

Optimization of Mycelial Culture Conditions for Six Isolates of Paddy Straw Mushroom from the Philippines

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Abstract

*This study evaluated the mycelial growth requirements of six *Volvariella volvacea* isolates. Three commercial isolates, San Jose, Nueva Ecija (SJ), Tarlac City (TC), and La Union (LU), and three wild isolates, Brgy. Rang-ayan, Muñoz, Nueva Ecija (VVA), CLSU (VVB), and San Mateo, Rizal (SP), were collected from Luzon Island, Philippines. Two isolates (SJ and VVA) showed the greatest mycelial growth and the highest mycelial density on PDA. The other four isolates (TC, LU, VVB, and SP) showed significant growth on CGDG with the shortest incubation period. The optimum pH for most isolates tested on PDA and CGDG was between pH 5.0 and 8.0. For the SJ isolate, the thickest mycelial growth was observed at pH 5, while both LU and SP isolates showed significant mycelial growth at pH 7. Efficient mycelial growth was recorded at pH 8 for TC, VVA, and VVB. All isolates showed maximum mycelial growth when incubated at room temperature (30-32 °C). The TC isolate showed maximum mycelial growth in the dark, while five isolates showed maximum growth under light conditions. For aeration, the SJ isolate showed maximum mycelial growth under unsealed conditions, while the remaining five isolates showed maximum growth under sealed conditions. Nonetheless, the efficient growth of all isolates at room temperature indicates that *V. volvacea* grows well in tropical countries at room temperature of 30-32 °C. Optimizing cultivation conditions for wild isolates may improve mycelial growth and potentially yield performance comparable to the commercial SJ isolate.*

Keywords: culture media, mycelial growth, pH, physical factors, *V. volvacea* Philippine isolates

1. Introduction

Volvariella volvacea (Bull.: Fr.) Singer, 1951, also known as the paddy straw mushroom, is an edible basidiomycetous mushroom in the family *Pluteaceae*. Locally, it is known as *kabuteng saging* or *kabuteng dayami* when found growing on decaying banana leaves or rice straw, respectively (Abon et al., 2020). The young fruiting body of *V. volvacea* has a grayish-to-black, egg-shaped volva that ruptures, expanding the pileus to nearly flat. The expanded pileus is attached centrally to a white-to-brown stipe. Local communities typically collect the fruiting bodies at the onset of the rainy season and use them as an ingredient in their dishes.

V. volvacea is regarded as a culinary ingredient due to its texture, umami taste, and strong aroma. Nutritionally, its fruiting bodies contain carbohydrates, sugars, proteins, crude fiber, and fats. It also contains vitamins and minerals and is rich in amino acids (Eguchi et al., 2015; Ahlawat & Arora, 2016; Sakinah et al., 2019). Secondary metabolites and other bioactive compounds, such as flavonoids, alkaloids, sterols, phenolic compounds, tannins, terpenoids, and lectins, have been reported to exhibit antioxidant, antibacterial, and radical scavenging activities in *V. volvacea* and other edible mushrooms (Hung & Nhi, 2012; Fan et al., 2006; Dulay et al., 2014). Active compounds recorded in *V. volvacea* include ascorbic acid, total flavonoids, β -carotene, and lycopene (Boonsong et al., 2016; Acharya et al., 2016). Mycelia cultured on rice bran had the highest phenolic content (23.19 mg AAE/g), while mycelia cultured on coconut water had 21.19% phenolic content (Dulay et al., 2016). *V. volvacea* also showed the highest DPPH radical scavenging activity (82.9%) and the highest total phenolic content (4122.7 μ g GAE/g) among edible mushrooms (Hung & Nhi, 2012).

V. volvacea was the first mushroom to be commercially cultivated in the Philippines. It is typically cultivated in indoor bed-type piles using dried banana leaves or rice straw, which require minimal aeration and illumination, with an average temperature of 30–35 °C for 14 days (Reyes, 2000). Previous research shows that yield and biological efficiency of fruiting bodies cultivated on different substrates, such as banana leaves and paddy straw, can vary (Belewu & Belewu, 2005; Sakib et al., 2022; Onuoha et al., 2009; Biswas, 2014). Other wastes found to have potential in *V. volvacea* cultivation include sawdust, cotton waste, waste paper and cardboard, wheat straw, and bamboo shoot shells, among others (Ajika et al., 2019; Dam et al., 2014; Htun & Shein, 2020; Philippoussis et al., 2001; Li et al., 2021).

