



Optimization of Culture Conditions for Mycelial Growth and Basidiocarp Production of *Cyclocybe cylindracea* (Maire)

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Submitted June 26, 2019. Accepted August 26, 2019. Published online July 31, 2020.

Abstract

To develop production technology to popularize the utilization of the mushroom, *Cyclocybe cylindracea*, the influence of culture media, pH, and physical factors such as aeration, illumination, and temperature for optimum mycelial growth and fruiting body production was investigated. Among the media evaluated, potato sucrose agar, rice bran decoction agar, and malt extract agar recorded the highest mycelial growth rate (9.00 mm/day), shortest incubation period (10.00 days) with very thick mycelial density. Potato sucrose agar (most common culture medium) at pH 6 incubated in either sealed or unsealed, lighted or dark, and in room temperature (28-33°C) or air-conditioned temperature (23-25 °C), registered the highest mean mycelial growth rate. The substrate formulation of 7:3 rice straw-sawdust ratio recorded the highest total yield of 106.94g, and biological efficiency of 12.89%. These findings are significant in the generation of practical production technology that can be adopted by Filipino mushroom growers and those from other countries with similar environmental conditions, to increase the number of cultivated mushrooms regardless of the strain origin.

Key Words: culture media, *Cyclocybe cylindracea*, grain spawn, mycelial growth, physical factors

Introduction

Although mushrooms have been domesticated for cultivation since early times, the most commonly grown strains are temperate species (Stamets, 2000; Muruke et al., 2002; Ferreira et al., 2010). In addition, numerous new species have been introduced to science, some being edible and others that are medicinal (Le et al., 2007; Sanmee et al., 2008; Zhao, 2003; Kerekes and Desjardin, 2009; Wannathes, Desjardin, & Lumyong, 2009; Wannathes, Desjardin, Hyde et al., 2009; Zhao et al., 2010; Zhao et al., 2011). However, tropical regions like the Philippines have the potential to be one of the

richest foundations of cultivatable fungal species. In fact, the country has acquired various native and exotic species of mushrooms such as *Schizophyllum commune*, *Lentinus tigrinus*, *Lentinus sajor-caju*, *Ganoderma lucidum*, *Collybia reinakeana*, and *Panaeolus antillarum* which exhibited antidiabetic, antibacterial, anti-inflammatory, antioxidant, antihypertensive, and anticoagulative properties (Reyes et al., 2013; Dulay et al., 2014; Eguchi et al., 2014; Dulay et al., 2015). In our continuous search for additional mushrooms for cultivation, we also studied the optimization and adaptation of exotic mushrooms such as *C. cylindracea*.

Cyclocybe cylindracea is an edible white rot basidiomycetes, which can be cultivated in different agricultural residues. The fruiting bodies are excellent, high quality mushroom with a pleasant aroma, delicious taste, and high nutritional value (Uhart & Alberto, 2007; Noonsong et al., 2016). The fruiting bodies of *C. cylindracea* are known for the biosynthesis of several secondary metabolites that have been studied in more detail for its anti-oxidative and antimicrobial activities as well as tumor-protective capacities (Zhao et al., 2003). Moreover, it was found to be medically active in several therapeutic effects such as antitumor, antifungal, nerve tonic, hypercholesterolemia, and hyperlipidemia (Wasser & Weis, 1999; Cheung et al., 2003; Lo & Cheung, 2005; Tsai et al., 2007). Presently, numerous studies are initiated in order to extend awareness of those properties as well as wider exploitation of *C. cylindracea* in medicine (Jasińska et al., 2012).

Interestingly, *C. cylindracea* is cultivated mainly on low-cost substrates derived from agricultural and forest wastes, such as barley and wheat straw, orange peel, grape stalks, rice husks, sawdust of broad leaf trees, as well as on the stumps of cottonwoods (Philippoussis et al., 2001). Depending on the substrate used, mycelium cultivation is enhanced by different environmental and nutritional factors (Watling, 1992). This work aimed to investigate the mycelial growth response to the nutritional and physical factors and fruiting body production using rice straw- and sawdust-based substrates. Mushroom growers and farmers can utilize the production technology that will be developed for *C. cylindracea*.

Materials and Methods

Source and Revival of Culture

The pure culture of *C. cylindracea* (Japan strain) was obtained from the culture collection of the Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Mycelia were sub-cultured on potato dextrose agar plate and incubated at room temperature for seven days. Mycelial discs were prepared using a flame-sterile 10 mm-diameter cork borer and served as inoculants.

Evaluation of Culture Media

The effects of indigenous and commercial culture media, namely: coconut (*Cocos nucifera*) water agar (CWA), potato (*Solanum tuberosum*) decoction sucrose agar (PSA), corn (*Zea mays*) grit decoction agar (CDA), and rice (*Oryza sativa*) bran decoction agar (RBDA); potato dextrose agar (PDA; 39g/1L distilled H₂O), malt extract agar (MEA; 47g/1L distilled H₂O), mycological agar (MA; 35g/1L distilled H₂O), and Sabouraud dextrose agar (SDA; 30g/1L distilled H₂O) were determined. Broth were prepared by separately boiling 250 g potato, 50 g corn grit, and 50 g rice bran in 1 L distilled water, reconstituted to 1 L and added with 20 g of agar and 10 g sucrose. On the other hand, 1 L of matured coconut water was boiled and added with 20 g of agar. These media were adjusted to pH 6 and transferred into flasks plugged with cotton and wrapped with aluminum foil. These were sterilized in an autoclave at 121 °C and 15 psi for 30 min. Media were pour-plated, allowed to solidify, and inoculated centrally with mycelial discs and incubated. Mycelial growth rate, incubation period, and

mycelial density were determined. Mycelial density was observed visually and rated as described by Reyes et al. (2009) and De Leon et al. (2017).

