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Original Research

Invasive Grass Species *Megathyrsus maximus* and *Cenchrus setosus* for the Preparation of Substrate Media for Mushroom Cultivation in Jun Cao Technology: Alternatives to Sawdust Medium

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ABSTRACT

Purpose: *This study was conducted to prepare mushroom substrate media using Megathyrsus maximus and Cenchrus setosus, two invasive grass species in Sri Lanka by Jun Cao technology, with an anticipation of addressing the issue of shortage of sawdust to prepare substrate mixtures and as a strategy for controlling the grass populations simultaneously.*

Research Method: *Commercial mushroom, Pleurotus floridanus, Pleurotus djamor, Pleurotus cystidiosus, Pleurotus sajor-caju and Calocybe indica and two mushrooms novel to Sri Lanka, Pleurotus citrinopileatus and Pleurotus eryngii were inoculated in substrate media bags prepared using selected Jun Cao media and sawdust media. Mycelial growth in each medium was measured, and growth rates, mean yield per substrate bag, and biological efficiencies were compared.*

Findings and Values: *The maximum yield of P. djamor [285.20(18.13) g per bag] and P. floridanus [375.6(25.4) g per bag] were from M. maximus based medium and sawdust medium, respectively. The yields of P. sajor-caju, P. cystidiosus, C. indica and P. citrinopileatus in at least one selected Jun Cao medium, M. maximus and C. setosus, were not significantly different from yields of the same mushroom when cultivated in sawdust. The biological efficiencies in sawdust were not significantly higher than those of Jun Cao media. The exotic mushroom P. eryngii was successfully cultivated in Nuwara Eliya, Sri Lanka, using Jun Cao and sawdust-based media while C. setosus based medium gave the highest yield [642.4(41.61) g per bag] and the highest biological efficiency [217.03(14.05) %]. These two grass species can successfully be utilized as alternatives substrates in mushroom cultivation.*

Keywords: *Grass media, Mushroom science, Novel mushrooms, Sri Lankan mushrooms*

INTRODUCTION

Megathyrsus maximus (Jacq.) B.K. Simon & S.W.L. Jacobs and *Cenchrus setosus* Sw. are two grass species belonging to the family *Poaceae*. *Megathyrsus maximus* is known as Guinea grass in English and Gini thana or Rata thana in Sinhalese. It is a tropical African plant (Tomaszewska *et al.* 2021) and has been introduced to Sri Lanka as a food source for cattle and cultivated in many areas of the country (Thwaites, 1864, Wisumperuma 2007). In the 19th century, the plant was naturalised and

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later considered invasive (Bambaradeniya 2002, Kariyawasam *et al.* 2019). *Cenchrus setosus* is also a native African plant (Parker 2022), which is identified as Mission grass in English and is currently considered an invasive species in Sri Lanka (Ranwala *et al.* 2011), and it is considered difficult to control the populations (Senarathne 2019). These two grass species are becoming a devastating issue for the natural ecosystems and agroecosystems of the country. According to Ranwala *et al.* (2011), these two species are the most troublesome alien invaders in the country.

A successful strategy to control the spread and existing populations has not been identified yet. Recently, several projects have been underway to check the possibility of manufacturing paper products using these grass species as a strategy to minimise the plant population and to maximise the economic benefits. Alternatively, there is a potential for using these plants in many industries, including as substrate media for mushroom cultivation, which may have promising potential. This method of mushroom cultivation, based on substrate media prepared using grasses, is known as the "Jun Cao" method of mushroom cultivation.

The Jun Cao grass technology of mushroom cultivation was invented by Lin Zanxi in 1983, and this technology was awarded at the 20th International Invention Exhibition in Geneva in 1993 (Zhanxi & Zhanzhua 1995). Jun Cao can be defined as "herbaceous plants that are suitable for cultivating edible and medicinal fungi".

This method is a successful alternative to mushroom cultivation substrates such as sawdust, which are in short supply in some areas. Different types of grasses such as *Dicranopteris dicnotoma*, *Phragmitas communis*, *Themba gigantea*, *Pennisetum purpureum*, and *Arundinella hirta* can be used to prepare substrates in the cultivation of mushroom species such as *Lentinula edodes*, *Auricularia polytricha*, *Flammulina velutipes*, *Tremella fuciformis*, and *Dictyophora indusiate* (Zhanxi & Zhanzhu 1995).

The primary significance of Jun Cao technology is the high biological conversion rate and the high quality of the mushrooms produced. Additionally, this technology can be applied for small-scale to large-scale mushroom production with a wide range of applications. The availability of various suitable grass species is also a favourable condition for Jun Cao cultivation.

There are several methods for processing Jun Cao for cultivation. Generally, grasses are cut into pieces nearly 2–5 cm in size and moistened with the required amount of water, then mixed with a nitrogen source (such as wheat or rice bran) and lime (Lin, 2004, Zhanxi & Zhanzhua 1995). Successful cultivation of *Ganoderma balabacense* (Liu *et al.* 2015), *G. lucidum* (Rolim *et al.* 2014), *Pennisetum* sp. (Zhu *et al.* 2022), and *P. sajor-caju* (da Paz *et al.* 2013) using Jun Cao technology has also been reported. In Sri Lanka, Rajapakse *et al.* (2007) have identified the possibility of cultivating oyster mushrooms on several Jun Cao-based media.

In Sri Lanka, several mushroom species, such as *P. ostreatus*, *P. cystidiosus*, *P. sajor-caju*, *P. djamor*, and *C. indica* are cultivated on a commercial scale. Currently, the cultivation of these mushroom species primarily relies on sawdust-based substrate media. However, due to the insufficient availability of sawdust to meet the demand, alternative media for mushroom cultivation need to be developed.

Jun Cao technology for mushroom cultivation is a solution for this, and in the present study, the possibility of cultivating a few commercial mushroom species (*P. floridanus* [Pearl oyster], *P. djamor* [Pink oyster], *P. sajor-caju* [Bhutan oyster], and *C. indica* [Milky mushroom]) on the substrate media prepared using the grasses *M. maximus* and *C. setosus* was studied. Apart from the current commercial mushroom species in Sri Lanka, the possibility of cultivating two mushroom species novel to Sri Lanka, namely, *P. citrinopileatus* (Golden oyster) and *P. eryngii* (King oyster) on the selected grass-based substrate media compared to the sawdust-based media, was identified.

