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Biophysiological profiling of *Ganoderma resinaceum* Boud., a newly recorded Philippine mushroom

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Abstract

Ganoderma resinaceum, a member of the family Ganodermataceae, is a saprophytic fungus that grows parasitically on living trees. It is considered an ethnomedicine as it contains different important bioactive compounds and exhibits various bioactivities. This study focused on establishing the optimal culture conditions for the mycelial growth of *G. resinaceum*. Luxuriant secondary mycelial growth of *G. resinaceum* was obtained using MEA and PDA as culture media. Meanwhile, a slightly alkaline medium with a pH of 8 favors the optimal growth of *G. resinaceum*, while no significant differences were observed for both aeration and illumination; *G. resinaceum* exhibited its best growth under both conditions. Moreover, a slightly lower temperature of 23 °C promotes the optimal growth of the secondary mycelia of this mushroom. On the other hand, by utilizing the 7:3 v/v rice straw-sawdust substrate formulation, *G. resinaceum* successfully produced fruiting bodies, yielding 11.40 g per bag equivalent to 2.40% biological efficiency. Overall, this study determined the optimum cultivation conditions and fructification of *G. resinaceum*, indicating the successful domestication of this wild mushroom. In addition, the successful production of fruiting bodies in *G. resinaceum* has paved the way for mass industrial cultivation. Subsequent studies that focus on optimizing substrate formulations to enhance the yields and efficiency of fruiting body production for *G. resinaceum* are recommended.

Keywords – fruiting body – indigenous medium – optimization – secondary mycelia

Introduction

Mushrooms from class Agaricomycetes have been widely studied due to their possession of biologically active compounds with various therapeutic potentials, including antimicrobial, antioxidative, anti-inflammatory, hypocholesterolemic, hypoglycemic, anti-hypertensive, fibrinolytic, and thrombolytic properties, with broad applications in the medical and pharmaceutical industries (Badalyan et al. 2021). The genus *Ganoderma* has undergone cultivation for dietary purposes and is employed in traditional medicinal practices across Asian nations, owing to its demonstrated therapeutic efficacy against a spectrum of life-threatening diseases (Bhardwaj &

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Misra 2018, Thakur & Singh 2013, Wasser 2005). In addition, *Ganoderma* is recognized as a wood-degrading macrofungus characterized by hard fruiting bodies, and the genus comprises more than 300 species distributed in tropical regions (Baby et al. 2015). In terms of biological properties, *Ganoderma* is renowned for its antibacterial, anticancer, antiviral, antioxidant, immune-modulating, and sedative properties, thus, many studies are being performed to harness its potential (Yu et al. 2020).

The spores, basidiocarp, and mycelia of *Ganoderma lucidum* contain approximately 400 different bioactive compounds; these are primarily triterpenoids, polysaccharides, sterols, steroids, fatty acids, proteins/peptides, and trace elements, all of which have been known to have a variety of pharmacological applications, including immunomodulation, anti-inflammatory, analgesic, chemo-preventive, antitumor, antibacterial, antiviral, hepatoprotective, anti-diabetic, anti-oxidative and radical-scavenging, anti-aging, and anti-ulcer properties (Sanodiya et al. 2009).

Furthermore, in terms of cultivation, solid-state cultivation is widely utilized in many mushroom cultivation processes due to its low contamination risks resulting from low water content, low-cost media, and its ability to yield higher and reproducible products (Letti et al. 2018). In addition, solid-state cultivation is utilized in various agricultural processes to reduce the environmental impact of residue while still producing a product that can be used for environmental purposes (Mitchell et al. 2010). Fungi are among the microorganisms cultivated in this technique to produce value-added products, as they can naturally grow on a solid substrate (Sadh et al. 2018). Many cost-efficient substrates, including cotton waste, corncobs, rice straw, urea, and even manure, have been employed in mushroom cultivation (Yang & Xue 2000).

