

STUDY ON PRIMARY SPAWN MEDIUM FOR SHIITAKE MUSHROOM (*Lentinula edodes* (Berk) Pegler) UNDER LABORATORY CONDITIONS

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ABSTRACT

This study aims to evaluate the effects of the composition and ratio of the medium/substrate on the mycelium growth of the shiitake mushroom (*Lentinula edodes* (Berk) Pegler) under laboratory conditions. Before production, shiitake mushrooms must go through two stages: primary and secondary varieties. Primary varieties are cultured on agar medium. Secondary varieties are propagated on substrates such as corn cobs or cereal grains. For primary varieties, we arranged 3 formulas. After experimentation, formula 3 (20 g sugar + 250 g potatoes + 0.5 g MnSO₄ + 1.0 g KH₂PO₄ + 20 g agar) gave the best results in terms of colony diameter, mycelium spreading speed and coverage. In formula 3, the colony diameters at the 5th, 10th, 15th, 20th days after inoculation were 6.3 mm, 13.5 mm, 27.0 mm, 40.5 mm, respectively; the mycelial spread rates were 1.26 mm/day, 1.44 mm/day, 2.70 mm/day, 2.70 mm/day, respectively; the mycelial coverage rates were 7.0%, 15.0%, 30.0%, 45.0%, respectively. Formula 3 had superior mycelial growth, so the time for mycelial coverage of the plate was the shortest, 22 days, and the average mycelial spread rate for the entire process was 2.03 mm/day. For secondary propagation, we have 3 formulas. The results show that formula 1 (35% boiled rice + 69% sawdust + 1% CaCO₃) is the best for secondary propagation. The average mycelium growth rate is 22.3 mm/day; on the substrate, the mycelium grows more slowly, taking 38–44 days to cover the bag; in the air on the agar medium, it only takes 22–28 days.

Keywords: Shiitake mushroom, *Lentinula edodes*, primary spawn, secondary spawn.

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INTRODUCTION

Lentinula edodes is one of the valuable edible and medicinal mushrooms, widely grown in many Asian countries such as China, Japan, Korea, which is increasingly popular in Vietnam. They are rich in nutrients, delicious in taste, and contain many important biological compounds such as lentinan, eritadenine which can enhance immunity and support the treatment of chronic diseases (Zhang et al., 2020).

Primary spawn strains are purebred, cultured on agar media under sterile conditions and used as starting sources. During this stage, the choice of culture medium plays an essential role, directly affecting the mycelium spreading rate, mycelium density, genetic stability and the ability to form fruit bodies in subsequent generations of strains (Pham et al., 2021). The common media currently used are PDA (Potato Dextrose Agar), MEA (Malt Extract Agar) or CDA (Corn Dextrin Agar). However, for each type of fungus, the medium is adjusted accordingly. In the study by Nguyen Thi Luyen et al. (2020), the authors added KH_2PO_4 (0.5 g/L) and MnSO_4 (0.5 g/L) to the PDA medium. Experimental results showed that this combination had a clear effect in promoting the speed of mycelium spreading and improving the quality of the mycelium, with the characteristics of whiter, thicker and more uniform mycelium compared to the control formula using only PDA. In Vietnam, we have not found any publications on the amount of MnSO_4 in the shiitake mushroom propagation medium. However, Potassium and Phosphorus are mineral elements involved in many important processes such as enzyme activation, ion exchange, ATP structure, etc. Therefore, shiitake mushrooms will need more KH_2PO_4 than MnSO_4 . Therefore, we conduct research on this topic with the aim of obtaining more information on this issue.

Secondary varieties are often propagated on substrates such as cereal grains, corn cobs. However, if only on the granular medium, the mycelium will develop very slowly because they need time to decompose the substrate. Therefore, depending on the type of

mushroom, the purpose and the practice, we add more nutrients to have the substance. We can use barley bran, rice bran, sawdust, etc. In this study, we chose sawdust and rice bran - by-products that are easy to find locally to conduct the research in order to reduce costs, increase production efficiency and sustainable development.

