Original Research Article

Evaluation ofdifferent Agro-based waste extract media for*Lentinula edodes*

Abstract

Shiitake mushrooms (*Lentinula edodes*), known for growing on decaying deciduous trees, are particularly notable for their health benefits.Recent advancements in cultivation techniques involve growing *Lentinula edodes* on sterile substrates to boost yield and shorten the growth cycle. In Telangana state, the climate and agricultural waste provide optimal conditions for mushroom cultivation, which can boost the local economy and nutrition. Thisstudy aims to determine the *invitro*performance of *L. edodes* using combinations of 17 types of agro-based wasteextract mediaincubated at temperatures of 20ºC and 24ºC,to evaluate the best substrate extract media for shiitake mushroom production. At 20°C, the maximum mycelium growth of *L. edodes*was recordedonS17 (PDA, 75.33mm) on7 DAI, while minimum mycelium growth was recorded on sawdust mixed with paddy straw (S8, 35.67mm). Similarlyat 24°C, the substrates S17 (PDA) and S10,S11, and S12 (eucalyptus bark supplemented with wheat grains and sorghum grains)recorded the maximum mycelium growth(90mm), and S8 (sawdust mixed with paddy straw)recorded minimum mycelium growth (30.33mm) on 7 DAI. The results from the Principal Component Analysis of recorded data of mycelial growth was shown in the form of Eigen values, which shows that the highest Eigen value was recorded for mycelial growth of *L. edodes* at 7 DAI and 14 DAI at 24°C indicates significant influence on substrates.

Keywords:*Lentinula edodes*, mushroom, substrates, mycelium growth, media, temperature

Introduction

Shiitake mushroom (*Lentinula edodes*) is one of the six popular edible mushrooms native to the East Asian region.The practice of cultivating shiitake mushrooms can be traced back to ancient times in the Far East, particularly in areas such as China, and Japan. The generic name *edodes* comes from Latin, meaning “edible”. *L. edodes* grows in groups on decaying deciduous trees, particularly on the *Castanopsiscuspidata* species. They are white wood rotting fungi capable of decomposing the cellulose and lignin structural components.(Annepu*et al*., 2019 and Kobayashi *et al*.,2020).Shiitake mushrooms are prized for being fat-free and having low cholesterol and sodium content, making them a healthy dietary choice. They are also rich in proteins, lipids, carbohydrates, fibers, ergosterols, antioxidants, and vitamins like provitamin D, which are not commonly found in other food sources(Paswal*et al*., 2024). Due to their exceptional nutritional value and potential medical benefits, this species is often referred as "the queen of plants."(Chen *etal*., 2017).Season based cultivation of these mushrooms can be taken up with favourable climatic conditions.

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Additionally, the abundance of agricultural wastes from local farms can be converted into prized mushroom production. Despite efforts to standardize its cultivation, this mushroom has not yet been commercially produced on large over in India, with no reports of its cultivation in Telangana. Mushroom cultivation helps recyclingagricultural waste and addresses nutritional deficiencies and health issues.The present *invitro* studies were taken up to evaluate the suitable mediaand growth performance of shiitake mushrooms at different temperatures prevailing in Telangana state.

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Experimental site

The experiment trial was conducted in the Mushroom Cultivation Scheme, Department of Plant Pathology, College of Agriculture, Rajendranagar, PJTSAU, Telangana State, India. This study was supported by the Central Instrumentation Cell, which provided essential resources and facilities for the research.

Materials and Methods:

Pure culture

The shiitake mushroom (*Lentinula edodes*) culture was procured from the IIHR, Bangalore. The culture was grown on sterilized Petri plates containing Potato Dextrose Agar (PDA)mediain a BOD incubator at a temperature of 25±2°C. The fully grown 7 days old culture was then transferred onto fresh PDAslants and after full growth,they are stored in a refrigerator at 10-12°C for future use.

