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# Optimizing Spawn Production and Yield of Shiitake Mushroom (*Lentinula edodes*) - A Comprehensive Study on Grain Substrates and Growth Supplements

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**Abstract**

The experiment was conducted during January to June, 2023 at the Mushroom Research Laboratory, Department of Plant Pathology, Palli Siksha Bhavana, Visva-Bharati University, West Bengal, India, focused on assessing various substrates and supplements for optimizing the cultivation of *Lentinula edodes* (Shiitake mushrooms). Analysis of spawn run durations across different grain substrates revealed sorghum as the most efficient, with strain LE-03 exhibiting the fastest colonization in just 13.67 days. Paddy and wheat substrates followed suit, while maize exhibited the longest colonization times across all strains. Rice bran emerged as the most efficient treatment for spawn run duration, surpassing wheat bran and groundnut oil cake, with the control group requiring the longest duration. Furthermore, examination of supplements highlighted wheat bran as consistently yielding the highest mushroom weight (750 grams of substrate$^{-1}$) and biological efficiency (46.48% to 54.71%) across all strains. Rice bran also showed competitive results, particularly in yield. Groundnut oil cake consistently lagged behind in both yield and biological efficiency compared to wheat and rice bran, while the control group consistently produced the lowest results. These findings underscore the importance of substrate and supplement selection in optimizing spawn run duration, yield, and biological efficiency in Shiitake mushroom cultivation. Wheat bran and rice bran emerged as superior supplements, maximizing both yield and biological efficiency, while groundnut oil cake and the control group exhibited comparatively inferior performance. This comprehensive analysis provides valuable insights for enhancing Shiitake mushroom cultivation practices, emphasizing the significance of substrate and supplement choices for achieving optimal results.

***Keywords:*** Shiitake, grain substrates, spawn, supplements, yield

## 1. Introduction

The term "mushroom" originates from the French "mousse" or "mousseron," reflecting its culinary significance. Revered as "God's flesh" in Romanian customs and the "Elixir of Life" in Chinese traditions, mushrooms hold cultural importance worldwide. Shiitake, scientifically known as *Lentinula edodes*, is a prized edible mushroom esteemed for its culinary and medicinal properties, originating in Japan, China, and Southeast Asia (Mata and Savoie, 2018). The term "shiitake" originates from the combination of "shii," denoting the Japanese chinquapin tree (*Castanopsis cuspidate*), and "take," meaning mushroom (Tian et al., 2016). This saprophytic white-rot fungus thrives on decaying wood from deciduous trees or sawdust, decomposing cellulose, hemicellulose, and lignin through lignocellulolytic enzymes. *L. edodes* stands as the second most cultivated mushroom species globally, trailing behind *Agaricus bisporus* (Leatham, 1986; Chang, 1999; Chang and Buswell, 2001; Szeto et al., 2008; Andrade et al., 2013; Kalaw et al., 2021; Abdullah et al., 2022; Guetarni et al., 2022). Predominantly found in Asian regions with warm, humid climates, shiitake contributes to approximately 25% of global mushroom production (Jiang et al., 2015), with China leading in production, export, and consumption (Ye et al., 2012). The study underscores the vital role of spawn production and yield optimization, focusing on grain substrates and growth supplements to enhance productivity in shiitake mushroom cultivation, crucial for its significant market potential.

The choice of substrates for spawn production is critical in optimizing yield and biological efficiency in cultivating shiitake mushrooms. Various grains and substrates have been explored, including wheat, sorghum, maize, millet, and rice, each exhibiting different colonization and yield characteristics (Phutela et al., 1986; Royse and Bahler, 1986; Kawai et al., 1996; Ashrafuzzaman et al., 2009; Narh et al., 2011; Puri, 2011; Sahu et al., 2014; Gaitan-Hernandez and Norberto Cortes, 2014; Kerketta et al., 2017; Shim et al., 2014; Joshi and Sugar, 2016; Pandey et al., 2017; Afzal et al., 2019; Chittaragi et al., 2020). Research compares solid and liquid spawn methods, revealing differences in colonization speed and fruiting duration (Kawai et al., 1996; Shim et al., 2014), while Puri (2011) investigates the effectiveness of local substrates for shiitake spawn production.

