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Evaluation of culture conditions for the mycelial growth of *Lentinula edodes* (Berk.) Pegler

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Lentinula edodes (Berk.) Pegler, commonly known as shiitake mushroom is globally renowned for its culinary and medicinal value, ranking first only to button mushrooms in global production (Singh *et al.,* 2021). Shiitake mushrooms stand out among edible fungi for their numerous health benefits, including anticancer, anticholesterolemic and antidiabetic properties. Their nutritional profile is rich in free sugars such as arabinose, arabitol, mannose, mannitol, trehalose, and glycerol, along with bioactive polysaccharides like â-D glucan, heteroglucan, xylomannan, lentinan, and eritadenine, as well as essential vitamins (B2, B12, and D2) and dietary fibre (Hobbs, 2002). Additionally, as a white rot fungus, shiitake mushrooms possess enzymes capable of degrading lignin and cellulose, thereby breaking down wood components and facilitating their growth on lignocellulosic substrates (Curvetto *et al.,* 2002).

One key challenge in shiitake cultivation is the lengthy spawn run period, which can be reduced by using various lignocellulosic substrates along with organic and inorganic additives. The pH and temperature of these substrates play crucial roles in supporting optimal growth. Deviations in pH can lead to decreased mycelial growth or unsuccessful fruiting, while excessive temperatures may cause the mushrooms to dry prematurely (Kumar *et al.*, 2019). Among environmental factors, pH is particularly critical; shiitake growth is optimal in slightly acidic to neutral conditions, which supports efficient enzyme production and nutrient absorption.

The availability of carbon and nitrogen sources also significantly impacts shiitake growth. Carbon sources provide essential energy, while nitrogen sources support protein synthesis and cellular structure, influencing biomass production. Supplementing shiitake cultures with easily available carbon sources (such as sucrose, dextrose and fructose) and nitrogen sources (such as peptone, ammonium chloride and yeast extract) has been shown to enhance mycelial growth and enzyme excretion. Moreover, the liquid nutrient medium and cultivation environment, along with the strain's biological traits, are crucial to improve yield and polysaccharide productivity (Scherba *et al.*, 1999; Elisashvili, 2012; Krasnopolskaya *et al*., 2012). Given the importance of these factors, this study investigates the optimal physiological and nutritional requirements for the vegetative growth of *L. edodes*, focusing on suitable pH, carbon and nitrogen sources.

The shiitake strain (DMRO 327) obtained from the CMRT Unit, Department of Plant Pathology, CSKHPKV, Palampur and was maintained by regular sub culturing on PDA slants and kept at 20±2ºC in the BOD incubator.

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Five liquid media *viz.,* malt extract broth (MEB), potato dextrose broth (PDB), potato sucrose broth (PSB), oat meal broth (OMB), yeast extract sucrose broth (YSB) were evaluated in 50 ml of sterilized medium and incubated at 22±2°C. Fresh and dry weights of the mycelium were recorded up to 25 days of incubation at an interval of 5 days. The average fresh and dry weights of the mycelial mat formed in each liquid broth were measured and presented in table 1 and fig 1. The maximum average fresh mycelial weight was achieved in Potato sucrose broth (18.8 mg/ml) and Oat meal broth (18.4 mg/ml). In contrast, the lowest biomass production was observed in Potato dextrose broth having average fresh weight (7.4 mg/ml) and dry weight (2.7 mg/ml). Lata and Sharma (2012) evaluated 17 liquid media for vegetative growth across five *L. edodes* strains. They identified Asthana and Hawker's broth as the most effective, supporting mycelial growth with a dry weight of 340–350 mg.

