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Evaluation of different culture media for *in vitro* mycelial growth of different strains of shiitake mushroom [*Lentinula edodes* (Berk.) Pegler]

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Abstract
The study was conducted to evaluate different solid/liquid media for *in vitro* mycelia growth of *L. edodes* strains. Three strain of *L. edodes viz*., DMR-356, DMR-35 and DMR- 410 were evaluated on solid culture media *viz*., potato dextrose agar, malt extract agar, yeast potato dextrose agar, wheat straw extract agar and poplar sawdust extract agar. Maximum radial growth (41.33mm) was observed in poplar sawdust extract agar with DMR-356 strain while as minimum radial growth(10.33 mm) of DMR-356 was observed in malt extract agar after 14 days of inoculation. Similar trend was observed with DMR-35 and DMR-410 strain where maximum mycelial growth (40.00 mm), (39.00 mm) was observed in poplar sawdust extract agar however minimum mycelium growth (4.33mm), (4.00mm) was obtained from malt extract agar. Three liquid media *viz*., Potato dextrose broth, Wheat straw extract broth and poplar sawdust extract broth were evaluated for mycelial biomass production of *L. edodes* strains. In case of DMR-356, highest fresh mycelium weight (6.02g) and dry mycelium weight (1.67g) was found from Poplar sawdust extract broth however the lowest fresh mycelium weight (3.43g) and dry mycelium weight (0.51g) was observed on potato dextrose broth. Similar trend was observed in case of DMR-35 and DMR- 410 where maximum fresh and dry mycelium weight was observed in poplar sawdust extract broth and minimum fresh and dry mycelium weight was found on potato dextrose broth.

Keywords: *Lentinula edodes* strains, liquid media, mycelia growth, solid media

Introduction
The mushroom *Lentinula edoes* (Berk.) Pegler, popularly known as Shiitake is a lignolytic, aerobic basidiomycete (Chen, 2005, minhoni *et al*., 2007) [5, 11]. The *in vitro* cultivation of *L. edodes* seeks to elucidate the optimal growth conditions for the fungus with regard to culture media, temperature, and incubation times (Hatvani, 2001) [9]. During a given period of time the fungus mycelia growth can be represented as a typical sigmodial curve, including several stages with distinctive physiological properties (Montini, 2006) [12]. Mycelial growth can be measured in different ways such as through radial growth, vigor, growth velocity and mycelia mass. Under experimental conditions the use of solid culture medium to evaluate fungal growth is considered adequate since in nature fungi normally develop on solid substrates, such as plant and animal residues or in the soil (Griffin, 1994) [8]. Production of liquid inoculums of shiitake is also an alternative to the spawn used in commercial mushroom production. The advantages of liquid inoculum are its uniform distribution in the substrate and growth time reduction (Song *et al.*, 1987) [13] In Brazil the most frequently used substrate for *L. edodes* commercial cultivation consists of *Eucalyptus* spp. Log or sawdust. Hence based on *in vitro L. edodes* mycelia growth evaluation in different solid and liquid culture media. It is possible to determine suitable type of solid and liquid media for development by stimulating the natural cultivation conditions of the fungus. Therefore the objective of this work was to evaluate the different solid and liquid media for mycelia growth of *L. edodes* strains-DMR-356, DMR-35 and DMR-410 and to assess critically the varied approaches published recently to establish convenient and reliable *in vitro* cultivation conditions for mycelia of the *L. edodes* strains as a source of inoculum. Which could also prove useful for commercial inoculum production.

Material and Methods
The experiment was conducted at spawn lab of the department of plant pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences & Technology, Chatha, Jammu.

Procurement and maintenance of culture

The pure culture of *Lentinula edodes* (berk.) Pegler. DMR-356, DMR-35 and DMR-410 used in the present investigation was procured from Directorate of Mushroom Research, Chambaghat, Solan. These pure cultures were multiplied further in slants on potato dextrose agar (PDA) medium. The slant cultures were stored at ambient temperature (25±2 ℃) for 7 days; after that the slants were kept in a refrigerator at 4 ± 2 °C.

