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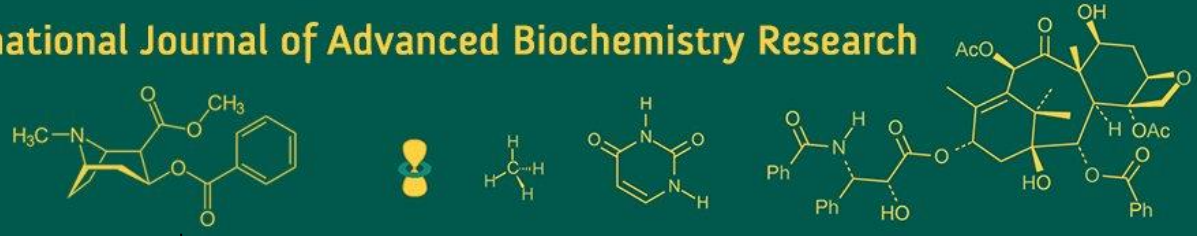

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Optimizing growth conditions for shiitake mushroom cultivation in Birbhum, West Bengal: A study on media, temperature, and ph variations

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Abstract
Shiitake mushroom (*Lentinula edodes* (Berk.) Pegler) is a valuable edible fungus known for its culinary and medicinal properties. Understanding the influence of media, temperature, and pH variations is crucial for the growth and productivity of *L. edodes*, offering insights into enhancing cultivation practices and yield optimization. The study investigated the influence of various substrates, temperature conditions, and pH levels on the mycelial growth of *L. edodes*. Potato Dextrose Agar exhibited the highest mycelial growth rate, whereas Rose Bengal Agar showed the lowest. Among the tested substrates, Potato Dextrose Agar was preferred by all three strains, followed by Malt Extract Agar, Sorghum Meal Agar, Saw Dust Extract Agar, and Czapek Dox Agar. Strain LE-02 demonstrated superior mycelial growth compared to the other strains. Regarding temperature, optimal growth was observed at 25 °C, while growth decreased at 15 °C. Deviations from the optimal temperature of 25 °C resulted in reduced mycelial growth. Regarding pH levels, pH 5.0 supported the highest mycelial growth compared to pH levels of 4.0, 6.0, and 7.0. These findings underscore the importance of substrate composition, temperature management, and pH regulation in optimizing the mycelial growth of *L. edodes* under laboratory conditions.

Keywords: Medicinal mushroom, Growth media, pH, temperature, strains

Introduction
Mushrooms hold significant cultural importance worldwide, often linked with celebrations and esteemed for their nutritional value. The term "mushroom" originates from the French "mousse" or "mousseron," reflecting their historical culinary significance. In Romanian tradition, mushrooms are revered as "God's flesh," while in Chinese culture, they are seen as the "Elixir of Life." The scientific study of fungi, known as mycology, explores the intricate world of mushrooms. Shiitake (*L. edodes*), also called Xiang-gu, is a prized edible fungus belonging to the Agaricales order of the Basidiomycota phylum. It is indigenous to Japan, China, and other Southeast Asian nations, valued for both its culinary and medicinal properties (Mata and Savoie, 2018) [16].

The name "shiitake" is a combination of "shii," referring to the Japanese chinquapin tree (*Castanopsis cuspidata*), and "take," meaning mushroom (Tian *et al*., 2016) [25]. It is a saprophytic white-rot fungus that thrives on decaying wood from deciduous trees or sawdust, breaking down cellulose, hemicellulose, and lignin with lignocellulolytic enzymes. *L. edodes* is the second most cultivated mushroom species globally, following *Agaricus bisporus* (Leatham, 1986) [14], (Chang, 1999) [5], (Chang and Buswell, 2001) [4], (Szeto *et al*., 2008) [24], Andrade *et al*., 2013) [2]. Naturally occurring in Asian regions with warm, humid climates, shiitake accounts for approximately 25% of global mushroom production (Jiang *et al*., 2015) [10], with China leading in production, export, and consumption (Ye *et al*., 2012) [27]. Shiitake mushrooms are dubbed "the queen of mushrooms" due to their significant market potential domestically and internationally. These mushrooms produce flavorful brown sporocarps known for their medicinal properties. In Chinese folklore, there's a belief that shiitake is an "elixir of life," capable of enhancing stamina, treating colds, improving circulation, and preventing premature aging (Puri, 2011) [19].

