeldahl distillation unit.

The total dietary fibre content was determined using the AOAC 985.29 method with slight modifications. Approximately 1.00 g of the mushroom sample was incubated with thermostable  $\alpha$ -amylase (150 U) in a phosphate buffer solution (50 mL, pH 6.0, 95–100°C) to hydrolyse starch. Protease (35 U) was then added to the sample to hydrolyse the proteins at pH 7.5 and 60°C for 30 min. Next, amyloglucosidase (40 U) was used to remove starch by incubating the sample at pH 4.0–4.6 for 30 min at 60°C. The soluble dietary fibre was precipitated by adding four volumes of 95% ethanol and then filtering through a pre-weighed dry crucible. The precipitates were sequentially washed with three 20 mL portions of 78% ethanol, two 10 mL portions of 95% ethanol, and two 10 mL portions of acetone. The crucibles were then dried at 105°C overnight in a hot air oven, cooled in a desiccator, and weighed to determine the weight of the crucibles with dietary fibre. The total dietary fibre content was calculated using Equation 5. The insoluble dietary fibre content was determined by continuing the same experimental procedure but removing the soluble dietary fibres by filtering after amyloglucosidase digestion and omitting the precipitation of soluble dietary fibre using 95% ethanol. Each mushroom sample was analysed in triplicate.

### Equation 5

$$\text{Dietary fibre percentage} = \frac{(W_R - W_P - W_A) - (W_{BR} - W_{BP} - W_{BA})}{W_S} \times 100\%$$

$W_R$  - residual weight (mg)

$W_P$  - protein weight of the sample (mg)

$W_A$  - ash weight of the sample (mg)

$W_{BR}$  - residual weight of the blank (mg)

$W_{BP}$  - protein weight of the blank (mg)

$W_{BA}$  - ash weight of the blank (mg)

$W_S$  - sample weight (mg)

The carbohydrate content of the *P. djamor* strain PL01-White mushroom was determined using the methods described by Sanmee (2003) and Srikrum & Supapvanich (2016). The sum of the percentage values of ash, crude protein, fat and total fibre was subtracted from 100 to obtain the carbohydrate content.

### Determination of the mineral content

The protocol by Yin et al. (2012) and Tinkov et al. (2020) was followed with slight modifications to determine the amount of minerals present in *P. djamor* strain PL01-White mushrooms by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). Approximately 1.000 g of dried mushroom powder was added to a 50 mL microwave digestion vessel and a mixture of 5 mL of 67% HNO<sub>3</sub> and 1 mL of 35% HCl was added. The mixture was allowed to stand in a fume hood for about 30 min. Then the vessels were placed in a microwave digestion system and subjected to a temperature of 170–180°C for one hour. After cooling, the solution was added to a 50 mL volumetric flask and topped with deionized distilled water. The major and trace elements Ca, Cu, Fe, Mg, Mn, K, Na and Zn were determined with heavy metals As, Cd, Pb, and Hg using Agilent® 7900 ICP-MS analyser.

### *Artemia salina* toxicity assay to determine the acute toxicity of the mushroom extract

Approximately 0.5 g of *Artemia salina* cysts were soaked in 1 L of tap water in a 2 L flask for approximately 1 hour. Next, approximately 37.0 g of artificial sea salt was added to the flask, mixed thoroughly, and aerated for 48 h (Banti & Hadjidakou 2021). *Artemia salina* toxicity assay was conducted based on the protocol of Ashtari et al. (2012), Wong et al. (2014), Adebayo-Tayo et al. (2019) and Banti & Hadjidakou (2021).

Approximately 2.0 g of *P. djamor* strain PL01-White and a commercial strain of *P. floridanus* were ground separately using a mortar and pestle, adding 20.0 mL of distilled water. The resulting extract was filtered through Whatman™ No. 1 filter paper, using suction filtration, after which the extract was filtered through cellulose syringe filters with a pore size of 0.22 µm. Several dilutions of the mushroom extracts from the above species were prepared: 10X, 100X, 1,000X and 10,000X. For each dilution, 1.0 mL of crude mushroom extract and 0.5 mL of filter-sterilized 2X artificial seawater were added to a well of a 24-well plate, and two other wells were filled with the same composition to replicate the experiment. Next, 10–20 *Artemia salina* nauplii were added to each well. Three wells were filled with 1.0 mL of distilled water and 0.5 mL of filter-sterilized 2X artificial seawater to serve as the negative control. The number of nauplii added to each well was recorded, and after 24 hours, the numbers of dead and live nauplii were counted. The half maximal inhibitory concentration (IC<sub>50</sub>) was obtained by probit analysis using the online tool Quest Graph™ IC<sub>50</sub> Calculator (Available at <https://aatbio.com/tools/ic50-calculator>).

Two grams of each of the mushrooms mentioned above were boiled in 10 mL of distilled water for approximately 20 minutes to prepare the mushroom extracts following the same protocol. Toxicity assays were performed for different dilutions (10X, 100X, 1,000X and 10,000X) of the extracts as described previously, and the IC<sub>50</sub> values were calculated.

## Statistical analyses

The means were compared for significant differences using Tukey's pairwise comparison of one-way ANOVA, with a significance level of 0.05, utilizing Minitab 17 Statistical Software. When the ANOVA conditions were not satisfied, non-parametric pairwise comparisons were conducted using SPSS Statistics software version 21.0.

## Results

### Morphological and molecular identification of the mushrooms

The *P. djamor* strain PL01-White is characterized by its distinct morphological features (Fig. 2). The cap of this mushroom typically measures between 3–14 cm in diameter, exhibiting a fan-shaped or false-bell shape that tapers toward its point of attachment. The colouration of the cap ranges from bright white to a creamy yellowish hue, sometimes displaying whitish-grey tones. Fine hairs are present toward the base of the cap, which is finely striated and features an incised margin. The texture of the cap is soft and fleshy. The gills of *P. djamor* strain PL01-White are deeply decurrent lamellae that appear white to yellowish white and are approximately 2–4 mm in width. The stipe of this mushroom can be either absent or significantly reduced. When present, it is cylindrical and may have fine hairs. The *P. djamor* strain PL01-White produces a white spore print, and its spores are cylindrical and hyaline, measuring approximately  $7.5\pm 0.5 \times 3.4\pm 0.4 \mu\text{m}$  in size, with a length-to-width ratio (Q value) of approximately 2.2. The mushroom emits a mild acidic smell, adding to its distinctive characteristics.

In BLAST searching for the ITS region of the *P. djamor* strain PL01-White as the mushroom query, many target sequences were identified with 100% query coverage and 100% identity belonging to the *P. djamor* species. However, a few targets were observed to have the same query coverage and identity but belonging to different species of the genus *Pleurotus*. Fig. 3 displays the phylogram generated for the strain PL01-White, with the highest log likelihood (-1886.39), which demonstrating that the strain was grouped with *P. djamor*, and confirms the species of the strain. In the respective analysis, 42 sequences with 357 positions were used.

