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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(10): 2110-2113
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www.thepharmajournal.com
Received: 15-08-2023
Accepted: 22-09-2023

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Studies on the culture media requirements, temperature and pH on the vegetative growth of Shiitake mushroom (*Lentinula edodes* (Berk.) Pegler)

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Abstract
Shiitake mushroom (*Lentinula edodes*) is one of the most important culinary and medicinal mushroom having anticancerous properties in the world. Traditionally, Shiitake has been cultivated on oak logs but recently there is a trend to cultivate it on sterilized or pasteurized substrates in order to increase yield and to reduce the time of its growth cycle. Mushroom cultivation has received little attention in most developing countries where millions of tons of lignocellulose rich wastes are unused. The present work aimed to determine the ideal culture medium, optimum temperature and pH for the invitro vegetative growth of Shiitake mushroom. The mushroom was found to grow best in Malt extract peptone dextrose agar medium followed by oat meal agar at an optimum temperature of 20 °C and pH of 6.00.

Keywords: *Lentinula edodes,* media, pH, temperature

1. Introduction
Mushrooms are one of the most promising sources of functional food, drug, dietary supplements, healthy beverages etc. *Lentinula edodes* (Berk.) Pegler commonly known as Shiitake mushroom, is one of the most widely grown species of mushrooms and a very efficient biodegrader. The fungus was first described scientifically as *Agaricus edodes* by Miles Joseph Berkeley (1877) [1]. It was placed in the genus Lentinula by David Pegler (1975) [5]. The mushroom is called 'elixir of life' capable of generating stamina, curing colds, improving circulation and preventing premature ageing**.** Shiitake is known to contain proteins, lipids, carbohydrates, fibres, minerals, Vitamin B1, B2, C, D, provitamin, Vitamin E and Selenium. It is a rich source of antioxidants. The mushroom is used as anticarcinogenic, anti-inflammatory, antifungal, antibacterial, antiviral and anti-cardiovascular disorders. Mushroom is well known for unique taste and aroma. Moreover it is a highly priced mushroom. In India, Shiitake cultivation is mainly reported from North Eastern regions of Meghalaya, Solan, Himachal Pradesh, Rajasthan etc. where the climatic conditions suited to the mushroom prevails. There is a vast scope of mushroom cultivation in Kerala where agricultural byproducts like sawdust, paddy straw and wood shavings of hardwood trees are available in plenty. The present work was conducted with an objective to study the different culture media and physiological attributes that enhance the vegetative growth of Shiitake mushroom.

2. Materials and Methods
2.1 Effect of different solid media on the mycelial growth of *Lentinula edodes*
The experiments were carried out with all the six strains of *L. edodes* in completely randomised design (CRD) on seven different media. The growth of *L. edodes* were evaluated in terms of radial mycelial growth (cm) to find out the best medium for the growth of the fungus.

1. Potato dextrose agar (PDA)
2. Oat meal agar (OMA)
3. Malt extract agar (MEA)
4. Malt extract peptone dextrose agar (MEPDA)
5. Carrot agar (CA)
6. Czapek's Dox agar (CDA)
7. Yeast extract agar (YEA) Kaur and Lakhanpal (1995) [4].

Each of the medium was prepared and transferred to 250 ml conical flasks at the rate of 150 ml per flask and plugged tightly with cotton plugs. The flasks were sterilized in an autoclave at 15 psi pressure and 121 °C for 20 minute. The molten media were poured into sterile petriplates of nine cm diameter and allowed to solidify. The mycelial disc of nine mm diameter from seven day old culture of each strain was inoculated at the centre of the dish. The dishes were properly labelled, sealed with parafilm and incubated at room temperature (28±2 ºC). Three replications were maintained for each treatment. Nature of mycelial growth and colony diameter, were recorded for each strain. Observations were taken till the mycelial growth covered the entire petriplate.

2.2 Effect of different liquid media on the mycelial growth of *Lentinula edodes*

The different liquid media *viz*., potato dextrose broth, oat meal broth, malt extract broth, malt extract peptone dextrose broth, carrot broth, Czapek's Dox broth and yeast extract broth were evaluated to find out the best medium that supported maximum biomass production of all the six strains of *L. edodes* (Jung *et al.* 2001) [2]. The composition was same as that of solid media used in the previous experiment except for the omission of agar maintaining three replications for each treatment.

The liquid media were prepared and fifty ml of each medium were dispensed in 100 ml conical flask and autoclaved at 15 psi pressure and 121 °C for twenty minute. The media were then inoculated aseptically with nine mm culture disc's of each strain obtained from actively growing culture. The flasks were incubated at room temperature. Observations were recorded 25 days after inoculation by filtering the mycelia through Whatman No: 1 filter paper and drying at 60 ºC. The dry weights were taken until a constant weight was obtained.

