Original Research Article

Performance of shiitake mushrooms (*Lentinula edodes)* on some selected agro-based waste extracts media under *in vitro*conditions

Abstract

Shiitake mushrooms (*Lentinula edodes*), known for growing on decaying deciduous trees, are particularly notable for their health benefits. In Telangana state, the climate and agricultural waste provide optimal conditions for mushroom cultivation, which can boost the local economy and nutrition. The present investigation was taken up in Completely randomized block design with 3 replications to evaluate the performance of shiitake mushroom (*Lentinula edodes*) on seventeen different agro-based waste extract media and maintained at two different temperatures*viz.*, 20°C and 24°C under *in vitro* conditions. Results revealed that at 20°C, the maximum mycelium growth of *L. edodes*was recordedintreatment T17 (PDA) (75.33mm, 90.00 mm) on7 DAI(Days After Inoculation) and 14 DAI respectively, while minimum mycelium growth was recorded in T8 (Sawdust + Paddy Straw)(22.00 mm, 35.00mm). Similarlyat 24°C treatment T17 (PDA) and T12 (Eucalyptus Bark+ Wheat Grains and Sorghum Grains)recorded the maximum mycelium growth(90.00 mm, 90.00 mm)and T8 (Sawdust + Paddy Straw)recorded minimum mycelium growth (30.33mm, 42.97 mm) on 7 DAI and 14 DAI respectively. The Principal Component Analysis of recorded values of the mycelial growth was shown in the form of eigen values, the highest eigen values were recorded for *L. edodes*mycelial growth on 7 DAI and 14 DAI at 24°C indicatingthe significant influence of temperature and media.

Keywords:*Lentinula edodes*, mushroom, mycelium growth, media, temperature, *in vitro* condition, agro-based waste.

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”. *Lentinula 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[1], [2].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, fibres, ergosterols, antioxidants and vitamins like provitamin D which are not commonly found in other food sources[3]. Due to their exceptional nutritional value and potential medical benefits, this species is often referred as "the queen of plants" [4]. Season-based cultivation of these mushrooms can be taken up with favourable climatic conditions. 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 in India, information regarding shiitake mushroom cultivation in

Telangana is not available in spite of its importance. Mushroom cultivation helps recyclingagricultural wastes and addresses nutritional deficiencies and health issues. This study was taken up to evaluate the suitable media prepared from abundantly available agricultural wastes and two different temperatures viz., 20ºC and 24ºCtemperatures for growth of shiitake mushroom and also for getting preliminary data for promotion of shiitake mushroom cultivation.

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:

Preparation of pure culture of *Lentinula edodes*

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)media in a BOD incubator at a temperature of 25±2°C. Five mm disc of 7 days old shiitake pure culture was then transferred onto fresh PDAslants,allowed tofully grow and thenstored in a refrigerator at 10-12°C for future use.

Preparation of Potato Dextrose Agar media

To prepare the Potato Dextrose Agar (PDA) mediumprocedure followed was given by Aneja [5]. 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 solution and 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 minutes in an autoclave.

Preparation of different agro based waste extract media

The extracts of the agro-based waste were prepared by following the procedure used by Singh [6].Boiling of each substrate composition separately in 1 litre of water for 25-30 minutes as listed (Table 1). The resulting substrate extract was strained through muslin cloth and the volume was adjusted to 1 litre. Then 20 g agar of and 20 g of dextrose wereadded into substrate extract. The prepared substrate extract media was make up to 1000ml and poured into conical flasks, plugged with non-absorbent cotton and sterilized at 121.6°C and 15 psi pressure for 20 minutes.

Pouring, incubation and data collection

Each of the 17 prepared media was filled up to two-thirds of 500 ml conical flask. The flask was then tightly plugged with non-absorbent cotton, covered with aluminium foil and secured with rubber bands. The flasks wereautoclaved for 20 minutes at 121.6ºC and 15 psi pressure. In three replicates, 20 ml of each of the prepared media was aseptically poured into 90 mm sterilized petri plates inside laminar air flow cabinet. The centre of the Petri plate were inoculated with 5mm discs of 7-day-old culture of

*Lentinula edodes*and incubated at two different temperatures *viz*., 20ºC and 24ºC in a BOD incubator. The mycelial growth was recorded after 7 DAI and 14 DAI.