MATERIALS AND METHODS

Authentication of the grass species

The grass species, *M. maximus* and *C. setosus*, were collected from the same geographical location in Polgahawela, Sri Lanka (7.382010 °N, 80.280150 °E). The authenticity of these plant species was verified at the Sri Lanka National Herbarium.

Morphological and molecular identification of the mushroom species

Cultures of industrially cultivated mushroom species, *P. floridanus*, *P. sajor-caju*, and *P. cystidiosus*, were obtained from "Sahan Mushroom Seeds", Maligamale, Panwilatenna, Sri Lanka. *Pleurotus djamor* and *C. indica* cultures were obtained from "Taru Biotech", Hunukumbura, Itanawatta, Sri Lanka. Tentative identification of the selected mushroom species was performed by analysing the morphological characteristics using keys described by Arora (1986) and Pegler (1986).

The cultures of *P. eryngii* and *P. citrinopileatus* were imported to Sri Lanka under the permission of Sri Lanka Plant Quarantine Service (Permit number – NPQS/PIP/2020/514). The confirmation of the species *P. citrinopileatus* was based on information from several sources, including Arora (1986), Pegler (1986), Ohira (1990), Nagasawa & Arita (2000), and Singer (1943). Similarly, the confirmation of the species *P. eryngii* was based on publications by Arora (1986), Pegler (1986), and Kim *et al.* (1997).

The obtained cultures were sub-cultured, and molecular identification was carried out to confirm the identity of the cultures of *P. floridanus*, *P. djamor*, *P. sajor-caju*, *P. citrinopileatus* and *C. indica*. DNA was extracted from fresh mycelia based on the protocol by Guo *et al.* (2000).

For DNA barcoding, the universal fungal barcoding region, the nuclear ribosomal Internal Transcribed Spacer (ITS) region, was amplified using Polymerase Chain Reaction (PCR) following the protocol by Manamgoda *et al.* (2012).

The nuclear ribosomal ITS region was amplified using PCR primers ITS1 and ITS4 (White *et al.* 1990). After electrophoresis, the PCR product was purified, and the nuclear ribosomal ITS region was sequenced using Sanger sequencing. The resulting sequences were used as the query for the BLAST® tool (Zhang *et al.* 2000; Okonechnikov *et al.* 2012) to identify the potential target species of the mushrooms.

Phylogenetic placement of the studied mushroom species was performed by phylogenetic analyses of the generated ITS sequences, retrieving some ITS sequences of several closely related species from the GenBank database (Benson *et al.* 2012) based on previous studies by Razaq *et al.* (2016) and Barbosa *et al.* (2018). The required adjustments to the retrieved sequences were made using the software Unipro UGENE v43.0 (Okonechnikov *et al.* 2012). Maximum likelihood phylogenetic analysis (Huelsenbeck & Crandall 1997) was conducted using the bootstrap method test of phylogeny (Felsenstein, 1985) and Kimura's 2-parameter model (Kimura, 1980) with 1000 bootstrap replicates using the software MEGA v11 (Tamura *et al.* 2021).

Preparation of substrate

To prepare the substrate medium for mushroom cultivation, the grasses were initially cut into small pieces measuring 1–3 cm, and those pieces were dried in direct sunlight for approximately five days. Subsequently, the grass pieces were soaked in well water overnight and drained. Afterwards, they were dried in the shade while being continuously mixed until the moisture percentage was reduced to 170% (dry weight basis).

The moisture content of the grass pieces was determined using the oven-dry method (Ahn *et al.* 2014), and the amount of dry grass was calculated. Based on the dry mass of grass, rice bran, $CaCO_3$ and $CaSO_4$ were added as 10 kg, 2.5 kg and 1 kg per 100 kg of dry grass pieces, respectively. The amount of well-water required to achieve 170% (w/w) moisture content was

added, with dissolved $MgSO_4$ at 200 g per 100 kg of dry grass pieces.

Standard cultivation of mushrooms

Polypropylene bags with a thickness of 300 µm were filled with approximately 800 g of *C. setosus*-based medium and about 1000 g of *M. maximus*-based medium separately while keeping the bag size constant (about 10 cm in diameter and 20 cm in height). Control substrate bags were prepared with rubber sawdust-based medium that had a moisture percentage of 170% (w/w), and the same contents of rice bran, $CaCO_3$, $CaSO_4$ and $MgSO_4$. The bags were filled up to a weight of approximately 1400 g using sawdust medium, keeping the same bag size as Jun Cao media (10 cm in diameter and 20 cm in height).

The substrate bags were sterilized by steaming for about 3–4 hours. After cooling, the bags were inoculated with 5–10 g of grain spawn inoculum of each mushroom species selected under aseptic conditions, making five replicates from each substrate type, and covered with a filter cap. They were incubated in an incubation chamber at 25 $\pm$ 2 °C for three to five weeks. The mycelial growth was measured from the inoculum point to the mycelial front at three-day intervals until the mycelia fully colonised the substrate medium. After the bags were fully colonised with mycelia, they were transferred to the fruiting chamber.

The bags were arranged according to Randomised Block Design (Dodge 2008). The filter caps were removed while the neck ring was retained, and the bags were sprayed with water three times a day. Using an ultrasonic mist maker, the relative humidity inside the fruiting chamber was maintained between 95–99%.

Fructification of the *P. eryngii* mushroom was performed in three different locations: Polgahawela, Sri Lanka (7.382010 °N, 80.280150 °E), Nanu-Oya, Nuwara Eliya, Sri Lanka (6.943391 °N, 80.751425 °E) and Sri Jayewardenepura, Sri Lanka (6.8541561 °N, 79.9041446 °E), while all other mushrooms were fructified in Polgahawela, Sri Lanka. The mushrooms were harvested daily, and their weight was recorded for each bag. Harvesting continued for a period of three months.

Statistical analysis

The mean yields were compared for significant differences using Tukey's pairwise comparison of one-way ANOVA, with a significance level of 0.05, utilising Minitab 17 Statistical Software (2010). When ANOVA conditions were not satisfied, non-parametric pairwise comparisons were conducted using SPSS Statistics software version 21.0 (IBM Corporation, 2012). The biological efficiency was calculated based on Chang (1993) using Equation 1.

