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Laboratory Screening of Different Nutrient Media for Mycelial Growth and Cultural Characteristics of Blue Oyster Mushroom [*Hypsizygus ulmarius* (Bull.: Fr.) Redhead]

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***Abstract*:** Study was conducted to observe the effect of different solid and liquid nutrient media on mycelial growth, cultural characteristics and growth rate of *Hypsizygus ulmarius*. Five different solid media *viz.,* Potato dextrose agar (PDA), Czapek's Dox agar (CDA), Malt extract agar (MEA), Carrot extract agar (CEA) and Asthana and Hawker's agar (A&HA) and their respective broths were evaluated under laboratory conditions to find out the best physiological conditions for the growth of *H. ulmarius*. Potato dextrose agar medium was found to be the best medium (76.20 mm) followed by malt extract agar medium (59.40 mm) and Czapek's Dox agar medium (57.00 mm). In liquid media, highest biomass was in carrot extract broth (0.52 g). However, minimum dry weight was in Asthana and Hawkers medium (0.04 g) broth. The mycelial growth in different media showed absolutely white, cottony and fluffy growth with circinate pattern but in Czapek's Dox agar and Asthana & Hawker's medium it was light thin transparent white and become visible only when seen under light.

***Keywords*:** *Hypsizygus ulmarius*, Mushroom, Mycelial growth, Nutrient media, Cultural characters

The elm oyster or blue oyster mushroom [*Hypsizygus ulmarius* (Bull.: Fr.) Redhead]; (Lyophyllaceae, Agromycetes) grows in clusters on elm trees. This mushroom is widely distributed in the temperate forests of North America, Japan, Europe and other countries. *H. ulmarius* is not only considered as food but also rich in bioactive compounds of high medicinal value (Chioza and Ohga 2014). Blue oyster mushroom being saprophytic can easily be introduced in any part of the world. Its fast growth and high resistance against competitive microorganisms is likely to make its cultivation more economical and less tedious. For successful introduction and cultivation its physiological studies are pre-requisite. Since nutrition is required by each and every living organisms for their growth and development. The physiological and nutritional studies become more vital factor in case of mushroom as success or failure in their cultivation is mainly dependent on the clear and correct understanding of nutritional and environmental needs. Oyster mushrooms are not an exception to it. It is therefore becomes necessary to add those compounds which are required for its growth and to accomplish other life processes. But the data pertaining to influence of physiological and nutritional aspects on the mycelial growth and cultural characteristics of test fungus is very meager. Hence in the present investigation, the study was conducted on the above mentioned aspects.

MATERIAL AND METHODS

The present investigation was carried out at Dr. Y S Parmar University of Horticulture and Forestry, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India during the year 2019-21.

Procurement, maintenance and preservation of culture: The pure culture of blue oyster mushroom (*Hypsizygus ulmarius*) was procured from Directorate of Mushroom Research, ICAR complex, Chambhaghat, Solan (H.P). The culture, thus obtained was maintained on potato dextrose agar (PDA) medium (sub cultured periodically at an interval of 30-45 days). Full grown culture was stored at 2-4^{0}C in the refrigerator until used further for the entire work (Plate 1).

Sterilization: All media were sterilized at 15 psi pressure for 20 min. in an autoclave. All glassware were sterilized in hot air oven at 180^{0}C for 2 h. However, the spawn substrates were sterilized in the autoclave at 22 psi pressure for 2 h and 20 min. The cork borer and inoculating needle were initially dipped in ethyl alcohol, finally flame sterilized and used only after complete cooling.

Cultural Studies: Cultural studies were conducted under *in vitro* conditions to find out the best physiological conditions for the growth of *H. ulmarius* with the standard procedure laid down by Lilly and Barnett (1951) and Tuite (1969) with some modifications, wherever necessary.

Screening of basal media: Five different media *viz.,* potato

dextrose agar (PDA), Czapek's Dox agar (CDA), malt extract agar (MEA), carrot extract agar (CEA) and Asthana and Hawker's agar (A&HA) were evaluated to find out the best suitable nutrient medium for diametric growth of *H. ulmarius.* Forty mililitre of each medium was poured in each sterilized Petri plate. After solidification of media, Petri plates were inoculated with culture disc (5.0 mm dia.) of actively growing mycelium of *H. ulmarius.* These Petri plates were then incubated at 25±1^{0}C for 5 days and the observations on diametric growth (mm) and growth characteristics of the test fungus were recorded at 24 h interval up to 120 h of incubation.