Effect of temperature on growth of *Lentinula edodes*

The 17 prepared media were poured into petri plates, allowed for solidification and inoculated with5mm disc of 7 days old mycelial culture.Inoculated petri plates were placed at two different temperatures*viz*., 20ºC and 24ºC. The mycelial growth of *Lentinula edodes* were recorded on 7 DAI and 14 DAI.

Table 1.Compositionsof agro-based waste extracts media used for *in vitro*evaluation of *Lentinula edodes*

Treatments	Details of extract media	Ratio and composition
T1	Sorghum Grains +Sawdust	(1:4) 40g +160g
T2	Wheat Grains+ Sawdust	(1:4) 40g+160 g
T3	Wheat Bran + Sawdust	(1:4) 40g+160 g
T4	Rice Bran + Paddy Husk and Sawdust	(1:1:3)40g+40g +120g
T5	Paddy Husk + Sawdust	(1:4) 40g+160 g
T6	Ashoka Woodchips + Paddy Husk + Wheat Bran	(3:1:1) 120g+40g+40g
T7	Ashoka Wood Chips +Paddy Husk + Rice Bran	(3:1:1) 120g+40g+40g
T8	Sawdust + Paddy Straw	(4:1) 160g+40 g
T9	Eucalyptus Bark + Sawdust	(4:1) 160g+40 g
T10	Eucalyptus Bark + Paddy Husk	(4:1) 160g+40 g
T11	Eucalyptus Bark + Rice Bran	(4:1) 160g+40 g
T12	Eucalyptus Bark + Wheat Grains + Sorghum Grains	(1:1:1) 66g+66g+66g
T13	Rice Bran +Sawdust	(1:4) 40g+160 g
T14	Only Sawdust	200g
T15	Only Sorghum Grains	200g
T16	Only Ashoka Wood Chips	200g
T17	PDA	200g

Statistical analysis

Experiment was carried out using aCompletely randomized block design (CRBD) with three replications of each treatment.The data obtained from the experiment were analysed using the analysis of variance (ANOVA) technique employedusing SPSS software.PCA analysis werecarried out using OPSTAT software by following the standard statistical procedure suggested by Gomez and Gomez [7].

Results and discussion

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

Under *in vitro* conditions, different agro-based waste extracts were evaluated for the growth of *Lentinula edodes*. The mycelial growth ranged from 39.59mm to 64.40mm ontwo days after inoculation *viz*., 7DAI and 14 DAIwere recorded.Maximum growth of myceliumwas recorded on 14 DAI (64.40mm) and lowest on 7 DAI (39.59mm). The impactof different treatments on mycelial growth was found to be significant.In treatmentT17 (82.67mm)maximum mycelial growth was recordedwhile minimum in T8 (28.50mm).Significant interaction effectsof DAI and treatments were observed on mycelial growth of *L. edodes*.The maximum interaction effect of mycelial meanwas recorded in T17 (90.00 mm) and T12 (90.00 mm)treatments on 14 DAI whileminimum was recorded in T8 (22mm) and T14 (23.67mm)treatment on7 DAI (Table 2 and Fig 1. Fig 2).

Similar findings were reported earlier by Gbolagade *et al.*[8]that PDA was the most suitable medium for culturing *Lentinula edodes* mycelium. Vermaand Singh[9] reported that the maximum mycelial growth was recorded on the PDA medium among all other media. Iqbal *et al.*[10] reported similar findings of this study themaximum mycelial growth of *L. edodes* was recordedon the PDA media. Similarly, Shanmugaraj*et al*.[11] found that PDA mediawasbest forthe maximum growth of *Lentinula edodes*.

