



Optimization of Mycelial Culture Conditions and Fructification of *Lentinus* Species Using Rice Straw and Sawdust Based Substrates

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Abstract

Lentinus (Basidiomycota, Polyporaceae) species are naturally-occurring mushrooms with nutritional and pharmacological importance. Unlike shiitake *Lentinula edodes*, a widely cultivated mushroom, *Lentinus* species are often ignored because they are underutilized and unpopular. However, like shiitake, *Lentinus* spp. could also be valuable resources of functional food and bioactive compounds. It is therefore of high interest to understand their cultural requirements leading to their efficient biomass production. Thus, this study established the optimal culture conditions for the maximum mycelial growth of seven wild strains under four *Lentinus* species. Their cultivation potentials were also determined using rice straw and sawdust (7:3 v/v) substrate formulation. Mycelia of all *Lentinus* strains evaluated favorably grew on coconut water *gulaman* (local crude agar) and other culture media for specific strain with suitable pH ranging from 5.0 to 8.0. Aeration was not a major physical factor for all *Lentinus* strains except *L. squarrosulus* strain 1, which favored sealed condition. Both lighted and dark conditions were found suitable for both strains of *L. sajor-caju*, *L. strigosus* strain 2 and *L. swartzii* whereas both strains of *L. squarrosulus* and *L. strigosus* strain 1 favorably grew in lighted condition. However, all *Lentinus* strains evaluated recorded the highest mycelial growth rates and thick mycelia at 30°C. In terms of fruiting body production, all *Lentinus* strains demonstrated cultivation potential. The seven *Lentinus* strains completely colonized the substrate for 18.0–25.6 days and initiated primordia for 21.6–33.5 days. The highest yield (52.5 g bag⁻¹) and biological efficiency (10.5%) was recorded in *L. strigosus* strain 1, whereas the lowest was noted in *L. strigosus* strain 2 with 21.1 g bag⁻¹ yield and 4.2% biological efficiency. *L. sajor-caju* strain 2 and *L. squarrosulus* strain 1 showed the widest cap diameter and longest stipe, respectively. The mycelial growth and fruiting body performance of *Lentinus* may vary depending on species and strains. Altogether, the seven new wild strains of *Lentinus* can be artificially cultivated by providing the nutritional and physical requirements.

Key words – fruit body – formation medicinal mushrooms – mycelia – nutritional requirements – physical factors

Introduction

Mushrooms are Ascomycetous or Basidiomycetous, epigeous or hypogeous, edible or poisonous macrofungi with distinctive fruiting bodies. They are rich sources of valuable nutrients, which are considered nutritious food. The dry matter of mushrooms contains 50%–65% carbohydrates, 19%–35% proteins, and 2%–6% fat (Dulay et al. 2014, Rathore et al. 2017). Mushrooms are also a good source of different dietary fiber with non-dietary carbohydrates, amino acids, vitamins, minerals and fatty acids (Rathore et al. 2017, Painuli et al. 2020, Bengu 2020). Apart from the nutritional values, mushrooms also produce various biologically-active compounds. *Lentinula edodes*, for instance, contain lenthionine, lentinan, other polysaccharides, amino acids, vitamins (B1, B2, C, D) and dietary fibers, which exhibit several biological activities including antimicrobial, cytotoxic, antitumor, anti-inflammatory, antioxidant, anti-aging, anti-hepatitis and immune-enhancing (Xu et al. 2014, Wang et al. 2015, Kupcova et al. 2018, Chung et al. 2019). Accordingly, mushroom is now being used as an ingredient to improve the nutritional and functional qualities of food products and dietary supplements.

The Philippine archipelago is a natural habitat of diverse macrofungal species. Several species listing and biodiversity studies have been conducted in the different areas of the country particularly in Luzon Island (De Leon et al. 2012, De Castro & Dulay 2015, Tadiosa & Arsenio 2014, Arenas et al. 2015, 2018, Dulay & Maglasang 2017, Culala & Dulay 2018, Dulay et al. 2020a). The most common macrofungal genera that have been recorded in the above-cited studies include *Ganoderma*, *Lentinus*, *Schizophyllum*, *Volvariella*, *Collybia*, *Polyporus*, *Trametes*, *Pycnoporus*, *Auricularia*, *Pleurotus*, *Panaeolus*, *Termitomyces*, *Coprinus*, *Dictyophora*, *Oudemansiella*, *Chlorophyllum*, *Marasmius* and *Xylaria*. *Lentinus* (Polyporaceae), also known as sawgill mushrooms, are wood-rotting basidiomycetous mushrooms that naturally grow in a wide temperature range, from the tropical to temperate regions. Species of this genus are relative to the widely cultivated and medicinal shiitake (*Lentinula edodes*) mushroom. Mushrooms such as *L. tigrinus*, *L. sajor-caju*, *L. strigosus* and *L. swartzii* contain carbohydrates, proteins, crude fibers, crude fat, vitamins, minerals and important bioactive metabolites such as alkaloids, flavonoids, phenols, coumarins, triterpenes, tannins, saponins, anthraquinones, athrones and steroids (Dulay et al. 2012, De Leon et al. 2017a, Dulay & Pamiloza 2018, Austria et al. 2021). Moreover, *Lentinus* species exhibit antioxidant, antibacterial, antidiabetic, anti-HIV, anticancer, and other biological properties (Dulay et al. 2012, Dulay et al. 2017a, Xu et al. 2012, Udchumpisai & Bangyeekhun 2019a, b).

