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EFFECTS OF SELECTED FACTORS ON THE MYCELIAL INCUBATION OF *Lentinula edodes* (Berk.) Pegler PEGLER UNDER LABORATORY CONDITIONS

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ABSTRACT

In this paper, we studied the effects of substrate composition and ratio on the mycelium growth of *Letinula edodes* (Berk) Pegler. The experiment was conducted in laboratory conditions. We monitored quantitative indicators, including mycelium spreading speed, coverage and observed mycelium characteristics. Then, we statistically analyzed to evaluate the influence of the experimental formulas. We arranged 4 different substrate formulas. The experimental results showed that formula 4 (69% sawdust, 15% corn cob, 15% rice bran, 1% $CaCO_3$) was best for the mycelium growth of shiitake mushrooms. The coverage of the mycelium in formula 4 at 5, 10, 15 and 20 days was 18.0, 40.0, 66.7, 94.7%, respectively. The mycelium spreading rate in formula 4 at 5, 10, 15 and 20 days was 5.4 mm/day, 6.6 mm/day, 8.0 mm/day, 8.4 mm/day, respectively. Substrate formula number 4 was used to conduct experiments on the influence of bag density. The bag density with the highest mycelium spreading rate was 25 bags/m^2. In addition, we also conducted research on the influence of temperature on the development of the mycelium of shiitake mushrooms. The experiment was conducted in laboratory conditions. The temperature was controlled by air conditioning. The most suitable temperature for mycelium development was 25–27 ^oC. The results of this study are the basis for making the best decisions in shiitake mushroom production, improving productivity and economic efficiency.

Keywords: Substrate, temperature, bag density, mycelium incubation process, shiitake mushrooms, *Letinula edodes.*

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INTRODUCTION

Lentinula edodes mushrooms are one of the valuable edible and medicinal mushrooms, widely grown in many Asian countries such as China, Japan, Korea, and increasingly popular in Vietnam. They are rich in nutrients, delicious in taste, contain many important biological compounds such as lentinan, eritadenine, which have the ability to enhance immunity and support the treatment of chronic diseases (Zhang et al., 2020). In the shiitake mushroom production chain, the mycelium incubation stage plays a particularly important role, directly affecting the speed of completing the growth cycle, the rate of fruiting body formation and the yield. Mycelium incubation is usually performed after the secondary or tertiary strains are inoculated into a wood substrate or a lignocellulose-rich mixture, to help the mycelium grow throughout the entire substrate mass before entering the fruiting body stage (Royse et al., 2024). The effectiveness of the mycelium incubation process depends closely on the composition and mixing ratio of the substrate, such as sawdust, rice bran, corn flour, lime, $CaCO_3$ or other organic additives. Optimizing the substrate composition will help shorten the mycelium incubation time, improve the mycelium spreading ability, and increase mycelium density to increase mushroom yield.

In Vietnam, the two main substrates used to grow shiitake mushrooms are rotten wood and sawdust with added nutrients (Nguyen Lan Dung, 2010; Dinh Xuan Linh et al., 2012; Trinh Tam Kiet, 2012). However, rotten wood is increasingly scarce, so research to find alternative substrates for wood is very necessary. According to Nguyen Thi Luyen et al. (2020) the most favorable substrate formula for the development of mycelium and the formation of shiitake mushroom fruiting bodies is 89% corn cob, 1% $CaCO_3$, 10% barley bran. In Phuc Yen, Vinh Phuc, sawdust is easier to find than corn cobs. Through observation, we found that many households in this area used sawdust as the main ingredient. Therefore, we conducted research to develop substrate formulas with the main ingredient being sawdust, supplemented with corn cobs, rice bran and $CaCO_3$. The goal was to find the most suitable substrate formula, taking advantage of the waste by-products at the research site. According to Dinh Xuan Linh et al. (2012) temperature and bag density also affect the growth, development and productivity of shiitake mushrooms. The mycelium incubation stage requires a temperature of 20−27 ^oC, higher than the fruiting body development stage (12−18 ^oC). To determine the temperature suitable for local cultivation conditions, we conducted a study on the effects of these two factors on the development of shiitake mycelium.