### Optimum culturing and cultivation conditions

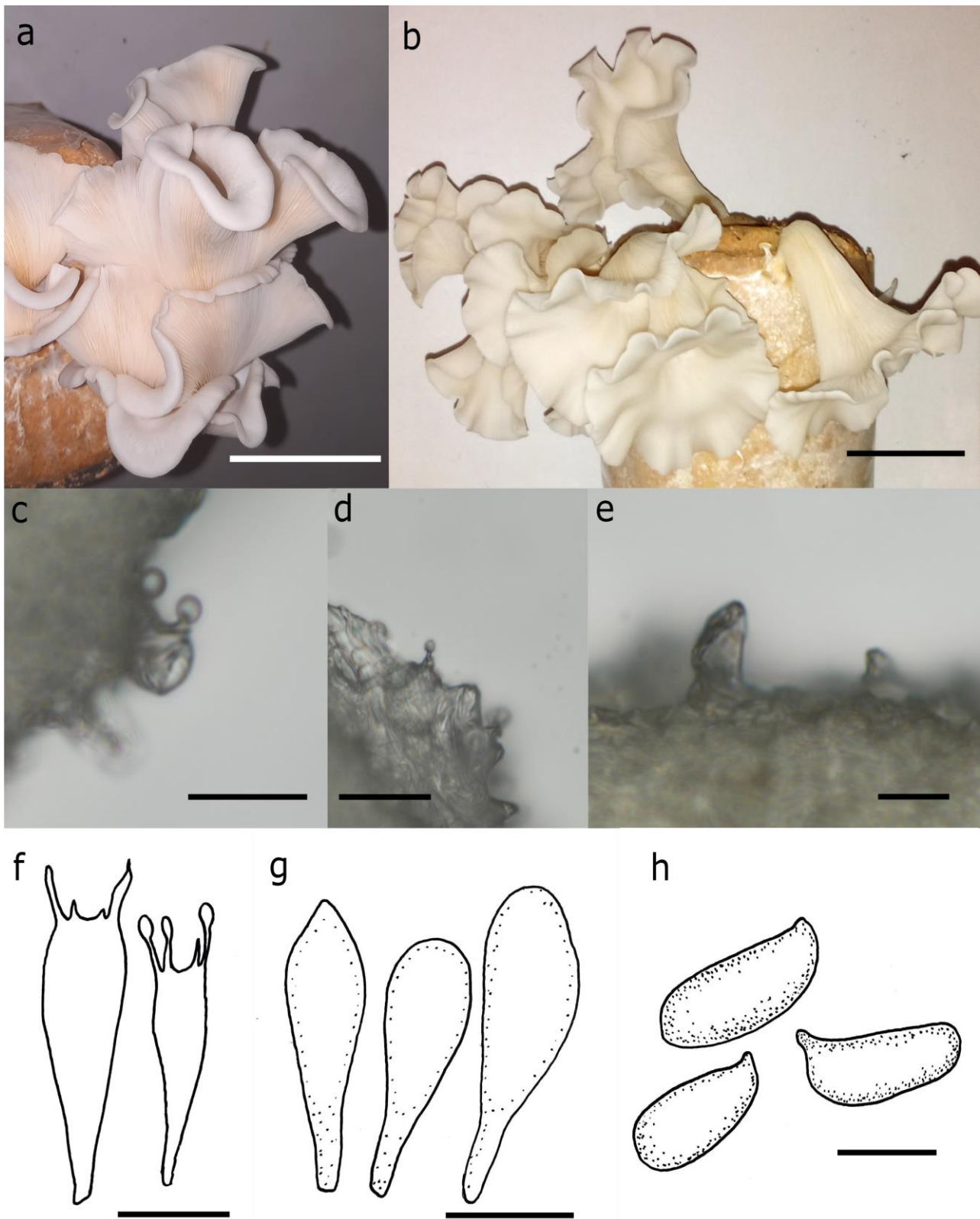
Among the tested temperatures, the growth rate of the *P. djamor* strain PL01-White mushroom was maximum at 25°C, and the minimum growth rate was observed at 35°C (Fig. 4a).

The growth rate of the *P. djamor* mushroom was tested at pH values ranging from 4.0 to 10.0. The results showed that mycelial growth was fastest at pH values of 6.0–9.0. The slowest growth was observed at pH 4.0 (Fig. 4b).

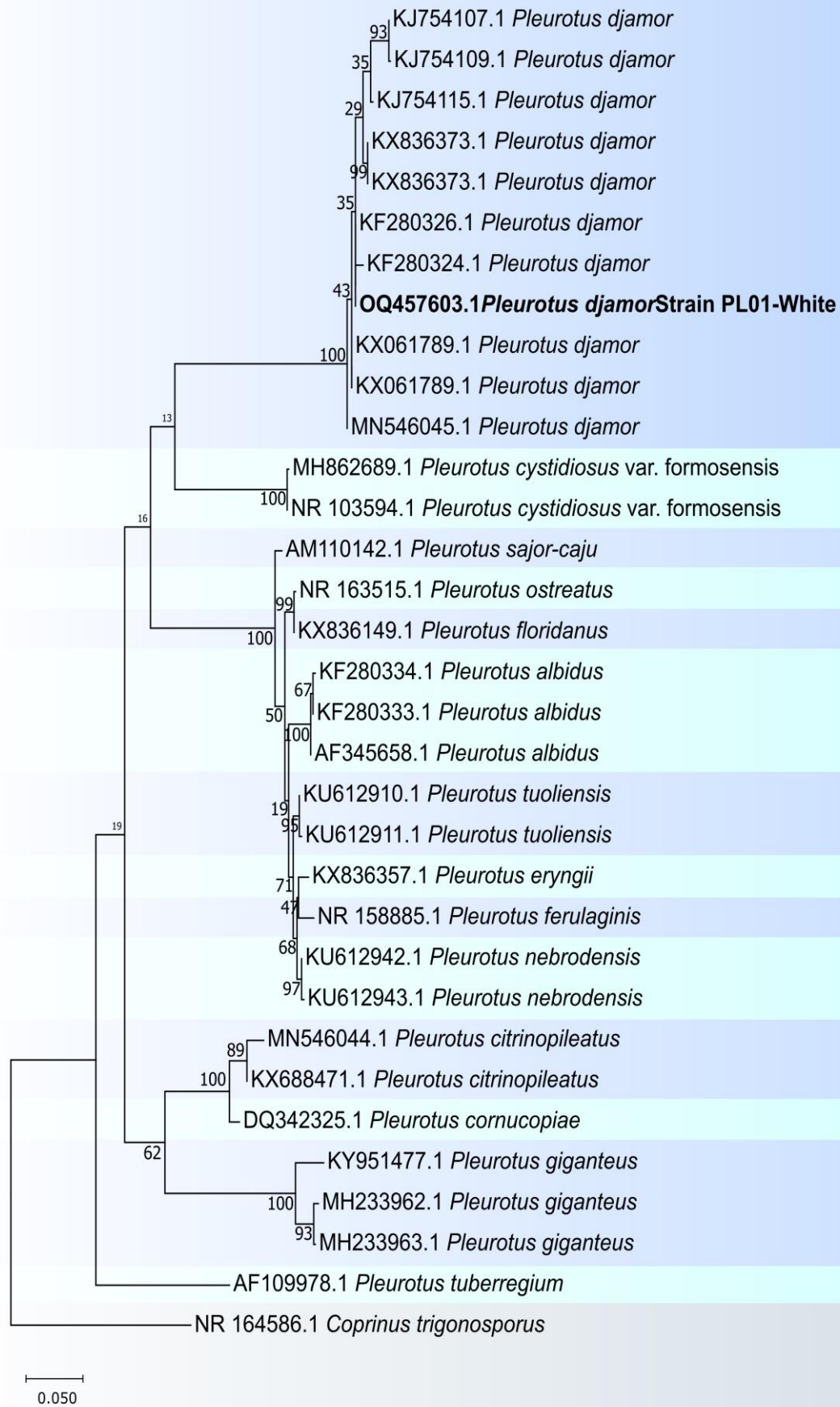
The growth rate of the *P. djamor* strain PL01-White in different culture media was observed. The results showed that the growth rate of the *P. djamor* strain PL01-White was significantly highest when it was cultured on CMA and OMA (Fig. 4c). The growth rate of the mycelia on SDA was also comparatively higher but lower than that on CMA. It did not significantly differ from OMA. The growth rate of the mycelia of *P. djamor* strain PL01-White on the other tested media was much lower. The growth of *P. djamor* strain PL01-White mushroom mycelia in different culture media after seven days of inoculation is shown in Fig. 5.