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Pouring, incubation, and data collection

In each 500 ml conical flask, 17 different types of prepared media was filled up to two-thirds of its volume. The flask was then tightly sealed with non-absorbent cotton plugs and covered with aluminium foil, which was secured with rubber bands. Further, thisflasks were sterilized at 121.6ºC and 15 psi pressure autoclaved for 20 minutes. After sterilization, the media was poured into 90mm sterilized petri plates, with 20 mlper plate for every treatment, three replications were donewithin a laminar air flow cabinet.Then plates were inoculated with 7mm discs of 7-day-old culture at the centreof the Petri plate within the laminar air flow chamber and incubated at two different temperatures (20ºC and 24ºC) in a BOD incubator. The growth of mushroom mycelium was recorded after 7 DAI along with the mycelium grown in the control plate.

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Preparation of Potato Dextrose Agar media

To prepare the Potato Dextrose Agar (PDA) medium, 200 g of peeled potatoes were boiled in water, and the extract was diluted to a total volume of 1 litre by adjusting with water. Then, 20 g of dextrose and 20 g of agar were dissolved in the solutionand dispensed into 250 ml conical flasks, which were plugged with non-absorbent cotton and sterilized at 121.6°C and 15 psi pressure for 20 minute in an autoclave.

Preparation of different agro based waste extract media

Various agro-based waste extract media were prepared by boiling each substrate composition separately as listed(Table 1) in 1 litre of water for 25-30 minutes. The resulting substrate extract was then strained through muslin cloth, and the volume was adjusted to 1 litre. Subsequently, 20 g of

dextrose and 20 g of agar was dissolved in the solution. The prepared substrate media was poured into 250 ml conical flasks, plugged with non-absorbent cotton, and sterilized at 121.6°C and 15 psi pressure for 20 minutes.

Effect of temperature on growth of *Lentinula edodes*

For the present study, 17 different types of media were prepared and poured into respective two different temperatures, 20ºC and 24ºC on the sterilized Petri plates, with 20 ml per plate. This study aimed to evaluate the effect of mycelial growth on the *Lentinula edodes*. Then Petri plates were allowed to solidify in aseptic conditions and inoculated with 7mm diameter mycelium discs in the center of the Petri plate containing media. Each treatment was done with three replications along with control plate at two temperatures. The mycelial growth of *Lentinula edodes* was observed and recorded on the 7 and 14 DAI.

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Table 1. List of different substrate compositionsused for evaluation of *Lentinula edodes* under *in vitro* conditions

S.No.	Substrate composition	Ratio and composition
S1	Sorghum Grains +Sawdust	(1:4) 40g +160g
S2	Wheat Grains+ Sawdust	(1:4) 40g+160 g
S3	Wheat Bran + Sawdust	(1:4) 40g+160 g
S4	Rice Bran + Paddy Husk and Sawdust	(1:1:3)40g+40g +120g
S5	Paddy Husk + Sawdust	(1:4) 40g+160 g
S6	Ashoka Woodchips + Paddy Husk + Wheat Bran	(3:1:1) 120g+40g+40g
S7	Ashoka Wood Chips +Paddy Husk + Rice Bran	(3:1:1) 120g+40g+40g
S8	Sawdust + Paddy Straw	(4:1) 160g+40 g
S9	Eucalyptus Bark + Sawdust	(4:1) 160g+40 g
S10	Eucalyptus Bark + Paddy Husk	(4:1) 160g+40 g
S11	Eucalyptus Bark + Rice Bran	(4:1) 160g+40 g
S12	Eucalyptus Bark + Wheat Grains + Sorghum Grains	(1:1:1) 66g+66g+66g
S13	Rice Bran +Sawdust	(1:4) 40g+160 g
S14	Only Sawdust	200g
S15	Only Sorghum Grains	200g
S16	Only Ashoka Wood Chips	200g
S17	PDA	200g

Statistical analysis

In this research, an experiment was carried out using a Factorial completely randomized design (FCRD) with three replications of each treatment by followingthe standard statistical procedure

suggested by Gomez and Gomez (1984).The data obtained from the experiment were analysed using the analysis of variance (ANOVA) technique employedusing SPSS software.

