Assessment of Various Substrates for Shiitake Mushroom (*Lentinula edodes*) Cultivation in the Agro-Climatic Conditions of West Bengal

ABSTRACT

Aim: This study aimed to evaluate the impact of different substrates on various stages of shiitake mushroom (*Lentinula edodes*) cultivation to optimize growth and yield.

Study Design: A Completely Randomized Design with three replications for each treatment was employed to ensure statistical reliability. Duncan's multiple range tests were utilized at a significance level of 5% to evaluate differences between treatments.

Place and Duration of Study: The research was conducted at the Mushroom Research Laboratory, Department of Plant Pathology, Palli Siksha Bhavana (Institute of Agriculture), located in Sriniketan, Birbhum district, West Bengal, throughout the year 2023.

Methodology: Different substrates including lentil husks, rice straw, wheat straw, sawdust, and mustard pods were evaluated for their impact on spawn run completion, mycelial bump formation, and sporocarp formation durations. Each substrate's performance was assessed in terms of sporocarp production and biological efficiency.

Results: Across the three strains (LE-01, LE-02, and LE-03), lentil husks consistently exhibited the shortest spawn run duration, averaging 34.59 to 37.00 days. Following closely, wheat straw displayed favorable performance with durations ranging from 39.20 to 41.23 days. Sawdust also proved efficient, albeit slightly longer than lentil husks and wheat straw. Conversely, maize cobs and sugarcane bagasse exhibited longer spawn run durations, averaging from 57.80 to 64.90 days. For mycelial bump formation, lentil husks again showed the shortest duration, averaging 42.67 to 45.77 days across the strains. Wheat straw followed suit with durations ranging from 48.93 to 50.20 days, while sawdust demonstrated favorable times albeit slightly longer. Conversely, maize cobs and sugarcane bagasse exhibited longer mycelial bump formation times, ranging from 68.11 to 74.40 days. For sporocarp formation, lentil husks and sawdust emerged as top performers with the shortest durations, averaging 76.66 to 81.52 days. Conversely, maize cobs exhibited the longest sporocarp formation time, averaging 97.43 days. In terms of sporocarp yield, rice straw yielded the highest, averaging 27.67 sporocarps per bag, followed by wheat straw with 24.33 sporocarps per bag. Lentil husks, while efficient in other aspects, produced an average of 21.78 sporocarps per bag, trailing slightly behind rice straw and wheat straw. Regarding yield and biological efficiency, sawdust displayed superior performance, yielding an average of 384.57 grams per 750 grams of substrate with a biological efficiency of 51.18%. Lentil husks and rice straw exhibited comparable performance in yield and biological efficiency, with lentil husks yielding 367.81 grams and rice straw yielding 333.09 grams per 750 grams of substrate, both with biological efficiencies around 47-48%. Conversely, mustard pods exhibited the lowest yield and biological efficiency, averaging 269.02 grams per 750 grams of substrate with a biological efficiency of 35.91%.

Conclusion: The study underscores the importance of substrate selection in optimizing various stages of *L. edodes* cultivation. Lentil husks emerged as a promising substrate choice for rapid mycelial growth, but its sporocarp production lagged behind other substrates. Sawdust proved to be the top-performing substrate in terms of yield and biological efficiency, indicating its efficacy in supporting robust mushroom growth.

Keywords: Medicinal mushroom; Substrates; Sporocarp; Yield; Biological Efficiency.

1. INTRODUCTION

Shiitake mushrooms, also known scientifically as (*Lentinula edodes*(Berk.) Pegler), and referred to by various names such as Xiang-gu, golden oak mushroom, Chinese black mushroom, or Oriental black mushroom, belong to the Agaricales order within the Basidiomycota phylum. Originating from Japan, China, and other Southeast Asian countries, shiitake mushrooms are highly valued for their culinary and medicinal uses.

The term "shiitake" is derived from the Japanese word "shii," which denotes the chinquapin tree, and "take," which means mushroom. This fungus thrives as a saprophytic white-rot species, predominantly found on decomposing wood from deciduous trees or sawdust. It breaks down cellulose, hemicellulose, and lignin using lignocellulolytic enzymes. *L. edodes* ranks as the second most cultivated mushroom species worldwide, after *Agaricusbisporus*[1, 2, 3]. It is commonly found in warm and humid climates, particularly in Asian regions, where it contributes to approximately a quarter of global mushroom production. China leads in the production, export, and consumption of shiitake mushrooms.