The growth rates of *P. djamor* strain PL01-White mushroom in grain spawn media with different moisture contents were measured. The results revealed that the mycelial growth rate was highest at 60% moisture of dry substrate weight (Fig. 4d). A trend was observed to increase the growth rate from 30% moisture spawn bottles to 60% moisture and then decrease. In the present study, the growth rate of *P. djamor* was measured in grain spawns prepared using different grain types such as chickpea, cowpea, finger millet, maize, mung bean, paddy sorghum, and wheat. Among these, the paddy seeds were identified as the substrate with the highest mycelial growth rate of *P. djamor* strain PL01-White (Fig. 4e). CaCO<sub>3</sub> was added to the grain spawn media, and the effect of the amount of CaCO<sub>3</sub> added to the grain spawn media on the growth rate of *P. djamor* strain PL01-White was tested. The growth rate of the mushroom mycelia did not significantly differ

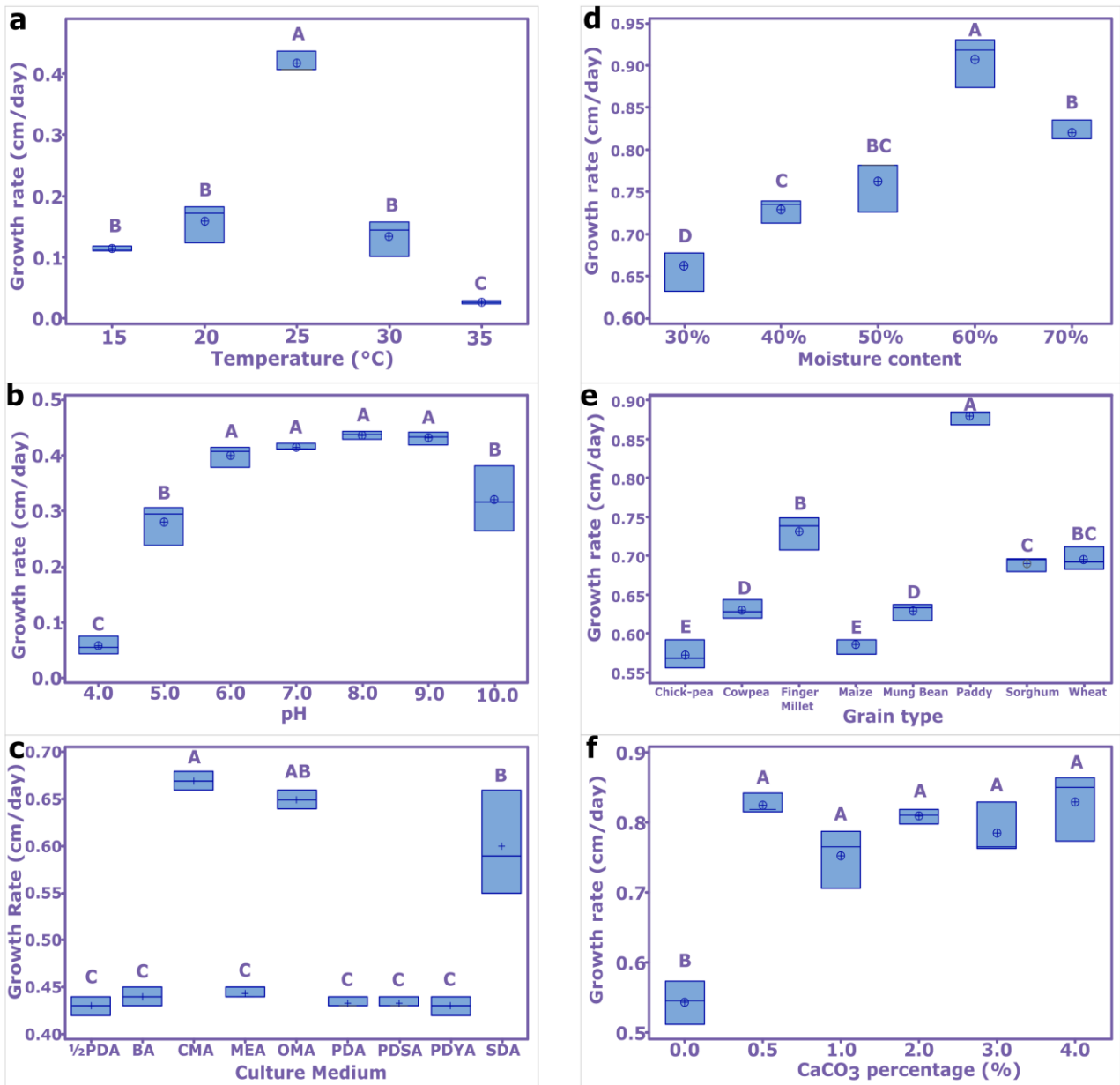
among the different substrate  $\text{CaCO}_3$  contents, but the growth rate in spawns prepared without the addition of  $\text{CaCO}_3$  was much lower (Fig. 4f).



**Fig. 2** – Morphological characteristics of *Pleurotus djamor* strain PL01-White. a Ventral view of the pilei. b Dorsal view of the mushroom pilei. c,d Basidia. e Cheilocystidia. f Illustration of basidia. g Illustration of cheilocystidia. h Illustration of basidiospores. Scale bars: a,b = 5 cm, c–g = 10 μm, h = 5 μm.

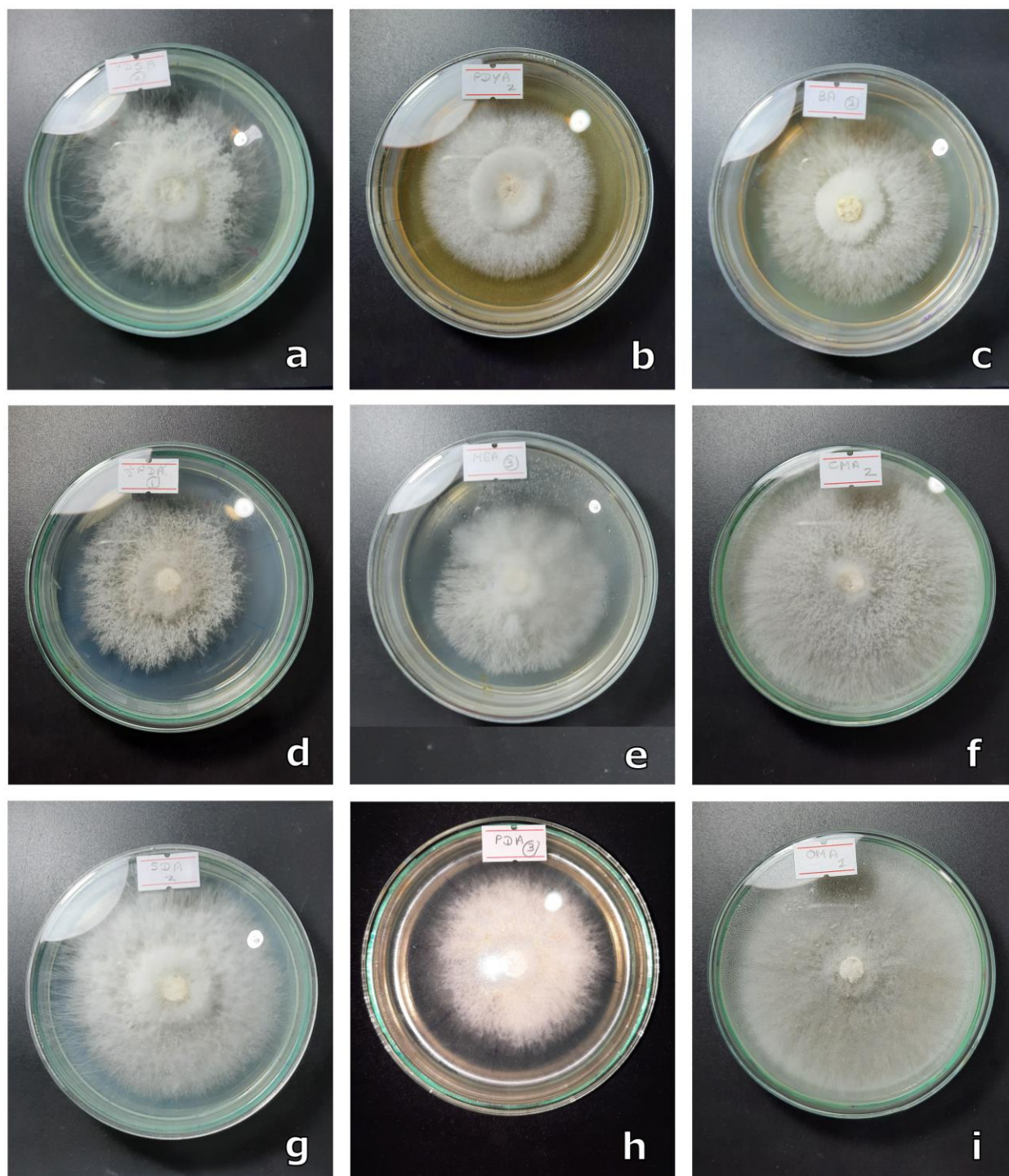


**Fig. 3** – Phylogram obtained for the *Pleurotus djamor* strain PL01-White from maximum likelihood analysis based on Internal Transcribed Spacer (ITS) sequences using MEGA v11. The tree is rooted with *Coprinus trigonosporus*. The species in the present study is in bold.



