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Original Article

Optimization of mycelial culture condition and biomass production of selected wild *Agaric* mushrooms from Luzon Island, Philippines

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Abstract

Agaricomycetous mushrooms are widely utilized as a source of food and or traditional medicine in the world as they exhibit both nutritional and pharmaceutical properties. In order to establish the optimal culture conditions of the 2 *Coprinopsis*, 2 *Leucoagaricus*, and 1 *Leucocoprinus* mushroom, we evaluated the optimum culture medium, pH condition, aeration, illumination, and temperature requirements for the luxuriant mycelial growth of these wild mushrooms. The fruiting body and mycelial biomass production were also carried out in this study to measure mushrooms' biological efficiency. Among commercially-available culture media, malt extract agar (MEA) was found to be the most suitable for the 4 mushrooms (except *C. cinerea*), followed by potato dextrose agar (PDA) for 3 mushrooms (*C. cinerea*, *C. verticillata*, *L. cretaceous*). Potato sucrose gulaman (local crude agar) or PSG and corn grit decoction gulaman or CGDG were also found favorable for the growth of *Coprinopsis verticilatta* and *Leucoagaricus americanus*, respectively. In terms of pH requirement, *L. americanus* (pH 5) and *Leucoagaricus meleagris* (pH 5-6) favored slightly acidic, while the other three mushrooms showed a wide range of pH requirements. *C. verticillata* and *L. meleagris* favored sealed condition, while *Coprinopsis cinerea* favored unsealed condition. However, *L. americanus* and *Leucocoprinus cretaceous* showed efficient mycelia growth in both sealed and unsealed conditions. Dark condition were found to be favorable for mycelial growth of both *C. cinerea* and *L. cretaceous*, whereas lighted condition was found appropriate for *L. americanus* mycelia. However, illumination was found to be not important factor for *C. verticillata* and *L. meleagris*. All evaluated mushrooms grew best at room temperature conditions (30-32ºC), but *C. cinerea*, *L. meleagris*, and *L. cretaceous* could also thrive at lower temperatures (23-25ºC). Three mushrooms namely, *L. amaericanus*, *L. meleagris*, and *L. cretaceous* successfully produced fruiting bodies in fruiting bags containing rice straw and sawdust at a 7:3 ratio by volume with biological efficiencies (BE) of 5.75%, 5.75%, and 2.3%, respectively. However, the mycelia of *C. cinerea* and *C. verticillata* were mass-produced in potato broth in submerged cultivation with 18.18% and 23.86% BE, respectively. Generally, the optimum culture condition for both mycelial growth and fruiting body production were found to be species-dependent.

Keywords: Biological efficiency, *Coprinospsis*sp., *Leucoagaricus*, *Leucocoprinus*, Mycelial biomass, Optimization study

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Introduction

Rich tropical rainforests in tropical countries like the Philippines provide an ideal growing environment for a diverse range of fungal species. As a result, various mushrooms have been studied, rescued, and optimized for the culture conditions, successfully identified their bioactivities, and utilized in various industries. A recent study by Dulay et al. (2021) reported a total of 447 wild mushroom species belonging to 193 genera in the Philippines. These mushrooms are derived from 9 research aspects namely ethnomycology/ biodiversity (19.88%), molecular identification (5.59%), developmental biology (2.48%), optimization/cultivation (23.36%), bioremediation (4.97%), chemical composition (15.53%), and bioactivity (29.19%). These data highlight the potential of mushrooms as underutilized natural resources with enormous potential for commercial utilization. However, the ability of mushrooms to be used as natural resources is the ability to domesticate wild mushrooms and optimize their culture condition for mass production and efficient utilization.