Shiitake mushrooms are often hailed as "the queen of mushrooms" due to their significant market potential both domestically and internationally. They are renowned for producing delicious brown sporocarps that are valued for their recognized medicinal properties. In Chinese folklore, shiitake mushrooms are even referred to as an "elixir of life," believed to enhance stamina, treat colds, improve circulation, and prevent premature aging. The history of shiitake mushroom cultivation dates back to the Sung dynasty era around 1100 A.D., with cultivation techniques potentially introduced to Japan by Chinese growers between the 15th and 16th centuries. Traditionally, shiitake cultivation involved growing them on hardwood logs, particularly oak trees, with advancements in cultivation techniques leading to the use of artificial sawdust substrate logs in the 19th century. Sawdust substrates have become the primary choice in recent years, accounting for about 75% of total production [4]. Sawdust serves as the main ingredient for cultivating *L. edodes*, although other wood-rot fungi can utilize alternative substrates such as rice straw, wheat straw, corncob, and bagasse [5, 6].

Several studies have delved into various aspects of shiitake cultivation, including optimizing substrates, cultivation techniques, and methods to improve yield. For instance, research conducted by Salmones et al. [7] investigated the cultivation of shiitake on different substrates, finding that sugarcane bagasse yielded the highest biological efficiency. Additionally, advancements such as utilizing pH indicators to assess culture maturity [8], and employing vacuum-soaking techniques for substrate preparation have been explored [9]. Furthermore, recommendations have been put forth regarding substrate formulations, such as substituting oak wood chips with ground wheat straw [10], and incorporating hazelnut husk as a substrate alongside commercial sawdust [11]. Shen et al. [12] conducted studies aimed at reducing production costs while improving yield by adjusting moisture content and porosity.Gaitan-Hernandez et al. [13] evaluated the production on vineyard prunings (VP), sorghum stubble (SS), sugarcane bagasse (SB), and oak shavings (OS). Baktemur et al. [14] investigated for cultivation of *L. edodes* by using different agricultural wastes (oak

sawdust, poplar sawdust, wheat stalk, peanut shell, corncob and vine pruning waste. Yu et al. [15] evaluated the effect of using corncob as a substrate for Shiitake cultivation. Kumar [16] evaluated the efficacy of wheat straw and saw dust and Mahdizadeh et al. [17] investigated the malt and wheat straw for the *L. edodes* cultivation.

Furthermore, researchers have explored the potential of non-traditional substrates and discarded materials for cultivating shiitake mushrooms. Puri et al. [18] demonstrated successful cultivation using agricultural residues, with wheat straw proving to be particularly productive in terms of yield and biological efficiency. Moreover, studies investigating the use of chipped alder [19], and analyzing the biochemical composition of substrates have offered valuable insights for optimizing cultivation techniques [20].

Overall, mushroom cultivation offers an environmentally sustainable solution for waste management while also providing a valuable food source. However, commercial shiitake cultivation in India remains largely untapped, indicating the necessity for further research and development focusing on locally available substrates [21]. Therefore, the aim of this research is to establish standardized cultivation methods for shiitake mushrooms (*L. edodes*) in the lateritic region of West Bengal by evaluating various substrates readily accessible in the local area.

2. MATERIAL AND METHODS

2.1 Cultures

In this investigation, the *L. edodes* cultures were sourced from two distinct origins: one strain was acquired from the Indian Institute of Horticultural Research (IIHR) located in Bengaluru, representing LE-01, while two additional strains (LE-02 and LE-03) were obtained from the Directorate of Mushroom Research (DMR) situated in Solan.

2.2 Spawn preparation

2.2.1 Preparation of mother and bed spawn

Disease-free wheat grains served as the substrate for both mother and bed spawn preparation in cultivating shiitake mushrooms. The grains underwent meticulous treatment, including soaking in water and boiling until soft, followed by air-drying to achieve a moisture content of approximately 30-35%. Addition of 2% gypsum and 2% calcium carbonate helped regulate moisture levels and prevent clumping. Filled into glass bottles, leaving two-thirds of volume, they underwent sterilization in an autoclave at 121.6°C and 15 psi for two hours. After cooling overnight, in aseptic conditions, bottles were inoculated with *L. edodes* pure culture. Incubation followed at 24±2°C for 10-15 days. It's imperative to maintain cleanliness and humidity control during spawn production. Substrates, raw or supplemented with wheat bran, are recommended with pH adjusted to 5.5-6.5. Perforated spawn containers ensure proper aeration, and careful inoculation evenly distributes the mycelium throughout the substrate.