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushrooms}}{\text{Dry weight of the substrate}} \times 100 \quad (1)$$

RESULTS AND DISCUSSION

Authenticity of the grass and mushroom species

Megathyrsus maximus is a robust perennial grass (60–200 cm) with hairy nodes, linear leaves, and branched panicles bearing oblong spikelets, producing fertile seeds with unequal glume (Fig.1). *Cenchrus setosus* is a tufted grass with fibrous roots and slender to stout culms (0.3–3 m). It has narrow, flat leaves with scattered hairs, a hairy ligule, and spike-like panicles. Spikelets are sessile, surrounded by bristles, with one fertile flower and shiny, yellow-brown grains enclosed by bristles (Fig.2).

Morphological characterization confirmed the identity of *P. eryngii* and *P. djamor* mushrooms, allowing for unambiguous identification. Fig. 3 depicts some morphological characteristics of the *P. eryngii* mushroom that were used to confirm the species identity. Even though the cultures of commercially cultivated mushroom species in Sri Lanka were obtained from government-registered spawn-producing institutes, the molecular identification based on BLAST® targets and the ITS region-based phylogenetic analysis were used to reveal the authenticity of *P. sajor-caju* (Fig. 4), *P. cystidiosus* (Fig. 4), *P. floridanus* (Fig. 5) and *C. indica* (Fig. 6). Bhutan oyster has been grouped

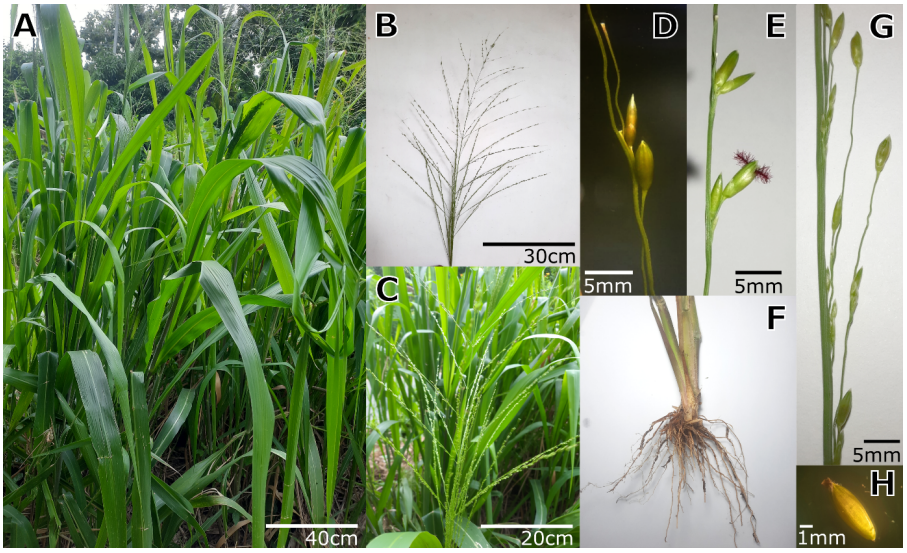

Figure 1. Morphology of *Megathyrsus maximus*; A. The habit; B,C. Inflorescence; D,H. Seeds; E. Flowers; F. Root system; G. Spikelets.)

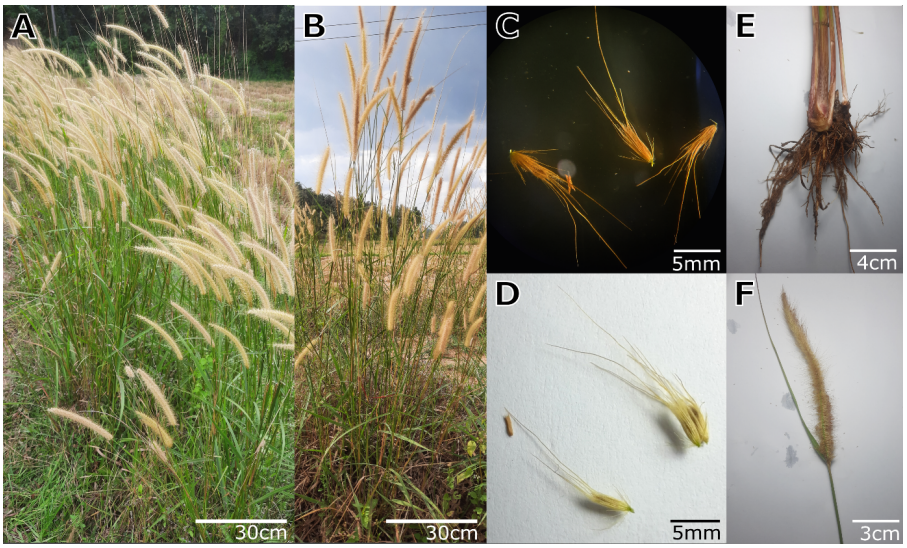

Figure 2. Morphology of *Cenchrus setosus*; A,B. The habit; C,D. Spikelets; E. Root system; F. Inflorescence.)

with both the species *P. pulmonarius* and *P. sajor-caju* (Fig. 4).

Two independent studies have been carried out by *Shnyreva et al.* (2012) and *Zmitrovich & Wasser* (2016) to differentiate the two species *P. sajor-caju* and *P. pulmonarius*, which are morphologically indistinguishable and whose molecular phylogenetic characteristics based on ITS regions are also similar. According to *Zmitrovich and Wasser* (2016), *P. sajor-caju* differs from *P. pulmonarius* in the tendency to form a large cluster of basidiocarps, fan-shaped pilei, bent stipe and an undulating margin. They describe *P. sajor-caju* as a new variety, *P. pulmonarius var. stechangii*. *Shnyreva et al.* (2012) distinguished *P. sajor-caju* from *P. pulmonarius* based on total reproductive isolation demonstrated by monokaryotic haploid sexual compatibility assays. Based on this information and the morphological traits, the strain used in the present study was identified as *P. sajor-caju*.

