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In vitro Evaluation of Different Nutritive Media for Mycelia Growth of *Pleurotus eryngii* (DC ex Fr.) Quel

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***Abstract*:** The study was conducted to evaluate the effect of different solid and liquid media for the mycelial growth, cultural characteristics and growth rate of *Pleurotus eryngii*. Five different solid media *viz.,* Potato dextrose agar (PDA), Carrot extract agar (CEA), Malt extract agar (MEA), Asthana and Hawker's agar (A&HA) and Czapek Dox Agar (CDA) as well as their broths were evaluated *in vitro* to find out the best nutritive media for the optimum mycelial growth of *Pleurotus eryngii*. Maximum diametric growth (56.27 mm) was in Potato dextrose agar followed by Malt extract agar (48.58 mm) and Asthana and Hawker's agar (41.84 mm). In liquid media, highest biomass (467.50 mg) was in Potato dextrose broth followed by Carrot extract broth (228.33 mg) and Malt extract broth (186.67 mg). The mycelial growth in different media was white, cottony growth having concentric rings or ray like pattern whereas, in Asthana and Hawker's as well as Czapek Dox agar, the mycelial growth was transparent white.

***Keywords*:** *Pleurotus eryngii*, Mycelial growth, Nutrient media, Mushrooms

Among all the species of mushroom grown in the world, oyster mushrooms represent the third largest group of edible mushrooms. *Pleurotus* species are considered as a good source of protein, fibre, carbohydrates, vitamins and minerals, and are low in calories, fats and sodium content (Cohen *et al.,* 2002). Among commercially cultivated species of oyster, King oyster mushroom (*Pleurotus eryngii*) belongs to phylum Basidiomycota, order Agaricales, family *Pleurotaceae*, genus *Pleurotus* and species *eryngii* (Kang 2004). Due to the excellent consistency of the cap and stem, cooking qualities and longest shelf life, this mushroom is regarded as one of the best among all the species of *Pleurotus*. The longer shelf life is due to the less content of water and firm flesh of the sporocarp (Moonmoon et al., 2010; Yildiz et al., 2002). It is commonly known as 'Afghani dhingri' or 'King trumpet mushroom' as it forms the largest fruiting bodies among the oyster species. Also, as its fruit bodies taste like almond and abalone; it is known as 'Almond abalone mushroom'. This species is characterized by its cream to ochraceous brown fruiting bodies with flabelliform to depressed pileus and eccentric to lateral stipe. Every organism needs nutrients as a source of energy and certain environmental conditions for its growth and development. For successful cultivation of mushrooms, growth medium plays a very vital role because it provides necessary nutrients for proper growth of mycelium. The first vital stage towards the success of spawn production is the maintenance and revival of pure culture mycelium with splendid quality (Kumar et al., 2018). All the microorganisms require a set of conditions under which they can flourish and sporulate best where culture medium is the major factor which influences the fungal cultivation (Dhingra and Sinclair 2014). In India, the cultivation of this mushroom is yet to be explored. Therefore, the present study was aimed out to determine the best nutritive media for *in vitro* cultivation of *Pleurotus eryngii* under subtropical zone of Himachal Pradesh.

MATERIAL AND METHODS

The current study was carried out in the department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh, India.

Procurement, maintenance and preservation of the culture: The pure culture of King oyster mushroom (*Pleurotus eryngii)* strain DMRP-135 was procured from Directorate of Mushroom Research, ICAR Complex, Chambhaghat, Solan. The culture thus obtained was maintained on potato dextrose agar (PDA) medium and sub-cultured periodically at an interval of 45 days. The full-grown culture was stored in the refrigerator at 2-4°C and was used for further studies.

Sterilization of the glassware and culture media: All media under study were sterilized in an autoclave at 121.5°C temperature and 15 psi pressure for 20 minutes. Glassware was sterilized in hot air oven at 180°C for 2 hours. The inoculating needle and cork borer were initially dipped in ethyl alcohol, flame sterilized and used after complete cooling.

