Original Research Article

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: Lentil husks exhibited the shortest time for spawn run completion (34.59 days), mycelial bump formation (43.13 days), and sporocarp formation (76.66 days), but with lower sporocarp production compared to rice straw and wheat straw. Sawdust demonstrated the highest sporocarp yield (27.67 per bag) and biological efficiency (51.18%), followed by lentil husks and rice straw. Mustard pods showed the lowest sporocarp production (16.89 per bag) and biological efficiency (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 *Agaricus bisporus(Quote …….. reference)*. 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.

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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 [1]. 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 [2, 3].

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. [4] 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 [5], and employing vacuum-soaking techniques for substrate preparation have been explored [6]. Furthermore, recommendations have been put forth regarding substrate formulations, such as substituting oak wood chips with ground wheat straw [7], and incorporating hazelnut husk as a substrate alongside commercial sawdust [8]. Shen et al. [9] conducted studies aimed at reducing production costs while improving yield by adjusting moisture content and porosity.

Furthermore, researchers have explored the potential of non-traditional substrates and discarded materials for cultivating shiitake mushrooms. Puri et al. [10] 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 [11], and analyzing the biochemical composition of substrates have offered valuable insights for optimizing cultivation techniques [12].

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 [13]. 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 spawn

Disease-free wheat grains were carefully chosen as the substrate for mother spawns production, unless specified otherwise. These grains underwent a series of steps, starting with soaking in water for one hour and then boiling until they became soft. Following boiling, the grains were air-dried in shaded areas until their moisture content reached approximately 30-35%. Then, they were combined with 2% gypsum ($CaSO_4$) and 2% calcium carbonate ($CaCO_3$) based on their dry weight to eliminate excess moisture and prevent clumping. These treated grains were subsequently filled into glass bottles, filling them to around two-thirds of their volume. The bottle openings were sealed with non-absorbent cotton, and the filled bottles were sterilized in an autoclave for two hours at a temperature of 121.6°C and a pressure of 15 psi. After sterilization, the bottles were allowed to cool overnight. In aseptic conditions within a laminar airflow chamber, the bottles were inoculated with a pure culture of *L. edodes*. Following inoculation, the bottles were placed in an incubator set at a temperature of 24±2°C for a period of 10-15 days. (this is the procedure in vouge ,, add specific measures in spawn preparation to this *L.edodes*)

Comment [S2]:

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2.2.2 Preparation of Bed spawn

Disease-free wheat grains were employed as the substrate for bed spawn preparation, following the identical procedure as the mother spawn preparation. The grains underwent boiling and sterilization processes akin to those in the mother spawn preparation, and the bottles were allowed to cool overnight. In aseptic conditions within a laminar airflow chamber, the bottles were inoculated with the *L. edodes* mother spawn. Following inoculation, the bottles were transferred to an incubator set at a temperature of 24±2°C for duration of 10-15 days.

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

The sterilized or pasteurized substrates, placed in grow bags, were allowed to cool overnight in the inoculation room. Subsequently, they were inoculated with 5% grain spawn in a sterile manner within the laminar airflow chamber.

2.5 Cropping

The spawned bags were placed in 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 was maintained at a temperature of approximately 20°C with 90% humidity, achieved by regularly spraying water on gunny bags attached to the room's walls and windows. Alternatively, an automated ultrasonic humidifier could be utilized to regulate humidity levels. Carbon dioxide levels were managed using an exhaust fan, while fluorescent lighting was used to maintain light intensity.

2.6 Observations

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.

2.7 Harvest

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.

2.8 Yield 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 [14], 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 Effect of different substrates on the growth & yield of shiitake mushroom

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

Comment [S4]: Scope to explain in a better manner

The results indicate significant variations in the time taken for complete spawn run across different substrate treatments. Among the substrates tested, lentil husks exhibited the shortest duration for spawn run completion, with an average of 34.59 days, followed closely by wheat straw and mustard pods at 39.33 days and 40.20 days, respectively. On the contrary, maize cob required the longest time for spawn run completion, averaging 61.29 days across all three strains. Sugarcane bagasse and sawdust also exhibited comparatively longer durations, with averages of 60.06 days and 48.31 days, respectively. These findings suggest that lentil husks represent a promising substrate choice for efficient spawn run completion, while maize cob presents a less favorable option due to its prolonged duration. Moreover, the consistently shorter durations observed with lentil husks across all three strains underscore its potential as a rapid substrate for mushroom cultivation (Table 1).

3.1.2 Days taken for mycelial bump formation

Comment [S5]: Scope to add a detailed explnation

The results demonstrate notable differences in the time required for mycelial bump formation across various substrate treatments. Lentil husks emerged as the substrate facilitating the quickest mycelial bump formation, with an average duration of 43.13 days, followed closely by rice straw at 54.9 days. Conversely, maize cob necessitated the longest period for mycelial bump formation, averaging 71.54 days across all three strains. Sugarcane bagasse and wheat straw also exhibited relatively longer durations, with averages of 70.07 days and 49.93 days, respectively. These findings suggest that lentil husks represent a favorable substrate choice for prompt mycelial bump formation, highlighting its potential for efficient mushroom cultivation. Conversely, maize cob presents a less optimal option due to its prolonged duration for mycelial bump formation (Table 1).

