J ISSAAS Vol. 28, No. 2: 42 – 51 (2022)

OPTIMIZATION OF MYCELIAL CULTURE CONDITIONS AND FRUCTIFICATION OF *Ganoderma* SPECIES IN RICE STRAW-BASED SUBSTRATES

Mark Adrian G. del Rosario*, Rich Milton R. Dulay, Sofronio P. Kalaw, Eduardo J. Damaso, Jr., Joshua P. Ramos, Marcelino A. Abon, Angeles M. De Leon, Renato G. Reyes
Center for Tropical Mushroom Research and Development, Tuklas Lunas Development Center, Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3120 Philippines
*Corresponding author: delrosario.markadrian@clsu2.edu.ph

(Received: May 30, 2022; Accepted: September 20, 2022)

ABSTRACT

Ganoderma (Basidiomycota, Ganodermataceae) species are saprophytic mushrooms that are widely known for their medicinal properties and are used in many Asian countries. This study focused on the effects of nutritional (culture media), environmental (pH, aeration, illumination, and temperature), and fructification of six *Ganoderma* species rescued from Luzon Island, Philippines. Mycelia of the tested *Ganoderma* species preferred coconut water agar as their basal culture media with pH levels ranging from acidic to slightly acidic medium (pH 5.0-6.0) while others were unaffected (pH 5.0-8.0). Aeration was not a major factor for three species whereas *Ganoderma applanatum* and *Ganoderma lucidum* strain 2 favored sealed conditions and *Ganoderma lucidum* strain 1 preferred unsealed. Light was proven to be beneficial for mycelial growth of *G. lucidum* strain 2 and dark conditions for *G. applanatum* though others were not affected. The majority of the *Ganoderma* species favored room temperature (30ºC) for optimum growth although *G. weberianum* preferred 23ºC. In conclusion, optimized and domesticated species/strains of *Ganoderma* showed variation among their fructification parameters and provide a data baseline for further studies.

Key words: conservation, domestication, Ganodermataceae, mushroom, sustainable production

INTRODUCTION

Diverse mushroom species can be found in temperate and tropical regions of the world and represent a large biodiverse group of organisms (Hawksworth 2001). Fungi play an important role as pathogens, decomposers, and symbionts in terrestrial ecosystems (Mueller et al. 2007). These are most visible at the start of the rainy season, but these can also be found in a variety of habitats and substrates, including rich soils, grassy ground, decaying plant litter, animal manure, and wooden logs in forests (López-Quintero et al. 2012). Over the years, mushrooms have been collected and consumed as a delicacy for their nutritional and medicinal properties (Nacua et al. 2018). These are low in calories and have a rich nutritional value with high protein content, vitamins, minerals, chitin, trace elements, cholesterol, and other bioactive mycochemicals (Wani et al. 2010). Medicinal activities can be observed among mushroom species, an example of which is *Ganoderma lucidum.* It contains triterpenoids, polysaccharides, mycins, steroids, and organic germanium (Ge) that contribute to its medicinal effects (Deepalakshmi and Mirunalini 2011; Taofiq et al. 2017).

The vast ecosystems in the Philippines provide a suitable habitat for various macrofungal species. Studies with regards to species listing, biodiversity, and distribution have been conducted on Luzon Island, the Philippines to identify the present mushrooms in the area. Common mushroom genera found in the wild include *Auricularia, Ganoderma, Lentinus, Marasmiellus, Phelinus, Pleurotus, Schizophyllum, Termitomyces,* and *Trametes* (Dulay et al. 2020; Licyayo 2018; Liwanag et al. 2017). *Ganoderma* (Ganodermataceae) are wood-rotting basidiomycetous fungi with a double-walled basidiospore that are common in tropical areas and have a close relationship to Asian traditional medicine (Donk 1946; Lin and Zhang 2004). The basidiocarp of this fungus has a laccate (shiny) or non-laccate (dull) pilear surface, and the basidiospores have an ovoid shape that is enlarged or truncated at the apex (Monclavo 2000). *G. lucidum* is a well-studied mushroom for its numerous bioactive compounds. It is mainly composed of polysaccharides, trace elements, fats, soluble proteins, and fiber, with domesticated varieties having similar nutritional values to wild types (Wasser 2005). Further studies show that several species of *Ganoderma* have antioxidant, antibacterial, and antidiabetic properties (Ma et al. 2015; Mohammadifar et al. 2020; Osińska-Jaroszuk et al. 2014).

Various *Ganoderma* spp. were documented, and identified from Luzon Island, the Philippines. Secondary mycelia of these mushrooms were successfully rescued and added to the culture collections of Philippine wild mushrooms at the Center for Tropical Mushroom Research and Development, Central Luzon State University. Knowing the bioactive compounds present in *Ganoderma*, it is of valuable interest to understand and determine the optimal culture conditions and fructification capabilities for mushroom biomass production that can lead to possible pharmacological utilization. This study sought to determine the optimal growth conditions of six collected *Ganoderma* species/strains.