**Fig. 4** – The variation of growth rate of *Pleurotus djamor* strain PL01-White mycelia at different conditions. a Temperature. b pH values. c Culture media. The variation of the mycelial growth rate of *P. djamor* strain PL01-White mushroom in spawns of different conditions. d Moisture. e Grain types. f CaCO<sub>3</sub> percentages.

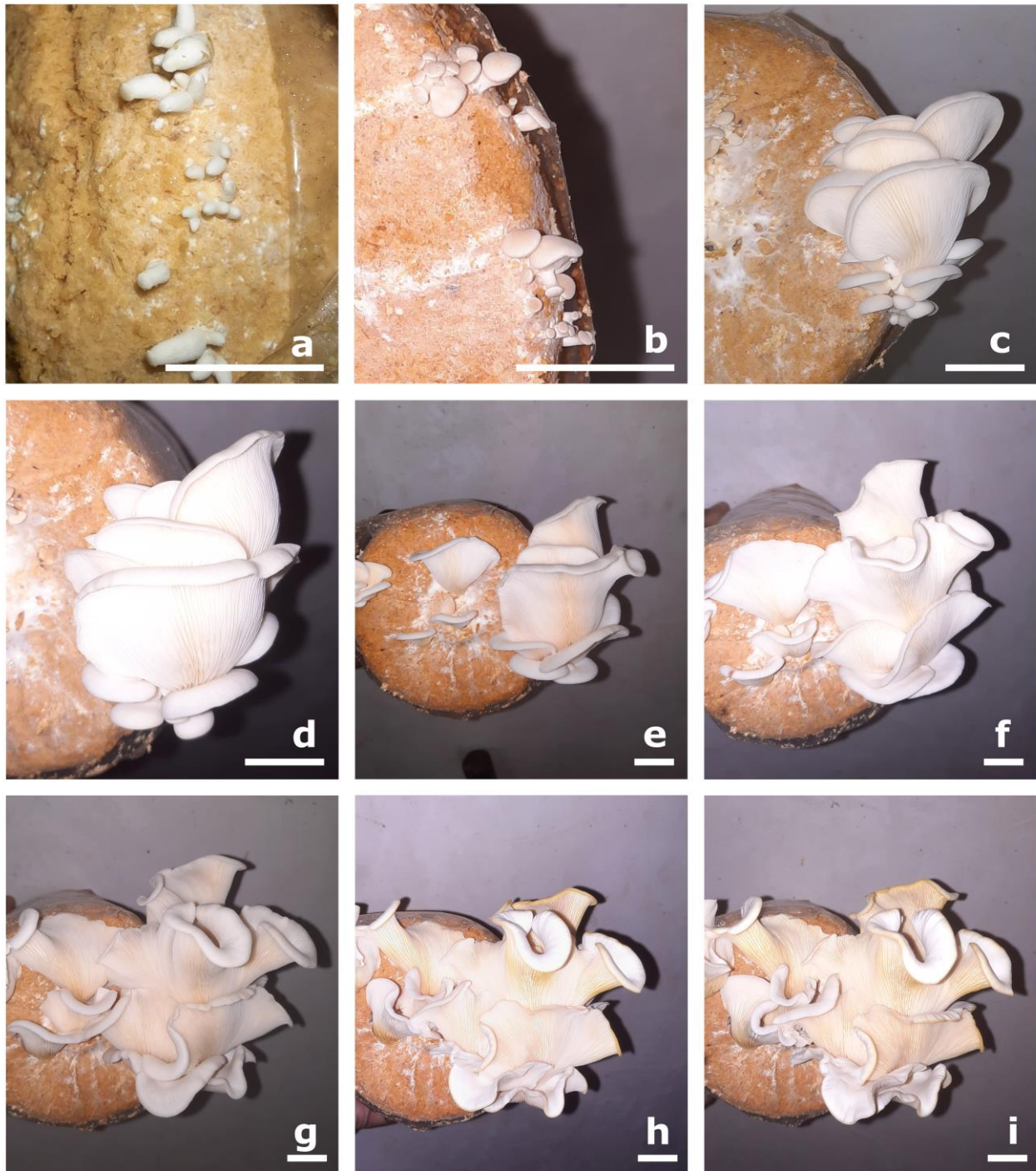
*Pleurotus djamor* strain PL01-White mushroom fructification occurred without requiring special conditions. Primordial initiation occurred after 2–7 days of introduction to the fruiting chamber. Temperature reduction was not required for fruiting induction, and exposure of the substrate to the outer environment was also observed to be nonessential. However, when the substrate bags are exposed and the temperature is reduced, primordia appear, covering the exposed area and leading to increased yields. Spraying water about 2–3 times a day is required for the optimum growth of this mushroom. These mushrooms take approximately 66 hours from primordia initiation for maximum development, and harvesting can be done within a time period of 55–78 hours from primordia initiation. Before this time interval, mushrooms are not fully developed, and harvesting is economically disadvantageous. Harvesting after this time period was also unsuccessful; hence, the mushrooms started degrading, losing their white colour and characteristic smell. Fig. 6 shows the growth stages of *P. djamor* strain PL01-White mushroom.



**Fig. 5** – Growth of *Pleurotus djamor* strain PL01-White mushroom mycelia in different culture media after seven days of inoculation. a Potato Dextrose Sucrose Agar (PDSA). b Peptone Dextrose Yeast Extract Agar (PDYA). c Benomyl containing Agar (BA). d ½ Potato Dextrose Agar (½ PDA). e Malt Extract Agar (MEA). f Corn Meal Agar (CMA). g Sabouraud Dextrose Agar (SDA). h Potato Dextrose Agar (PDA). i Oat Meal Agar (OMA).

The mushroom yield did not depend on the substrate moisture content in the tested range (Fig. 7a). However, the harvesting can be performed for a longer period of time at higher moisture content such as 65.5% and 67.7%. Moreover, the requirement for moistening the substrate bags during fructification is lower when the substrate moisture content is high. Therefore, 65.5% substrate moisture was selected to prepare substrate media for *P. djamor* strain PL01-White.

Rice bran is added to cultivation substrate media as a nitrogen supplement, and the optimum rice bran content needs to be determined to obtain maximum economic benefits from mushroom cultivation. The media prepared either without rice bran or with 5 kg of rice bran per 100 kg of saw-dust had the lowest yield indicating that rice bran was needed for a better yield. When 5, 10, 15 or 20 kg of rice bran per 100 kg of saw-dust was used to prepare substrate bags, a significant difference in yield was not observed (Fig. 7b). Based on these results, it can be recommended to use 10 kg of rice bran per 100 kg of saw-dust to obtain maximum economic benefits in the cultivation of this mushroom.



**Fig. 6** – Developmental stages of *Pleurotus djamor* strain PL01-White mushroom in cultivation bags after different periods of primordia initiation. a After 6 h. b After 18 h. c After 30 h. d After 42 h. e After 54 h. f After 66 h. g After 78 h. h After 90 h. i After 126 h. Scale bars = 2 cm.

The effects of different CaCO<sub>3</sub> contents in substrate bags on yield were tested, and the addition of 1.5, 2.5 or 4.5 kg of CaCO<sub>3</sub> per 100 kg of saw-dust in substrate media led to the maximum yield (Fig. 7c). Among them, yields in 1.5 and 4.5 kg of CaCO<sub>3</sub> per 100 kg of saw-dust were not significantly different from the yields of the other CaCO<sub>3</sub> amounts tested. Therefore, adding 2.5 kg of CaCO<sub>3</sub> to 100 kg of dry saw-dust when preparing substrate media for cultivating *P. djamor* strain PL01-White is recommended.

In the fruiting chamber, the mushroom substrate bags should be opened to the outer environment since higher CO<sub>2</sub> concentrations prevent mushroom primordia formation during the incubation of substrate bags (Miles 2018). Different methods can be used to open substrate bags in a fruiting chamber, and an optimum method should be identified to obtain maximum economic benefits. The yield of *P. djamor* strain PL01-White was tested via three different bag opening methods, and the results revealed that the yields obtained for those three methods were not significantly different (Fig. 7d). Therefore, any of the three bag opening methods in which mushrooms are obtained, through the plastic neck by removing only the filter cap, by removing the neck by cutting around the neck or by removing the whole circular face of the bag, can be used in commercial cultivation.