Agaricomycetes mushrooms contain biologically active compounds with antimicrobial, antioxidative, anti-hypertensive, anti-inflammatory, hypoglycemic, fibrinolytic, hypocholesterolemic, and thrombolytic properties, which offer a wide variety of potential medicinal and pharmaceutical use (Badalyan et al., 2021). However, as many studies claim the advantageous potential of the wild mushrooms under class agaricomycetes, few mushroom species are well elucidated. Accordingly, the available study in the Philippines for the *Coprinopsis* only focuses on species listing, ethnomycological studies, and diversity surveys (Dulay et al., 2020; Torres et al., 2020; Kalaw et al., 2016) with exception to *Coprinopsis cinerea* which was already subjected in optimization study upon collection for secondary mycelial growth utilizing the indigenous culture medium (Kalaw et al. 2016). Moreover, in the ethnomycological survey of De Leon et al. (2017a), they found out that *Leucoagaricus cepaestipes* locally known as "Gum-gumot" are being utilized as food by the Kalanguya community in Brgy. General Luna, Sitio Binbin, Caranglan, Nueva Ecija, Philippines. Likewise, *Leucocoprinus* available study in the Philippines was its diversity and taxonomy and was found to thrive in leaf litter and decayed plant matter in Southern Luzon, Philippines (Brazas et al., 2020). Therefore, in order to provide additional information focusing on the optimization studies of these wild mushrooms we determined the established optimum culture condition for mycelial growth and biomass production of these wild mushrooms for the same goal of efficient commercial utilization of natural resources.

Material and Methods

Source of inoculants

Five wild mushrooms used in this study, the *Coprinopsis cinerea* (MP11), *Coprinopsis vericillata* (DQS7), *Leucoagaricus amaericanus* (CAG10), *Leucoagaricus meleagris* (CAG9), and *Leucocoprinus cretaceous* (MP20) were collected from three collection points of Luzon Island, Philippines particularly in Dolores, Quezon, and Mango Plantation and College of Agriculture of Central Luzon State University, Philippines (PHL), 12.8797º N, 121.7740º E (Figure 1). Pure culture of the said mushrooms was acquired from the culture collection of the Center for Tropical Mushroom Research and Development (CTMRD), CLSU, PHL. Using sterile potato dextrose agar (PDA) plates, mushroom cultures were aseptically sub-cultured and were incubated at room temperature (30◌ºC) for 7 days. After incubation, the inoculant of each isolate was prepared by acquiring mycelial discs from PDA plates using a 10-mm cork borer.

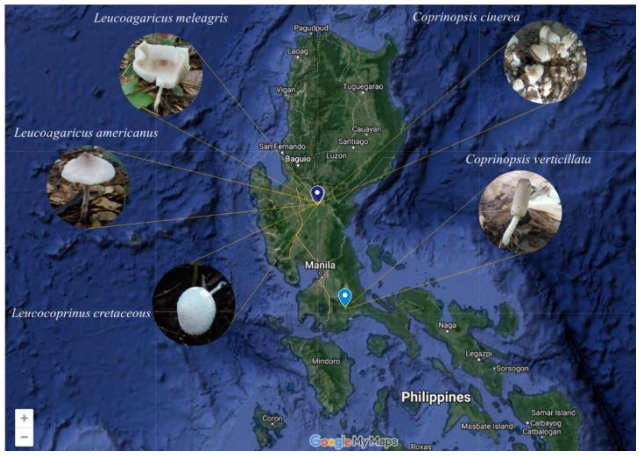

Figure-1: Wild fruiting bodies of *Agaric* mushrooms collected from Luzon Island, Philippines

Optimization of culture medium and pH

Four indigenous culture media, such as coconut water gulaman (*Cocos nucifera*) CWG, potato sucrose

gulaman (*Solanum tuberosum*) PSG, corn grit decoction gulaman (*Zea mays*) CGDG, and rice bran decoction gulaman (*Oryza sativa*) RBDG, four commercially-available media, namely malt extract agar (MEA), mycological agar (MA), potato dextrose agar (PDA), and Sabouraud dextrose agar (SDA) were utilized to evaluate the mycelial growth of the 5 wild mushrooms. The indigenous media (CWG, RBDG, CGDG, PSG) were prepared following the protocol of Dulay et al. (2015) and the commercially-available media (MEA, MA, PDA, SDA) were prepared according to the instruction of the manufacturer. Each medium was prepared in triplicates.