MATERIALS AND METHODS

Evaluation of culture medium and pH. Four indigenous culture media namely: coconut water gelatin (CWG), potato sucrose gelatin (PSG), rice bran decoction gelatin (RBDG), and corn grit decoction gelatin (CGDG), and four commercially dehydrated media, such as potato dextrose agar (PDA), malt extract agar (MEA), Sabouraud dextrose agar (SDA) and mycological agar (MA) were used. Indigenous cultural media decoctions were prepared separately in 1L of distilled water using 250g of evenly sliced potato, 50g of rice bran, and 50g of corn grit. These decoctions were boiled separately until homogenized and strained to remove large particles and impurities. This mixture and coconut water were added discretely with 10% agar (local crude agar), 1% sucrose (for PSG, RBDG, and CGDG), and brought to boiling point to further homogenize the media. The dehydrated media were prepared according to the manufacturer's instructions: PDA; 39g/1000ml of distilled water (HIMEDIA), SDA; 65g/1000ml of distilled water (HIMEDIA), MA; 35g/1000ml of distilled water (HIMEDIA), and MEA; 33.6g/1000ml of distilled water (CONDA). All media were transferred into Erlenmeyer flasks with cotton plugs, sterilized in an autoclave at 121°C for 15 minutes, pour-plated, and solidified. Three replicates of the medium were prepared. A seven-day-old pure culture of secondary mycelia of the mushrooms was used. Each culture medium was aseptically inoculated with 10 mm-diameter mycelial discs centrally in the plated medium, sealed, and incubated at room temperature. The mycelial growth diameter of each mushroom species was measured every 24 hours for seven days and mycelial density was also recorded.

The optimum pH of the medium was determined once the optimal culture medium was established. Preparation of the media was done under the same procedure with the exception of adjusting the pH of the culture media from pH 5.0 to 8.0 at 0.5 intervals. Mycelial growth rate and thickness were also observed.

Evaluation of aeration, illumination, and temperature. The mycelial plate cultures with optimized medium and pH were subjected to three physical environmental factors, namely: aeration, illumination,

and temperature, to elucidate the effects on the secondary mycelial growth and density. The plate cultures were incubated in two aeration conditions (sealed and unsealed), two illumination conditions [artificial light (322.92 lumens/m^2) and complete darkness], and three temperature conditions (6, 23, and 30°C). Three replicates were prepared for each condition. Mycelial growth rate and mycelial thickness were noted.

Grain spawn preparation. The substrate used for the spawn production of *Ganoderma* species was unmilled rice. Unmilled rice seeds were thoroughly washed and boiled in water until partial cracking of grains was observed, drained, and packed into 6 x 12 x 0.3 polypropylene bags (PP bags). The grains were then sterilized in an autoclave at 121 °C for 1 hour. An agar block of mushroom mycelia with the optimum culture conditions was then inoculated in sterilized grains and allowed for full mycelial ramification. After full ramification with mycelia, the grain spawns were used as inoculants for the production of mushrooms.

Fruiting body production. The main substrates used to determine the fruiting body production and bio-efficiency of *Ganoderma* species were rice straw and sawdust. Aseptically, 40 g of fully ramified mushroom grain spawns were inoculated into pasteurized fruiting bags (500g) using 6 x 12 x 0.3 (inches) PP bags containing the formulated rice straw-based substrate (7 rice straw: 3 sawdust v/v), composed of soaked rice straw for 5 to 7 days for partial degradation of lignin as well as other components and sawdust obtained from local lumber mills. These two were mixed and maintained a moisture level of 65% prior to pasteurization (60–100°C) for 8 hours, developed by the CLSU Center for Tropical Mushroom Research and Development (CLSU-CTMRD). Inoculated fruiting bags were then incubated at room temperature until the substrate is completely ramified by mycelia. The fully ramified bags were opened at one end to allow fruiting initials to develop into mature basidiocarps. Matured fruiting bodies were harvested, evaluated by measuring the pileus and stipe and weighed to determine the yield per bag and biological efficiency. Periods of incubation and primordial development were also recorded.

Statistical analysis. The data were analyzed using Analysis of Variance at a 5% level of significance and compared using Tukey's honestly significant difference and t-test.

RESULTS AND DISCUSSION

Culture media. In general, mushrooms require sources of carbon and nitrogen to facilitate normal metabolic processes essential for survival. The different *Ganoderma* species statistically favored CWG as their suitable culture media, with the capability to colonize the substrate rapidly (Table 1).

Table 1. Mycelial growth of *Ganoderma* species on different culture media, pH, aeration, illumination and temperature.