The present study used substrate bags in five different sizes to cultivate *P. djamor* strain PL01-White, and the yields obtained were compared. The results revealed that bags 1,300 g in weight, 10 cm in diameter and 20 cm in height yielded the most significant maximum yield (Fig. 7e) and can be considered the optimum bag size for cultivating *P. djamor* strain PL01-White.

When cultivated under different light illuminances, *P. djamor* strain PL01-White mushroom, did not exhibit significant differences in yield (Fig. 7f). However, light illuminance in the range of 500–5,000 lx can be recommended since the mushroom colour and shape are more uniform within that range.

### **The shelf life of the spawn**

In the present study, the spawn shelf-life was assessed after storage at 4°C and 25°C. *Pleurotus djamor* strain PL01-White spawns prepared under the optimized conditions were observed to be successfully stored for approximately three months at 25°C and four months at 4°C during which their viability was retained.

### **Nutrient content**

The proximate composition of the *P. djamor* strain PL01-White is shown in Table 4. When the fatty acid content was analysed, eight fatty acids were identified, two of which were unsaturated fatty acids (Table 5).

### **Mineral composition**

The major and trace mineral elements identified in the anhydrous *P. djamor* strain PL01-White mushroom are shown in Table 6. Potassium is the most abundant mineral element and varies in the order K > Mg > Ca > Na. The concentrations of the microelements are in the descending order of Zn > Fe > Cu > Mn. Among the heavy metals tested, As was not detected and Cd > Pb > Hg were in descending order.

### **In-vitro cytotoxicity of mushrooms**

Although this mushroom species is a known edible, acute toxicity was compared with that of a commonly cultivated mushroom, *P. floridanus*. Fig. 8 shows the variation in the percentage mortality of *Artemia salina* nauplii after exposure to the raw and boiled mushroom extracts of *P. djamor* strain PL01-White and *P. floridanus* respectively. The LC<sub>50</sub> values of the raw mushroom extracts of *P. djamor* strain PL01-White and *P. floridanus* were 0.0003 g/mL and 0.0035 g/mL, respectively, while those of the boiled mushroom extracts were 0.0460 g/mL and 0.0253 g/mL, respectively. Considering the LC<sub>50</sub> values, the acute toxicity of the raw *P. djamor* strain PL01-

White is higher than *P. floridanus* but when boiled, the toxicity of *P. djamor* strain PL01-White became less than that of *P. floridanus*, and the toxicity of both mushrooms reduced with boiling.

## Discussion

The optimization of cultivation conditions for the mushroom species employed the “One Factor at a Time Method” to determine the optimum conditions for various selected parameters (Czitrom 1999). This method was chosen to streamline the investigation by focusing on one variable at a time. However, a drawback is its inability to reveal correlations among different parameters, potentially leading to false-optimum outcomes. Despite this limitation, the method was selected based on its successful application in optimization research, as demonstrated in prior studies (Singh et al. 2011, Irfan et al. 2014, Khusro 2016, Karamba et al. 2016, Nor et al. 2017, Othman et al. 2022).

In the BLAST search of the molecular authentication of the species, it was observed that several sequences available in the GenBank database, with 100% query coverage and 100% sequence identity to the ITS sequence of *P. djamor* in the present study, were of different species. According to Zervakis et al. (2019), there is high intra-specific polymorphism in the *P. djamor* complex, both phenotypically and genotypically, in which genomic variations are indicated by divergence of sequences, genetic distance values, and the cosmopolitan distribution of the species. The specimens previously identified as *P. flabellatus*, *P. opuntiae*, *P. ostreatoroseus*, *P. parsonsiae*, and *P. salmoneostramineus* are probably *P. djamor* with ambiguous identity. This could also explain why the BLAST results revealed target sequences of some *Pleurotus* species with 100% identity and 100% query coverage; these species were not named *P. djamor*.

In Sri Lanka, the pink *P. djamor* strain, which is of non-Sri Lankan origin, is occasionally cultivated on a commercial scale. In the present study, the *P. djamor* strain PL01-White was selected for optimisation of cultivation conditions, which has many morphological differences from the commercial strain (Fig. 9). The mushrooms of the local strain are predominantly white in colour, while the commercial strain is reddish-pink. The shape of the pilei under mature conditions of local strain is a false bell shape without a distinct stipe, whereas the pilei of the commercial strain are mostly petaloid with a distinct but reduced stipe. Furthermore, the pilei size of the local strain is larger than that of the commercial strain. The local strain has a lemony, mild acidic smell, while the commercial strain has an earthy smell.

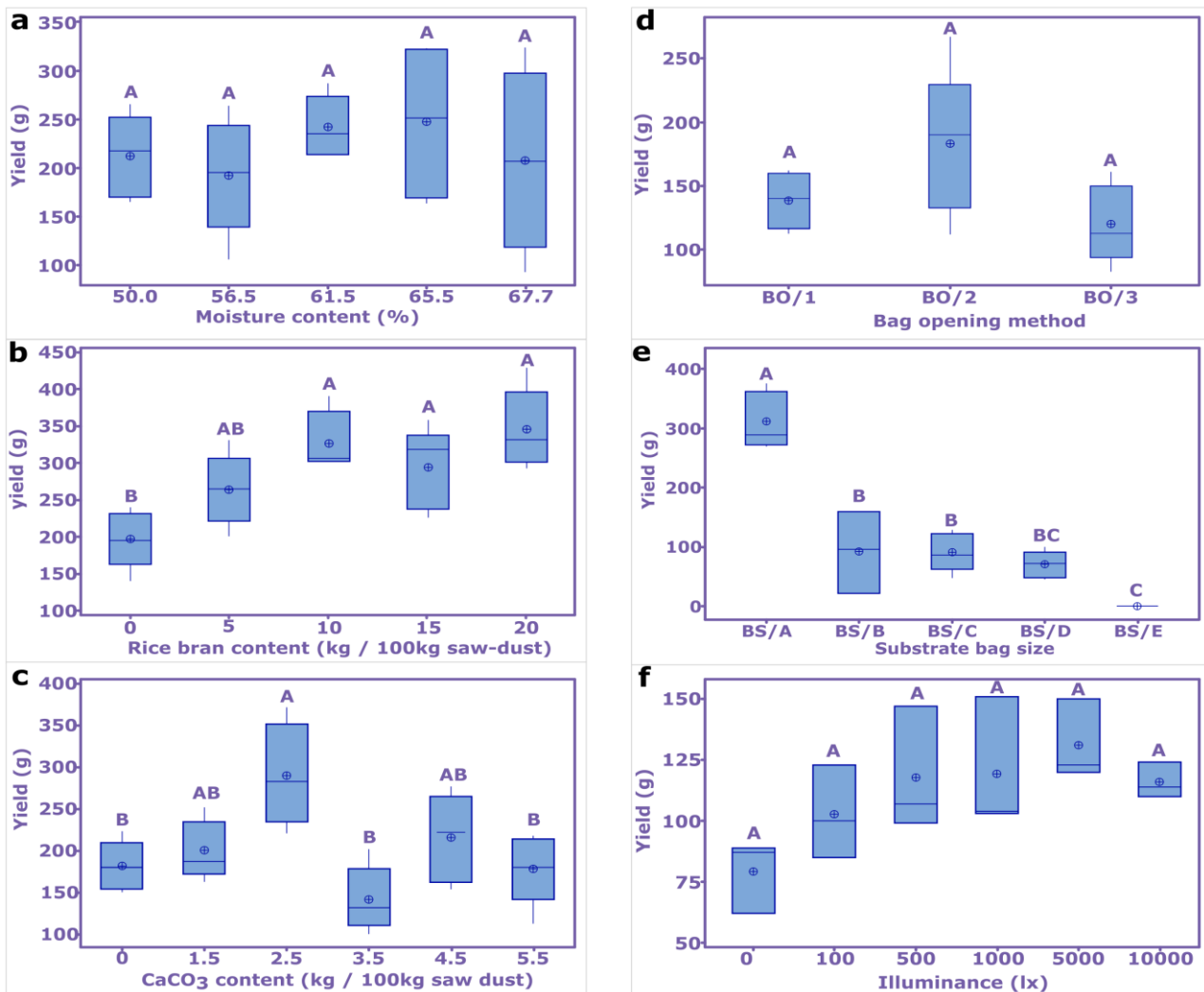
The optimum temperature for the mycelial growth and fruiting of a mushroom should be identified since the mushrooms should be cultivated for maximum economic benefit. Therefore, it can be recommended that these mushrooms can be incubated in laboratory culturing, spawn production or cultivation at the identified optimum temperature. According to Singh & Singh (2018), the optimum temperature for the growth of *P. djamor* is 28°C which is slightly greater than the optimum temperature for the strain in the present study. Stamets (2000) reported that the *P. djamor* mycelial growth rate is optimum at the temperature range of 24–30°C. Some *Pleurotus* species growth rate is optimal at temperatures of 20–30°C, where *P. ostreatus* growth rate is optimum at 21–24°C (Jafarpour & Eghbalsaeed 2012), *P. sajor-caju* growth rate is optimum at 20–25°C (Rajak et al. 2011) and *P. florida* growth rate is optimum at 21–25°C (Ahmed et al. 2009). When optimizing the pH, agar degrades at low pH, and it is challenging to prepare agar media at pH values less than 4.0 (Kanazawa & Kunito 1996). For this reason, the mushroom’s growth rate at pH values less than 4.0 was not tested. Benomyl containing Agar has been used as a culture medium to initially culture mushroom mycelia to avoid the growth of unnecessary fungal contaminants. Benomyl and 2-phenylphenol present in this medium inhibit all other fungi except basidiomycetes ensuring the growth of mushroom mycelia.