Evaluation on the Influence of pH

Potato sucrose agar was used as basal medium in the evaluation of pH and physical factors. The medium was adjusted to different pH levels (6.0–8.0) using 1 M NaOH and HCl, sterilized, pour-plated, inoculated with mycelial discs, and incubated. Mycelial growth rate, incubation period, and mycelial density were determined.

Evaluation of Physical Factors for Mycelial Growth

The best culture medium with optimum pH plates were prepared, sterilized, inoculated, and incubated in two aeration conditions: (1) the sealed condition in which the petri plates were covered with parafilm, and (2) unsealed condition in which petri plates were left without parafilm. To evaluate illumination, mycelial discs were inoculated in the best medium using pH plates and incubated in required illumination conditions (lighted; 137 lux, full dark conditions; covered with black cover and in alternating light and dark condition; 12 hr light, 12 hr dark). Lastly, the effect of temperature was evaluated by incubating the plates in three different temperatures, 28–33°C (room temperature), 23–25°C (air-conditioned), and 9°C (refrigerated). Mycelial growth rate, incubation period, and mycelial density were determined.

Evaluation of Grain Spawn

Five different spawn materials, namely: unmilled rice, corn grit, wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), and millet (*Panicum miliaceum*) were evaluated. The grains were boiled until tender, drained, and 40 grams of each were dispensed into petri plates and were sterilized at 15 psi or 121°C for 45 min. After sterilization and cooling, the plates were inoculated with a 10-mm fungal disc from 7-day old pure culture and incubated at room temperature (32°C) to allow ramification of the mycelia. The mycelial growth rate, incubation period, and mycelial density were determined.

Evaluation of Fruiting Body Production

Rice straw was soaked in a water tank overnight and then washed with running water to remove undesirable odor. The washed rice straw was hauled out from the tank, piled, and covered with plastic sheets to stimulate the growth of native decomposers. The pile was turned every day for three consecutive days until materials became pliable and dark brown. After the decomposition process, the rice straw was chopped at about 50–75 mm in size and mixed with parts of sawdust with 65–70% moisture content. Approximately 750 g of formulated substrates was bagged and compacted in a 15.24 x 30.48-cm polypropylene bag. Bagging of each treatment was replicated 5 times. The bags were plugged with cotton and pasteurized at 80–100°C for 6 hr. The bags were allowed to cool before inoculation with 40 g of best grain spawn of mushrooms from the previous sub-study. The inoculated bags were incubated in the mushroom house at 32°C to allow full ramification of mycelia. The number of days of total mycelia ramification and primordial initiation on different formulated rice straw-based substrates were recorded. Bags with fruiting initials (primordia) were opened to allow maturation of basidiocarp. Matured fruiting bodies were harvested and weighed to determine the total yield and its biological efficiency. Percent biological efficiency was calculated as $BE = (\text{total fresh weight of harvested mushrooms} / \text{initial weight of the substrate}) \times 100$.

Statistical Analysis

All the treatments were laid out in complete randomized design under laboratory conditions. Tukey's HSD Test was used to determine significant differences between treatment means at 5% level of significance. The SAS 9.1 program was used for the analysis (SAS Institute Inc. Cary, NC, USA).

Results and Discussion

Effect of Culture Media

The choice of appropriate culture medium is an important consideration in growing mushrooms. The ideal culture medium should contain the necessary ingredients that will support the growth of the mushroom. At present, there are indigenous and commercial culture media that can be used in mushroom production. The suitable culture medium will produce the densest mycelial growth in the shortest time (Zubano et al., 2017; Magday et al., 2014). In this present study, four indigenous and four commercial culture media were evaluated. All culture media supported mycelial growth of *C. cylindracea* (Table 1; Figure 1). However, among the different culture media evaluated, PSA, RBDA, and MEA recorded the highest mean mycelial growth rate at 9.00 mm/day, and the shortest incubation period of 10.00 days with very thick mycelial density. Conversely, CWA and PDA recorded the lowest mean mycelial growth rate (value?). These results correspond to the findings reported by Dulay and Garcia (2017) which showed that the highest mycelial growth rate of Philippine strain of *L. strigosus* was obtained in potato sucrose agar and lowest in coconut water agar. Likewise, potato sucrose agar favored mycelial growth of other Philippine wild mushrooms including *Volvariella volvacea* CLSU and Rang-ayan strains and *Coprinopsis cinerea* Sto. Domingo strain (Kalaw et al., 2016). In addition, the results obtained agreed with the findings of Nasim et al. (2001) and Muthu and Shanmugasundaram (2017) which stated that mycelial growth of *P. ostreatus*, *P. sajor-caju*, *P. cystidiosus*, *V. volvacea*, and *A. aegerita* were maximum in malt extract agar medium. Moreover, Dulay et al. (2015) revealed that *L. sajor-caju* and *L. tigrinus* efficiently grew on rice bran decoction.