MATERIALS AND METHODS

L. edodes mushroom strain is collected and isolated from nature. Substrate: acacia wood sawdust, corn cob, rice bran. We use equipment and tools in the Biology department laboratory, including sterile incubators, autoclaves, air conditioners, temperature sensors, scales, test tubes, petri dishes, triangular flasks, sterile cotton, alcohol lamps, etc. Chemicals: Agar gum, 70% alcohol, $CaCO_3$. Implementation time from June 2024 to April 2025.

We arranged 4 different substrate formulas. Each formula will plant 10 bags (4 formulas correspond to 40 bags). Repeat the experiment 3 times. The total of 3 times is 120 bags (Nguyen Chi Thanh, 2001). The ingredients and ratios of the formulas are presented in the following table.

After determining the most suitable substrate formula, taking advantage of local by-products. We continued to experiment to study the effects of 2 factors: room temperature and bag density on the development of the shiitake mushroom mycelium. These 2 factors were arranged on the most suitable substrate formula that was evaluated. For the density factor, we arranged 4 experimental formulas corresponding to 4 density levels of 25, 50, 75, 100 bags/m^2, respectively.

The parameters monitored included mycelium emergence time, mycelium length, mycelium spreading speed, mycelium coverage, and mycelium characteristics. Monitoring was carried out every 5 days. We

inoculated the seeds at the neck of the bag, then placed them upright in mycelium incubation conditions (neck facing up). The mycelium began to grow from the inoculation position and spread along the length of the bag to the bottom.

Table 1. Ingredients and proportions of growing medium formulas

Materials / Formula	Acacia wood sawdust	Corncob	Rice bran	$CaCO_3$
Formula 1	69	20	10	1
Formula 2	64	15	20	1
Formula 3	74	15	10	1
Formula 4	69	15	15	1

Mycelium emergence time (days) is the number of days from the time of inoculation until the first mycelium is observed on the surface of the substrate. Mycelium length is the distance measured from the point of inoculation to the point where the mycelium has spread to (mm). The ruler is placed vertically parallel to the straight line connecting the neck of the bag to the bottom of the bag. Fiber spreading rate (mm/day) is calculated by the following formula:

$$\text{Spreading rate}\left(\text{mm/day}\right) = \frac{D_2 - D_1}{T_2 - T_1}$$

D_1: The lengths of mycelial colonization measured at the initial time (mm); D_2: The lengths of mycelial colonization measured at the later time (mm). Mycelium length is the distance measured from the inoculation point to the nearest point where the mycelium extends (mm). The ruler is placed vertically parallel to the line connecting the neck of the bag to the bottom of the bag; $(T_2 - T_1)$: Interval between measurements.

Coverage is calculated by the following formula: Coverage (%) = $(D_n/D_0) \times 100$

D_n: The length of mycelial colonization at the nth measurement. D_0: The total length of the substrate bag. The length of the growing medium bag is the length of the straight line connecting from the neck of the bag to the bottom of the bag.

Data were processed using Microsoft Excel software, using the average calculation functions. Means were compared using the ANOVA Single Factor tool.

RESULTS

Effect of Corn Cob Proportion

We performed measurements four times at intervals of five days, from the 5^{th} to the 20^{th} day after inoculation, when the mycelial spreading rate had become relatively stable. A total of 60 samples were evaluated (2 formulas × 3 replications × 10 bags each). The averaged results are presented in Table 2.

Table 2. Effect of corn cob proportion in the Substrate on the mycelial coverage of *Lentinula edodes*

Creteria	Mycelial coverage (%)			
	Attempt 1	Attempt 2	Attempt 3	Attempt 4
Formula 1	14^b	32.7^b	56.0^b	82.0^b
Formula 3	16.7^a	37.3^a	62.7^a	90.0^a
$LSD_{0.05}$	0.25	1.56	2.65	2.90

Note: Different letters within the same column indicate statistically significant differences at the 0.05 confidence level (95%).