Growth rate (mm/h):

$r_g = dgt_2\text{-}dgt_1/t_2\text{-}t_1$

Where, dgt_2 = Diametric growth (mm) at time t_2; dgt_1 = Diametric growth (mm) at time t_1.

Screening of liquid medium: Seventy five ml broth of each of above media was taken in 250 ml Erlenmeyer flasks. These flasks were inoculated with 5.0 mm disc of actively growing mycelium of *H. ulmarius.* The inoculated flasks were incubated at 25±1 ^{0}C for 5, 10 and 15 days. Thereafter, dry mycelial weight of the test fungus was recorded after each interval. The broths were filtered through Whatmann's No. 1 filter paper and fresh mycelial mat was collected and weighted on electric top pan balance with sensitivity of 0.01 g. For dry weight of mycelium, mat was continuously dried in an oven at 60^{0}C overnight and the dry weight of the test fungus was calculated.

Data analysis*:* The experiments were conducted in completely randomized design having four replications in each treatment. The observations were recorded in *in vitro* conditions by culturing the fungus in Petri plates. The average diametric growth (mm/day) was recorded after 24 h of interval up to five days of incubation. The data thus obtained were statistically analysed by using statistically package of program OPSTAT (Sheoran 2006).

RESULTS AND DISCUSSION

Physiological Studies

Effect of different solid nutrient media on the growth of *H. ulmarius*: PDA exhibited significantly mean maximum (41.44 mm) mycelial growth, followed by MEA (32.63 mm) and CDA (32.32 mm, Table 1). However, latter two treatments were statistically at par with each other. Significantly mean minimum diametric growth was in A&HA (27.04 mm) followed by CEA medium, irrespective of different durations of incubation. However, irrespective of the nutrient media under study, average mean diametric growth was maximum (56.24 mm) after 120 h of incubation followed by that after 96 h. Minimum growth was after 24 h (11.60 mm) followed by 48 and 72 h. Significantly higher diametric growth (76.20 mm) was in PDA after 120 h of incubation, which was followed by growth in MEA after same duration of incubation. Minimum diametric growth (11.00 mm) was in A&HA after 24 h of incubation which was statistically at par with the diametric growth in CDA (11.40 mm) after same duration of incubation. An intermediate range of diametric growth was in rest of the test media after varying duration of incubation. Mycelial characteristics of *H. ulmarius* were also recorded in different media (Table 1). The colour of the mycelium was white in PDA, MEA and CEA, while it was transparent in CDA and A&HA media. The growth in PDA was observed to be cottony white but having suppressed ray pattern while, in case of MEA it was cottony and fluffy ray with circinate pattern. Cottony compact growth was noticed in CEA while, thin and transparent growth was observed in CDA and A&HA media which was visible only when seen under light (Plate 2). The maximum mean growth rate of *H. ulmarius* was recorded in

Table 1. Evaluation of different solid nutrient media and cultural characteristics for the growth of *Hypsizygus ulmarius*

Nutrient medium	Average diametric growth (mm) after different incubation duration (h)					Overall Mean	Colour of mycelium	Type of growth
	24	48	72	96	120			
Potato dextrose agar	12.60	23.60	38.20	56.62	76.20	41.44	White	Cottony but suppressed, ray pattern
Malt extract agar	11.80	20.35	29.00	42.60	59.40	32.63	White	Cottony and fluffy, ray with circinate pattern
Carrot extract agar	11.20	17.50	25.20	33.40	42.60	25.98	White	Cottony and compact
Czapex's Dox agar	11.40	19.20	30.60	43.40	57.00	32.32	Transparent	Thin and transparent
Asthana and Hawker's agar	11.00	17.60	25.40	35.20	46.02	27.04	Transparent	Thin and transparent
Overall Mean	11.60	19.65	29.68	42.24	56.24			
	Nutrient medium		Duration		Interaction			
CD (p=0.05)	0.19		0.19		0.42			

