GROWTH PREFERENCES OF SECONDARY MYCELIA, OPTIMUM SPAWNING MATERIAL, AND FRUITING BODY PRODUCTION OF *Auricularia polytricha* (Mont.) Sacc. UTILIZING A RICE STRAW-BASED SUBSTRATE FORMULATION

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ABSTRACT

Auricularia polytricha (Mont.) Sacc. is a highly demanded species of mushroom with considerable culinary applications. However, there is very limited technology for the cultivation of this mushroom in the Philippines. Thus, this study was conducted to develop an efficient and sustainable cultivation technology to consistently harness the potential of this valuable macrofungus. This research was conducted at the Center for Tropical Mushroom Research and Development in Central Luzon State University, Nueva Ecija, Philippines from January to July 2023. The growth performances of secondary mycelia in indigenous materials and the most suitable spawning material were determined. The best substrate formulation using rice straw and sawdust for fruiting body production with regards to incubation period, primordia formation, cap size, yield, and biological efficiency was established to be rice straw and sawdust (80:20). Mycelial growth of *A. polytricha* (Mont.) Sacc. is favored in potato sucrose agar with pH 6-7. It prefers sealed conditions in either dark or with light at 32°C. Cracked corn seed is the best spawning material. This production technology could therefore secure a consistent and abundant supply of *A. polytricha* (Mont.) Sacc. in the local market.

Key words: mushroom cultivation, conservation, sustainability, biological efficiency

INTRODUCTION

Auricularia polytricha (Mont.) Sacc., commonly called ear rat mushroom, is a basidiomycetous fungus belonging to the family Auriculariaceae within the order Auriculariales. It features a soft, reddish-brown fruiting body that typically emerges on decaying wood and twigs. This gelatinous mushroom is highly popular in the Philippines and is frequently utilized as a culinary ingredient in various Filipino dishes, including chop suey, pansit (noodles), and other vegetable-based delicacies. Beginning in the 17th century, *Auricularia* mushrooms were being utilized for the treatment of thrombosis, cold and fever, jaundice, pharyngitis, constipation, and menstrual problems (Berch et al. 2007; Jamtsho and Wangchuk 2023). Renowned for its nutritional value, this mushroom contains significant amounts of protein, fiber, ash, and carbohydrates, while maintaining low levels of fat (Hassan and Medany 2012; Rawiningtyas et al. 2023; Vidyaresmi 2008). Moreover, compounds that exhibit antioxidant properties (Chen and Xue 2018), anti-hypercholesterolemia effects (Li et al. 2022), anti-proliferative activity (Yu et al. 2014), hepatoprotective potential (Chellappan et al. 2016), anti-

inflammatory attributes (Hou et al. 2020), antidiabetic characteristics (Xiang et al. 2021), and significant constipation-relieving effects (Jia et al. 2020) are present in this mushroom. Furthermore, it has hypolipidemic effects, aiding in the reduction of liver fat accumulation and promoting the enrichment of gut microbiota diversity (Fang et al. 2021). Despite its significance, its commercial cultivation within the country remains limited, leading to substantial imports from China and Taiwan, where large quantities of *A. polytricha* (Mont.) Sacc. are produced (Jia et al. 2017). Consequently, it is important to develop localized cultivation techniques for this mushroom for its continuous production.

Cultivation practices for *Auricularia* mushrooms include the log method, the artificial log method, and the poly bag method which is the most commonly used (Priya et al. 2016). Depending on the availability of waste materials, different regions have developed unique technologies for successfully cultivating various *Auricularia* species (Regis and Geosel 2024). Sawdust and corn straw were used for *A. cornea* (Chen et al. 2021; Zhang and Riskowski 2020). *A. auricula-judae* is cultivated in a wide range of substrates such as sawdust, various bran, oil palm wastes, sugarcane, and grasses (Onyango et al. 2011; Siwulski et al. 2011; Zhang and Riskowski 2020) while sago waste, acacia, birch, beech sawdust and palm oil wastes, are applicable for growing *A. polytricha* (Mont.) Sacc. (Razak et al. 2013; Siwulski et al. 2011). The combination of rice straw, dried banana leaves, and coco husk supplemented with rice bran is ideal for the basidiocarp production of *A. polytricha* (Mont.) Sacc. (Zurbano 2018). Spent mushroom sawdust wastes can also be used as a base substrate (Wu et al. 2020).

In the country, rice straw and sawdust are the most practical and economically viable choices for cultivating mushrooms. Currently, the Center for Tropical Mushroom Research and Development (CTMRD) in Central Luzon is promoting zero rice waste technology wherein rice straw is utilized as a base material in cultivating mushrooms. This technology is widely adopted since it is cost-efficient and high-yielding. It also promotes organic farming which not only generates livelihoods but also helps in the reduction of waste materials. Species of mushrooms that have been successfully grown using these substrates include those from the genus *Pleurotus* and *Lentinus*, *Ganoderma lucidum*, *Panaeolus antillarium*, *Panaeolus cyanescens*, *Cyclocybe cylindracea*, and *Pycnoporus sanguineus* (Bustillos et al. 2014; Dulay and Damaso 2020; Dulay et al. 2021; Kalaw et al. 2021; Landingin et al. 2020; Magday et al. 2014). The appropriateness of these substrates for a wide variety of mushrooms might also be suitable for *A. polytricha* (Mont.) Sacc. Although it has been successfully grown in palm oil waste, sawdust, and stalks of grass (Liang et al. 2019; Razak et al. 2013; Zurbano 2018), studies have not been conducted on the utilization of rice straw and coconut sawdust for *A. polytricha* (Mont.) Sacc. Hence, this study was conducted. However, to achieve its optimum fruiting performance, it is crucial to determine the exact proportion of these materials since the nutrient preferences of mushrooms vary depending on the species.