2.3 Evaluation of different substrates for the growth and yield of *Lentinula edodes*

2.3.1 Preparation of substrates

In this study, a variety of organic agricultural residues including sawdust, rice straw, wheat straw, lentil husks, sugarcane bagasse, maize cobs, and mustard pods were employed for

cultivating shiitake mushrooms. Each substrate, comprising approximately 85% of the total, was supplemented with 0.5% calcium carbonate ($CaCO_3$) and 14.5% wheat bran, unless stated otherwise. The substrates underwent thorough cleaning and processing. Paddy straw, wheat straw, sugarcane bagasse, etc., were cut into small pieces measuring around 3cm. All substrates were soaked in water for about 16-18 hours, while wheat bran was soaked for 3 hours. Following soaking, the substrates were drained of excess water and evenly spread on a clean plastic sheet in shaded conditions. Once the moisture content reached 60-65%, wheat bran was incorporated into the substrate. The mixture was then filled into polypropylene grow bags, with their openings sealed using non-absorbent cotton and heat-resistant rubber bands. To prevent fermentation, the bags were promptly sterilized, primarily through autoclaving, unless specified differently for the particular investigation.

2.4 Spawning and Cropping

The sterilized or pasteurized substrates, housed in grow bags, were allowed to cool overnight in the inoculation room before inoculation with 5% grain spawn in a sterile manner within the laminar airflow chamber. The spawned bags were then transferred to a designated spawn run room with a temperature ranging from 24±2°C, where they underwent the spawn run process without specific attention. Throughout this phase, observations were conducted on the development of the mycelial coat, mycelial bump formation, and browning, as well as the presence of yellowish metabolites secreted by the fungus. Once approximately three-fourths of the substrate had turned brownish, the grow bags were moved to a refrigerator overnight to induce cold shock and initiate the fruiting process. Following the cold shock treatment, the polypropylene cover was removed, and the substrate blocks were transferred to a cropping room for fruiting. The cropping room, maintained at a temperature of approximately 20°C with 90% humidity, utilized water sprayed on gunny bags attached to the room's walls and windows or an automated ultrasonic humidifier to regulate humidity levels. Carbon dioxide levels were managed using an exhaust fan, while fluorescent lighting maintained appropriate light intensity.

2.5Observations

Observations were systematically documented during the cultivation process, covering aspects such as the duration of spawn run, the growth of mycelial coat and bumps, browning, average number of fruit bodies per bag, and the weight of fruit bodies.

2.6Harvest

During harvesting, the base of the stipe is cut with a sharp knife to minimize harm to the fruiting blocks, which might otherwise attract pests and competing molds. Subsequent to harvesting, the fruiting blocks are left without water spray for a period of 10 days. Following this resting period, the blocks are rehydrated and transferred to the fruiting chamber for the next flush. Usually, two to three flushes can be achieved from a single block. However, the yield of subsequent flushes tends to decline due to elevated contamination levels.

2.7Yield and Biological Efficiency

The overall yield from each replication was determined by assessing the fresh weight at each harvest, expressed as weight (in grams) per unit weight of the substrate. Biological efficiency, as described by Miles and Chang [22], was computed using the subsequent formula.

$$\text{Biological Efficiency (\%)} = \frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrate (g)}} \text{X } 100$$

2.9 Statistical analysis

A Completely Randomized Design was utilized in the study, with each treatment replicated three times to ensure statistical reliability. Data analysis was performed using the statistical software WASP 1.0 (Web Agri Stat Package), which was accessed from https://ccari.icar.gov.in/waspnew.html on 2 April 2024. ANOVA was employed to investigate potential differences in parameter values. Duncan's multiple range tests were utilized at a significance level of 5% to evaluate differences between treatments.

3. RESULTS AND DISCUSSION

3.1 Impact of diverse substrates on shiitake mushroom growth and yield

The effects of different substrates, including rice straw, wheat straw, sawdust, lentil husks, sugarcane bagasse, maize cobs, and mustard pods, were examined to assess their impact on spawn run duration, mycelial bump formation period, sporocarp formation time, number of fruiting bodies harvested, yield, and biological efficiency.