Several studies have investigated the nutritional and physical requirements for preserving *V. volvacea* mycelial culture. Two strains of *V. volvacea* isolated from selected areas in Central Luzon, Philippines, grow best in potato sucrose gulaman with a pH of 6 to 7 and are incubated under dark conditions, while Sabouraud dextrose agar at pH 6.5 to 7.5 was recorded as optimal with a temperature range of 26 °C to 32 °C for the *V. volvacea* Ecuador isolate (Abon et al., 2020; Kalaw et al., 2016). Furthermore, Aldave et al. (2021) found that at 28.6°C, using 50% decocted banana leaves, *V. volvacea* showed the highest mycelial run and fastest mycelial maturity.

In the Philippines, research on the optimum conditions for *V. volvacea* mycelial growth from wild isolates is limited, and to the best of the authors' knowledge, no studies have focused on commercial isolates. As research on wild macrofungi and their bioactive compounds grows, maintaining a ready supply of macrofungal cell lines is necessary to assess their nutraceutical and pharmacological importance. Therefore, establishing the optimum conditions for the mushroom's mycelial growth is crucial.

In this study, we optimized the mycelial culture conditions for efficient growth of three commercially cultivated isolates and three wild isolates of *V. volvacea* using locally available culture media from indigenous resources. This research aimed to generate essential information for developing practical production technology to harness its promising potential and fully realize its position in the Philippine mushroom industry.

2. Methodology

2.1 Pure Cultures of Mushrooms

Pure cultures of three commercially cultivated isolates were purchased from mushroom growers in San Jose City, Nueva Ecija; Tarlac City; and La Union, and were deposited at the Center for Tropical Mushroom Research and Development (CTMRD), College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Pure cultures of three wild isolates were obtained from the culture collections of the CTMRD. Culture codes of commercial *V. volvacea* isolates are SJ (San Jose City), TC (Tarlac City), and LU (La Union), while for wild isolates, VVA (isolated from Brgy.

Rang-ayan Muñoz, Nueva Ecija), VVB (isolated from CLSU, Nueva Ecija), and SP (isolated from San Mateo, Rizal).

2.2 Culture Inoculant

Agar blocks of mycelium were sub-cultured on potato dextrose agar (PDA) plates and incubated for seven days to promote mycelial growth. To allow mycelial growth, culture plates were incubated at room temperature for 5–8 days. The prepared culture plates served as the inoculum source or stock culture for evaluating the optimum nutrient and physical conditions required for efficient mycelial growth (mycelial expansion).

2.3 Evaluation of Culture Media and pH

Six culture media were evaluated for their ability to support the secondary mycelial growth performance of each *V. volvacea* isolate. Three commercially available culture media were purchased from Asiagel Corporation, Quezon City: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Sabouraud Dextrose Agar (SDA), and were prepared according to the manufacturer's instructions. Three other culture media were made from indigenous resources: coconut water from mature coconut (*Cocos nucifera*), rice bran decoction (50 g of *Oryza sativa* bran/L of water), and corn grit decoction (50 g of *Zea mays* grit/L of water). For preparing the indigenous culture media, 250g of each sample (rice bran D1 and corn grit) was measured and boiled in 1 L of distilled water for 30 minutes at approximately 100 °C. The broth was filtered using a muslin cloth and the filtrate was adjusted to a final volume of 1 L. The mixture was reboiled, and 15 g of agar was added until dissolved and homogenized. Meanwhile, 1 L of coconut water from freshly cracked mature coconuts was boiled, and 15 g of agar was added. The pH of the media was adjusted to pH 6 before sterilization. In each medium, a 10 mm mycelial disc from the prepared stock culture was inoculated at the center of the plate. Triplicates were prepared for each setup and for each isolate, and the plates were incubated at room temperature. Based on the evaluation of culture media, the optimum media for each isolate were evaluated at different pH levels. The prepared media were sterilized for 20 minutes at 121 °C and 15 psi. After sterilization, pH of the media was adjusted to 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 using sterile 1 M NaOH or 1 M HCl. A 10 mm mycelial disc from a 7-day-old pure culture was inoculated at the center of each plate, and the plates were incubated at room temperature.