To establish efficient production technology for this mushroom, a comprehensive study was conducted to determine its optimal conditions from mycelial growth to fruiting body production. The growth preferences of *A. polytricha* (Mont.) Sacc. mycelia through the utilization of indigenous culture media were understood as well as its responses to variations in temperature, light exposure, and aeration conditions. Furthermore, the study also determined the most appropriate spawning material. In terms of fruiting body production, the utilization of locally accessible resources and the development of a substrate formulation composed of rice straw and coconut sawdust were emphasized. The establishment of an alternative production technology for *A. polytricha* (Mont.) Sacc. will therefore secure a consistent and abundant supply of this mushroom in the local market.

MATERIALS AND METHODS

Time and place of the study. This research was carried out at the Center for Tropical Mushroom Research and Development (CTMRD) at Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines from January to July 2023.

Source of *A. polytricha* (Mont.) Sacc. culture. Pure culture of *A. polytricha* (Mont.) Sacc. was obtained from the Center for Tropical Mushroom Research and Development, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines.

Preparation of culture inoculants. All methods employed in this optimization study were adopted from the study of Dulay et al. (2015). Potato dextrose agar (PDA) was used to sub-culture *A. polytricha* (Mont.) Sacc. It was prepared by dissolving 39 grams of PDA in 1 liter of distilled water. The mixture was boiled until completely dissolved. The media was placed in a clean bottle, then plugged with cotton, and covered with paper to prevent the cotton from absorbing moisture. The bottles were sterilized for 30 minutes (121°C, 15 psi) using an autoclave (Vertical Pressure Steam Sterilizer, Model: LS-B7SL-I). After sterilization, the media was poured into a sterilized petri dish and allowed to cool. Mycelial block of *A. polytricha* (Mont.) Sacc. were obtained from the source culture and aseptically transferred into the center of the plate-containing media. It was then stored at room temperature (32°C) to facilitate mycelial ramification.

Effect of culture media and pH. Indigenous materials such as potato (*Solanum tuberosum*), corn (*Zea mays*), coconut (*Cocos nucifera*) water, and rice (*Oryza sativa*) bran were used to prepare solid culture media. Following the method used by Dulay et al. (2015), potato sucrose agar (PSA) was prepared by boiling 250 grams of peeled potato cubes in 1 liter of distilled water. Then, the broth was obtained, filtered, and reconstituted back to 1 liter. It was then re-boiled and 10 grams of white table sugar and 20 grams of shredded white agar were added. The mixture was stirred until all the agar was dissolved. For corn grit sucrose agar (CGSA) and rice bran sucrose agar (RBSA), 50 grams of corn and rice bran were used following the above-mentioned procedure. Coconut water agar (CWA) was prepared by filtering the coconut water; it was boiled and 20 grams of agar was added per liter. All the prepared media was adjusted to pH 6 using 1M NaOH and 1 M HCl before sterilization to ensure uniformity. Sterilization was done using an autoclave for 30 minutes at 121°C and 15 psi. The media was cooled and then poured onto sterilized petri plates. Once solidified, a 10-mm mycelial disc was aseptically inoculated at the center of each plate. The plates were incubated at room temperature (32°C).

A one-factor-at-a-time optimization approach was used wherein the optimal condition determined in the previous setup was used in succeeding experiments. The best media was prepared and the pH was adjusted to 4, 5, 6, 7, and 8. This was followed by sterilization, pour plating, inoculation, and incubation at room temperature.

Effects of physical factors. The most appropriate culture medium adjusted to the best pH level was used to determine the influence of physical factors such as aeration, illumination, and temperature on the secondary mycelial growth of *A. polytricha* (Mont.) Sacc. The media was prepared, sterilized, and inoculated with a 7-day-old mycelial disc. Then the plates were incubated under two aeration conditions (sealed and unsealed). The plates were wrapped with parafilm for the sealed condition while the other plates were left unsealed and were incubated at room temperature. After determining the aeration preference, the effect of light was determined. The plates inoculated with mycelial discs were sealed with parafilm. The plates were exposed to a continuous lighted condition using artificial light at 137 lux for the lighted condition. The culture plates under dark condition were wrapped with black paper and placed in a black glass chamber to ensure that no light would pass through. After determining the best illumination condition, the influence of temperature on the secondary mycelial growth of *A. polytricha* (Mont.) Sacc. was evaluated. The culture plates were inoculated with mycelial discs, covered with parafilm, incubated at room temperature (32°C), air-conditioned (26°C), and refrigerated (8°C) under either lighted or dark conditions.