Factors	**Mycelial growth rate (day)**					
Culture media	*G. applanatum*	*G. gibbosum*	*G. australe*	*G. lucidum* strain 1	*G. lucidum* strain 2	*G. weberianum*
CWG	13.04±0.32^{ab}	15.00±0.00^{a}	15.00±0.00^{a}	12.28±0.55^{a}	12.86±0.00^{a}	15.00±0.00^{a}
PSG	8.21±2.34^{d}	10.74±0.60^{cd}	14.68±0.35^{a}	4.45±1.39^{f}	7.21±0.32^{cd}	8.83±1.20^{e}
CGDG	11.47±0.54^{bc}	10.01±0.17^{cd}	13.26±0.32^{b}	5.57±0.45^{ef}	6.33±0.35^{de}	12.34±0.52^{bcd}
RBDG	15.00±0.00^{a}	13.27±0.14^{b}	15.00±0.00^{a}	10.97±0.48^{ab}	9.53±0.85^{b}	13.76±1.47^{ab}
PDA	13.13±0.31^{ab}	11.05±0.38^{c}	14.44±0.45^{a}	8.37±0.49^{cd}	7.48±0.40^{cd}	10.65±0.11^{cde}
MEA	10.05±0.78^{cd}	7.58±0.28^{e}	13.05±0.72^{b}	7.01±0.75^{de}	3.69±0.76^{f}	10.25±1.10^{de}
SDA	14.54±0.40^{a}	9.82±0.78^{d}	14.61±0.39^{a}	10.03±0.91^{bc}	8.11±0.77^{bc}	13.13±0.66^{abc}
MA	9.75±1.20^{cd}	6.95±0.18^{e}	12.43±0.28^{b}	5.35^{e}±0.31^{f}	5.27±0.15^{e}	8.81±0.85^{e}

Factors	**Mycelial growth rate (day)**					
Culture media	*G. applanatum*	*G. gibbosum*	*G. australe*	*G. lucidum* strain 1	*G. lucidum* strain 2	*G. weberianum*
			pH			
5.0	13.50±1.49^{ab}	12.84±0.41^{c}	15.00±0.00^{a}	11.87±0.44^{a}	11.81±0.42^{a}	12.30±0.03^{ab}
5.5	12.37±0.49^{bc}	14.27±0.44^{ab}	13.47±0.38^{b}	11.66±1.20^{ab}	12.46±0.68^{a}	12.55±0.15^{a}
6.0	14.83±0.30^{a}	13.97±0.04^{abc}	13.32±0.32^{b}	11.32±0.71^{ab}	12.86±0.00^{a}	12.29±0.05^{ab}
6.5	9.58±0.00^{d}	13.53±0.84^{bc}	12.99±0.05^{b}	8.75±0.84^{ab}	11.98±0.97^{a}	11.90±0.31^{b}
7.0	11.23±0.39^{cd}	14.77±0.11^{ab}	13.42±0.19^{b}	6.87±1.20^{b}	12.27±0.46^{a}	11.13±0.09^{c}
7.5	10.42±0.71^{cd}	14.46±0.56^{ab}	11.26±0.13^{c}	8.60±2.30^{ab}	11.61±0.18^{a}	10.75±0.33^{c}
8.0	10.09±0.65^{d}	15.00±0.00^{a}	13.19±0.17^{b}	8.86±3.36^{ab}	11.52±0.08^{a}	10.07±0.34^{d}
			Aeration			
Sealed	18.00±0.00^{a}	15.00±0.00^{a}	12.78±0.14^{a}	11.67±1.29^{a}	15.00±0.00^{a}	12.03±0.56^{a}
Unsealed	14.58±0.75^{b}	13.91±1.20^{a}	12.39±0.81^{a}	6.69±0.69^{b}	13.01±0.56^{b}	11.14±0.34^{a}
			Illumination			
Light	4.73±0.77^{b}	15.00±0.00^{a}	12.86±0.00^{a}	10.40±0.39^{b}	15.00±0.00^{a}	11.44±0.98^{a}
Dark	18.00±0.00^{a}	15.00±0.00^{a}	12.86±0.00^{a}	12.86±0.00^{a}	13.50±0.15^{b}	11.57±0.89^{a}
			Temperature			
6°C	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
23°C	15.22±0.24^{b}	14.26±0.66^{a}	14.52±0.83^{a}	14.93±0.12^{a}	12.52±0.61^{b}	12.51±0.08^{a}
30°C	18.00±0.00^{a}	15.00±0.00^{a}	15.00±0.00^{a}	15.00±0.00^{a}	15.00±0.00^{a}	11.90±0.27^{b}

Mature coconut water contains minerals such as K, Ca, Na, Mg, P, Cu, and Fe (Uphade et al. 2008). These elements are necessary for enhanced optimum growth of mycelia and directly affect the yield of several mushrooms (Jonathan and Fasidi 2001; Siwulski et al. 2019). In addition, most of the soluble solids found in coconut water are sugars; glucose, fructose, and sorbitol (Shubhashree et al. 2014). These high concentrations of sugar provide the secondary mycelia with large amounts of energy for substrate colonization. While the aforementioned minerals and sugar constituents are present in other culture media used in the experiment, the distinct growth rates are accounted for by the concentration availability of these components from one medium to another.