2.4 Evaluation of Physical Factors

To evaluate the optimum temperature for mycelial growth, plates were incubated in three temperature-controlled environments: refrigerated (6–9 °C), air-conditioned (23–25 °C), and room temperature (30–32 °C). Petri plates inoculated with *V. volvacea* were prepared using the most appropriate medium and pH determined from previous evaluations. After determining the optimum medium, pH, and temperature, these conditions were used to evaluate the influence of illumination on mycelial growth. Plates were incubated under lighted and dark conditions. For the lighted condition, plates were incubated in a chamber with fluorescent light providing an illuminance of 322.92 lux, as specified by the manufacturer, under a 24-hour light cycle. Plates were placed in the chamber approximately 0.6 meters from the light source. For the dark condition, the culture plates were covered with black plastic bags to prevent light exposure for 24 hours. All plates were maintained under the same room temperature conditions, where microenvironmental factors such as humidity, gas exchange, and possible changes in temperature caused by the covering with a plastic bag, were not measured during the experiment. Therefore, the observed influence of light and dark conditions was limited to the presence or absence of light. Finally, the influence of aeration on mycelial growth was evaluated under the optimum nutritional and physical conditions. Inoculated plates of *V. volvacea* were sealed with parafilm, while other plates were left unsealed to allow aeration. Daily mycelial growth diameters (mm) were measured using digital vernier caliper by measuring the diameter of the growing mycelial colony. The initial inoculum of 10-mm mycelial disc was not subtracted from the measured colony diameter, consistent with the measurement approach described by Abon *et al.* (2020) and Kalaw *et al.* (2016). Three technical replicates were prepared for all evaluations with each replicate assessed independently. The prepared cultured plates were monitored until full mycelial growth of one treatment was recorded. Following Aldave *et al.* (2021), daily monitoring was terminated after full mycelial growth in one treatment to compute daily mycelial growth diameter (Mycelial diameter in mm divided by the number of days of monitoring).

The optimum condition was determined after the daily monitoring of mycelial growth. Adapting the method of Kalaw *et al.* (2016) with minor modifications, the optimal condition was determined using mycelial growth rates (mm/day). Treatments with statistically comparable growth rates were compared and the treatment exhibiting the highest mycelial growth rate was identified as the

preferred condition. Mycelial thickness was used as the secondary criterion if treatments showed no statistical difference. The treatment producing the thickest mycelia was identified as the preferred condition. In the present study, mycelial density or mycelial thickness was qualitatively assessed by determining the thickest mycelia through visual comparison.

The optimum culture conditions were determined using a sequential screening approach to allow the identification of favorable conditions for each factor under the previously selected conditions. Therefore, interactions among the culture factors were not evaluated.

2.5 Statistical Analysis

Statistical analyses were performed using Minitab statistical software. For each treatment, three technical replicates were independently monitored and measured. The experimental unit for statistical analysis was the stock culture rather than the individual plates, and measurements from the three technical replicates were summarized for each experimental unit before statistical analysis. Differences among treatment means were evaluated for experiments involving more than two treatment levels using analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test at a significance level of $\alpha = 0.05$ when the ANOVA indicated a significant treatment effect. For experiments involving only two treatment levels, an independent (unpaired) t-test was used to compare treatment means at $\alpha = 0.05$.

3. Results and Discussion

3.1 Effect of Culture Media and pH

Optimization studies are typically conducted to determine the most suitable conditions, such as culture media, pH, and physical factors, for the luxuriant growth of mushroom mycelia (Kalaw *et al.*, 2016). One of the primary considerations in growing the mycelia is the culture medium that will support mycelial ramification. The growth and development of mushrooms depend on the nutrients present in the culture media. Different media may have varying quantities and types of nutrients. In this study, three commercial media (PDA, MEA, and SDA) and three indigenous culture media (CWG, CGDG, and

RBDG) were used to grow six isolates of *V. volvacea*. Figure 1 shows the mycelial growth performance of the different isolates on the six culture media.