Nevertheless, mushrooms must first be domesticated from the wild and establish the optimum culture condition prior to bioactivity study. In the Philippines, several *Ganoderma* species, including *G. applanatum*, *G. australe*, *G. lucidum*, *G. gibbosum*, and *Ganoderma weberianum*, have been successfully cultivated and their culture conditions optimized (Magday et al. 2014, del Rosario et al. 2022). In this study, *Ganoderma resinaceum*, previously known as *Ganoderma sessile* according to Species Fungorum, was domesticated from Baywatch, CLSU, PHL (15.7400358 N, 120.9364438 E), and its mycelial culture condition and fruiting body production were optimized using locally-produced commodities and agricultural waste (Figure 1). *Ganoderma resinaceum* is a rare poroid fungus that exhibits persistent presence throughout the annual cycle. During its juvenile stage, the fungus is covered with reddish-brown spore dust, occasionally forming tiers of brackets that may merge. The cap of the fruiting body shows a light yellowish margin and an orange-tinged rufous-brown apex, engaging in a parasitic symbiosis with living broadleaf tree trunks (Mattheck 2003, O'Reilly 2016, Kirk 2008).

Optimizing the culture conditions for large-scale production will enable to cultivate this mushroom ex situ and will reveal the untapped potential of this mushroom as a valuable natural resource with significant applications in medicine, pharmaceuticals, and agriculture. Additionally, it opens up many research opportunities that could lead to the development of immense medical resources, including anti-inflammatory medicines, antibacterial pharmaceuticals, dietary supplements, and the potential contribution of mushrooms to cancer research.

Materials & Methods

Fungal Source

The pure culture of *Ganoderma resinaceum* (BW5) was obtained from the mycological repository at the Center for Tropical Mushroom Research and Development (CTMRD), CLSU, PHL. Prior to the optimization study, young isolates were secured from the stock culture of *G. resinaceum* by aseptically sub-culturing it onto new sterile potato dextrose agar (PDA). Incubation occurred for 7 days at 30 °C. Subsequently, 10-mm mycelial discs were obtained from the 7-day-old culture using a 10-mm cork borer.

Fig. 1 – Wild fruiting body of *Ganoderma resinaceum* (property of CTMRD, CLSU and DOST, PCHRD)

Optimization of culture medium and pH condition

The optimization of intrinsic factors, culture medium, and pH for the mycelial growth of *G. resinaceum* was performed. Following the study by Kalaw et al. (2016), four indigenous media, namely matured coconut water agar (CWA), potato sucrose agar (PSA), corn grit decoction agar (CGDA), and rice bran decoction agar (RBDA), were prepared and sterilized. In addition, three commercial media, specifically malt extract agar (MHA), potato dextrose agar (PDA), and saboraud dextrose agar (SDA), were prepared and sterilized, according to the manufacturer's instructions. The optimum culture medium was determined by evaluating seven different culture media (both indigenous and commercial). After identifying the best culture medium, the optimal pH condition for the growth of *G. resinaceum* was determined through four different pH levels (pH 5, pH 6, pH 7, and pH 8). The incubation was conducted at room temperature (30ºC), with daily measurements and recordings of the mycelial growth diameter.

Optimization of aeration, illumination, and temperature condition

Upon determining the intrinsic factors influencing the mycelial growth of *G. resinaceum*, the effects of extrinsic factors, including aeration, illumination, and temperature, were determined based on the study of Fabros et al. (2023). Simultaneously, two different sets of aeration conditions (sealed and unsealed) and illumination conditions (lighted and dark) were evaluated using the ideal culture medium and pH. Subsequently, three temperature conditions (9ºC, 23ºC, and 32ºC) were evaluated systematically. Consistent with the procedure applied to intrinsic factors, incubation occurred at room temperature (except for the assessment of temperature conditions), and measurements and recordings of both mycelial growth and density were conducted.

Spawn preparation

The production of *G. resinaceum* spawn involved using unmilled rice for the cultivation of fruiting bodies. The spawn preparation followed the method outlined by Fabros et al. (2023) for preparing, sterilizing, inoculating, and incubating the grains. Subsequently, the fully ramified mycelia served as an inoculant for the fructification of *G. resinaceum*.