Similar studies revealed *Ganoderma* species prefer coconut water gelatin as their basal medium for optimum growth and biomass production (Bellere 2018; Magday et al. 2014). The outcome of this experiment also showed that different species of the same genus can favor the same nutritional requirements. Likewise, *Pleurotus citrinopileatus, P. djamor,* and *P. salmoneostramineus* cultured in CWG had luxuriant growth and thick density (Jacob et al. 2015). The aforementioned findings support the results from this study that CWG is the best medium for the optimum growth of *Ganoderma* mycelia.

Effect of pH. Different species of *Ganoderma* in this study grew favorably in acidic to basic conditions. *G. applanatum, G. australe,* and *G. weberianum* had luxuriant mycelial growth on pH 5.0–6.0 indicating that these are pH sensitive while *G. lucidum* strain 1, *G. lucidum* strain 2, and *G. gibbosum* were not pH sensitive, hence having a broad pH range (Table 1). According to research, the optimal pH range for *Ganoderma* species is between 5.0 and 9.0 (Jayasinghe et al. 2008; Jo et al. 2009). Moreover, a Thailand strain of *G. australe* exhibited optimal growing conditions at pH 7.0–8.0 (Hyde 2017). This is not consistent with the pH 5.0 requirement of *G. australe* (Philippine strain) used in this study. This indicates that optimal pH conditions are species/strain dependent.

Influence of aeration. The presence or absence of air proves to be important for normal mycelial activity and growth. Table 1 shows no significant difference between *G. gibbosum, G. australe,* and *G. weberianum* under both aeration conditions (sealed and unsealed). Similar conditions were beneficial

to three strains of *Volvariella volvacea* (Abon et al. 2020). *G. applanatum* registered the highest mycelial growth rate under sealed conditions. This response was observed in *G. lucidum, Lentinus strigosus* BIL 1324, and *Lentinus swartzii*, (Dulay et al. 2017; Dulay et al. 2021; Magday et al. 2014). Interestingly, the two strains of *G. lucidum* favored distinct aeration conditions, *G.lucidum* strain 1 under unsealed conditions and *G.lucidum* strain 2 under sealed conditions. This outcome is similar to the study of Kalaw et al. (2021) wherein sealed conditions was ideal for *Lentinus squarrosulus* strain 1 while *Lentinus squarrosulus* strain 2 grew luxuriantly in both sealed and unsealed conditions.

Influence of light. In the present study, mycelial growth of *G. lucidum* strain 2 statistically preferred lighted conditions while *G. gibbosum, G. australe, G. lucidum* strain 1, and *G. weberianum* were unaffected in both conditions shown in Table 1. *Chlorophyllum molybdites* and *V. volvacea* incubated under lighted and dark conditions displayed no discrepancies in their mycelial growth, indicating similar responses in other species as well (Abon et al. 2020; Garcia et al. 2020). Interestingly, *G. applanatum* favored dark environment as well as *Fomitopsis feei, L. swartzii,* and *L. strigosus* (De Leon et al. 2020; Dulay et al. 2020).

Influence of temperature. The results from this study imply that the *Ganoderma* species are grouped as tropical mushrooms due to statistically optimal growing conditions at 30^{o}C. Likewise, two oyster mushroom species have the same optimal temperature of 28^{o}C followed by 32^{o}C and has improved growing conditions in autumn and summer seasons of tropical regions (Hoa and Wang 2015). However, *G. weberianum* has statistically optimal growth under air-conditioned (23^{o}C). No growth was observed under refrigerated conditions (6^{o}C) due to reduced enzymatic activity at low temperatures seen in Table 1.

The outcome of this study indicates that optimal temperatures are species/strain dependent. Other findings show that environmental temperatures between 20-30^{o}C proved beneficial for the mycelial growth of *V. volvacea, P. salmoneostramineus, P. cornicopae, P. eryngii, P. giganetus, Laetiporus sulphureus* and *Lentinus conatus* (Akinyele et al. 2020; Karunarathna et al. 2014; Luangharn et al. 2014).

Fructification on rice straw and sawdust-based substrate. Different morphological parameters of the successfully cultivated *Ganoderma* species on rice-straw and sawdust-based formulation are shown in Table 2 and Figure 1. Various *Ganoderma* species respond differently upon cultivation on the same formulated substrate. *G. australe* and *G. gibbosum* registered the shortest incubation period with 21.3-21.7 days, followed by *G. applanatum, G. lucidum* strain 1, *G. lucidum* strain 2 and *G. weberianum* having 24.5-26.8 days. Although these mushroom species were grown under the same conditions, substrate colonization depends on the carbon and nitrogen concentration requirements of the species/strain (Hoa et al. 2015). Similar incubation periods can be observed in *Lentinus strigosus* (23.67 days) (Dulay et al. 2017). The variations may be due to the differences in temperature, species, and humidity (Suwannarach et al. 2022).