In mushroom cultivation, the choice of substrates and supplementation significantly influences the yield and quality of various mushroom species, including *L. edodes* (shiitake), *Agaricus*, *Pleurotus* spp., *Auricularia*, and *Flammulina* (Royse, 1985; Petre and Teodorescu, 2009; Kapoor et al., 2009; Ramkumar et al., 2011; Moonmoon et al., 2011; Chioza and Ohga, 2013; Mathieu et al., 2015; Alemu, 2014a, 2014b, 2015; Pardo-Gimenez et al., 2016; Ranjbar and Olfati, 2017; Ranjbaret al., 2017; Dayani et al., 2018; Carrasco et al., 2018; Valenzuela-Cobos et al., 2019; Chen et al., 2020; Shuangshuang et al., 2020; Chen et al., 2021; Kumar, 2022; Desisa et al., 2023; Atila and Cetin, 2024; Paswal et al., 2024). Longer spawn run periods and substrate supplementation correlate positively with mushroom yield, often leading to increased biological efficiencies (Royse, 1985). The composition of supplements, including organic and inorganic materials, significantly influences mycelial growth and enzyme production in mushrooms (Kapoor et al., 2009; Ramkumar et al., 2011). Local substrates like coffee husk and teff straw are found to enhance mushroom cultivation (Alemu, 2014a, 2014b, 2015). This study aims to contribute to the understanding of optimal substrate selection and supplementation strategies for improving shiitake mushroom cultivation efficiency and yield.

## 2. Materials and Methods

The research trials took place at the Mushroom Research Laboratory, situated within the Department of Plant Pathology at Palli Siksha Bhavana (Institute of Agriculture), situated in Sriniketan, Birbhum district, West Bengal, India during January to June 2023.

### *2.1. Cultures*

The three *L. edodes* strains (LE-01, LE-02, LE-03) employed in this research were obtained from the Indian Institute of Horticultural Research (IIHR), Bengaluru and the Directorate of Mushroom Research (DMR), Solan.

### *2.2. Evaluation of different grain substrates for the spawn preparation*

The assessment involved the use of various disease-free and healthy grain substrates, including paddy, wheat, sorghum, bajra, and maize grains, for spawn preparation of shiitake mushrooms. The grains underwent a process of soaking in water for an hour, followed by boiling until they reached a softened state. Afterward, the grains were shade-dried to achieve a moisture content of approximately 30–35%. To regulate moisture levels and prevent clumping, the grains were supplemented with 2% gypsum ($CaSO_4$) and 2% calcium carbonate ($CaCO_3$) based on their dry weight. Subsequently, the grains were filled into glass bottles, filling about two-thirds of their capacity, and the bottle openings were sealed with non-absorbent cotton. Sterilization of the filled bottles was carried out in an autoclave for duration of 2 hours at a temperature of 121.6°C and a pressure of 15 psi. Following sterilization, the bottles were left to cool overnight. Inoculation of the bottles with a pure culture of *L. edodes* was conducted under sterile conditions within a laminar airflow chamber, after which the bottles were placed in a BOD chamber at a temperature of 24±2°C. The duration required for complete mycelial coverage was then documented.Top of Form

### *2.3. Effect of the different supplements on the growth and yield of shiitake mushroom*

For this study, the conventional substrate, sawdust, was utilized. Various supplements such as rice bran, wheat bran, and groundnut oilcake were soaked in water for 3–4 hours. After processing, these supplements were mixed with sawdust at a rate of 14.5%. The mixture was then filled into grow bags and subjected to sterilization in an autoclave for 2 hours at a temperature of 121.6°C and a pressure of 15 psi. Following sterilization, the substrates were allowed to cool down. They were then inoculated with 5% wheat grain spawn under sterile conditions within a laminar airflow chamber. The inoculated bags were placed in a spawn run room with a constant temperature of 24±2°C. Observations such as the time required for complete spawn run, as well as yield and biological efficiency, were periodically recorded.

### *2.4. Cropping*

The spawned bags were placed in a spawn run room with a temperature set at 24±2°C, where they underwent the spawn run process without specific care. As the mycelial coat formed and mycelial bump and browning occurred, observations were made. During growth, the fungus secreted yellowish metabolites into the substrates. Upon noticing approximately three-fourths of the substrate turning brownish, the grow bags underwent overnight refrigeration for cold shock to stimulate fruiting. Following cold shock treatment, the polypropylene cover was removed, and the substrate blocks were transferred to a cropping room for fruiting. The cropping room maintained a temperature of around 20°C with 90% humidity, achieved by frequent water spraying on gunny bags attached to room walls and windows. Alternatively, an automated ultrasonic humidifier could be utilized for humidity maintenance. Carbon dioxide levels were regulated by an exhaust fan, while light intensity was controlled by fluorescent lighting.

