Plant Archives Vol. 24, No. 2, 2024 pp. 235-238 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.030

STUDIES ON MYCELIUM PERFORMANCE OF SHIITAKE MUSHROOM (*LENTINULA EDODES*) ON DIFFERENT pH AND TEMPERATURE

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(Date of Receiving-16-02-2024; Date of Acceptance-30-04-2024)

ABSTRACT

Shiitake (*Lentinula edodes)* Mushrooms is most valuable fungi in the world. Many factors influenced in artificial cultivation of Shiitake during its growth period. So in this study a range of pH (5.5-8.5) and temperature (16 - 28°C) was used with the aim to find out most favourable pH and temperature for mycelia growth and dry matter weight. The Optimum pH for mycelium growth and dry matter weight of *Lentinula edodes* was 5.5, while optimum temperature for mycelium growth and dry matter weight was 28°C.

Key words : *Lentinula edodes*, Mycelial growth, Dry matter weight, pH, Temperature.

Introduction

Lentinula edodes is best known by its Japanese name (Shiitake), Chinese name (*Xiangu*) and French name (*Lentin*). In China, it was originated and called as *Xiangu*, *Xiangu* means mushroom with great aroma (*Xian* means aroma, *gu* means mushrooms) (Chang and Miles, 1989). The word 'shiitake' was originally derived from Japanese words: *shii* which means *oak* and *take* which means mushroom, reflecting the importance of oak wood as the natural host of the fungus (Davis, 1993; Royse, 2001). Shiitake (*Lentinula edodes*) is the most important culinary medicinal mushroom (Belong from Basidiomycetes), which ranks at number two in terms of total mushroom production in the world only next to button mushroom. It is used medicinally for diseases involving depressed immune function including cancer, AIDS, environmental all allergies, *Candida* infections and frequent flu and colds. In the last decades, indoor Shiitake production was introduced in USA and in Europe substituting wood logs by synthetic media in which sawdust is the basic ingredient for substrate formulation (Pryzbylo Wicz and Donoghue, 1990). Very strict controlled conditions of cultivation are required for both mycelial propagation and fruiting and therefore very costly investment is required. Present study has been done with the aim to examine the effect of different temperature and pH on the mycelia growth of Shiitake.

Materials and Methods

Experimental site

The experiments were conducted in Mushroom Laboratory, Department Plant of Pathology, S. V. P. University of Agriculture and Technology, Meerut, U.P. (India) during year 2018-20, which is situated on the Western side of the Delhi-Dehradun high way (NH-58) at distance of 10.0 km away in the north of Meerut city. The district Meerut is situated between 29° 01'N latitude and 77° 45'E longitude at an altitude of 237 meters above the mean sea level

Establishment of pure culture

Culture of Shiitake (*Lentinula edodes*) were purified and maintained by single hyphal tip method. For this purpose, the cultures were grown in sterilized Petri plate on Potato Dextrose Agar Medium (PDA) for 8-10 days. Single branched hyphae from the periphery of the growing colony were marked under low power (10 x) in the compound microscope and transferred to PDA slants. These tubes were incubated at 21-24^{0}C for about a week, again sub cultured on PDA and then stored in a refrigerator at 5-10^{0}C for further use (Dlamini *et al.*,

2012). Optimizations of Culture Conditions on redial Growth of Shiitake (*Lentinula edodes*) are as given below:

Effect of different pH on mycelial growth and dry matter weight

For studies of suitable pH, the culture of *Lentinus* spp. were incubated at seven different pH media viz. 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. Required pH of the culture media (PDA) was adjusted with N/10 solutions of NaOH or HCl, used before adding of agar, it was measured by a digital pH meter. After sterilization at 121^{0}C (15 lbs pressure) for 20 minutes in autoclave, the test media (PDA) were poured into the Petri plates (90 mm @ 20 ml/plate). The plates were inoculated centrally with a 9 mm diameter disc of 7 days old culture of *Lentinus* spp. and incubated at 23±2^{0}C in B.O.D with three replications of each treatment. The observations of radial growth were taken at each 98 hrs until the colony covered the full plate.