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

Treatments	Details of treatments	Mycelial growth(mm) *At 20ºC		
		7 DAI	14 DAI	MEAN
T1	Sorghum Grains +Sawdust	34.00	67.75	50.88
T2	Wheat Grains+ Sawdust	26.33	58.00	42.17
T3	Wheat Bran + Sawdust	30.33	58.75	44.54
T4	Rice Bran + Paddy Husk and Sawdust	26.00	56.75	41.38
T5	Paddy Husk + Sawdust	25.33	55.75	40.54
T6	Ashoka Woodchips + Paddy Husk + Wheat Bran	49.33	66.50	57.92
T7	Ashoka Wood Chips +Paddy Husk + Rice Bran	31.33	56.50	43.92
T8	Sawdust + Paddy Straw	22.00	35.00	28.50
T9	Eucalyptus Bark + Sawdust	43.67	65.25	54.46
T10	Eucalyptus Bark + Paddy Husk	56.00	82.00	69.00
T11	Eucalyptus Bark + Rice Bran	56.33	83.25	69.79
T12	Eucalyptus Bark + Wheat Grains + Sorghum Grains	64.00	90.00	77.00

T13	Rice Bran +Sawdust	24.00	51.25	37.63
T14	Only Sawdust	23.67	52.00	37.84
T15	Only Sorghum Grains	57.67	80.00	68.84
T16	Only Ashoka Wood Chips	27.67	46.00	36.84
T17	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**	
	Treatment (B)	**1.359**	**0.482**	
	Interaction (A X B)	**1.922**	**0.681**	

Note: * Average of 3 replications

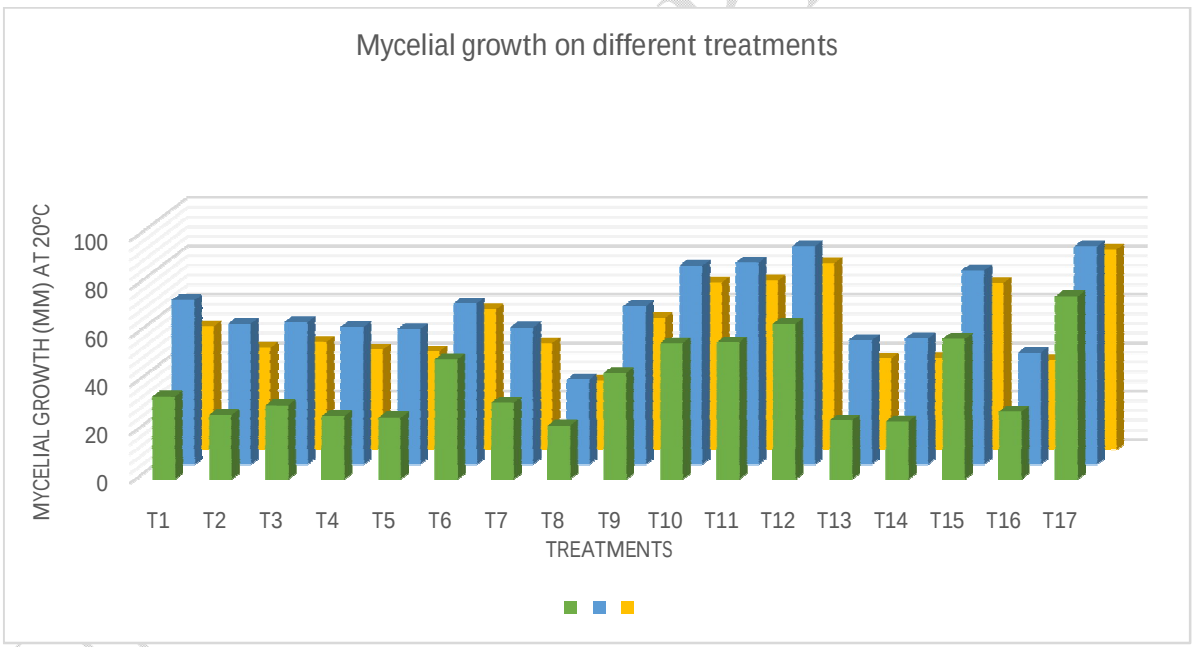

Fig.1 Mycelial growth of *Lentinula edodes* on differenttreatments at 20ºC temperature