MATERIALS AND METHODS

L. edodes mushroom strain is collected and isolated from nature. Primary spawn culture medium: PDA (Potato Dextrose Agar), composed of 200 g potato, 20 g agar, 20 g glucose, and distilled water (1 liter), with pH 6. Equipment: Laminar flow cabinet, autoclave, pH meter, analytical balance, test tubes, Petri dishes, Erlenmeyer flasks, sterile cotton, alcohol lamp, etc. Chemicals: Agar, 70% ethanol, MnSO_4 , KH_2PO_4 , glucose/sucrose. Secondary spawn: sawdust, rice grains, and CaCO_3 . Scope of the study: Assessment of the effects of substrate composition and ratios on the mycelial development of *L. edodes* under laboratory conditions with controlled temperature and humidity ($t = 25\text{--}27^\circ\text{C}$ & $\text{RH} = 65\text{--}70\%$).

Location: Laboratory of the Faculty of Biology, Hanoi Pedagogical University 2 (21.28113683780571, 105.72619916236548); From June 2024 to May 2025. With the aim of investigating the impact of primary spawn medium composition and ratios on the growth and development of shiitake mushroom mycelium, we arranged 3 different formulas, with 10 petri dishes for each formula (3 formulas correspond to 30 petri dishes). The experiment is repeated 3 times, with a total of 90 petri dishes (Nguyen, 2001). Formula 1 (F1): 20 g sugar + 200 g potato + 0.5 g MnSO_4 + 0.5 g KH_2PO_4 + 20 g agar; Formula 2 (F2): 20 g sugar + 200 g potato + 1.0 g MnSO_4 + 0.5 g KH_2PO_4 + 20 g agar; Formula 3 (F3): 20 g sugar + 200 g potato + 0.5 g MnSO_4 + 1.0 g KH_2PO_4 + 20 g agar.

In the study on the effect of substrate composition and proportion in secondary spawn media on the mycelial growth and development of *L. edodes*, we arranged 3 different formulas, with 10 bags for each

formula (3 formulas correspond to 30 bags). The experiment is repeated 3 times, with a total of 90 bags (Nguyen Chi Thanh, 2001). Formula 1: 35% boiled rice grains + 64% sawdust + 1% CaCO_3 ; Formula 2: 64% boiled rice grains + 35% sawdust + 1% CaCO_3 ; Formula 3: 99% boiled rice grains + 1% CaCO_3 . Experimental design: Each treatment consisted of 10 bags per replicate, with three replications. The substrate height in each bag was maintained at 9–10 cm.

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.

Mycelium emergence time 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.

Monitoring and Evaluation

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

D_1 : Colony diameter/The length of mycelial colonization measured at the initial time (mm); D_2 : Colony diameter/The length of mycelial colonization measured at a later time (mm); ($T_2 - T_1$): Interval between measurements.

$$\text{Coverage (\%)} = (D_n / D_0) \times 100$$

D_n : Colony diameter/The length of mycelial colonization at the n^{th} measurement; D_0 : Petri dish diameter/The total length of substrate bag. The length of the growing medium bag is

the length of the straight line connecting 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

Study on the effects of substrate composition and ratio on the primary spawn production of shiitake mushroom (*Lentinula edodes*)

The results in Table 1 reveal a clear difference in colony diameter across three media formulas over four incubation points (days 5, 10, 15, and 20). On day 5, formula 3 showed a colony diameter of 6.3 mm, which was higher than that of formula 1 (4.5 mm) and formula 2 (3.6 mm). By day 20, formula 3 reached 40.5 mm, outperforming formula 1 (22.5 mm) and formula 2 (11.7 mm). This indicates that formula 3 supported faster and more stable mycelial growth compared to the other two formulations. This superiority is attributed to the doubled KH_2PO_4 content in formula 3. KH_2PO_4 provides phosphorus and potassium, both critical for nucleic acid structure, ATP synthesis, and pH regulation-factors that enhance metabolic activity and cell division.