Mycelial growth performance on different spawning materials. The mycelial growth performance of *A. polytricha* (Mont.) Sacc. in different indigenous spawning materials (rice seeds, cracked corn seeds, and sorghum seeds) was determined. The spawning materials were thoroughly washed and boiled separately then the water was drained. Forty grams of the cooled seeds were separately placed

in sterile petri plates. The plates were then wrapped in clean paper and placed in a 6" x 12" polypropylene bag secured with a rubber band. It was followed by sterilization at 121°C and 15 psi for 45 minutes. Once cooled, the plates were inoculated aseptically with 10-mm mycelial discs from 7-day-old mycelia grown using the best nutritional factors and incubated under the optimum environmental conditions. The plates were incubated at room temperature (32°C).

The mycelial diameter and density were used as a basis to determine the optimum nutritional and physical growth preferences and the best spawning material. Mycelial density was observed visually and rated as very thick, thick, thin, and very thin following the procedures of Reyes et al. (2009) and De Leon et al. (2017).

Fruiting body production on different rice straw-sawdust-based substrate formulations.

Preparation of grain spawn. The most suitable spawning material was used to prepare grain spawn. Cracked corn was washed and boiled until tender. Seeds (40 grams) were placed in a 3" x 4" polypropylene bag, plugged with cotton, and sterilized in an autoclave for 45 minutes at 121°C and 15 psi. The bags were cooled before inoculation. Ten millimeters of mycelial disc was inoculated aseptically into each bag and stored at room temperature (32 °C) until full mycelial ramification.

Evaluation of fruiting body production. The fruiting body production of *A. polytricha* (Mont.) Sacc. as affected by the different formulations of rice straw (RS) and coconut sawdust (SD) was determined. A different ratio of rice straw and coconut sawdust was prepared and the treatments were designated as follows: T1= 0 RS:10 SD, T2= 1 RS:9 SD, T3=2 RS:8 SD, T4=3 RS:7 SD, T5=4 RS:6 SD, T6=5 RS:5 SD, T7=6 RS:4 SD, T8=7 RS:3 SD, T9=8 RS: 2 SD, T10=9 RS:1 SD, T11=0 RS:10 SD.

Rice straw was soaked in tap water for three days, washed thoroughly, and transferred to a strainer to allow the excess water to drain. It was covered for three to five days using a plastic sheet. to facilitate the decomposition process. After five days, it was chopped (1-3 inches long) and then mixed with coconut (*Cocos nucifera*) sawdust. The formulation of the different treatments was prepared in terms of volume using a 10-liter pail. Five hundred grams of each substrate formulation were placed in a 6" x 12" polypropylene bag. The fruiting bags were pasteurized in a box-type pasteurizer for 6-8 hours at 90°C -100°C. After cooling, the bags were aseptically inoculated with 40 grams of spawn and incubated in a room with a temperature range of 30-32°C. The number of days of full mycelial ramification and initiation of primordia from the date of inoculation was recorded. The mature fruiting bodies were harvested and weighed using an analytical weighing balance (BEL Engineering ES20S, Italy). The diameter of the cap/pileus and the length of the stipe were measured using a digital Vernier caliper. Yield per bag was recorded as the total fresh weight of fruiting bodies obtained throughout the fruiting period. The biological efficiency (BE) was computed by dividing the total fresh weight of the fruiting body by the fresh weight of the substrate (De Leon et al. 2017). This experiment was replicated 6 times.

Statistical analysis. All treatments were laid out in a Completely Randomized Design (CRD). Analysis of Variance (ANOVA) was used to analyze the data and the treatment means were compared using Tukey's HSD at a 5% level of significance. Experiments with only two treatments were analyzed using T-test.

RESULTS AND DISCUSSION

Influence of nutritional factors. The mean mycelial growth per day of *A. polytricha* (Mont.) Sacc. as influenced by the different indigenous culture media is presented in Table 1. Among the five media used, the highest mean daily mycelial diameter was recorded in PSA with 12.76 mm. A significantly lower mycelial diameter was obtained in control (PDA) with a mean of 8.40 mm. However, the mean mycelial diameters in CGSA, CWA, and RBSA are comparable to each other. In terms of mycelial density, all media produced thick mycelia except for CWA which produced thin mycelia (Fig 1).

Table 1. Mycelial growth per day of *A. polytricha* (Mont.) Sacc. grown on different indigenous culture media.

Culture Media	Mycelial growth per day (mm)
PSA	12.76±0.05[a]
CGSA	11.43±0.51[b]
CWA	10.86±0.28[b]
RBSA	11.14±0.08[b]
PDA (control)	8.40±0.52[c]

Means with the same letter superscript are not significantly different from each other at a 5% level of significance using Tukey's HSD.