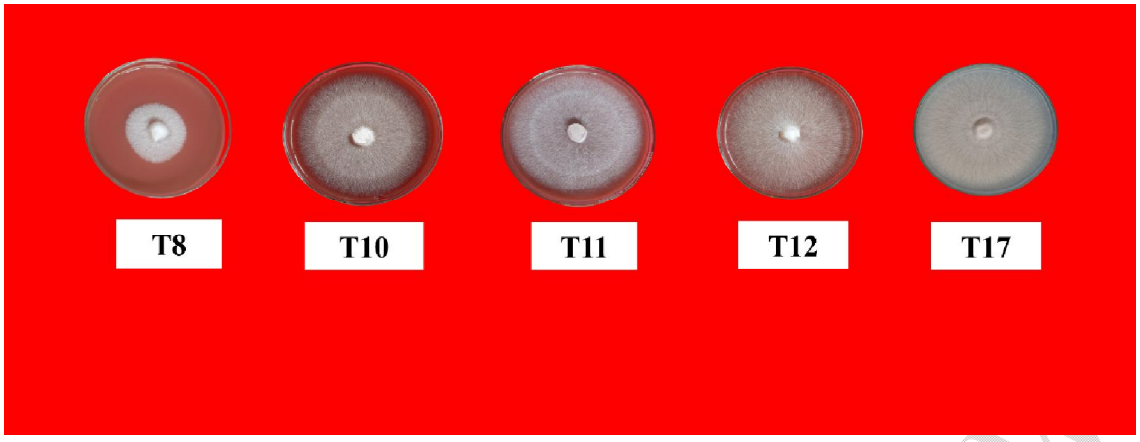

Fig.2. Mycelial growth of *Lentinula edodes* on different treatments at 20ºC temperature

Note: T8-Sawdust+Paddy Straw, T10-EucalyptusBark +Paddy Husk, T11-Eucalyptus Bark +Rice Bran, T12- Eucalyptus Bark +Wheat Grains +Sorghum Grains, - Potato Dextrose Agar

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

Growthof *Lentinula edodes* mycelium among two DAIwere recorded and ranged from 55.86mm to 80.45mm. Maximum mycelial growthwas recorded on 14 DAI (80.88mm) and minimum on 7 DAI (55.86mm). There was an significant effect of different treatments on mycelial growth which varied from 36.00mm to 90.00 mm. The maximum mycelial growth was recorded in treatment T17 (90.00 mm) while the lowest was recorded in T8 (36.00 mm).Further there was a significant interaction effect of two DAI and 17 treatments on mycelial growth of *L. edodes*at a temperature of 24°C. The maximum interaction effect was recorded with mycelial growth of90.00 mmon 7 DAI and 14 DAI intwo treatments T17 and T12treatments. T11 and T10 treatments were also recorded maximum growth (90.00 mm) on 14 DAI. While the minimumgrowth was recorded in T8 (30.33mm)on 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 mushrooms under *invitro* conditions.

These findings are in accordance with the results of Vermaand Singh [9] reported 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 and provided evidence supporting the suitability of PDA as an effective medium for the growth of *Lentinula edodes* mycelium. Similarly, Kumar*et al.*[12] reported that the maximum growth of mycelium at 24°C.In our findings, Eucalyptus bark extracts media showed maximum mycelium growth after PDA medium. Similar observations were also reported by Andrade *et al.*[13] fastest mycelial growth of *Lentinula* was recorded when eucalyptus sawdust extract was used as growing media.