2.3 Effect of different temperature on the mycelial growth and biomass production of *Lentinula edodes*

Disc of nine mm taken from the actively growing mycelium of six strains of *Lentinula edodes* were inoculated into the sterilised malt extract peptone dextrose agar medium poured on sterile petriplates. The discs were placed at the centre of the medium and incubated at 5, 10, 15, 20, 25 °C and room temperature (28±2 °C) (Khan *et al*, 1995) [5]. These experiment was conducted in three replications. Measurements of radial growth of each strain of *L. edodes* was taken when the mycelial growth of any of the tested strains completely covered the entire petriplate.

Fifty millilitres of malt extract peptone dextrose broth were taken in 100 ml conical flask and autoclaved at 121 °C and 15 lbs pressure for twenty minutes. The medium were then inoculated with nine mm disc of seven day old culture of six strains of *Lentinula edodes* and incubated at 5, 10, 15, 20, 25 and room temperature (28±2 °C). Observations were taken 25 days after inoculation. The mycelial mat was then filtered, dried at 60 ºC until constant weights were obtained.

2.4 Effect of different pH on the mycelial growth and biomass production of *Lentinula edodes*

Malt extract peptone dextrose agar medium was used for studying the effect of pH on mycelial growth of different strains of *L. edodes*. The medium was prepared and pH was adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 by adding 0.1 N Hydrochloric acid (HCl) or 0.1 N sodium hydroxide (NaOH). Sterilization was done by autoclaving at 15 lbs pressure and 121 °C for twenty minutes. After cooling, the media was poured into sterile petriplates and allowed to solidify. All the six strains of *L. edodes* were used for the experiment. Seven day old culture disc of nine mm dia. of all the six strains of *L. edodes* were inoculated at the centre of the dishes that were then sealed with parafilm and incubated at room temperature. (28±2 ºC). Three replications were maintained for each treatment in CRD. Measurements of radial growth of each strain of *L. edodes* was taken when the mycelial growth of any of the tested strains completely covered the entire petriplate.

Malt extract peptone dextrose broth was prepared with different pH concentration as given above. Fifty ml of the media was taken in 100 ml conical flask and autoclaved at 15 lbs pressure and 121 °C for twenty minutes. The medium were then inoculated with nine mm disc of seven day old culture of *Lentinula edodes* strains and incubated at room temperature. (28±2 ºC) for 25 days. The mycelial mat was filtered, dried at 60 ºC until constant weights were obtained.

3. Results and Discussion

3.1 Effect of different solid media on the mycelial growth of *Lentinula edodes*

The growth of *L. edodes* on petriplate was completed on 12th day after inoculation (Fig 1). Among the various media tested, malt extract peptone dextrose agar was found to be the best, inducing maximum radial growth (9.00 cm) for all the strains. Oat meal agar and malt extract agar were found to be on par with MEPDA in supporting their mycelial growth. The findings concur with studies by Lata and Sharma (2012) [6], where malt extract peptone dextrose agar medium supported maximum radial growth followed by potatao dextrose agar.

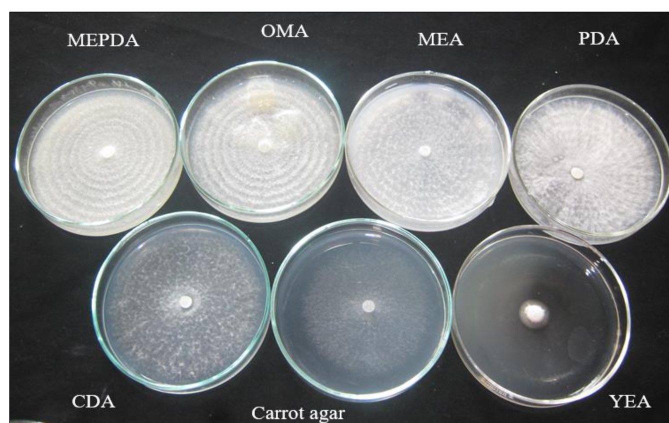

Fig 1: Growth of *Lentinula edodes* strains in different solid media

From the above studies, it was observed that mycelial growth of 9.00 cm was attained in strains LE-1, 2, 3 and 6 on malt extract peptone dextrose agar media twelve days after inoculation. Mycelial growth of LE-4 and LE-5 recorded 8.63 and 8.80 cm which were on par with other strains. LE-1 also showed highest growth of 9.00 cm in oat meal agar. LE-2 and LE-6 showed 9.00 cm growth in malt extract and potato dextrose agar media. On oat meal agar, LE- 2,3,4,5 and 6 produced 8.80, 8.63, 8.96, 8.62 and 8.90 cm of radial growth respectively which was on par with LE-1 (9.00 cm). On malt extract, LE-3 and LE-5 showed mycelial growth of 8.33 and 8.53 cm respectively which were on par with LE-1, 2 and 3. Mycelial growth of all strains except LE-1 and LE-2 were

