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Optimization of Culture Conditions and Forced Wood Decay Assessment of Wood-rotting Mushrooms of Central Luzon, Philippines

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Abstract

This paper reports the optimal conditions of six wood-rotting mushrooms of Central Luzon, Philippines. The wood degradation of the six mushrooms on selected tropical trees was also investigated. Coconut water agar was the most suitable culture medium for efficient mycelial growth. The optimal initial pH of coconut water agar for the six species was found to be 6 to 7. Four species preferred sealed conditions while the other two species showed comparable yields in both conditions. Light conditions were found to be more favorable than dark conditions for all species, except for *Trametes cubensis*, which preferred dark conditions. All macrofungal species prefer 30°C as the optimum temperature. In the forced wood decay assay, the highest weight loss was found in mango and gmelina wood blocks. Among the fungal species, *Physisporinus lineatus* showed the highest average percentage of weight loss.

Keywords – White Rot – Brown Rot – Polypores – Hymenochaetales – Culture Media

Introduction

Wood-rotting mushrooms, like other mushrooms, play a crucial role in various ecosystems as decomposers. They thrive in diverse environments, from decomposing logs and living trees to soil and animal manure, significantly contributing to energy cycling and decomposition. Some also establish symbiotic relationships with plants, as seen in mycorrhizal fungi. Additionally, it is also worth noting that some of these mushrooms are actively utilized by humans for both food and medicinal purposes (Reyes et al. 2013, Kalaw & Albinto 2015, Devkota et al. 2023, Dulay et al. 2023). Wood-rotting mushrooms function as decomposers with the capability to break down carbohydrate polymers, lignin, and cellulose (Binder et al. 2013). They also play a destructive role by attacking plant cells, specifically the cambium cells, inducing plant diseases. This process leads to alterations in the tree's physiology, leading to tree mortality. Moreover, these decomposers possess

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enzymes which play a pivotal role in penetrating plant cells, absorbing nutrients, disrupting the septum for enhanced fungal infection, and dismantling cell walls, leading to protoplast destruction and immediate plant cell death (Dyakov 2007, Salvatore & Andolfi, 2021, Rigling et al. 2021).

Polypores, whether parasitic or saprotrophic, predominantly establish themselves at the biotope level, serving as indicators of ecosystem health (Leite 2008). However, understanding the dynamics of wood-rotting polypores and their interactions with host plants is essential for sustainable land management and targeted ecological monitoring of forest resources in the context of climate change (Nankone 2020). Fischer et al. (2012) highlight the increasing emergence of fungal threats, a trend often overlooked due to limitations in detection methods. This phenomenon may be attributed to various factors, including the expansion of fungal threats beyond their natural geographic ranges due to globalization, international trade, adaptation, ecological changes, climate change, and modern agricultural practices, such as widespread use of antifungal chemicals and altered land usage (El-Sayed & Kamel 2020). Despite posing a significant threat to the environment, global economy, public health, and food security, these emerging fungal pathogens have received insufficient attention and remain understudied (Avery et al. 2019). The consequences of unknown and undetected fungal pathogens and their management strategies could be severe, leading to disruptive impacts on the global economy and food security (Avery et al. 2019, Fones et al. 2020).

The Philippines boasts a diverse and abundant ecosystem of fungi flourishing on various substrates throughout the country (Reyes et al. 2013). Within this richness, parasitic and wood-rotting mushrooms also exhibit ecological diversity (Pampolina et al. 2023). Central Luzon, particularly its mountainous sites, stands as a haven for economically vital and noteworthy trees, thanks to its favorable topography and geographical location. These trees play a multifaceted role, contributing to clean air, water, wildlife habitat, regional protection from typhoons, and, crucially, serving as livelihood sources for nearby communities by providing food, lumber, charcoal, medicines, and other valuable products (Alberto and Galvez 2004, Chokkalingam et al. 2006, Alberto 2011).

To our knowledge, comprehensive reports on the optimization of culture conditions and wood-rotting capacities of Philippine local mushroom species, particularly in Central Luzon, are lacking. Herein, we focused on optimizing the culture conditions of six previously identified wood-rotting mushrooms, *Ganoderma applanatum*, *G. lucidum*, *Phellinus linteus*, *Physisporinus lineatus*, *Rhodofomitopsis feei*, and *Trametes cubensis*, as influenced by culture medium, pH, aeration, illumination, and temperature. The study also lays the groundwork for a wood-rotting assessment through the forced-wood decay assay, which assessed the effectiveness of these mushrooms in decomposing wood substrates commonly found in Central Luzon. This method allowed for a systematic evaluation of the wood-rotting abilities of the selected mushrooms under controlled laboratory conditions, providing valuable data for understanding their potential ecological roles and practical applications.

Materials & Methods

Fungal source

The pure cultures of *G. applanatum*, *G. lucidum*, *P. linteus*, *R. feei*, and *T. cubensis* were obtained from the mycological repository of the Center for Tropical Mushroom Research and Development (CTMRD), Central Luzon State University (CLSU). Isolates of the six wood-rotting mushrooms were obtained by aseptically transferring the stock culture onto potato dextrose agar (PDA). Cultures were then incubated at 30°C for seven days. Following incubation, 10-mm mycelial discs were extracted from the cultures using a 10-mm cork borer. Table 1 presents detailed information on each isolate, including its taxonomic identity, culture code, and geographic origin.