Fruiting body production

After determining the optimal cultural conditions for both intrinsic (medium and pH) and extrinsic factors (aeration, illumination, temperature) for the mycelial growth of *G. resinaceum*, an assessment of the fruitification performance of the mushroom was conducted. Following the procedures described by Kalaw et al. (2021) and utilizing a substrate formulation with a ratio of 7 parts rice straw to 3 parts sawdust (v/v), the evaluation proceeded to determine the fructification performance and assess the biological efficiency of *G. resinaceum*. The experimental procedures encompassed the formulation of the fruiting bag, sterilization, inoculation processes, controlled incubation, and subsequent quantification of biological efficiency (BE).

Statistical Analysis

At 5% significance level, the data were analyzed using ANOVA and compared using Tukey's HSD and t-test.

Results and Discussion

Continuous research and studies on the potential of mushrooms enable the development of immense medical resources, such as anti-inflammatory medicines, antibacterial pharmaceuticals, dietary supplements, and the possible contribution of mushrooms to cancer research. The diverse bioactivities of various mushrooms have already been studied and successfully explored for their utilization in different industries (Zhong & Tang 2004).

In this study, the optimal culture conditions for the secondary mycelial growth and fructification of *Ganoderma resinaceum* were established. *Ganoderma resinaceum*, an agaric microfungus belonging to Ganodermataceae, is a saprophytic fungus that mainly grows parasitically on living trees and is characterized by a smooth, irregular rugose and concave surface pileus with a rounded margin that is light yellowish-brown buff and a liver-brown colored stipe with rounded pores (Mattheck 2003, O'Reilly 2016, Kirk 2008). Additionally, *G. resinaceum* has white-colored mycelia at an early age, which turns cream-colored with a few light-yellow zones (Mohanty et al. 2011). *Ganoderma resinaceum* is considered an ethnomedicine with various medicinal applications (Chen et al. 2017b). A phytochemical investigation of the fruiting body of *G. resinaceum* resulted in the isolation of five new meroterpenoids, fifteen nortriterpenoids, and eight triterpenoids (Chen et al. 2017a, Chen et al. 2017b, Huang et al. 2020). In addition, ethyl acetate extracts of *G. resinaceum* exhibit antimicrobial activity against 30 strains of MRSA and MSSA, with a maximum inhibitory zone of 14.29 ± 0.14mm. The bioactive compounds, coupled with the bioactivity of *G. resinaceum*, can find applications in the pharmaceutical and medical industries. Recognizing *G. resinaceum* as an underutilized natural resource with enormous potential in medicine and pharmaceuticals opens up numerous research opportunities. Thus, we optimized the mycelial culture condition and fruiting body production of *G. resinaceum*, focusing on aspects such as culture media, pH, oxygen requirements, light conditions, and temperature conditions. This optimization aims to facilitate the mass production of this mushroom for industrial utilization (Table 1).

Effect of culture medium and pH

In nature, lignocellulosic culture substrates, such as dead branches and trunks of trees, fallen leaves, woods, branches, soils, and other agro-industrial waste, serve as nutrient sources for macrofungi. Moreover, for in vitro mycelial growth, carbon and nitrogen sources are crucial compounds in a culture medium, serving as essential nutrients (Itoo & Reshi 2014). Both macro- and micronutrients are essential for establishing the optimum mycelial growth of various macrofungi (Mortada et al. 2020, Gençcelep et al. 2009). In this study, we determined the suitability of four locally modified media (CWA, PSA, CGDA, RBDA) and three commercial media (PDA, MEA, SDA). Figure 2 shows the mycelial growth of *G. resinaceum* in different conditions, including various culture media.

Based on the data obtained in this study, MEA and PDA demonstrated robust support for the luxuriant mycelial growth of *G. resinaceum*, yielding average growth rates of 12.32 mm and 11.09 mm, respectively. Similar findings were reported by Nasreen et al. (2005) and del Rosario et al. (2022), where malt extract media exhibited high mycelial growth among four tested media. In their studies, malt extract media showed a mycelial growth of 0.26 g/100 mL dry weight after 3 days of incubation. The PDA also proved favorable for the growth of *G. applanatum* (13.13 mm) and *G. australe* (14.44 mm) in solid-state cultivation. In addition, different isolates of *G. lucidum* exhibited maximum average mycelial growth in MEA, with isolate GA reaching full growth (9.0 cm) after eight days of incubation (Rawat 2018). Meanwhile, other *Ganoderma* species, such as *G. gibbosum*, *G. lucidum*, and *G. weberianum*, showed positive mycelial growth in SDA, and two locally modified media, such as coconut water gelatin (CWG), and rice bran decoction gelatin

(RBDG) (del Rosario et al. 2022). Moreover, acceptable mycelial growth rates were observed in locally modified media like CWA, PSA, and RBDA, with an average growth rate ranging from 7.084 –7.96 mm in the present study.