comparatively low in potato dextrose agar, Czapek's Dox and carrot agar. Radial growth of all the strains were lowest on yeast extract agar (Table 1).

Table 1: Growth of *Lentinula edodes* strains in different solid media

Media	Radial growth 12 DAI (cm)*					
	Strains					
	LE-1	LE-2	LE-3	LE-4	LE-5	LE-6
MEPDA	9.00a	9.00 a	9.00 a	8.63 a	8.80 a	9.00 a
OMA	9.00 a	8.80 a	8.63 a	8.96 a	8.62 a	8.90 a
MEA	9.00 a	9.00 a	8.33 a	8.13 b	8.53 a	9.00 a
PDA	8.83 a	9.00 a	8.23 a	6.36 c	8.23 b	8.36 b
CDA	6.50 c	8.36 b	5.93 c	5.20 d	6.20 d	6.90 c
CA	8.36 b	6.90 c	7.66 b	6.20 c	8.13 b	7.93 b
YEA	1.00 d	0.96 d	1.00 d	1.50e	1.56e	1.43d
CD (0.05)	0.23	0.25	0.43	0.28	0.36	0.31

* Average of three replications

MEPDA: Malt extract peptone dextrose agar, OMA- Oat meal agar
MEA: Malt extract agar, PDA- Potato dextrose agar
CDA: Czapek's Dox agar, CA- Carrot agar, YEA- Yeast extract agar

3.2 Effect of different liquid media on the mycelial growth of *Lentinula edodes*: Biomass production of all the strains of *L. edodes* in liquid media were taken 25 days after inoculation (Fig 2). There was significant difference between each liquid media in influencing biomass production of *L. edodes.* Maximum biomass production of all the six strains were obtained in oat meal broth ranging from 145 mg/ 50 ml to 405 mg/ 50 ml. LE-2 and LE-4 strains also produced highest biomass in PDB with 188.46 mg/ 50 ml and 118.66 mg/ 50 ml respectively which were on par with oat meal broth. Strain LE-3 produced biomass of 132.66 mg/ 50 ml on MEPDB, followed by LE-4 (74.40 mg/50 ml), LE-2 (63.80 mg/ 50 ml), LE-1 (48.13 mg/ 50 ml), LE-6 (40.30 mg/ 50 ml) and LE-5 (34.16 mg/ 50 ml). On malt extract broth, LE-2 produced maximum biomass of 71.40 mg/50ml which was followed by 49.93 mg/ 50ml (LE-4), 39.30 mg/50ml (LE-6), 39.06 mg/ 50 ml (LE-3), 31.00 mg/50 ml (LE-1) and 18.46 mg/ 50 ml (LE-5). In carrot broth, maximum biomass of 48.16 mg/ 50 ml was produced by LE-2 strain followed by 38.96 mg/ 50 ml (LE-4), 34.66 mg/ 50 ml (LE-3), 30.70 mg/ 50 ml (LE-6) and 30.40 mg/ 50 ml (LE-5). Lowest biomass production of *L. edodes* strains were recorded (3.59 mg/ 50 ml to 14.96 mg/ 50 ml) on yeast extract broth.