Results and discussion

***Lentinula edodes* mycelial growth on different agro-basedwaste extract media at 20º C**

Under in vitro conditions, various agro-based waste extracts were evaluated as media for the growth of *Lentinula edodes*. The mycelial growth ranged from 39.59mm to 64.40mm on different days after inoculation (DAI) recorded significant results.Maximum growth was recorded at 14 DAI (64.40mm) and lowest at 7 DAI (39.59mm). The impact of different substrates on mycelial growth was found to be significant.On S17 (82.67mm) media maximum growth was recordedwhile minimum on S8 (28.50mm) medium.Significant interaction effects of DAI and substrates evaluated were observed on the mycelial growth of shiitake mushroom.The maximum interaction effect was recorded on S17 (90mm), and S12 (90mm) media on 14 DAI, the minimum was recorded on S8 (22mm), and S14 (23.67mm) mediaat 7 DAI was on par with the minimum growth (Table 2 and Fig 1. Fig 2). Similar findings were reported earlier by Gbolagade*et al.* (2006)that PDA was the most suitable medium for culturing *Lentinula edodes* mycelium. Verma (2021) and his co-workersreported that the maximum mycelial growth was recorded on the PDA media. Iqbal(2009)and his associates reported similar findings, maximum mycelial growth of *L. edodes* was on the PDA media. Similarly, Shanmugaraj*et al*. (2024) found that PDA mediawasbest forthe maximum growth of *Lentinula edodes.*Similar observations were also reported by Andrade *et al.* (2008). The mycelial growth of *Lentinula edodes* was assessed using various sawdust extracts from eucalyptus species. The results showed that the fastest growth was recorded when eucalyptus sawdust extractwas used as growingmedia.

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Table 2. Mycelial growth of *Lentinula edodes* on different agro-based waste extract media at 20ºCtemperature

S.No.	TREATMENTS	Mycelial growth(mm) *At 20ºC		
		7 DAI	14 DAI	MEAN
S1	Sorghum Grains +Sawdust	34.00	67.75	50.88
S2	Wheat Grains+ Sawdust	26.33	58.00	42.17
S3	Wheat Bran + Sawdust	30.33	58.75	44.54

S4	Rice Bran + Paddy Husk and Sawdust	26.00	56.75	41.38
S5	Paddy Husk + Sawdust	25.33	55.75	40.54
S6	Ashoka Woodchips + Paddy Husk + Wheat Bran	49.33	66.50	57.92
S7	Ashoka Wood Chips +Paddy Husk + Rice Bran	31.33	56.50	43.92
S8	Sawdust + Paddy Straw	22.00	35.00	28.50
S9	Eucalyptus Bark + Sawdust	43.67	65.25	54.46
S10	Eucalyptus Bark + Paddy Husk	56.00	82.00	69.00
S11	Eucalyptus Bark + Rice Bran	56.33	83.25	69.79
S12	Eucalyptus Bark + Wheat Grains + Sorghum Grains	64.00	90.00	77.00
S13	Rice Bran +Sawdust	24.00	51.25	37.63
S14	Only Sawdust	23.67	52.00	37.84
S15	Only Sorghum Grains	57.67	80.00	68.84
S16	Only Ashoka Wood Chips	27.67	46.00	36.84
S17	PDA	75.33	90.00	82.67
	MEAN	**39.59**	**64.40**	
	CV	**2.24**		
		CD @5%	**SE(m)**	
	DAI (A)	**0.466**	**0.165**	
	Substrate (B)	**1.359**	**0.482**	
	Interaction (A X B)	**1.922**	**0.681**	

Note: * Average of 3 replications

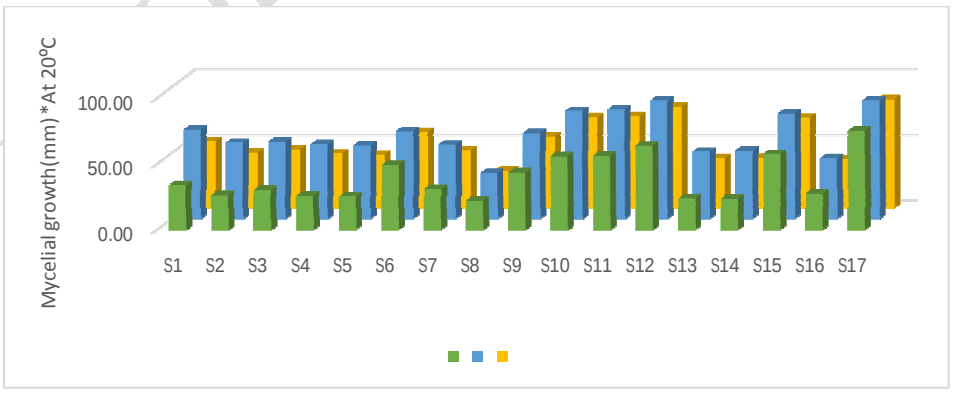

Fig.1 Mycelial growth of *Lentinula edodes* on different agro-based waste extract media at 20°C temperature

