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Studies on culture media preference of three *Lentinus edodes* strains

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Abstract
Lentinula edodes is known as the "Shiitake mushroom" and is one of the most cultivated mushroom species in the world. Three strains of *Lentinus edodes* namely DMR-327, Le-18-01 and Le-18-02 were evaluated for culture media preference studies. Different synthetic culture media *viz*. Malt extract agar (MEA), Potato dextrose agar (PDA), Oatmeal agar (OMA), and agro residues extract media namely Sawdust dextrose agar (SDA) and Walnut dextrose agar (WDA) were investigated for the effective colony diameter of three strains of Shiitake mushroom [*Lentinus edodes* (Berk) Pegler]. Strain DMR-327 exhibited significantly higher mycelial growth of 82.6 mm with a growth rate of 6.36 mm/day in walnut dextrose agar (WDA) media. The lowest mycelial colonization (39.50 mm) and growth rate (3.04 mm/day) were recorded in sawdust agar media. The other strain Le-18-01 colonized faster (85.73 mm) with a growth rate (6.59 mm/day) in walnut dextrose agar (WDA) media with the whitish colony and thin mycelial strands whereas slower colonization (57.30 mm) and growth rate (4.41 mm/day) was recorded in potato dextrose agar media with white thickish growth. Third strain Le-18-02 exhibited the highest colony diameter of 85.52 mm and growth rate (6.58 mm/day) in walnut dextrose media (WDA) with whitish thin mycelium and the least colony diameter (60.90 mm) and growth rate (4.64 mm/day) potato dextrose agar media (PDA).

Keywords: *Lentinus edodes*, culture media, mycelial colonization, growth rate

Introduction
Mushrooms constitute one of the major groups of Kingdom Fungi that are chlorophyll deficient and derive nutrition as saprophytes from lignocellulosic materials. In India, commonly cultivated species include white button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* spp.), paddy-straw mushroom (*Volvariella volvacea*) and white milky mushroom (*Calocybe indica*) (Tewari, 2004) [11].
Mushrooms are considered not only as spice and food ingredients but also as a nutritional supplement in the human diet which also plays the role of functional foods (Drewnowska *et al*., 2012) [3]. Mushroom cultivation is an eco-friendly method of solid waste management. It is evident that mushroom cultivation helps in the biological degradation of natural resources and is an eco-friendly, protein-rich food and the recent developments in the scientific understanding of mushroom cultivation have aided in the improvement of its cultivation technology (Puri, 2011) [8]. Mushrooms are nutritionally a very good food and physiologically an important potential source of biologically active compounds of medicinal value much more recent (Chang, 1996) [2].
Lentinula edodes is known as the "Shiitake mushroom" and is one of the most cultivated mushroom species in the world. World total mushroom production is 8,993,280 or 89.932 lakh tons and the ranking is as follows: China with 6,664,606 or 66.460 lakh tons, the USA with 4, 160, 50 or 4.160 lakh tons, the Netherlands 30,00,00 or 3 lakh tons, Poland with 28,02,32 or 2.80 lakh tons and Spain 16,62,50 or 1.66 lakh tons (Anonymous, 2018) [1]. Li *et al*. (2019) [5] emphasized that 70% of the shiitake mushroom production has been provided by China.
Shiitake mushrooms contribute around 22% (approximately seven billion kg) of the five main cultivated mushrooms which contribute around 85% of the total World‟s mushroom supply (Royse *et al*., 2017) [10]. *Pleurotus* sp., mushrooms with a wide variety placed second contributing about 19% of the World‟s production.
According to 2021-22 estimates, the overall mushroom production of India accounts for 242,85,000 tonnes. Bihar state produces 2,80,00 tonnes of mushrooms annually with a major share of 10.82% of total Indian production.

Union Territory of Jammu and Kashmir accounts for 2,65,000 tonnes of mushroom production and a total share of 1.02% of India's total production (Anonymous, 2021) [1].

Synthetic substrates include sawdust of hardwoods. This methodology lends itself to faster and greater productivity by mixing the spawn thoroughly with the substrate which produces more flush of mushrooms in much shorter growing cycles (Royse, 2004; Oei, 2003) [9, 6].