Screening of appropriate indigenous culture media for mycelial growth

The nutritional requirements of the secondary mycelia of wood-rotting mushrooms were determined using various indigenous culture media. Secondary mycelial discs (10 mm) from a sevenday old pure culture were prepared through a cork borer and inoculated centrally on the indigenous

culture media. The indigenous culture media used were as follows: Potato Sucrose Agar (PSA), Coconut Water Agar (CWA), Corn Grit Decoction Agar (CGDA), and Rice Bran Decoction Agar (RBDA). Potato Dextrose Agar served as the control. All the culture media were sterilized by autoclaving at 121 °C (15 psi) for 15 min and pour-plated aseptically. Incubation was done at room temperature. The diameter of mycelial ramification was measured every 24 hours until each plated medium was fully ramified. Each set-up was replicated three times.

Table 1. Different isolates of wood-rotting mushrooms from the different areas of Central Luzon, Philippines.

Wood-rotting mushroom species	Code	Place of origin
Rhodofomitopsis feei	CLSU01	Science City of Munoz, Nueva Ecija
Ganoderma applanatum	BPP01	Magalang, Pampanga
Ganoderma lucidum	BNB01	San Miguel, Bulacan
Tropicoporus linteus	BPC02	Carranglan, Nueva Ecija
Physisporinus lineatus	BNB02	San Miguel, Bulacan
Trametes cubensis	BPP02	Magalang, Pampanga

Influence of physical factors (pH, aeration, illumination, and temperature) on mycelial growth

To determine the optimum pH, the mycelia of the different wood-rotting mushrooms were cultured on the best medium. The pH of the selected culture media from indigenous sources were adjusted to pH 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 using 0.1M HCl or 0.1M NaOH, and were pour plated, then inoculated with species of bracket mushrooms. Mycelial diameter and density were measured every 24 hours, after incubation at room temperature. The best culture media and its optimum pH were then used to determine the effects of aeration.

In the evaluation of the aeration, the best indigenous culture medium at the optimum pH were inoculated with the mycelia of the different species of wood-rotting mushrooms. Some inoculated plates were double sealed with cling wrap, while the others were left unsealed. Plates were incubated for six days. The mycelial growth diameter and density were measured every 24 hours.

The influence of light on the mycelial growth of wood-rotting mushrooms was assessed. Mycelial discs of 10 mm diameter from cultures grown on the best indigenous culture medium at optimum pH and aeration were prepared aseptically using a cork borer and were utilized to inoculate the plates. The plates that were inoculated with different wood-rotting mushrooms were exposed to either full light condition or full dark condition and incubated at room temperature. For light conditions, the plates were incubated in a well-lit room with artificial white light (322.92 lumens/m²). For full dark conditions, the plates were wrapped in black paper. Mycelial diameter and density were recorded.

To evaluate the influence of temperature, 10 mm diameter mycelial plugs of the different wood-rotting mushrooms from cultures grown on the best indigenous culture media, at optimum pH, aeration, and illumination, were prepared aseptically using a cork borer and were utilized for inoculation. The inoculated plates were incubated at the following temperatures: room temperature (30 °C - 35 °C), air-conditioned (18 °C - 23 °C), and refrigerated conditions (7 °C - 11 °C). Mycelial diameter and density were assessed at every 24 hours for seven days.

Wood decay assay

The ability of wood-rotting mushrooms to decompose the different wood blocks, each measuring $1.5 \times 1.5 \times 1$ inches, was assessed. Wood blocks were obtained from the following trees: coconut (*Cocos nucifera*), gmelina (*Gmelina arborea*), mahogany (*Swietenia macrophylla*), and mango (*Mangifera indica*). The wood blocks were air dried in a drying oven for 24 hours at 75 °C (Alpinun Austria, Drying Oven). The weight of the air-dried wood blocks was recorded. Two hundred milliliter jars were half-filled with dry white sand. The half-filled white sand was moistened to approximately 65% with the best culture medium. The jars were sealed with polypropylene plastic

and sterilized at 121°C and 15 psi for 30 minutes. The previously sterilized jars with the substrate were allowed to cool and were inoculated with two milliliters of a 3-day old liquid culture of woodrotting mushroom prepared using the best medium. The inoculated jars with substrates were incubated until full ramification of mycelia for fourteen days at room temperature (30 °C–35 °C). After 14 days of incubation, the wood blocks were air-dried in a drying oven for 24 hours at 75 °C (Alpinun Austria, Drying Oven), and the surface mycelia of the wood-rotting mushrooms were carefully scraped off. The wood blocks were further air-dried. Percentage weight loss was computed.

Statistical analysis

A randomized complete design was employed to structure the experiment. The data collected underwent analysis using the Minitab Statistical Software version 21.0.1 program. The statistical evaluation was conducted through the One-Way Analysis of Variance (ANOVA). Means were subsequently compared utilizing Tukey Pairwise Comparison, employing a 95% confidence level and a 5% error rate.

Results

Culture media

The mycelial growth of each mushroom varies for each type of culture medium, as presented in Figure 1. Significant differences in mycelial growth diameter and growth rate were exhibited among the various indigenous culture media. Coconut water agar (CWA) consistently demonstrated the highest mycelial growth for all mushroom isolates. On the other hand, the smallest mycelial growth diameter was observed on CGDA for *R. feei, G. applanatum, T. linteus,* and T. *cubensis*.

Optimum pH

Under varying pH conditions, the mycelial growth of six wood-rotting mushrooms was evaluated over a six-day incubation period, as depicted in Figure 2. Coconut water with a pH of 6–7 exhibited the largest mycelial growth diameter among all the mushroom isolates. Nevertheless, the lowest biomass yields were significantly recorded at pH 4.0 and 5.0 for all the mushroom species. The findings show that fungal isolates thrive most effectively in a culture medium with a pH of neutral to slightly basic condition.