The data show that mycelial spreading rates differed significantly between treatments and over incubation periods. Formula 3 consistently exhibited the highest spreading rates, significantly outperforming formula 1 and formula 2 at days 5, 10, and 15. On day 5, formula 3 reached 1.26 mm/day, higher than formula 1 (0.90 mm/day) and formula 2 (0.72 mm/day). This trend continued and peaked at day 15 with formula 3 achieving 2.70 mm/day, 1.5 times higher than that of formula 1 and nearly five times higher than that of formula 2. This reflects the enhanced nutrient utilization of formula 3 after the initial adaptation phase.

Remarkably, formula 3 maintained this rate at day 20 (2.70 mm/day), while that of formula 1 and formula 2 declined significantly (0.90 and 0.54 mm/day, respectively). This demonstrates that formula 3 enables not only rapid but also

sustained growth, avoiding the stagnation often caused by nutrient depletion or imbalance in pH and humidity. F3 (20 g sugar + 200 g potato + 0.5 g MnSO_4 + 1.0 g KH_2PO_4 + 20 g agar) contains higher phosphate, supporting cell division and ATP, DNA, and enzyme synthesis, explaining its superior and lasting growth. F1 has similar ingredients but with only 0.5 g KH_2PO_4 , limiting its phosphate and pH buffering capacity. Growth slows after day 15, indicating medium instability. F2 has reduced potato (200 g) and low KH_2PO_4 , leading to carbon and phosphorus deficiency, slow mycelial expansion, and little growth

improvement during the experiment. These growth rates, especially in formula 3, reach 2.70 mm/day on days 15 and 20, in accordance with previous studies. Furlan et al. (1997) found *L. edodes* spreading at 1.0–2.3 mm/day on PDA and MEA media, while Nguyen et al. (2023) reported 2.5–2.7 mm/day using enhanced PDA with 1.0g/L KH_2PO_4 and yeast extract-matching formula 3 formulation. Royse et al. (2024) suggested that a spreading rate ≥ 2.5 mm/day between days 10–20 is ideal for primary spawn production. In this study, only these growth rates, especially formula 3 have 2.70 mm/day on days 15 and 20, align with previous studies.

Table 1. Effect of medium composition and ratio in primary spawn on the mycelial colony diameter of shiitake mushroom under laboratory conditions

Time \ Formula	Mycelial colony diameter (mm)			LSD _{0.05}
	Formula 1	Formula 2	Formula 3	
Day 5	4.5 ^b	3.6 ^c	6.3 ^a	0.92
Day 10	9.0 ^b	6.3 ^c	13.5 ^a	0.87
Day 15	18.0 ^b	9.0 ^c	27.0 ^a	1.25
Day 20	22.5 ^b	11.7 ^c	40.5 ^a	3.05

Note: Different superscript letters within the same row indicate statistically significant differences at the 95% confidence level.

Table 2. Effect of medium composition and ratio in primary spawn on the mycelial spreading rate of shiitake mushroom under laboratory conditions

Time \ Formula	Mycelial spreading rate (mm/day)			LSD _{0.05}
	Formula 1	Formula 2	Formula 3	
Day 5	0.90 ^b	0.72 ^c	1.26 ^a	0.12
Day 10	0.90 ^b	0.54 ^c	1.44 ^a	0.25
Day 15	1.80 ^b	0.54 ^c	2.70 ^a	0.57
Day 20	0.90 ^b	0.54 ^c	2.70 ^a	0.22

Note: Different superscript letters within the same row indicate statistically significant differences at the 95% confidence level.