Variation of growth rate, yield, and biological efficiency of mushrooms cultivated on grass-based media compared to sawdust-based media

The growth rates of mycelia of selected mushroom species on the two grass-based media were compared to sawdust (Fig. 7). *Pleurotus*

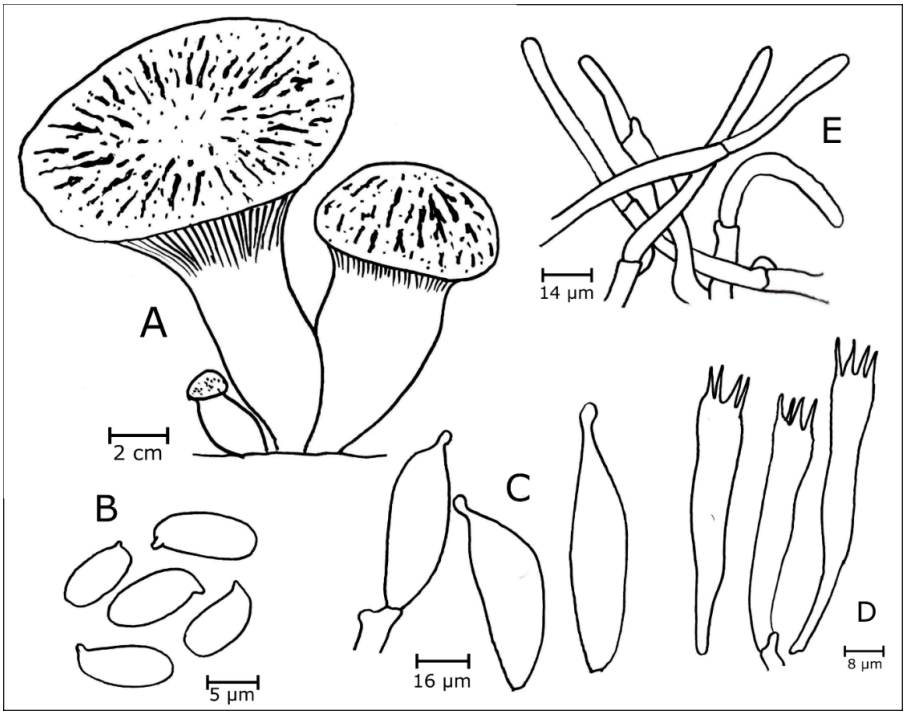

Figure 3. Morphological characteristics of *Pleurotus eryngii* mushroom; A. Habit of the basidiocarp; B. Basidiospores; C. Cheilocystidia; D. Basidia; E. Pileipellis.)

djamor growth was significantly faster on *C. setosus*-based medium than on *M. maximus*-based medium ($P = 0.003$), however, not significantly differed from that on sawdust. The growth rates of *P. floridanus* and *P. sajor-caju* were significantly high both on *C. setosus*-based medium and sawdust medium. The growth rates of *P. citrinopileatus* and *P. cystidiosus* were not significantly different in any tested media. The fastest growth of *C. indica* mycelia were observed in *C. setosus*-based medium which is significantly faster than that in sawdust medium ($P = 0.036$). According to Ogidi *et al.* (2017), the growth rate of *P. ostreatus*, *P. pulmonarius*, *P. cornucopiae*, and *P. djamor* do not show significant difference in the growth rate when cultivated in *Urochloa decumbens* grass medium. Liang *et al.* (2009) have identified that medium prepared with *Zea mays* facilitates mycelial growth than two other grass media prepared using *Panicum repens*, *Pennisetum purpureum* in the cultivation of *P. citrinopileatus*. However, comparisons of growth rates in Jun Cao media with sawdust media are rarely documented in studies.

The yields (Fig. 8) and biological efficiencies (Fig. 9) of selected mushroom species grown on *C. setosus* and *M. maximus* grass-based media was compared to those grown on sawdust. Fig. 10 displays the appearance of mushrooms on grass and sawdust-based substrates.

Yield and biological efficiency of mushrooms on grass-based and sawdust media

Pleurotus djamor yielded significantly more in *M. maximus*-based medium, and biological efficiencies were greater when grown on two grass-based media rather than sawdust. *Pleurotus floridanus* yield was highest in sawdust medium, whereas biological efficiency was highest in *C. setosus*-based medium, with no significant difference between the other two media. Although the yield in sawdust media was much higher than the yield in *M. maximus*-based medium ($P = 0.000$), the biological efficiencies of the *P. sajor-caju* mushroom cultivated on all three types of media were not significantly different. *Pleurotus citrinopileatus* biological efficiencies in grass and sawdust media were not statistically different, while the yield in sawdust medium was significantly higher than the yield in *C. setosus*-based medium ($P = 0.039$). The yield in the *M. maximus*-based medium did not differ considerably from the yield in the other two media. The yields and biological efficiencies obtained by cultivating