Fully ramified fruiting bags were opened at one end and the days to primordia formation or fruiting initials were observed. The two strains of *G. lucidum* displayed significant differences with strain 1 having the earliest primordia after 19.6 days and strain 2 after 41.1 days. This is followed by the primordia of *G. weberianum, G. australe, G. applanatum,* and *G. gibbosum* after 34.4, 37.9, 39.6, and 40.0 days, respectively. Subsequently, the opened fruiting bags with emerged fruiting initials were watered allowing the primordia to further develop into mature fruiting bodies. Matured fruiting bodies of the *Ganoderma* species grown on rice straw substrates were measured shown in Table 2. With regards to the cap diameter, *G. australe* measures 33.36 mm, *G. gibbosum* at 30.88 mm, *G. applanatum* at 28.10 mm, and *G. weberianum* at 25.6 mm. Interestingly, *G. lucidum* strain 1 had a wider cap diameter (32.70 mm) when compared with strain 2 (17.12mm) and was the narrowest among the species. In terms of the stipe, *G. lucidum* strain 1 demonstrated the longest stipe (14.21 mm) among the species. This is

followed by *G. australe* (8.82 mm), *G. gibbosum* (6.96 mm), *G. lucidum* strain 2 (3.56 mm), *G. applanatum* (3.72 mm), and lastly, *G. weberianum* (3.14 mm).

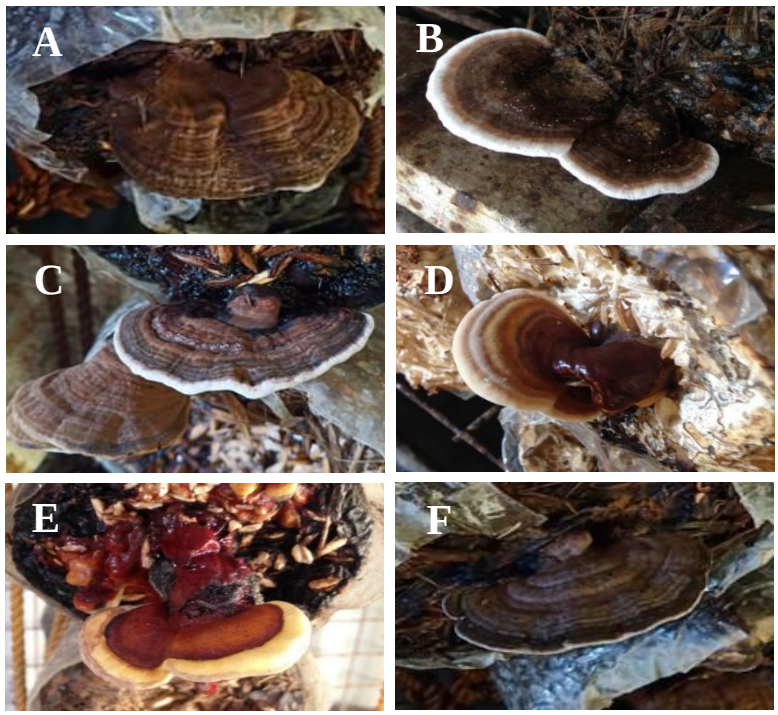

Fig. 1. Successfully domesticated *Ganoderma* species from Luzon Island, Philippines, *Ganoderma applanatum* AS4 (A), *Ganoderma gibbosum* AS10 (B), *Ganoderma australe* SS54 (C), *Ganoderma lucidum* strain 1 CPS6 (D), *Ganoderma lucidum* strain 2 C006 (E), *Ganoderma weberianum* DQS74 (F).

The yield and biological efficiency (BE) of the six culturally optimized *Ganoderma* species grown on rice-straw and saw dust formulation are displayed in Table 2. It can be perceived that *G. australe* had the lowest yield and a conforming BE while the highest recorded yield and BE among the *Ganoderma* species tested was that of *G. applanatum*. This was followed by *G. weberianum, G. lucidum* strain 1, *G. lucidum* strain 2, and *G. gibbosum.* These outcomes are very low when compared to a different cultivation method of *Ganoderma leucocontextum, G. resinaceum,* and *G. gibbosum* in soil casing layer (60.43% BE and 253.82 g/kg; 26.94% BE and 7.02 g/kg; 73.80% and 284.15 g/kg, respectively) and non-casing layer cultivation methods (13.60% BE and 58.18 g/kg; 109.26% BE and 27.75g/kg; 40.26% BE and 172.08g/kg, respectively) (Luangharn et al. 2020). *G. lucidum* grown in three different sawdust as basal substrates, on the other hand, yielded 15.05g/400gm with 15.69% BE, 1.5g/400gm with 0.512% BE, and no yield due to poor mycelial growth (Gurung et al. 2012). This indicates that the basal substrate affects greatly the development and morphology of fruiting bodies in mushrooms. The variation in the growth performance and morphology can be attributed to the genotype and physiological differences among the species and strains (Kalaw et al. 2021). Furthermore, different species of mushrooms require different carbon/nitrogen ratios in the substrate to obtain the highest yield possible (Kumla et al. 2020). Prevailing environmental conditions also affects the yield and morphological characteristics of the mushroom species (Chang and Miles 2004; Sher et al. 2010).