***In-vitro* evaluation of mycelial growth of *Lentinula edodes* on different media**

During the present investigation five solid agar media *viz.*, Potato dextrose agar, Malt extract agar, Yeast potato dextrose agar, Wheat straw extract agar and Sawdust extract agar, and three liquid broth media *viz.*, Potato dextrose broth, Wheat straw extract broth and Sawdust extract broth were tested for the evaluation of mycelia biomass production of *L. edodes.*

The constituents of the solid agar media are given as under:

i. Potato Dextrose Agar (PDA) medium

Potato (Peeled and sliced)	200 g
Dextrose	20 g
Agar-agar	20 g
Distilled water	1000 ml

ii. Malt Extract Agar (MEA) medium

Malt extract	20 g
Agar-agar	20 g
Distilled water	1000 ml

iii. Yeast Potato Dextrose Agar (YPDA) medium

Yeast	20g
Potato (peeled and sliced)	200g
Dextrose	20g
Agar-agar	20g
Distilled water	1000ml

iv. Wheat Straw Extract Agar (WSEA) medium

Wheat straw	200g
Dextrose	20g
Agar-agar	20g
Distilled water	1000ml

v. Sawdust Extract Agar (SEDA) medium

Paddy straw	200g
Dextrose	20g
Agar-agar	20g
Distilled water	1000ml

Preparation of media

All the above mentioned media were prepared by weighing the prescribed quantities of the ingredients in to the distilled water in order to make the requisite quantity of each medium (Aneja, 2002) [3]. In case of wheat straw extract agar, wheat straw (200g) was boiled in a container using 500 ml of distilled water for 45 minutes. The extract of wheat straw was strained through double layered muslin cloth and the extract was kept in a beaker separately. Twenty grams of agar-agar was boiled in 500 ml of water followed by the addition of 20 g of dextrose. Wheat straw extract and agar liquid were mixed together with constant stirring using a glass rod and the contents were made up to 1000 ml in a conical flask by adding more distilled water (Singh *et al.*, 2009). Same procedure was followed for the preparation of sawdust extract agar medium. Media thus prepared were sterilized in the autoclave at 15 psi for 20 minutes.

Pouring of media in Petri plates

Sterilized petri plates were used for pouring the medium. A set of petri plates were maintained for each treatment and 20 ml of each medium was aseptically poured in each sterilized petri plate. The medium in the plates was allowed to solidify before inoculating with *L. edodes* Strains.

Radial growth of *Lentinula edodes* strains on solid media

Five solid media *viz*. potato dextrose agar, wheat extract agar, malt extract agar, sawdust extract agar and yeast extract agar were selected to find the suitable medium for maximum radial growth of *L. edodes* strains. Twenty milliliter of each medium was aseptically poured in set of sterilized petri plates. The plates were inoculated with 10 mm disc of actively growing mycelium of *L. edodes*. Thereafter, plates were incubated at 25±1 ºC for incubation in a B.O.D incubator and the observations on radial growth and morphological characters were recorded when the growth of *L. edodes* strains completely covered the plates in any of the treatments after 14 days of incubation. Each treatment was replicated thrice under CRD factorial design.

Growth of *Lentinula edodes* strains in liquid media

Three liquid broth media *viz*., potato dextrose broth, wheat extract broth and sawdust extract broth were studied to find out the suitable medium for yielding maximum biomass of *L. edodes*. In a 100 ml conical flask, 50 ml of selected medium (potato dextrose broth, wheat extract broth and sawdust extract broth) was sterilized in an autoclave. The media were then inoculated with 10 mm mycelial disc of actively growing *L. edodes* strains individually and incubated at 25±1ºC for 35 days. Thereafter, the broth was filtered and mycelial mat was collected on Whatman filter paper No.1 and fresh weight of mycelium was recorded on electronic top pan balance. The mycelial mat was dried in an oven at 60 ºC and its dry weight was recorded as given below (Arey, 2010) [4].

Weight of mycelium = (Weight of filter paper + Weight of Mycelium) – (Weight of filter paper)

Statistical analysis: All the data of laboratory experiments were statistically analyzed using factorial CRD (completely randomized design).

Results and Discussion

The mycelia growth results for the *L.edodes* strains after cultivation of 14 days in the culture media. *In- vitro* growth of the *L. edodes* seeks to elucidate the optimal growth conditions for the fungus with regard to culture media, temperature and incubation times (Andrade *et al*., 2008) [2]. Media, temperature and pH plays an important role mycelial colony proliferation of mushroom (Amita and Atri, 2017) [1]. Quite a large number of experiments have been carried out globally to find out the suitable media for growth of mushroom. Several factors such as quality of spawn, culture media, temperature, pH, moisture content and light intensity are reported to alter the mushroom growth (Kadiri and Kehinde, 1999) [10]. Considering obvious prospective, the present experiments were designed to ascertain the effect of solid and liquid media for the growth as well as mycelial biomass of the *L. edodes* strains under *in-vitro* conditions.