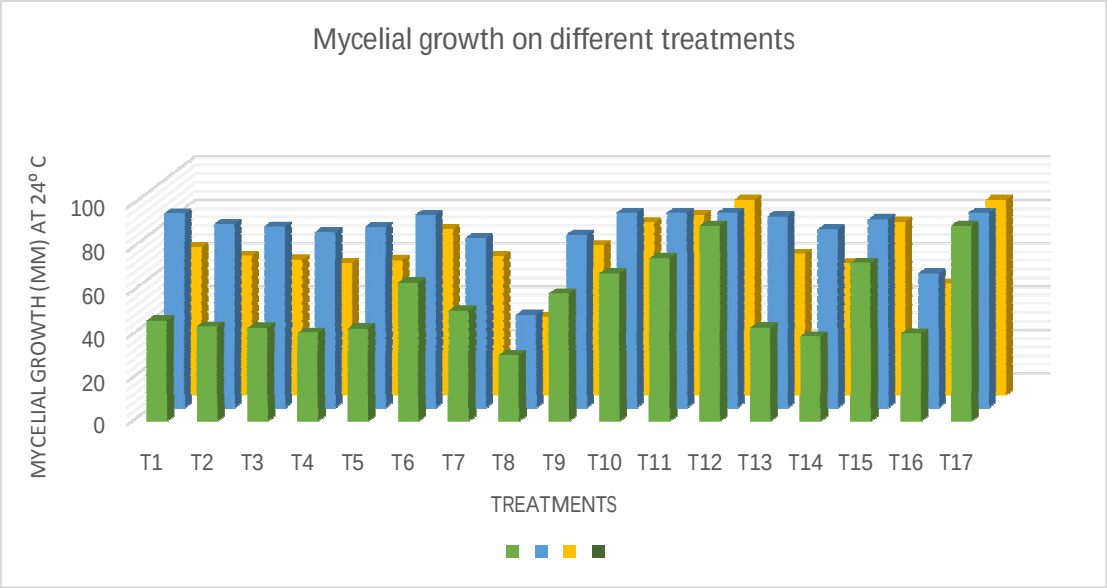

Fig 3. Mycelial growth of *Lentinula edodes* on different treatments at 24ºC temperature

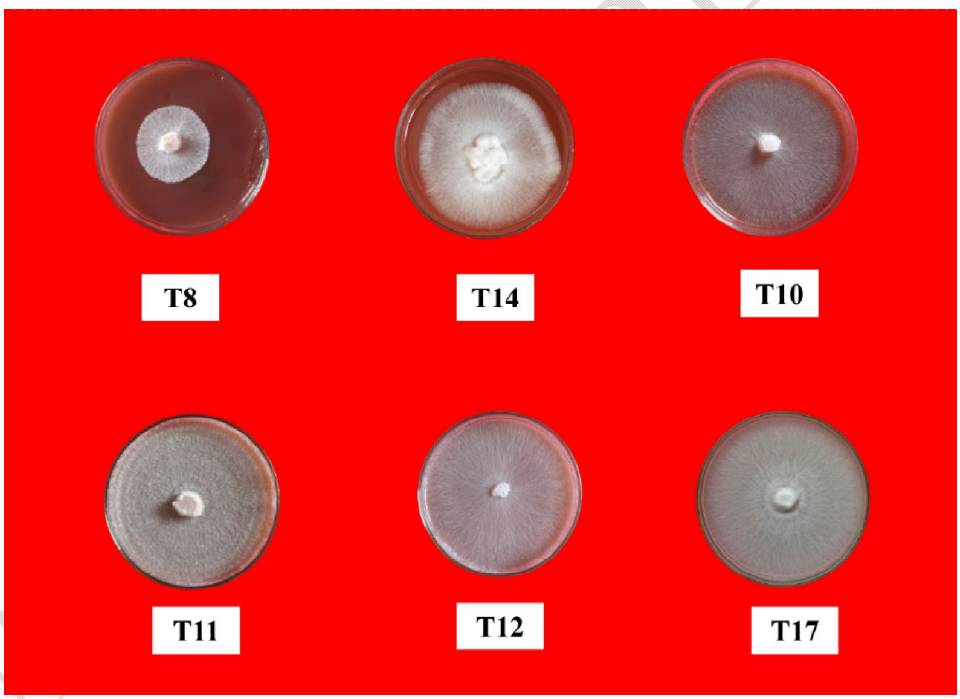

Fig 4.Mycelial growth of *Lentinula edodes* on different treatments at 24ºC temperature