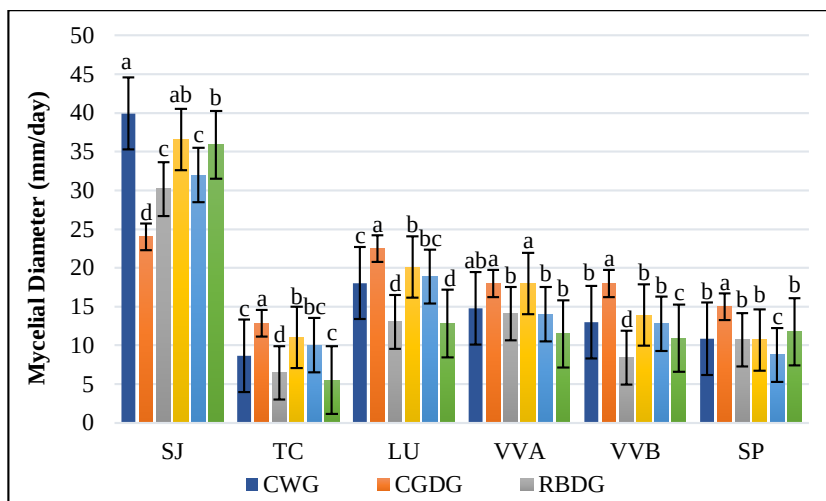


Figure 1. Mycelial growth rate (mm/day) of *V. volvacea* strains in different culture media. In each isolate, mean values with the same letter assignment indicate no significant difference at the 5% level of significance using Tukey's HSD

CGDG supported faster growth of four isolates (TC, LU, VVB, and SP), while 2 isolates (VVA and SJ) grew efficiently on PDA. The SJ isolate exhibited faster mycelial growth on CWG, which is not significantly different from PDA. Therefore, PDA was selected as the optimal medium for growth because mycelia were much thicker on it. This study showed a variation in the mycelial growth performance of *V. volvacea* across different media. Differing nutrient contents in the media, such as carbohydrates, sugars, proteins, fibers, vitamins, and minerals, strongly influence growth performance. These nutrients are available in culture media in varying amounts, resulting in different growth performance of different strains of mushrooms. In the study of Abon *et al.* (2020), different *V. volvacea* strains grew differently on different culture media. Similarly, different strains of *Lentinus* spp. observed to grow differently on different media primarily due to their unique nutritional requirements (Dulay *et al.*, 2021; Kalaw *et al.*, 2021). The best mycelial growth of *V. volvacea* isolates in this study was mainly attributed to the nutrient content of the optimum media. PDA contains dextrose as its carbon source and potato infusion as its nutrient source, while corn grit contains various nutrients, including fiber, starch, sugar, protein, fat, and several B vitamins (Kalaw *et al.*, 2021; Tremblay, 2018). Therefore, mycelial growth

performance depends not only on species but also on isolate. In a similar study, maximum mycelial growth of two wild strains of *V. volvacea* was observed separately in corn grit gulaman (CGG) and potato sucrose gulaman (Kalaw *et al.*, 2016), while Abon *et al.* (2020) found that SDA supported the best mycelial growth for three wild strains of *V. volvacea*. Nutritional components vary across culture media, and the carbon-to-nitrogen ratio, which strongly influences mushroom mycelial growth (Dulay *et al.*, 2021), can play a significant role. Reyes *et al.* (1998) found that starch, cellulose, and sorbitol best support *V. volvacea* mycelial growth, while soytone, peptone, and glycine are efficient nitrogen sources. On the other hand, *V. volvacea* was found to grow effectively in the presence of nitrate nitrogen and alkali-hydrolyzed nitrogen as the nitrogen sources, while microbial biomass carbon as the carbon source (Wang *et al.*, 2005). The importance of carbon and nitrogen sources was also reported in various mushrooms. In the study of Jo *et al.* (2010), carbon-to-nitrogen ratios of 2:1, 5:1, and 10:1 were found to be efficient for the mycelial growth of *C. versicolor*. Similarly, Jo *et al.* (2006) reported that carbon-to-nitrogen ratios of 10:1 and 5:1 were efficient for *Phellinus* spp. Additionally, Jo *et al.* (2010) found that selecting suitable carbon and nitrogen sources in media contributes to efficient mycelial growth of *C. versicolor*. For *C. versicolor*, dextrin and yeast extract as carbon and nitrogen sources, respectively, were optimal for growth. Rana and Dahot (2017) also stated that certain carbon and nitrogen sources help mushrooms produce secondary metabolites, with higher levels of tannins and flavonols achieved using glucose as the carbon source and casein as the nitrogen source. Because secondary metabolites are essential to the biological properties of mushrooms, such as antioxidant and antibacterial activity, identifying suitable carbon and nitrogen sources for *V. volvacea* mycelial culture could be a promising area for future research.