Recently, a number of geographical wild species/strains of *Lentinus* have been documented and collected from different regions of Luzon Island including Isabel, Cagayan Valley, Ilocos Sur, Nueva Vizcaya, Nueva Ecija, Zambales, Tarlac, Quezon Province, and Camarines Sur. Mycelia from the wild *Lentinus* fruiting bodies have been successfully rescued and included in the list of our culture collections of Philippine wild mushrooms at the Center for Tropical Mushroom Research and Development, Central Luzon State University. Since *Lentinus* is one of the promising mushroom genus for their nutritional and pharmacological properties, it is necessary to understand their cultural requirements that would lead to the generation of the practical technology for mushroom biomass production for culinary and medicinal applications, thus, this study was conducted. The optimal cultural conditions for mycelial growth of seven strains under four *Lentinus* species, namely *L. sajor-caju*, *L. squarrosulus*, *L. strigosus*, and *L. swartzii* from the Philippines are reported herein.

Materials & Methods

Lentinus cultures

The mycelial cultures of *Lentinus* species were acquired from the Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. The identification of the mushroom species was previously confirmed using rDNA-ITS sequence analysis. To prepare mycelial inoculant, the mycelial blocks of each

mushroom was inoculated on potato dextrose agar plate and incubated at 30°C to allow fungal growth. After seven days of incubation, mycelial discs were prepared using a flame-sterile cork borer and were used as inoculants in the growth evaluation.

Evaluation of culture media and pH

A total of eight culture media were used in this study. Four indigenous culture media, namely coconut water gulaman (CWG), potato sucrose gulaman (PSG), corn grit decoction gulaman (CGDG), and rice bran decoction gulaman (RBDG), and four commercially-available dehydrated media, namely potato dextrose agar (PDA), malt extract agar (MEA), Sabouraud dextrose agar (SDA), and mycological agar (MA) were evaluated. Decoctions of 250 g potato cubes, 50 g rice bran, and 50 g corn grit in 1 L distilled water were separately prepared. Coconut water and the three decoctions were separately boiled and added with 10% gulaman (local crude agar) and 1% sucrose for PSG only. Boiling was maintained until homogenized. Dehydrated media were prepared according to the instructions provided by the manufacturer. All media were dispensed into Erlenmeyer flask with cotton plugged, sterilized at 15 psi, 121°C for 20 min, pour-plated and solidified. Three replicates of each medium were prepared. Each plated culture media was aseptically inoculated with a 10 mm-diameter mycelial disc and incubated at 30°C. Mycelial growth diameter was measured and the mycelial growth rate was calculated for each strain. Mycelial thickness was also noted.

After determining the optimal medium for each mushroom, the optimum pH of the medium was determined. pH of the culture media was adjusted from 5.0 to 8.0 with 0.5 interval. Media were sterilized in the same conditions, pour-plated and solidified. These were inoculated with mycelial discs, incubated at 30°C. The mycelial growth rate and thickness were also recorded.

Evaluation of aeration, illumination and temperature

The effects of the three physical environmental factors, namely aeration, illumination and temperature were also investigated in this study. Mycelial plate cultures of *Lentinus* species were prepared using the optimal medium with the optimum pH of each mushroom. These plate cultures were incubated in two aeration conditions (sealed and unsealed), two illumination conditions [artificial light (322.92 lumens/m²) and dark], and temperature conditions (9, 23, 30°C). Three replicate plates were done in each condition. Mycelial growth rate and mycelial thickness were determined.

Grain spawn preparation

Unmilled rice was used as the substrate for the spawn production of *Lentinus* species. The preparation, sterilization, inoculation and incubation of grain spawn described in the previous work of Dulay et al. (2017b) were adopted with minor modification. Once fully ramified with mycelia, grain spawn was used as inoculant in the production of mushrooms.