Table 1. Effect of liquid media on the mycelial growth of *Lentinula edodes*

S. No.	Medium	Av. Fresh weight of mycelial mat (mg / ml)	Av. Dry weight of mycelial mat (mg / ml)
1.	Potato dextrose broth	7.4	2.7
2.	Potato sucrose broth	18.8	7.8
3.	Malt extract broth	9.4	3.0
4.	Oat meal broth	18.4	7.4
5.	Yeast sucrose broth	12.8	4.0
	CD (0.05)	0.4	0.2

*Average of three replication

The Potato sucrose broth was used to study the effect of pH on mycelial growth of *L. edodes*. The pH of the medium was adjusted to 4.0, 5.0, 6.0, 7.0 and 8.0 using either 0.1N hydrochloric acid (HCl) or 0.1N sodium hydroxide (NaOH). The medium was inoculated and observations on fresh and dry mycelia weight were taken after 25 days. The highest mycelial growth of was recorded in the medium with pH 6.00 (103.12 mg/50 ml) (Table 2 and Fig 2). It was well evident from this experiment that acidic pH favours the growth of *L. edodes* while, increasing pH towards alkalinity decreases mycelial growth of the mycelium. Similar results were also reported by Rani *et al*.

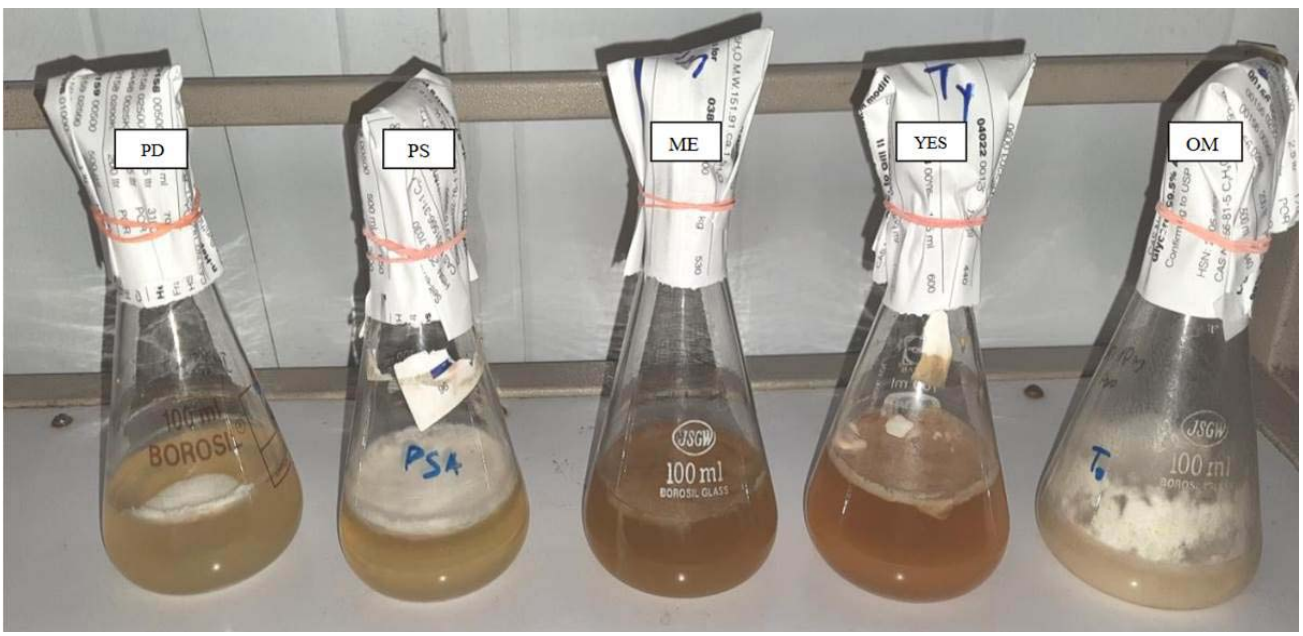

Fig. 1. Biomass production of *Lentinula edodes* on different liquid media

Table 2. Effect of pH, carbon and nitrogen sources on the mycelial growth of *Lentinula edodes*after 25 days of incubation