Medium pH is another important parameter to consider when growing mushroom mycelia. In this study, PDA and CGDG were used as the basal media, and the pH was adjusted from 4 to 9. However, PDA with a pH of 4 did not solidify, so this pH was excluded from the evaluation. The mycelial growth performance of six *V. volvacea* isolates at different pH levels is shown in Figure 2. All isolates grew across the tested pH range. However, the SJ isolate exhibited faster mycelial growth at pH 5, while three isolates (TC, VVA, and VVB) showed optimum mycelial growth at pH 8. Two isolates (LU and SP) showed maximum mycelial growth at pH 7.

Along with the optimum medium, pH is an important factor to consider when growing mushroom mycelia. The data show that different isolates have varying pH requirements for optimum mycelial growth. Some prefer slightly acidic conditions, while the majority prefer slightly alkaline pH levels. Researchers also reported differences in pH requirements among *V. volvacea* strains such as Montalvo, La Clemencia, and Vines, as well as among six strains of *Pleurotus djamour* (Abon et al., 2020; Kalaw et al., 2022). Kalaw et al. (2016) found that media with a pH of 6 to 7 supported maximum mycelial growth and the thickest mycelial density of *V. volvacea*, while Reyes et al. (1998) found that pH 8 is suitable for the growth of *V. volvacea*. Variation in pH requirements was also observed in other mushrooms such as different *Ganoderma* species (Del Rosario et al., 2022). Optimum pH is critical because it was found to affect growth of mushrooms such as *Xylaria papulis* by influencing cell structure and function, nutrient uptake, and product synthesis (Lopez et al., 2022). pH strongly affects enzymes involved in vegetative mycelial growth. For instance, when the medium is too alkaline for *Ganoderma lucidum*, it can greatly affect enzyme activity and other cellular functions, potentially denaturing cells and rendering them nonfunctional (Chang & Miles, 2004; Magday et al., 2014). In the present study, the tolerance of *V. volvacea* mycelia to different pH levels aligns with the fact that many mushroom species can grow across a wide pH range (Dulay et al., 2021; Garcia et al., 2020).

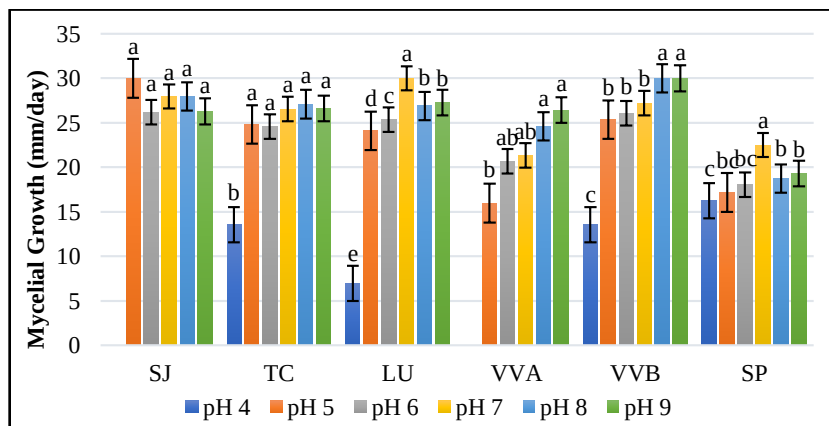


Figure 2. Mycelial growth rate (mm/day) of six *V. volvacea* isolates at different pH levels. In each isolate, mean values with the same letter assignment indicate no significant difference at 5% level of significance using Tukey's HSD

3.2 Effect of Physical Factors

Temperature, illumination, and aeration are crucial environmental conditions to consider for efficient mycelial growth. Temperature is an essential factor for mushroom growth and development, determining the distribution and survival of mushrooms in particular areas (Chang & Miles, 2004). Three temperature conditions—room temperature, air-conditioned, and refrigerated conditions—were used to determine the optimum temperature for the mycelial growth of six *V. volvacea* isolates. Figure 3 shows the mycelial growth performance of the isolates at different temperatures.