Cultural studies: *In vitro* studies were carried out to

determine the best nutritive media for the growth of *Pleurotus eryngii* as per the standard method given by Lilly and Barnett (1951).

Screening of solid and liquid media: Five different solid media *viz*., potato dextrose agar (PDA), malt extract agar (MEA), carrot extract agar (CEA), Czapek's dox agar (CDA) and Asthana and Hawker's agar (A&HA) were evaluated for mycelial growth of *P. eryngii*. With the help of a sterilized cork borer, mycelial bits of 5.0 mm diameter were cut from the actively growing areas of pure culture plate and inoculated in the centre of Petri plate of the respective media and then incubated in a BOD incubator at 25±1°C.Data were recorded in terms of average diametric growth (mm), cultural characteristics (type of growth and colour of mycelium) and growth rate (mm/h) up to 240 h of incubation. Growth rate (mm/h) was calculated with the help of following formula:

$$r_g = \frac{dgt_2 - dgt_1}{t_2 - t_1}$$

where, r_g = growth rate(mm/h), dgt_2= Diametric growth (mm) at time t_2, dgt_1= Diametric growth (mm) at time t_1

Five different liquid media potato dextrose broth (PDB), malt extract broth (MEB), carrot extract broth (CEB), Czapek's dox broth (CDB) and Asthana and Hawker's broth (A&HB) were evaluated for mycelial growth of *P. eryngii*. A 5.0 mm diameter bit of test fungus was taken with the help of sterilized cork borer from the pure culture plate and inoculated in respective broth of 75 ml (150 ml capacity Erlenmeyer's flasks). After inoculation, the flasks were incubated in a BOD incubator at 25±1°C. Data were recorded in terms of dry mycelial weight (mg) after 7, 14 and 21 days of inoculation. For determination of fungal biomass in liquid media, mycelial mat of the test fungus was filtered through Whatman's No. 1 filter paper disc and dried at 50°C overnight. The dry weight of the fungus was calculated by using the following formula:

Dry weight of the fungus= (weight of filter paper + mycelium) – (weight of filter paper)

Data analysis: The experiments were conducted in completely randomized design with four replications in each treatment and statistically analysed by using statistics package program OPSTAT (Sheoran, 2006).

RESULTS AND DISCUSSIONS

Effect of different solid media on the growth of *Pleurotus eryngii*: Significantly mean maximum (56.27 mm) diametric growth was in PDA followed by MEA (48.58 mm) while, mean minimum (32.36 mm) was in CEA (Table 1). Irrespective of different media under study, significantly mean maximum (70.85 mm) diametric growth was after 240 h of incubation followed by 192 h (61.30 mm) while, mean minimum (12.03 mm) diametric growth was recorded after 48 h of incubation (Plate 1). Significantly maximum (89.64 mm) diametric growth was in PDA after 240 h of incubation followed by 192 h (80.65 mm) on same medium while, minimum (10.13 mm) diametric growth was in CEA after 48 h of incubation which was statistically at par with CDA (10.19 mm) after same duration of incubation. Rest of the treatments exhibited intermediate level of diametric growth.

Colour of mycelium was white in PDA, MEA and CEA while transparent white in A&HA and CDA (Table 1). The thick cottony growth was in PDA while dense compact growth with ray like pattern in MEA. In CEA, fluffy growth having concentric rings was recorded with progression of time while, thin and transparent growth was recorded in A&HA and CDA. Significantly mean maximum growth rate (0.35 mm/h) was in PDA followed by MEA (0.30 mm/h) and A&HA (0.26 mm/h) (Table 2). However, significantly mean minimum growth rate (0.21 mm/h) was in CEA. Irrespective of different nutrient media under investigation, significantly mean maximum growth rate (0.40 mm/h) was between 96-144 h of incubation

Table 1. Effect of different solid media on mycelial growth and mycelial characteristics of *Pleurotus eryngii*