Corn cobs are an easily accessible by-product in Vietnam and are commonly incorporated into the growing substrate for shiitake cultivation. They are rich in cellulose

and hemicellulose, which serve as nutrient sources for mycelial growth, while also improving the porosity and structure of the substrate. Our results demonstrate that the proportion of corn cob in the substrate has a pronounced effect on the growth rate and coverage of *L. edodes* mycelium during the incubation stage. In particular, the treatment containing 15% corn cob (Treatment 3) produced significantly better growth outcomes compared to the treatment with 20% corn cob (Treatment 1). Although some non-experimental factors could not be entirely controlled in this study, the findings nonetheless provide useful insights for selecting an optimal corn cob ratio.

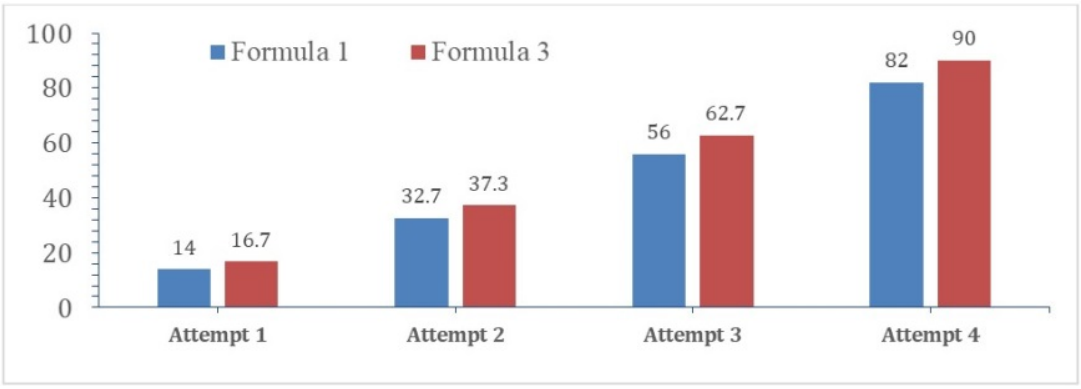

Figure 1. Comparison chart of the effect of corn cob ratio in the substrate on the coverage of shiitake mushroom mycelium

Table 3. Effect of corn cob proportion in the substrate on the spreading rate of *Lentinula edodes*

Creteria	Spreading rate (mm/day)			
	Attempt 1	Attempt 2	Attempt 3	Attempt 4
Formula 1	4.2^{b}	5.6^{b}	7.0^{b}	7.8^{b}
Formula 3	5.0^{a}	6.2^{a}	7.6^{a}	8.2^{a}
$LSD_{0.05}$	0.25	0.15	0.20	0.22

Note: Different letters within the same column indicate statistically significant differences at the 0.05 confidence level (95%).

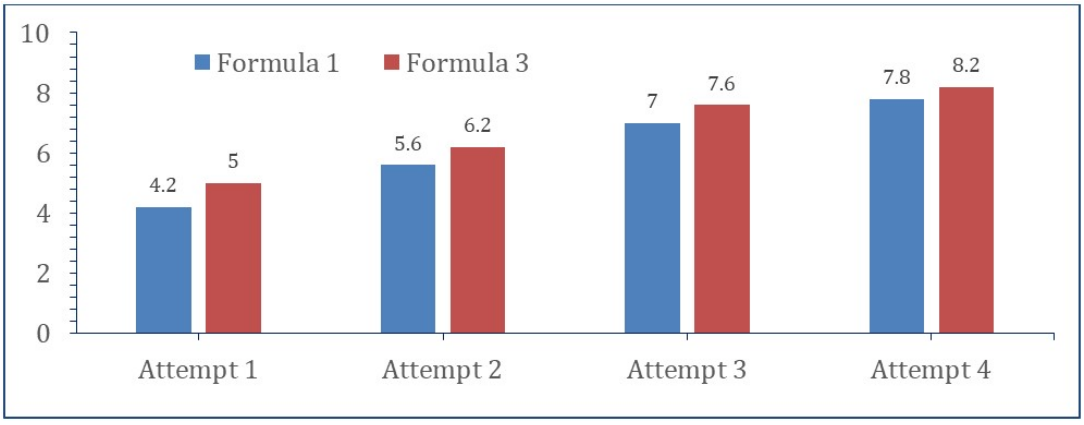

Figure 2. Comparison chart of the effect of corn cob ratio in the substrate on the spreading rate of shiitake mushrooms

In Treatment 3, the mycelial growth rate ranged from 5.0 to 8.2 mm/day, consistently higher than that observed in Treatment 1 (4.2 to 7.8 mm/day) across all measurement intervals. Likewise, mycelial coverage in Treatment 3 reached 90% by day 20, whereas Treatment 1 achieved only 82%, indicating that Treatment 3 supported superior mycelial development.