Different agricultural wastes have been investigated in studies on shiitake mushroom cultivation, such as hazelnut husk-wheat straw-wheat bran-beech sawdust, hazelnut husk- wheat straw-beech wood chip-wheat bran mixtures, wheat straw-corn cobs-oak wood sawdust mixtures (Philippoussis *et al*., 2007), cocoa husk- cotton waste-oak sawdust-wheat bran mixtures (Escobar *et al*., 2007),

Materials and Methods

Five different media were used to evaluate the colony diameter of three strains of *L.edodes.* Studies were conducted on different media *i.e.*Walnut shells dextrose extract agar, Sawdust agar (SDA), Oatmeal agar (OMA), Malt extract agar (MEA), and Potato dextrose agar (PDA). Some media are available as synthetic media and are prepared as per the recommended compositions and some of them were prepared using raw ingredients.

Preparation of Agro-Residue Extract Media

Different agro-residues like sawdust and walnut shells were used for the media evaluation studies of *Lentinus edodes.* The medium was prepared by boiling the sawdust or walnut shells in the water for 20 minutes and filtered through a cheese cloth. Dextrose (20 grams) and agar-agar (20grams) were added to the filtrate and boiled the filtrate to avoid clod formation by agar-agar. The filtrate is finally made to 1000 ml by adding distilled water followed by sterilizing the media at 121ºC,15 lbs psi for 20 minutes. The Petri plates were poured with the above-prepared media and inoculated with 5mm discs of *Lentinus edodes* and incubated at 25±1 ºC in BOD. The radial growth of the mycelium was recorded at an interval of 5 DAI.

Results and Discussion

Three strains namely DMR-327, Le-18-01, and Le-18-02 were evaluated on five different media *viz*., Walnut dextrose agar, Oatmeal agar, malt extract agar, Sawdust agar, and Potato dextrose agar (PDA). The media includes both synthetic and agro-waste residue media.

Results from Table 1 and Plate 1, unveiled that the strain DMR-327 exhibited significantly higher mycelial growth of 82.6 mm and growth rate of 6.36 mm/day in WDA followed by OMA (80.6 mm) (6.20 mm/day), PDA (79.00 mm) (6.07mm/day) and SDA (71.60 mm) (5.51 mm/day). However, the lowest mycelial colonization and growth rate of 39.50 mm and 3.04 mm/day respectively were recorded in Sawdust agar media (SDA). Walnut dextrose agar (WDA) and Sawdust agar media (SDA) exhibited whitish thin mycelial strands. Moderate mycelial growth was observed in the Sawdust agar medium (SDA). Whereas the profuse thick whitish mycelial colonization was observed in Oatmeal agar media (OMA) and Potato dextrose agar media (PDA).

In the case of strain Le-18-01, significant results were observed as mentioned in Table 1. The faster colonization (85.73 mm) and growth rate (6.59 mm/day) in walnut dextrose agar media with the whitish colony and thin mycelial strands and least mycelial colonization (57.30 mm) and growth rate (4.41 mm/day) were recorded in potato dextrose agar media with white thickish growth. The remaining media *viz.*, oatmeal agar (67.50 mm) and growth rate (5.19 mm/day), malt extract agar (64.66 mm)(4.97 mm/day) and sawdust agar (73.60 mm) (5.66 mm/day) significantly varied in both colony diameter, growth rate per day and type of mycelium.

Likewise in strain Le-18-02, the mycelial colonization of 85.52 mm and growth rate (6.58 mm/day) were observed significantly higher in walnut dextrose media with whitish thin mycelium followed by malt extract agar (77.04 mm) (5.99 mm/day), sawdust agar media (71.96 mm) (5.54 mm/day). The mycelium type is whitish and thin in the case of sawdust agar media and moderately thick in malt extract agar media. Mycelial colonisation and growth rate are significantly on par in the case of oatmeal agar (61.73 mm) (4.75 mm/day) and potato dextrose agar media (60.90 mm) (4.64 mm/day). A graphical representation of the present study is depicted in Fig.1. The mycelium type is similar *i.e.* thick and whitish in Oatmeal agar and potato dextrose agar medium.

The mycelial growth depends mainly on genetic parameters, nutrition and growing conditions like temperature, pH etc., Culture media helps in the growth and multiplication of fungi. The cellulose and hemicellulose present in the agro-residue extracts along with sugar support the fungal growth.