Ten-millimeter mycelial discs were inoculated aseptically in the prepared medium and incubated at room temperature (30°C) after media preparation. For each strain, the mycelial growth diameter was measured and the mycelial growth rate was determined. The thickness of the mycelium was also evaluated visually.

The optimum pH of the medium was determined after determining the ideal culture media for each mushroom species. The culture medium was similarly sterilized, pour-plated, and solidified while the pH was adjusted in 1.0 increments from 5.0 to 9.0. Afterward, mycelial discs were inoculated prior to 30°C incubation. The rate and thickness of mycelial growth were also determined visually.

Optimization of aeration, illumination, and temperature conditions

This study also identified the 3 physical factors using the best culture medium and suitable pH conditions, namely aeration, illumination, and temperature. Prior to optimization, mycelial plate cultures of the 5 mushrooms were prepared using the optimal culture medium and pH. Following the plate incubation of Kalaw et al. (2022), the plate cultures were incubated in three physical conditions (aeration, illumination, temperature) and the mycelial thickness and growth rate were measured.

Grain spawn preparation

Spawn production of the 3 wild mushrooms (*L. amaericanus* (CAG10), *L. meleagris* (CAG9), and *L. cretaceous* (MP20) utilized unmilled rice. Following the study of Dulay and Garcia (2017), grain spawn preparation, sterilization, inoculation, and incubation were performed in this study with slight modifications. The grain spawn was utilized as an inoculant in the fructification of the three mushrooms once the full ramification of mycelia was obtained.

Fruiting body production

The fructification and biological efficiency of the 3 mushrooms (*L. amaericanus* (CAG10), *L. meleagris* (CAG9), and *L. cretaceous* (MP20)) were achieved using the substrate composition of rice straw and sawdust. The fruiting bag formulation and preparation followed the previous work of Kalaw et al. (2021) with slight modifications. The prepared fruiting bags were sterilized using an autoclave at 121ºC, 15 psi for 1 h. After cooling, each fruiting bag was aseptically inoculated with 30-40 grams of grain spawn prior to incubation under optimum culture conditions until full ramification of mycelia was observed in the fruiting bags. The incubation period and the development period of primordia were recorded. Following incubation, the bags were opened and watered prior to getting transferred to the growing house at 80-90 relative humidity and 27-29ºC. Afterward, the fruiting body was harvested, weighed, and the determination of biological efficiency (BE) was done.

Mycelial biomass production

Mycelial biomass production of the two *Coprinopsis* species, *C. cinerea* (MP19), and *C. verticillata* (DQS7)) were evaluated using a potato broth culture medium. The culture broth was prepared in a beaker containing 4 liters of distilled water, and 1 kg of freshly sliced potato boiled using a hot plate. After boiling, the suspension was filtered using a cheesecloth and reconstituted to its final volume (4L) prior to the addition of 40 g of sugar. Afterward, the culture broth was dispensed into a culture vessel (100mL/container) and sterilized in an autoclave at 121ºC, 15 psi for 30 mins. After cooling, each culture vessel was inoculated with the mycelial disc (20 culture vessels for *C. cinerea;* 20 culture vessels for *C. vericillata*) and incubated at 32ºC for 2 weeks to allow mycelial mat production. The mycelial mats were harvested and the incubation period and the yields per bottle were recorded.

Statistical analysis

At a 5% level of significance, data were evaluated using analysis of variance (ANOVA) and compared using Tukey's HSD and t-test.

Results

Effect of culture medium

The effect of the culture media on the mycelial growth of the 5 wild mushrooms was determined (Table 1). MEA was found suitable for the efficient growth of the 5 wild mushrooms except for *C. cinerea*, while PDA was found suitable for the efficient growth of the 5 mushrooms except for the 2 mushrooms of *Leucoagaricus.* Specifically, *C. cinerea* preferred PDA and MA, *C. vericillata* exhibited excellent mycelial growth and density with PSG, PDA, MEA, and MA, *L. amaericanus* favored CGDG, and MEA, while only MEA was found suitable for the mycelial growth of *L. meleagris.* In the case of *L. cretaceous*, the 2 commercial media PDA and MEA were found suitable for its growth. Overall, the MEA was found to be the most suitable culture medium for all 5 wild mushrooms tested. These media show high mycelial growth with excellent mycelial density.