The PD broth media was poured into 150 ml conical flasks @ 50 ml per flask and after adjusting of pH, broth sterilized in autoclave at 121^{0}C, 15 lbs psi for 20 minutes and allowed to cool. The 9 mm diameter disc cut by cork borer from the periphery of 7 days old culture of *Lentinus* spp. was inoculated in the flask with the help of sterilized inoculation needle. The flasks were incubated at 23±2^{0}C in B.O.D with three replications of each treatment. The culture was then filtered with Whatman filter paper No. 1 and the mycelium was dried at 60^{0}C in oven for 48 hours before measuring the dry weight of mycelium mat, on an electronic balance. The dry weight of mycelia mat was recorded after 10 days.

Effect of different temperature on mycelial growth and dry matter weight

For studies of suitable temperature, the culture of *Lentinus* spp was incubated at seven different temperature *viz.* 16, 18, 20, 22, 24, 26 and 28^{0}C in BOD. Petri plates containing 20 ml of sterilized PDA medium were inoculated at the centre with 9 mm diameter disc of 7 days old actively growing culture of *Lentinus* spp. under aseptic conditions. Inoculated plates were incubated in different B.O.D and three replications for each treatment were maintained. Observations on radial growth were taken at each 98 hrs until the colony covered the first full plate.

The PD broth was poured into 150 ml conical flasks @ 50 ml per flask and sterilized in autoclave at 121^{0}C, 15 lbs psi for 20 minutes and allowed to cool. The 9 mm diameter disc was cut by cork borer from the periphery of 7 days old culture of *Lentinus* spp. and inoculated in the flask with the help of inoculation needle. The flasks were incubated at seven different temperature *viz.* 16, 18, 20, 22, 24, 26 and 28^{0}C in different B.O.D with three replications of each treatment. The culture was then filtered with Whatman filter paper No. 1 and the mycelium was dried at 60^{0}C in oven for 48 hours before measuring the dry weight of mycelium mat, on an electronic balance. The dry weight of mycelial mat was recorded after 10 days.

Results and Discussion

Effect of different pH on mycelial growth and dry matter weight

Results of effect of pH range (6.9-8.1) on the mycelial growth and dry matter weight revealed that, maximum mycelial growth (89.00 mm) of Shiitake (*Lentinula edodes*) was found at pH 5.5 and it was followed by pH 6.0 (85.67 mm). Minimum mycelial growth (47.67 mm) was recorded at pH 8.5. It was followed by pH 8.0 (54.33 mm). Maximum dry matter weight (1.38 mg/50ml) of Shiitake (*Lentinula edodes*) was also observed at pH 5.5 and followed by pH 6.5 (1.27 mg/ 50ml) dry matter weight. Minimum dry matter weight (1.05 mg/50ml) was observed at pH 8.5. It was followed by pH 7.5 (1.20 mg/50ml) dry matter weight as shown in Table 1, Fig. 1, Plates 1 and 2.

The results were in accordance with the findings of Khan *et al.* (1995) observed that colony diameter was

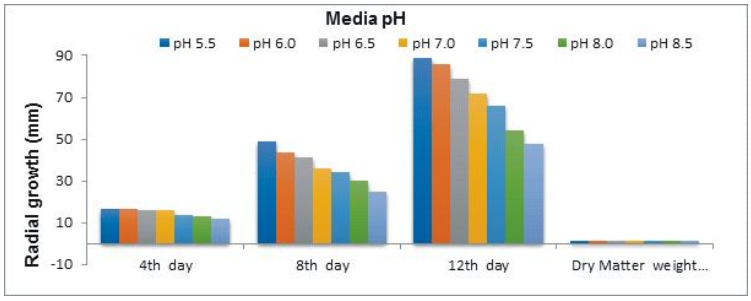

Fig. 1 : Effect of different pH on mycelial growth (mm) and dry matter weight (mg) of *Lentinula edodes*.

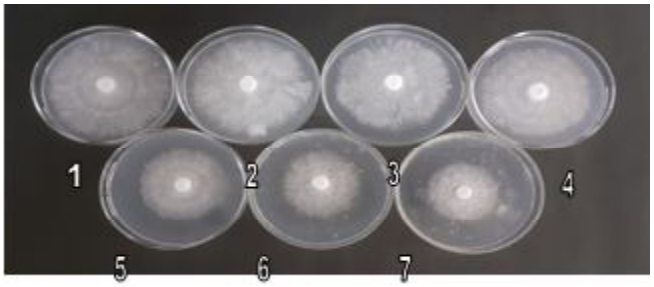

Plate 1 : Effect of different pH on mycelial growth (mm) of *Lentinula edodes*. 1. pH 5.5, 2. pH 6.0, 3. pH 6.5, 4. pH 7.0, 5. pH 7.5, 6. pH 8.0, 7. pH 8.5.