The study also revealed that from the 5th to the 15th day after inoculation, the mycelium exhibited vigorous growth, with clear differences between the two formulas. For instance, in Formula 1, the spreading rate increased from 4.2 mm/day at the first measurement to 5.6 mm/day at the second, a difference of 1.2. Between the second and third measurements, the difference rose to 1.4 mm, but between the third and fourth it declined to only 0.8 mm. A similar trend was observed for overall growth dynamics. These findings suggest that after the 20th day, mycelial spreading gradually slowed as the system stabilized in preparation for the initiation and development of fruiting bodies.

Effect of rice bran

The results indicate that the rice bran content in the substrate has a substantial effect on the development of shiitake mycelium. Rice bran, as well as other cereal brans, can be added to substrates to enrich them with proteins, trace minerals, and vitamins. Among these, barley bran and rice bran are the most commonly used. In the localities of Phuc Yen and Vinh Phuc, however, rice bran is both more accessible and more economical than barley bran.

Table 4. Effect of rice bran proportion in the substrate on the mycelial coverage of *Lentinula edodes*

Creteria	Coverage (%)			
	Attempt 1	Attempt 2	Attempt 3	Attempt 4
Formula 2	16.2^{b}	36.3^{b}	60.8^{b}	88.7
Formula 3	16.7^{b}	37.3^{b}	62.7^{b}	90.0^{b}
Formula 4	18.0^{a}	40.0^{a}	66.7^{a}	94.7^{a}
$LSD_{0.05}$	0.65	1.12	2.25	1.95

Note: Different letters within the same column indicate statistically significant differences at the 0.05 confidence level (95%).

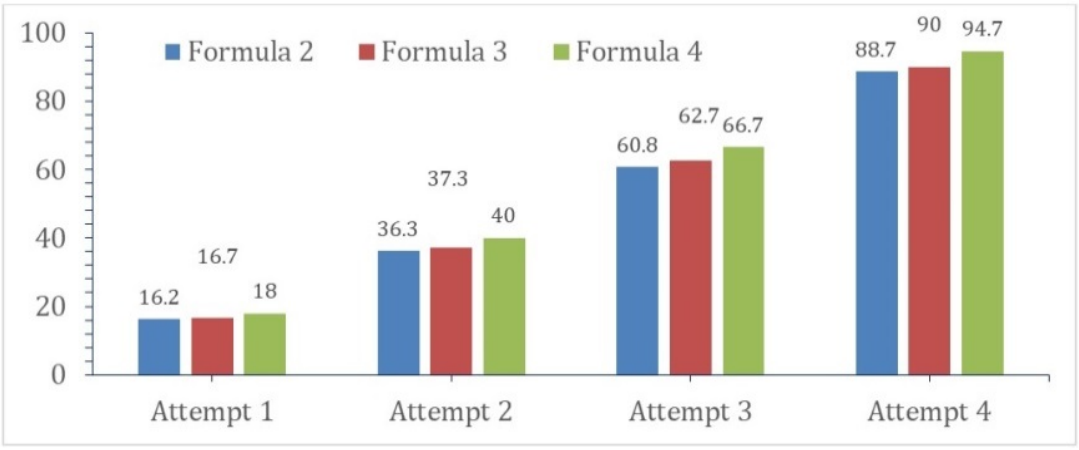

Figure 3. Comparison chart of the effect of rice bran proportion in the substrate on the coverage of shiitake mushroom mycelium

Across all four observation periods, Formula 4 consistently exhibited the highest growth rate, showing statistically significant differences compared with Formulas 2 and 3.