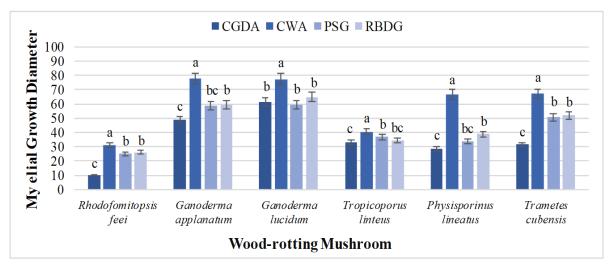


Fig. 1 – Mycelial growth diameter of six wood-rotting mushrooms in four locally available culture media after six days of incubation. The same superscript letters indicate that the mean values of each isolate are not significantly different according to Tukey's HSD at a 5% level of significance. The following abbreviations indicate (CWA) coconut water agar, (RBDA) rice bran decoction agar, (CGDA) corn grit decoction agar, and (PSA) potato sucrose agar.

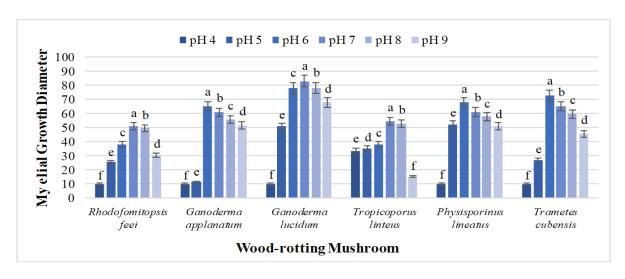


Fig. 2 – Mycelial growth diameter of six wood-rotting mushrooms in coconut water agar at varying pH levels after six days of incubation. The same superscript letters indicate that the mean values of each isolate are not significantly different according to Tukey's HSD at a 5% level of significance.

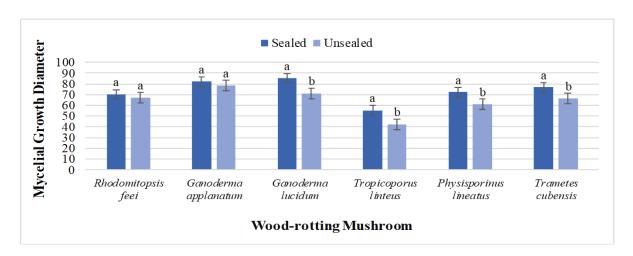


Fig. 3 – Mycelial growth diameter of six wood-rotting mushrooms in coconut water agar at sealed and unsealed conditions after six days of incubation. The same superscript letters indicate that the mean values of each isolate are not significantly different according to Tukey's HSD at a 5% level of significance

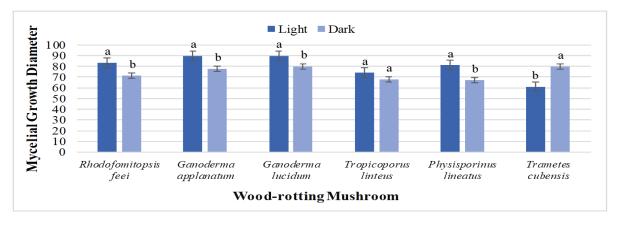


Fig. 4 – Mycelial growth diameter of six wood-rotting mushrooms in coconut water under light and dark conditions after six days of incubation. The same superscript letters indicate that the mean values of each isolate are not significantly different according to Tukey's HSD at a 5% level of significance.

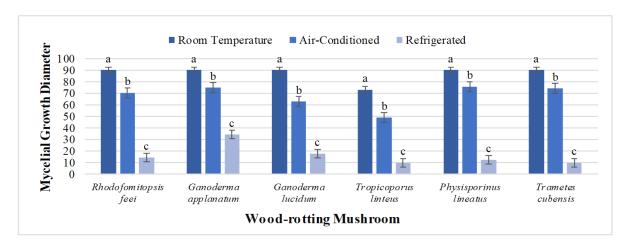


Fig. 5 – Mycelial growth diameter of six wood-rotting bracket mushrooms in coconut water agar at varying temperature conditions after six days of incubation. The same superscript letters indicate that the mean values of each isolate are not significantly different according to Tukey's HSD at a 5% level of significance.

Effects of aeration

As shown in Figure 3, secondary mycelia of *R. feei* and *G. applanatum* thrive well in both aerated and sealed conditions. This suggests that there are mushroom species which are flexible and can grow optimally in the absence or presence of oxygen. On the other hand, the T-test revealed a significant difference between the two culture conditions, as observed in the mycelial growth diameter of *G. lucidum*, *T. linteus*, *P. lineatus*, and *T. cubensis*. Thus, light has the potential to stimulate the mycelial growth of these mushroom species.

Effects of illumination

The second extrinsic factor evaluated is the effects of illumination, where six macrofungal isolates were subjected to light and dark incubation. Based on the data gathered, as presented in Figure 4, there is a significant difference in the mycelial growth observed in both light and dark conditions. The T-test revealed a significant difference between the two culture conditions. This suggests that the illumination, primarily the lighted condition, is substantial for the mycelial growth of six macrofungal isolates.

Effects of temperature

Temperature was the last extrinsic factor evaluated in this study. As presented in Figure 5, all six wood-rotting macrofungal isolates produce significant mycelial growth at room temperature. On the other hand, zero to minimal mycelial growth was recorded in refrigerated condition for all six species of wood-rotting mushrooms in the duration of six-day incubation.