Table 1 : Effect of different pH on mycelial growth (mm) and dry matter weight (mg) of *Lentinula edodes*. (Average of three replications).

pH	Radial Growth (mm)			12th days growth rate (mm/day)	Dry Matter weight (mg/50ml)	Dry Matter growth rate (mg/day)
	4th day	8th day	12th day			
5.5	16.67	49.00	89.00	7.41	1.38	0.13
6.0	16.67	43.33	85.67	7.13	1.30	0.13
6.5	15.67	41.00	79.00	6.58	1.27	0.12
7.0	15.67	35.67	71.67	5.97	1.22	0.12
7.5	13.33	34.00	65.67	5.47	1.20	0.12
8.0	13.00	30.00	54.33	4.52	1.17	0.11
8.5	12.00	25.00	7.67	3.97	1.05	0.10
CD at 5 %	1.28	3.51	2.80	-	0.13	-
SE(m)	0.41	1.14	0.91	-	0.04	-

Table 2 : Effect of different temperature on mycelial growth (mm) of *Lentinula edodes*.

Temperature (^{0}C)	Radial Growth (mm)			12th days growth rate (mm/day)	Dry matter weight (mg/50ml)	Dry matter growth rate (mg/day)
	4th day	8th day	12th day			
16	12.00	25.33	42.33	3.52	1.00	0.10
18	14.00	30.00	48.33	4.02	1.19	0.11
20	21.33	37.00	53.00	4.41	1.21	0.12
22	23.67	41.33	70.33	5.86	1.22	0.12
24	25.33	50.00	76.67	6.38	1.79	0.17
26	26.00	51.33	83.67	6.63	2.27	0.22
28	27.00	57.00	89.33	7.44	3.01	0.30
CD at 5 %	2.21	2.31	3.03	-	0.15	-
SE(m)	0.72	0.75	0.99	-	0.02	-

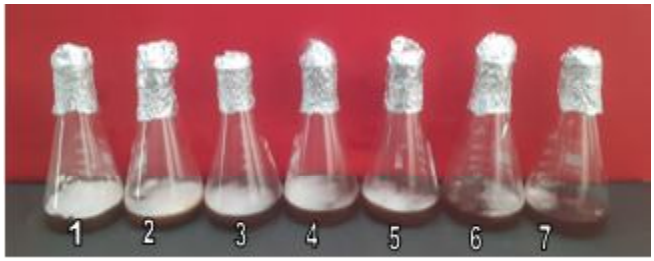

Plate 2 : Effect of different pH on dry matter weight (mg) of *Lentinula edodes*. 1. pH 5.5, 2. pH 6.0, 3. pH 6.5, 4. pH 7.0, 5. pH 7.5, 6. pH 8.0, 7. pH 8.5.

25.6 mm at pH 5, followed by 22.3, 17.6, 12.0 and 8.0 mm at 5.5, 6.0, 6.5 and 7.0 pH. Fungal colony diameter decreased with an increase in pH but the most suitable pH for the growth of shiitake recorded was 5.

Balazs *et al*. (1996) also studied on the effect of pH on shiitake mycelium and he recommended that the pH should be kept below 6. And Michael *et al*. (2001) evaluated the effect of pH on the mycelia growth of shiitake; pH 4 – 7 seemed to give the best yields of mycelia. Krupodorova *et al*. (2019) also studied the mycelia growth of shiitake on different pH and recorded maximum growth of mycelia at pH 3.5-4.0 of shiitake.

Effect of different temperature on mycelial growth and dry matter weight

A range of temperature (16-28^{0}C) was observed to know its effect on the mycelial growth and dry matter weight of Shiitake (*Lentinula edodes*) results revealed that, maximum mycelial growth (89.33 mm) of Shiitake (*Lentinula edodes*) was found at temperature 28^{0}C. It was followed by temperature 26^{0}C (83.67 mm). Minimum mycelial growth (42.33 mm) was recorded at temperature 16^{0}C. It was followed by temperature 18^{0}C (48.33 mm). Maximum dry matter weight (3.01 mg/50ml) of Shiitake (*Lentinula edodes*) was also observed at temperature 28 ^{0}C and followed by temperature 26^{0}C (2.27 mg/50ml). Minimum dry matter weight (1.00 mg/50ml) was observed at temperature 16^{0}C and followed by temperature 18^{0}C (1.19 mg/50ml) as presented in Table 2, Fig. 2, Plates 3 and 4.