Mushrooms require specific nutrients for their optimal growth. Although various culture media formulations can support the growth of different mushroom species, the quality and efficiency of mushroom mycelial growth are dependent on the nutrient composition of the media. Corn, rice bran, potato, and coconut water all contain sugar, vitamins, nitrogenous compounds, amino acids, and some phytohormones (Kaul et al. 2019; Sapwarobol et al. 2021; Yong et al. 2009). Altogether, these compositions support the mycelial development of mushrooms which was proven in previous studies. The results obtained in this study can be attributed to the differences in the quantities of the components present in the media evaluated. Sucrose, together with the minerals, vitamins, and nitrogen present in potato extract favors the rapid mycelial growth of *A. polytricha* (Mont.) Sacc. Similarly, among various carbon sources, sucrose was found to be the most suitable for the efficient mycelial biomass production of *A. polytricha* (Mont.) Sacc. (Xu and Yun 2003). Other potato-based media facilitate excellent mycelial growth of this mushroom, including the *A. polytricha* (Mont.) Sacc. Japan strain which exhibited luxurious growth in PSA supplemented with 0.5% magnesium sulfate and magnesium chloride (Tabata and Ogura 2003). Moreover, PDA also demonstrated superior suitability for the mycelial growth of *A. polytricha* (Mont.) Sacc. compared to malt extract agar, carrot extract, and oatmeal agar (Priya and Geetha 2016). In addition, the best media for the biomass production of *A. polytricha* (Mont.) Sacc. in liquid condition was 20% potato, 2% sucrose, and 1.5% corn flour supplemented with peptone, potassium, and magnesium (Tang 2014). In this study, PSA was found to be the most suitable medium for *A. polytricha*, which aligns with other findings. Although other strains exhibit a preference for commercially available PDA and PSA with supplementation, the common constituent of the above-mentioned media suitable for *A. polytricha* is potato or nutrients derived from potatoes. Other species under the genus *Auricularia* that prefer potato-based media include *Auricularia delicata* (Devi and Singh 2008) and *Auricularia villosula* (Kejariwal 2023). Interestingly, all media were found suitable for *A. polytricha* (Mont.) Sacc., implying its ability to utilize media with different carbon and nitrogen sources. Glucose, sucrose, and maltose are effective carbon compounds capable of enhancing the biomass production of *A. polytricha* (Mont.) Sacc. in submerged fermentation (Hassan and Medany 2012; Jonathan 2011). On the other hand, *A. polytricha* (Mont.) Sacc. obtained from Lopez, Quezon, Philippines grew well in CWA with a very thick mycelial density (Zurbano 2018). Differences in the media preference of *A. polytricha* (Mont.) Sacc. could be influenced by the variation in the genetic composition among different strains. The genetic composition of a species can indeed contribute to variations in its physiological and metabolic characteristics, including its preference for different growth media. Based on the results gathered, PSA can be used as the most suitable alternative media for *A. polytricha* (Mont.) Sacc.

The influence of varying pH of PSA on the mycelial growth performance of *A. polytricha* (Mont.) Sacc. is presented in Table 2. The optimum mycelial growth per day was recorded at pH 6 with a mean diameter of 12.99 mm which is comparable to pH 7.0 (12.09 mm). Mycelial growth decreased in media with pH concentrations of 4, 5, and 8, with 9.38 mm, 9.87 mm, and 11.37 mm diameters, respectively. Thick mycelia were produced at all pH concentrations (Fig. 1).

Table 2. Mycelial growth per day of *A. polytricha* (Mont.) Sacc. grown on PSA with varying pH levels.

pH Levels	Mycelial growth per day (mm)
4	9.38±0.96^{c}
5	9.87±0.37^{c}
6	12.99±0.11^{a}
7	12.09±0.37^{ab}
8	11.37±0.17^{b}

Means with the same letter superscript are not significantly different from each other at a 5% level of significance using Tukey's HSD.

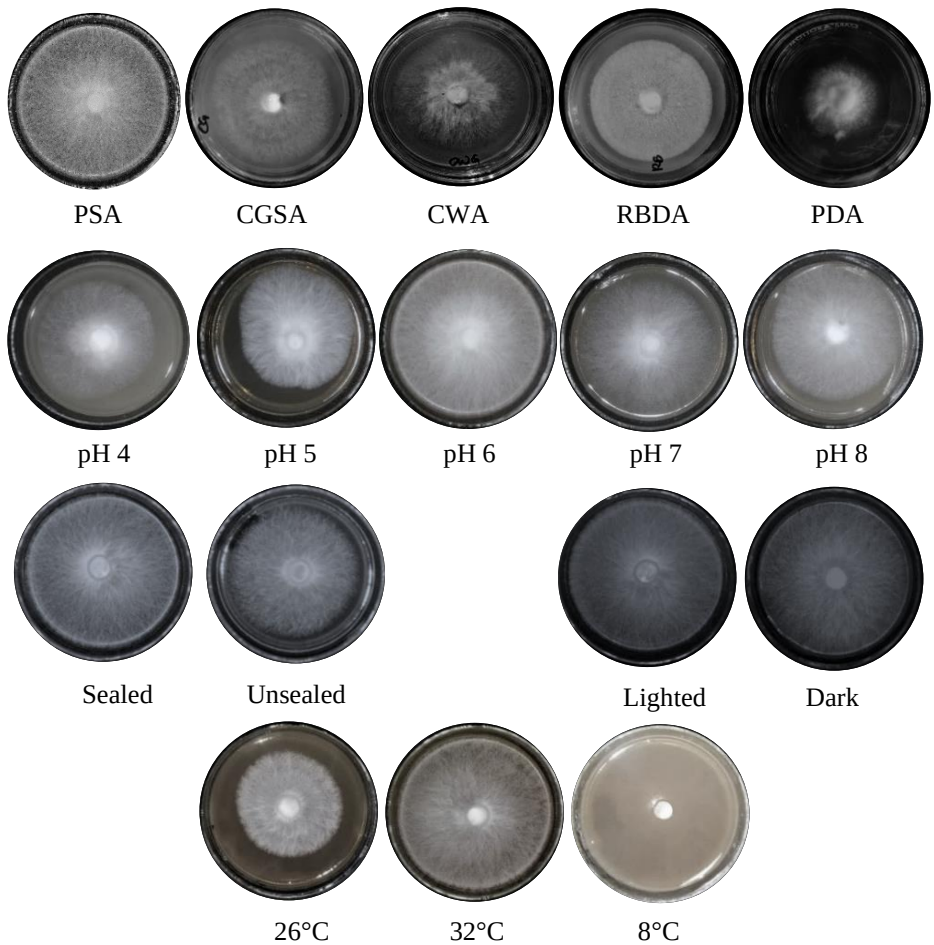

Figure 1. Mycelial growth performance of *A. polytricha* (Mont.) Sacc. on different nutritional and physical factors.