Table 1

Mycelial Growth, Incubation Period, and Mycelial Density of C. cylindracea on Different Culture Media

Culture Media	Mycelial Growth Rate (mm/day)	Incubation Period (days)	Mycelial Density
Coconut Water Agar	6.66 ^c	13.00 ^a	++++
Potato Sucrose Agar	9.00 ^a	10.00 ^c	++++
Corn Grit Decoction Agar	8.18 ^b	11.00 ^b	++
Rice Bran Decoction Agar	9.00 ^a	10.00 ^c	++++
Potato Dextrose Agar	6.66 ^c	13.00 ^a	++++
Malt Extract Agar	9.00 ^a	10.00 ^c	++++
Mycological Agar	6.81 ^c	13.00 ^a	++++
Saboraud Dextrose Agar	6.92 ^c	13.00 ^a	++++

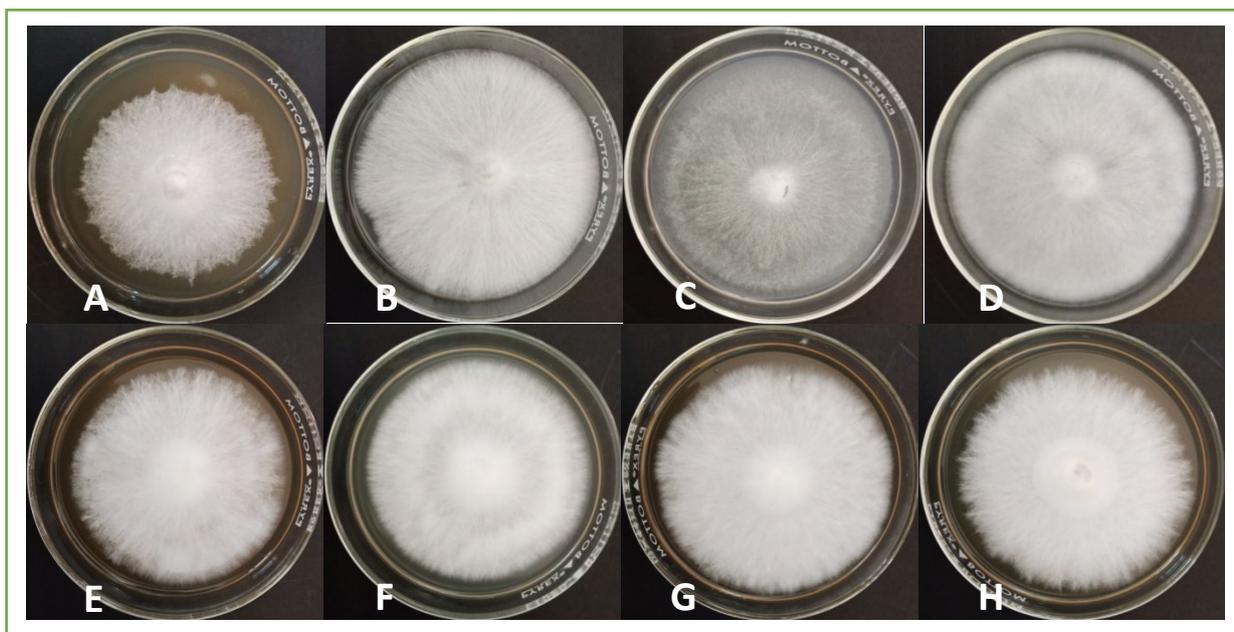
Note: Means with the same superscript are not significantly different from each other at 5% level of significance +++++ (very thick), +++ (thick), ++ (thin), + (very thin)

The substantial variation in the mycelial density response of *C. cylindracea* could be due to differences in the nutrient composition of culture media examined. The dense growth of mycelia in PSA can be attributed to the nutritional content of potato. Based on previous studies, potato contains nutrients such as fructose, sucrose, amylase protein, vitamins (such as thiamine, niacin, folic acid), and

minerals (such as potassium, phosphorus, and magnesium). Furthermore, potato supplies suitable sources and quantities of nitrogen and growth factors. The good growth of mycelia frequently observed on PSA suggested that such stimulation is widespread (Borlingame et al., 2009; Kalaw et al., 2016). On the other hand, MEA provides carbon, protein, and nitrogen source required for mycelial growth and is recommended for the cultivation, isolation, and maintenance of different mushroom species. It also contains digests of animal tissues (peptones) which provide sources of amino acids, nitrogenous compounds, and polysaccharides, which are used as energy source for mycological growth (Atlas, 1997; Stevens, 1981). Rice bran is also a valuable source of carbohydrates (49.69 g), dietary fiber (21 g), sugar (0.9 g), sucrose (0.5 g), glucose (0.2 g), fructose (0.2 g), protein (13.35 g), and fat (21g) which could be essential to mycelial growth (USDA, 2012). It also has significant amounts of proteins, lipids, edible fiber, minerals (magnesium, potassium, phosphorus, iron, manganese, and zinc), and vitamins (thiamin, riboflavin, niacin, pyridoxine, pantoic acid, biotin, and tocopherol) (Oliveira et al., 2012). Since PSA was the most commonly used culture medium, it was used as an assay medium in the evaluation of pH and physical parameters such as aeration, illumination, and temperature.