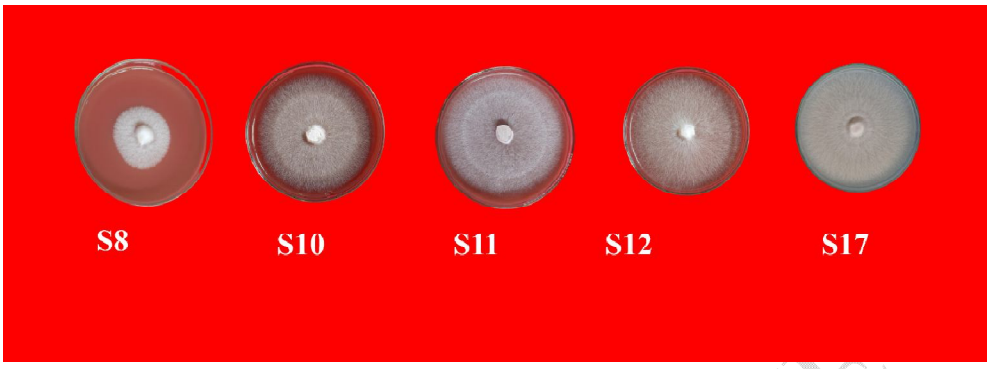

Fig.2. Mycelial growth of *Lentinula edodes* on different agro-based waste extract media at 20ºC temperatureat 14 DAI

Note: SDPS-Sawdust and Paddy Straw, EBPH-EucalyptusBark and Paddy Husk, EBRB-Eucalyptus Bark and Rice Bran, EBWGSG- Eucalyptus Bark and Wheat Grains and Sorghum Grains, PDA-Potato Dextrose Agar

***Lentinula edodes* mycelial growth at 24º C temperature**

Growthof *Lentinula edodes* mycelium was significant among two DAI evaluated and,ranged between 55.86mm to 80.45mm. Maximum growth was recorded on 14 DAI (80.88mm) and minimum on 7 DAI (55.86mm). The impact of different substrates on mycelial growth was significant, varying from 90mm to 36mm. The maximum growth was recorded on S17 (90mm) media while the lowest was recorded on S8 (36mm) medium. Additionally, the interaction effect of two DAI and among 17 various substrates, mycelial growth of *L. edodes* was significant at a temperature of 24°C. The maximum interaction effect was recorded 90mm at 7 DAI and 14 DAI onS17 and S12media. On S11, and S10 media also recorded maximum growth (90mm) at 14 DAI. While the minimumgrowth was recorded on S8 (30.33mm) mediumat 7 DAI and 14 DAI(Table 3 and Fig 3, Fig 4.).The experimental results revealed a significant effect of temperature on the mycelial growth of shiitake mushroom under *invitro* conditions. These findings are in accordance with the results of Verma, I.K. and Singh, P. (2021)who reported that PDA served as the most conducive medium for the growth of *Lentinula edodes* mycelium and provided evidence supporting the suitability of PDA as an effective media. The study revealed that the optimal temperature for the *L. edodes* growth was24°C, where maximum mycelial growth was recorded within 7 DAI when compared to other agro-based waste extract media.Similarly, Kumar and his co-workers (2019) reported that the maximum growth of mycelium was recorded at 24°C.