### *2.5. Observations*

Observations, including the duration of spawn run, development of mycelial coat, formation of mycelial bumps and browning, as well as metrics such as the average number and weight of fruit bodies bag$^{-1}$, were systematically documented throughout the experiment.

*2.6. Harvest*

The harvest procedure involved using a sharp knife to cut the base of the stipe, ensuring minimal damage to the fruiting blocks to prevent pest infestation and mold competition. Following harvest, the fruiting blocks were left untouched without water spraying for a period of 10 days. After this resting period, the blocks were rehydrated and placed back in the fruiting chamber for subsequent flushes. Typically, two to three flushes could be obtained from a single block, although subsequent yields were generally lower due to increased contamination levels.

*2.7. Yield of mushroom*

The total yield from each replication was assessed by calculating the fresh weight at each harvest, expressed as weight (in grams) unit weight of the substrate$^{-1}$.

*2.8. Biological Efficiency*

Biological efficiency, as defined by Miles and Chang (2004), was computed using the subsequent equation,

Biological Efficiency (%)=(Fresh weight of mushroom (g)/Dry weight of substrate (g))×100

*2.9. Statistical analysis*

The study employed a Completely Randomized Design, with each treatment being replicated three times for statistical reliability. The data analysis was conducted using the statistical software WASP 1.0 (Web Agri Stat Package), accessible at https://ccari.icar.gov.in/waspnew.html, and accessed on 2 April 2024. ANOVA was utilized to examine potential differences in parameter values. Duncan's multiple range tests were employed at a significance level of 5% to assess variations between treatments.

## 3. Results and Discussion

*3.1. Evaluation of different grain substrates for spawn production*

The study aimed to assess the suitability of various grain substrates (Paddy, Wheat, Sorghum, Maize, and Bajra) for spawn production of shiitake mushrooms, utilizing three strains (LE-01, LE-02, and LE-03) and incubating the inoculated grains at a temperature of 25±2°C in a BOD incubator. The duration required for complete mycelial coverage on the grains was recorded, aiming to identify the optimal combination of substrate and strain. Analysis of the data revealed notable differences in the time taken for complete spawn run among the strains across different grain substrates.

The analysis of days required for complete spawn run across different grain substrates reveals notable variations. Among the substrates, sorghum stands out as the most efficient, with significantly shorter durations for spawn run compared to others for all three strains. Specifically, sorghum records the shortest times for spawn run across all three strains, with strain LE-03 showing the fastest colonization, completing in just 13.67 days, closely followed by strains LE-01 and LE-02. Following sorghum, paddy and wheat substrates demonstrate competitive performance, with strain LE-03 showing the fastest colonization on both substrates, completing in 15.32 days. However, while paddy substrate exhibits slightly longer durations for strains LE-01 and LE-02, wheat substrate records consistent durations across all three strains. Conversely, maize substrate consistently exhibits the longest durations for spawn run for all three strains, with strain LE-01 requiring the longest time, followed by strains LE-02 and LE-03. This suggests that sorghum substrate offers the most favorable conditions for rapid mycelial colonization, while maize substrate presents as the least conducive environment (Table 1).

Table 1: Evaluation of different grain substrates for spawn production of shiitake mushroom

| Grain substrates | Number of days taken for complete spawn run | | |
|---|---|---|---|
| | LE-01 | LE-02 | LE-03 |
| Paddy | 16.92c | 15.97d | 15.32b |
| Wheat | 18.35ab | 17.93c | 15.32b |
| Sorghum | 15.83d | 13.98e | 13.67c |
| Maize | 19.25a | 20.65a | 20.33a |
| Bajra | 17.67bc | 18.67b | 15.31b |
| SEm± | 0.248 | 0.038 | 0.125 |
| CD (*p*=0.05) | 0.907 | 0.357 | 0.643 |

Similar trends regarding spawn production of *L. edodes* on different grain substrates have been reported by previous studies (Puri, 2011; Narh et al., 2011; Pandey et al., 2017; Chittaragi et al., 2020). Phutela et al. (1986) highlighted the influence of grain availability on spawn production, suggesting that in regions abundant with paddy grains like West Bengal; they could serve as a viable alternative to sorghum grains for shiitake mushroom spawn production. The findings from our study resonate with previous research, notably Chittaragi et al. (2020), which highlights the superior performance of sorghum as a spawning substrate due to its rapid mycelial colonization, contrasting sharply with the sluggish progress observed with pearl millet. This aligns with the investigation of Puri (2011), which underscores the suitability of sorghum for Shiitake spawn production, attributed to its conducive environment for *L. edodes* growth and minimal susceptibility to contamination. Our results affirm the significance of substrate selection in spawn run efficiency, echoing the preference for sorghum due to its ability to support faster mycelial growth and ultimately maximize yield and biological efficiency. Thus, incorporating sorghum into spawn production protocols emerges as a prudent strategy, bolstered by its proven track record in promoting optimal colonization and productivity in Shiitake cultivation.