Figure 1

Mycelial Growth of C. cylindracea on the Different Indigenous Media at Ninth Day of Incubation



Note: (A) Coconut Water Agar, (B) Potato Sucrose Agar, (C) Corn Grit Decoction Agar, (D) Rice Bran Decoction Agar, (E) Potato Dextrose Agar, (F) Malt Extract Agar, (G) Mycological Agar, and (H) Sabouraud Dextrose Agar

Effect of pH

The pH of a culture medium is an important parameter that has been described to affect fungal morphology (Gibbs et al., 2000). Mushroom mycelia have a good tolerance to low water activity, pH, and high osmotic pressure (Atlas, 1997). Although the effect of pH on the growth of mycelia depends to some extent on the species, pH 4–7 seems to give the best yields of mycelia (Carlie & Watkinson, 2000). To determine the optimum pH requirement of *C. cylindracea*, the initial pH of the medium was adjusted to different pH levels ranging from 6.0 to 8.0 at intervals of 0.5.

The mycelial growth rate at different pH levels varied significantly (Table 2). Among pH levels, pH 6 recorded the highest mean diameter of 9.85mm, followed by those grown at pH 6.5 and pH 7 (Figure 2). Treatments had the same incubation period of 10 days.

The result of this study agreed with the findings of Aminuddin et al. (2013), who reported that the most favorable pH range for the growth of mushrooms is pH 6–7. However, it does not conform to the results obtained by Dulay et al. (2012) which registered the fastest mycelial growth of *L. tigrinus* at pH 8. Consequently, mushroom mycelia develop in optimum pH ranges which are related to enzymatic systems, essential vitamin entry in the cell, surface metabolic reactions, and mineral capture. Some fungi, however, are able to adjust to the pH of the medium, optimizing it for their better development (Barros et al., 2006).

Table 2

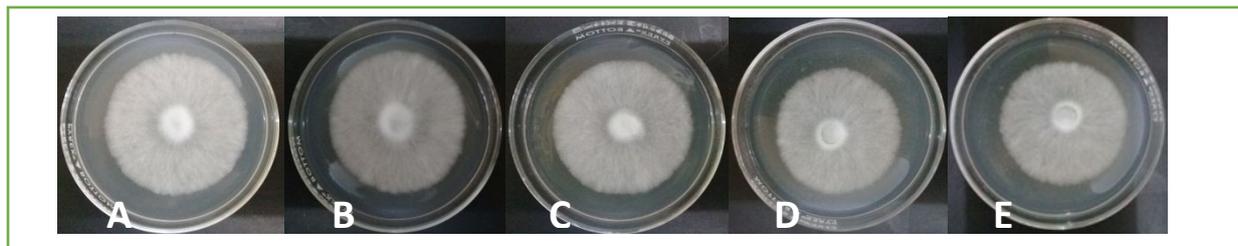
Mycelial Growth, Incubation Period, and Mycelial Density of C. cylindracea on Different pH Levels on PSA

pH Levels	Mycelial Growth Rate (mm/day)	Incubation Period (days)	Mycelial Density
6.0	9.85 ^a	10.00	++++
6.5	9.42 ^{ab}	10.00	+++
7.0	9.48 ^{ab}	10.00	+++
7.5	9.28 ^b	10.00	++++
8.0	9.35 ^b	10.00	++++

Note: Means with the same superscript are not significantly different from each other at 5% level of significance +++++ (very thick), +++ (thick), ++ (thin), + (very thin)

Figure 2

Mycelial Growth of C. cylindracea on Different pH Levels at Seventh Day of Incubation



Note: (A) 6, (B) 6.5, (C) 7.0, (D) 7.5, and (E) 8

Influence of Physical Growth Factors

Mycelium cultivation is enhanced by different environmental and nutritional factors, and the propagation of mycelia is an earlier and essential step to artificial cultivation of mushrooms (Watling, 1992). After the establishment of the suitable nutritional requirements for the successful cultivation of the mycelia, environmental conditions including aeration, illumination, and temperature were evaluated on the growth of *C. cylindracea* (Table 3).

Concentration of oxygen and carbon dioxide are important factors for the mycelial growth of mushrooms (Belletini et al., 2019). Most fungi are considered aerobes, although many can also grow in oxygen-deprived habitats or hypoxic conditions (Leatham & Stahmann, 1987). The influence of

aeration on the mycelial growth rate, incubation period, and mycelial density of *C. cylindracea* were evaluated. It can be seen that aeration is not an important factor since no significant difference was found between sealed and unsealed conditions in terms of mycelial diameter and incubation period (Figure 3). Both conditions showed very thick mycelial density. Similarly, both *L. tigrinus* and *P. djamor* favor both sealed and unsealed conditions (Dulay et al., 2012; Bumanlag et al., 2017). However, it does not conform to the findings of Reyes et al. (2009) and Tolentino (2016) who reported that *C. comatus*, *P. florida*, *P. sajor-caju*, and *P. pulmonarius* cultivated best in sealed conditions.