pH	Mycelial dry weight (mg/50ml)	Carbon sources	Mycelial dry weight (mg/50ml)	Nitrogen sources	Mycelial dry weight (mg/50ml)
4	44.35	Sucrose	101.34	Peptone	117.21
5	50.13	Lactose	86.74	Ammo. Chloride	101.35
6	103.12	Maltose	68.20	Yeast	111.23
7	58.41	Mannose	77.24	Potassium nitrate	103.15
8	49.83	Dextrose	98.82	Ammo. Sulphate	97.13
		Fructose	78.45	Asparagine	110.35
CD(p=0.05)	1.06	CD(p=0.05)	1.41	CD(p=0.05)	0.65

*Average of three replications

(2023) who reported pH 6.00 as ideal for *in vitro* growth of Shiitake mushrooms. However, Kumar *et al*. (2019) suggested that pH levels of 5.00 to 6.00 are ideal, while Sharma *et al.* (2013) identified pH 7.00 as optimal for maximal mycelial growth. Aminuddin *et al*. (2013) found pH 6.00 to be most effective in potato dextrose broth, whereas Lata and Sharma (2012) suggested an optimal range of pH 5.00–7.50. Balazs *et al*. (1996) further highlighted that a pH of 5.00 is favourable for *L. edodes* mycelia growth.

Effect of different carbon sources was studied using different carbon sources (dextrose, lactose, galactose, mannitol, fructose and sucrose) replacing sucrose in the media keeping the carbon content stable. Results were calculated after 25 days of incubation. Observations are presented in table 2 and fig 3. The carbon sources demonstrated significant