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).

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).

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).

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

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

Comment [S6]: Better to have some photo graphs of the experimental set up

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 [15-18]. 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. 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 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.

REFERENCES

1. Takabatake K. Current trends and future prospects for mushroom production inJapan.Mokuzai Gakkaishi. 2015;61(3):243–249. https://doi.org/10.2488/jwrs.61.243
2. Alananbeh KM, Bouqellah NA, AlKaff NS. Cultivation of oystermushroom *Pleurotus ostreatus* on date-palm leaves mixed with other agro-wastesinSaudi Arabia. Saudi J

Biol Sci. 2014;21(6):616–625. https://doi.org/10.1016/j.sjbs.2014.08.001

3. Miao R, Zhou J, Tan W, Peng W, Gan B, Tang L, et al. Apreliminaryscreeningofalternativesubstrateforcultivationof*Flammulinavelutipes*.Mycosystema. 2014;33(2):411–424.
4. Salmones D, Mata G, Ramos LM, Waliszewski KN. Cultivation ofshiitakemushroom,*Lentinulaedodes*,inseverallignocellulosicmaterialsoriginatingfrom thesubtropics.Agronomie. 1999;19(1):13–19.
5. OhgaS.Evaluation ofmaturitybyuseof pH indicatorsin sawdust-basedculturesof *Lentinulaedodes*.J WoodSci. 1999;45(5):431–434.http://dx.doi.org/10.1007/BF01177917
6. Royse D, Rhodes T, Sanchez J. Vacuum-soaking of wood chip shiitake(*Lentinula edodes*) logs to reduce soak time and log weight variability and tostimulate mushroom yield. Appl Microbiol Biotechnol. 2002;58(1):58–62.https://doi.org/10.1007/s00253-001-0870-y
7. Royse DJ, Sanchez JE. Ground wheat straw as a substitute for portions ofoakwoodchipsusedinshiitake(*Lentinulaedodes*)substrateformulae.BioresourTechnol. 2007;98(11):2137–2141.https://doi.org/10.1016/j.biortech.2006.08.023
8. OzcelikE,PeksenA.Hazelnuthuskasasubstrateforthecultivationofshiitakemushroom (*Lentinulaedodes*).BioresourTechnol. 2007;98(14):2652–2658.https://doi.org/10.1016/j.biortech.2006.09.020
9. Shen Q, Liu P, Wang X, Royse DJ. Effects of substrate moisture content,log weight and filter porosity on shiitake (*Lentinula edodes*) yield. BioresourTechnol. 2008;99(17):8212–8216.https://doi.org/10.1016/j.biortech.2008.03.067
10. PuriS,BhattR,MishraKK.Cultivationof*Lentinulaedodes*(Berk.)Pegleronsawdustsubstratesandagriculturalwastes.IntJSciNat. 2011;2(4):752–756.
11. Tad-awanBS.ProfitabilityofChippedAlderasSubstrateforShiitakeProduction.MountainJournalofScienceandInterdisciplinaryResearch(FormerlyBenguet StateUniversityResearch Journal). 2014;71:57–63.
12. Barshteyn V, Krupodorova T. Utilization of agro-industrial waste by highermushrooms: Modern View and Trends. J Microbiol Biotechnol Food Sci. 2016;5(6):563–577.https://doi.org/10.15414/jmbfs.2016.5.6.563-577
13. Ramkumar L, Ramanathan T, Nedumaran T. In vitro effect of organic andinorganicadditivesfromtheproductionofradialmycelialgrowthandlignocellulolytic enzyme in *Lentinus edodes* (Berk.) Sing. Emir J Food Agric. 2011;23(1):71–79.

14. MilesPG,ChangST.Mushrooms:Cultivation,nutritionalvalue,medicinaleffect,and environmental impact. 2nded. CRCpress; 2004.
15. AshrafuzzamanM,KamruzzamanAKM, IsmailMR,ShahidullahSM,Fakir SA. Substrate affects growth and yield of shiitake mushroom. AfrJ Biotechnol.2009;8(13):2999-3006.https://doi.org/10.4314/ajb.v8i13.60972
16. Moonmoon M, Shelly NJ, Khan MA, Uddin MN, Hossain K, Tania M,et al. Effects of different levels of wheat bran, rice bran and maizepowder supplementation with saw dust on the production of shiitake mushroom(*Lentinusedodes*(Berk.)Singer).SaudiJBiolSci. 2011;18(4):323–328.https://doi.org/10.1016/j.sjbs.2010.12.008
17. LinY,GeX,LiuZ,LiY.IntegrationofShiitakecultivationandsolid-stateanaerobic digestion for utilization of woody biomass. Bioresour Technol. 2015;182:128–135. https://doi.org/10.1016/j.biortech.2015.01.102
18. Ranjbar ME, Olfati JA. Evaluation of Substrate Components on ShiitakeMushroomProperties.IntJVegSci. 2017;23(2):145–150. https://doi.org/10.1080/19315260.2016.1220438

Comment [S7]: Please refer latest refrence the latest one quoted wasf from the year 2014