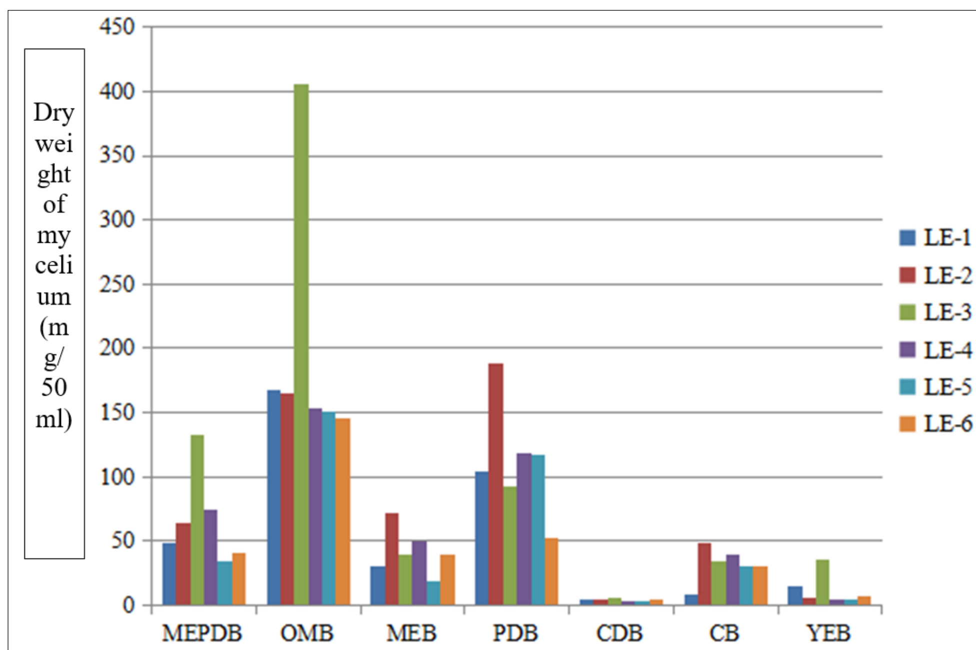

Fig 2: Growth of *Lentinula edodes* strains in different liquid media

3.3 Effect of different temperature on the mycelial growth and biomass production of *Lentinula edodes*

In solid medium, all strains of *L. edodes* attained maximum growth at 20 ºC (8.63 cm to 9.00 cm). This was closely followed by growth at 25 ºC temperature where LE-6 showed maximum radial growth (8.30 cm) followed by LE-1(8.03 cm), LE-2 (8.17 cm), LE-3 (8.00 cm), LE-4 (7.60 cm) and LE-5 (7.43 cm). Sharma *et al.* (2013) [8] also reported maximum mycelial growth of *L. edodes* strains at 20 ºC. Radial growth was comparatively high at room temperature (28 ±2 °C) (5.23 cm to 6.03 cm) when compared to lower temperatures of 15 °C and 10 °C. Lowest mycelial growth was observed at 5 °C (5.23 cm to 6.03 cm) (Table2).

Table 2: Growth of *L. edodes* strains in different temperature in solid media

Temperature (°C)	Radial growth 12 DAI (cm)*					
	LE-1	LE-2	LE-3	LE-4	LE-5	LE-6
5	0.90 f	0.90 d	0.90 e	0.90 e	0.90 f	0.90 f
10	1.50 e	1.30 d	1.50 e	1.26 e	1.70 e	1.77 e
15	4.20 d	3.73 c	4.03 d	3.96 d	3.70 d	3.97 d
20	8.76 a	8.73 a	8.63 a	8.67 a	8.70 a	9.00 a
25	8.03 b	8.17 b	8.00 b	7.60 b	7.43 b	8.30 b
Room temperature	5.27 c	5.26 c	5.43 c	5.60 c	5.23 c	6.03 c
CD (0.05)	0.37	0.40	0.60	0.58	0.42	0.27

*Average of three replications

In liquid medium (Fig 3) highest mycelial biomass of all the strains was obtained in broth at 20 ºC (58.73 mg/ 50 ml to 81.90 mg/ 50 ml) (Fig 2). LE-6 produced maximum biomass of 56.90 mg/ 50 ml at 25 °C, which was followed by LE-5 (55.60 mg/ 50 ml), LE-1 (52.40 mg/ 50 ml), LE-3 (50.23 mg/ 50 ml), LE-2 (46.87 mg/ 50 ml) and LE-4 (43.67 mg/ 50 ml). The biomass production at room temperature (28 =-2 °C) (22.66 mg/ 50 ml to 34.67 mg/ 50 ml) was high compared to 15 °C (11.93 mg/50 ml to 17.10 mg/ 50 ml) and 10 °C (4.20 mg/ 50 ml to 9.63 mg/ 50 ml). The biomass production was lowest at 5 °C of all the strains of *Lentinula edodes*.