Table 3

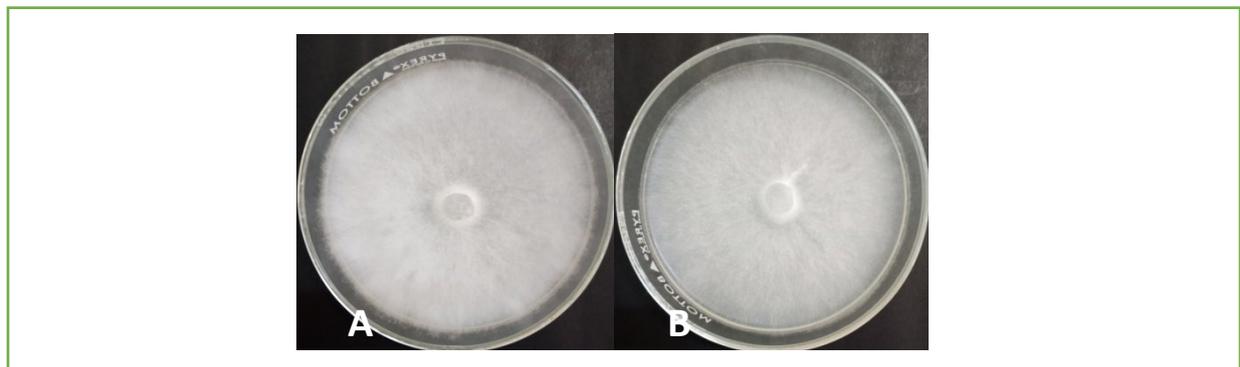
Mycelial Growth, Incubation Period, and Mycelial Density of C. Cylindracea Exposed to Different Physical Factors

Physical Factors	Mycelial Growth (mm/day)	Incubation Period (days)	Mycelial Density
Aeration			
Sealed	10.85 ^a	9.00	++++
Unsealed	10.51 ^a	9.00	++++
Illumination			
Lighted	9.06 ^a	9.00	++++
Alternate	9.16 ^a	9.00	++++
Dark	9.20 ^a	9.00	++++
Temperature			
Room temperature	8.40 ^a	10	+++
Air-conditioned	8.41 ^a	10	+++
Refrigerated	0.00 ^b	---	---

Note: Means with the same superscript are not significantly different from each other at 5% level of significance +++++ (very thick), +++ (thick), ++ (thin), + (very thin)

Figure 3

Mycelial Growth of C. cylindracea on PSA as Influenced by Aeration at Ninth day of Incubation



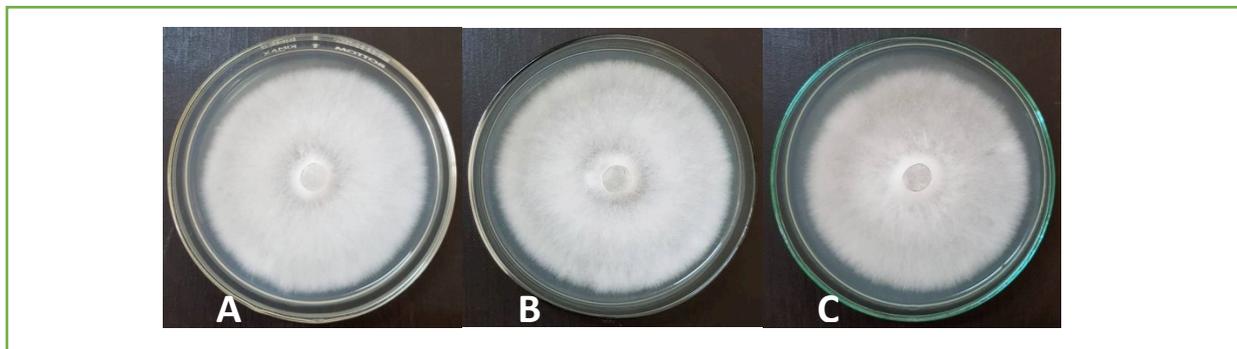
Note: (A) Sealed and (B) Unsealed

Light is of great importance for many aspects of basic and applied mycology, particularly for mushroom cultivation (Kurtzman & Martinez-Carrera, 2013). Some fungi require light for particular stages of development although the situation varies between species. Responses of individual mycelia may be moderated by different environmental factors (Isaac, 1995). Depending on the fungus and developmental stage, light may stimulate or inhibit development (Leatham & Stahman, 1987).

Furthermore, there are certain species of fungi that are cultivated optimally in the dark, whereas others in restricted light (Bellettini et al., 2019). The effect of illumination was also evaluated by incubating the inoculated plates to three conditions, namely: lighted, alternating light and dark, and dark condition (Table 3). No significant difference in mycelial diameter response and mycelial density was observed with similar incubation periods (Figure 4). The findings of the present study indicate that the mycelial growth of *C. cylindracea* is not directly affected by illumination which agreed with the mycelial growth of *S. commune* and basidiospore germination of *V. volvacea* obtained by Reyes et al. (2009), and *P. djamor* (Bumanlag et al., 2018). On the other hand, it does not agree with the findings reported by Bustillos et al. (2014) in *P. antillarum* and *P. cyanescens* which exhibits better mycelial growth in dark conditions.