Table 3. Effect of medium composition and ratio in primary spawn on the mycelial surface coverage of shiitake mushroom under laboratory conditions

Time \ Formula	Mycelial coverage (%)			LSD _{0.05}
	Formula 1	Formula 2	Formula 3	
Day 5	5.0 ^b	4.0 ^c	7.0 ^a	0.55
Day 10	10.0 ^b	7.0 ^c	15.0 ^a	1.25
Day 15	20.0 ^b	10.0 ^c	30.0 ^a	2.65
Day 20	25.0 ^b	13.0 ^c	45.0 ^a	3.75

Note: Different superscript letters within the same row indicate statistically significant differences at the 95% confidence level.

Mycelial surface coverage reflects the spatial expansion of the mycelium across the medium, directly related to biomass density and nutrient efficiency. Table 3 shows increasing coverage over time for all formulas, but with statistically significant differences.

On day 5, formula 3 achieved 7.0% coverage, higher than formula 1 (5.0%) and formula 2 (4.0%). By day 20, formula 3 reached 45.0%, nearly double formula 1 (25.0%) and more than triple formula 2 (13.0%). Superior performance of formula 3 is attributed to its nutrient composition 1.0 g KH_2PO_4 and 200 g potato, providing sufficient phosphorus, potassium, and carbon-crucial for cell division, ATP synthesis, nucleic acid formation, and mycelial elongation. As a result,

formula 3 promoted rapid and uniform mycelial spread, achieving optimal coverage. In contrast, formula 1, despite having the same potato content, contained only 0.5 g KH_2PO_4 , offering less phosphorus. Its coverage plateaued at 25.0% by day 20. Formula 2, with the least nutrient content (reduced potato and 0.5 g KH_2PO_4), resulted in poor growth and only 13.0% coverage.

These findings are consistent with Nguyen et al. (2023), who noted that enhanced PDA with ≥ 1.0 g KH_2PO_4 and high carbon sources achieved 40–50% coverage in 20 days similar to formula 3. Zhang et al. (2020) also showed phosphate-deficient media only reached 18–25% coverage, underlining phosphorus's central role in early-stage mycelial expansion.

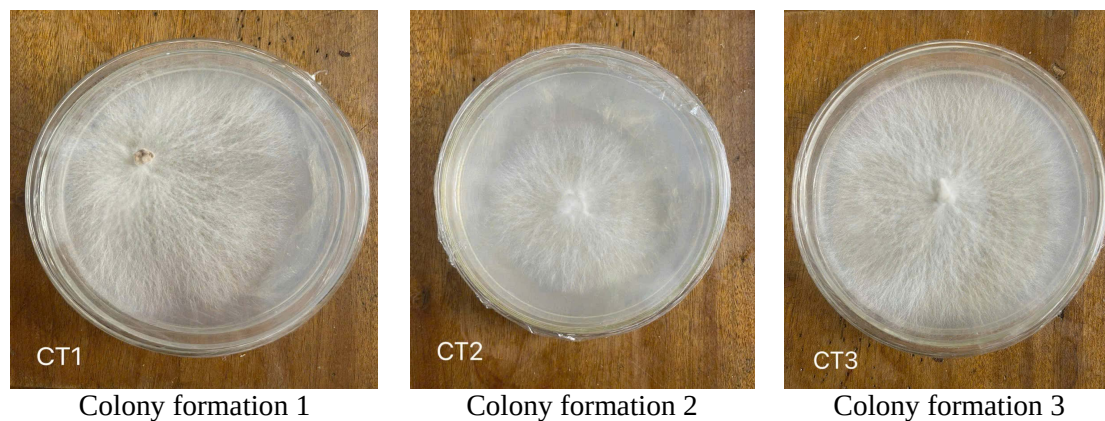


Figure 1. Mycelial growth of *Lentinula edodes* after maximum development on primary spawn medium

Notably, Royse et al. (2024) recommend $\geq 40\%$ coverage at day 20 as a benchmark for suitable primary spawn media. Only formula 3 met this criterion, confirming the synergistic effect of KH_2PO_4 and potato in creating optimal growth conditions for *L. edodes* in lab environments.