Effect of pH

The 5 wild mushrooms' (*C. cinerea*, *C. verticillata*, *L. amaericanus*, *L. meleagris*, and *L. cretaceous*) mycelial growth in response to varying pH levels are presented in Table 1. There is a wide range of pH requirements shown for all five wild mushrooms. Accordingly, *C. cinerea* favored pH 5-9, *C. verticillata* shows excellent mycelial growth and density at pH 6-8, *L. americanus* showed mycelial suitability at pH 5, *L. meleagris* at pH 5-6, and *L. cretaceus* at pH 4-7 and 9.

Effect of aeration

This study also evaluated the effect of aeration on the five wild mushrooms. The conditions set for aeration sealed (with parafilm) and unsealed conditions are shown in Table 1. Based on the data obtained, the two aeration conditions' various effects on mycelial growth rates have a slightly significant difference in which *C. cinerea* prefers unsealed condition, while *C. verticillata* and *L. meleagris* favored sealed conditions. There is no statistically significant difference observed for *L. americanus* and *L. cretaceous* for the two aeration conditions.

Effect of illumination

The presence and absence of artificial light were evaluated for the illumination condition. Incubation of culture plates was done in both light and dark conditions. The effect of two illumination conditions for the five wild mushrooms is presented in Table 1. Both conditions (lighted and dark) were found to be favorable for the luxuriant mycelial growth of *C. verticillata* and *L. meleagris*, while the mycelial growth of *C. cinerea* and *L. cretaceous* were found suitable in dark conditions. However, in the case of *L. americanus* higher growth rates were recorded in lighted conditions.

Effect of temperature

This study also evaluated the effect of temperature on the mycelial growth of five wild mushrooms. The growth rates of these five wild mushrooms under three temperature conditions are presented in Table 1. The three wild mushrooms, *C. cinerea*, *L. meleagris*, and *L. cretaceous* have no significant difference for the air conditioning (23-25ºC) and room temperature (30-32ºC) conditions. However, *C. verticillata* and *L. americanus* only favored slightly higher temperatures of 30-32ºC. No mycelial growth is recorded in refrigerated condition (9ºC) for all the five mushrooms evaluated.

Fruiting body production on rice straw and sawdust-based substrate

After assessing the nutritional and physical conditions for the five wild mushrooms' mycelial growth, three wild mushrooms, namely *L. americanus*, *L. meleagris*, and *L. cretaceus* were subjected to fructification utilizing 70% rice straw and 30% sawdust formulation developed by the Center for Tropical Mushroom Research and Development (CTMRD). Table 2 shows the fructification parameters of the three wild mushrooms. Incubation days for mycelial colonization were observed for inoculated fruiting bags under suitable environmental conditions. Based on the data obtained, the shortest incubation period of 30.7 days was recorded in *L. cretaceus* followed by the two strains of *Leucoagaricus*, *L. meleagris* (31.7 days), and *L. americanus* (51.4 days).

After obtaining the complete ramification of mycelia in the fruiting bags, the primordia initiation (also known as fruiting initiation) was observed. Similarly, *L. cretaceus* showed the earliest primordia after 32.5 days followed by *L. meleagris* (34.7 days) and *L. americanus* (54.2 days).

Table-1: Influence of intrinsic and extrinsic factors on the mycelial growth of five wild mushrooms