At the 0.05 significance level, Formulas 2 and 3 did not differ from each other (both ranked at the same statistical level b), whereas Formula 4 achieved the highest mycelial coverage (level a), with values of 18, 40, 66.7, and 94.7% at days 5, 10, 15, and 20, respectively. By contrast, Formula 2 reached 16.2, 36.3, 60.8, and 88.7% at the same time points, while Formula 3 reached 16.7, 37.3, 62.7, and 90.0%.

Table 5. Effect of rice bran proportion in the substrate on the spreading rate of *Lentinula edodes*

Creteria	Spreading rate (mm/day)			
	Attempt 1	Attempt 2	Attempt 3	Attempt 4
Formula 2	4.9^{b}	6.2^{b}	7.5^{b}	8.1^{b}
Formula 3	5.0^{b}	6.2^{b}	7.6^{b}	8.2^{b}
Formula 4	5.4^{a}	6.6^{a}	8.0^{a}	8.4^{a}
$LSD_{0.05}$	0.20	0.19	0.25	0.11

Note: Different letters within the same column indicate statistically significant differences at the 0.05 confidence level (95%).

The proportion of rice bran in the substrate exerts a clear influence on both mycelial surface coverage and spreading speed. As shown in Table 5 and Figure 4, the optimal range of rice bran is between 10 and 20%, with 15% proving to be the most effective. Ratios above 20% or below 10% were less favorable.

At all observation points, substrates containing 20% rice bran (CT2) and 10% rice bran (CT3) showed no significant differences in spreading speed, both ranking at the same statistical level (b) at the 95% confidence level. Specifically, Formula 2 recorded growth rates of 4.9, 6.2, 7.5, and 8.1 mm/day, while Formula 3 produced values of 5.0, 6.2, 7.6, and 8.2 mm/day across the same time intervals. In contrast, Formula 4, with 15% rice bran, achieved the highest rates-5.4, 6.6, 8.0, and 8.8 mm/day-ranking at level (a).

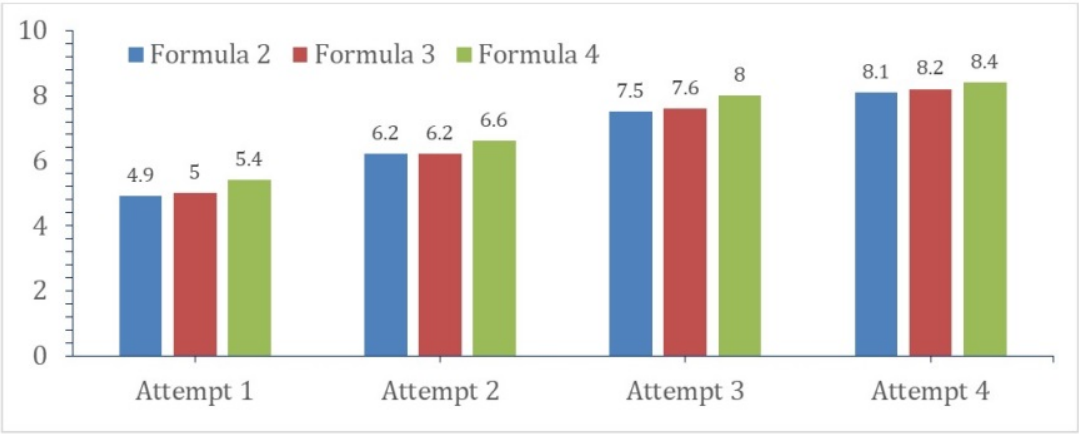

Figure 4. Comparison chart of the effect of rice bran proportion in the substrate on the spreading rate of shiitake mushroom mycelium

From a practical standpoint, Formula 4 represents the most advantageous choice for laboratory mycelial incubation, as it balances nutritional requirements effectively. This formulation is well-suited not only for small-scale spawn production but also for advanced research in edible mushroom biotechnology.

