



Influence of Different Environmental Conditions on *Schizophyllum commune* Spawn Cultivation

Darwin A. Garbeles

College of Arts and Sciences, Partido State University, Goa, Camarines Sur, Philippines

Abstract— *Schizophyllum commune* (Split-gill mushroom) is a wild mushroom utilized as food, especially in the Philippines. In this study, the optimal environmental conditions for the mycelial growth of *S. commune* spawn were studied to develop a protocol for efficient spawn cultivation. Mycelial increment and density of *S. commune* incubated under varying illumination (24hrs light, 24hrs darkness, and 12hrs light-dark cycle) and temperature (32-36oC, 28-32oC, and 16-23oC) conditions were evaluated. Results showed that *S. commune* spawn cultures can grow vigorously in any of the illumination conditions. However, an equal amount of light and dark cycles significantly improved the mycelial density of *S. commune* spawn. The result also revealed that incubating under 16-23oC significantly increased *S. commune* mycelial increment at 0.92 ± 0.03 cm day⁻¹. Moreover, spawn incubated under 28-32oC significantly improved the mycelial density of *S. commune*.

Keywords— density, increment, kurakding, temperature, illumination

I. INTRODUCTION

Schizophyllum commune or "Common Split-gill Mushroom" is a tiny fan-shaped and edible wood-decaying macrofungi (Kuo, 2020; Ortega, 2012), and is one of the many fungal species abundant in the Philippines (Acanto et al., 2022). *S. commune* could be found and harvested naturally on the bark of decaying hardwood trees. It is known for its distinct taste profile, high nutritional content, and high medicinal value (Wu et. al, 2015; Rajaei and Mohammadi, 2017). However, despite the potential uses of Kurakding in culinary and medicine, there are limited records of its commercial cultivation (Dasanayaka and Wijeyaratne, 2017). The supply of Kurakding is limited and seasonal because it can only be harvested during rainy seasons on rotten wood such as bamboo and mango. Thus, this problem prevents this type of mushroom from entering the market chain (Aminah et al., 2020).

Spawn is the mushroom mycelium growing on a given pasteurized medium such as grain and serves as the planting material in mushroom cultivation (Stanley and Awi-Waadu, 2010). Spawn quality is counted as the most important part of mushroom production (Mohammadi and Purjam, 2003) and is considered the bedrock of the mushroom industry and the limiting factor to mushroom cultivation or production all over the world. Environmental factors such as temperature and light have been reported to affect mycelia growth in spawn preparation (Stanley and Awi-Waadu, 2010). Therefore, the study was undertaken to evaluate the most efficient environmental conditions in *S. commune* spawn cultivation.

II. MATERIALS AND METHODS

A. Research Design and Treatments

This study utilized the one-shot case study design type of Experimental Research. Two (2) sets of experimental groups (Table 1) were established representing two different environmental conditions to be tested such as

temperature and illumination. A total of three (3) experimental treatments were established for illumination conditions such as (AT1) 24hrs total light, (AT2) 24hrs total darkness, and (AT3) 12hrs light and 12hrs dark cycle. For the temperature conditions, a total of three (3) experimental treatments were established, which include (BT1) 32-36OC, (BT2) 28-32OC, and (BT3) 16-23OC. All of the experimental treatments were replicated three (3) times.

Table 1: Experimental Groups

ENVIRONMENTAL CONDITION	GROUP	TREATMENT
Illumination	AT1	12hrs light and 12hrs dark cycle
	AT2	24hrs total light
	AT3	24hrs total darkness
Temperature	BT1	28-32OC
	BT2	16-24OC
	BT3	34-38OC

B. Schizophyllum commune collection and isolation

Wild strain of *Schizophyllum commune* was collected from Brgy. Napawon, Goa, Camarines Sur. This type of macrofungi is a wood-decaying, white-brown colored fungus that usually thrives in groups and is mostly seen in decaying logs of mango and bamboo and measures 0.5-1.5 inches wide with fawn gills radiating from the point of attachment to an outward direction. *S. commune* samples were prepared and surface-sterilized following the protocol of De Leon et al., (2013). A total of twenty (20) pieces of uniformly sized *S. commune* were collected and washed to remove wood debris. The samples were soaked in 10% sodium hypochlorite for 1 minute and washed three times. Using a sharp and sterile scalpel, the outermost part of *S. commune* was removed to expose the sterile inner tissues and was further placed on previously prepared sterile Rice Flour Agar (RFA) culture media and incubated at 28-32OC until full ramification.

