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In vitro evaluation for optimum growth conditions of shiitake mushroom (*Lentinula edodes*)

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ABSTRACT

The mushrooms are commercially cultivated for its nutritional and medicinal importance. Among all the cultivated mushrooms, shiitake holds a special place due to its medicinal importance. The current study was undertaken to explore the best media among potato dextrose agar (PDA), sawdust extract agar (SDEA), wheat straw extract agar (WSEA), paddy straw extract agar (PSEA), malt extract agar (MEA), wheat straw extract agar + paddy straw extract agar (WSEA + PSEA) in 1:1, maize straw extract agar (MSEA) and litchi leaf extract agar (LLEA) media. The potato dextrose agar media was found best among all the tested media at 7 and 14 days after inoculation. However, maize straw extract agar and paddy straw extract agar were the least effective media at 7 and 14 DAI. The 25°C temperature was most preferred among various temperature regimes of 15°C, 20°C, 25°C and 30°C while 15°C was least preferred temperature for mycelial proliferation of the shiitake.

Keywords: Culture media, culture maintenance, shiitake, temperature

The mushrooms are being cultivated from centuries for its nutritional and medicinal attributes. The mushroom is a macrofungus with a prominent basidiocarp, which are easily visible with naked eyes (Chang and Miles, 1992) and it comes under either basidiomycota or ascomycota. Mushrooms are highly nutritious as well as possess therapeutic properties (Gupta *et al.,* 2018). The five mushroom genera namely *Agaricus bisporus, Pleurotus* spp., *Lentinula edodes*, *Auricularia* and *Flammulina* are contributing to the 75 % of the world's total mushroom production. *Lentinula edodes* is commonly known as shiitake, ranked second among cultivated edible mushroom in the world (Royse, 2001). It originated in the East Asia and well known for its pleasant flavor, medicinal and nutritional importance. Shiitake possess good amount of vitamins like B_1, B_2, B_3, D, phosphorus, calcium, magnesium and essential amino acids. It is commonly cultivated on the wood logs and research is going on alternative substrates for its cultivation (Annepu *et al.,* 2018, 2019). This method of cultivation on wood logs does not meet the expected demand due to limited supply of hardwoods and long spawn-run period. In India, much work has not been done on this mushroom and hence there is a need for alternative substrate for its cultivation to meet the market demand. The present research work was carried out *in vitro* to evaluate the effect of various media and temperature for its faster mycelial proliferation.

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MATERIALS AND METHODS

Source of culture and its maintenance

The present research work was carried out at Advance Centre of Mushroom Research, Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar. The five strains of shiitake namely LE-1501, LE-1502, LE-1503, LE-1504 and LE-1505 were obtained from the above mushroom center. These shiitake strains were maintained on basal media potato dextrose agar and kept in refrigerator for further use.

Screening of various culture media

The various culture media viz., potato dextrose agar (PDA), sawdust extract agar (SDEA), wheat straw extract agar (WSEA), paddy straw extract agar (PSEA), malt extract agar (MEA), wheat straw extract agar + paddy straw extract agar (WSEA + PSEA) in 1:1, maize straw extract agar (MSEA) and litchi leaf extract agar (LLEA) media were prepared. The Petri plates were poured with 20 ml of the media and 5 mm disk of seven days old culture of *L. edodes* were placed in center of the Petri plates. The Petri plates were incubated at 25±1°C in triplicates. The colony diameter was recorded at 7 and 14 days after inoculation.

Effect of various temperature regimes

The study was carried out in PDA media and PDA was poured @ 20 ml/Petri plates and 7 days old culture of *L. edodes* was inoculated. The plates were incubated at different temperature regimes namely 15°C, 20°C, 25°C and 30°C. The colony diameters were recorded at 7 and 14 days after inoculation.

RESULTS AND DISCUSSION

Screening of various culture media

The data shown in table 1, table 2 and fig. 1 clearly revealed that potato dextrose agar was most preferred media for the maximum mycelial proliferation of all the strains followed by malt extract agar and sawdust extract agar both at 7 and 14 DAI. At 7 DAI, the maximum colony diameter of 39.07 mm was recorded with potato dextrose agar medium which was followed by 38.80 mm on malt extract agar, 35.53 mm on sawdust extract agar, 34.60 mm on wheat straw extract agar, 33.27 mm on wheat straw extract agar

Table 1. Evaluation of different media for mycelial growth of *L. edodes* strains at 7 DAI