The pH level of the growth medium plays a vital role in the mycelial growth of mushrooms. Different mushroom species have specific pH requirements for their optimal growth. The activity of enzymes that facilitate the breakdown of nutrients may be influenced by the pH of the medium. The pH preference of *A. polytricha* (Mont.) Sacc. observed in this study is similar to the pH requirement of *A. polytricha* (Mont.) Sacc. collected in China and India which exhibited efficient mycelial ramification in pH 6.5 to 7.0 (Priya and Geetha 2016; Hassan and Medany 2012). *A. polytricha* (Mont.) Sacc. in Malaysia grew best at pH 5.5 to 6.5 (Razak et al. 2013). However, pH preferences may vary, other strains and *Auricularia* species prefer a slightly acidic pH. As an example, the mycelial biomass production of *A. polytricha* (Mont.) Sacc. from Korea peaked at pH 5 (Xu and Yun 2003) and *Auricularia auricula* grew best in media with a pH of 4.5 to 5.5 (Khaskheli et al. 2017). In addition, pH 6.5 favors the growth of *Auricularia delicata* (Devi and Singh 2008). Lower mean values of mycelial diameter obtained from media with pH concentrations of 4, 5, and 8 further proved that the activity of enzymes during mycelial development is directly affected by the pH concentration of the medium. Adjustment of the pH of the media to 6 and 7 created a favorable environment that promotes the production and activity of these enzymes, thereby enhancing mycelial growth. Differences in the pH preferences of mushrooms can be attributed to their adaptation to the specific environmental conditions of their respective origins. By maintaining the appropriate pH condition of the media, this mushroom can optimize the uptake of essential nutrients present in the media, allowing the mycelia to grow efficiently.

Influence of physical factors. Table 3 presents the mean mycelial diameter per day of *A. polytricha* (Mont.) Sacc. as influenced by the different physical factors. Sealed cultures produced a significantly wider mycelial diameter of 13.53 mm compared to unsealed cultures with a 9.18 mm mycelial diameter. In terms of illumination, the mean mycelial diameter of cultures incubated in lighted condition was statistically comparable with that in a dark condition. Among the three temperature conditions, the longest mycelial diameter was recorded at 32°C (13.57 mm) while a significantly shorter mycelial diameter was recorded at 26°C (7.49°C). Cultures incubated at 8°C did not show mycelial growth. All culture plates produced thick mycelia (Fig. 1).

Table 3. Daily mycelial growth of *A. polytricha* (Mont.) Sacc. grown on PSA at pH 6 as affected by varying temperature, illumination, and aeration conditions.

Physical factors	Conditions	Mycelial growth per day (mm)
Aeration	Sealed	13.53±0.07[a]
	Unsealed	9.18±0.14[b]
Illumination	Lighted	13.47±0.09[a]
	Dark	13.32±0.23[a]
Temperature	8 °C	0.00±0.00[c]
	26°C	7.49±0.60[b]
	32°C	13.57±0.00[a]

Means with the same letter superscript are not significantly different from each other at a 5% level of significance using Tukey's HSD.

Aeration, illumination, and temperature are critical physical factors influencing the growth and development of mushrooms. Mycelia need oxygen for respiration, which is indispensable for energy generation and metabolic processes (Bellettini et al. 2019; Umar et al. 2023). In this study, sealed plate cultures of *A. polytricha* (Mont.) Sacc. exhibited superior mycelial growth compared to unsealed plates, suggesting that direct exposure to air is not favorable for *A. polytricha* (Mont.) Sacc. The shorter mycelial diameter observed in unsealed cultures indicates a slower rate of mycelial growth. Thus, the presence of air negatively impacts the development of *A. polytricha* (Mont.) Sacc. mycelia. Similar aeration preferences have been observed in other mushroom species, including various *Lentinus*

isolates (Dulay et al. 2021; Kalaw et al. 2021). However, it is important to note that certain mushrooms do require oxygen for their growth and development.

Light is an important factor to consider as it can influence various stages of mushroom growth, including mycelial colonization, fruiting body formation, and morphology. Based on statistical analysis, illumination is not a critical factor for the mycelial growth of *A. polytricha* (Mont.) Sacc. since there is no significant difference in the daily mycelial diameter between lighted and dark conditions. Although mushrooms are generally considered to be non-photosynthetic organisms, some of them respond to light. This was observed for another strain of *A. polytricha* (Mont.) Sacc. and *A. auricula* which exhibited efficient mycelial growth when exposed to lighted condition (Priya and Geetha 2016).