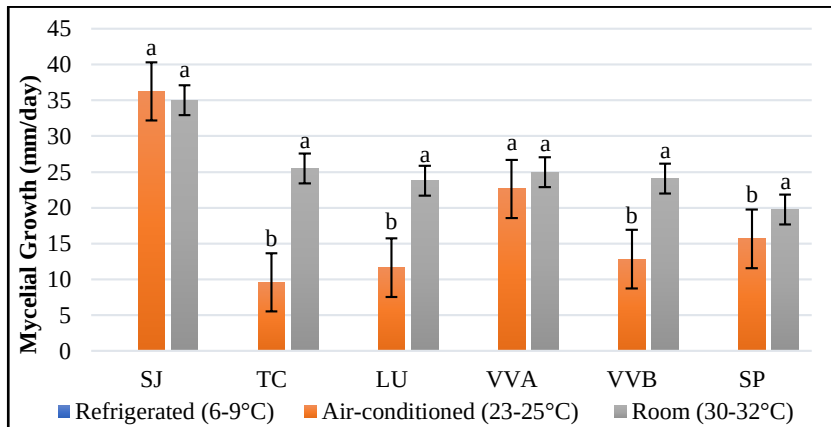


Figure 3. Mycelial growth rate (mm/day) of different *V. volvacea* strains at different temperatures. In each isolate, mean values with the same letter assignment indicate no significant difference at 5% level of significance using Tukey's HSD

Four isolates (TC, LU, VVB, and SP) exhibited faster mycelial growth at room temperature, while comparable growth for VVA and SJ was observed at both room temperature and air-conditioned conditions. No measurable mycelial growth was observed in the initial 10 mm mycelial disk under refrigerated conditions. Several studies have reported that mycelial growth of certain mushrooms is drastically suppressed at lower temperatures, typically below 10 °C (Garcia *et al.*, 2020; Magday *et al.*, 2014). Jayasinghe *et al.* (2008) found that at lower temperatures, essential enzymes involved in metabolic processes denature and become inactive. In the present study, different isolates can be cultivated at slightly higher temperatures, which contradicts previous reports that *V. volvacea* requires 35 °C for maximum mycelial growth (Kumar *et al.*, 2016). Moreover, previous studies reported that *V. volvacea* can grow

between 25 °C and 40 °C, with no growth observed when the temperature was raised to 45 °C or lowered to 20 °C (Chang & Steinkraus, 1982). Separate studies have assessed the maximum mycelial performance of different *V. volvacea* strains at various temperatures, finding the optimum range to be between 26 °C and 32 °C. This temperature range supports the conclusion that *V. volvacea* is a tropical mushroom (Abon et al., 2020; Aldave et al., 2021), similar to other tropical mushrooms like *Lentinus*, which has an optimum temperature of 30 °C (Dulay et al., 2021).

Although mushrooms are heterotrophic, light can stimulate mycelial growth. In their natural environment, mushrooms experience shifting light and dark conditions. This study assessed the influence of illumination on mycelial growth in *V. volvacea* isolates. The results are shown in Figure 4.