Note: T8-Sawdust+ Paddy Straw, T14-Ashoka Wood chips + Paddy Husk + Wheat Bran, T10-Eucalyptus Bark + Paddy husk, T11-Eucalyptus Bark + Rice Bran, T12- Eucalyptus Bark + Wheat Grains + 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

		7DAI	14 DAI	MEAN
T1	Sorghum Grains +Sawdust	46.33	89.60	68.167
T2	Wheat Grains+ Sawdust	43.67	84.90	64.333
T3	Wheat Bran + Sawdust	42.94	83.67	62.333
T4	Rice Bran + Paddy Husk and Sawdust	40.60	81.00	60.867
T5	Paddy Husk + Sawdust	42.67	83.33	62.167
T6	Ashoka Woodchips + Paddy Husk + Wheat Bran	64.00	89.00	76.50
T7	Ashoka Wood Chips +Paddy Husk + Rice Bran	51.00	78.33	64.167
T8	Sawdust + Paddy Straw	30.33	42.97	36.00
T9	Eucalyptus Bark + Sawdust	58.67	79.83	69.00
T10	Eucalyptus Bark + Paddy Husk	68.00	90.00	79.50
T11	Eucalyptus Bark + Rice Bran	75.00	90.00	83.00
T12	Eucalyptus Bark + Wheat Grains + Sorghum Grains	90.00	90.00	90.00
T13	Rice Bran +Sawdust	43.00	88.33	65.167
T14	Only Sawdust	39.00	82.37	60.66
T15	Only Sorghum Grains	73.00	87.00	80.00
T16	Only Ashoka Wood Chips	40.37	62.00	51.33
T17	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**	
	Treatments (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

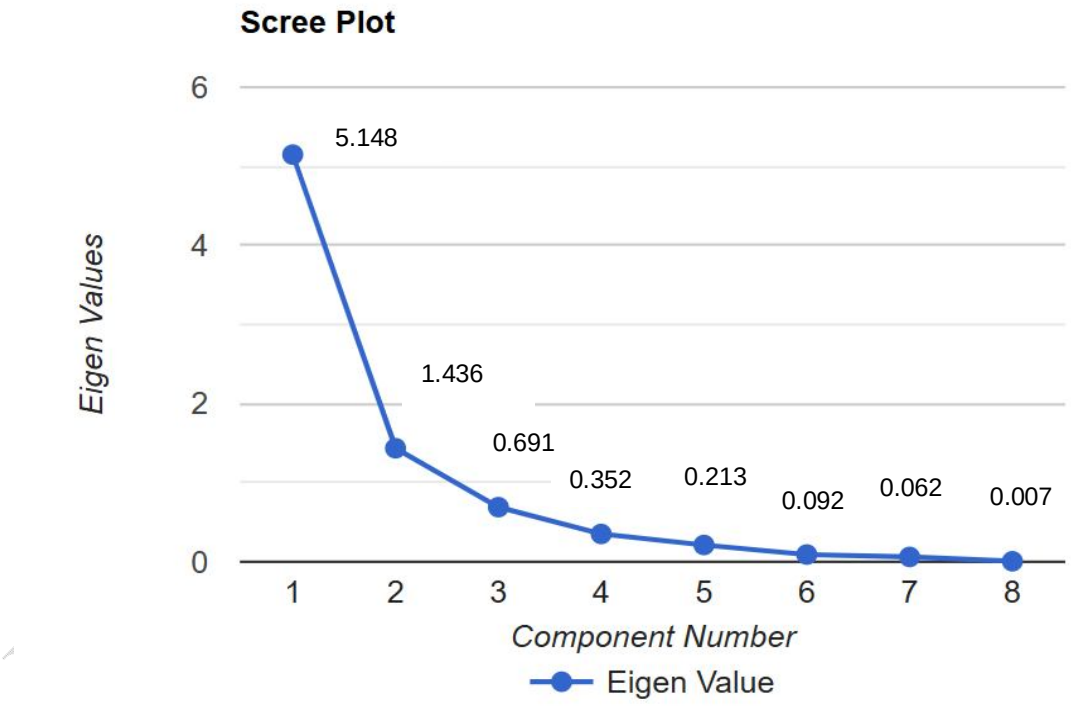

Fig 5. Screen plot for growth of *Lentinula edodes* by using Principal Component Analysis