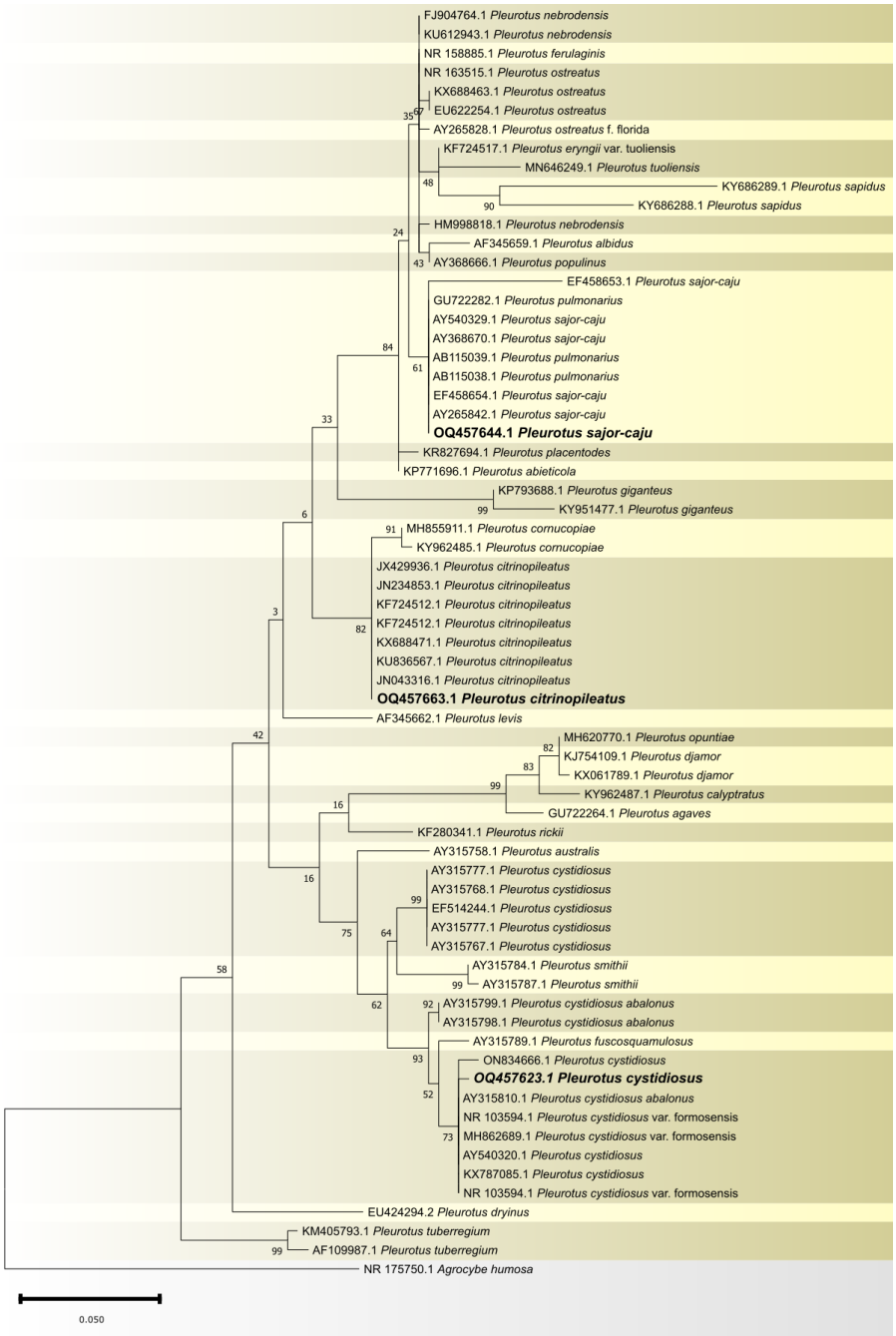

Figure 4. Phylogram obtained for the *Pleurotus sajor-caju*, *Pleurotus cystidiosus* and *Pleurotus citrinopileatus* used in Jun Cao cultivation from maximum likelihood analysis based on Internal Transcribed Spacer (ITS) sequences using MEGA v11. The tree is rooted with *Agrocybe humosa*. The species in the present study are shown in bold letters

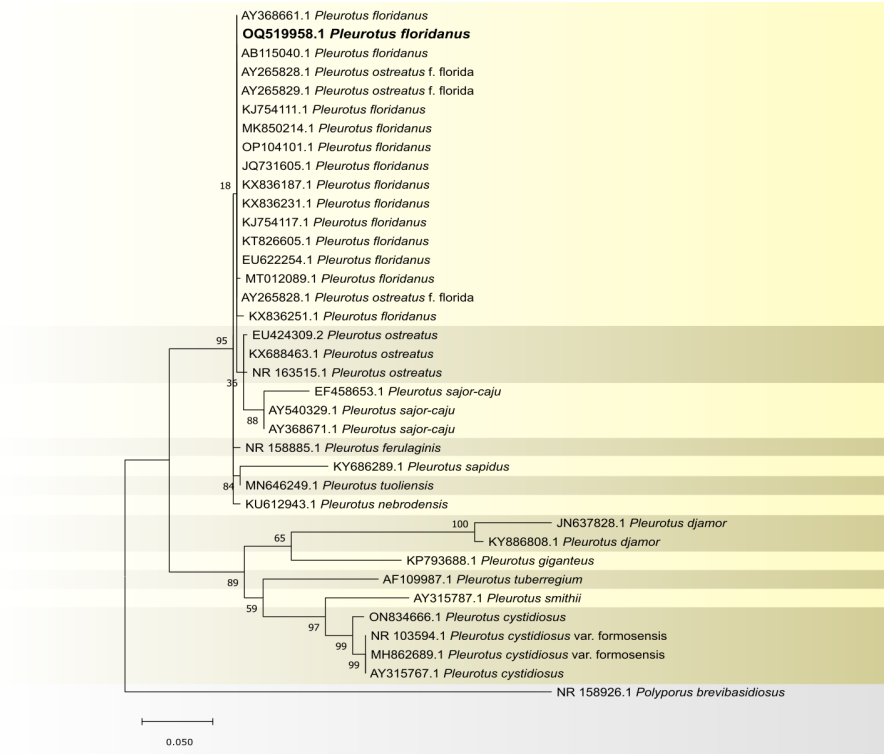

Figure 5. Phylogram obtained for the *Pleurotus floridanus* used in Jun Cao cultivation from maximum likelihood analysis based on Internal Transcribed Spacer (ITS) sequences using MEGA v11. The tree is rooted with *Polyporus brevibasidiosus*. The species in the present study is shown in bold letters.

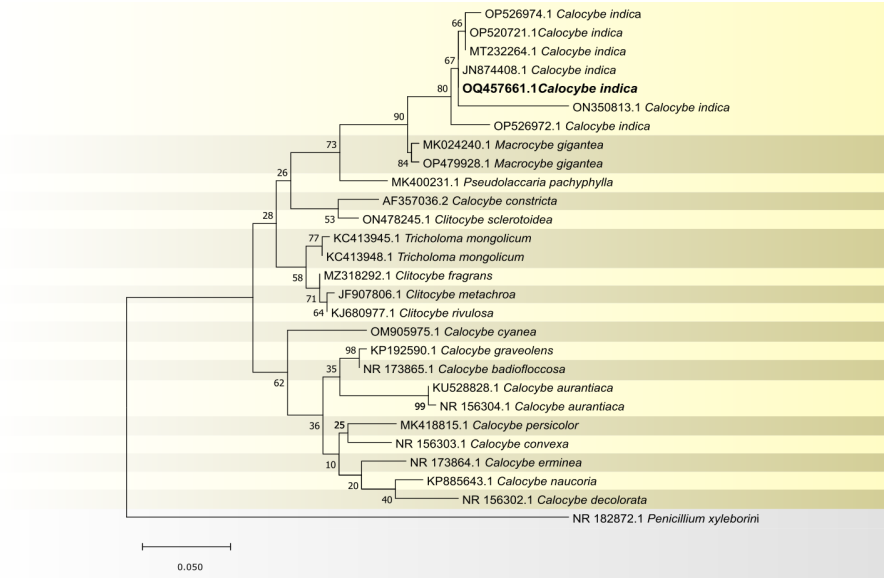

Figure 6. Phylogram obtained for the *Calocybe indica* used in Jun Cao cultivation from maximum likelihood analysis based on Internal Transcribed Spacer (ITS) sequences by using MEGA v11. The tree is rooted with *Penicillium xyleborini*. The species in the present study are in bold letters.