### 3.2. Effect of different supplements on the growth and yield of shiitake mushroom

#### 3.2.1. Days taken for complete spawn run

The analysis reveals notable differences in the time taken for complete spawn run among the various treatments for all three strains. Among the treatments, rice bran demonstrates the shortest duration for complete spawn run across all three strains, with values ranging from 46.33 to 50.97 days, followed closely by wheat bran and groundnut oil cake. Specifically, wheat bran exhibits competitive performance, with durations ranging from 44.33 to 52.00 days, while groundnut oil cake shows slightly longer durations, ranging from 49.67 to 56.67 days. In contrast, the control group consistently requires the longest time for spawn run across all three strains, with durations ranging from 60.00 to 65.90 days. These findings underscore the importance of substrate selection in optimizing spawn run duration, with rice bran emerging as the most efficient treatment, followed by wheat bran and groundnut oil cake, while the control group necessitates the longest duration for spawn run across all three strains (Table 2).

Table 2: Days taken for complete spawn run

| Supplements | Time taken for complete spawn run (days) | | |
|---|---|---|---|
| | LE-01 | LE-02 | LE-03 |
| Rice bran | 46.33c | 50.97c | 47.59bc |
| Wheat bran | 49.33bc | 52.00c | 44.33c |
| Groundnut oil Cake | 52.25b | 56.67b | 49.67b |
| Control | 60.00a | 61.77a | 65.90a |
| SEm± | 8.613 | 5.663 | 7.412 |
| CD (p=0.05) | 5.526 | 4.481 | 5.126 |

This aligns with previous studies, reinforcing the critical role of substrate supplementation in enhancing spawn run efficiency. Sardar et al. (2020) reported that substrates supplemented with rice and wheat bran required significantly fewer days for complete mycelial colonization compared to control treatments. Similarly, Deb et al. (2021) found that rice bran supplementation significantly reduced the spawn run duration in Oyster mushroom cultivation, while Jana et al. (2022) observed that rice bran-supplemented substrates required less time for spawn run in paddy straw cultivation. These consistent findings highlight the efficacy of rice bran as a superior supplement for optimizing spawn run duration across different fungal strains and cultivation contexts.

The efficiency of rice bran in reducing spawn run duration can be attributed to its rich nutrient profile, which provides an optimal environment for rapid mycelial growth. Wheat bran, although slightly less effective than rice bran, also offers a substantial nutrient supply that supports quick colonization. Groundnut oil cake, while beneficial, appears to be less effective than rice and wheat bran, possibly due to differences in nutrient composition and availability. The significantly longer spawn run duration observed in the control group underscores the necessity of substrate supplementation for enhancing fungal growth and productivity. The control group's performance highlights the limitations of non-supplemented substrates, which lack the enriched nutrient environment provided by rice bran, wheat bran, and groundnut oil cake.

#### 3.2.2. Performance on yield and biological efficiency

The primary focus of assessing various supplements in the cultivation of *L. edodes* was to evaluate their impact on yield and biological efficiency. Analysis of the data highlighted significant differences in the yield and biological efficiency of shiitake mushrooms across different supplements.

Among the supplements tested for Shiitake mushroom cultivation, wheat bran consistently demonstrates the highest yield and biological efficiency across all three strains (LE-01, LE-02, and LE-03). Specifically, wheat bran yields the highest average weight of mushrooms 750 grams of substrate$^{-1}$, with values ranging from 391.80g to 410.34g, and achieves the highest biological efficiency ranging from 46.48% to 54.71%. Following closely behind, rice bran also exhibits competitive results, particularly in terms of yield, with values ranging from 348.46g to 374.85g, and biological efficiency ranging from 46.46% to 49.98%. Conversely, groundnut oil cake consistently shows lower yields and biological efficiency compared to rice bran and wheat bran across all strains, with values ranging from 305.78g to 341.18g in yield and 40.76% to 45.49% in biological efficiency. The control group consistently produces the lowest yields and biological efficiency, with values ranging from 269.52g to 291.63g in yield and 35.94% to 38.88% in biological efficiency across all strains. These findings underscore the effectiveness of wheat bran and rice bran as supplements for maximizing both yield and biological efficiency in Shiitake mushroom cultivation, while groundnut oil cake and the control group demonstrate comparatively inferior performance (Table 3, 4).