Temperature directly affects the metabolic activity, growth rate, and distribution of mushrooms. The results obtained in this study suggest that *A. polytricha* (Mont.) Sacc. can be categorized as a tropical species, as it exhibited the fastest mycelial growth at 32°C. A broader temperature range of 20 to 35°C was suitable for optimal growth of *A. polytricha* (Mont.) Sacc. (Vidyaresmi 2008). It is good to note that *A. polytricha* (Mont.) Sacc. can also survive at lower temperatures (26°C), although it exhibits a reduction in mycelial diameter. Incubation of *A. polytricha* (Mont.) Sacc. at 32°C possibly favors the metabolic processes that occur during mycelial development, leading to increased enzymatic activity and faster growth.

Influence of spawning material. The mean mycelial diameter per day of *A. polytricha* (Mont.) Sacc. in cracked corn seeds, sorghum seeds, and rice seeds is presented in Table 4. Among the three spawning materials evaluated, cracked corn was found to be the most suitable for the luxurious growth of *A. polytricha* (Mont.) Sacc. which recorded the highest mycelial diameter (6.54 mm) and very thick mycelia. However, the lowest mean mycelial growth was recorded in rice seeds (4.33 mm) with very thin mycelia (Fig. 2).

Table 4. Mycelial growth per day of *A. polytricha* (Mont.) Sacc. grown on different spawning materials.

Spawning material	Mycelial growth per day (mm)
Cracked corn seeds	6.54 ± 0.25^{a}
Sorghum seeds	5.28 ± 0.21^{b}
Rice seeds	4.33 ± 0.48^{c}

Means with the same letter superscript are not significantly different from each other at 5% level of significance using Tukey's HSD.

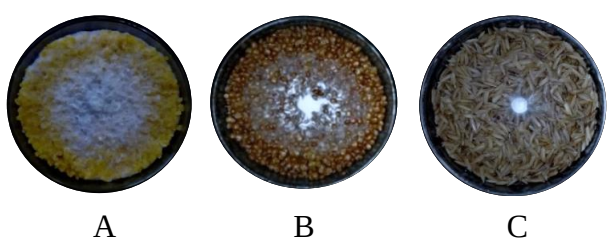

Figure 2. Mycelial growth of *A. polytricha* (Mont.) Sacc. *A. polytricha* on different spawning materials (A) cracked corn seeds (B) sorghum seeds and (C) rice seeds after 13 days of incubation.

Rapid mycelial growth and the development of a dense mycelial network observed in cracked corn seeds suggest that the nutrients present in corn are favorable to the growth of *A. polytricha* (Mont.)

Sacc. Corn contains high amounts of carbohydrates, proteins, vitamins, and minerals that support the optimal growth of *A. polytricha* (Mont.) Sacc. (Loy and Lundy 2019). Contrastingly, it demonstrated fast mycelial growth in sorghum compared to corn and rice seed with 17.67 mm (Zurbano 2018).

Fruiting body production in different rice straw and sawdust-based substrate formulations. The fruiting body production *of A. polytricha* (Mont.) Sacc. using rice straw and coconut sawdust as substrates were investigated. The different parameters gathered for fruiting body production are shown in Table 5. *A. polytricha* (Mont.) Sacc. successfully produced fruiting bodies in different formulations of rice straw and coconut sawdust. Among the different formulations, T1, T2, and T3 displayed the shortest incubation period of 29 days, which is significantly comparable to T4, T4, T6, and T7. Conversely, T11 exhibited the longest incubation period of 35 days. The earliest formation of primordia was observed in T1 after 53 days, which is significantly comparable to T2-T6. However, T9-T11 took 59 days for primordia to form. In terms of cap diameter, no significant difference was found between T1-T5, which showed wider diameters compared to the other treatments. The optimum yield was obtained from T3, with a mean of 75.57g per 500g of substrate, while the lowest yield was obtained from T11 with a mean of 15.95g.

Table 5. Fructification parameters of *A. polytricha* (Mont.) Sacc. using different formulations of rice straw (RS) and sawdust (SD).

Rice straw (RS): Sawdust (SD) ratio		Incubation period (day)	Primordia formation (day)	Cap diameter (mm)	Yield Per bag (g)	BE (%)
T1	10 RS:0 SD	29±0.52^{e}	53±0.41^{d}	43.02±6.86^{ab}	66.31±8.78^{a}	13.26±1.75^{a}
T2	9 RS:1 SD	30±0.41^{de}	54±0.00^{d}	44.97±6.34^{a}	65.75±3.19^{a}	13.15 ±0.63^{a}
T3	8 RS:2 SD	30±0.41^{de}	54±0.41^{d}	43.27±4.95^{ab}	75.57±7.40^{a}	15.11±1.48^{a}
T4	7 RS:3 SD	30±0.00^{de}	54±0.41^{d}	39.90±4.24^{ab}	65.19±4.97^{a}	13.03±0.99^{a}
T5	6 RS:4 SD	30±0.00^{de}	54±0.00^{d}	39.90±3.61^{ab}	54.00±6.39^{b}	10.80±1.27^{b}
T6	5 RS:5 SD	30±0.00^{de}	54±0.52^{d}	36.12±3.57^{b}	36.76±5.86^{c}	7.35±1.17^{c}
T7	4 RS:6 SD	30±0.52^{de}	55±0.52^{c}	30.72±3.10^{c}	35.04±4.82^{c}	7.00±0.96^{c}
T8	3 RS:7 SD	33±0.41^{c}	57±0.55^{b}	28.40±3.32^{c}	27.52±5.44^{c}	5.50±1.08^{c}
T9	2 RS:8 SD	34±0.41^{bc}	59±0.00^{a}	29.33±2.05^{c}	26.00±5.9^{cd}	5.43 ±1.28^{c}
T10	1 RS:9 SD	34±0.00^{ab}	59±0.00^{a}	28.98±1.19^{c}	14.17±3.26^{de}	2.83 ±0.65^{d}
T11	0 RS:10SD	35±0.00^{a}	59±0.00^{a}	28.61±1.27^{c}	15.95±3.13^{e}	3.19±0.62^{d}