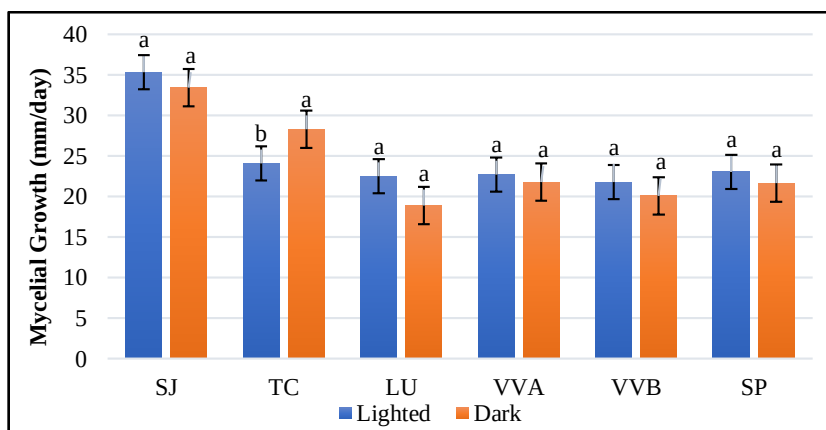


Figure 4. Mycelial growth rate (mm/day) of different *V. volvacea* isolates at optimum media and pH incubated under light and dark conditions at the optimum temperature. Within each isolate, mean values with the same letter assignment indicate no significant difference at the 5% level of significance by t-test

Mycelial growth differed significantly only in the TC isolate, which grew faster in the dark. Efficient mycelial growth under light conditions is consistent with Chang and Miles (2004), who found that mushrooms can grow unaffected by light, and that mycelia may be inhibited or killed by exposure to strong light. Moreover, Magday et al. (2014) found that the combination of carbon and nitrogen sources in the medium and the presence of light stimulates mycelial growth. In the present study, most *V. volvacea* isolates could be cultured under either of the 2 conditions. This result is inconsistent with the

findings of Reyes *et al.* (1998), who noted that *V. volvacea* grew best under lighted conditions, and Kalaw *et al.* (2016), who reported that *V. volvacea* isolate from Central Luzon, Philippines, also grew best under lighted conditions. Moreover, Vines and Montalvo *V. volvacea* strains favored dark and lighted conditions, respectively, while the La Clementina strain favored both (Abon *et al.*, 2020). In *V. volvacea*, exposure to light may not be a crucial factor, since light is essential for fruiting body formation rather than vegetative growth.

The last physical factor assessed is aeration, which is a significant factor for efficient mycelial growth. Reyes *et al.* (1998) reported that proper sealing provides the necessary gaseous requirements for growing *V. volvacea* mycelia. Similarly, Chang and Miles (2004) found that proper aeration benefits mycelial growth by ensuring continuous development. In Petri plates, adequate aeration prevents an excessive increase in carbon dioxide concentration around the respiring mycelium.

In this study, the mycelial growth of six isolates was investigated in terms of aeration requirements. No significant growth differences were observed between sealed and unsealed plates for commercial isolates and the VVB isolate. However, significant differences were observed for the VVA and SP isolates. Figure 5 illustrates the mycelial growth of the six isolates under sealed and unsealed conditions.

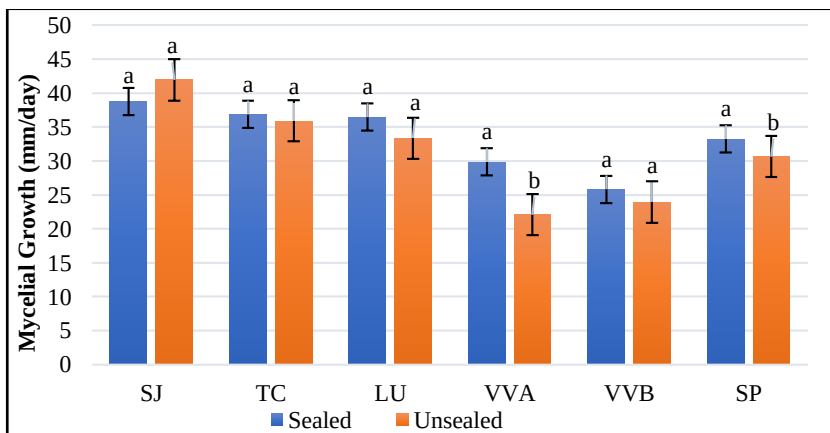


Figure 5. Mycelial growth rate (mm/day) of different *V. volvacea* strains under sealed and unsealed conditions. In each isolate, mean values with the same letter assignment indicate no significant difference at 5% level of significance using t-test

The *V. volvacea* SJ isolate exhibited the best mycelial growth under unsealed conditions, though its growth under sealed conditions was comparable. Similarly, no significant differences were observed between the sealed and unsealed conditions for the TC, LU, and VVB isolates, although maximum mycelial growth was observed under sealed conditions. In contrast, the VVA and SP isolates showed significant growth differences between the two conditions. Previous studies have reported that some mycelial species grow best on unsealed plates, while others perform better on sealed plates (Reyes *et al.*, 1998).