Forced wood decay assessment

The present study explored the effectiveness of wood-rotting mushroom isolates in decomposing wood substrates, with an emphasis on the percentage weight loss recorded on coconut (*Cocos nucifera*), gmelina (*Gmelina arborea*), mahogany (*Swietenia macrophylla*), and mango (*Mangifera indica*) wood blocks (Figure 8). The percentage weight loss induced by six wood-rotting mushroom isolates is presented in Figure 6. Interestingly, the highest average percentage of weight loss of 24.73% was recorded in gmelina and mango wood blocks (p<0.05), while *P. lineatus* induced the highest average percentage of weight loss. However, the average percentage weight loss induced by the six wood-rotting mushroom isolates was not significantly different (p>0.05). On the contrary, the lowest percentage of weight loss across the wood blocks was recorded in coconut and mahogany wood blocks (p<0.05). The lowest average percentage of weight loss was shown by *Trametes cubensis* (p>0.05).

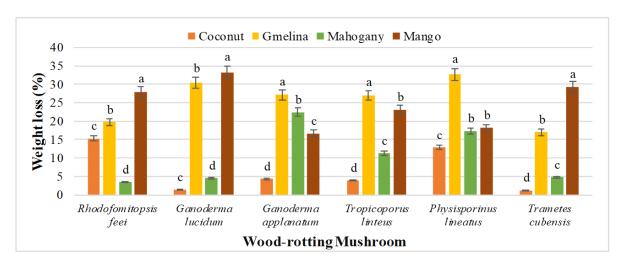


Fig. 6 – Weight loss (%) induced by six wood-rotting mushrooms in wood blocks after 14 days of incubation.

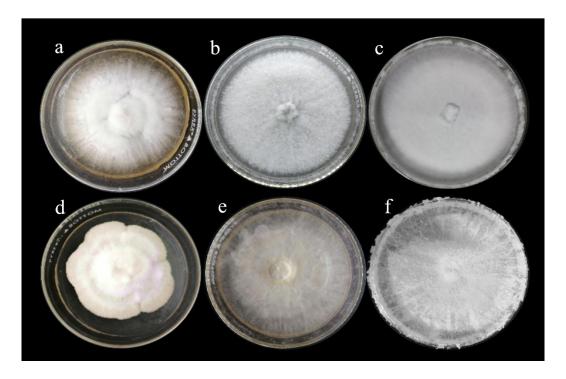


Fig. 7 – Mycelia of six wood-rotting macrofungi on their optimized culture condition after seven days of incubation. a *R. feei*. b *G. applanatum*. c *G. lucidum*. d *T. linteus*. e *P. lineatus*. f *T. cubensis*.

Discussions

The production of active mycelia, the luxuriance and the rapid fungal growth depend mainly on the appropriate culture medium during its proliferation (Reyes et al. 2009). Mushrooms have specific nutritional requirements, which is why they tend to exhibit a host specificity (Rustøen et al. 2023). Coconut water agar (CWA) is the optimal medium which favors the efficient secondary mycelial growth of *R. feei, G. applanatum, G. lucidum, P. linteus, T. lineatus,* and *T. cubensis*. The significant differences in mycelial growth could be a result of the varying nutrient compositions present in the indigenous culture media that were examined (Dulay et al. 2021). Thus, different types of mushroom species have unique nutritional requirements for mycelial growth. Coconut water is a natural and inexpensive medium that is commonly utilized in the cultivation of fungi. Its nutrient content and high water content make it an excellent culture medium for wood-rotting mushrooms. The natural nutrient composition of coconut water (CW) contributes to its suitability for mycelial growth. A 100 g portion of coconut water contains essential nutrients, such as 3.71 g of carbohydrates,

2.61 g of sugars, 1.1 g of dietary fibre, 0.72 g of protein, and 0.2 g of fat. It also offers minerals like 250 mg of potassium, 105 mg of sodium, 25 mg of magnesium, 24 mg of calcium, and 20 mg of phosphorus. Trace elements, such as 0.29 mg of iron, 0.14 mg of manganese, 0.1 mg of zinc, and 0.04 mg of copper, are present. Additionally, coconut water is a source of various vitamins, including ascorbic acid, thiamine, riboflavin, niacin, pantothenic acid, pyridoxine (B6), cobalamin (B12), folate, and choline (Food Data Central, USDA, 2019). Moreover, coconut water is rich in several essential substances, such as amino acids, carbohydrates, enzymes, inorganic ions, minerals, phytohormones, vitamins, as well as trace elements (Yong et al. 2009). Therefore, this observation strengthens the notion that the nutritional composition of culture media is critical in the growth and proliferation of various types of these wood-rotting mushroom species. The study by De Leon et al. (2020) investigated the use of coconut water as a culture medium for the secondary mycelial growth of F. feei. The researchers found that coconut water provided a superior environment for secondary mycelial growth, compared to other commonly used culture media. The high water and nutrient content in coconut water were cited as key factors for its effectiveness as a culture medium. Similar results were obtained by the study of Magday et al. (2014), in which coconut water agar was the most suitable culture medium for the growth of G. lucidum.