The results were in accordance with the findings of Quaicoe *et al*. (2014) were investigated, three strains of *Lentinula edodes* (Le 75, Le P and Quagu) for their ability to grow under different temperatures. Optimal

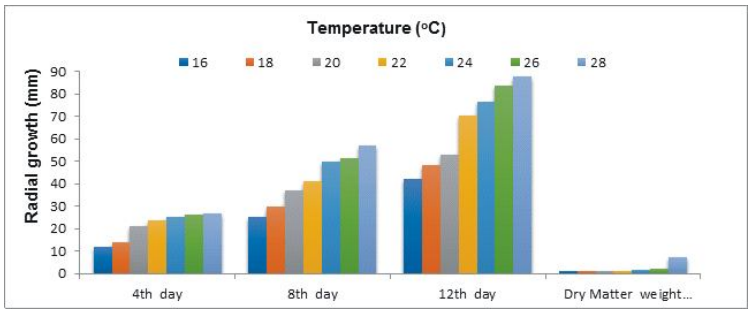

Fig. 2 : Effect of different temperature on mycelial growth (mm) and dry matter weight (mg) of *Lentinula edodes*. Average of three replications.

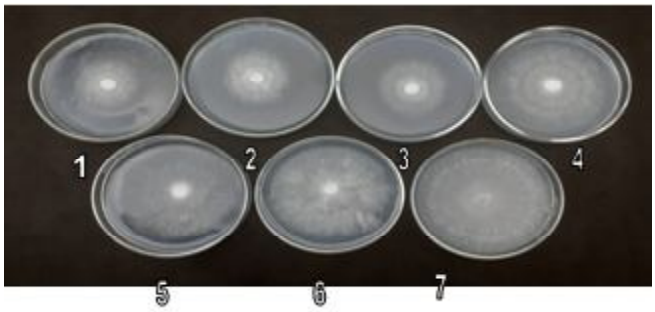

Plate 3 : Effect of different temperature on mycelial growth (mm) of *Lentinula edodes*. 1. 16^{0}C, 2. 18^{0}C, 3. 20^{0}C, 4. 22^{0}C, 5. 24^{0}C, 6. 26^{0}C, 7. 28^{0}C.

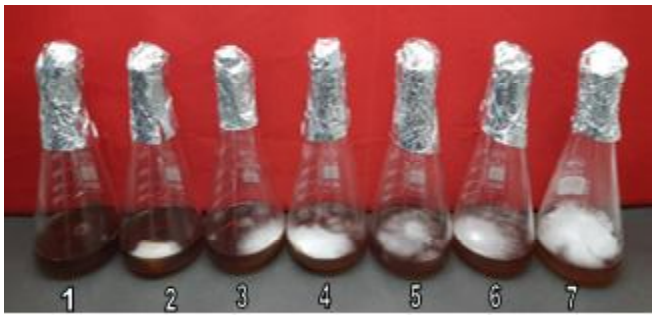

Plate 4 : Effect of different temperature on dry matter weight (mg) of *Lentinula edodes*. 1. 16^{0}C, 2. 18^{0}C, 3. 20^{0}C, 4. 22^{0}C, 5. 24^{0}C, 6. 26^{0}C, 7. 28^{0}C.

temperature for the development of all the three strains of *L. edodes* was at 25 ^{o}C.

Furlan *et al.* (2017) also tested a range of temperature (20-40 ^{0}C) for the mycelial growth of Shiitake and other mushroom and he also observed the maximum mycelial growth of Shiitake between the temperatures 25-30^{0}C. There results were almost similar with our finding.

Conclusion

Thus, it can be concluded that maximal mycelium growth and dry matter weight of Shiitake mushroom was observed at pH 5.5. Regarding temperature, it was observed that, maximum mycelial growth and dry matter weight of shiitake was found at 28^{0}C and thus it's recommended for shiitake culture conditions to be use.

Acknowledgement

Author would like to thanks to Head of plant pathology, SVPUA&T, Meerut, India, for the authentication of specimens and its culture and dean college of agriculture for providing the lab facilities during the research work.

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