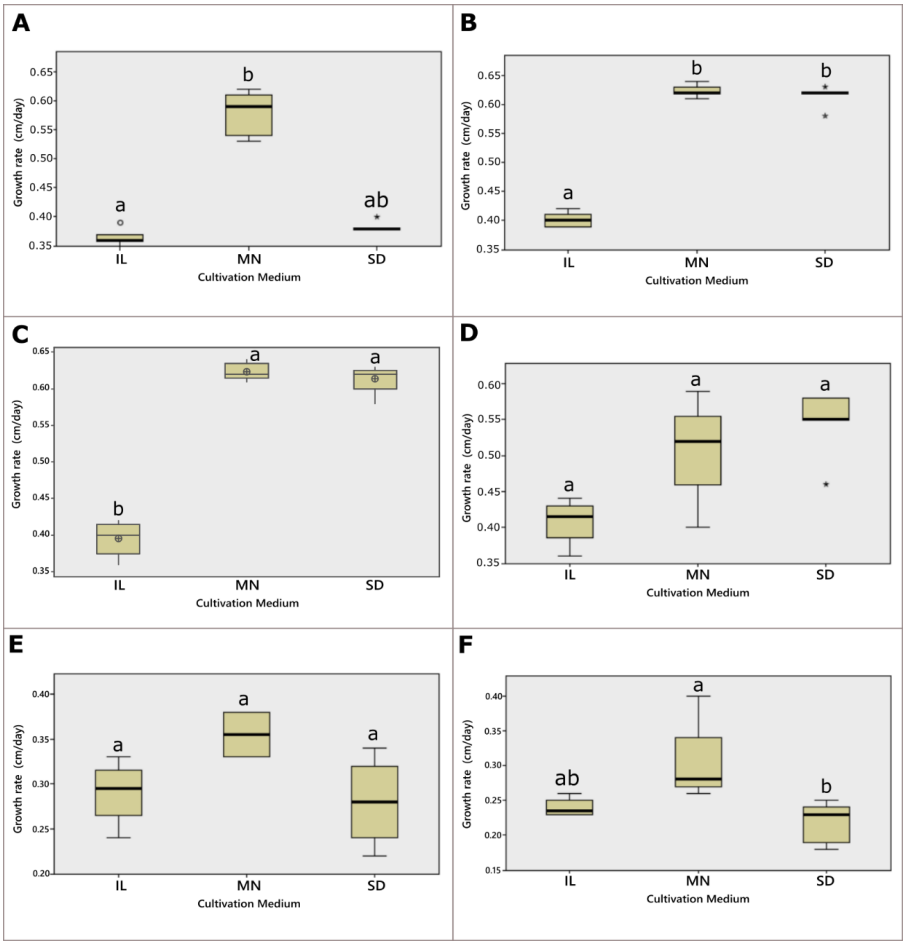

Figure 7. The variation of growth rate of mushroom mycelia when cultivated in sawdust-based medium (SD) and grass-based media, *Megathyrsus maximus*-based medium (IL) and *Cenchrus setosus*-based medium (MN); A. *Pleurotus djamor*; B. *P. floridanus*; C. *P. sajor-caju*; D. *P. citrinopileatus*; E. *P. cystidiosus*; F. *Calocybe indica*. The graph 'C' was generated by Analysis of Variance (ANOVA) using Minitab 17 software. The graphs 'A', 'B', 'D', 'E' and 'F' were generated by non-parametric pairwise comparison using IMB® SPSS® Statistics v21 software. Means that do not share a letter are significantly different ($P < 0.05$).

P. cystidiosus mushrooms on sawdust-based media versus grass-based media did not differ significantly. *Calocybe indica* was grown with greater biological efficiency ($P = 0.006$) and yield ($P = 0.031$) on *M. maximus*-based medium than on sawdust-based medium. The yields and biological efficiencies obtained for this mushroom on *C. setosus*-based medium were not significantly different from those obtained on the other two media. A similar trend was reported by Liang *et al.* (2009) during the cultivation of *Auricularia polytricha* on various grass-based media composed of *Panicum repens*, *Pennisetum purpureum*, and *Zea mays*. The study found that biological efficiencies were higher when the mushroom was cultivated on these grass-based media compared to sawdust. According to Argaw *et al.* (2023), *Cordia africana* leaves emerged as a highly favorable substrate for cultivation of oyster mushrooms, demonstrating the fastest primordial initiation, highest mean yield and biological efficiency over sawdust medium.

Pleurotus eryngii is a species that thrives in subtropical and temperate climates and is extensively cultivated worldwide. This mushroom had not previously been commercially cultivated in Sri Lanka, and the cultivation potential of the mushroom in Sri Lanka was identified in this study.

The fructification of the mushroom was tested in three different locations in Sri Lanka within the month of October. The mean temperatures in