3.1.1 Days taken for complete spawn run

Understanding the duration required for complete spawn colonization is crucial for efficient mushroom cultivation. Spawn run duration refers to the time taken for the mycelium to fully colonize the substrate before fruiting body formation begins. The findings reveal significant variations in spawn run duration among the different substrates. This discrepancy may be attributed to variations in substrate composition, nutrient availability, and structural properties affecting mycelial growth rate.Understanding the factors influencing spawn run duration is essential for optimizing cultivation practices and maximizing yield.In the study evaluating the impact of different substrates on the spawn run duration of three strains of shiitake mushroom (LE-01, LE-02, and LE-03), it was observed that lentil husks consistently exhibited the shortest time for complete spawn run across all three strains, with an average of 31.43, 37.00, and 34.59 days, respectively. Following lentil husks, wheat straw showed relatively shorter spawn run durations, ranging from 39.20 to 41.23 days for LE-02 and LE-03, respectively. Sawdust also displayed relatively favorable spawn run durations for LE-03 and LE-01, although slightly longer compared to lentil husks and wheat straw. Conversely, substrates such as maize cobs and sugarcane bagasse demonstrated longer spawn run durations, with average times ranging from 57.80 to 61.74 days for maize cobs and 59.22 to 63.78 days for sugarcane bagasse across the three strains. These results suggest that lentil husks and wheat straw are promising substrates for accelerating spawn run duration in shiitake mushroom cultivation, while maize cobs and sugarcane bagasse may require longer periods to complete the spawn run (Table 1 and 3).

3.1.2 Days taken for mycelial bump formation

Mycelial bump formation refers to the stage in shiitake mushroom cultivation where the mycelium, the vegetative part of the fungus, begins to aggregate and form visible bumps or knots on the substrate surface. This process marks an important transition towards the formation of fruiting bodies. The time taken for mycelial bump formation can vary significantly depending on the substrate used. In our study, we observed that the days taken for mycelial bump formation varied across the different substrates tested. This variation can be attributed

to several factors, including the composition and nutrient content of the substrate, as well as its physical characteristics such as porosity and moisture retention capacity.Substrates rich in lignocellulosic materials tend to promote faster mycelial growth and bump formation due to their higher availability of carbon and nitrogen sources for fungal metabolism. Conversely, substrates with lower nutrient content or less favorable physical properties may result in slower mycelial growth and bump formation.Understanding the dynamics of mycelial bump formation is crucial for optimizing shiitake mushroom cultivation practices. Analyzing the data specifically for mycelial bump formation time across the three strains of shiitake mushroom (LE-01, LE-02, and LE-03), lentil husks consistently exhibited the shortest duration for this stage, with averages ranging from 42.67 to 45.77 days across the strains. Following lentil husks, wheat straw showed relatively shorter mycelial bump formation times, ranging from 48.93 to 50.20 days for LE-03 and LE-01, respectively. Sawdust also demonstrated favorable mycelial bump formation times, particularly for LE-03 and LE-01, though slightly longer compared to lentil husks and wheat straw. Conversely, substrates such as maize cobs and sugarcane bagasse exhibited longer mycelial bump formation times, with averages ranging from 70.80 to 74.40 days for maize cobs and 68.11 to 72.70 days for sugarcane bagasse across the three strains. These results indicate that lentil husks and wheat straw are conducive substrates for promoting quicker mycelial bump formation in shiitake mushroom cultivation, while maize cobs and sugarcane bagasse require longer periods to reach this critical stage of growth (Table 1 and 3).

3.1.3 Days taken for sporocarp formation

The data reveals significant variations in the time required for sporocarp formation across different substrate treatments. Lentil husks emerged as the substrate fostering the quickest sporocarp formation, with an average duration of 76.66 days, closely followed by sawdust at 81.52 days. Conversely, maize cob exhibited the longest period for sporocarp formation, averaging 97.43 days across all three strains. Sugarcane bagasse and rice straw also displayed relatively longer durations, with averages of 98.1 days and 87.09 days, respectively. These findings underscore the importance of substrate selection in influencing the timing of sporocarp formation in mushroom cultivation. Lentil husks stand out as a promising substrate for accelerating sporocarp formation, while maize cob presents a less favorable option due to its prolonged duration (Table 1 and 3).