The evaluation of different media, temperature, and pH for the mycelial growth of *L. edodes* is crucial for optimizing cultivation conditions and maximizing yield in commercial mushroom production. Understanding the ideal environmental parameters such as media composition, temperature, and pH levels can significantly enhance mycelial growth rates, leading to improved productivity and quality of *L. edodes* mushrooms. Several studies have investigated the optimal growth conditions for various edible mushrooms. Furlan *et al*. (1997) [7] examined seven mushroom species on different agar media, finding wheat dextrose agar to be the most conducive for mycelial growth. Vargas-Isla and Ishikawa (2008) [26] reported that PDA supported the highest radial mycelial growth of *L. strigosus* among several media tested. Imtiaj *et al*. (2009) [9] identified PDA, Hamada, Lilly, and YM media as most suitable for mushroom mycelial growth, while Quaicoe *et al*. (2014) [21] found sorghum meal agar to promote the highest growth of shiitake mycelium. Additionally, observed varying growth rates of *L. edodes* on different culture media, with potato dextrose agar supporting maximum growth (Mata and Mishra, 2015) [17]. Arif *et al.* (2015) [3] noted that neutral to slightly acidic pH favored *L. edodes* growth on potato dextrose agar. Various alternative substrates, such as swine wastewater (Lee *et al.*, 2017) [15] and rice wastewater (Setiati *et al*., 2019) [23], have also been explored for mushroom cultivation. These studies collectively contribute to understanding optimal conditions for mushroom mycelial growth and cultivation techniques.

Materials and Methods

Cultures: The three *L. edodes* utilized in this study, *viz*., (LE-01) was sourced from the Indian Institute of Horticultural Research (IIHR), Bengaluru, while (LE-02, LE-03) were acquired from the Directorate of Mushroom Research (DMR), Solan.

Preparation of different culture media

The media used in this study include Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Sorghum Meal Agar (SMA), Rose Bengal Agar (RBA), Saw Dust Extract Agar (SDEA), and Czapek Dox Agar (CDA). These media were prepared following specific protocols, including ingredient quantities and sterilization methods, outlined in the literature (Overcast and Weakley, 1969) [18].

Potato dextrose agar (PDA): Preparation: Boil 200 g peeled potatoes in 500 ml distilled water, filter, and mix with 20 g dextrose, 20 g agar-agar, adjust to 1000 ml with water.

Malt Extract Agar (MEA)
Preparation: Dissolve 20 g malt extract, 20 g agar-agar in 1000 ml distilled water.

Sorghum Meal Agar (SMA)
Preparation: Mix 200 g ground sorghum grains with 500 ml water, add 12 g agar, 60 g dextrose, boil, and adjust to 1000 ml.

Rose Bengal Agar (RBA)
Preparation: Mix mycological peptone, dipotassium phosphate, magnesium sulphate, rose Bengal, chloramphenicol, agar in water; adjust pH to 7.2 ± 0.2.

Sawdust Extract Agar (SDEA)
Preparation: Boil 20 g sawdust in 500 ml water, filter, mix with 20 g dextrose, 20 g agar-agar, adjusts to 1000 ml.

Czapek Dox Agar (CDA)
Preparation: Mix sucrose, sodium nitrate, dipotassium phosphate, magnesium sulphate, potassium chloride, ferrous sulphate in water, add agar, and adjust pH to 7.3 ± 0.2.

Statistical analysis

The research utilized a Completely Randomized Design, with each treatment replicated three times to ensure statistical reliability. Data analysis was performed using the statistical software WASP 1.0 (Web Agri Stat Package), which was accessed via https://ccari.icar.gov.in/waspnew.html on 2 April 2024. ANOVA was employed to investigate potential disparities in parameter values. Duncan's multiple range tests were utilized at a significance level of 5% to evaluate differences among treatments.

Results and Discussion

Effect of different media on mycelial growth of *L. edodes*

The growth of three distinct strains of shiitake mushroom (LE-01, LE-02, and LE-03) was examined across various media, including Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Sorghum Meal Agar (SMA), Rose Bengal Agar (RBA), Sawdust Extract Agar (SDEA), and Czapek Dox Agar (CDA), maintained at 25±2 °C (Fig 1). Colony diameter measurements were taken on days 6 and 12 post-inoculation, averaging two perpendicular diameters.