In each column, means with the same letter superscript are not significantly different from each other at a 5% level of significance using Tukey's HSD.

In terms of BE, T1-T4 significantly exhibited the highest BE ranging from 13.03% to 15.11%, followed by T5 with 10.80%. The lowest BE was obtained from T10 and T11. The fruiting body performance of *A. polytricha* (Mont.) Sacc. on the different rice straw-sawdust substrate formulations is shown in Fig. 3.

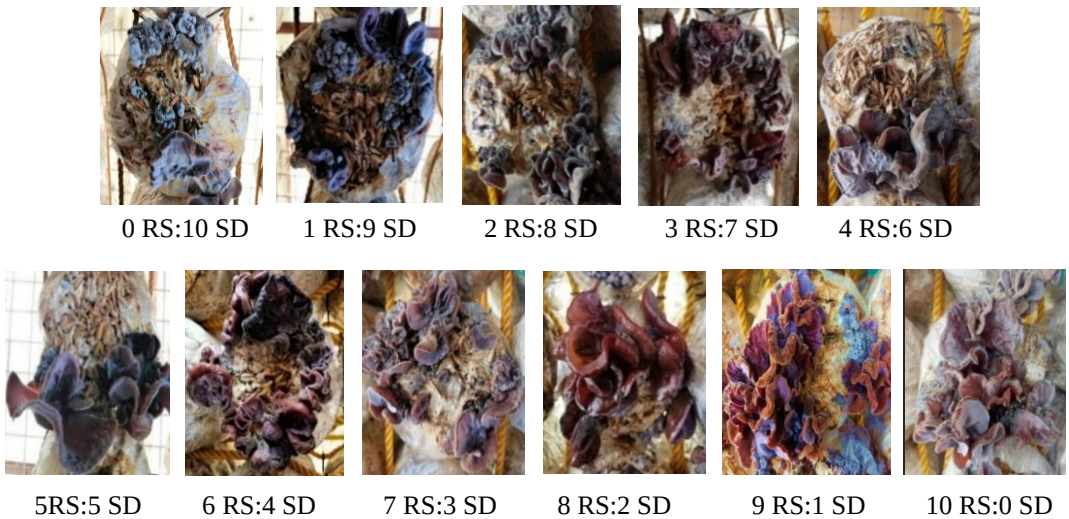

Figure. 3. Fruiting body production of *A. polytricha* (Mont.) Sacc. on different rice straw and sawdust substrate formulations.

The ability of a mushroom to produce a fruiting body using formulated substrates indicates that it can be cultivated and used for commercialization (Kalaw et al. 2021). The right formulation of the substrate is critical in mushroom cultivation as it affects the yield and quality of the fruiting body (Suwannarach et al. 2022). Three-part wheat and rice bran and one-part paddy straw were the best substrates for fast mycelial growth of *A. polytricha* (Mont.) Sacc. (Devi et al. 2013).

The incubation period refers to the time it takes for the mycelia to fully colonize the substrate. During this period, mycelia breaks down the organic material present in the substrate, utilizing it for continuous development. Results show that *A. polytricha* (Mont.) Sacc. can colonize the different substrate formulations within 29 to 35 days. Similarly, it fully colonized the substrate made up of sawdust and stalks of the grass plant *Pennisetum purpureum* within 32 days (Liang et al. 2019) while it took 29−38 days for the *A. polytricha* (Mont.) Sacc. Malaysia strain to fully colonize its palm oil waste substrate (Razak et al. 2013). A. *polytricha* (Mont.) Sacc. Taiwan strain grown on different ratios of various spent mushroom sawdust supplemented with 9.5% rice bran and 0.5% calcium carbonate took 19.6 to 37.4 days to fully colonize the substrates (Wu et al. 2020).

Primordia are the initial structures that develop into mature basidiocarps once the substrate is fully colonized by mycelia. In this study, these structures were observed from about 53 to 59 days after inoculation. This observation is aligned with the incubation period wherein the formation of primordia appeared earlier on substrates with higher percentages of rice straw. Rice straw has higher levels of hemicellulose and lower levels of lignin compared to sawdust, which possibly allows faster mycelial growth on these substrates. Since mushrooms have many cellulase enzymes, they can easily degrade and utilize these materials leading to faster mycelial colonization (Afsar et al. 2024; Suwannarach et al. 2022). This fast decomposition of the substrate initiates the formation of the primordia as the mycelia mature enough to produce fruiting initials. Palm oil waste-based substrate supplemented with rice bran took 43-45 days for primordia formation (Razak et al. 2013) while it only took 25.1−38.1 days to form fruiting initials in mushroom spent sawdust enriched with rice bran and calcium carbonate (Wu et al. 2020). Paddy straw and sawdust with a 3:1 ratio required 52.3 days to form primordia (Devi et al. 2013).