Table 2. Morphological characteristics of successfully cultivated *Ganoderma* species on rice straw-based substrate.

Mushroom Species	Incubation Period (days)	Days to Primordia Initiation (days)	Cap Diameter (mm)	Stipe Length (mm)	Yield per bag (g)	Biological Efficiency (%)
Ganoderma applanatum (AS4)	24.5 ±3.10	39.6 ±2.36	28.10 ±2.75	3.72 ±1.35	10.61 ±3.32	2.12 ±0.66
Ganoderma gibbosum (AS10)	21.7 ±1.25	40.0 ±2.0	30.88 ±3.73	6.96 ±1.69	7.00 ±1.15	1.40 ±0.23
Ganoderma australe (SS54)	21.3 ±1.25	37.9 ±2.38	33.36 ±6.96	8.82 ±1.68	4.58 ±1.69	0.91 ±0.34
Ganoderma lucidum strain 1 (CPS6)	24.6 ±1.51	19.6 ±0.97	32.70 ±2.96	14.21 ±2.71	8.7 ±1.63	1.74 ±0.33
Ganoderma lucidum strain 2 (C006)	25 ±2.44	41.1 ±1.66	17.21 ±2.36	3.56 ±1.13	7.3 ±2	1.46 ±0.4
Ganoderma weberianum (DQS74)	26.8 ±0.91	34.4 ±1.63	25.6 ±1.82	3.14 ±1.46	9.7 ±2.21	1.94 ±0.44

CONCLUSIONS

The optimal nutritional (culture media) and physical (pH, aeration, illumination, and temperature) requirements for the secondary mycelial growth of *Ganoderma* species and cultivation capacities using rice straw and sawdust-based substrate has been established. These findings prove that mycelia of *Ganoderma* species/strains respond differently when cultured under various conditions and favor different factors per species/strain. The incubation period, primordia initiation, cap diameter, stipe length, yield, and biological efficiency varied in performance among the species. *G. applanatum* exhibited the best cultivation performance among the species evaluated. However, further studies on the effect of substrate supplementation need to be investigated to improve the yield and biological efficiency of each species, especially for *G. australe* and *G. gibbosum*.

ACKNOWLEDGEMENT

This work was funded by the Philippine Council for Health Research and Development, Department of Science and Technology.

REFERENCES CITED

Abon, M. D., R. M. R. Dulay, S. P. Kalaw, M. E. Romero Román, L. P. Arana Vera, W. O. Reyes Borja, and R. G. Reyes. 2020. Effects of culture media and physical factors on the mycelial growth of the three wild strains of *Volvariella volvacea* from Ecuador. J. Appl. Biol Biotech. 8(6): 60-63.

Akinyele, B.J., and F.C. Adetuyi. 2005. Effect of agrowastes, pH and temperature variation on the growth of *Volvariella volvacea*. Afr. J. Biotechnol, 4(12): 1390-1395.

Bellere, A.D. 2018. Mycelial growth of *Ganoderma curtissii* in locally indigenous media. KnE Soc. Sci, 3(6): 118-124.

Chang, S.T., and P.G. Miles. 2004. Mushroom Formation: Effects of Environmental, Nutritional, and Chemical Factors, pp 129-143. In S.T. Chang and, P.G. Miles (eds.). Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact. CRC press.

De Leon, A.M., A.R. Dulay, A.L. Villanueva, and S.P. Kalaw. 2020. Optimal culture conditions and toxicity assessment of *Fomitopsis feei* (Fr.): a newly documented macro fungus from Philippines. Studies in Fungi. 5(1): 491-507.

Deepalakshmi, K., and S. Mirunalini. 2011. Therapeutic properties and current medical usage of medicinal mushroom: *Ganoderma lucidum*. Int. J. Pharm. Sci. Res. 2(8): 1922.

Donk, M.A. 1964. A conspectus of the families of Aphyllophorales. Pers.: Mol. Phylogeny Evol. Fungi. 3(2): 199-324a.

Dulay, R.M.R., A.G.C. Rivera, and E.J.B. Garcia. 2017. Mycelial growth and basidiocarp production of wild hairy sawgill *Lentinus strigosus*, a new record of naturally occurring mushroom in the Philippines. Biocatal. Agri. Biotechnol. 10: 242-246.

Dulay, R.M.R., E.C. Cabrera, S.P. Kalaw, and R.G. Reyes. 2021. Optimization of culture conditions for mycelial growth and fruiting body production of naturally-occurring Philippine mushroom *Lentinus swartzii* Berk. J. Appl. Biol. Biotechnol. 9(3): 1-5.

Dulay, R.M.R., J.S.C. VI, S.P. Kalaw, and R.G. Reyes. 2020. Distribution and species listing of wild macrofungi in sitio Canding, Barangay Maasin, San Clemente, Tarlac Province, Philippines. J. Appl. Biol. Biotechnol, 8(5): 7-5.