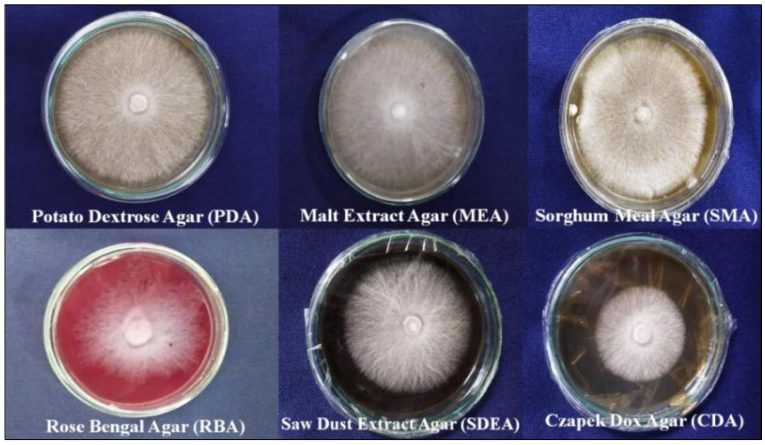

Fig 1: Effect of different media on mycelial growth of *L. edodes*

The mycelial growth of *L. edodes* was assessed on various media at 6 and 12 days after inoculation (DAI). Among the media tested, Potato Dextrose Agar (PDA) consistently supported the highest colony diameter across all three strains (LE-01, LE-02, and LE-03) at both 6 and 12 DAI. At 6 DAI, the highest colony diameter was observed on PDA with values ranging from 30.62 to 36.19 mm, while at 12 DAI, the colony diameter increased substantially, ranging from 66.06 to 74.46 mm, indicating robust mycelial growth. Conversely, the lowest colony diameters were consistently recorded on Rose Bengal Agar (RBA) at both time points for all three strains, indicating poorer growth conditions on this medium. At 6 DAI, colony diameters ranged from 23.83 to 30.22 mm on RBA, while at 12 DAI, the diameters varied from 44.47 to 50.83 mm, significantly lower compared to other media. These findings underscore the importance of selecting appropriate growth media to promote optimal mycelial growth of *L. edodes*, with PDA being particularly favorable for cultivation (Table 1 and 2).

Table 1: Effect of different media on mycelial growth of *L. edodes* on 6 DAI

Media	Colony diameter (mm) at 6 days after Inoculation		
	LE-01	LE-02	LE-03
PDA	36.19^{ab}	30.62^{bc}	32.85^{a}
MEA	34.50^{bc}	32.91^{ab}	28.6^{bc}
SMA	33.72^{bc}	26.09^{d}	28.02^{bc}
RBA	30.22^{c}	23.83^{d}	26.48^{c}
SDEA	39.19^{a}	35.62^{a}	29.96^{ab}
CDA	33.25^{bc}	27.17^{cd}	26.59^{c}
CD @ 5%	4.477	3.639	3.191
S.Em ±	6.333	4.183	3.217
CV (%)	7.292	6.963	6.239

PDA – Potato Dextrose Agar; MEA – Malt Extract Agar; SMA – Sorghum Meal Agar; RBA – Rose Bengal Agar; SDEA – Saw Dust Extract Agar; CDA – Czapek Dox Agar; CD – Critical Difference; S.Em – Standard Error of mean; CV – Coefficient of Variation; Different letters after values are significantly different at $p \leq 0.05$

Table 2: Effect of different media on mycelial growth of *L. edodes* on 12 DAI

Media	Colony diameter (mm) at 12 days after Inoculation		
	LE-01	LE-02	LE-03
PDA	68.86^{a}	74.46^{a}	66.06^{a}
MEA	68.44^{a}	67.18^{b}	60.44^{b}
SMA	64.61^{ab}	64.29^{bc}	56.99^{bc}
RBA	50.83^{d}	44.47^{e}	45.31^{e}
SDEA	60.05^{bc}	60.52^{c}	55.02^{cd}
CDA	55.47^{cd}	52.68^{d}	52.09^{d}
CD @ 5%	4.884	4.705	4.160
S.Em ±	7.535	6.994	5.466
CV (%)	4.472	4.364	4.176