3.1.4 Number of sporocarps

The data illustrates notable differences in the number of sporocarps produced across various substrate treatments. Among the substrates tested, rice straw emerged as the top performer, consistently yielding the highest number of sporocarps across all three strains, with an average of 27.67 sporocarps per bag. Following closely behind is wheat straw, with an average of 24.33 sporocarps per bag. Conversely, mustard pods exhibited the lowest sporocarp production, averaging only 16.89 sporocarps per bag across all three strains. Sugarcane bagasse also displayed relatively lower yields, with an average of 17.56 sporocarps per bag. These findings underscore the significant impact of substrate choice on sporocarp production in mushroom cultivation. Rice straw stands out as a particularly favorable substrate for maximizing sporocarp yields, while mustard pods present a less optimal option due to their lower sporocarp production. While lentil husks exhibited favorable performance in terms of spawn run duration, mycelial bump formation, and sporocarp formation, yielding relatively quicker results compared to other substrates, its sporocarp production trailed behind some of the other treatments. Despite taking less time for spawn run completion, mycelial bump formation, and sporocarp formation, lentil husks produced an average of 21.78 sporocarps per bag, placing it behind rice straw, wheat straw, and sawdust in terms of sporocarp yield (Table 2 and 4).

3.1.5 Performance on Yield and Biological efficiency

In terms of mushroom yield and biological efficiency, sawdust emerged as the top-performing substrate, consistently producing the highest yield and biological efficiency across all three strains. With an average yield of 384.57 grams per 750 grams of substrate and a biological efficiency of 51.18%, sawdust demonstrated its effectiveness as a substrate for mushroom cultivation. Following closely behind, lentil husks and rice straw exhibited comparable performance in terms of yield and biological efficiency, with lentil husks yielding an average of 367.81 grams and rice straw yielding 333.09 grams per 750 grams of substrate, both with biological efficiencies ranging around 47-48%. Conversely, mustard pods displayed the lowest yield and biological efficiency, averaging only 269.02 grams per 750 grams of substrate with a biological efficiency of 35.91%. Maize cobs and wheat straw also demonstrated relatively lower yields and biological efficiencies compared to sawdust, lentil husks, and rice straw, further highlighting the influence of substrate choice on mushroom cultivation success. Overall, these results emphasize the importance of selecting suitable substrates to optimize yield and biological efficiency in mushroom cultivation endeavors (Table 2 and 4, Figure 1).

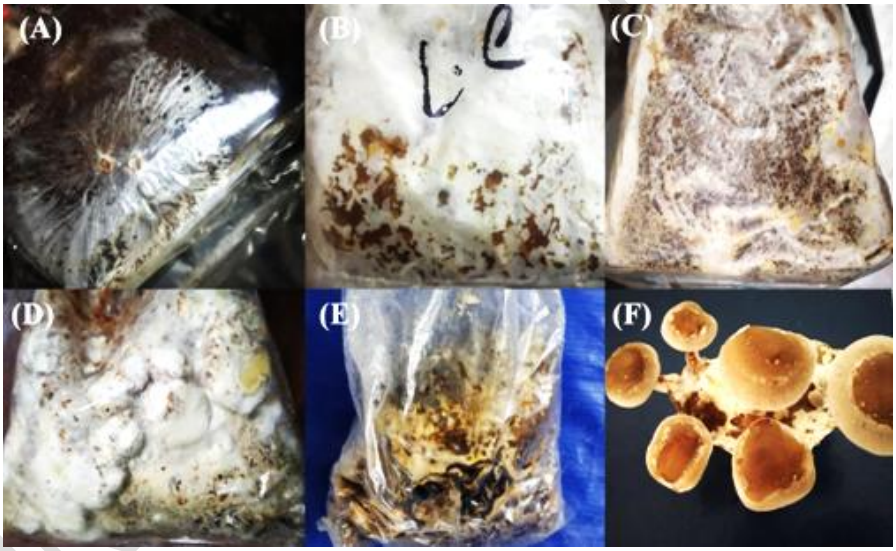

Figure 1. Different growth stages of *L. edodus*. (A) Initial spawn run (B) Complete spawn run (C) Mycelial coat formation (D) Mycelial bump formation (E) Coat hardening and browning (F) Fruiting