The results in Table 4 reveal clear differences in the growth rate and morphological characteristics of *L. edodes* mycelia among the culture formulations on primary spawn media. Although the initial mycelial emergence was observed on day 5 in all treatments, the time required for full colonization of the Petri dish and the growth rates differed significantly

($\text{LSD}_{0.05}$). Specifically, formula 3 exhibited the highest growth rate (2.03 mm/day), producing dense, cottony, white mycelia that colonized the dish completely within 22 days, outperforming formula 1 and formula 2. In contrast, formula 2 showed the slowest growth (0.59 mm/day), with thin and sparse mycelia requiring up to 28 days to cover the dish surface. Formula 1 displayed intermediate performance, with a rate of 1.13 mm/day and ivory-white mycelia of moderate density, reaching full colonization in 24 days.

Compared with previous reports, Nguyen et al. (2020) documented that the growth rate of *L. edodes* on PDA medium typically ranges from

1.0 to 2.1 mm/day, which is consistent with the results of formula 1 and formula 3 in this study. Similarly, Royse et al. (2024) recommended that a growth rate of ≥ 2.0 mm/day, along with dense

and bright-white mycelia, should be considered key indicators of high-quality primary spawn. These criteria were well met by formula 3, highlighting its suitability for spawn production.

Table 4. Growth and development of *Lentinula edodes* mycelium on primary spawn media

Formula	Mycelial initiation time (days)	Full colonization time (days)	Growth rate (mm/day)	Mycelial characteristics
Formula 1	5	24 ^b	1.13 ^b	Ivory-white, sparse mycelium with moderate growth rate
Formula 2	5	28 ^a	0.59 ^c	White, sparse mycelium with slow growth rate
Formula 3	5	22 ^c	2.03 ^a	White, dense, cottony mycelium with rapid growth rate
LSD _{0,05}		0.57	0.35	

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

The effects of substrate composition and ratio on the secondary spawn production of shiitake mushroom (*Lentinula edodes*)

Table 5 demonstrates clear differences in the growth rate of *L. edodes* mycelia among treatments during the extended incubation period. While no statistically significant differences were observed among treatments during the first 10 days, from day 15 onward formula 1 exhibited a marked advantage, reaching a mycelial length of 78.2 mm after 35 days-significantly higher than that of formula 2 (69.4 mm) and formula 3 (60.5 mm). This

indicates that formula 1 is capable of sustaining a more stable and consistent growth rate throughout the spawn run. These findings are consistent with Zhang et al. (2020), who reported that substrates with a balanced ratio of readily degradable carbon and crude fiber promote improved mycelial development. Similarly, Royse et al. (2024) confirmed that an appropriate substrate composition not only accelerates growth but also enhances spawn quality. Therefore, formula 1 can be regarded as the optimal formulation under laboratory conditions, ensuring both growth rate and mycelial density.

Table 5. Effect of medium composition and ratio in secondary spawn on the mycelial colony diameter of shiitake mushroom under laboratory conditions

Time \ Formula	Mycelial colony diameter (mm)			LSD _{0.05}
	Formula 1	Formula 2	Formula 3	
Day 5	10.5 ^a	11.3 ^a	12.4 ^a	1.5
Day 10	18.6 ^a	19.4 ^a	20.8 ^a	1.9
Day 15	31.3 ^a	26.5 ^b	24.3 ^c	0.9
Day 20	48.2 ^a	37.3 ^b	32.6 ^c	1.7
Day 25	60.1 ^a	47.5 ^b	41.8 ^c	2.5
Day 30	72.6 ^a	59.9 ^b	51.5 ^c	3.2
Day 35	78.2 ^a	69.4 ^b	60.5 ^c	3.7

Note: Different superscript letters within the same row indicate statistically significant differences at the 95% confidence level.