Similar results were also recorded in the findings of Kalmis and Kalyoncu (2006) [4] who evaluated different media for mycelial growth of *Lentinus edodes* and found potato dextrose agar media as the best culture media.

The studies of Paswal (2019) [7] postulated that sawdust extract agar media is a suitable media among the five different media namely sawdust extract agar, malt extract agar, potato dextrose agar, wheat straw extract agar and yeast potato dextrose agar for the growth of L. edodes. In another study from the literature, Bilay *et al*., (2000) reported a common suitable media for medicinal mushrooms as malt extract agar media. The results of the present study reported negative results with the literature mentioned.

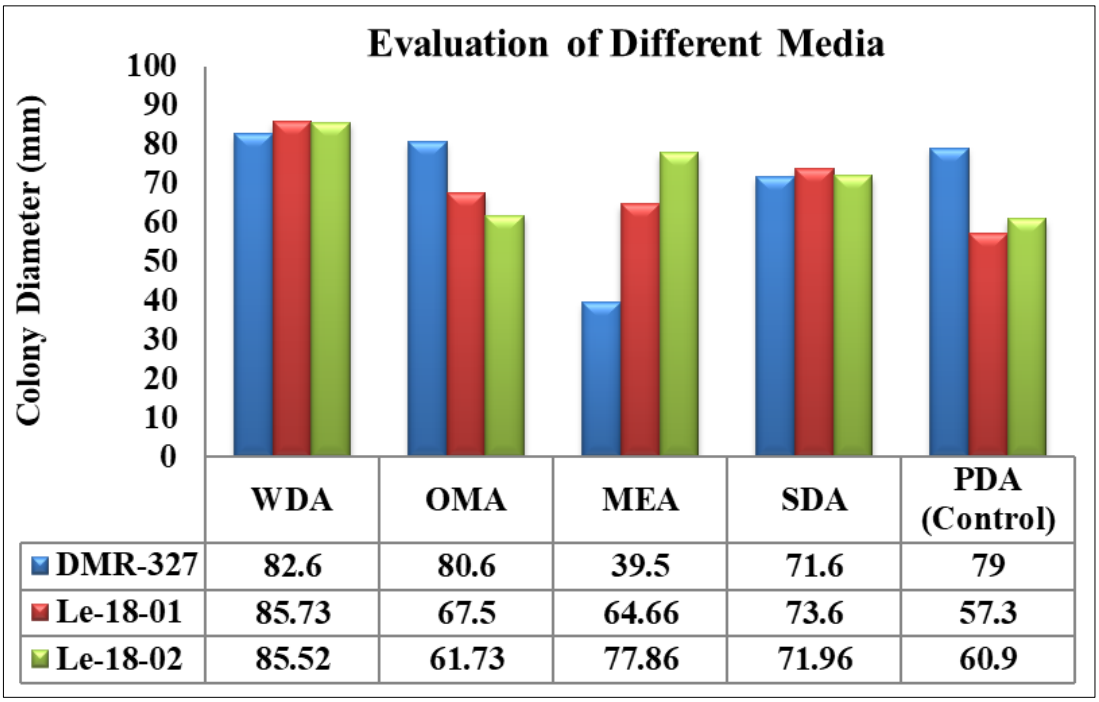

Fig 1: Comparative evaluation of different media for mycelial growth of different strains of *Lentinus edodes*

Table 1: Evaluation of different media on mycelial growth of *Lentinus edodes*

S. No.	Media	Colony Diameter of Strains (mm)			Growth Rate (mm/ day)		
		DMR-327	Le-18-01	Le-18-02	DMR-327	Le-18-01	Le-18-02
1.	Walnut dextrose agar	82.6	85.73	85.52	6.36	6.59	6.58
2.	Oatmeal agar	80.6	67.5	61.73	6.20	5.19	4.75
3.	Malt extract agar	39.5	64.66	77.86	3.04	4.97	5.99
4.	Sawdust agar	71.6	73.6	71.96	5.51	5.7	5.54
5.	Potato dextrose agar (Control)	79	57.3	60.9	6.07	4.41	4.68
	CD (p<0.05)	3.63	2.00	1.76			
	SE (mean)	1.14	0.63	0.55			

*mean of 3 replications

Plate 1: Evaluation of different media on radial growth of *Lentinus edodes* (DMR-327)

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