Nutrient medium	Average diametric growth (mm) after different duration of incubation (h)					Overall mean	Colour of mycelium	Type of growth
	48	96	144	192	240			
Potato dextrose agar	13.38	35.32	62.38	80.65	89.64	56.27	White	Thick cottony
Malt extract agar	15.31	33.38	51.64	66.19	76.38	48.58	White	Dense compact growth having ray-pattern
Carrot extract agar	10.13	19.57	31.90	45.06	55.13	32.36	White	Fluffy growth having concentric rings
Asthana and Hawker's agar	11.13	26.22	45.75	58.54	67.56	41.84	White	Thin, transparent growth
Czapek's dox agar	10.19	23.82	41.14	56.06	65.57	39.35	White	Thin, transparent growth
Overall mean	12.03	27.66	46.56	61.30	70.85			
$CD_{p\geq0.05}$	Medium 0.08		Duration 0.08		Interaction 0.19			

followed by 48-96 h (0.33 mm/h) while, significantly mean minimum growth rate (0.15 mm/h) was between 0-48 h of incubation. Interaction between nutrient media and time of incubation reveals that maximum average growth rate (0.56 mm/h) of the test fungus was in PDA between 96-144 h of incubation followed by 48-96 h (0.46 mm/h) on same medium. However, minimum average growth rate (0.11 mm/h) was in CEA and CDA between 0-48 h of incubation which was statistically at par with A&HA (0.13 mm/h) between same duration of incubation. Rest of the treatments exhibited intermediate levels of growth rate.

Effect of different liquid media on mycelial growth of *Pleurotus eryngii*: irrespective of different durations of incubation, significantly maximum (467.50 mg) biomass was in PDB followed by CEB (228.33 mg) and MEB (186.67 mg) (Table 3). However, significantly mean minimum (87.50 mg) biomass of the test fungus was in A&HB. Irrespective of different liquid media under study, significantly mean maximum (295.00 mg) biomass was recorded after 21 days of incubation followed by 14 days (237.00 mg) while, mean minimum (147.50 mg) biomass was recorded after 7 days of incubation (Plate 2). Interaction between nutrient broth and time interval reveals that significantly maximum average biomass (577.50 mg) was recorded in PDA after 21 days of

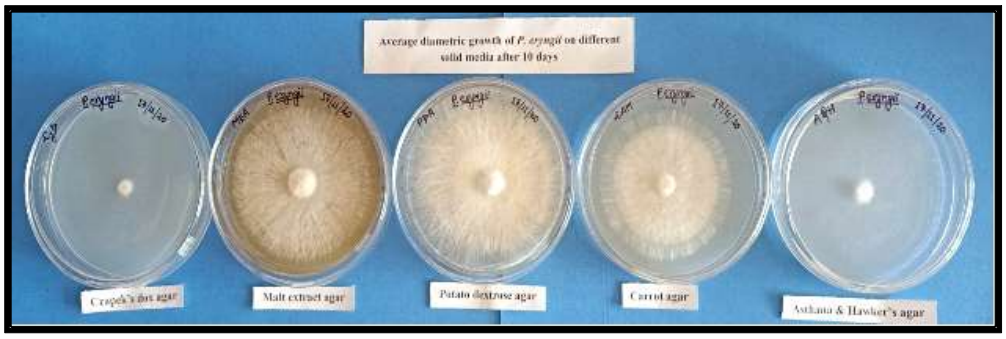

Plate 1. Mycelial growth of *Pleurotus eryngii*on different solid media

Table 2. Effect of different solid media on growth rate of *Pleurotus eryngii*

Nutrient medium	Growth rate (mm/h) between different duration of incubation (h)					Overall mean
	0-48	48-96	96-144	144-192	192-240	
Potato dextrose agar	0.18	0.46	0.56	0.38	0.19	0.35
Malt extract agar	0.21	0.38	0.39	0.31	0.21	0.30
Carrot extract agar	0.11	0.20	0.26	0.27	0.21	0.21
Asthana and Hawker's agar	0.13	0.32	0.41	0.27	0.19	0.26
Czapek's dox agar	0.11	0.29	0.36	0.31	0.20	0.25
Overall mean	0.15	0.33	0.40	0.31	0.20	
CD (p=0.05)	Nutrient medium 0.01		Duration 0.01		Interaction 0.03	