C. Spawn preparation and incubation

The seed-based spawn preparation followed the protocol used by Onyango et al., (2011). Red Sorghum seeds were used as a substrate for seed-based spawn preparation. Five grams of dry sorghum seeds were boiled in 100C distilled water for 15 minutes. After boiling, the seeds were transferred into sterile test tubes and autoclaved at 121OC and 15psi for 15 minutes. Using an aseptic technique, a 1x1 cm mycelial block obtained from the previously tissue-cultured *S. commune* was inoculated to the prepared sorghum-based spawn substrates. Successfully inoculated kurakding spawns were incubated at different illumination and temperature conditions. For the varying illumination conditions, a total of nine Kurakding spawns were distributed to three varying light-dark conditions such as (AT1) 24hrs total light, (AT2) 24hrs total darkness, and (AT3) 12hrs light and 12hrs dark cycle. For the varying temperature conditions, a total of nine (9) Kurakding spawns were distributed to three (3) varying temperature conditions such as (BT1) 32-36OC, (BT2) 28-32OC, and (BT3) 16-23OC. All of the experimental setups were replicated three (3) times.

D. Evaluation of mycelial growth

The mycelial growth of *S. commune* in varying environmental conditions was evaluated by measuring its mycelial increment and density and counting the incubation period before initial mycelial growth, the total number of days until full ramification. The mycelial increment was determined by measuring the length of the mycelial run daily for 5 days and was represented as an average unit increase per day. Mycelial density was evaluated by observing the thickness of mycelial ramification visually for 10 days and rated using a scale of 1-5 to represent its thickness and percent cover. The following scale values were used 1- Very thin, <20% cover; 2-Thin, 20-40% cover; 3- Average, 41%-60% cover; 4- Thick, 61%-80% cover; and 5-Very Thick, >80% cover. All of the data obtained throughout the study were expressed as means of replicates \pm standard error of the means (SEM) and were statistically analyzed using the One-way Analysis of Variance (ANOVA) procedure followed by Tukey's test for multiple comparisons. P values <0.05 was considered significant.

III. RESULTS AND DISCUSSION

A. Mycelial performance under varying illumination conditions

Mushrooms are photosensitive, and the presence or absence of light may affect the growth and development of various mushrooms (Chang and Miles, 1989). In this study, *S. commune* spawn cultures were subjected to different light and dark cycles. The result of the study (Table 2) showed that all of the treatments obtained an incubation period of 1 day before mycelial growth and attained maximum mycelial ramification (6.5cm) on the 5th day after inoculation.

The *S. commune* incubated under 24hrs total darkness (AT3) recorded the highest average daily gain of 0.93 ± 0.04 cm day⁻¹ followed by the *S. commune* spawn incubated under 12hrs light/dark cycle (AT1) with an average daily gain of 0.88 ± 0.08 cm day⁻¹. Lastly, the *S. commune* incubated under 24hrs total light (AT2) attained the lowest average daily gain of 0.84 ± 0.09 cm day⁻¹. However, statistical analysis revealed that there were no significant differences ($p > 0.05$) in the mycelial increment of *S. commune* under varying illumination conditions indicating that *S. commune* spawn cultures could grow vigorously in any light or dark conditions.

Table 2: Mycelial growth performance of *S. commune* on various illumination conditions

Illumination	Incubation Day(s)	Average Daily Gain (cm day⁻¹)	Days to Full Ramification	Density
Light and Dark	1.00a	$0.88 \pm 0.08b$	5.00c	Very Thick
Full Light	1.00 a	$0.84 \pm 0.09b$	5.00c	Average
Full Dark	1.00 a	$0.93 \pm 0.04b$	5.00c	Thick

Note: Means with the same letter are not significantly different

Furthermore, the *S. commune* under 12hrs light/dark (AT1) showed the thickest density at 4.67 ± 0.00 mean density score (MDS) followed by the *S. commune* incubated under 24hrs total darkness (AT3) with 3.56 ± 0.11 MDS. Lastly, the *S. commune* incubated at 24hrs total light showed the thinnest density at 2.89 ± 0.11 MDS. Statistical analysis showed that there were significant differences ($p < 0.05$) in the mycelial density of all the

treatments incubated under varying light/dark conditions, implicating that an equal amount of light and dark incubation conditions for *S. commune* spawn culture is more suitable in thickening mycelial density.