Fruiting body production

Rice straw and sawdust were the basal substrates used to determine the fruiting body production and bio-efficiency of the *Lentinus* species. The substrate with formulation of 7:3 v/v ratio of rice straw and sawdust was prepared, bagged (500 g) and compacted in 6×12 inches polypropylene bag with cotton plugs. Three substrate bags were prepared for each mushroom. Bags were sterilized at 15 psi, 121°C for 1 h. After cooling, 40 g of grain spawn was aseptically inoculated into each bag and subsequently incubated in the optimal culture conditions until full colonization. The period of incubation and primordial development were recorded. After incubation, bags were transferred into the growing house with 26–28°C and 80–90 relative humidity, opened and watered to allow fruiting body emergence. Fruiting bodies were harvested and weighed. The biological efficiency was determined.

Statistical analysis

Data were analyzed using Analysis of Variance and compared using Turkeys HSD and t-test at 5% level of significance.

Results

Suitable culture media

The effect of different culture media on the mycelial growth of seven strains of four *Lentinus* species was investigated (Table 1). Among eight culture media evaluated, CWG showed the highest mycelial growth rate in all strains of *Lentinus* and thick mycelia were observed. Aside from CWG, other culture media were also found suitable for the growth of specific strains. For instance, *L. sajor-caju* strain 1 favored MEA and SDA, *L. squarrosulus* strain 1 favored RBDG, and *L. swartzii* preferred PSG, CGDG and MEA. All culture media were found suitable for *L. strigosus* strain 2 and *L. sajor-caju* strain 2, except CGDG and MEA for the latter strain.

Effect of pH

The effects of varying pH levels on the mycelial growth of seven *Lentinus* strains are presented in Table 1. Apparently, culture media with pH 5–8 were found suitable for the mycelial growth of all *Lentinus* strains. However, the seven *Lentinus* strains exceedingly favoured pH 5.0–5.5 for *L. sajor-caju* strain 1, pH 5.0–6.5 for *L. sajor-caju* strain 2, pH 5.0–6.0 for *L. squarrosulus* strain 1, pH 5.0–7.0 for *L. squarrosulus* strain 2, pH 5.0–5.5 for *L. strigosus* strain 1, pH 6.0–7.5 for *L. strigosus* strain 2, and pH 5.0–8.0 for *L. swartzii*, which significantly recorded the highest growth rates. All strains produced thick mycelia in all tested pH levels.

Influence of aeration

The effects of sealed (with parafilm) and unsealed conditions on the mycelial growth of seven *Lentinus* strains were also evaluated in this study. The growth rates of *Lentinus* strains incubated in two aeration conditions are shown in Table 1. It can be seen that the differences in the mycelial growth rates between two aeration conditions were not statistically significant in all *Lentinus* strains except *L. squarrosulus* strain 1.

Table 1 Effect of culture media, pH, aeration, illumination and temperature on the mycelial growth of the seven *Lentinus* strains

Factors	Mycelial growth rate (day ⁻¹)						
	<i>L. sc</i> strain1	<i>L. sc</i> strain 2	<i>L. sq</i> strain1	<i>L. sq</i> strain2	<i>L. st</i> strain 1	<i>L. st</i> strain 2	<i>L. sw</i>
CWG	12.75 ± 0.18 ^a	18.00 ± 0.00 ^a	12.86 ± 0.00 ^a	12.84 ± 0.02 ^a	15.00 ± 0.00 ^a	13.87 ± 1.97 ^a	18.00 ± 0.00 ^a
PSG	8.37 ± 0.08 ^{cd}	17.22 ± 0.28 ^{ab}	10.37 ± 0.10 ^b	4.27 ± 0.26 ^e	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	18.00 ± 0.00 ^a
CGDG	6.80 ± 1.55 ^d	15.85 ± 0.75 ^{bc}	9.57 ± 0.75 ^{bc}	8.70 ± 0.05 ^c	13.80 ± 0.84 ^{ab}	14.81 ± 0.33 ^a	18.00 ± 0.00 ^a
RBDG	8.79 ± 0.06 ^c	17.22 ± 0.76 ^{ab}	12.86 ± 0.00 ^a	8.44 ± 0.81 ^{cd}	13.20 ± 0.07 ^b	15.00 ± 0.00 ^a	17.23 ± 0.27 ^b
PDA	10.25 ± 0.61 ^c	17.61 ± 0.03 ^a	6.89 ± 0.24 ^d	7.30 ± 0.76 ^d	14.11 ± 0.35 ^{ab}	14.56 ± 0.13 ^a	16.27 ± 0.41 ^c
MEA	11.46 ± 0.56 ^{ab}	14.88 ± 0.67 ^c	6.05 ± 0.20 ^d	11.20 ± 0.33 ^b	11.87 ± 0.74 ^c	14.49 ± 0.49 ^a	18.00 ± 0.16 ^a
SDA	10.91 ± 0.59 ^{ab}	17.99 ± 0.42 ^a	9.19 ± 0.19 ^c	10.14 ± 0.25 ^b	14.66 ± 0.17 ^a	14.93 ± 0.88 ^a	8.00 ± 0.00 ^e
MA	8.78 ± 0.61 ^c	17.83 ± 0.29 ^a	4.43 ± 0.19 ^e	5.17 ± 0.12 ^e	9.44 ± 0.43 ^d	15.00 ± 0.00 ^a	11.81 ± 0.12 ^d