The quality of the cap, or pileus, is an important characteristic of mushrooms. Larger caps obtained in treatments with a high percentage of rice straw indicate that the mushroom is well

developed. The nutrients present in the substrate are in the right amount supporting the cap to develop and expand fully. Smaller caps obtained from substrates with less rice straw content could possibly due to the insufficient amount of nutrients, thus- resulting in underdeveloped caps.

Another indicator that *A. polytricha* (Mont.) Sacc. is highly favored in these substrate formulations is the yield, which refers to the total number of fruiting bodies that can be harvested from a fruiting bag. The optimum yield produced in T3 suggests that this kind of substrate formulation best supports the growth and development of the fruiting bodies from primordia initiation up to fruit maturation. Similar to sawdust which contains cellulose and lignin, rice straw is composed primarily of cellulose, hemicellulose, lignin, minerals, vitamins, and nitrogen (Boadu et al. 2023; Sharma et al. 2023). Together, these components of rice straw serve as energy sources for the mycelia during colonization up to fruiting body development and maturity (Sarnklong et al. 2010).

However, the yield of *A. polytricha* obtained in this study is lower compared to prior investigations. This observed variation may be attributed to the specific strain of mushroom used, as different strains exhibit varying responses to substrates. Another reason could be the variations in the combinations of the substrates and the addition of supplements to enhance the nutritional attributes of the substrate. In this study, pure coconut sawdust and rice straw were used to grow *A. polytricha* whereas in other studies, supplementation of either sugar, pH regulators, or rice or wheat bran to any well-composted substrate was employed which generated a higher yield of *A. polytricha* (Mont.) Sacc. (Onyango et al. 2011). Rubber sawdust enhanced with rice bran, sugar, and calcium carbonate can produce maximum yield (Vidyaresmi 2008). Throughout the fruiting period, a total of 254 grams of *A. polytricha* (Mont.) Sacc. was obtained in a substrate consisting of good lumber sawdust, rice bran, and lime (Zurbano 2018).

Total yield ranging from 107.3−147.6 grams was obtained from paddy straw-based substrates combined with either rice bran, wheat bran, or sawdust (Devi et al. 2013). The variance in yield between our study and that of Devi et al. (2013) can be attributed to environmental conditions. In their study, the temperature (24°C) and relative humidity (85%) were maintained whereas in this study, temperature and humidity tend to be relatively higher given the fact that the Philippines is a tropical country. Another reason could be the different sizes of fruiting bags, the amount of substrate used, and the ratio of each component of the substrate. These variations during cultivation have a significant impact on the growth of mushrooms which consequently affects the overall yield.

BE measures the ability of a mushroom to convert a certain amount of substrate into fruiting bodies. Among the 11 substrate formulations, the optimum BE was obtained from 8 parts rice straw and 2 parts coconut sawdust (T3) which indicates it to be the best substrate formulation that can support the optimum activity of the mycelia to convert the nutrients into fruiting bodies. *A. polytricha* (Mont.) Sacc. showed higher BE when grown in *F. moluccana* sawdust enhanced with rice bran (Irawati et al. 2012). Moreover, 100% BE was achieved from 78% sawdust, 20% bran, 1% CaCO3, and 1%, sucrose (Rai, 2004), and 80.9% was obtained from the combination of rubberwood sawdust, rice bran, and calcium carbonate (Teoh et al. 2018).

The *A. polytricha* (Mont.) Sacc. strain used in this study had a lower BE compared to previous studies. These variations could be due to differences in the substrate composition, weight of the substrate, as well as the technique of BE calculation. Previous studies calculated BE using the dry weight of the substrate, whereas, in this study, the original weight of the substrate was used. Furthermore, another contributing factor is the supplementation of substrates. Earlier investigations employed the addition of supplements to the substrates to enhance nutrient availability, while this study focused on utilizing pure coconut sawdust and rice straw without any supplementation. In terms of nutrient availability, substrate with supplements is advantageous for mycelial development. Since only the nutrients naturally present in rice straw and coconut sawdust were used by the mycelia, a lower BE was obtained in this study. The presence of supplemental nutrients might have a positive effect on the

efficiency of mycelial growth and, consequently, the biological efficiency of the mushroom. The low BE in pure coconut sawdust (treatment 11) is in congruence with the findings for other *A. polytricha* (Mont.) Sacc. strains that did not show mycelial growth in pure good lumber sawdust (Zurbano 2018).

CONCLUSION

The mycelial growth of *A. polytricha* (Mont.) Sacc. is favored when grown in potato sucrose agar with a pH concentration of 6-7. Optimum mycelial growth can be achieved in an unaerated condition at 32°C. Cracked corn seeds are the best spawning material and the optimum substrate formulation for fruiting body production is 8 parts rice straw and 2 parts sawdust. This established cultivation technology for *A. polytricha* (Mont.) Sacc. can be adapted and utilized by local growers specifically in regions where there is a sufficient supply of rice straw and sawdust. This could lead to a consistent mushroom supply, benefiting the agricultural sector of the country. It is recommended that the substrate be supplemented with other materials to enhance the fruiting body production of *A. polytricha* (Mont.) Sacc.

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