Media	Colony diameter (mm) at 7 DAI					Mean
	LE-l501	LE-l502	LE-l503	LE-l504	LE-l505	
SDEA	35.00	34.00	33.33	40.00	35.33	35.53
PDA	42.67	44.33	40.00	34.00	34.33	39.07
WSEA	40.33	35.00	35.00	30.33	32.33	34.60
MEA	41.00	42.67	41.67	34.67	34.00	38.80
WSEA+PSEA	39.00	30.33	33.33	30.00	33.67	33.27
MSEA	33.33	33.33	31.00	31.33	30.67	31.93
PSEA	30.67	32.67	31.67	32.67	32.67	32.07
LLEA	38.00	35.00	30.67	30.67	31.33	33.13
Mean	37.50	35.92	34.58	32.96	33.04	
	Media (M)		Strains (S)		M × S	
CD (5%)	1.32		1.04		2.96	
SE(m)±	0.47		0.37		1.05	

Table 2. Evaluation of different media for mycelial growth of *L. edodes* strains at 14 DAI

Media	Colony diameter (mm) at 14 DAI					Mean
	LE-l501	LE-l502	LE-l503	LE-l504	LE-l505	
SDEA	81.00	83.00	82.33	81.00	80.00	81.47
PDA	86.67	90.00	86.33	84.33	84.00	86.27
WSEA	80.33	75.00	70.67	75.00	71.67	74.53
MEA	83.00	88.67	85.67	83.33	81.67	84.47
WSEA+PSEA	72.00	68.00	69.00	71.00	66.67	69.33
MSEA	77.67	79.33	75.00	75.67	69.00	75.33
PSEA	62.00	68.33	60.33	70.33	66.33	65.47
LLEA	73.67	69.00	64.67	67.67	64.67	67.93
Mean	77.04	77.67	74.25	76.04	73.00	
	Media (M)		Strains (S)		M × S	
CD (5%)	0.87		0.69		1.96	
SE(m)±	0.31		0.25		0.69	

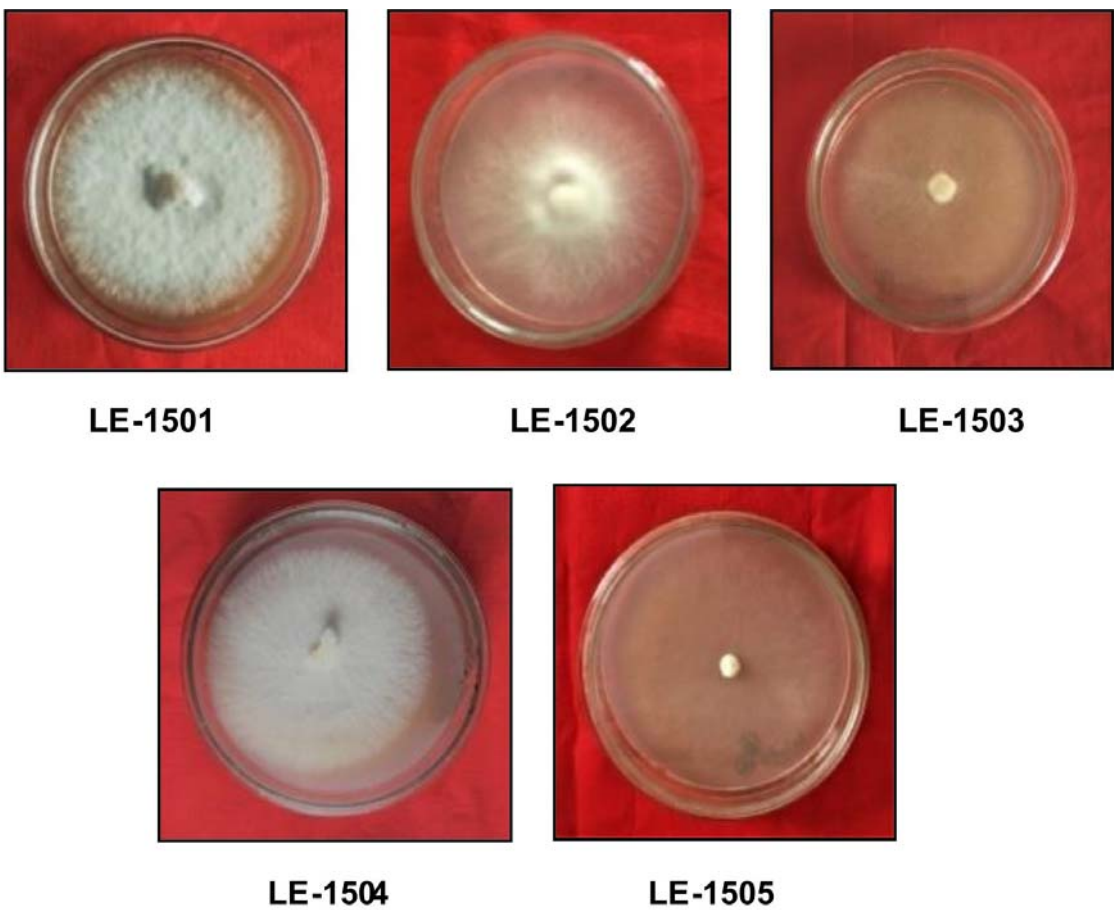

Fig. 1. Growth of *Lentinula edodes* strains on potato dextrose agar medium at 14 DAI