Figure 4

Mycelial Growth of C. cylindracea as Affected by Illumination at Seventh Day of Incubation



Note: (A) Lighted, (B) Alternate, and (C) Dark

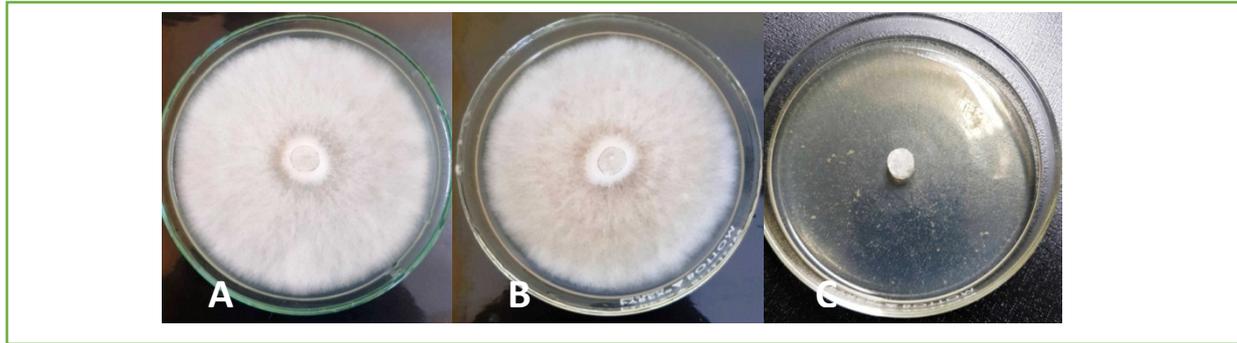
Temperature is a very significant environment factor for the mycelia growth of fungi (Hoa & Wand, 2015). Fungi can be classified based on their temperature requirement (Bumanlag et al., 2018). To determine optimal temperature for rate of mycelial growth, *C. cylindracea* was cultivated in PSA medium and incubated at three different temperatures: 32°C (room temperature), 23°C (air-conditioned), and 9°C (refrigerated). The mycelial growth rate, incubation period, and mycelial density of *C. cylindracea* are presented in Table 3. There was no significant difference on mycelial growth response of *C. cylindracea* incubated at 32°C and 23°C with mean mycelial growth rate of 8.40 mm/day and 8.41 mm/day, respectively. Moreover, both conditions have thick mycelial density (Figure 5) and the same incubation period of 10 days to full ramification. Interestingly, no growth was observed in the refrigerated condition. The results of the present study agreed with the findings of Klomklung et al. (2014) which recounted that mushroom mycelia were able to grow at temperatures ranging from 20–30 °C, with optimal growth temperatures of 30 °C and 25 °C for *Lentinus* and *Pleurotus* species, respectively. Though, the results of Jacob et al. (2015) contradict the findings of the present experiment which stated that *P. citrinopileatus*, *P. djamor*, *P. salmoneostramineus* are best grown in room temperature only. Zharare et al. (2010) also found that *P. sajor-caju* can tolerate high 35°C temperature. The insufficiency of growth in the refrigerated temperature denoted that low temperature can inhibit the mycelial growth of some fungi. Moreover, the sensitivity of these species to cold temperatures proves that it can be grown on tropical conditions.

Mycelial Growth on the Spawn Materials

Grain spawn has been a vital concern in mushroom cultivation which is accomplished by developing mushroom mycelia on supporting medium under controlled environmental conditions. In almost all cases, the supporting matrix is sterilized grain which is preferred due to its biochemical

Figure 5

Mycelial Growth of C. cylindracea as Affected by Temperature at Ninth Day of Incubation



Note: (A) Room Temperature, (B) Air-Conditioned, and (C) Refrigerated

properties and practical performance over others (Siddhant et al., 2013). It facilitates the rapid colonization of the mushroom substrate, hence reducing the days of the mycelial incubation period. Different grains rich in protein, carbohydrates, and other essential elements were used as grain spawning material in mushroom cultivation. Table 4 shows the mean mycelial growth, incubation period, and mycelial density of *C. cylindracea* on different grains. All the grains favored the growth of mycelia (Figure 6). However, corn grit had the highest growth rate of 9.01 mm/day and shortest incubation period which is significantly different to all other grains evaluated. De Leon et al. (2017) specified that corn contains nutrients best for the development of mycelia and substrates which benefits higher yield and denser weight of fruiting bodies of *L. squarrosulus*. In addition, Dulay et al. (2017) reported that cracked corn was the best granulated spawning material for mass production of mycelial inoculants for Philippine (CLSU) strain of *L. strigosus* (BIL1324). Environmental factors such as temperature, light, O₂, CO₂, humidity, and pH have also been described to affect mycelial growth in grain spawn preparation (Nwanze et al., 2005).

Table 4

Mycelial Growth, Incubation Period, and Mycelial Density of C. cylindracea on Different Grains

Spawning Material	Mycelial Growth Rate (mm/day)	Incubation Period (days)	Mycelial Density
Unmilled Rice	7.75 ^b	13.00 ^b	+++
Corn grit	9.01 ^a	11.00 ^c	++++
Wheat	7.90 ^b	13.00 ^b	++++
Sorghum	7.70 ^b	14.00 ^a	+++
Millet	7.48 ^b	13.00 ^b	+++

Note: Means with the same superscript are not significantly different from each other at 5% level of significance++++ (very thick), +++ (thick), ++ (thin), + (very thin)

Fruiting Body Production

Generating a practical production technology is a vital factor for mushroom cultivation. *C. cylindracea* is an edible white rot basidiomycetes which can be cultivated in different agricultural residues. In this study, rice straw-sawdust-based substrate formulations were evaluated for the fruiting body production of *C. cylindracea*. The number of days of incubation period, primordial initiation, and

diameter of pileus, length of stipe, total yield, and biological efficiency of *C. cylindracea* on the different substrate formulations are shown in Table 5.