Garcia, B.L., J.R. Undan, R.M.R. Dulay, S.P. Kalaw, and R.G. Reyes. 2020. Molecular identification and optimization of cultural conditions for mycelial biomass production of wild strain of *Chlorophyllum molybdites* (G. Mey) Massee from the Philippines. J. App. Biol. Biotechnol. 8(6): 1-6.

Gurung, O.K., U. Budathoki, and G. Parajuli. 2012. Effect of different substrates on the production of *Ganoderma lucidum* (Curt.: Fr.) Karst. Our Nature. 10(1): 191-198.

Hawksworth, D.L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 105: 422-1432

Hoa, H.T., and C.L Wang. 2015. The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). Mycobiology. 43(1): 14-23.

Hoa, H.T., C.L. Wang, and C.H. Wang. 2015. The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). Mycobiology. 43(4): 423-434.

Hyde, K.D. 2017. Antibacterial activity, optimal culture conditions and cultivation of the medicinal *Ganoderma australe*, new to Thailand. Mycosphere. 8(8): 1108-1123.

Ivone, H.A., M.T. Jorge, G.R.M. Guadalupe, and Y.J. Berenice. 2016. Total polyphenols and antioxidant activity of *Ganoderma curtisii* extracts. J of Med Plants Studies. 4(4): 136-141.

Jacob, J.K.S., S.P. Kalaw, and R.G. Reyes. 2015. Mycelial growth performance of three species of *Pleurotus* on coconut water gelatin. Curr. Res. Environ. Appl. Mycol. 5(3): 263-268.

Jayasinghe, C., A. Imtiaj, H. Hur, G.W. Lee, T.S. Lee, and U.Y. Lee. 2008. Favorable culture conditions for mycelial growth of Korean wild strains in *Ganoderma lucidum*. Mycobiology. 36(1): 28-33.

Jo, W.S., Y.J. Cho, D.H. Cho, S.D. Park, Y.B. Yoo, and S.J. Seok. 2009. Culture conditions for the mycelial growth of *Ganoderma applanatum*. Mycobiology. 37(2): 94-102.

Jonathan, S.G., and I.O. Fasidi. 2001. Effect of carbon, nitrogen and mineral sources on growth of *Psathyerella atroumbonata* (Pegler), a Nigerian edible mushroom. Food Chem. 72(4): 479-483.

Kalač P. 2013. A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. J. Sci. Food Agric. 93(2): 209-218.

Kalaw, S.P., R.M.R Dulay, E.J. Damaso, J.C. Ramos, M.A.G. del Rosario, M.D. Abon, A.M. De Leon, J.R. Undan, and R.G. Reyes. 2021. Optimization of mycelial culture conditions and fructification of *Lentinus* species using rice straw and sawdust based substrates. Studies in Fungi. 6(1): 519-530.

Karunarathna, S.C., K.D. Hyde, E. Chukeatirote, and N. Klomklung. 2014. Optimal conditions of mycelial growth of three wild edible mushrooms from northern Thailand. Acta Biol. Szeged. 58(1): 39-43.

Kim, Y.S., S.K. Eo, K.W. Oh, C.K. Lee, and S.S. Han. 2000. Antiherpetic activities of acidic protein bound polysaccharide isolated from *Ganoderma lucidum* alone and in combinations with interferons. J. Ethnopharmacol. 72(3): 451-458.

Kumla, J., N. Suwannarach, K. Sujarit, W. Penkhrue, P. Kakumyan, K. Jatuwong, S. Vadthanarat, and S. Lumyong. 2020. Cultivation of mushrooms and their lignocellulolytic enzyme production through the utilization of agro-industrial waste. Molecules. 25(12): 2811.

Licyayo, C.M. 2018. Gathering practices and actual use of wild edible mushrooms among ethnic groups in the Cordilleras, Philippines, pp 71-86. In A. Niehof, H.N. Gartaula, and M. Quetulio-Navarra (Eds.). Diversity and change in food wellbeing: Cases from Southeast Asia and Nepal. Wageningen Academic Publishers.

Lin, Z.B., and H.N. Zhang. 2004. Anti-tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanisms. Acta Pharmacol. Sin. 25: 1387-1395.

Liwanag, J.M.G., E.E. Santos, F.R. Flores, R.F. Clemente, and R.M.R. Dulay. 2017. Species listing of macrofungi in Angat watershed reservation, Bulacan province, Luzon Island, Philippines. Int. J. Biol. Pharm. Allied. Sci. 6(5): 1060-8.

López-Quintero, C.A., G. Straatsma, A.E. Franco-Molano, and T. Boekhout. 2012. Macrofungal diversity in Colombian Amazon forests varies with regions and regimes of disturbance. Biodivers. Conserv. 21(9): 2221-2243.

Luangharn, T., P.E. Mortimer, S.C. Karunarathna, K.D. Hyde, and J. Xu. 2020. Domestication of *Ganoderma leucocontextum*, *G. resinaceum*, and *G. gibbosum* collected from Yunnan Province, China. Biosci. Biotechnol. Res. Asia. 17(1): 7-26.