Table 6 shows that the mycelial spreading rate of *L. edodes* fluctuated over time and differed significantly among treatments after day 15. In the early stage (days 5–10), the spreading rates of formula 1 - formula 3 were relatively uniform with no significant differences. However, from day 15 onward, formula 1 exhibited a clear advantage, reaching 2.54 mm/day-nearly 3.6 times higher than formula 3 (0.70 mm/day). Notably, on day 20, formula 1 achieved the highest spreading rate of the entire experiment (3.38 mm/day), while formula 2 and formula

3 reached only 2.16 mm/day and 1.66 mm/day, respectively. These results indicate that formula 1 sustained stable development, whereas formula 2 and formula 3 showed a gradual decline in spreading rate after 15 days. This finding is consistent with Atila (2017), who emphasized that a balanced supply of readily available carbon and minerals is crucial for efficient mycelial extension. Similarly, Royse et al. (2024) confirmed that nutrient-balanced formulations help maintain uniform growth throughout the secondary spawn stage.

Table 6. Effect of medium composition and ratio in secondary spawn on the mycelial spreading rate of shiitake mushroom under laboratory conditions

Time	Mycelial spreading rate (mm/day)			LSD _{0.05}
	Formula 1	Formula 2	Formula 3	
Day 5	2.10 ^a	2.26 ^a	2.48 ^a	0.21
Day 10	1.62 ^a	1.62 ^a	1.68 ^a	0.25
Day 15	2.54 ^a	1.42 ^b	0.70 ^c	0.36
Day 20	3.38 ^a	2.16 ^b	1.66 ^c	0.38
Day 25	2.38 ^a	2.04 ^b	1.84 ^c	0.18
Day 30	2.50 ^a	2.48 ^a	1.94 ^b	0.25
Day 35	1.12 ^a	1.90 ^a	1.80 ^a	0.79

Note: Different superscript letters within the same row indicate statistically significant differences at the 95% confidence level.

Table 7. Effect of medium composition and ratio in secondary spawn on the mycelial surface coverage of shiitake mushroom under laboratory conditions

Time	Mycelial coverage (%)			LSD _{0.05}
	Formula 1	Formula 2	Formula 3	
Day 5	11.67 ^a	12.56 ^a	13.78 ^a	1.30
Day 10	20.67 ^a	21.56 ^a	23.11 ^a	1.70
Day 15	34.78 ^a	29.44 ^b	27.00 ^c	1.52
Day 20	53.56 ^a	41.44 ^b	36.22 ^c	3.58
Day 25	66.78 ^a	52.78 ^b	46.44 ^c	4.25
Day 30	80.67 ^a	66.56 ^b	57.22 ^c	5.50
Day 35	86.89 ^a	77.11 ^b	67.22 ^c	5.22

Note: Different superscript letters within the same row indicate statistically significant differences at the 95% confidence level.

Table 7 shows that the mycelial coverage of *L. edodes* during secondary spawn development was markedly influenced by the composition and ratio of the substrate. In the first 10 days, the differences among treatments (formula 1 - formula 3) were not

significant, with coverage ranging from 11.67% to 23.11%. However, from day 15 onward, formula 1 demonstrated a clear advantage, with coverage increasing much faster than that of formula 2 and formula 3. Specifically, at day 20, formula 1 reached

53.56%, compared with 41.44% for formula 2 and 36.22% for formula 3; by day 35, formula 1 achieved 86.89%, while formula 2 and formula 3 reached only 77.11% and 67.22%, respectively. This indicates that formula 1 provided a more favorable nutrient supply and substrate structure, enabling faster and more uniform colonization. These results are consistent with Zhang et al. (2020), who

reported that a balance between readily available carbon and organic nitrogen is a key determinant of mycelial spreading and coverage. Similarly, Royse et al. (2024) highlighted that substrates with an appropriate ratio of lignocellulose and nutritional supplements optimize surface colonization, ensuring uniform spawn quality and reducing the risk of contamination.