Furthermore, Nussbaum et al. (2023) demonstrated the mycelial growth of both *G. lucidum and G., resinaceum* corresponding to the malt extract concentration, based on mycelial network density. The results showed that as malt concentration increases, the mycelium density also increases, contributing to the mechanical strength of the mycelium (Nussbaum et al. 2023).

pH is another intrinsic factor that affects the physiological state of mushrooms affecting their growth. The pH level, measuring the acidity or alkalinity of a substrate, plays a crucial role in modulating enzymatic activities, nutrient availability, and microbial interactions during mycelial growth. In the present study, the optimal mycelial growth of *G. resinaceum* was achieved in MEA adjusted to pH 8, resulting in an average mycelial density of 11.62 mm. This slightly alkaline condition, favoring the growth of *G. resinaceum*, aligns with the observations in *G. gibbosum* and *G. lucidum* (del Rosario et al. 2022).

According to Lee et al. (2008), the optimal pH range for the best growth of *Ganoderma* species is between 5.5 to 6.0. Similar findings were observed by Magday et al. (2014), where *G. lucidum* luxuriantly grew at pH 6.0 to 6.5. However, in the study of Subedi et al. (2021), a slightly acidic substrate between 4.5 to 5.5 favored the best growth of the *G. lucidum* strain from Philippines. Moreover, in the submerge cultivation study of Kapoor & Sharma (2014), it was found that *G. lucidum* effectively grew in a wide pH range between 3.0 to 11.0, with pH 5.0 recorded as the optimal pH for mycelium growth.

In conclusion, both the culture medium and pH are crucial factors influencing the growth of mushrooms. Variations in the ideal conditions for different mushroom species and strains indicate a dependency on specific factors. Further research to pinpoint the exact nutritional requirements and hydrogen ion concentrations for optimal growth is worth considering. Overall, the findings from this study provide additional insights for future studies on optimizing the cultivation of the beneficial macrofungus, *G. resinaceum*.

Effects of aeration, illumination, and temperature

In addition to the intrinsic factors, extrinsic factors such as oxygen requirement, light conditions, and temperature also affect mushroom growth. Oxygen availability plays a crucial role in various physiological and morphological aspects of fungal development, affecting the physiology and productivity of the mushrooms (Kalaw et al. 2021). According to Reid & Seifert (1982), an increased oxygen partial pressure stimulates carbohydrate consumption by most fungi. They also noted that fungi grow optimally in submerged cultivation using oxygen as an air source. In terms of fungal morphology, controlled dissolved oxygen (DO) conditions result in a dispersed mycelium with highly branched hyphae, while uncontrolled DO leads to clumps of mycelia (Fazenda et al. 2009).

In this study, no significant differences were observed at different aeration conditions, suggesting that the presence or absence of oxygen has no substantial effect on the growth of *G. resinaceum*. The average mycelial growth rate was 11. 44 mm for sealed conditions (absence of oxygen) and 11.38 mm for unsealed (presence of oxygen) conditions. Similar findings were reported in three *Lentinus* species (*Lentinus sajor-caju*, *L. squarrosulus*, and *L. swartzii*), and five strains of *Pleurotus djamor* where no significant differences were observed in aerated conditions (Kalaw et al. 2021, Kalaw 2022). However, two strains of *G. lucidum* and *Trametes versicolor* favored sealed conditions for luxuriant mycelial growth (del Rosario et al. 2022; Fabros et al. 2023). Moreover, Tang & Zhong (2002) studied the effects of oxygen supply on the submerged fermentation of *G. lucidum* and revealed that an increased initial volumetric oxygen transfer coefficient led to larger mycelial aggregates, higher production, and productivity of ganoderic acid. Additionally, increased aeration and dissolved oxygen in the culture medium were observed to enhance laccase production in *Ganoderma multistipitatum* (Umar et al. 2023). Thus, oxygen

requirements vary among mushroom species and strains, as well as the type of cultivation, based on the data obtained in the present study and supporting literature.