This study's findings align with those of Dulay *et al.* (2021), who reported that different mushroom strains and species may exhibit distinct illumination preferences. This could also be influenced by illumination conditions during their fruiting body development.

Based on the study's results, isolates of the same species exhibit different growth responses to aeration. The *V. volvacea* SP isolate showed optimal growth when aeration was restricted. However, overall, aeration does not appear to have a significant impact on the mycelial growth of the six isolates, as growth was observed under both sealed and unsealed conditions. This conclusion is consistent with previous research, which found that aeration conditions did not significantly influence the growth of three strains of *V. volvacea* and that both conditions were suitable for the mycelial growth of different strains of *P. djamor* (Abon *et al.*, 2020; Kalaw *et al.*, 2022) as shown in Figure 6.

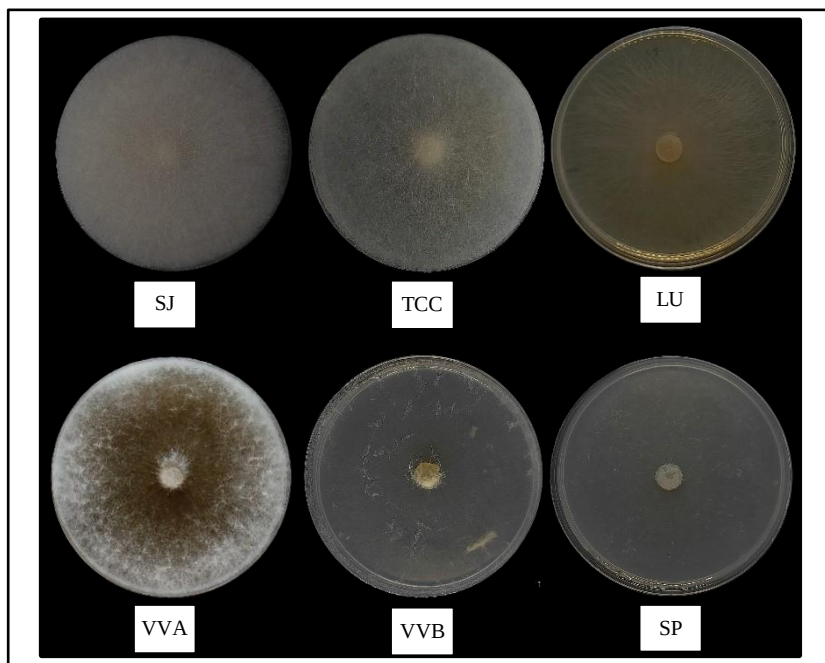


Figure 6. Mycelial growth of the six isolates of *V. volvacea* on their most suitable culture media and optimal pH, incubated under optimal temperature, illumination, and aeration

4. Conclusion and Recommendation

This study highlighted the requirements of six *V. volvacea* isolates for efficient mycelial growth. The mycelial growth of the different isolates was influenced by both commercial and indigenous media. Isolates SJ and VVA showed optimal growth on PDA, while CGDG was found to be the best medium for the commercial isolates TC and LU, as well as for the wild isolates VVB and SP. The optimal pH for mycelial growth was also determined for each isolate, with some preferring slightly acidic conditions, while others thrived in slightly alkaline environments. Mycelial growth was observed to be efficient under both lighted and dark conditions, as well as in both sealed and unsealed plates. Room temperature (30–32 °C) supported the best mycelial growth for all six isolates. This finding is consistent with previous reports describing *V. volvacea* as a tropical mushroom that grows well under warm conditions. Thus, the room temperature (30–32 °C) conditions typical of tropical countries

such as the Philippines may provide suitable temperatures for its mycelial growth. Overall, the results indicate that the optimal conditions for mycelial growth vary among *V. volvacea* isolates, demonstrating isolate-specific growth responses. Future research could explore the composition of culture media, particularly focusing on its carbon and nitrogen content and the C and N ratio. Investigating the biological activities and identifying important bioactive compounds of *V. volvacea* mycelia are potential areas of research that may contribute to advancements in the pharmaceutical and food industry.

5. Acknowledgement

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