Table 1. Effect of different substrates on spawn run, mycelial bump formation and sporocarp formation in shiitake mushroom cultivation

Substrates	Daystakenforcomplete Spawnrun			Daystakenformycelial Bumpformation			Daystaken for Sporocarpformation		
	LE-01	**LE-02**	**LE-03**	**LE-01**	**LE-02**	**LE-03**	**LE-01**	**LE-02**	**LE-03**
Rice straw	44.30^{d}	52.27^{c}	47.33^{c}	50.60^{c}	56.97^{b}	55.13^{b}	88.23^{c}	85.67^{d}	91.37^{b}
Wheat straw	41.23^{de}	39.20^{e}	39.33^{e}	50.20^{c}	49.67^{cd}	48.93^{c}	81.33^{de}	86.33^{d}	85.10^{d}
Sawdust	49.33^{c}	51.80^{c}	43.80^{cd}	56.10^{b}	60.67^{b}	53.47^{b}	84.96^{cd}	91.47^{c}	79.13^{e}
Lentil husks	31.43^{f}	37.00^{e}	34.59^{f}	42.67^{d}	45.77^{d}	43.96^{d}	76.67^{e}	74.87^{e}	77.44^{e}
Sugarcane bagasse	57.80^{b}	60.60^{b}	63.78^{a}	68.11^{a}	70.73^{a}	72.70^{a}	98.33^{a}	102.00^{a}	99.96^{a}
Maizecob	61.74^{a}	64.90^{a}	59.22^{b}	70.80^{a}	71.27^{a}	74.40^{a}	94.33^{ab}	98.10^{ab}	89.77^{bc}
Mustardpods	38.26^{e}	43.27^{d}	40.20^{de}	53.40^{bc}	50.67^{c}	56.27^{b}	90.00^{bc}	95.90^{b}	86.54^{cd}
CD @ 5%	3.922	3.192	3.628	3.239	3.995	3.922	5.183	4.418	4.551
SEm±	5.015	3.321	4.291	3.421	5.203	5.014	8.759	6.364	6.752
CV (%)	4.837	3.655	4.418	3.304	3.935	3.871	3.375	2.784	2.985

Different letters after values are significantly different at (*P* = .05)

Table 2. Effect of different substrates on yield and biological efficacy of shiitake mushroom cultivation

Substrates	Numberofsporocarps perbag			Yield(g)/750gramsof Substrate			Biologicalefficiency		
	LE-01	LE-02	LE-03	LE-01	LE-02	LE-03	LE-01	LE-02	LE-03
Ricestraw	26.00^{a}	29.00^{a}	28.00^{a}	359.55^{b}	316.33^{d}	323.40^{c}	47.92^{b}	42.17^{d}	43.12^{c}
Wheat straw	26.00^{a}	24.00^{b}	23.00^{b}	318.25^{c}	346.02^{c}	318.08^{c}	42.43^{c}	46.44^{c}	42.41^{c}
Sawdust	23.00^{ab}	19.00^{d}	20.67^{b}	388.23^{a}	366.17^{b}	397.31^{a}	51.76^{a}	48.82^{b}	52.97^{a}
Lentil husks	21.67^{ab}	23.33^{bc}	19.33^{bc}	358.19^{b}	386.59^{a}	343.66^{b}	47.75^{b}	51.54^{a}	45.81^{b}
Sugarcanebagasse	18.00^{bc}	19.67^{cd}	16.00^{cd}	280.07^{d}	315.95^{d}	275.66^{d}	37.34^{d}	42.12^{d}	36.75^{d}
Maizecobs	19.00^{bc}	21.00^{bcd}	19.33^{bc}	346.70^{b}	380.36^{a}	353.86^{b}	46.22^{b}	50.71^{ab}	47.17^{b}
Mustard pods	16.00^{c}	19.00^{d}	14.67^{d}	271.31^{d}	297.60^{e}	239.16^{e}	36.17^{d}	39.68^{e}	31.88^{e}
CD @ 5%	5.630	4.273	4.152	19.368	13.779	12.434	2.581	1.969	1.659
SEm±	10.333	5.952	5.619	122.289	61.900	50.405	2.172	1.263	0.898
CV (%)	15.035	11.018	11.768	3.33	2.286	2.208	3.332	2.447	2.210