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

Conclusion

This laboratorystudy taken up for evaluating theperformance of the Shiitake mushroom (*Lentinula edodes*) using seventeen different agro-based waste extracts as growth media. Results revealed that among all treatments ofagro-based wastes extracts eucalyptus bark supplemented with wheat grains, sorghum grains, rice bran and paddy husk and PDArecorded the maximum mycelial growth at temperature of 24°C. This finding suggests that *Lentinula edodes* has the potential to be cultivated using locally available residues for commercial spawn production which can also be further tested for fresh shiitake mushroom cultivation.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Acknowledgment

The authors would like to thank the Mushroom Cultivation Scheme, Department of Plant Pathology, College of Agriculture, Rajendranagar, PJTSAU, Central Instrumentation Cell, Hyderabad for financial aid and other resources in conducting the research and the opportunity to carry out this research.

References

1. Kumar, A.S., Sharma, V.P., Satish, K. and Anupam, B. 2019. Cultivation techniques of Shiitake (a medicinal mushroom with culinary delight), ICAR-directorate of mushroom research, chambaghat, solan-173213 (HP). Technical Bulletin.
2. Kobayashi, T., Oguro, M., Akiba, M., Taki, H., Kitajima, H and Ishihara, H. 2020. Mushroom yield of cultivated shiitake (Lentinula edodes) and fungal communities in logs. Journal of Forest Research. 25 (4): 269-275.
3. Paswal, S., Kakraliya, S.S. and Fogawat, V. 2024. Effect of Different Substrates on Nutritional Composition of Shiitake Mushroom (Lentinula edodes). Archives of Current Research International. 24(4): 156-161.
4. Chen, A.W.2005.What is shiitake? In: Mushroom growers Handbook. 2nd ed. Mushroom world, Seoul. Korea: 1-11
5. Aneja. K. R. 1993. Experiments in microbiology, plant pathology and tissue culture. Indian Journal of Genetics and Plant Breeding. 53 (4): 448-449.
6. Singh, M.P. and Singh, V.K., 2011, October. Yield performance and nutritional analysis of Pleurotus citrinopileatus on different agrowastes and vegetable wastes. In *Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7)* : 4-7.

7. Gomez, K.A. and Gomez, A.A. 1984. Statistical procedures for agricultural research. John wiley& sons.
8. Gbolagade, J.S., Fasidi, I.O., Ajayi, E.J. and Sobowale, A.A. 2006. Effect of physico-chemical factors and semi-synthetic media on vegetative growth of Lentinus subnudus (Berk.), an edible mushroom from Nigeria. Food chemistry. 99(4): 742-747.
9. Verma, I.K. and Singh, P. 2021. In vitro evaluation for optimum growth conditions of shiitake mushroom (Lentinula edodes). Mushroom Research. 29(2): 203-207.
10. Iqbal, S.M., Arif, Z., Zahid, N.Y. and Abbasi, N.A. 2015. Effect of different culture medium and pH on the mycelial growth of shiitake mushroom. MYCOPATH. 13(1): 25-28.
11. Shanmugaraj, C., Saranraj, K. and Biswas, M.K. 2024. Optimizing growth conditions for shiitake mushroom cultivation in Birbhum, West Bengal: A study on media, temperature, and ph variations. International Journal of Advanced Biochemistry Research. 8(5): 219-223.
12. Kumar, V., Mishra, S.K and Kaur, M. 2019. Effect of different media, temperature and pH on radial mycelial growth of Lentinula edodes strain Le-17-04. Journal of Pharmacognosy and Phytochemistry. 8(1):345-348.
13. Andrade, M.C.N.D., Silva, J.H.D., Minhoni, M.T.D.A. and Zied, D.C.2008. Mycelial growth of two Lentinula edodes strains in culture media prepared with sawdust extracts from seven eucalyptus species and three eucalyptus clones. Acta Scientiarum. Agronomy. 30: 333-337.