It can be recommended to use grains with 60% moisture, which is the optimum moisture content for grain spawn preparation of *P. djamor* strain PL01-White. Bilal et al. (2014) compared the growth rate of *P. ostreatus* on different grain types such as corn, wheat, barley and millet. Nguyen & Ranamukhaarachchi (2020) reported the growth rate of *P. ostreatus* and *P. eryngii* on

grain spawn prepared from wheat, rye, barley and oat grains. The use of paddy seeds to prepare spawns of *P. djamor* strain PL01-White can be considered optimum based on the growth rate of mycelia. Since paddy seeds are widely available in Sri Lanka and are comparatively cost-effective, the use of paddy seeds is economically beneficial.

The results suggest that the optimum amount of CaCO<sub>3</sub> might be between 0 kg/100 kg of grains and 0.5 kg/100 kg of grains, which should be further analysed. Based on the obtained results, it can be recommended that 0.5 kg CaCO<sub>3</sub>/100 kg of grains be used to prepare grain spawn for *P. djamor* strain PL01-White.

The shelf-life of spawns depends on various internal and external factors, and the storage temperature is the most crucial factor. According to Lee et al. (1999), spawn stored at 5°C, are much less contaminated after 120 days of storage than spawn stored at 20°C. Furthermore, reported that the re-culture period is longer when the spawn storage time period is longer. The same pattern of growth time variation when re-cultured, as reported by Lee et al. (1999) was observed in the present study. The viability of the spawns can be maintained for a longer period of time when the spawns are stored at 4°C than when stored at 25°C. Furthermore, Lee et al. (1999) reported that mushroom yield is also higher in substrate media prepared from spawn stored at a lower temperature than in the substrate from spawn stored at higher temperatures, which were not tested in the present study.



**Fig. 7** – The variation in the yield of *Pleurotus djamor* strain PL01-White mushroom in different substrate and environmental conditions. a Substrate moisture. b Substrate rice bran percentage. c Substrate CaCO<sub>3</sub> content. d different substrate bag opening methods. e different substrate bag sizes. f different illuminance levels.

**Table 4** Proximate composition of *Pleurotus djamor* strain PL01-White (% of dry weight) compared with few other strains of *P. djamor*. \* Represented as a percentage of fresh weight

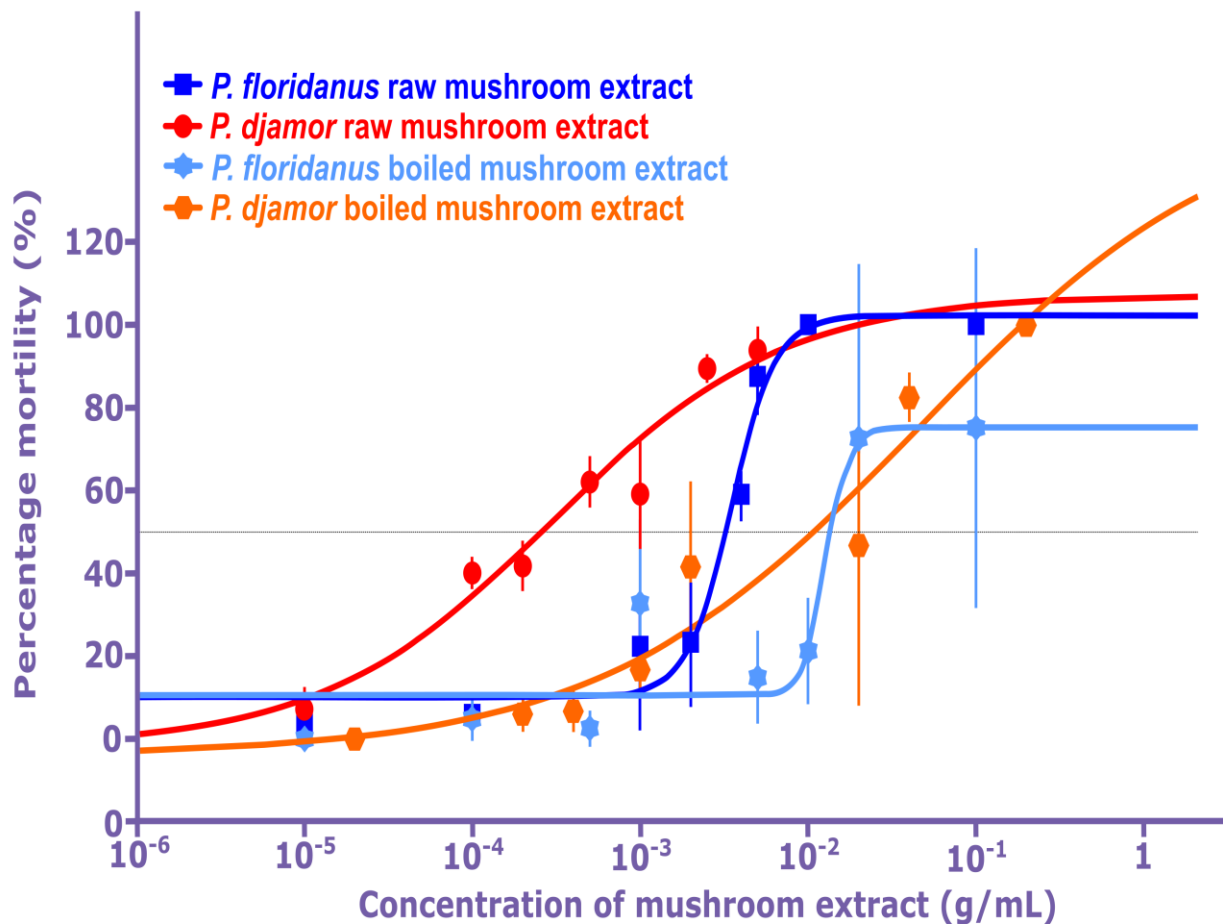
Reference	Moisture (%) *	Crude protein (%)	Crude fat (%)	Carbohydrate (%)	Dietary fibre / Crude fibre (%)	Ash (%)
Present study	90.118 ± 1.629	26.038 ± 0.183	0.881 ± 0.128	28.0	37.2 ± 2.0	7.864 ± 0.195
Capetillo Leala et al. (2010)	85.96 ± 0.70	20.18 ± 0.35	2.81 ± 0.05	-	39.77 ± 0.16	8.69 ± 0.18
Hasan et al. (2015)	89.85	23.5	6.343	49.01	22.65	8.757
Zurbano et al. (2017)	90.15	1.21	1.72	-	31.5	8.83
Jegadeesh et al. (2018)		35.50 ± 1.78	1.72 ± 0.03	44.75 ± 2.02	14.60 ± 0.43	5.90 ± 0.05
Raman et al. (2021)	86.81	24.10	4.73	45.59	15.91	9.84
N. A. Khan et al. (2013)	84.55	21.89	0.80	-	8.92	7.65

**Table 5** Fatty acids present in *Pleurotus djamor* strain PL01-White identified using Gas Chromatography-Mass Spectrometry (GC-MS) and their chemical structures