Another extrinsic factor affecting mycelial growth, illumination was established in this study. Idnurm & Heitam (2005) emphasized that light plays a crucial role in regulating asexual and sexual reproduction, pigment formation, and the direction of growth, which are important for the dissemination and survival of fungal species, In addition, light influences changes in gene expression and morphology in fungi, a phenomenon known as photomorphogenesis. According to Corrochano (2011), fungal morphogenesis is particularly associated with blue light, which activates metabolic pathways and directs the growth of fungal structures. Accordingly, determining the effect of light on the mycelial growth of *G. resinaceum* is vital for establishing optimal illumination conditions for its growth.

Based on the gathered data, no significant differences in the illumination condition were observed in the mycelial growth of *G. resinaceum.* Accordingly, thick mycelial density was recorded, with an average growth rate of 12.09 mm and 12.07 mm for light and dark conditions, respectively. Similar findings were observed in *G. lucidum* strain B, *L. tigrinus* CLSU strain, and *P. cystidiosus* after three days of incubation (Kalaw et al. 2016). Moreover, some mushrooms prefer total light conditions, as seen in *G. lucidum,* while others favor total dark conditions, as observed in *G. applanatum* (del Rosario et al. 2022, Magday et al. 2014, Alcazar et al. 2021).

Furthermore, the study by Alcazar et al. (2021), investigated the effects of light-emitting diodes (LED) on the mycelial biomass production of *G. lucidum*. The data revealed that red LED shows higher yields of 7.20 g (fresh weight) and 0.50 g (dry weight). On the other hand, Amano et al. (2007) reported a notable increase in the mycelial growth rate of *G. lucidum* when transitioning from light to dark conditions.

The last intrinsic factor optimized in this study is temperature. According to Bakar et al. (2020), temperature is a crucial factor for fungal growth, directly influencing the protein dynamics responsible for crop performance. In addition, temperature conditions can serve as markers to differentiate whether a mushroom species is tropical or temperate. In the present studies, we investigated the mycelial growth of *G. resinaceum* under three sets of temperature conditions; refrigerated (9 °C), air conditioned (23 °C), and room temperature (32 °C). Based on the data obtained, air-conditioned favored the luxuriant mycelial growth of *G. resinaceum*, with an average growth rate of 11.12 mm and thick mycelial density. Similarly, slightly low-temperature conditions of 22 °C were found suitable for the optimal growth of *P. ostreatus* and *G. lucidum* (Fletcher et al. 2019). In contrast, in the study of Kalaw et al. (2016), two strains of *G. lucidum* favored high-temperature conditions of 32 °C. Meanwhile, a wide range of temperature requirements was observed in *G. applanatum* and *Ganoderma boninense*, which thrived between 25–32 °C (Jo et al. 2009, Peng et al. 2019).

Overall, the present study successfully identified the optimum culture conditions for the growth of *G. resinaceum*. The data indicate that MEA and PDA adjusted at pH 8 represent the optimal intrinsic conditions for the growth of this mushroom. Additionally, no significant differences were observed for both aeration and illumination, while a slightly lower temperature of 23 °C emerged as the ideal extrinsic condition for the growth of *G. resinaceum.*

Fruiting body production using 7:3 v/v rice straw-sawdust substrate

After determining the optimal conditions for the secondary mycelial growth of *G. resinaceum*, the mushroom underwent fructification using the substrate formulation developed by the Center for Tropical Mushroom Research and Development (CTMRD), incorporating rice straw and sawdust (see Fig 2). Fructification parameters for *G. resinaceum* are presented in Table 2. Under optimal environmental conditions, we observed the incubation days for mycelial colonization of fruiting bags. Key parameters, including the duration of incubation, days of primordia initiation, cap diameter, stipe length, and yield per bag, were subsequently assessed. Figure 3 showcases the fruiting body of *G. resinaceum* cultivated using the rice straw-sawdust substrate formulation.