Environmental temperature is a key ecological factor that strongly influences the development of *L. edodes* mycelium during

the incubation stage. Across all treatments, both spreading speed and coverage increased markedly within the range of 23–27 ^{o}C. At 20–22 ^{o}C, the spreading rate was limited to 4.1–5.8 mm/day and coverage remained below 20%, reflecting a slowdown in mycelial metabolism. In contrast, at 25–27 ^{o}C, growth indicators reached their optimum, with CT4 showing the highest performance-a spreading rate of 7.2 mm/day and coverage of 66.3%. However, when the temperature rose toward the upper end of this range, a slight decline in spreading speed was observed, accompanied by the appearance of excess moisture at the edges of the culture bags.

Table 6. Effect of environmental temperature on the mycelial growth of *Lentinula edodes*

Indicator / Formula	Temperature 20−22 ^{o}C		Temperature 23–25 ^{o}C		Temperature 25−27 ^{o}C		$LSD_{0.05}$
	Spreading rate (mm/day)	Mycelial coverage (%)	Spreading rate (mm/day)	Mycelial coverage (%)	Spreading rate (mm/day)	Mycelial coverage (%)	
F1	4.2^{c}	14.0^{c}	5.6^{b}	32.7^{b}	7.8^{a}	58.7^{a}	$LSD_{0.05}(1) = 0.6$ $LSD_{0.05}(2) = 5.5$
F2	4.1^{c}	13.7^{c}	5.6^{b}	32.3^{b}	6.5^{a}	54.0^{a}	$LSD_{0.05}(1) = 0.6$ $LSD_{0.05}(2) = 5.5$
F3	4.5^{c}	15.0^{c}	6.7^{b}	37.3^{b}	7.3^{a}	61.7^{a}	$LSD_{0.05}(1) = 0.6$ $LSD_{0.05}(2) = 5.5$
F4	5.8^{c}	19.3^{c}	6.9^{b}	42.3^{b}	7.2^{a}	66.3^{a}	$LSD_{0.05}(1) = 0.6$ $LSD_{0.05}(2) = 5.5$

Note: All treatments were conducted under the same humidity condition (70–80%). $LSD_{0.05}$ (1): Least Significant Difference at 5% significance level for comparing the mean mycelial growth rates at different temperatures within the same treatment. $LSD_{0.05}$ (2): Least Significant Difference at 5% significance level for comparing the mean mycelial coverage at different temperatures within the same treatment.

Table 7. Effect of bag density on the mycelial development of *Lentinula edodes*

Bag Density (bags/m²)	Time to Full bag (days)	Spreading rate (mm/day)
25 bags/m^2	14^{d}	7.1^{a}
50 bags/m^2	16^{c}	6.2^{b}
75 (bags/m²)	18^{b}	4.8^{c}
100 (bags/m²)	20^{a}	3.8^{d}
$LSD_{0.05}$	1.25	0.30

Note: The bags are incubated under the same conditions at the same time.

To further refine cultivation practices for shiitake mushrooms, we also investigated the effect of bag density. Using the optimal substrate formulation (Formula 4), we conducted experiments at four different density levels to assess their impact on mycelial development.

Bag density had a pronounced effect on both the spreading rate and coverage of *L. edodes* mycelium during the incubation period. At the lowest density of 25 bags/m², mycelial growth was rapid, reaching 7.1 mm/day and covering 66% of the substrate surface within 14 days - the highest

values among all treatments. When density was increased to 50 bags/m², growth parameters remained relatively favorable, with a spreading rate of 6.2 mm/day and 63.7% coverage, though the time required to achieve this level extended to 16 days. At higher densities of 75–100 bags/m², growth performance declined markedly: spreading rates dropped to 4.8–3.8 mm/day, and coverage decreased to 58% and 50.7% after 18–20 days. These results suggest that excessive bag density intensifies competition for space, light, gas exchange, and temperature regulation, ultimately hindering mycelial development.

Figure 5. Mycelial growth of *Lentinula edodes* on different substrate formulations under laboratory conditions

DISCUSSION

Previous studies (Nguyen Thi Luyen et al., 2020; Pham Nu Kim Hoang et al., 2021) demonstrated that incorporating corn cobs at an appropriate ratio (15–25%) accelerates mycelial spreading, produces whiter and denser fibers, and shortens the time required to fully colonize the bag compared to substrates composed solely of sawdust. Building on these findings, our study sought to further refine the optimal ratio. The results indicate that a 15% corn cob addition is the most suitable for promoting shiitake mycelial development.