Factors	**Mycelial growth rate (day^{-1})**				
Culture media	*C. cinerea*	*C. verticillata*	*L. americanus*	*L. meleagris*	*L. cretaceous*
CWG	2.78±0.11^{e}	10.17±0.62^{c}	2.55±0.07^{e}	3.44±0.12^{f}	3.36±0.15^{e}
PSG	10.12±0.52^{bc}	15.00±0.00^{a}	3.67±0.09^{cd}	4.68±0.05^{e}	4.35±0.47^{d}
CGDG	2.74±0.05^{e}	12.15±0.19^{b}	5.56±0.37^{a}	2.60±0.02^{g}	1.90±0.02^{f}
RBDG	9.30±0.11^{c}	7.39±0.11^{d}	4.37±0.18^{b}	2.84±0.08^{g}	3.34±0.09^{e}
PDA	11.37±0.30^{a}	15.00±0.00^{a}	4.08±0.24^{bc}	6.13±0.18^{b}	5.41±0.08^{ab}
MEA	10.14±0.47^{bc}	15.00±0.00^{a}	5.47±0.13^{a}	6.49±0.17^{a}	5.67±0.13^{a}
SDA	8.07±0.69^{d}	10.93±0.61^{c}	3.12±0.09^{de}	5.48±0.15^{c}	5.05±0.24^{bc}
MA	10.91±0.42^{ab}	15.00±0.00^{a}	4.33±0.29^{b}	5.06±0.08^{d}	4.69±0.09^{cd}
pH					
4.0	6.72±0.16^{b}	4.23±0.29^{c}	5.69±0.15^{b}	5.47±0.20^{b}	6.95±0.42^{ab}
5.0	11.97±0.47^{a}	13.35±0.53^{b}	6.64±0.18^{a}	6.39±0.43^{a}	6.92±0.32^{ab}
6.0	12.27±0.20^{a}	15.00±0.00^{a}	5.12±0.14^{c}	6.57±0.27^{a}	7.22±0.19^{a}
7.0	12.20±0.09^{a}	15.00±0.00^{a}	4.79±0.07^{d}	5.15±0.17^{b}	6.59±0.33^{ab}
8.0	11.84±0.13^{a}	15.00±0.00^{a}	4.79±0.07^{d}	5.36±0.29^{b}	6.28±0.29^{b}
9.0	11.85±0.08^{a}	12.78±0.15^{b}	4.72±0.02^{d}	4.90±0.24^{b}	6.75±0.11^{ab}
Aeration					
Sealed	10.75±0.27^{b}	15.00±0.00^{a}	5.69±0.25^{a}	7.03±0.10^{a}	5.37±0.33^{a}
Unsealed	11.96±0.20^{a}	14.09±0.27^{b}	5.29±0.62^{a}	6.80±0.09^{b}	5.45±0.03^{a}
Illumination					
Lighted	8.77±0.91^{b}	15.00±0.00^{a}	6.47±0.09^{a}	7.06±0.26^{a}	6.92±0.23^{b}
Dark	10.60±0.04^{a}	15.00±0.00^{a}	6.02±0.10^{b}	7.00±0.19^{a}	7.74±0.28^{a}
Temperature	*C. cinerea*	*C. verticillata*	*L. americanus*	*L. meleagris*	*L. cretaceous*
6-9^{o}C	No Growth	No Growth	No Growth	No Growth	No Growth
23-25^{o}C	10.40±0.23^{a}	16.87±0.35^{b}	5.88±0.15^{b}	6.78±0.06^{a}	7.12±0.58^{a}
30-32^{o}C	9.72±1.21^{a}	18.00±0.00^{a}	6.39±0.14^{a}	7.02±0.46^{a}	7.22±0.46^{a}

The primordia were then allowed to mature into full fruiting bodies by opening and watering the fruiting bags. Figure 2 shows the fruiting bodies of three wild mushrooms cultivated on a sawdust and rice straw-based substrate formulation. With regards to cap diameter, *L. americanus* had a wider cap diameter (79.29 mm) followed by *L. meleagris* (61.41%), and *L. cretaceous* (56.24 mm). In terms of stipe length, no significant difference was found between the two species of *Leucoagaricus* (80.72). Meanwhile, *L. cretaceus* shows a longer stipe length with a mean of 128.97 mm.

Moreover, the yield and biological efficiency of the three mushrooms, *L. americanus, L. meleagris,* and *L. cretaceus* are also presented in Table 2. There is no significant difference was found between the two species of *Leucoagaricus.* Both of the species show excellent yields of 28.78 g bag^{-1}. Moreover, the *Leucocoprinus* has a lesser yield of 11.5 g bag^{-1}. Furthermore, both the *Leucoagaricus* mushrooms show excellent biological efficiency (BE) of 5.75%, while *Leucocoprinus cretaceus* show BE of 2.3%.