The findings from our study align closely with previous research, particularly Kapoor et al. (2009), which highlights the significant impact of organic supplements on mycelial growth and enzyme activity in *L. edodes* cultivation. Our

Table 3: Effect of supplements on yield of shiitake mushroom

| Supplements | Yield (g) 750 g of substrate$^{-1}$ | | |
|---|---|---|---|
| | LE-01 | LE-02 | LE-03 |
| Rice bran | 348.46b | 374.85b | 349.04a |
| Wheat bran | 391.80a | 410.34a | 348.61a |
| Groundnut oil Cake | 341.18b | 335.70c | 305.78b |
| Control | 280.32c | 269.52d | 291.63c |
| SEm± | 26.141 | 28.098 | 24.797 |
| CD (*p*=0.05) | 9.627 | 9.980 | 9.376 |

Table 4: Effect of supplements on biological efficiency

| Supplements | Biological efficiency (%) | | |
|---|---|---|---|
| | LE-01 | LE-02 | LE-03 |
| Rice bran | 46.46b | 49.98b | 46.53a |
| Wheat bran | 52.24a | 54.71a | 46.48a |
| Groundnut oil Cake | 45.49b | 44.76c | 40.76b |
| Control | 37.38c | 35.94d | 38.88c |
| SEm± | 0.465 | 0.500 | 0.443 |
| CD ($p$=0.05) | 1.285 | 1.331 | 1.254 |

results corroborate their findings, as we observed maximum mycelial extension rates with 10% supplementation of rice bran and 20% supplementation of wheat bran. Interestingly, similar trends were noted by Dayani et al. (2018), who emphasized the importance of supplementation levels in achieving higher biological efficiency, particularly with wheat-milling residues. The addition of organic nitrogen supplements, such as wheat bran or rice bran, to sawdust is crucial for supporting the mycelial growth and yield of shiitake mushrooms (Kumar et al., 2019). Among the various substrates used alone or in combination with different supplements for cultivating shiitake mushrooms, wheat straw combined with 20% wheat bran was found to be the most effective, followed by wheat straw combined with 20% rice bran (Paswal et al., 2024). In oyster mushroom cultivation, the highest fruit yield and biological efficiency were recorded when wheat straw was supplemented with rice bran at 2% of the substrate weight, corn flour at 25 g $kg^{-1}$ of prepared straw, and gypsum at 20 g $kg^{-1}$ of straw (Kumar et al., 2020). The total yield of milky mushrooms per bag significantly varied with the type of supplementing material used. The maximum total yield and the highest biological efficiency were achieved with wheat bran supplementation, followed by rice bran supplementation. In contrast, the lowest efficiency was observed in non-supplemented substrates (Sardar et al., 2020). Similarly, supplementation with both wheat bran and rice bran significantly improved the yield and biological efficiency of various *Pleurotus* species (Deb et al., 2021; Rout and Mohapatra, 2022). Additionally, rice bran and wheat bran supplementation significantly increased the yield and biological efficiency of paddy straw mushroom cultivation (Jana et al., 2022). Our findings align with previous studies, emphasizing that supplementation significantly enhances the yield and biological efficiency of mushroom cultivation. This suggests a consistent pattern across studies, indicating the efficacy of certain organic supplements like rice bran and wheat bran in enhancing mushroom cultivation parameters. However, it's noteworthy that peanut meal exhibited slower growth, emphasizing the variability in supplement effects. Overall, our findings contribute to the growing body of evidence supporting the strategic use of organic supplements to optimize *L. edodes* cultivation, with implications for improving yield and biological efficiency.

## 4. Conclusion

The study found sorghum to be the best grain substrate for Shiitake mushroom (*Lentinula edodes*) cultivation, with strain LE-03 colonizing the fastest. Maize was the least effective. Among supplements, wheat bran yielded the highest mushroom weight and biological efficiency, followed by rice bran. Groundnut oil cake performed poorly, and the control group had the lowest yields. Sorghum and wheat bran were identified as optimal for enhancing Shiitake production, offering insights for maximizing productivity and efficiency.

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