Another intrinsic factor measured in the present study is the pH. The pH of coconut water was adjusted to five different levels to ascertain the optimal pH for the mycelial growth of six woodrotting mushroom isolates. These results suggest that the mycelial growth of six wood-rotting mushroom isolates favorably grew at pH conditions ranging from 6.0-9.0 and showed a greater preference for slightly acidic to basic pH levels. The pH of the medium plays a pivotal role in the cultivation process, influencing the ionic state of the medium and consequently affecting the structure, morphology, and physiological functions of fungal cells. Furthermore, it has implications for nutrient uptake and the biosynthesis of products (Deacon 2006). Maintaining an appropriate pH level is essential for fostering lush growth and efficient mycelial production of mushrooms, as highlighted by previous research (Dulay et al. 2015). In general, a certain range of initial pH values of the medium can optimize fungal growth (Kashangura 2008). According to Dulay et al. (2012), the most appropriate pH offers favorable conditions for luxuriant mycelial growth. Hence, mushroom species utilized in this study grew optimally in pH 6.0 to 7.0. Most of the mushrooms produced maximum mycelial growth and very thick mycelial density at pH 6 to 7. These findings are congruent with the observation of De Leon et al. (2020) that R. feei can grow optimally in pH levels that range from pH 5.0 to 8.0. Also, Magday et al. (2014) showed in their study that a pH of 6.0 to 9.0 was tolerable for G. applanatum. Similarly, Jayasinghe et al. (2008) found G. lucidum can grow at pH 5.0 to 9.0.

The study also considered several extrinsic factors that could impact the growth of six woodrotting mushroom species, including the effect of aeration by implementing two treatments: sealed (oxygen-deprived) and unsealed (aerated) conditions. Tisdale (2004) highlights the production of carbon dioxide during fungal growth as it decomposes substrates. Introducing 'outside air' serves to decrease carbon dioxide accumulation, thus elevating oxygen levels. Fungal mycelium exhibits a high tolerance to CO₂, thriving even at approximately 20% CO₂ levels, yet oxygen remains essential for fruiting body formation (Tisdale, 2004). Stamets (2000) emphasizes the crucial role of decreased ambient CO₂ levels and increased oxygen for the initiation and development of primordia. Therefore, adequate air circulation within mushroom fruiting sites is imperative. Since the plates were double sealed with cling wrap, it can be assumed that oxygen cannot freely enter and similarly, carbon dioxide would not be easily dissipated. Therefore, CO₂ accumulated on sealed plates and the mycelial growth of macrofungal isolates can be performed in the absence of oxygen (Kashangura 2008). Leatham and Stahmann (1987) revealed that for mushrooms to produce fruiting bodies, it is crucial to have enough oxygen supply. It was observed that covering cultures with polypropylene membranes did not stop the formation of pigmented buried primordia or exudation, meaning oxygen was not solely responsible for these processes. Based on their experiments, it was suggested that carbon dioxide (CO₂) could be a possible regulator of fruiting. High levels of CO₂ could inhibit the formation of fruiting bodies. When a primordium expands rapidly, it requires a lot of energy in the form of ATP

to create important components like UDP-glucose and UDP-N-acetylglucosamine, which is necessary for cell wall formation. The buildup of CO₂, which is a byproduct of energy metabolism, reduces the ATP regeneration necessary for primordium expansion, based on Leatham & Stahmann (1987).

In general, mushroom species tend to have larger mycelial diameters when incubated in a light condition compared to those incubated in a dark condition. This finding is consistent with previous studies on *Lentinus tigrinus* and *Volvariella volvacea* by Dulay et al. (2012). However, when both strains of *L. tigrinus*, *V. volvacea*, and *C. cinerea* are incubated in a dark condition, they register wider mycelial growth than those cultured in a light condition. Similar findings were also reported in *Paneolus antillarium* and *Paneolus cyanescens* by Bustillos et al. (2014). In addition, Damaso et al. (2018) found that the most favorable illumination condition for mycelia and fruiting body production, as well as for the antioxidant properties of *L. tigrinus*, was blue and red LED. Considering the findings of both the current investigation and previous studies, it is evident that mushrooms necessitate varied types, wavelengths, coherences, and intensities of light throughout different developmental stages—from basidiospore germination to fruiting body formation. These light factors may significantly influence the growth, biomass yield, quality attributes, and biochemical properties of mushrooms. However, the present study showcases the luxuriant growth of six wood-rotting mushroom isolates in light conditions, excluding *T. cubensis*, rendering the illumination as a significant factor for the mycelial growth of these mushroom isolates.

According to Hudson (1986), temperature is one of the cardinal factors that determine the distribution of fungi in different ecological niches. Temperature, as noted by Mswaka and Magan (1999), can influence a fungus largely via its effects on enzyme-catalyzed reactions. The combined effects of numerous different chemical reactions are represented by the overall response of a fungus to varying temperature conditions, whereby each of which exhibits its own characteristic relationship to temperature (Rayner & Boddy 1988). Fungi can be classified as either temperate, semi-temperate or tropical depending on the mycelial growth. Temperature requirements for mycelial cultures have been extensively studied across various mushroom species. Similar preferences have been reported in submerged mycelial cultures of Coprinopsis cinerea, Flammulina velutipes, Ganoderma lucidum, Lentinus sajor-caju, L. tigrinus, Lentinula squarrosulus, Pleurotus ostreatus and Volvariella volvacea (Kim et al. 2002, Dulay et al. 2015a, b, 2016, Kupradit et al. 2020). This trend is also evident in semi-solid mycelial cultures of Lentinus conatus, L. roseus, L. subnudus, and L. tigrinus (Gbolagade et al. 2006, Dulay et al. 2012, Klomklung et al. 2014). In contrast, widely cultivated mushrooms, such as Agaricus blazei, Cordyceps militaris, Cordyceps jiangxiensis, Flammulina velutipes, Hericium erinaceus, and Pleurotus ostreatus, exhibit distinct temperature preferences for submerged cultivation, ranging from 20 °C to 27.89 °C (Park et al. 2001, Lee et al. 2010, Hassan et al. 2012, Xiao et al. 2004, Lin & Yang, 2006, Wahyudi et al. 2015).