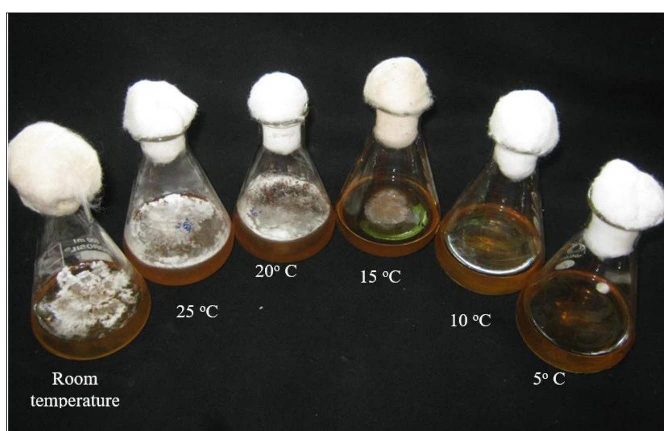

Fig 3: Growth of *L. edodes* strains in different temperature in liquid media

3.4 Effect of different pH on the mycelial growth and biomass production of *Lentinula edodes*

All the six strains of *L. edodes* showed highest radial growth (9.00 cm) at pH 6 which was followed by pH 7. Radial growth of LE- 2, 3, 4 and 5 at pH 7 was on par with the radial growth of all the strains at pH6. At pH 8 (8.59 cm to 8.77 cm) and pH9 (8.40 cm to 8.63 cm) there was a decrease in trend in the mycelial growth of the strains. Lowest growth was observed at pH 5 (4.20 cm to 5.50 cm) and 4 (3.13 cm to 3.73 cm) in supporting the mycelial growth of *L. edodes* (Table 3).

Table 3: Growth of *L. edodes* strains in different pH in solid media

pH	Radial growth 12 DAI (cm) *					
	LE-1	LE-2	LE-3	LE-4	LE-5	LE-5
4	3.37 d	3.57 d	3.70 e	3.73 e	3.54 d	3.13 d
5	4.93 c	5.50 c	5.17 d	5.33 d	4.20 c	5.50 c
6	9.00 a	9.00 a	9.00 a	9.00 a	9.00 a	9.00 a
7	8.68 b	8.73 ab	8.90 ab	8.80 ab	8.68 b	8.82 ab
8	8.59 b	8.67 b	8.63 bc	8.63 c	8.60 b	8.77 ab
9	8.50 b	8.50 b	8.53 c	8.40 bc	8.50 b	8.63 b
CD (0.05)	0.27	0.29	0.27	0.33	0.38	0.36

*Average of three replications

In liquid medium (Fig 4) highest mycelial biomass of all the strains was obtained at pH 6 (50.66 mg/50 ml to 154.00 mg/ 50 ml) (Fig 3). At pH 7, maximum biomass (114.86 mg/50ml) was produced by LE-5 strain followed by LE-3 (78.03 mg/ 50 ml), LE-2 (61.10 mg/50 ml), LE^{-1} (56.53 mg/ 50 ml) and LE-6 (50.26 mg/50 ml broth). Comparatively less biomass production was noticed at pH 8 and 9. Maximum biomass production at pH 5 was obtained in LE-5 (50.93 mg/ 50 ml) followed by LE-2 (50.90 mg/ 50 ml), LE-6 (50.86 mg/ 50 ml), LE-1 (50.76 mg/ 50 ml). Lowest biomass production was recorded in LE-4 (35.63 mg/ 50 ml broth). Singh *et al* (2000) [9] and Cristina *et al* (2001) [2] reported that when various *Lentinula edodes* strains cultivated in PDA medium at different pH's were tested the most suitable pH was reported to be 6.00.

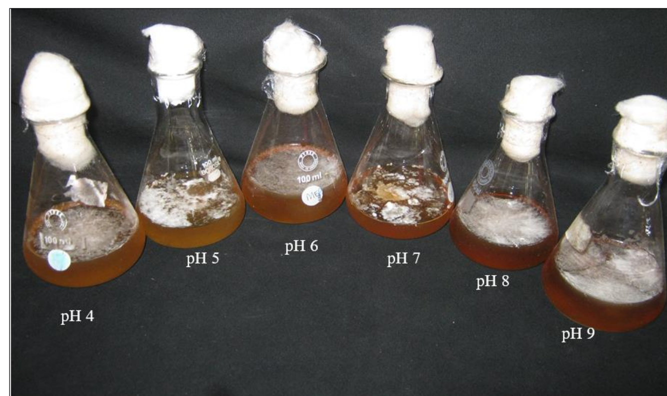

Fig 4: Growth of *L. edodes* strains in different pH in liquid media

4. Conclusion

In the present study it can be concluded that Malt extract peptone dextrose agar was found to be the best culture medium for the mycelial growth of Shiitake. The optimum temperature and pH for the mycelial growth of Shiitake in both solid and liquid medium proved to be 20 ºC and 6 respectively.

5. Acknowledgement

The facilities and support provided by Kerala Agricultural University has been duly acknowledged.

6. References

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