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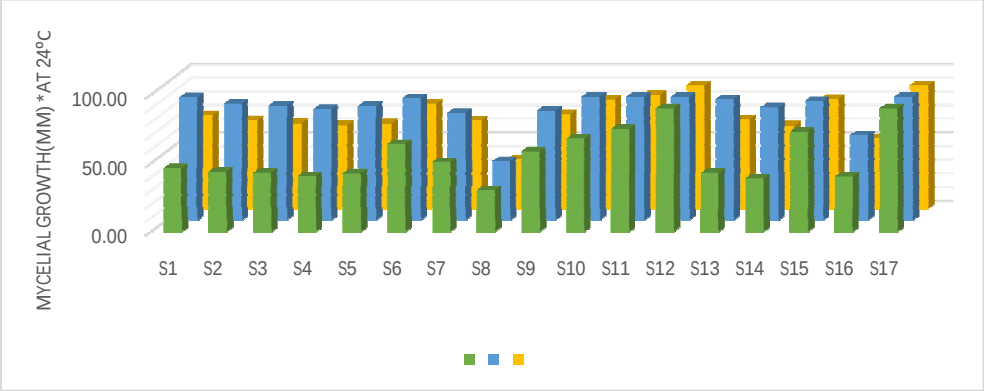

Fig 3. Mycelial growth of *Lentinula edodes* on different agro-based waste extract media at 24ºC temperature

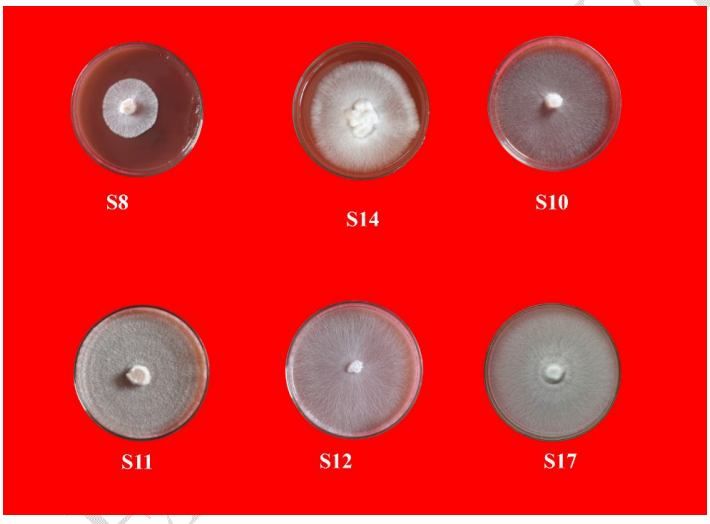

Fig 4.Mycelial growth of *Lentinula edodes* on different agro-based waste extract media at 24ºC temperature at 14 DAI

Note: SDPS-Sawdust and Paddy Straw, AWPHWB-Ashoka Wood chips and Paddy Husk, Wheat Bran, EBPH-Eucalyptus Bark and Paddy husk, EBRB-Eucalyptus Bark and Rice Bran, EBWGSG-Eucalyptus Bark and Wheat Grains and Sorghum Grains, PDA- Potato Dextrose Agar

Table 3. Mycelial growth of *Lentinula edodes* on different agro-based waste extract media at 24ºC temperature

S.No	TREATMENTS	Mycelial growth(mm)* At 24ºC		
		7 DAI	14 DAI	MEAN
S1	Sorghum Grains +Sawdust	46.33	89.60	68.167
S2	Wheat Grains+ Sawdust	43.67	84.90	64.333
S3	Wheat Bran + Sawdust	42.94	83.67	62.333

S4	Rice Bran + Paddy Husk and Sawdust	40.60	81.00	60.867
S5	Paddy Husk + Sawdust	42.67	83.33	62.167
S6	Ashoka Woodchips + Paddy Husk + Wheat Bran	64.00	89.00	76.50
S7	Ashoka Wood Chips +Paddy Husk + Rice Bran	51.00	78.33	64.167
S8	Sawdust + Paddy Straw	30.33	42.97	36.00
S9	Eucalyptus Bark + Sawdust	58.67	79.83	69.00
S10	Eucalyptus Bark + Paddy Husk	68.00	90.00	79.50
S11	Eucalyptus Bark + Rice Bran	75.00	90.00	83.00
S12	Eucalyptus Bark + Wheat Grains + Sorghum Grains	90.00	90.00	90.00
S13	Rice Bran +Sawdust	43.00	88.33	65.167
S14	Only Sawdust	39.00	82.37	60.66
S15	Only Sorghum Grains	73.00	87.00	80.00
S16	Only Ashoka Wood Chips	40.37	62.00	51.33
S17	PDA	90.00	90.00	90.00
	MEAN	**55.86**	**80.88**	
	CV	**1.58**		
		CD @5%	**SE(m)**	
	DAI (A)	**0.427**	**0.151**	
	Substrate (B)	**1.244**	**0.441**	
	Interaction (A X B)	**1.76**	**0.624**	

Note: * Average of 3 replications

Table 4. Principal Component Analysis of *Lentinula edodes* on different substrates at various temperatures.