In-vitro evaluation for mycelia growth of *L. edodes* on solid media

In the present study, the composition of different media had significant effect on the overall mycelia growth of different *L. edodes* strains presented in Table 1. And plate-1. Among the five solid media (potato dextrose agar, malt extract agar, yeast potato dextrose agar, wheat straw extract agar and sawdust extract agar) evaluated, sawdust extract agar showed the maximum mycelia growth for DMR-356 strain (41.33 mm), DMR-35 strain (40.00 mm) and DMR-410 strain (39.00 mm) after 14 days of incubation. Malt extract agar proved to be least effective media having mycelia growth of 10.33, 4.33 and 4.00 mm for DMR-356, DMR-35 and DMR-410, respectively, after 14 days of incubation at 23±1ºC. The above finding are in agreement to the finding of Andrade *et al*. (2008) [2] who reported that the culture medium prepared from extract of *Eucalyptu citriodora* recorded the fastest mycelia growth of 53.58 and 61.40 mm for LE-95/01 and LE96/18, respectively, after ten days of incubation at 25±1ºC, which may be due to some specific nutrients present in the medium. There were significant differences for the interaction between culture media and *L. edodes* strains. These results are in accordance with Donini *et al*. (2005) who also observed the variation in the mycelia growth of *Pleurotus* spp.

Table 1: *In- vitro* evaluation of different solid media on the mycelial growth (mm) of *Lentinula edodes* strains

Strains	Radial growth(mm) on solid media					
	*PDA	MEA	YPDA	WSEA	SDEA	Mean
DMR-356	15.00	10.33	13.00	34.66	41.33	22.86
DMR-35	14.00	4.33	8.33	32.00	40.00	19.73
DM- 410	12.60	4.00	6.66	18.66	39.00	16.18
Mean	13.89	6.22	9.33	28.44	40.11	
C.D ($p \leq 0.05$)	Strains = 0.89 Media = 1.15 Strains × Media = 2.00					
S.Em (±)	Strains = 0.31 Media = 0.40 Strains × Media = 0.69					

*PDA=Potato dextrose agar, MEA=Malt extract agar, YPDA=Yeast potato dextrose Agar, WSEA=Wheat straw extract agar, SDEA=Sawdust extract agar

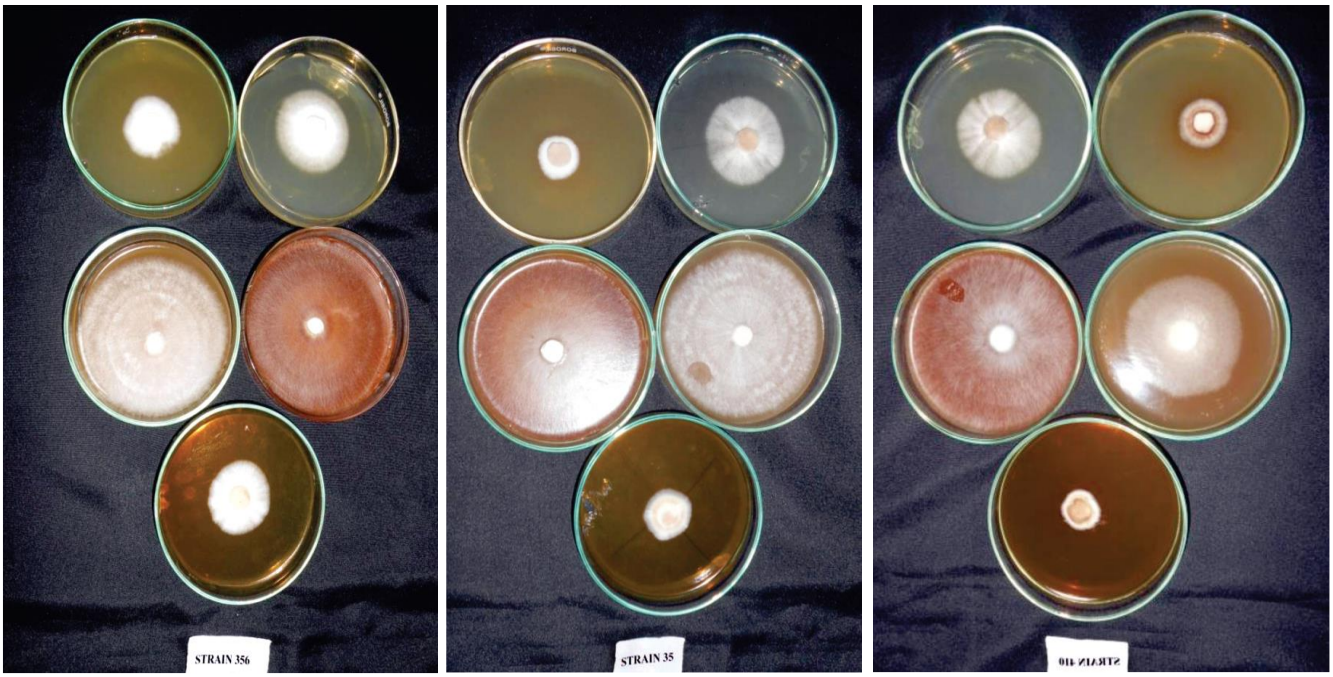

Plate 1: *In-vitro* effect of different solid media on the mycelial growth (mm) of *L. edodes* strains a) Potato Dextrose Agar (PDA), b) Malt Extract Agar(MEA), c) Wheat Straw Extract Agar (WSEA), d) Sawdust Extract Agar(SDEA) and e) Yeast Potato Dextrose Agar (YPDA)