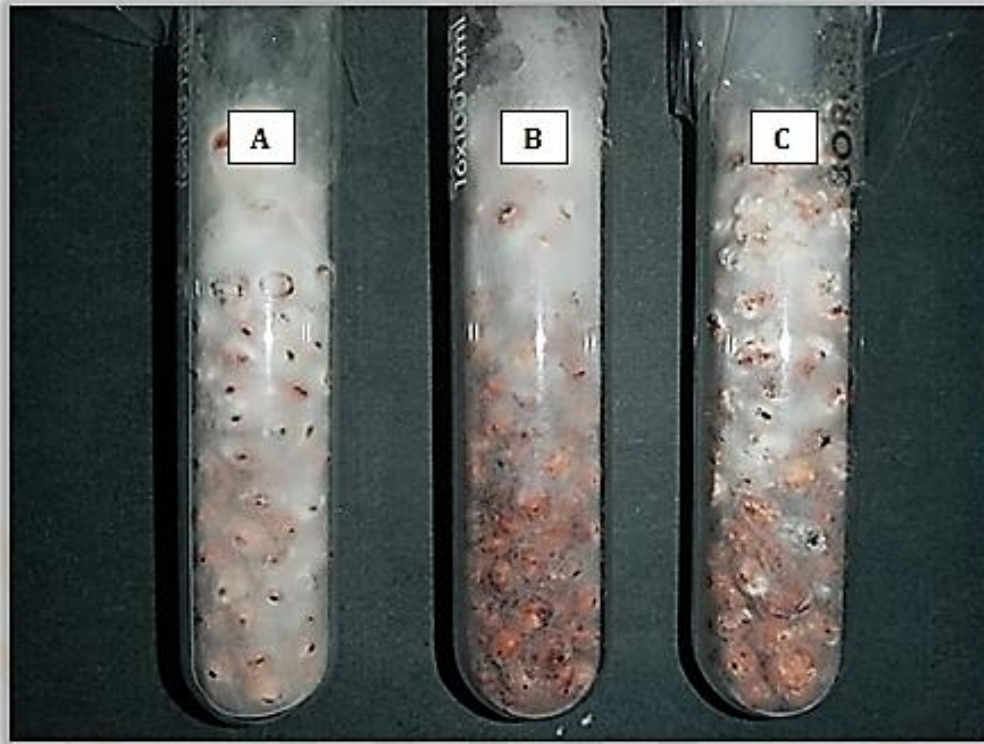


Figure 01: Mycelial density of *S. commune* under various illumination conditions: (A) Alternating Light and Dark Conditions, (B) Full light, (C) Full dark 10 days after incubation

B. Mycelial performance under varying temperature conditions

The mycelial increment of *S. commune* under varying temperature conditions was determined by evaluating the average daily gain of mycelial run on the seed-based spawn cultures. The result of the study (Table 3) showed that all of the *S. commune* spawn cultures incubated in varying illumination conditions attained similar initiation of mycelial growth on 1st day after inoculation.

Moreover, the *S. commune* spawn culture incubated at 16-22oC (BT2) significantly attained the shortest period to fully ramify (6.5cm) on the 5th day after incubation followed by the *S. commune* spawn culture incubated at 28-32oC (BT1) which attained maximum mycelial run at the 8th day after incubation. While *S. commune* spawn culture incubated at 34-38oC (BT3) attained the longest duration to fully ramify on the 9th day after incubation.