Table 1 Continued.

Factors	Mycelial growth rate (day ⁻¹)						
pH							
5.0	18.00 ± 0.00 ^a	18.00 ± 0.00 ^a	12.49 ± 0.63 ^a	12.86 ± 0.00 ^a	15.00 ± 0.00 ^a	13.87 ± 0.14 ^c	17.86 ± 0.24 ^a
5.5	17.66 ± 0.23 ^a	18.00 ± 0.00 ^a	11.89 ± 0.10 ^a	12.86 ± 0.00 ^a	13.86 ± 0.14 ^{abc}	14.21 ± 0.36 ^{bc}	17.91 ± 0.16 ^a
6.0	16.65 ± 0.09 ^b	17.53 ± 0.43 ^a	11.37 ± 0.15 ^a	12.86 ± 0.00 ^a	13.17 ± 0.21 ^{bcd}	14.63 ± 0.39 ^{ab}	18.00 ± 0.00 ^a
6.5	16.02 ± 0.07 ^c	17.24 ± 0.43 ^a	7.99 ± 0.62 ^b	12.86 ± 0.00 ^a	12.87 ± 0.04 ^{cd}	15.00 ± 0.00 ^a	18.00 ± 0.00 ^a
7.0	14.82 ± 0.29 ^d	16.13 ± 0.43 ^b	8.20 ± 0.66 ^b	12.86 ± 0.00 ^a	14.19 ± 0.23 ^{ab}	15.00 ± 0.00 ^a	18.00 ± 0.00 ^a
7.5	14.11 ± 0.13 ^e	15.26 ± 0.65 ^b	8.44 ± 0.22 ^b	11.89 ± 0.28 ^b	12.53 ± 0.06 ^d	15.00 ± 0.00 ^a	18.00 ± 0.00 ^a
8.0	13.50 ± 0.18 ^f	15.14 ± 0.09 ^b	7.84 ± 0.21 ^b	12.19 ± 0.04 ^b	12.51 ± 1.18 ^d	13.59 ± 0.28 ^c	18.00 ± 0.00 ^a
Aeration							
Sealed	18.00 ± 0.00 ^a	18.00 ± 0.00 ^a	15.00 ± 0.00 ^a	12.86 ± 0.00 ^a	14.59 ± 0.37 ^a	15.00 ± 0.00 ^a	18.00 ± 0.00 ^a
Unsealed	18.00 ± 0.00 ^a	18.00 ± 0.00 ^a	13.78 ± 0.13 ^b	12.86 ± 0.16 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	18.00 ± 0.00 ^a
Illumination							
Lighted	18.00 ± 0.00 ^a	18.00 ± 0.00 ^a	15.00 ± 0.84 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	18.00 ± 0.00 ^a
Dark	18.00 ± 0.00 ^a	18.00 ± 0.00 ^a	13.10 ± 0.00 ^b	14.58 ± 0.00 ^b	13.10 ± 0.99 ^b	15.00 ± 0.00 ^a	18.00 ± 0.00 ^a
Temperature							
9°C	ng	ng	ng	ng	ng	ng	ng
23°C	18.00 ± 0.00 ^a	20.31 ± 0.24 ^b	15.00 ± 0.00 ^a	14.58 ± 0.14 ^b	13.19 ± 0.11 ^b	13.98 ± 0.19 ^b	16.86 ± 0.24 ^b
30°C	18.00 ± 0.00 ^a	22.50 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	18.00 ± 0.00 ^a

Means with similar superscripts are statistically comparable from each other using Tukeys HSD and t-test at 5% level of significance. *L. sc*, *Lentinus sajor-caju*; *L. sq*, *Lentinus squarrosulus*; *L. st*, *Lentinus strigosus*; *L. sw*, *Lentinus swartzii*; CWG, coconut water gulaman; PSG, potato sucrose gulaman; CGDG, corn grit decoction gulaman; RBDG, rice bran decoction gulaman; PDA, potato dextrose agar; MEA, malt extract agar; SDA, Sabouraud dextrose agar; MA, mycological agar; ng, no growth.