Different letters after values are significantly different at (*P* = .05)

Table 3. Analysis of Variance on spawn run, mycelial bump formation and sporocarp formation in shiitake mushroom cultivation

Source of Variation	Degree of Freedom	Mean of Squares								
		Days taken for complete Spawn run			Days taken for mycelial Bump formation			Days taken for Sporocarp formation		
		LE-01	LE-02	LE-03	LE-01	LE-02	LE-03	LE-01	LE-02	LE-03
Treatments	6	347.48	336.69	350.15	306.55	309.49	397.91	166.13	252.62	175.94
Error	14	5.015	3.321	4.291	3.421	5.203	5.014	8.759	6.364	6.741
CV (%)		4.837	3.655	4.418	3.304	3.935	3.871	3.375	2.784	2.983
CD (0.05)		3.922	3.192	3.628	3.239	3.995	3.922	5.183	4.418	4.547

Table 4. Analysis of Variance on yield and biological efficacy of shiitake mushroom cultivation

Source of Variation	Degree of Freedom	Mean of Squares								
		Number of sporocarps per bag			Yield(g)/750gramsof Substrate			Biological efficiency		
		LE-01	LE-02	LE-03	LE-01	LE-02	LE-03	LE-01	LE-02	LE-03
Treatments	6	45.71	39.54	59.31	5695.53	3668.34	8091.16	101.18	65.30	143.85
Error	14	10.33	5.952	5.619	122.289	61.900	50.405	2.172	1.263	0.898
CV (%)		15.035	11.018	11.768	3.333	2.286	2.208	3.332	2.447	2.210
CD (0.05)		5.630	4.273	4.152	19.368	13.779	12.434	2.581	1.969	1.659

In the discussion of the obtained results, it's essential to contextualize them within existing literature to provide a comprehensive understanding. Consistent findings regarding the yield and biological efficiency of *L. edodes* (shiitake mushroom) across various substrates have been reported by several researchers [16, 18, 23-27]. These studies, akin to our findings, have highlighted the significant influence of substrate choice on mushroom cultivation outcomes. Specifically, they underscore the importance of selecting substrates conducive to optimal yield and biological efficiency.

Our results align with the observations made by these researchers, demonstrating that certain substrates, such as sawdust, lentil husks, and rice straw, consistently yield higher mushroom yields and biological efficiencies compared to others.Our result align with the findings of Moonmoon et al. [24], Kumar [16] reported that Sawdust substrate gained as a superior substrate for shiitake cultivation followed by wheat straw substrate and Kalaw et al. [27] found that, rice straw and saw dust formulation gained as a superior substrate.Sawdust, in particular, emerged as a standout substrate, consistently producing the highest yield and biological efficiency across all three strains tested. Lentil husks and rice straw also exhibited promising results, further corroborating previous findings viz., Kumar [16], Puri et al. [18]; Kalaw et al. [27] regarding their suitability for shiitake mushroom cultivation.

Conversely, substrates like mustard pods displayed lower yields and biological efficiencies, emphasizing their limited effectiveness in supporting robust mushroom growth. These consistent findings underscore the practical implications for mushroom cultivators, highlighting the importance of informed substrate selection to optimize production outcomes.Overall, our study contributes to the growing body of literature on substrate selection for shiitake mushroom cultivation, reaffirming the importance of selecting substrates that foster optimal yield and biological efficiency. By building upon the findings of previous researchers, we provide further insights into the factors influencing mushroom cultivation success and offer valuable guidance for practitioners in the field.

4. CONCLUSION

In Summary, the study underscores the pivotal role of substrate selection in determining various stages of *L. edodes* (shiitake mushroom) cultivation. Lentil husks emerged as a promising substrate choice, exhibiting efficient spawn run completion, mycelial bump formation, and sporocarp formation durations. However, its sporocarp production lagged behind other substrates, indicating the need for a balanced consideration of multiple factors. Sawdust proved to be the top-performing substrate in terms of yield and biological efficiency, highlighting its effectiveness in supporting robust mushroom growth. These findings provide valuable insights for mushroom cultivators, emphasizing the significance of informed substrate choices for maximizing yield and biological efficiency in commercial mushroom production. Future research may delve deeper into optimizing substrate formulations and cultivation practices to further enhance shiitake mushroom cultivation outcomes.

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