Mycelial biomass production

Aside from the fruiting body production of the three wild mushrooms, two species of *Coprinopsis* mushrooms (*C. cinerea* and *C. verticillata*) were subjected to mycelial biomass production. The mycelial biomass production parameters of the two species of *Coprinopsis*are shown in Table 3. The incubation period for mycelial ramification was recorded for inoculated culture bottles under the required environmental conditions. Both species of *Coprinopsis* have an incubation period of 14 days. However, the yield per bottle of *C. verticillata* (11.93 g bottle^{-1}) is higher than *C. cinerea* (9.09 g bottle^{-1}). Likewise, the biological efficiency of *C. verticillata* (23.86%) is higher than that of *C. cinerea* (18.18%).

Table-2: Fructification parameters of the *Leucoagaricus* and *Leucocoprinus* species

Mushroom Species	Incubation period (day)	Days to Primordia Initiation (day)	Cap Diameter (mm)	Stipe Length (mm)	Yield per bag (g bag^{-1})	Biological Efficiency (%)
L. americanus	51.4±0.69	54.2±0.42	79.29±17.29	89.72±7.97	28.78±11.95	5.75±2.39
L. meleagris	31.7±0.48	34.7±0.48	61.41±11.36	80.72±7.97	28.78±11.95	5.75±2.39
L. cretaceous	30.7±4.42	32.5±4.00	56.24±6.98	128.97±21.30	11.5±4.85	2.3±0.97

Table-3: Mycelial biomass production of two *Coprinopsis* species

Mushroom Species	Incubation Period (days)	Yield per bottle (g bottle^{-1})	Biological Efficiency (%)
C. cinerea	14.00	9.09±1.02	18.18±2.04
C. verticillata	14.00	11.93±6.26	23.86±12.53

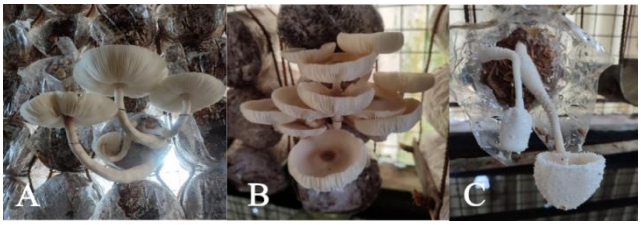

Figure-2: Cultivated fruiting bodies of (A) *L. americanus*, (B) *L. meleagris*, and (C) *L. cretaceus* on (7:3 v/v) rice straw sawdust-based substrate in growing house condition

Discussion

The growth of mushrooms differs from species to species. This is because of the different factors affecting mushroom growth. Such factors have been reviewed and categorized as intrinsic and extrinsic factors (Bellettini al., 2019). These factors play an important role in determining the best cultivation conditions which favor the efficient growth of specific mushrooms. In this study, five wild mushrooms collected in Luzon Island, Philippines were subjected to optimization studies, namely *Coprinopsis cinerea*, *Coprinopsis verticillata*, *Leucoagaricus americanus*, *Leucoagaricus meleagris*, and *Leucoagaricus cretaceous* to determine the best culture medium as well as pH, aeration, illumination, and temperature conditions favors the luxuriant secondary mycelial growth for a specific mushroom.