Influence of illumination

The influence of the presence and absence of light on the mycelial growth of *Lentinus* strains was investigated. Culture plates were incubated under artificial light and in dark conditions. The mycelial growth rates of seven *Lentinus* mushrooms as affected by the two illumination conditions are presented in Table 1. Both conditions were found suitable for the luxuriant growth of mycelia of two strains of *L. sajor-caju*, *L. strigosus* strain 2 and *L. swartzii*. However, mycelia of two strains of *L. squarrosulus* and *L. strigosus* strain 1 grown in the lighted condition significantly recorded higher growth rates than those incubated in the dark.

Influence of temperature

The influence of temperature on the mycelia growth of seven *Lentinus* strains was studied. Table 1 shows the growth rates of *Lentinus* mushrooms as affected by the three temperature conditions. All *Lentinus* strains evaluated recorded the highest mycelial growth rates and very thick mycelia at 30°C. However, mycelial growth rates of *L. sajor-caju* strain 1 and *L. squarrosulus*

strain 1 incubated at 30°C were not significantly different from those incubated at 23°C. No mycelial growth was observed at 9°C.

Fructification on rice straw and sawdust-based substrate

After determining the nutritional and physical requirements for mycelial growth of the seven *Lentinus* strains, their fructification abilities by using lignocellulosic agro-industrial as substrates in fruiting bags in growing house conditions were also investigated. The Center for Tropical Mushroom Research and Development (CTMRD) formulation consisting of 7:3 v/v ratio of rice straw and sawdust was used in this study. The fructification parameters of the seven *Lentinus* strains are shown in Table 2. Inoculated fruiting bags were incubated in the required environmental conditions and the incubation days for mycelial colonization was recorded. Both strains of *L. strigosus* and sole strain of *L. swartzii* displayed the shortest incubation periods of 18–18.8 days followed by both strains of *L. sajor-caju* with 20.9–21.3 days. On the other hand, both strains of *L. squarrosulus* registered the most extensive incubation period of 25.5–25.6 days. Comparing the two strains of each *Lentinus* species, t-test revealed no significant difference.

Once fully ramified with mycelia, the next cultivation phase observed was the initiation of primordia or also known as fruiting initials. Similarly, both strains of *L. strigosus* and sole strain of *L. swartzii* showed the earliest primordia after 21.6–22.3 days, whereas both strains of *L. squarrosulus* showed the latest primordia after 31.9–33.5 days. Again, no significant difference was found between two strains of each species according to t-test.

The fruiting bags were opened and watered allowing the primordia to develop into mature fruiting bodies. The fruiting bodies of different *Lentinus* strains grown on rice straw and sawdust-based substrate formulation are shown in Fig. 1. In terms of cap diameter, t-test revealed a significant difference between strains of *L. strigosus*. Strain 2 had a wider cap diameter (51.97 mm) than strain 1 (28.52 mm). However, no significant difference regarding the cap diameter was found between strains of *L. sajor-caju* and strains of *L. squarrosulus*. Among species, *L. sajor-caju* showed the widest cap diameter. Looking at the stipe length, significant difference was only found between strains of *L. squarrosulus*, while no significant difference was noted between strains of both *L. sajor-caju* and *L. strigosus*. The longest stipe was recorded in *L. squarrosulus* strain 1 with a mean of 53.54 mm while the shortest stipe was registered in *L. squarrosulus* strain 2 with a mean of 8.09 mm.

Table 2 Fructification parameters of the seven strains of *Lentinus*

<i>Lentinus</i>	Incubation period (day)	Primordia initiation (day)	Cap diameter (mm)	Stipe length (mm)	Yield (g bag ⁻¹)	Biological efficiency (%)
<i>L. sc</i> strain 1	20.9 ± 0.7	24.0 ± 0.8	60.7 ± 6.6	14.5 ± 1.3	44.2 ± 6.7	8.8 ± 1.3
<i>L. sc</i> strain 2	21.3 ± 1.1	24.5 ± 1.1	62.7 ± 4.4	14.8 ± 1.6	50.9 ± 6.3	10.2 ± 1.3
<i>L. sq</i> strain 1	25.6 ± 2.3	31.9 ± 2.8	45.8 ± 7.6	53.5 ± 8.3*	36.4 ± 10.9*	7.3 ± 2.2*
<i>L. sq</i> strain 2	25.5 ± 2.6	33.5 ± 2.9	45.5 ± 6.8	8.1 ± 1.9	23.5 ± 8.6	4.7 ± 1.7
<i>L. st</i> strain 1	18.0 ± 0.8	21.6 ± 0.5	28.5 ± 5.1	19.6 ± 1.9	52.5 ± 12.7*	10.5 ± 2.6*
<i>L. st</i> strain 2	18.8 ± 2.8	22.3 ± 2.9	51.9 ± 9.3*	19.7 ± 2.6	21.1 ± 7.4	4.2 ± 1.5
<i>L. sw</i>	18.4 ± 1.4	22.0 ± 1.6	40.6 ± 1.71	36.5 ± 3.3	34.6 ± 5.9	6.9 ± 1.2