There are two main types of fungi that cause wood rot: brown-rot fungi and white-rot fungi: Brown-rot fungi typically break down the carbohydrates of wood, leaving behind a brownish residue (Goodell 2003). Carbohydrates are broken down into simple sugars and are used as a source of energy for fungi, which causes wood to become dry, brittle, and cracked (Byrde & Willetts 2013). Examples of brown-rot fungi include R. feei (Han et al. 2016). White-rot fungi, on the other hand, break down both carbohydrates and lignin, which is a complex polymer that makes up roughly 30% of wood and provides structural support (Wesenberg et al. 2003). The fungi break down lignin into its individual components, which are then used as a source of energy. The breakdown of lignin leads to a whitish residue, hence the name white-rot fungi; Wood decay caused by white-rot fungi is usually soft and spongy (Wesenberg et al. 2003). Examples of white-rot fungi include G. lucidum, G. applanatum, T. linteus, P. lineatus, and T. cubensis (Parihar et al. 2012, Srivilai et al. 2013, Kaur et al. 2016, Sułkowska-Ziaja et al. 2023). Both brown-rot and white-rot fungi have evolved various enzymes that help them to break down wood components (Wesenberg et al. 2003). Some of the enzymes produced by fungi include lignin peroxidases, manganese peroxidases, and cellulases (Chander & Arora 2007). Lignin peroxidases and manganese peroxidases are enzymes that are specialized in breaking down lignin, and these enzymes break down the complex bonds within the lignin molecule, causing it to

fragment into smaller units (Wang et al. 2023). Cellulases are enzymes that break down cellulose, which is a polysaccharide that makes up roughly 40–50% of wood. These cellulases can hydrolyze the β -glucosidic bonds within cellulose, leading to the formation of glucose monomers (Uber et al. 2023). The glucose molecules can then be used as a source of energy by the fungi. Additionally, some fungi produce oxalic acid, which can cheat the calcium ions in wood, leading to a breakdown in the wood fibres (Graz et al. 2023).



Fig. 8 – Fully ramified wood blocks by wood-rotting mushroom mycelia after 14 days of incubation.

Conclusion

This study elucidates the optimized culture conditions and wood-rotting capabilities of six mushroom species isolated from specific sites across Central Luzon. Our findings underscore the pivotal role of intrinsic and extrinsic environmental factors, such as culture medium, pH, aeration, illumination, and temperature, in influencing the mycelial growth of wood-rotting mushrooms. Additionally, our research highlights mango (*Mangifera indica*) and gmelina (*Gmelina arborea*) woodblocks as particularly susceptible to decay caused by these mushrooms. Notably, among the isolates studied, *Physisporinus lineatus* emerges as the most potent, as evidenced by the highest recorded average percentage of weight loss. These insights deepen our understanding of the intricate interplay between environmental conditions and fungal activity, shedding light on potential avenues for further research and practical applications in areas such as bioremediation, bioconversion, sustainable forestry, and ecological restoration.

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References

Alberto AMP. 2011 – Basic Ecology. Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines.

Alberto AMP, Galvez CT. 2004 – Handbook on Trees. Rex Book Store.

Avery SV, Singleton I, Magan N, Goldman GH. 2019 – The fungal threat to global food security. Fungal Biology 123(8), 555–557.

Binder M, Justo A, Riley R, Salamov A et al. 2013 – Phylogenetic and phylogenomic overview of the Polyporales. Mycologia 105(6), 1350–1373.

Bustillos RG, Dulay RMR, Kalaw SP, Reyes RG. 2014 – Optimization of culture conditions for mycelial growth of *Paneolus antillarium* and *Paneolus cyanescens*. Mycosphere 5(3), 1–7.

Byrde RJW, Willetts, HJ. 2013 – The brown rot fungi of fruit: their biology and control. Elsevier.

Chander M, Arora DS. 2007 – Evaluation of some white-rot fungi for their potential to decolourise industrial dyes. Dyes and Pigments 72(2), 192–198.