Table 8. Growth and development of *Lentinula edodes* mycelium on secondary pawn media

Formula	Mycelial initiation time (days)	Full colonization time (days)	Growth rate (mm/day)	Mycelial characteristics
Formula 1	Mycelial initiation time (days)	38 ^c	2.23 ^a	Dense, cottony, white mycelium with rapid growth rate
Formula 2	8	39 ^b	1.98 ^b	Sparse, ivory-white mycelium with moderate growth rate
Formula 3	10	44 ^a	1.72 ^c	Thin, sparse, white mycelium with slow growth rate
LSD _{0.05}		1.68	0.15	

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



Figure 2. Mycelial growth of *Lentinula edodes* (Berk.) Pegler on different secondary spawn media

Table 8 highlights the pronounced differences in growth rate and colonization time of *L. edodes* mycelia among secondary spawn formulations. Formula 1 exhibited the most favorable performance, with initial mycelial emergence after only 7 days, a growth rate of 2.23 mm/day, and dense, cottony white mycelium. Formula 2 showed intermediate results, with emergence delayed by one day compared to formula 1 and a growth rate of 1.98 mm/day, while formula 3 achieved the

slowest result, requiring 10 days for emergence, with a lower growth rate (1.72 mm/day) and sparse mycelial development. These differences indicate that formula 1 provides a more balanced nutrient composition and substrate structure, making it more suitable for the spawn multiplication phase. The findings are consistent with Atila (2017), who emphasized that the balance between carbon and nitrogen is critical for determining mycelial spread and density. Similarly, Zhang et al. (2020) reported that

spawn media supplemented with readily degradable organic matter shortened the time to mycelial emergence and improved substrate colonization. Taken together, these results confirm formula 1 as the most suitable formulation for practical secondary spawn production.

CONCLUSION

The results indicate that the primary spawn propagation substrate significantly affects the growth rate and characteristics of the shiitake mycelium. Among them, formula 3 exhibited a rapid growth rate, with white, dense, and uniform mycelium, outperforming formula 1 and formula 2. This confirms that a well-balanced substrate composition is a decisive factor for the quality of the initial spawn. At the secondary spawn propagation stage, formula 1 continued to show an advantage, producing thick, white mycelium that covered the substrate quickly and uniformly, reducing the time needed to fully colonize the bag compared to formula 2 and formula 3. In contrast, formula 3 displayed slower mycelial expansion and a lower density. These results suggest that formula 1 is a suitable substrate for level-2 propagation under laboratory conditions.

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REFERENCES

- Zhang H., Chen Y., & Wang Z., 2020. Influence of carbon and phosphate sources on mycelial growth of shiitake (*Lentinula edodes*). *International Journal of Medicinal Mushrooms*, 22(6): 541–549.
- Pham M. T., Le H. A., & Ngo T. M., 2021. Role of primary culture media in quality of mushroom strains: a case of *Lentinula edodes*. *Asian Journal of Mycology*, 4(2): 89–96.
- Nguyen T. L., Nguyen V. H., & Pham N. K. H., 2020. Effects of KH_2PO_4 and MnSO_4 supplementation on PDA medium for *Lentinula edodes*. *Journal of Agricultural Sciences*, 18(2): 55–62.
- Nguyen C. T., 2001. *Experimental design and data analysis in biological research*. Agricultural Publishing House, Hanoi.
- Furlan S. A., Virmond L. J., de Souza C. G. M., Bonatti M., Gern R. M. M., & da Costa S. M. G., 1997. Mushroom production on coffee industrial residues in Brazil. *Bioresource Technology*, 82(2): 117–120.
- Nguyen V. A., Tran Q. T., & Do H. M., 2023. Optimization of nutrient medium for *Lentinula edodes* in primary spawn production. *Vietnam Journal of Biotechnology*, 21(3): 105–114.
- Royse, D. J., Baars, J., & Tan, Q., 2024. *Cultivation of Edible Mushrooms* (3rd ed.). Elsevier.
- Atila, F., 2017. Effect of different substrates on mycelial growth and spawn quality of edible mushrooms. *Journal of Food and Agricultural Research*, 5(1): 45–52.