Fatty acid	
Hexadecanoic acid	Saturated long-chain fatty acid.
9,12-Octadecadienoic acid (z,z) [Linoleic acid]	Unsaturated fatty acid two double bonds are at positions 9 and 12 and have Z (cis) stereochemistry
9-Octadecenoic acid	Unsaturated fatty acid with a double bond at C-9
Octadecanoic acid	Straight-chain saturated fatty acid
Tetracosanoic acid	Straight-chain saturated fatty acid.
Pentadecanoic acid	A straight-chain saturated fatty acid
Tetradecanoic acid	Long-chain saturated fatty acid
20-Methyl-heneicosanoic acid	A methyl-branched fatty acid

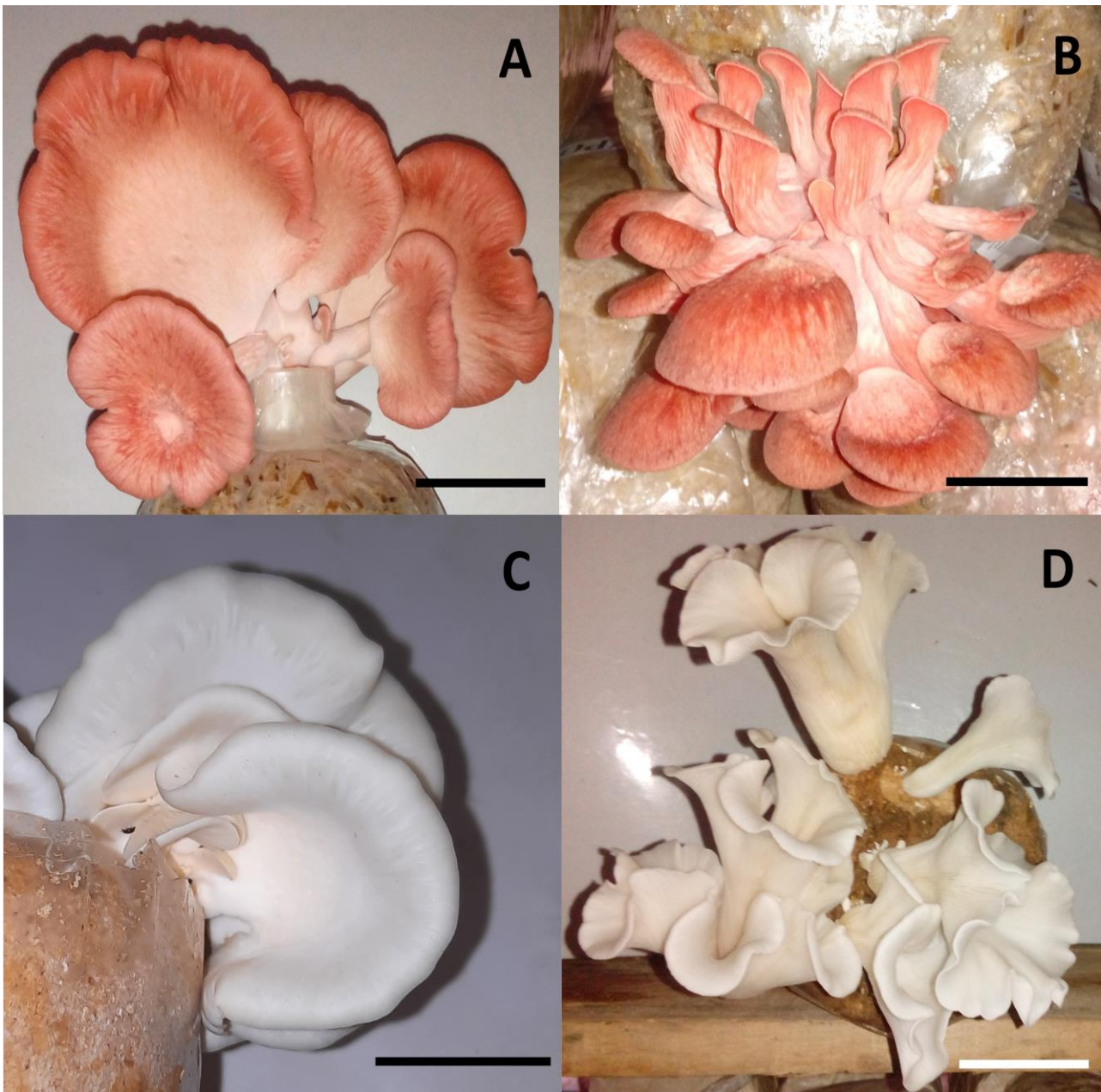
**Table 6** Mineral content of *Pleurotus djamor* strain PL01-White mushrooms based on dry weight (mg/kg). ND = Not detected

Macroelement	Quantity	Microelement	Quantity	Heavy metal element	Quantity
Ca	248 ± 16.2	Cu	23.8 ± 3.96	As	ND
Mg	1095 ± 120.2	Fe	74.1 ± 0.28	Cd	2.1 ± 0.35
K	18950 ± 7.0	Mn	8.7 ± 1.27	Pb	0.43 ± 0.01
Na	39.5 ± 3.54	Zn	85.0 ± 0.85	Hg	0.13 ± 0.02



**Fig. 8** – The variation in the percentage mortality of *Artemia salina* cysts with the concentration of raw and boiled mushroom extract of *Pleurotus djamor* Strain PL01-White and *P. floridanus*.

The mean yield obtained per mushroom bag when cultivated in the identified optimum medium was  $410.6 \pm 41.9$  g, with a biological efficiency of  $91.6 \pm 9.3\%$ . The biological efficiency observed in the cultivation of *P. djamor* on substrates of different compositions of sugar cane bagasse and wheat bran by Hasan et al. (2015) was in the range of 16.32% – 50.94%. Satpal et al. (2017) reported biological efficiencies in the range of 38% – 45% when *P. djamor* mushroom were cultivated on wheat straw, pigeon pea straw, and paddy straw with different combinations of these materials. Zurbano et al. (2017) cultivated *P. djamor* on media of different combinations of paddy straw, lumber saw-dust, rice bran, coconut peat, coconut husk, and banana leaf litter and have reported biological efficiencies in the range of 13.7% – 31.1%. A biological efficiency of 75.11% was reported by Barh et al. (2021) when this mushroom was cultivated on wheat straw with 0.5% urea. Jasinska et al. (2022) reported  $39.30 \pm 8.72\%$  biological efficiency when *P. djamor* was cultivated on media containing dairy manure and food waste digestate. According to each of these former cultivation records, the biological efficiency *P. djamor* cultivation was lower than that in the present study, validating the cultivation conditions introduced in the present study for commercial application. The optimized cultivation medium, characteristics of the mushroom strain, and cultivation of the strain within the same biogeographical range of natural occurrence may be the reasons for the greater biological efficiency observed in the present study. Contrary to the findings of the present study, Jegadeesh et al. (2018) have reported higher biological efficiencies when this mushroom was cultivated on paddy straw, ragi straw, corn straw and sugarcane bagasse, which were  $120.07 \pm 5.4\%$ ,  $119.37 \pm 5.74\%$ ,  $95.12 \pm 5.39\%$  and  $98.12 \pm 4.54\%$  respectively. Meanwhile, the biological efficiency of cultivating on coir pith was  $56.55 \pm 4.2\%$ , which was comparatively lower than that of the present study.



**Fig. 9** – Differences in the morphology of the *Pleurotus djamor* commercial strain and the PL01-White local strain. a,b Mushrooms of the *P. djamor* commercial strain cultivated in Sri Lanka. c,d Mushrooms of the *P. djamor* PL01-White local strain cultivated in the present study. Scale bars = 5cm.