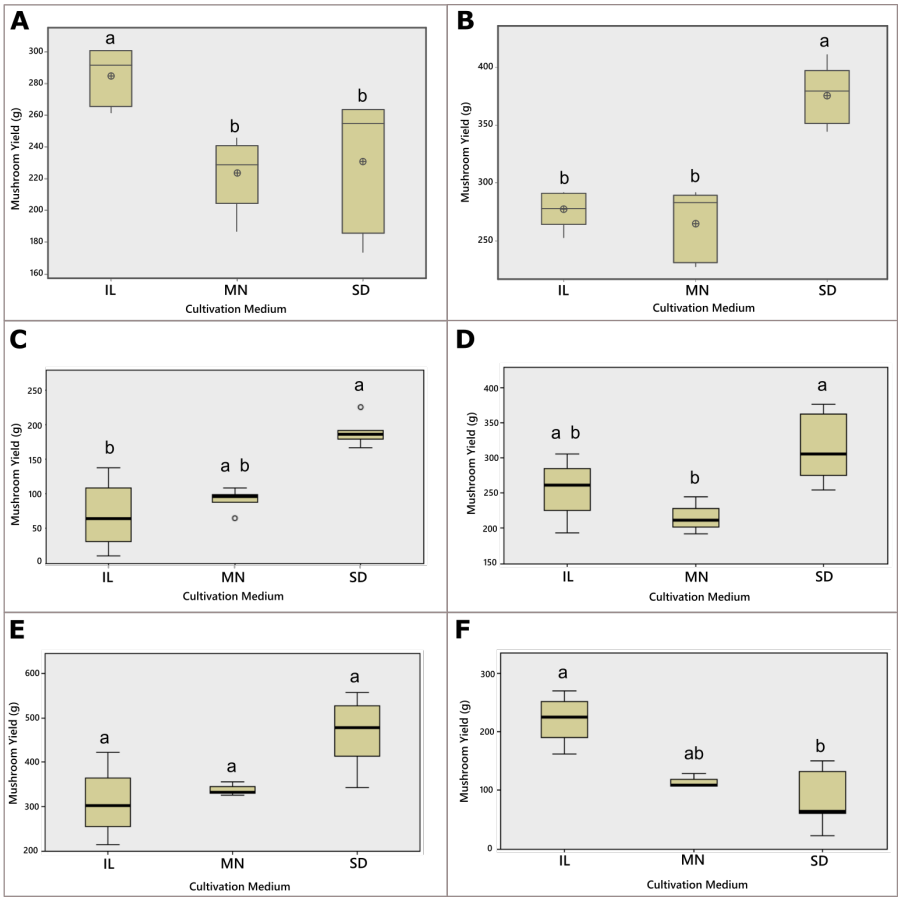

Figure 8. The variation of yield of mushrooms when cultivated in sawdust-based medium (SD) and grass-based media, *Megathyrsus maximus*-based medium (IL) and *Cenchrus setosus*-based medium (MN); A. *Pleurotus djamor*; B. *P. floridanus*; C. *P. sajor-caju*; D. *P. citrinopileatus*; E. *P. cystidiosus*; F. *Calocybe indica*. The graphs 'A' and 'B' were generated by Analysis of Variance (ANOVA) using Minitab 17 software. The graphs 'C', 'D', 'E' and 'F' were generated by non-parametric pairwise comparison using IMB® SPSS® Statistics v21 software. Means that do not share a letter are significantly different ($P < 0.05$).

these three locations were 26.1 °C, 24.9 °C and 18.9 °C in Sri Jayewardenepura, Polgahawela and Nuwara Eliya respectively. Among these three locations, mushrooms appeared only in substrate bags in Nuwara Eliya, Sri Lanka. Stamets (2000) has mentioned that the primordia formation of this mushroom occurs at 10–15 °C. Before sunrise in Nuwara Eliya, Sri Lanka, the temperature reaches this level. Fruiting body development occurs at a temperature of 15–25 °C (Stamets, 2000), which corresponds to the daytime temperature range in Nuwara Eliya.

Among the various substrate media types tested, *C. setosus*-based medium gave significantly the highest yield of *P. eryngii* (Fig. 11). Conversely, the sawdust-based medium yielded significantly lower results, as evidenced by the lowest mean yield. The trend in mean biological efficiency also aligned with these findings (Fig. 11). When filling these substrates into the bags, the weight of the bags varies due to the different densities of the media used. It is important to note that the size of the bags remained constant throughout the study keeping the substrate volume constant, as commercial cultivation typically involves the use of substrate bags of the same size.

In the process of filling grass-based media into polypropylene bags, there is a risk of tearing due to the presence of grass pieces. To address this issue, thicker polypropylene bags were utilized. However, it is worth considering that by employing a machine to pulverize the grass media, this problem can be largely avoided. Furthermore, the use of pulverization reduces the particle size, enabling a greater weight of media to be filled into each bag. This has the potential

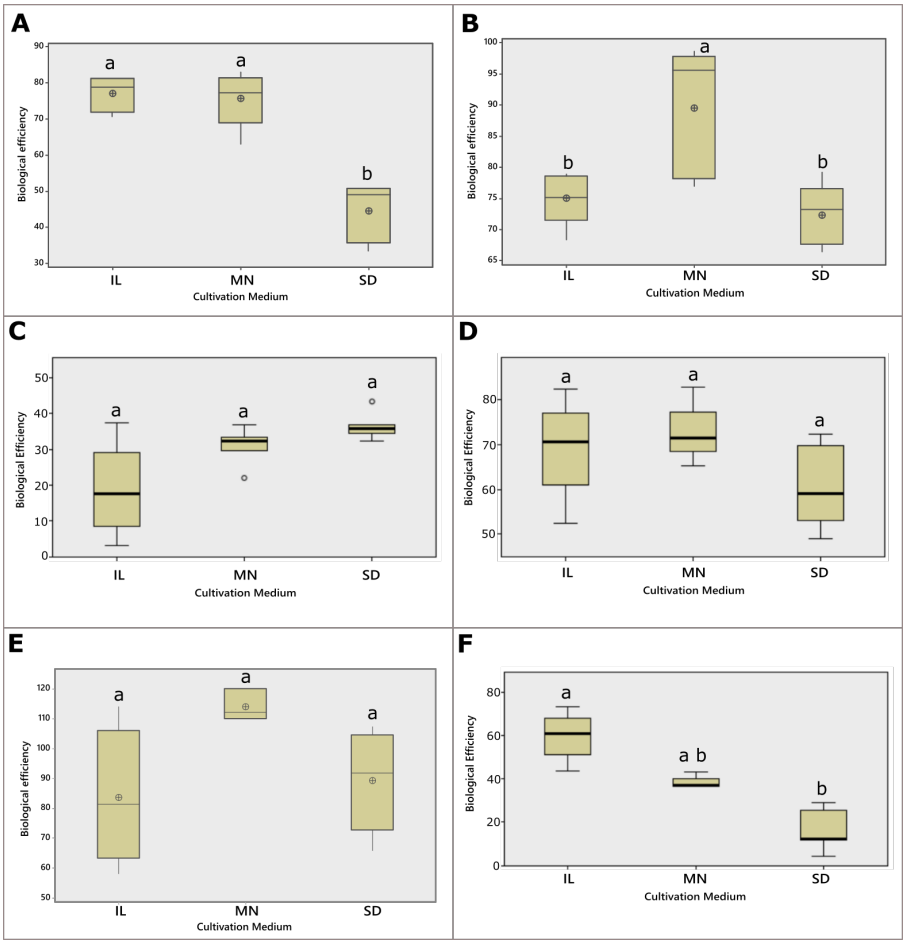

Figure 9. The variation of biological efficiency of mushrooms when cultivated in sawdust-based medium (SD) and grass-based media, *Megathyrsus maximus*-based medium (IL) and *Cenchrus setosus*-based medium (MN); A. *Pleurotus djamor*; B. *P. floridanus*; C. *P. sajor-caju*; D. *P. citrinopileatus*; E. *P. cystidiosus*; F. *Calocybe indica*. The graphs 'A', 'B' and 'E' were generated by Analysis of Variance (ANOVA) using Minitab 17 software. The graphs 'C', 'D' and 'F' were generated by non-parametric pairwise comparison using IMB® SPSS® Statistics v21 software. Means that do not share a letter are significantly different ($P < 0.05$).