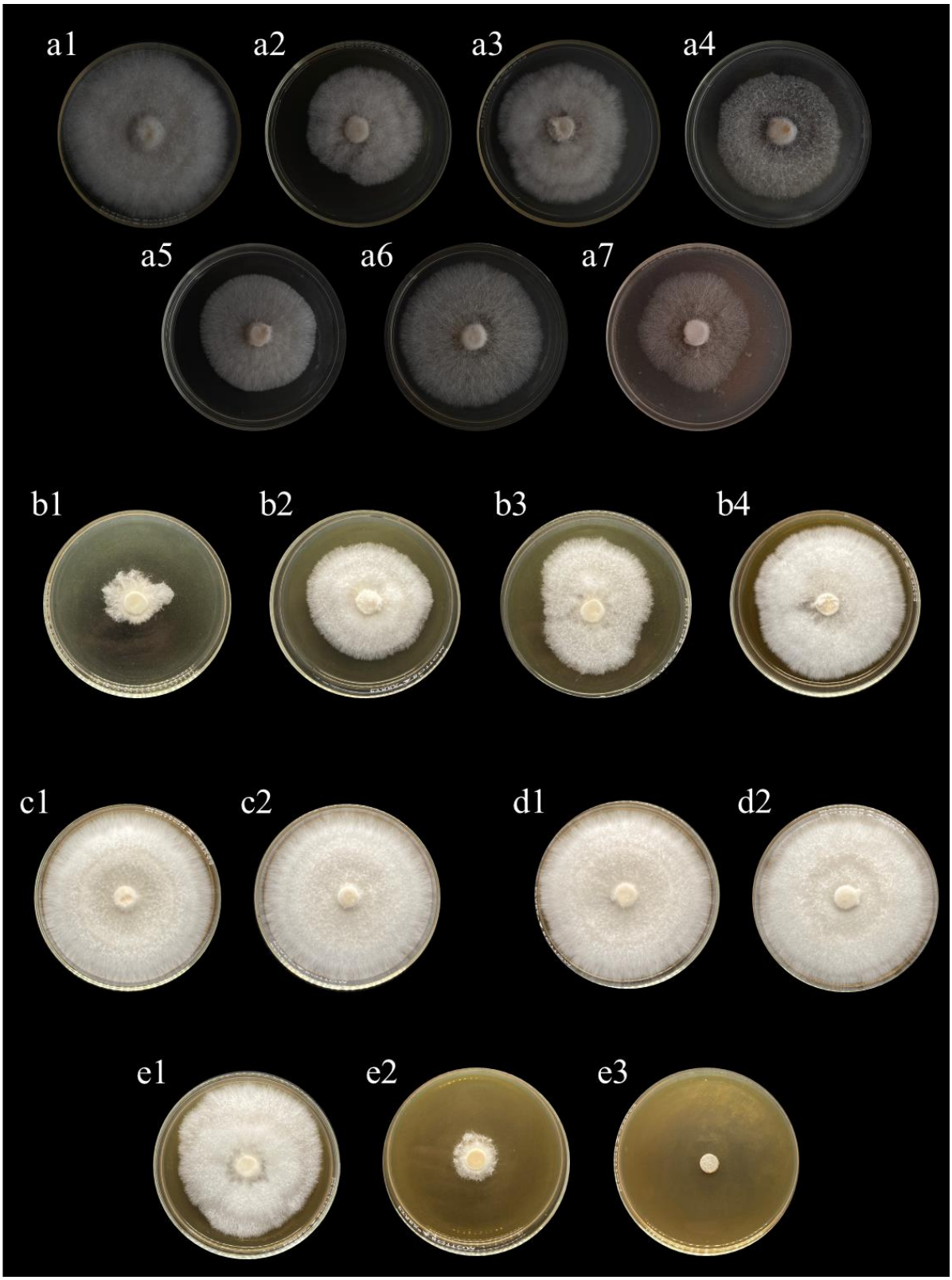

Fig. 2 – Secondary mycelial growth of *G. resinaceum* on different culture conditions. a Culture media (a1, MEA; a2, SDA; a3, PDA; a4, RBDA; a5, CWA; a6, PSA; a7, CGDA). b pH (b1, pH5; b2, pH6; b3, pH7; b4, pH8). (c) Aeration condition (c1, sealed; c2, unsealed). (d) illumination condition (d1, lighted; d2, dark). (e) temperature condition (e1, air condition; e2, room temperature; e3, refrigerated).