Mushrooms are good sources of nutrients, and their nutrient composition varies with the species of mushroom and growth conditions (Singh, 2017). A comparison of the proximate composition of *P. djamor* strain PL01-White from the present study with the proximate compositions of *P. djamor* mushroom from several previous studies is shown in Table 4. The moisture content of the mushroom strain in the present study was  $90.118 \pm 1.629\%$ , while the moisture content of this mushroom in previous studies ranged from 84.55% to 90.15% (Capetillo Leala et al. 2010, Khan et al. 2013, Hasan et al. 2015, Zurbano et al. 2017, Jegadeesh et al. 2018). The crude protein content of the novel mushroom strain was  $26.038 \pm 0.183\%$ , which is only lower than the value identified by Jegadeesh et al. (2018). The lowest fat content was observed in the present study among the selected previous studies. The higher protein content and the low-fat content of the novel mushroom strain can be considered nutritionally beneficial (Johnston et al. 2004). The dietary fibre content is also high, and it can be considered nutritionally important in

many aspects, such as gut motility and reducing constipation, maintaining body weight and abdominal adiposity, increasing insulin sensitivity, reducing colorectal carcinoma, and reducing cardiovascular diseases (Barber et al. 2020).

When identifying the moisture content of a mushroom, the mushroom should be harvested before humidifying the fruiting chamber to avoid errors from external water deposition on the mushroom pileus. Furthermore, the weight of the mushroom should be measured soon after removal from the desiccator because desiccated mushrooms tend to absorb atmospheric moisture rapidly. The moisture content of a mushroom is a highly variable parameter based on external conditions such as the amount of watering, relative humidity, temperature during fructification, and internal factors such as substrate moisture content and metabolic water production (Crisan & Sands 1978).

When determining the crude protein content, the total nitrogen content should be multiplied by a Nitrogen to Protein conversion factor (NP factor). Therefore, the measurement of the protein content depends on this, and the net protein conversion factor should be highly specific for the calculated material. For most biological samples, the value 6.25 is applied as the universal NP factor (Fujihara et al. 1995). However, when analysing the total protein content of a mushroom sample, it is not reasonable to use this value because of the presence of a high percentage of non-proteinaceous nitrogen in chitinous cell walls and amino acids in the cytoplasm (Mattila et al. 2002), and only 60–77% of the total nitrogen is proteinaceous (Danell & Eaker, 1992, Fujihara et al. 1995). Mattila et al. (2002) identified NP factors for *P. ostreatus*, *Agaricus bisporus* (Brown – Portobello) *Agaricus bisporus* (White – Button mushroom) and *Lentinula edodes* as 4.97, 4.55, 4.70, and 4.50, respectively. In the present study, 4.7 was used as the NP factor, which was recommended by Mattila et al. (2002) for use universally in mushrooms.

According to Crisan & Sands (1978), mushroom crude fat content can range from 1% to 20%, and mushrooms can contain all types of lipids, such as free fatty acids, monoglycerides, diglycerides, triglycerides, phospholipids, sterols and sterol esters. In the present study, the crude fat content obtained for the *P. djamor* strain PL01-White mushroom was less than the minimum value mentioned by Crisan & Sands (1978). When considering the fat contents of a few *Pleurotus* species, *P. eosmus* is 1.1% (Stamets 2000), *P. pulmonarius* is 2% (Stamets 2000), *P. ostreatus* is 3.1% (Yang et al. 2001), *P. sajor-caju* is 2.29% (Alam et al. 2008), *P. eryngii* is 7.5% (Akyüz & Kirbag 2010) and *P. djamor* is 4.73% (Kortei & Wiafe- Kwagyan 2015). The fat contents of all these *Pleurotus* species were greater than 1%, while the fat content obtained for *P. djamor* in the present study was less than 1%.

The mushroom *P. djamor* strain PL01-white contains several types of saturated and unsaturated fatty acids. Eight distinct types of fatty acids were identified in the mushrooms by GC-MS analysis of the fatty acids derivatized to their methyl esters. Hexadecanoic acid (palmitic acid) is the fatty acid with the highest peak area resembling the highest concentration, and it is the most common type of saturated fatty acid found in living organisms (Kien et al. 2013). Raman et al. (2020) identified several other fatty acids from the *P. djamor* variety *roseus* in addition to the fatty acids identified in the present study, such as 12-methyl tetradecanoic acid, 6-octadecenoic acid, heptadecenoic acid, 9-hexadecanoic acid, 9-oxo-nonanoic acid, dodecanoic acid, hexanoic acid, and octanoic acid. However, 20-methyl-heneicosanoic acid was identified in the *P. djamor* strain PL01-white but not in the *P. djamor* variety *roseus*. This difference in fatty acid composition may be due to differences in the mushroom strain, growth conditions, or analytical procedure.

The dietary fibre content of different mushroom species varies between 14.4% and 70.2% (Yu et al. 2020). According to Nile & Park (2014), the total dietary fibre content of *P. djamor* is  $37 \pm 2.6\%$  which is similar to the total dietary fibre content of the novel *P. djamor* strain. According to Raman et al. (2020), the crude fibre content or insoluble fibre content is  $14.60 \pm 0.43$ , which is lower than that of the novel strain. The crude fibre content of *P. djamor* varies in the range of  $13.32 \pm 0.72$  to  $19.10 \pm 0.48\%$  based on the cultivated medium (Vega et al. 2022).

The total carbohydrate content in mushrooms refers to the total amount of digestible and non-digestible carbohydrates, many of which are dietary fibres. In the present study, the carbohydrate

content was determined by calculating the remaining percentage after subtracting the percentages of protein, lipids, fibre, and ash from the desiccated mushroom samples. Although this method yields erroneous results, it is widely used in proximate analysis because the direct determination of carbohydrates is a costly and time-consuming process. The total soluble carbohydrate content of liquids can be measured easily using a method such as the phenol-sulphuric acid method, but applying this method to solids leads to erroneous results.

Based on these findings it can be concluded that the novel mushroom strain *P. djamor* strain PL01-white is a rich source of major and trace metal elements. Among the analysed heavy metals, As was not detected. The mineral element content of mushrooms mainly depends on the substrate, and the amounts of mineral elements vary with the substrate (Hoa et al. 2015, El Sebaaly et al. 2019, Elkanah et al. 2022). The Cd content of the *P. djamor* strain PL01-White mushroom was slightly higher than the recommended value based on Codex Alimentarius (1995), but the As, Pb, and Hg contents were lower.

Some species of mushrooms contain different types of mycotoxins, such as hepatotoxic cyclopeptides, hydrazines, muscarinic agents, isoxazoles, and psilocin, and psilocybin (Puschner 2018). These toxins cause different health issues, such as acute gastroenteritis, hallucinations, cholinergic toxicity, disulfiram-like reactions, liver toxicity, nephrotoxicity, and seizures (Tran & Juergens 2022). Therefore, extreme precautions should be taken when consuming unknown mushroom species. Some toxins in mushrooms, such as hydrazines, are heat-labile and volatile (Michelot & Toth 1991), while psilocin and psilocybin are also heat sensitive (Puschner 2018). By thorough cooking of the mushrooms, the effects of these toxins can be avoided. But, the consumption of toxin containing mushrooms is not recommended. To resemble cooking, the mushrooms in this study were boiled in water after which the toxicity of the mushroom extract was tested. The median Lethal Concentration (LC<sub>50</sub>) or the concentration at which 50% of *Artemia salina* nauplii died revealed a reduction in cytotoxicity when the mushrooms were boiled in water. Therefore, the consumption of well-cooked mushrooms of these species is recommended.

Identification of the edibility of a mushroom species is a complex process due to the lack of any known experiments for identification of edibility (Chang & Miles 2004). Identifying mushroom toxicity is time-consuming, and advanced technology is required for this process. Since threshold concentrations for toxicity levels of edible mushrooms were unavailable, the toxicity was compared to that of known edible mushrooms. With these results, the absolute toxicity and edibility of the selected mushroom species cannot be predicted. Since the selected mushroom species have been consumed by the local people of Sri Lanka for generations, it can be assumed that they are safe for consumption; however advanced toxicity evaluations can be conducted. Among the cytotoxicity assays, the *Artemia* lethality bioassay is the most straightforward and cost-effective (Sarah et al. 2017). This assay is a high throughput and efficient method of toxicity identification (Wu 2014) and can be performed as a preliminary study for mammalian model toxicity studies. Mushroom water extracts of fresh mushrooms were used in this assay to maximize the distinct types and quantity of compounds extracted. Water extracts contain a high proportion of polysaccharides and other nutrients, thus facilitating microbial growth when the assay is conducted. Therefore, precautions should be taken to minimize microbial growth. Antibiotics can be added to the liquid medium used in the assay at concentrations that are not toxic to *Artemia salina* nauplii. In the present study, only acute toxicities were identified, and chronic toxicities should also be identified, which is time-consuming.

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