Figure 6

Mycelial Growth of *C. cylindracea* on Grains at Seventh Day Of Incubation



Table 5

Incubation Period, Primordial Initiation, Diameter of the Pileus, Length of Stipe, Total Yield of Fruiting Bodies, and Percent Biological Efficiency of *C. cylindracea* Grown on the Different Substrate Formulations of Rice Straw and Sawdust

Substrates (Rice straw: sawdust)	Incubation Period (day)	Primordial Initiation (day)	Diameter of the Pileus (mm)	Length of Stipe (mm)	Total Yield/Bag	Biological Efficiency (%)
10:0	43.07 ^d	54.93 ^{de}	35.82 ^{ab}	40.49 ^a	98.17 ^{abc}	12.13
9:1	43.13 ^d	54.33 ^{de}	36.13 ^{ab}	41.70 ^a	96.79 ^{abc}	12.03
8:2	43.53 ^{dc}	55.60 ^{cd}	35.97 ^{ab}	41.72 ^a	94.99 ^{bcd}	11.30
7:3	42.33 ^d	52.40 ^e	36.81 ^{ab}	41.97 ^a	106.94 ^a	12.89
6:4	42.93 ^d	52.40 ^e	35.14 ^{abc}	37.04 ^b	101.21 ^{ab}	12.21
5:5	43.53 ^{dc}	55.26 ^{cd}	33.68 ^{bcd}	32.56 ^c	94.27 ^{bcd}	11.55
4:6	44.87 ^{bc}	56.33 ^{bcd}	32.33 ^{cde}	31.28 ^{cd}	93.12 ^{bcd}	12.12
3:7	45.73 ^b	56.73 ^{abcd}	31.35 ^{def}	29.45 ^{de}	87.28 ^{cdef}	11.35
2:8	48.33 ^a	57.8 ^{abc}	30.06 ^{efg}	28.87 ^{def}	83.83 ^{def}	11.05
1:9	49.4 ^a	58.87 ^{ab}	28.91 ^{fg}	27.75 ^{ef}	82.66 ^{ef}	10.81
0:10	49.53 ^a	59.47 ^a	28.01 ^g	26.52 ^f	78.38 ^f	10.34

Note: Means with the same superscript are not significantly different from each other at 5% level of significance

Incubation period was recorded as the number of days from inoculation of the substrate to complete mycelial ramification of the fruiting bags (Kalaw et al., 2017). The rate of mycelial colonization of the substrate is dependent on the nutrients present in the substrates and prevailing environmental conditions (Kalaw & Albinto, 2015). The shortest incubation period was recorded in 7:3 rice straw-sawdust (42.33 days) which is comparable to 10:0, 9:1, 8:2, 6:4, and 5:5. On the other hand, the most extensive incubation period was noted in pure sawdust (49.53 days) which is comparable to 1:9 and 2:8.

Results revealed a significant influence of higher amount of rice straw in mycelial colonization of *C. cylindracea*. This response was congruent with the results obtained by Dulay et al. (2017), who showed a shorter incubation period of *L. strigosus* in 60% rice straw + 40% sawdust. In addition, this result was also supported by Magday et al. (2014) who showed faster mycelial growth of *G. lucidum* in 7:3 rice straw-sawdust. Conversely, it does not conform to the findings observed by Obodai et al.

(2003), who revealed that *P. ostreatus* has a shorter mycelial proliferation in pure sawdust. The observed alterations among treatments used could be due to the proportions of the substrates. It has been perceived that a greater amount of sawdust could prolong the incubation period due to the small particle size of the sawdust. A small particle size results to a more compacted substrate that can hinder mycelial growth of the mushroom (Bumanlag et al., 2018; Stamets, 2000). In addition, the rice straw structures are hollow and contain pores where mushroom mycelium could proliferate properly. The presence of the pores provides enough oxygen (O₂) that is required by mushrooms in the early growth of the mycelium (Utami & Susilawati, 2017).

The occurrence of the primordial indicates the start of fruiting body development. On this scale, the number of days to formation of primordia was determined from the inoculation of the substrate to the appearance of the fruiting initials (Bumanlag et al., 2018). The substrate formulation consisting of 7:3 rice straw-sawdust had the shortest period with a mean of 52.4 days which is not significantly different to 6:4 rice straw-sawdust, while pure sawdust registered the longest period with a mean value of 59.47 days. These results revealed significant influence of the substrate formulations on the duration of primordial initiation of *C. cylindracea*. This finding implies that different proportions of rice straw and sawdust could considerably impact the appearance of the pin heads. The appearance of primordia is prolonged with an increase in the amount of sawdust. This is parallel to the results obtained by Girmay et al. (2016) with the longest period of primordial initiation in *P. ostreatus* using sawdust as substrate. However, this result does not conform to the observation of Dulay et al. (2017) who reported faster primordial initiation of *L. strigosus* in sawdust compared to rice straw. The small particle size of the sawdust made the substrates more compressed which lessens the amount of air present in the substrate and difficulty of the mycelia to penetrate the substrate.