- Chokkalingam U, Carandang AP, Pulhin JM, Lasco RD et al. 2006 One century of forest rehabilitation in the Philippines: approaches, outcomes and lessons. Center for International Forestry Research Jl. CIFOR, Situ Gede, Sindang Barang Bogor Barat 16680, Indonesia.
- Damaso Jr EJ, Dulay RMR, Kalaw SP, Reyes RG. 2018 Effects of color light-emitting diode (LED) on the mycelial growth, fruiting body production, and antioxidant activity of *Lentinus tigrinus*. CLSU International Journal of Science & Technology 3(2), 9–16.
- De Leon AM, Dulay AR, Villanueva AL, Kalaw SP. 2020 Optimal culture conditions and toxicity assessment of *Fomitopsis feei* (Fr.): a newly documented macrofungus from the Philippines. Studies in Fungi 5(1), 491–507.
- Deacon JW. 2006 Fungal Biology. Blackwell Publishing Ltd., New York, USA, 110–121.
- Devkota S, Fang W, Arunachalam K, Phyo KMM et al. 2023 Systematic review of fungi, their diversity and role in ecosystem services from the Far Eastern Himalayan Landscape (FHL). Heliyon 9, 1–15.
- Dulay RMR, Batangan JN, Kalaw SP, De Leon AM et al. 2023 Records of wild mushrooms in the Philippines: A review. Journal of Applied Biology and Biotechnology 11(2), 11–32.
- Dulay RMR, Cabrera EC, Kalaw SP, Reyes RG. 2021 Optimization of submerged culture conditions for mycelial biomass production of fourteen *Lentinus* isolates from Luzon Island, Philippines. Biocatalyst and Agricultural Biotechnology 38, 102–226.
- Dulay RMR, Cardona EMG, Kalaw SP, Reyes RG. 2016 Optimization of liquid culture conditions of *Coprinopsis cinerea* as natural source of bioactive compounds. Der Pharma Chemica 8(18), 313–319.
- Dulay RMR, Ray K, Hou CT. 2015a Optimization of liquid culture conditions of Philippine wild edible mushrooms as potential source of bioactive lipids. Biocatalysis and Agricultural Biotechnology 4(3), 409–415.
- Dulay, RMR, Flores KS, Tiniola RC, Marquez DHH et al. 2015b Mycelial biomass production and antioxidant activity of *Lentinus tigrinus* and *Lentinus sajor-caju* in indigenous liquid culture. Mycosphere 6(6), 659–666.
- Dulay RMR, Kalaw SP, Reyes RG, Cabrera EC et al. 2012 Optimization of culture conditions for mycelial growth and basidiocarp production of *Lentinus tigrinus* (Bull.) Fr., a new record of domesticated wild edible mushroom in the Philippines. Philippine Agricultural Scientist 95(3), 278–285.
- Dulay RMR, Barcelar EB, Oclarit JM. 2012 Effect of light on mycelial growth of *Lentinus tigrinus* (Bull. ex Fr.) Fr. and *Schizophyllum commune* (Fr.) Quél. Philippine Journal of Science 141, 89–95.
- Dyakov IT, Dzhavakhiya VV, Korpela T. 2007 Comprehensive and molecular phytopathology. Elsevier eBooks, Elsevier BV.
- El-Sayed A, Kamel MS. 2020 Climatic changes and their role in the emergence and re-emergence of diseases. Environmental Science and Pollution Research 27(18), 22336–22352.
- Fones HN, Bebber DP, Chaloner TM, Kay WK et al. 2020 Threats to global food security from emerging fungal and Oomycete crop pathogens. Nature Food 1(6), 332–342.
- Fischer MPA, Henk DA, Briggs CJ, Brownstein JS et al. 2012 Emerging fungal threats to animal, plant, and ecosystem health. Nature 484(7393), 186–194.
- Food Data Central, USDA, 2019 Nuts, Coconut Water (Liquid from Coconuts). Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/170174/nutrients.
- Gbolagade JS, Fasidi IO, Ajayi EJ, Sobowale AA. 2006 Effect of physicochemical factors and semi-synthetic media on the vegetative growth of *Lentinus subnudus* (Berk.), an edible mushroom from Nigeria. Food Chem 99, 742–749.
- Goodell B. 2003 Brown-rot fungal degradation of wood: our evolving view. American Chemical Society 845, 97–118.
- Graz M, Ruminowicz-Stefaniuk M, Jarosz-Wilkołazka A. 2023 Oxalic acid degradation in wood rotting fungi: searching for a new source of oxalate oxidase. World Journal of Microbial Biotechnology 39(1), 13.

- Han ML, Chen YY, Shen LL, Song J et al. 2016 Taxonomy and phylogeny of the brown-rot fungi: *Fomitopsis* and its related genera. Fungal Diversity 80, 343–373.
- Hassan FRH, Medany GM, El-Kady ATM. 2012 Mycelial biomass production of enoke mushroom (*Flammulina velutipes*) by submerged culture. Australian Journal of Basic Applied Science 6(7), 603–610.
- Hudson HJ. 1986 Fungal Biology. Edward Arnold Publishers, London, 24–33.
- Jayasinghe C, Imtiaj A, Hur H, Lee GW et al. 2008 Favorable culture conditions for mycelial growth of Korean wild strains in *Ganoderma lucidum*. Mycobiology 36(1), 28–33.
- Kalaw SP, Albinto RF. 2015 Growth performance and nutritional attributes of *Pleurotus* species grown on rice straw-based formulations. Advances in Environmental Biology 9, 72–81.
- Kashangura C. 2008 Optimisation of the growth conditions and genetic characterisation of Pleurotus species. A Doctor of Philosophy Dissertation. Department of Biological Sciences. University of Zimbabwe.
- Kaur H, Kapoor S, Kaur G. 2016 Application of lignolytic potentials of a white-rot fungus *Ganoderma lucidum* for degradation of lindane. Environmental Monitoring Assessment 188(10), 1–10.
- Kim JM, Ra KS, Noh DO, Suh HJ. 2002 Optimization of submerged culture conditions for the production of angiotensin converting enzyme inhibitor from *Flammulina velutipes*. Journal of Indian Microbial Biotechnology 29(5), 292–295.
- Klomklung N, Karunarathna SC, Hyde KD, Chukeatirote E. 2014 Optimal conditions of mycelial growth of three wild edible mushrooms from Northern Thailand. Acta Biologica Szegediensis 58(1), 39–43.
- Kupradit C, Ranok A, Mangkalanan S, Khongla C et al. 2020 Effects of natural carbon sources and temperature on mycelium cultivations of *Lentinus squarrosulus* (Mont.), *Lentinus polychrous* Lev., *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. and *Volvariella volvacea* (Bull.) Singer. Thai Journal of Science and Technology 9, 539–553.
- Leatham GF, Stahmann MA. 1987 Effect of light and aeration on fruiting of *Lentinula edodes*. Transactions of the British Mycological Society 88(1), 9–20.
- Lee JS, Wee JW, Lee HY, An HS, Hong EK. 2010 Effects of ascorbic acid and uracil on exopolysaccharide production with *Hericium erinaceus* in liquid culture. Biotechnology and Bioprocess Engineering 15(11), 453.
- Leite S, 2008. La bio-indication mycologique de la forêt domaniale sainte-croix-volvestre, Mémoire de master 2. Université Paul Sabatier Toulouse.
- Lin JH, Yang, SS. 2006 Mycelium and polysaccharide production of *Agaricus blazei* Murrill by submerged fermentation. Journal of Microbiology, Immunology, and Infections 39(2), 98–108.
- Magday Jr JC, Bungihan ME, Dulay RMR. 2014 Optimization of mycelial growth and cultivation of fruiting body of Philippine wild strain of *Ganoderma lucidum*. Current Research in Environmental and Applied Mycology 4(2), 162–172.
- Mswaka AY, Magan N, 1999 Temperature and water potential relations of tropical *Trametes* and other wood-decay fungi from the indigenous forest of Zimbabwe. Mycological Research 103(1), 1309–1317.
- Nankone SR, Sawadogo B, Sanon E, Dabiré K et al. 2022 Diversity of phytopathogenic polypores and their interaction with host plants in three biotopes in western Burkina Faso. Mycopath 20(2), 75–84.
- Pampolina NM, Tadiosa ER, Ata JP, Soriano JKR et al. 2023 Species and functional diversity of forest fungi for conservation and sustainable landscape in the Philippines. Elsevier EBooks, 89–135.
- Parihar J, Tiwari CK, Ayachi A, Verma RK. 2012 Biodegradation of cellulose by wood decaying fungi. Journal of Applied Science in Environmental Sanitation 7(3), 209–214.
- Park JP, Kim SW, Hwang HJ, Yun JW. 2001 Optimization of submerged culture conditions for the mycelial growth and exo-biopolymer production by *Cordyceps militaris*. Letters in Applied Microbiology 33(1), 76–81.