Table 3: Mycelial growth performance of *S. commune* on various illumination conditions

Temperature	Incubation Day(s)	Average Daily Gain (cm day-1)	Days to Full Ramification	Density
28-32oC	1.00a	0.84 ±0.05	8.00	Very Thick
16-22oC	1.00a	0.92 ±0.03	5.00	Thick
34-38oC	1.00a	0.75 ±0.01	9.00	Very Thin

Note: Means with the same letter are not significantly different

Moreover, the *S. commune* spawn culture incubated at 16-22oC recorded the highest average daily gain of 0.92 ± 0.03 cm day⁻¹ followed by the *S. commune* spawn culture incubated at 28-32oC with an average daily gain of 0.84 ± 0.05 cm day⁻¹. Lastly, *S. commune* spawn culture incubated at 34-38oC attained the lowest average daily gain of 0.75 ± 0.01 cm day⁻¹. Statistical analysis revealed that there were significant differences ($p < 0.05$) in the mycelial increment of *S. commune* incubated under varying temperature conditions, implying that temperature condition affects the growth of *S. commune* spawn culture and may grow vigorously under low temperature.

S. commune spawn cultures incubated under varying temperature conditions were observed for their mycelial density. It can be observed from the result of the study (Fig. 02) that the *S. commune* under temperature ranges of 28-32oC showed the thickest density at 5.00 ± 0.00 mean density score (MDS) followed by *S. commune* incubated under 16-22oC with 4.56 ± 0.11 MDS. Lastly, the *S. commune* incubated at 34-38oC showed the thinnest density at 1.00 ± 0.0 MDS. Statistical analysis showed that there were significant differences ($p < 0.05$) in the mycelial density of all the treatments incubated under varying temperature conditions, implicating that incubating *S. commune* under room temperature is more suitable for thickening the mycelial density.

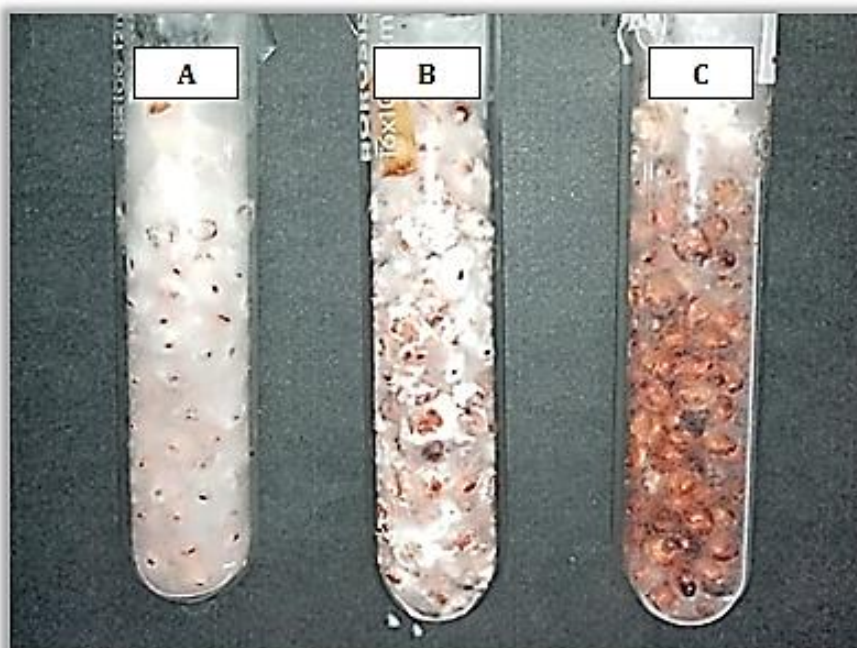


Figure 02: Mycelial density of *S. commune* incubated under various temperature conditions: (A) 28-32oC, (B) 16-22oC, (C) 34-38oC

IV. CONCLUSION AND RECOMMENDATION

Based on the data obtained throughout the study, it is concluded that *S. commune* spawn cultures can grow in any light conditions. However, an equal amount of light and dark cycles could improve the thickness and percent cover of *S. commune* spawn mycelium. It can also be concluded that a temperature range of 16-22oC could significantly increase *S. commune* mycelial growth and shorten the period to attain full ramification. However, incubating *S. commune* in a temperature range of 28-32oC could significantly improve the mycelial density of *S. commune* spawn.

Furthermore, based on the result of this study, it is recommended that *S. commune* seed-based spawn should be incubated under 16-22°C at the earlier stage of its growth and should be transferred under 28-32°C with equal light and dark cycle to further improve mycelial colonization. It can also be recommended that a further study on the yield performance of *S. commune* incubated under varying environmental conditions should be evaluated to verify the claims of this study.

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