The pileus is the upper cap-shaped part of a mushroom or similar spore-producing body. The diameter of the pileus of *C. cylindracea* as influenced by the different substrate preparations is also presented in Table 5. A significant difference was observed in the pileus diameter in different rice straw-sawdust substrate formulations. The highest diameter was recorded in 7:3 rice straw-sawdust with a mean value of 36.81 mm which is comparable to pure rice straw, 9:1 rice straw-sawdust, 8:2 rice straw-sawdust, and 6:4 rice straw-sawdust formulations; whereas the lowest was noted in pure sawdust with a mean value of 28.01 mm. The significant difference in the diameter of the pileus may possibly be affected by the nutrient content of the substrates. This result is congruent to the findings exhibited in *P. ostreatus* (Sitaula et al., 2018) and *P. florida* (Neupane et al., 2018) with higher diameter of the pileus using paddy rice straw compared to sawdust as mushroom substrate.

The stipe is the stem or stalk-like feature supporting the cap of a mushroom. The length of the stipe differs from species to species of mushrooms. The length of the stipe as affected by different rice straw-sawdust substrate formulations is also showed in Table 5. Significant difference was also observed on the length of the stipe in different substrate formulations. The highest value was obtained in 7:3 rice straw-sawdust with a mean value of 41.97 mm which is comparable to pure rice straw, 9:1 rice straw-sawdust, and 8:2 rice straw-sawdust; whereas the lowest value was recorded in pure sawdust with a mean of 26.52 mm. This finding suggested that the length of the stipe is affected by substrate proportions.

In the Philippines, rice straw- and sawdust-based formulations were used in the cultivation of *S. commune* (Bulseco et al., 2005), *L. sajor-caju* (Cuevas et al., 2009), *L. tigrinus* (Dulay et al., 2012), and *G. lucidum* (Tayamen et al., 2004). In this study, rice straw-sawdust-based substrate formulations were evaluated for the fruiting body production of *C. cylindracea* in order to generate a vital cultivation technology comparable to its natural environment.

The weight of fruiting bodies is the most substantial factor in mushroom production. The total yield of *C. cylindracea* on the different substrate formulations is shown in Table 5. The highest total

yield was found on 7:3 rice straw-sawdust with a mean value of 106.94 g/bag. This was followed by 6:4 rice straw-sawdust (101.21 g/bag). Conversely, the lowest yield was recorded in pure sawdust with 78.38 g/bag. Statistical analysis revealed a significant effect of substrate formulations on the yield of *C. cylindracea*. The variations in the yield could be ascribed to the nutrients present in the formulated substrates. This finding is congruent with the results obtained by Magday et al. (2014) which showed the highest total yield of *G. lucidum* on 7:3 rice straw-sawdust substrate formulations and lowest total yield on pure sawdust. In addition, Dulay and Rivera (2017) also showed highest total yield of *Polyporus grammacephalus* in 6:4 rice straw-sawdust substrate formulations. However, De Leon et al. (2017) showed that *L. squarrosulus* produced the highest total yield in 2:8 rice straw-sawdust substrate formulation.

Biological efficiency is the measure of effectiveness of the mushroom organism to convert the substrate into fruiting bodies. This parameter is significant in selecting the substrate to be used in the cultivation of a specific mushroom. The biological efficiency of *C. cylindracea* on different substrate proportions is likewise presented in Table 5. Results revealed that 70% rice straw: 30% sawdust formulation has the highest percentage biological efficiency with a mean value of 12.89% which is comparable to all other treatments except from 100% sawdust which registered the lowest percent biological efficiency with a mean value of 10.34%. The biological efficiency obtained in this study is relatively higher than *S. commune* (5.69%) and *G. lucidum* (6.26%) grown on the same substrate formulation reported by Kalaw et al. (2017). However, the value obtained in the present study is lower than the conveyed biological efficiency of *P. djamor* (16.22%) by Bumanlag et al. (2018). It was also supported by the values obtained by Dulay et al. (2017) in *L. strigosus* (13.84%) using 80% rice straw: 20% sawdust substrate formulation. Furthermore, the result of the study was far lower than the values obtained by Kalaw and Albinto (2015) in *P. cystidiosus* (27.72%), *P. florida* (66.98%), *P. ostreatus* (53.75%), *P. pulmonarius* (60.78%), and *P. sajor-caju* (50.56%) using 70% rice straw: 30% sawdust substrate formulation.

Conclusion

In conclusion, *C. cylindracea* mycelia can be grown on indigenous media and the fruiting body can be cultivated on the rice straw-sawdust-based substrate under the tropical conditions of the Philippines. The substrate formulation of 70% rice straw: 30% sawdust is found to be the most suitable substrate for the cultivation. These findings are significant in the generation of practical production technology that can be adopted by the Filipino mushroom growers to increase the number of cultivated mushrooms in the Philippines regardless of the strain origin. However, enriched cultivation should be done in order to improve the biological efficiency.

Acknowledgements

The authors would like to acknowledge the Academic Research Council (ARC), Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines for the financial support.

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