PDA – Potato Dextrose Agar; MEA – Malt Extract Agar; SMA – Sorghum Meal Agar; RBA – Rose Bengal Agar; SDEA – Saw Dust Extract Agar; CDA – Czapek Dox Agar; CD – Critical Difference; S.Em – Standard Error of mean; CV – Coefficient of Variation; Different letters after values are significantly different at $p \leq 0.05$

These findings align with previous studies (Vargas-Isla and Ishikawa, 2008) [26], (Mata and Mishra, 2015) [17], (Arif *et al*., 2015) [3], (Kumar *et al*., 2019) [12], which similarly found Potato Dextrose Agar to be superior for culturing *L. edodes*. Additionally, Cobos *et al*. (2017) [6] noted Malt Extract Agar as an effective medium for *L. edodes*, performing comparably well to Potato Dextrose Agar in this study.

Effect of different temperatures on mycelial growth of *L. edodes*: The optimal temperature for *L. edodes* growth was investigated on Potato Dextrose Agar (PDA) medium using three strains (LE-01, LE-02, and LE-03) across various temperature conditions: 15 °C, 20 °C, 25 °C, and 30 °C.

The mycelial growth of *L. edodes* was significantly influenced by temperature treatments at both 6 and 12 days after inoculation (DAI). At 6 DAI, the highest colony diameter was observed at 25 °C for all three strains (LE-01: 35.26 mm, LE-02: 29.83 mm, LE-03: 29.04 mm), indicating optimal growth at this temperature. Following 25 °C, 20 °C exhibited the next highest colony diameters across all strains, while 30 °C showed comparatively lower growth. Conversely, at 12 DAI, the highest colony diameters were consistently recorded at 25 °C, demonstrating its continued favorable effect on mycelial development (LE-01: 71.24 mm, LE-02: 68.92 mm, LE-03: 67.81 mm). Following 25 °C, 20 °C showed the next highest colony diameters, while 30 °C exhibited lower growth, similar to the pattern observed at 6 DAI. Notably, at both time points, the lowest colony diameters were consistently observed at 15 °C for all three strains, indicating suboptimal growth conditions at this temperature. This suggests that while 25 °C fosters the most robust mycelial growth over time, 20 °C also provides favorable conditions for *L. edodes*, with 15 °C representing the least conducive temperature for mycelial development (Table 3 and 4).

Table 3: Effect of different temperatures on mycelial growth of *L. edodes* on 6 DAI

Temperature (°C)	Colony diameter (mm) at 6 days after inoculation		
	LE-01	LE-02	LE-03
15	15.37^{c}	14.00^{b}	17.79^{b}
20	21.81^{b}	28.40^{a}	20.75^{b}
25	35.26^{a}	29.83^{a}	29.04^{a}
30	19.99^{b}	27.55^{a}	19.44^{b}
CD @ 5%	3.577	3.577	3.114
S.Em ±	3.610	3.610	2.735
CV (%)	8.222	7.617	7.602

CD – Critical Difference; S.Em – Standard Error of mean; CV – Coefficient of Variation; Different letters after values are significantly different at $p \leq 0.05$

Table 4: Effect of different temperatures on mycelial growth of *L. edodes* on 12 DAI

Temperature (°C)	Colony diameter (mm) at 12 days after inoculation		
	LE-01	LE-02	LE-03
15	35.51[c]	30.94[c]	32.24[d]
20	57.32[b]	56.09[b]	61.84[b]
25	71.24[a]	68.92[a]	67.81[a]
30	56.21[b]	53.92[b]	57.33[c]
CD @ 5%	3.614	4.303	3.254
S.Em ±	3.685	5.222	2.988
CV (%)	3.486	4.356	3.154

CD – Critical Difference; S.Em – Standard Error of mean; CV – Coefficient of Variation; Different letters after values are significantly different at $p \leq 0.05$

These findings align with previous studies (Quaicoe *et al*., 2014) [21], (Aminuddin *et al*., 2013) [1], (Krupodorova *et al*., 2019) [11], which identified 25 °C as the optimal temperature for *L. edodes* culture. Conversely, Gbolagade *et al*. (2006) [8] suggested a temperature range of 15 °C to 40 °C for *L. subnudus* growth.