+ paddy straw extract agar, 33.13 mm on litchi leaf extract agar and 32.07 mm on paddy straw extract agar medium. The least effective solid medium was maize straw extract agar having colony diameter of only 28.87 mm. However, at 14 DAI the highest colony diameter of 86.27 mm was observed on potato dextrose agar medium. This was followed by 84.47 mm on malt extract agar, 81.47 mm, 75.33 mm, 74.53 mm, 69.33 mm and 67.93 mm on sawdust extract agar, maize straw extract agar, wheat straw extract agar, wheat straw extract agar + paddy straw extract agar and litchi leaf extract agar medium while the least mycelial growth of 65.47 mm was recorded on paddy straw extract agar medium.

These results are also reported by the earlier worker Kalmis and Kalyoncu (2006) who observed variation in the mycelial growth of *L. edodes* on different media and potato dextrose agar media was best among all the tested media. Bilay *et al.* (2000) found malt extract agar media as most favoured media for growth of various medicinal mushrooms. These variations in mycelial growth of *L. edodes* on various media are due to the presence of a specific nutrient in a particular media.

Effect of various temperature regimes

The statistical analysis of the data presented in table 3 and table 4 clearly showed that 25°C was the

Table 3. Evaluation of temperature for mycelial growth of *L. edodes* strains at 7 DAI

Temperature	Colony diameter (mm) at 7 DAI					Mean
	LE-l501	LE-l502	LE-l503	LE-l504	LE-l505	
15±1 °C	29.67	24.33	29.00	28.00	20.67	26.33
20±1°C	48.33	51.00	45.00	49.00	47.00	48.07
25±1°C	62.33	64.67	57.00	60.67	58.00	60.53
30±1°C	42.67	44.67	43.67	46.33	42.67	44.00
Mean	45.75	46.17	43.67	46.00	42.08	
	Temperature (T)		Strains (S)		T × S	
CD (5%)	1.02		1.14		2.28	
SE(m)±	0.36		0.40		0.80	

Table 4. Evaluation of temperature for mycelial growth of *L. edodes* strains at 14 DAI

Temperature	Colony diameter (mm) at 14 DAI					Mean
	LE-l501	LE-l502	LE-l503	LE-l504	LE-l505	
15±1 °C	38.00	44.33	35.00	36.00	37.00	38.07
20±1°C	64.33	75.00	66.33	65.67	67.33	67.73
25±1°C	78.67	90.00	85.33	82.00	84.33	84.07
30±1°C	65.33	67.33	62.33	65.67	64.33	65.00
Mean	61.58	69.17	62.25	62.33	63.25	
	Temperature (T)		Strains (S)		T × S	
CD (5%)	0.92		1.02		2.04	
SE(m)±	0.32		0.36		0.71	

optimum temperature for maximum proliferation of all the strains followed by 20°C, 30°C and 15°C temperature. The average colony diameter of 60.53 mm was observed at 25°C followed by 48.07 mm, 44.00 mm and 26.33 mm at 20°C, 30°C and 15°C, respectively at 7 DAI. However, at 14 DAI the colony diameter of 84.07 mm followed by 67.73 mm, 65.00 mm and 38.07 mm were observed at 25°C, 20°C, 30°C and 15°C respectively. However, the growth of the isolates decreased with either increase or decrease in the temperature from 25°C. These results are in line with the findings of Imtiaj *et al.* (2008) who reported 25ºC was the most ideal temperature among the five different temperatures (15ºC, 20ºC, 25ºC, 30ºC and 35ºC) for the growth of *L. edodes*. Zagrean *et al*. (2017) studied the effect of various temperatures such as 18°C, 24°C and 30°C and observed that the temperature range of 24 - 30°C was most favourable for the maximum mycelial colonization of *Pleurotus eryngii* and *L. edodes* strains. Quaicoe *et al*. (2014) also conducted a similar study at four different temperatures namely 16ºC, 20ºC, 25ºC and 30ºC and showed that 25ºC was the optimum temperature for the growth of *L. edodes* strains.

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