Table 3. Effect of different liquid media on biomass production of *Pleurotus eryngii*

Nutrient medium	Average dry weight (mg) after different duration of incubation (Days)			Overall mean
	7	14	21	
Potato dextrose broth	360.00	465.00	577.50	467.50
Malt extract broth	132.50	180.00	247.50	186.67
Carrot extract broth	135.00	242.50	307.50	228.33
Asthana and Hawker's broth	60.00	80.00	122.50	87.50
Czapek's dox broth	50.00	217.50	220.00	162.50
Overall Mean	147.50	237.00	295.00	
CD (p=0.05)	Nutrient broth 17.53	Duration 13.58	Interaction 30.37	

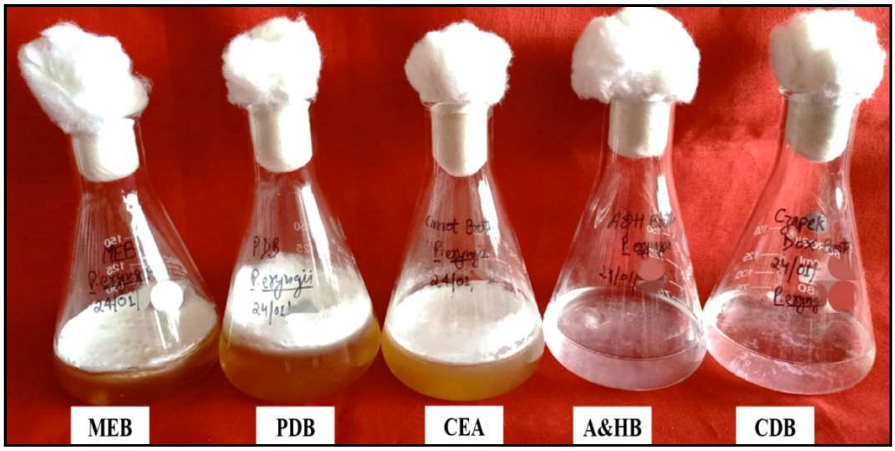

Plate 2. Effect of different liquid media on biomass production of *Pleurotus eryngii*

inoculation followed by 465.00 mg after 14 days and 360.00 mg after 7 days of inoculation in the same medium. However, significantly minimum average biomass (50.00 mg) of the test fungus was recorded in CDB after 7 days of inoculation which was statistically at par with A&HB (60.00 mg) after 7 days of inoculation and 14 days of incubation (80.00 mg). Rest of the treatments exhibited intermediate levels of biomass production.

Shrestha et al. (2006) observed that media which is rich in nutrients produced mycelium in abundance. This may be due to the sufficiency of all the nutritional requirements and optimal physical conditions for the vegetative growth of mycelium. Sardar et al. (2015) also reported maximum growth rate (0.52 cm/day) of *Pleurotus* spp. including *P. eryngii* in PDA medium. Nguyen and Ranamukhaarachchi (2020) observed maximum mycelial growth and growth rate (5.89 cm and 0.84 cm/day) of *P. eryngii* in potato dextrose agar medium. Different workers also reported potato glucose liquid medium and potato dextrose broth as the best medium for maximum biomass production of different species of *Pleurotus* (Diwan and Rawte 2011, Abd El Zaher et al., 2020).

CONCLUSION

Potato dextrose agar medium exhibited maximum mycelial growth followed by malt extract agar medium. Mean minimum mycelial growth was observed in carrot extract agar medium. Growth rate was maximum in potato dextrose medium followed by malt extract agar while was least in carrot extract agar medium. Mean maximum biomass was observed in potato dextrose broth followed by carrot extract broth and malt extract broth. However, mean minimum biomass was recorded in Asthana and Hawker's broth. In all the media under study, the colour of the mycelium varied from white to transparent white. The type of growth was observed as cottony, fluffy, thin and transparent having ray pattern and concentric rings.

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