Asterisk (*) indicates significant difference between two strains of each *Lentinus* species (except *L. swartzii*) using t-test at 5% level of significance. *L. sc*, *Lentinus sajor-caju*; *L. sq*, *Lentinus squarrosulus*; *L. st*, *Lentinus strigosus*; *L. sw*, *Lentinus swartzii*.

The yield and biological efficiency of seven *Lentinus* strains are presented in Table 2. Significant difference between strains of the same species was observed in *L. squarrosulus* and *L. strigosus*. In both species, strain 1 produced higher yields of 36.4 g bag⁻¹ and 52.5 g bag⁻¹ with corresponding biological efficiency of 7.27% and 10.50%, respectively. On the other hand, strain 2 of both *Lentinus* species produced the lowest yields of 23.5 g bag⁻¹ (4.7% bio-efficiency) and 21.1

g bag⁻¹ (4.22% bio-efficiency) among *Lentinus* strains evaluated, respectively. However, no significant difference was found between strains of *L. sajor-caju*. Strain 1 displayed a yield of 44.2 g bag⁻¹ (8.84% bio-efficiency) whereas strain 2 showed a yield of 50.9 g bag⁻¹ (10.18% bio-efficiency). The sole strain of *L. swartzii*, however, recorded a yield of 34.6 g bag⁻¹ with 6.92% biological efficiency.

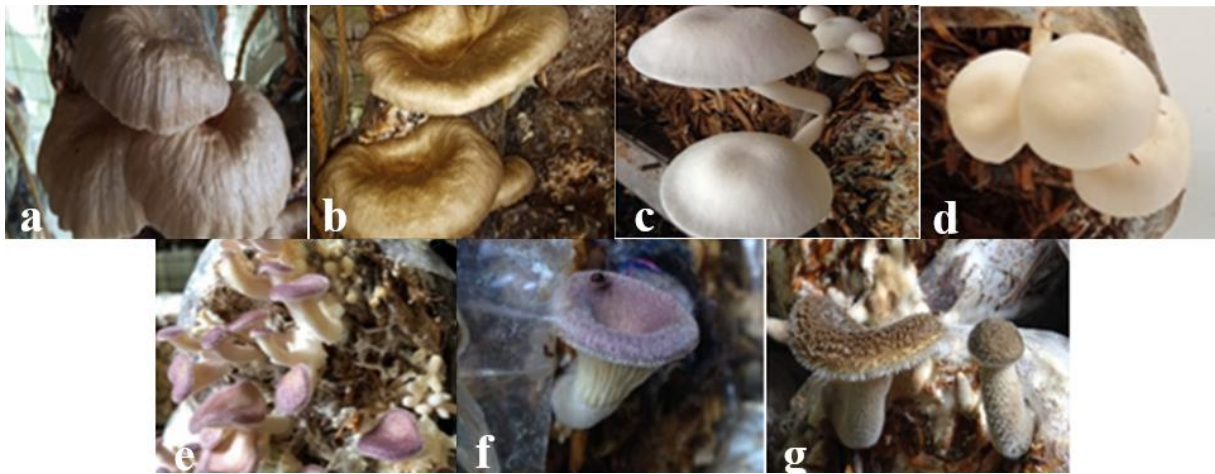


Fig. 1 – Fruit bodies of *Lentinus* species/strains growing on fruiting bags containing 7:3 rice straw–sawdust substrate formulation. a *L. sajor-caju* strain 1. b *L. sajor-caju* strain 2. c *L. squarrosulus* strain 1. d *L. squarrosulus* strain 2. e *L. strigosus* strain 1. f *L. strigosus* strain 2. g *L. swartzii*.