Table 1. Effects of intrinsic and extrinsic factors on the mycelial growth of *Ganoderma resinaceum*

Factors	Mycelial Growth Rate (mm day-1)	Mycelial Density
Culture Media		
CWA	7.084 ± 1.08^{b}	++++
PSA	7.75 ± 0.10^{b}	++++
CGDA	4.63 ± 0.63^{c}	+
RBDA	7.96 ± 0.93^{b}	++++
PDA	11.09 ± 0.61^{ab}	++++
MEA	12.32 ± 0.64^{a}	++++
SDA	9.01 ± 0.68^{b}	++++
pH		
5.0	4.76 ± 0.78^{c}	++++
6.0	6.90 ± 1.97^{bc}	++++
7.0	9.12 ± 1.33^{ab}	++++
8.0	11.62 ± 0.38^{a}	++++
Aeration		
Sealed	11.44 ± 0.47^{a}	++++
Unsealed	11.38 ± 0.10^{a}	++++
Illumination		
Lighted	12.09 ± 0.01^{a}	++++
Dark	12.07 ± 0.10^{a}	++++
Temperature		
Refrigerated (9 °C)	1.43 ± 0.00^{c}	No growth
Air-conditioned (23 °C)	11.12 ± 0.51^{a}	++++
Room Temperature (32 °C)	3.43 ± 0.37^{b}	++++

At the 5% level of significance, means with similar superscripts are statistically comparable to one another using Tukey's HSD and t-test.
Mycelial density, (+) very thin, (++) thin, (+++) thick, (++++) very thick

After 83 days of incubation in fruiting bags, the initiation of primordia was documented, with an average cap diameter of 20.13 mm and stipe length of 24.56 mm. A total yield of 11.40 g/bag, averaging 2.40% biological efficiency (BE), was also recorded. In comparison with the study of del Rosario et al. (2022), which examined six species/strains of *Ganoderma*, the *G. resinaceum* in our present study recorded the highest BE. Moreover, using the same substrate formulation, the *G. lucidum* yielded a total of 17.76 g/bag, accounting for 6.260% BE, which is higher than the present study (Magday et al. 2014). Therefore, it is safe to mention that the substrate formulation is species-dependent, as different species require different nutritional requirements. De Leon et al. (2017) stated that the organic matter of the substrate's nutritional value affects mushroom growth. As a result, determining the medium that supports the luxuriant growth of the mushroom species requires assessing the nutritional content.

Overall, the study successfully identified optimal culture conditions for the mycelial growth and fructification of *G. resinaceum*. It was observed that intrinsic factors, such as culture medium and pH, along with extrinsic factors, including aeration, illumination, and temperature, significantly influenced the mycelial growth of this mushroom. The substrate formulation, particularly one comprising rice straw and sawdust, proved effective in supporting the fructification of *G. resinaceum*. However, it is crucial to highlight the need for further investigations into the impact of substrate formulation and supplementation to enhance yield and biological efficiency. These additional studies are essential to collectively achieve mass production of this valuable wild mushroom. Nevertheless, the successful fruiting body production of *G. resinaceum* enables mass production for industrial utilization, such as in pharmaceutical and medical applications.

Table 2. Morphological characteristics of *Ganoderma resinaceum* on rice-straw-sawdust-based substrate

Incubation Period (day)	Primordia initiation (day)	Cap diameter (mm)	Stipe length (mm)	Yield (g/bag)	Biological efficiency (%)
95.02	83.4	20.13 ± 2.45	24.56 ± 4.23	11.40	2.40 ± 1.48

Fig. 2 – Fruiting body of domesticated *G. resinaceum* using rice straw-sawdust substrate formulation

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