In-vitro evaluation for mycelia growth of *L. edodes* on liquid media

To standardize the bio-mass production of *L. edodes*, on different liquid media *viz*., potato dextrose broth (PDB), wheat straw extract broth (WSEB) and sawdust extract broth (SDEB) were evaluated and depicted in fig 1. Fresh and dry mycelial weight of *L. edodes* differed significantly on different liquid culture media and strains selected. Maximum fresh and dry mycelium weight of 6.02 g and 1.67g was recorded in DMR-356 strain after 42 days of incubation period on sawdust extract broth while minimum fresh and dry mycelium weight of 3.43g and 0.51g was observed on potato dextrose broth. In case of DMR-35 strain, fresh and dry mycelium weight of 4.50g and 0.99g was recorded on sawdust extract broth, while minimum fresh and dry mycelium weight of 2.84g and 0.21g was observed on potato dextrose broth. Similarly, maximum fresh and dry mycelium weight of 3.69 g and 0.86g was recorded on sawdust extract broth while minimum fresh and mycelium weight of 1.44g and 0.05g was observed on potato dextrose broth for DMR-410. Earlier experiment also supports our findings in which maximum fresh and dry mycelial weight (13.12 and 0.53g) of *L. edodes* (American strain) was recorded on wheat extract broth (Dewangan, 2005) [6]. Variation in mycelial biomass on different media have also been observed in earlier studies of Gapinski *et al*. (2007) [7] who reported that the variation in the mass of dry mycelium grown on liquid medium depended on the medium composition.

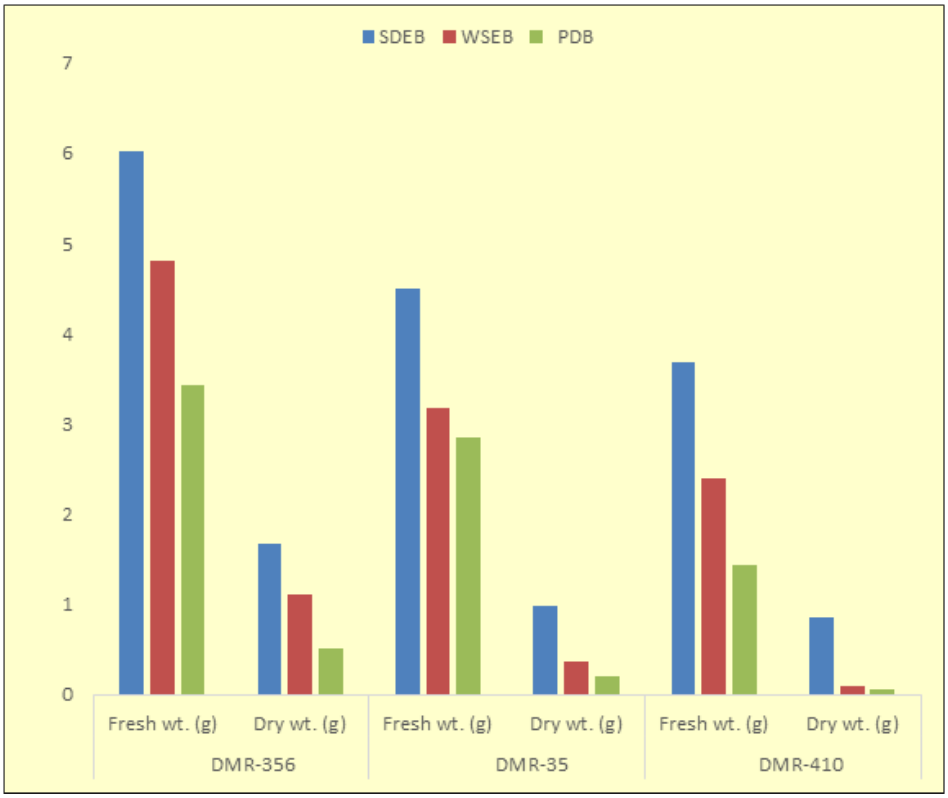

Fig 1: Effect of different liquid media on mycelial biomass (g) of *Lentinula edodes* strains

Conclusion

The results obtained in this study will be useful for evaluating solid and liquid media to enhance *L. edodes* mycelia biomass production and reduction time for production.

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