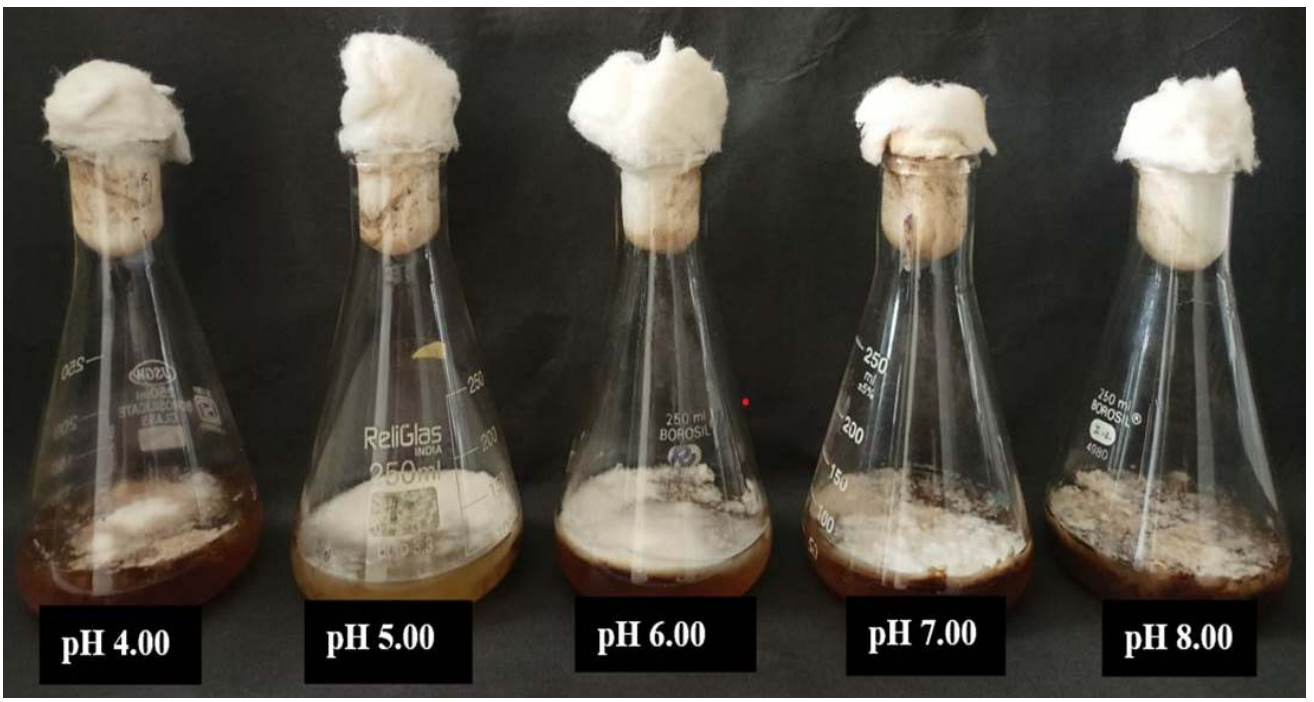

Fig. 2. Effect of different pH on mycelia growth of *L. edodes*

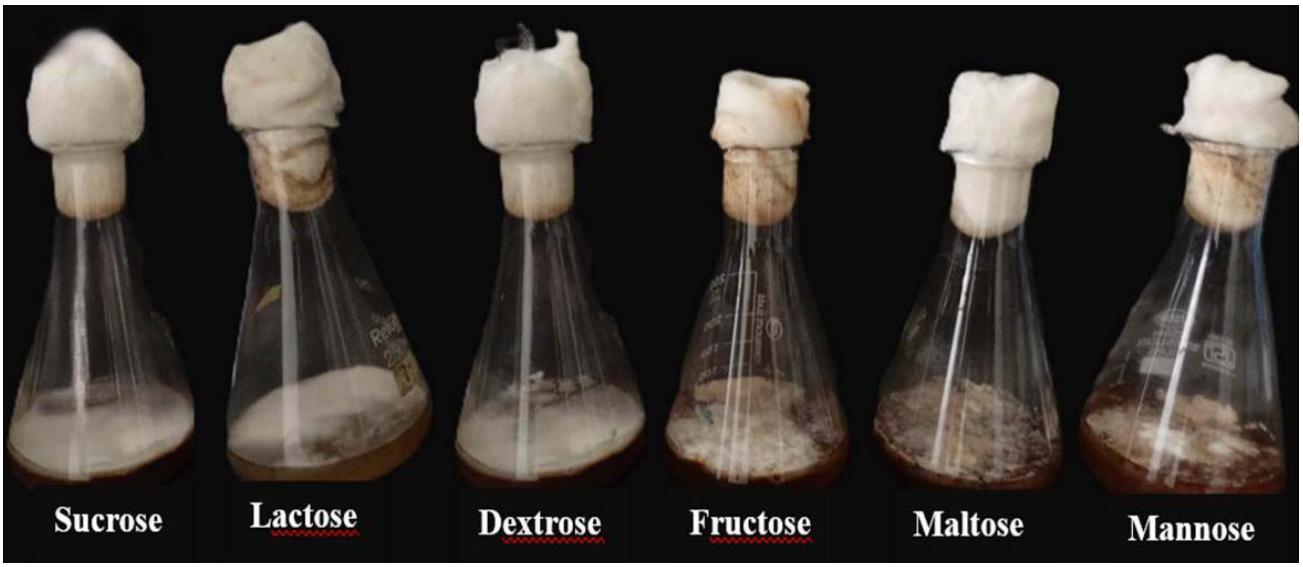

Fig. 3. Effect of different carbon sources on mycelia growth of *L. edodes*

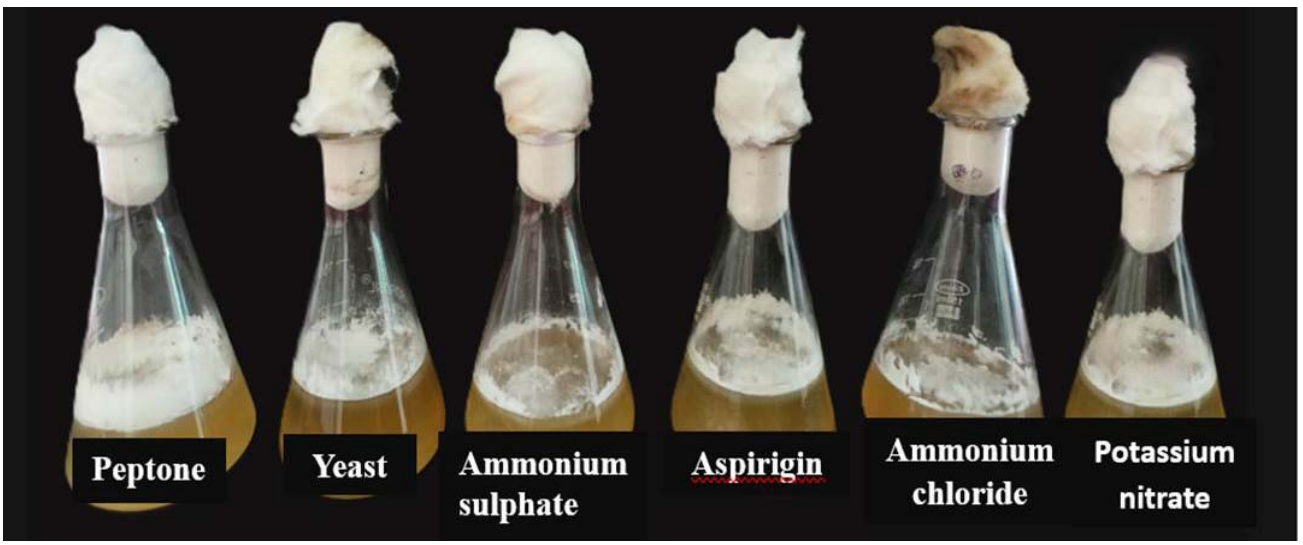

Fig. 4. Effect of different nitrogen sources on mycelia growth of *L. edodes*

differences in their influence on biomass production. Media supplemented with sucrose as the carbon source exhibited the highest biomass production at 101 mg/ 50 ml of broth followed by dextrose with 98 mg/ 50 ml. The results of the present study align with Jung *et al*. (2001), who reported sucrose as the best carbon source, followed by D-galactose and D-fructose. Song *et al*. (1987) found glucose to be the most effective carbon source for submerged *L. edodes* mycelial cultures. Similarly, Kaur and Lakhanpal (1995) demonstrated that glucose supported the greatest mycelial growth, followed by fructose and sucrose.

The nitrogen source utilization of *L. edodes* mycelium was evaluated by supplementing various nitrogen sources keeping same composition in all the treatments. Results were calculated after 25 days of incubation. Six different nitrogen sources i.e. peptone, ammonium chloride, yeast extract, potassium nitrate, ammonium sulfate and asparagine were used in the

study (Table 2 and Fig 4). Among the nitrogen sources, peptone demonstrated the highest biomass production (117.21 mg/50 ml), followed by yeast extract (111.23 mg/50 ml). The findings of the present study is supported by previous studies by Kaur and Lakhanpal (1995) and Balazs *et al*. (1998).

An analysis of the literature and the present study highlights the significant impact of medium composition, pH, and nutrient sources on the growth and biomass yield of the edible and medicinal mushroom *L. edodes*. The findings demonstrate that optimizing the selection of carbon and nitrogen sources, their ratios, the pH of the nutrient medium, and the cultivation medium can result in a three-fold increase in biomass production. Acidic conditions were found to favor mycelial growth, with the highest growth observed at pH 6.00, while increasing alkalinity reduced growth. Among carbon sources, sucrose was the most effective, followed by dextrose. Regarding nitrogen sources, peptone yielded the highest biomass production, followed by yeast extract. These results underscore the importance of tailored nutrient and environmental conditions for maximizing *L. edodes* biomass production.

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