- Rayner ADM, Boddy L. 1988 Fungal Decomposition of Wood. Forest Science 35(2), 647–648.
- Reyes RG, Imbernon M, Pardo-Gimenez A. 2009 Comparison of mycelial growth and fruiting body initiation in various strains of *Coprinus comatus*. Mycoscience 50, 135–140.
- Reyes RG, Kalaw SP, Dulay RMR, Yoshimoto H et al. 2013 Philippine native and exotic species of edible mushroom grown on rice straw-based formulation exhibit nutraceutical properties. Philippine Agricultural Science 96, 198–204.
- Rigling D, Robin C, Prospero S. 2021 Mycovirus-Mediated Biological Control. Elsevier eBooks, 468–477.
- Rustøen F, Høiland K, Heegaard E, Boddy L et al. 2023 The substrate affinities of wood decay fungi are foremost structured by wood properties not climate. Fungal Ecology 63, 101–231.
- Salvatore MC, Andolfi A. 2021 Phytopathogenic Fungi and Toxicity. Toxins 13(10), 689.
- Srivilai P, Chaiseana W, Loutchanwoot P, Dornbundit P. 2013 Comparison of differences between the wood degradation by monokaryons (n) and dikaryons (2n) of white rot fungus (Cambodian *Phellinus linteus*). Journal of Biological Science 13(3), 131–138.
- Stamets P. 2000 Growing gourmet and medicinal mushrooms. Ten Speed Press, Berkeley, CA. 139–147
- Sułkowska-Ziaja K, Galanty A, Szewczyk A, Paśko P et al. 2023 Effect of Methyl Jasmonate Elicitation on Triterpene Production and Evaluation of Cytotoxic Activity of Mycelial Culture Extracts of *Ganoderma applanatum* (Pers.) Pat. Plants 12(2), 294.
- Tisdale T. 2004 Cultivation of the Oyster Mushroom (*Pleurotus* sp.) on Wood substrates in Hawaii. World Journal of Microbiology and Biotechnology 22(3), 201–206.
- Uber TM, Backes E, Saute VMS, da Silva BP et al. 2023 Chapter 6 Enzymes from basidiomycetes—Peculiar and efficient tools for biotechnology. In: Brahmachari G, Biotechnology of Microbial Enzymes (2nd Edition), Academic Press, p. 129–164.
- Wahyudi P, Mangunwardoyo W, Sumaryono W, Gandjar I. 2015 Optimization of submerged culture for biomass and polysaccaharide of *Pleurotus ostreatus* BPPTCC 6017 using response surface methodology. Malaysian Journal of Microbiology 11(1), 27–39.
- Wang L, Li Y, Du X, Wu J et al. 2023 Performance enhancement of white rot fungi extracellular enzymes via new hydrogel microenvironments for remediation of benzo [a] pyrene contaminated soil. Journal of Hazardous Materials 454, 131–505.
- Wesenberg D, Kyriakides I, Agathos SN. 2003 White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnology Advances 22(1–2), 161–187.
- Xiao JH, Chen DX, Liu JW, Liu ZL et al. 2004 Optimization of submerged culture requirements for the production of mycelial growth and exopolysaccharide by *Cordyceps jiangxiensis* JXPJ 0109. Journal of Applied Microbiology 96(5), 1105–1116.
- Yong JWH, Ge L, Ng YF, Tan SN. 2009 The Chemical Composition and Biological Properties of Coconut (*Cocos nucifera* L.). Molecules 14(12), 5144–5164.