Discussion

The suitable culture medium and its natural nutritional components are the most critical requirement for the growth of mushroom mycelia. CWG was found to be the most suitable medium for the efficient mycelial growth of the seven strains of *Lentinus*. However, other culture media were also suitable for the mycelial growth of *Lentinus* species. These suggest that the mycelial growth performance of *Lentinus* on the different culture media may vary depending on the species and strain. This could be due to the unique inherent characteristics of individual mushroom formed in heterogeneous natural habitat. Accordingly, species and even strain could be categorized as slow-growing and fast-growing mushrooms.

Similarly, CWG was the preferred medium for the efficient mycelial growth of Central Luzon strain of *L. tigrinus*, Botolan strain of *L. squarrosulus*, Ifugao strain of *L. sajor-caju*, BIL1324 strain of *L. strigosus*, and Central Luzon strain of *L. swartzii* (De Leon et al. 2013, 2017a, Dulay et al. 2012, 2017b, 2021a). The results of the present study and these previous findings clearly suggest that CWG is the best medium for the maximum growth of *Lentinus* mycelia. Moreover, CWG is also the best medium for the luxuriant mycelial growth of other mushroom species including *Ganoderma lucidum* and *Auricularia polytricha* in semi-solid state, and *Schizophyllum commune* and *Volvariella volvacea* in liquid state (Magday et al. 2014, Kalaw et al. 2016, Dulay et al. 2016). The better performance of mushroom mycelia on CWG could be accounted to the natural nutrient components of coconut water, which are very essential for the growth of fungal cells. Coconut water contains carbohydrates, sugars, dietary fiber, protein, fat, vitamins and minerals (USDA 2019). Although these nutrients are also available in other culture media evaluated, the types (e.g. sugar constituents) and their corresponding amount vary from one medium to another, which greatly influence on the growth of mycelia. Dulay et al. (2020b) evaluated the nutritional requirements of the three *Lentinus* species and found that the mycelia of *L. swartzii*, *L. strigosus*, and *L. tigrinus* favorably grew on starch, fructose, and sucrose as carbon sources and ammonium chloride, yeast extract, and malt extract as nitrogen sources at C/N ratio of 10:1, 10:1, and 40:1, respectively.

In addition, Itoo & Reshi (2014) reported that *Cortinariusflexipes* and *Cortinariusfulvoconicus* exhibited maximum mycelial growth at >20 g/L glucose, while *Tricholoma aurantium*, *Suillus luteus*, *Laccarialaccata* and *Scleroderma citrinum* showed maximum growth at 20 g/L glucose. Di-ammonium hydrogen orthophosphate at 5–10 g/L was the optimum nitrogen source of the six ectomycorrhizal species. Apart from carbon and nitrogen sources, minerals such as KH_2PO_4 for *L. swartzii*, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ for *L. strigosus*, and all minerals tested except $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ for *L. tigrinus* and organic acids such as lactic acid, acetic acid and citric acid also enhanced the mycelial growth (Dulay et al. 2020b). Accordingly, mushrooms have specific nutrient type and quantity requirements, which are naturally present in their most suitable culture medium. Therefore, it is of high interest to also determine the specific nutrient sources of the seven *Lentinus* strains evaluated in the present study.

The optimal pH of the most suitable medium for each *Lentinus* strain was also determined. It was established that *Lentinus* mycelia requires a slightly acidic to neutral pH of the culture medium to produce superior mycelial growth. This finding of the present study conforms to our previous reports that the optimum pH for the luxuriant growth of *L. strigosus* BIL1324 strain, *L. swartzii* Central Luzon strain, and *Ganoderma lucidum* is pH 6 (Dulay & Garcia 2017, Dulay et al. 2021a, Magday et al. 2014). However, Botolan strain of *L. squarrosulus* and Central Luzon strain of *L. tigrinus* grew best at pH 6.5–7 and 7–8, respectively (Dulay et al. 2012, De Leon et al. 2017b), whereas Ifugao strain of *L. sajor-caju* exceedingly favoured pH 5 (De Leon et al. 2017a). Therefore, it is safe to mention that a pH range of 5–8 is the optimal pH for the growth of different species and even strains of *Lentinus* mycelia.

Aeration refers to the air composition or level of oxygen and carbon dioxide in the culture. The results of the present study showed that aeration is not a major physical factor that affects the growth of mycelia of the six strains, in which they could grow in either aerobic or anaerobic state. This response to aeration conditions is also observed in *L. sajor-caju* Ifugao strain and *L. tigrinus* Central Luzon strain (Dulay et al. 2012, De Leon et al. 2017a). However, *L. squarrosulus* strain 1 significantly favored sealed condition indicating that this strain does not require direct exposure to air. The same condition is the requirement of *L. strigosus* BIL 1324 strain and *L. squarrosulus* Botolan strain and *L. swartzii* Central Luzon strain (Dulay et al. 2021a, Dulay & Garcia 2017, De Leon et al. 2017b). In submerged cultivation of mycelia, agitation is applied to introduce aeration in the culture effectively. Agitation at 100 rpm improves the biomass yield of *G. lucidum* in shake-flask culture, whereas static culture condition is found suitable for the mycelial production of *Schizophyllum commune*, *Pleurotuscystidiosus*, and *Volvariellavolvacea* (Dulay et al. 2015).