For the first part of the optimization study, the best culture medium was evaluated. The culture medium provides essential nutrients for the luxuriant mycelial growth of mushrooms in pure culture. Therefore, it is important to determine the nutritional requirements of specific mushrooms to establish the optimal growing condition for mass production and to enable the mushroom to produce desired compounds and target products. According to Dulay and Garcia (2017), the nutritional content of the culture medium influences the luxuriant growth of mycelia in pure culture. In addition, the nutritional composition of the organic matter of the substrates affects mycelia growth; hence, nutritional evaluation is needed for establishing the medium that favors the efficient growth of mushroom species (De Leon et al., 2017a). In this study, a total of eight culture media were used to determine the optimum culture medium of the five wild mushrooms. Four indigenous culture media (CWG, RBDG, CGDG, PSG) and four commercially available media (PDA, MEA, SDA, MA) were used. Data showed that MEA was the best culture medium and was found suitable for the luxuriant mycelial growth of the four wild mushrooms, followed by PDA for three wild mushrooms. Additionally, indigenous medium, PSG, and CGDG were also found suitable for the efficient secondary mycelial growth of *C. verticillata* and *L. americanus*, respectively. Similarly, in the study of Rizal et al. (2014), the MEA and PDA were also recorded as the best culture medium among the nine media used in the optimization study of *Macrolepiota deters*. In addition, the shortest incubation period (10 days) and thickest mycelial density were both achieved on MEA, which also recorded the greatest mycelial growth rate (9.00 mm/day) in the optimization study of *Cyclocybe cylindracea* (Landingin et al., 2020). Furthermore, in the optimization study utilizing the indigenous culture medium of Kalaw et al. (2016) on wild macrofungi collected from Central Luzon, Philippines, the PSG was found suitable for the largest mycelial diameter for the two strains of *Volvariella volvacea*. Likewise, MEA, MA, and PSG were recorded as the best medium for the secondary mycelial growth of the different strains of *Pleurotus djamor* (Kalaw et al., 2022). Overall, the suitability

of culture media depends on the mushrooms being utilized given that different mushrooms require different nutritional requirements for their efficient growth.

On the other hand, mushroom morphology and physiology as well as the medium ionic state are greatly influenced by the pH concentration of the culture medium (Dulay et al., 2021). In this study, results showed a wide range of pH requirements (4-9) for the five wild mushrooms. Likewise, the recent review of Fabros et al. (2022) also shows a wide range of pH requirements (pH 4.5-8) for the growth of *Lentinus* mushrooms. However different mushrooms have specific pH for their optimum mycelial growth. This confirmed the claims of Michael et al. (2001) that the best yields were obtained at pH 4-7, but the effect of pH on mycelial growth still depends on which mushroom organism is being utilized. Accordingly, *L. americanus* (CAG10) shows a specific pH requirement of 5.0 for its secondary mycelial growth. Likewise, the study by Dulay et al. (2015) also evaluated the specificity of the optimum pH in the optimization study of the five Philippine wild mushrooms. Based on their findings the *Volvariela volvacea, Pleuroteus cyctidiosus, Ganoderma lucidum, and Schizophyllum commune* grown in Sabouraud Dextrose Broth (SDB) has an optimum pH of 6, 7, 7, and 8, respectively.

Aside from intrinsic factors such as culture medium and pH, extrinsic factors such as aeration, illumination, and temperature also play an important role in the optimization study. The first physical condition that was determined in this study is aeration. Five wild mushrooms were incubated in sealed (with parafilm) and unsealed plates to satisfy the anaerobic and anaerobic conditions. According to Leatham and Stahmann (1987), although many fungi may thrive in oxygen-depleted or hypoxic environments, most fungi are classified as aerobes. However, based on the data obtained, the five mushrooms preferred anaerobic conditions except for *C. cinerea* which favorably grows in unsealed plates (aerobic). Meanwhile, there is no significant effect shown for *L. cretaceous* and *L. americanus* for both aeration conditions. Similarly, the two *Lentinus* species, *Lentinus squarrosulus*, and *Lentinus swartzii* show excellent mycelial growth and mycelial density when grown in sealed (anaerobic) conditions (De Leon et al., 2017b; Dulay et al., 2021). Moreover, *C. cylindracea*, *Ganoderma gibbosum*, *Ganoderma australe*, and *Ganoderma weberianum* incubated in two different aeration conditions show no significant differences (Landingin et al., 2020; del Rosario et al., 2022).