Luangharn, T., S.C. Karunarathna, K.D. Hyde, and E. Chukeatirote. 2014. Optimal conditions of mycelia growth of *Laetiporus sulphureus* sensu lato. Mycology. 5(4): 221-227.

Ma, H.T., J.F. Hsieh, and S.T. Chen. 2015. Anti-diabetic effects of *Ganoderma lucidum*. Phytochemistry. 114: 109-113.

Magday Jr, J.C., M.E. Bungihan, and R.M.R. Dulay. 2014. Optimization of mycelial growth and cultivation of fruiting body of Philippine wild strain of *Ganoderma lucidum*. Curr. Res. Environ. Appl. Mycol. 4(2): 162-172.

Mohammadifar, S., S. Fallahi Gharaghoz, M.R. Asef Shayan, and A. Vaziri, A. 2020. Comparison between antioxidant activity and bioactive compounds of *Ganoderma applanatum* (Pers.) Pat. and *Ganoderma lucidum* (Curt.) P. Karst from Iran. Iran. J. Plant Physiol. 11(1): 3417-3424.

Moncalvo, J.M. 2000. Systematics of *Ganoderma*. *Ganoderma* diseases of perennial crops, pp 23-45. In J. Flood, P.D. Bridge, and M. Holderness (Eds.). Ganoderma Diseases of Perennial Crops. CABI Publishing.

Mueller, G.M. and J.P. Schmit. 2007. Fungal biodiversity: what do we know? What can we predict? Biodivers Conserv. 16*:* 1-5

Nacua, A.E., H.Y.M. PACIS, J.R. Manalo, C.J.M. Soriano, N.R.N. Tosoc, R. Padirogao, K.J.E. Clemente, and C.C Deocaris. 2018. Macrofungal diversity in Mt. Makiling forest reserve, Laguna, Philippines: with floristic update on roadside samples in Makiling Botanic Gardens (MBG). Biodiversitas. 19(4): 1579-1585.

Osińska-Jaroszuk, M., M. Jaszek, M. Mizerska-Dudka, A. Błachowicz, T.P. Rejczak, G. Janusz, J. Wydrych, J. Polak, A. Jarosz-Wilkołazka, and M. Kandefer-Szerszeń. 2014. Exopolysaccharide from *Ganoderma applanatum* as a promising bioactive compound with cytostatic and antibacterial properties. BioMed Res. Int. 2014: 1-10.

Öztürk, M., G. Tel-Çayan, F. Çayan, and M.E. Duru. 2021. Promising small molecules against cancer from *Ganoderma* Genus, pp. 139-174. In M. Öztürk, V. Altay, and R. Efe (Eds). Biodiversity, Conservation and Sustainability in Asia. Springer, Cham.

Sher, H., M. Al-Yemeni, A.H. Bahkali, and H. Sher. 2010. Effect of environmental factors on the yield of selected mushroom species growing in two different agro ecological zones of Pakistan. Saudi J. Biol. Sci. 17(4): 321-326.

Shubhashree, M.N., G. Venkateshwarlu, and S.H. Doddamani. 2014. Therapeutic and nutritional values of Narikelodaka (tender coconut water)-A review. J. of Pharmacogn. Phytochem. 6(4): 195.

Siwulski, M., P. Rzymski, A. Budka, P. Kalač, S. Budzyńska, L. Dawidowicz, E. Hajuk, L. Kozak, J. Budzulak, K. Sobieralski, and P. Niedzielski. 2019. The effect of different substrates on the growth of six cultivated mushroom species and composition of macro and trace elements in their fruiting bodies. Eur. Food Res. Technol. 245(2): 419-431.

Suwannarach, N., J. Kumla, Y. Zhao, and P. Kakumyan. 2022. Impact of Cultivation Substrate and Microbial Community on Improving Mushroom Productivity: A Review. Biology. 11(4): 569.

Taofiq, O., S.A. Heleno, R.C. Calhelha, M.J. Alves, L. Barros, A.M. González-Paramás, M.F. Barreiro, and I.C. Ferreira. 2017. The potential of *Ganoderma lucidum* extracts as bioactive ingredients in topical formulations, beyond its nutritional benefits. Food chem. Toxicol. 108: 139-147.

Thampan P.K., and P. Rethinam. 2004. Coconut products for health and medicine, Indian Coconut J. 35: 6-15.

Uphade B.K., S.S. Shelke, and D.G. Thorat. 2008. Studies on some physico-chemical characteristics of coconut water near sugar and chemical factory, Kopergaon (M.S.), Int. J. Chem. Sci. 6 (4): 2052-2054.

Wani B.A., R.H. Bodha, and A.H. Wani. 2010. Nutritional and medicinal importance of mushrooms. J. Med. Plants Res. 4(24): 2598-2604.

Wasser, S.P. 2005. Reishi or ling zhi (*Ganoderma lucidum*). Encyclopedia of dietary supplements. 1: 603-622.