Effect of different pH on mycelial growth of *L. edodes*

The effect of different pH levels on the mycelial growth of *L. edodes* was investigated on Potato Dextrose Agar (PDA) medium using three strains (LE-01, LE-02, and LE-03), incubated at 25±2 °C.

The mycelial growth of *L. edodes* was significantly influenced by different pH levels at both 6 and 12 days after inoculation (DAI). At 6 DAI, the highest colony diameters were observed at pH 5 for all three strains (LE-01: 31.36 mm, LE-02: 34.24 mm, LE-03: 30.58 mm), indicating optimal growth under slightly acidic conditions. Following pH 5, pH 6 exhibited the next highest colony diameters across all strains. Conversely, at 12 DAI, pH 5 demonstrated the most conducive environment for mycelial growth, with the largest colony diameters recorded among all pH treatments for all strains (LE-01: 63.92 mm, LE-02: 69.69 mm, LE-03: 58.72 mm). This suggests that while pH 5 initially promoted robust growth, it continued to sustain superior mycelial development over time. In contrast, pH 7 consistently exhibited the lowest colony diameters at both 6 and 12 DAI for all three strains, indicating its limited ability to support *L. edodes* mycelial growth compared to other pH levels (Table 5 and 6).

Table 5: Effect of different pH on mycelial growth of *L. edodes* on 6 DAI

pH	Colony diameter (mm) at 6 days after inoculation		
	LE-01	LE-02	LE-03
4	26.74[b]	29.40[b]	24.53[b]
5	31.36[a]	34.24[a]	30.58[a]
6	27.48[b]	33.09[a]	24.23[b]
7	25.29[b]	26.12[b]	23.78[b]
CD @ 5%	3.685	3.497	3.271
S.Em ±	3.830	3.450	3.017
CV (%)	7.061	6.048	6.738

CD – Critical Difference; S.Em – Standard Error of mean; CV – Coefficient of Variation; Different letters after values are significantly different at $p \leq 0.05$

Table 6: Effect of different pH on mycelial growth of *L. edodes* on 12 DAI

pH	Colony diameter (mm) at 12 days after inoculation		
	LE-01	LE-02	LE-03
4	54.29[b]	55.00[c]	50.71[b]
5	63.92[a]	69.69[a]	58.72[a]
6	62.96[a]	62.05[b]	60.00[a]
7	52.47[b]	55.79[c]	52.85[b]
CD @ 5%	3.872	3.992	3.607
S.Em ±	4.230	4.496	3.670
CV (%)	3.521	3.497	3.447

CD – Critical Difference; S.Em – Standard Error of mean; CV – Coefficient of Variation; Different letters after values are significantly different at $p \leq 0.05$

These results are consistent with earlier research findings (Puri, 2012) [20], identified pH 5.0 as the ideal pH level for the growth of *L. edodes* strains L1 and L2. Similarly, Reddy *et al*. (2017) [22] and Kumar (2018) [13] concluded that the optimal pH range for *L. edodes* growth falls between pH 4.5 and 6.5.

Conclusion

In summary, the study investigated the impact of different media, temperature regimes, and pH levels on the mycelial growth of *L. edodes*. Potato Dextrose Agar demonstrated the highest mycelial growth rate, while Rose Bengal Agar exhibited the lowest. Among the tested media, Potato Dextrose Agar was favored by all three strains, followed by Malt Extract Agar, Sorghum Meal Agar, Saw Dust Extract Agar, and Czapek Dox Agar. Strain LE-02 displayed higher mycelial growth compared to other strains. Regarding temperature, 25 °C facilitated the maximum mycelial growth, whereas 15 °C resulted in the lowest growth. Mycelial growth tended to decrease with deviations from the optimal temperature of 25 °C. In terms of pH levels, pH 5.0 was found to be superior, resulting in the highest mycelial growth compared to pH levels of 4.0, 6.0, and 7.0. These findings highlight the significance of media composition, temperature control, and pH regulation in optimizing the mycelial growth of *L. edodes* under *in-vitro* conditions.

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