Light regulates the growth, and reproduction of fungi which is necessary for their distribution and survival (Idnurm & Heitman 2005). Illumination is another important environmental factor that influences the growth of mushrooms. It was found that each *Lentinus* strain has a unique illumination preference. Previously, the lighted condition was found to stimulate the growth of mycelia of *L. sajor-caju* Ifugao strain (De Leon et al. 2017a). Contrastingly, the dark condition is more suitable for *L. tigrinus* Central Luzon strain, *L. strigosus* BIL 1324 strain and *L. swartzii* Central Luzon strain (Dulay et al. 2012, 2021a, Dulay & Garcia 2017). Since some mushrooms positively respond to lighted condition, it is also necessary to evaluate the effects of different light wavelengths and intensities on the mycelial growth. In our previous work, we found that blue LED enhances the mycelial growth, fruiting body yield and bio-efficiency of *L. tigrinus* (Damaso et al. 2018). However, in liquid culture, red LED stimulates mycelial growth and improves the fruiting body yield of *L. swartzii* (Tiniola et al. 2021). In addition, the positive effects of blue light on the metabolism, growth and development, fruiting body induction, and primordium differentiation into fruiting body in mushrooms have been widely reported (Kojima et al. 2015, Sakamoto et al. 2018, Sakamoto 2018).

Lentinus grows in a wide range of temperatures, from tropical to temperate regions of the Earth. Hence, temperature is very vital in the survival and distribution of mushrooms. The results suggest that the seven *Lentinus* strains are classified as tropical mushrooms. The 30°C is also the reported temperature requirement for the mycelial growth of other *Lentinus* species/strains

including *L. sajor-caju* Ifugao strain, *L. swartzii* Central Luzon strain (De Leon et al. 2017a, Dulay et al. 2021a) and other Philippine basidiomycetes such as *G. lucidum*, *V. volvacea* and *Trametes elegans* (Dulay et al. 2015, 2021b). However, *L. tigrinus* Central Luzon strain, *L. squarrosulus* Botolan strain, *L. strigosus* BIL1324 strain preferred 32°C (Dulay et al. 2012, Dulay & Garcia 2017, De Leon et al. 2017b). Contrastingly, their relative *L. edodes* is a semi-temperate species, which demonstrates optimal mycelial growth at 25°C (Chen 2004).

Fructification is an indicative of the cultivation potential of wild mushrooms prior to the generation of production technology necessary for mass production and even commercialization. The present study suggests that the different strains and species of *Lentinus* respond differently in terms of fruiting body production when cultivated on rice straw and sawdust-based substrate formulation. Variation in fruiting body production performance is also observed among five strains of *L. edodes* (Gaitan-Hernandez & Mata 2004) and three strains of *Pleurotus eryngii* (Moonmoon et al. 2010). This variation could be attributed to the genotype and physiological differences among mushroom strains. The yields and biological efficiencies of the seven *Lentinus* strains presented in this study are comparatively lower than the reported biological efficiencies of wild mushrooms such as *L. tigrinus* Central Luzon strain (15.93%) and *L. strigosus* BIL1324 strain (20.97%) (Dulay et al. 2012, 2017b), and commercially-cultivated *L. edodes* (46–80%) (Royse 1995). In our previous work, with the intention to improve the biological efficiency of *L. strigosus* BIL1324 strain, we reported that the addition of 20% rice bran and 20% corn grit to rice straw stimulates the formation of primordia, produces higher yield and improves the biological efficiency by 41.96% in rice bran and 40.86% in corn grit (Dulay & Garcia 2017).

This work has established the suitable culture media, optimal pH, and physical environmental requirements such as aeration, illumination and temperature for the luxuriant growth of mycelia of seven wild *Lentinus* strains. Moreover, the fructification abilities of seven *Lentinus* strains were also established, suggesting their promising cultivation potential using rice straw and sawdust-based substrate formulation in growing house conditions. Variation between strains is evident in the mycelial growth and fruiting body performance of each species, indicating that strain selection is also very important in mushroom production. However, further investigation on the effect of supplemented substrates in fruiting body cultivation must be done in order to improve the yield and biological efficiency.

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