Illumination condition is another extrinsic factor that affects mycelial growth. According to Rangel et al. (2011), fungi are known to be influenced by light conditions during mycelial development in a variety of ways, such as in the mycelial biomass production and antioxidant activity of *Ganoderma lucidum* (Alcazar et al., 2021), fumonisin production of *Fusarium verticillioides* (Fanelli et al., 2012), as well as in the fructification of *Lentinus tigrinus* (Damaso et al., 2018). In this study, the five wild mushrooms' mycelial growth and mycelial density were not affected by light with two species, *C. cinerea* and *L. cretaceus* favoring dark conditions. This is similar to the *Collybia reinakeana* (Reyes et al., 1997), *Ganoderma applanatum* (del Rosario et al., 2022), and *Lentinus swartzii* (Dulay et al., 2021) which also favored dark conditions for their mycelial growth. Meanwhile, lighted conditions were found suitable for the luxuriant mycelial growth of *Lentinus sajor-caju* (De Leon et al., 2017a) and *Schizophyllum commune* (Kalaw et al., 2016). Interestingly, the two strains of *Volvariella volvacea* (Vinces and Montalvo) and *Ganoderma lucidum* (strains 1 and 2) differ in their illumination conditions even if they are in the same species (Abon et al., 2020; del Rosario et al., 2022). This suggests that illumination condition is not only species-dependent but can also be strain dependent. Furthermore, light emitting diode (LED) is also being utilized in the optimization study of many mushrooms, such as *Aspergillus ficuum, Lentinus tigrinus,* and *Ganoderma lucidum* to promote higher yields, production of bioactive compounds, mycelial growth, and fructification (Cheng et al., 2012; Damaso et al., 2018).

The last physical factor that was established is the temperature condition. Temperature is crucial in the distribution of the fungal species as mushrooms thrive in tropical and others in temperate conditions (Thawthong et al., 2014; Sobal et al., 2007). According to Abu Bakar et al. (2020), temperature has a direct impact on fungal development and protein dynamics. Additionally, Dix and Webster (1995), claimed that the majority of the fungal species are mesophiles that thrive at temperatures ranging from 5 to 35 degrees Celcius with optimal growth temperatures between 25-30ºC. Accordingly, results obtained in this study show that the three wild mushrooms, *C. cinerea, L. meleagris,*

and *L. cretaceous* favored both the room temperature (30-32ºC) and air-conditioned (23-25ºC), while *C. vericillata* and *L. americanus* only favored room temperature conditions. Moreover, no growth was recorded for refrigerated conditions. These findings are similar to the study of Kalaw et al. (2022) and the review of Fabros et al. (2022) for the six strains of *P. djamor* and *Lentinus* species, respectively which favorably grow at room temperature (30ºC) and air-conditioned (23ºC). Additionally, the majority of the *Ganoderma* species in the optimization study of del Rosario et al. (2022) also favored the room temperature (30ºC) except for *Ganoderma webarianum* which favorably grows in air-conditioned (23ºC). However, some of the species favored lower temperatures like in the case of *Cordyceps militaris* (Park et al., 2001) and *Collybia maculate* (Lim et al., 2004) which thrive in slightly lower temperatures of 20ºC.

After determining the optimized culture condition of the five wild mushrooms, we evaluated the yield performance of these mushrooms utilizing the substrate formulation of CTMRD 70% rice straw 30% sawdust for fructification and potato broth for mycelial biomass production to satisfy the need for mass-producing these wild mushrooms. A variation in the mycelial growth, fructification, and mycelial biomass production, namely the incubation period, days of primordia initiation, cap diameter, stipe length, yield per bag/bottle, and biological efficiency was determined. Interestingly, the substrate formulation utilized was able to produce a fruiting body for the three wild mushrooms, and mycelial biomass for the two *Coprinopsis* mushrooms.

Conclusion

In conclusion, wild mushrooms show different responses to intrinsic and extrinsic factors for both mycelial growth, fructification, and mycelial biomass production. With this data, wild mushrooms can be further utilized for other studies such as proximate analysis, chemical and nutritional composition determination, and tests for different bioactivities, among others.

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Contribution of Authors

Fabros JA, Dulay RMR, Kalaw SP & Reyes RG: Conceptualization of the study, literature review, experimental design and data interpretation, manuscript writing and critiquing and final approval.

Ganarael KCO & del Rosiario MAG: Experimentation, data collection, statistical analysis and interpretation.