When the corn cob content exceeds 15–18%, the proportion of indigestible fiber increases, which can restrict gas exchange,

inhibit the activity of enzymes responsible for breaking down fungal substrates, and ultimately reduce nutrient absorption efficiency. In addition, excessive corn cob content tends to retain water, creating favorable conditions for pathogenic microorganisms to proliferate and raising the risk of contamination during incubation.

Our results align closely with those of Nguyen Thi Luyen et al. (2020), who showed that reducing the corn cob ratio from 20% to 15% enhanced mycelial growth rate and uniformity, stabilized substrate pH, and lowered contamination risk. These findings highlight the importance of carefully adjusting the proportion of slow-degrading components in substrate formulations as a key technical measure to improve seed production efficiency.

According to Pham Nu Kim Hoang et al. (2014), rice bran is a valuable source of organic nitrogen, amino acids, and B vitamins, and is particularly effective when incorporated into nutrient-poor substrates such as sawdust or wood chips. The recommended supplementation rate is 10–15%. Similarly, Nguyen Thi Luyen et al. (2020) reported that the most effective substrate formula for shiitake cultivation under Vietnamese conditions consists of 89% corn cob, 10% barley bran, and 1% $CaCO_3$, with sawdust serving as a complementary component. In this formulation, the addition of 10% barley bran yielded the highest biological efficiency. Thus, the authors concluded that 10% barley bran represents an appropriate supplementation level to enhance shiitake mushroom yield—a conclusion that is both reasonable and consistent with our findings.

Nguyen Thi Luyen et al. (2020) also determined that the optimal temperature range for mycelial incubation is 24–25 ^oC. Likewise, Pham Nu Kim Hoang et al. (2014) identified 23–27 ^oC as the most suitable range for this stage. The slight 1–2 ^oC difference between these studies can be attributed to the interaction of multiple environmental and methodological factors.

In terms of bag density, Zhang et al. (2020) emphasized that excessive culture density increases localized humidity and restricts gas exchange, thereby impairing mycelial growth. Atila (2017) similarly observed that densities exceeding 70 bags/m² often reduce the rate of mycelial colonization due to localized heat and moisture accumulation, which can result in slowed growth or contamination. Complementing these findings, Royse et al. (2024) recommended an optimal density of 30–50 bags/m² for small-scale production, balancing efficient growth with effective use of space and maintenance of favorable microclimatic conditions.

CONCLUSION

The proportions of rice bran and corn cob exerted not only individual effects but also combined influences on the growth of shiitake mycelium. Among the tested treatments, Formula 4 (69% sawdust, 15% corn cob, 15% rice bran, 1% $CaCO_3$) proved to be the most effective for mycelial development. In this formula, mycelial coverage at 5, 10, 15, and 20 days reached 18.0, 40.0, 66.7, and 94.7%, respectively, while the corresponding spreading rates were 5.4, 6.6, 8.0, and 8.4 mm/day. Since sawdust, corn cob, and rice bran are readily available by-products at the study site, the adoption of this formula offers both economic and environmental benefits. Specifically, it can lower raw material costs, improve production efficiency, and support sustainable development in shiitake mushroom cultivation.

Assuming other conditions remain constant, a substrate containing 15% corn cob and 15% rice bran provides the most favorable environment for shiitake mycelial growth. Corn cob contributes cellulose and enhances substrate porosity, while rice bran supplies essential proteins, vitamins, and minerals to support vigorous development.

The optimal temperature range for mycelial growth and development is 23–27 ^oC, with 25–27 ^oC identified as the most suitable. Temperatures above 27 ^oC or below 23 ^oC not only slow growth but also increase the risk of excess moisture and contamination. Maintaining this range consistently is therefore critical during production. In addition, a moderate bag density

of 25 bags/m² was found to be the most effective for the incubation process under laboratory conditions, ensuring rapid colonization and stable growth performance.

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