Component		Eigenvalues	Proportion	Cumulative (%)
Mycelial growth of *L.edodes*at 24ºC	7DAI	**5.148**	0.644	0.644
	14 DAI	**1.436**	0.179	0.823

Mycelial growth of *L.edodes*at 20ºC	7DAI	0.691	0.086	0.909
	14 DAI	0.352	0.044	0.953
Colony characters of *L.edodes*mycelium at 24ºC	Colony colour	0.213	0.027	0.980
	Thickness of mycelium	0.092	0.011	0.991
Colony characters *L.edodes* mycelium at 20ºC	Colony colour	0.062	0.008	0.999
	Thickness of mycelium	0.062	0.001	1.000

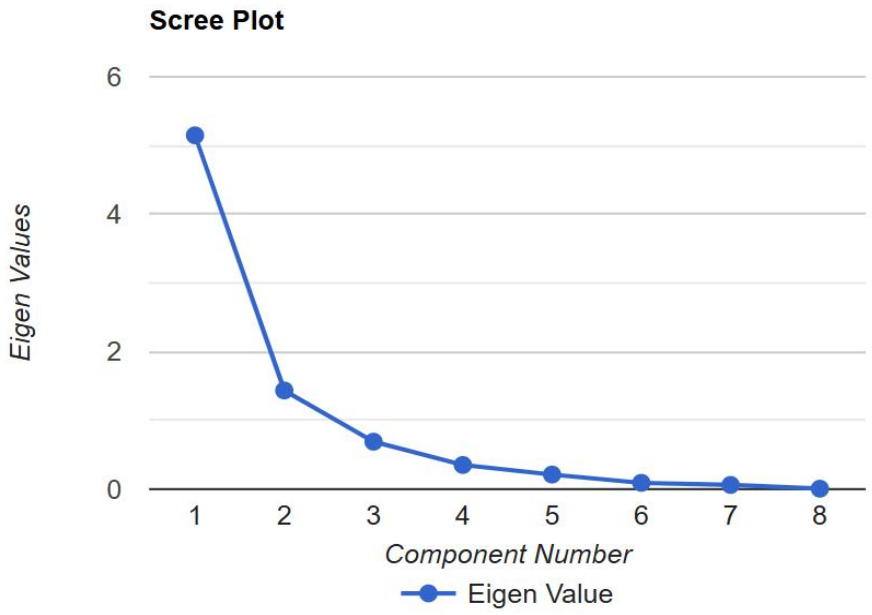

Fig 5. Screen plot for growth of *Lentinula edodes* by using Principal Component Analysis

According to the Principal Component Analysis, Eigen value ≥ 1 *i.e.*, Mycelial growth of *L. edodes* 7 DAI and 14 DAI at 24ºC temperature components was considered for the existence of influences of mycelial growth of *L. edodes*among 17 substrates extract media as illustrated in (Table 4 and Fig 5). The results from the Principal Component Analysis of recorded data of mycelial growth was shown in the form of Eigen values, which shows that the highest Eigen value was recorded for mycelial growth of *L. edodes* at 7 DAI and 14 DAI indicates significant influence of substrates.

Conclusion

This laboratory study aimed to asses growth performance of the Shiitake mushroom (*Lentinula edodes*) using various agro-based waste extracts as media. Among different agro-based

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waste extract substrates eucalyptus bark supplemented with wheat grains, sorghum grains, rice bran, and paddy husk and PDA recorded the maximum mycelial growth at temperature of 24°C. These findings suggest that *Lentinula edodes* has potential be cultivated using locally available residues for spawn production. Further research is needed for commercial production of shitake mushroom spawn.

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