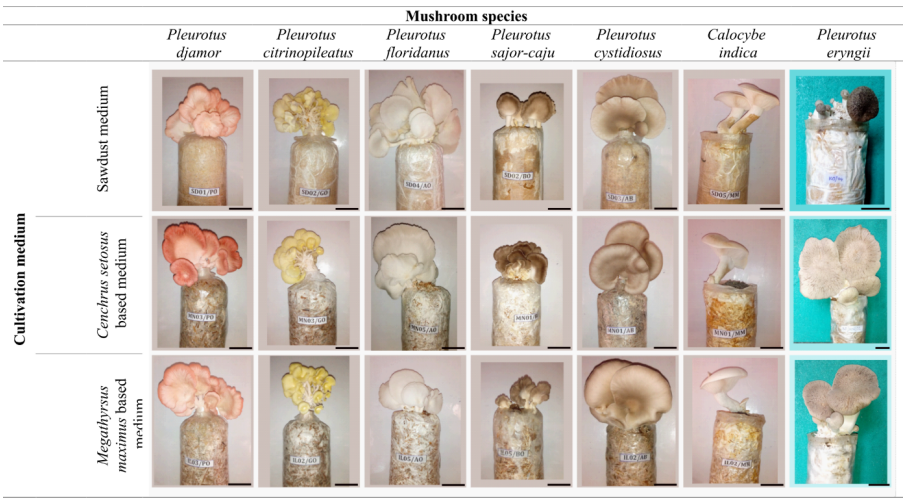

Figure 10. *Pleurotus djamor*, *P. citrinopileatus*, *P. floridanus*, *P. sajor-caju*, *P. cystidiosus*, *Calocybe indica* and *P. eryngii* in *Cenchrus setosus* and *Megathyrsus maximus* grass media and sawdust medium. Bars: 5 cm.

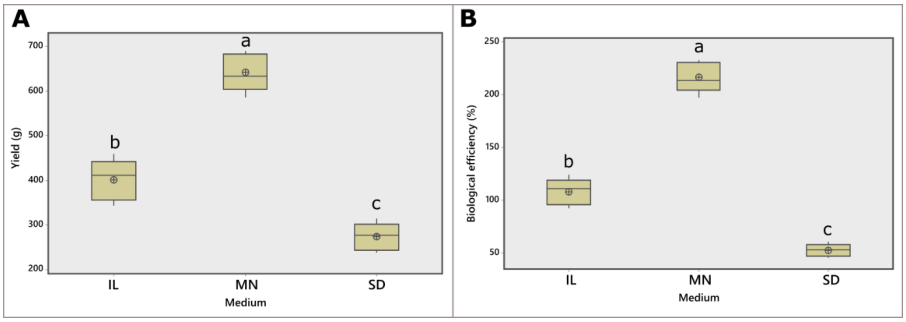

Figure 11. The variation of the yield (A) and biological efficiency (B) of *Pleurotus eryngii* mushroom when cultivated on sawdust-based medium (SD), *Cenchrus setosus*-based medium (MN) and *Megathyrsus maximus*-based medium (IL). The graph was generated by Analysis of Variance (ANOVA) using Minitab 17 software. Means that do not share a letter are significantly different ($P < 0.05$).

to increase the yield per bag. As a result, the need for thick bags may be reduced, leading to cost savings.

The moisture content of the substrate is a crucial factor affecting both the appearance and yield of mushrooms. In the current study, a moisture content of 170% (w/w) was employed, calculated based on the dry mass of the substrate. However, it is important to take into account the residual moisture content of the substrate during these calculations.

The moisture content is primarily influenced by the properties of the substrate, including particle size and water retention ability. Moreover, the particle size of the substrate can significantly impact the moisture content.

Bernardi *et al.* (2007) have tried cultivation of *P. ostreatus*, *P. ostreatoroseus*, and *P. citrinopileatus* on the substrate media prepared using *P. purpureum*, but the substrate preparation was different from the present study. They have used powdered dry grass instead of the grass pieces, which resembles the sawdust-based cultivation and more biomass could be filled to substrate bags which leads to higher yields per substrate bag.

André *et al.* (2017) have cultivated *Ganoderma lucidum* on various Jun Cao media prepared with *P. purpureum*, *Brachiaria brizantha*, *Panicum maximum*, *Brachiaria decumbens*, and *Brachiaria humidicola*.

They have observed that the biological efficiency is higher in Jun Cao media. Rolim *et al.* (2014) have successfully cultivated *Ganoderma lucidum* on media prepared by Jun Cao technology mainly using the grass *Pennisetum purpureum* and has achieved a biological efficiency ranging from 12–72%. Masevhe *et al.* (2016) have tested various grass-based media to cultivate *P. ostreatus*. Siqueira *et al.* (2011) have identified that *P. sajor-caju* show 74.4% and 74.12% biological efficiencies respectively when cultivated on banana stalks and Bahia grass. A maximum biological yield of 103% was obtained by Mshandete (2011) when *P. sapidus* cultivated on grass medium *Typha domingesis*.

CONCLUSION

The invasive grass species *M. maximus* and *C. setosus* can be successfully used to prepare substrate media for mushroom cultivation. Commercially cultivated mushroom strains in Sri Lanka, including *P. floridanus*, *P. djamor*, *P. cystidiosus*, *P. sajor-caju* and *C. indica* can be successfully cultivated on these media.

For optimal economic benefits, it is recommended to focus on cultivating *P. djamor*, *P. sajor-caju*, and *P. cystidiosus* using either of the two grass-based media at the commercial level. Meanwhile, cultivating *P. floridanus* on *C. setosus*-based medium and *C. indica* on *M. maximus*-based medium can be economically viable based on the results obtained from this

study.

Additionally, this study identified that *P. eryngii*, an exotic mushroom species, can be successfully cultivated in Nuwara Eliya, Sri Lanka, where relatively low environmental temperature conditions prevail throughout the year. Both *P. citrinopileatus* and *P. eryngii*, as exotic mushroom species, showed successful growth on *M. maximus* and *C. setosus* grass-based media. Commercial cultivation of these two mushroom species is recommended either on sawdust-based media or grass-based media.

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