PDA (0.58 mm) followed by MEA and CDA which differ significantly with each other (Table 2). Least growth rate was in CEA (0.31 mm) and A&HA (0.33 mm) media between 0-24 h of incubation. Irrespective of different nutrient media under study, growth rate of *H. ulmarius* was minimum (0.27 mm) between 0-24 h of incubation which increased significantly after each 24 h of incubation and reached its maximum (0.58 mm) between 96-120 h of incubation. Fungus attained maximum growth rate in PDA (0.81 mm) between 96-120 h of incubation, which was followed by PDA between 72-96 h of incubation. Minimum growth rate was recorded in A&HA (0.24 mm) medium between 0-24 h of incubation. Rest of all the treatments exhibited intermediate range of growth rate.

Effect of different liquid nutrient media on the growth of *H. ulmarius*: The mean maximum biomass of *H. ulmarius* was in CEB (0.52 g) which was statistically at par with the biomass in PDB irrespective of different days of incubation

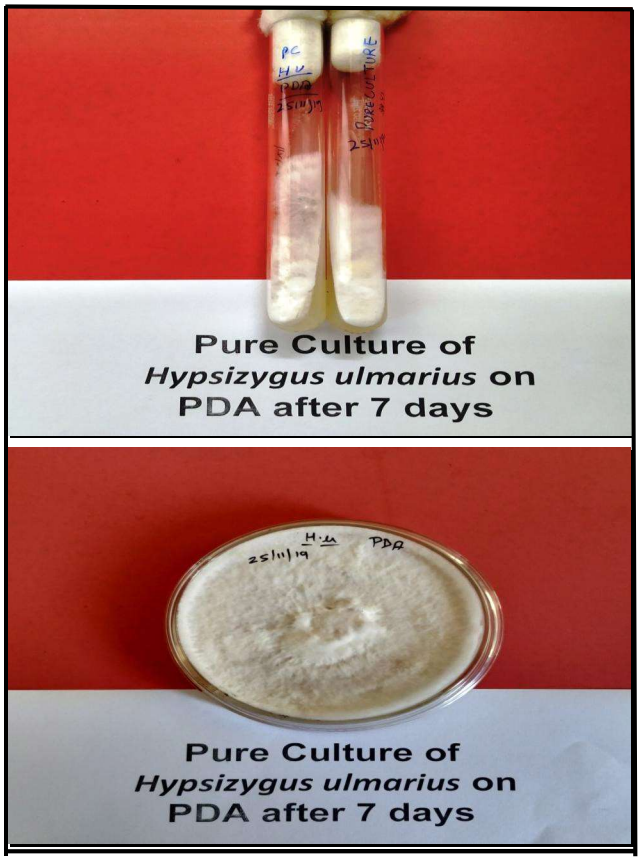

Plate 1. Pure culture of *Hypsizygus ulmarius*

Plate 2. Petri plates exhibiting mycelial growth of *Hypsizygus ulmarius* on different soild media

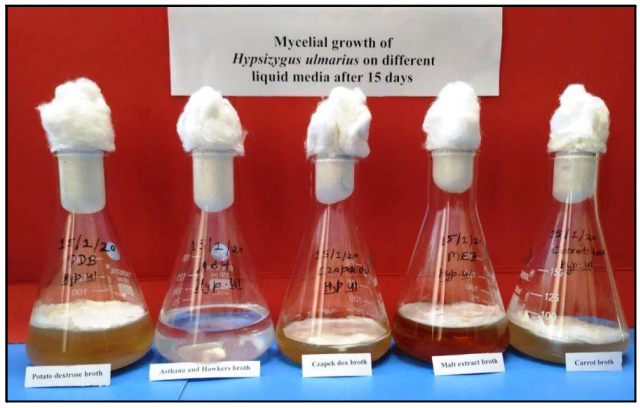

Plate 3. Erlenmeyer's flasks exhibiting biomass production of *Hypsizygus ulmarius* on different liquid media

Table 2. Effect of different nutrient media on the growth rate of *Hypsizygus ulmarius*

Nutrient medium	Average growth rate (mm/h) between duration of incubation (h)					Overall Mean
	0-24	24-48	48-72	72-96	96-120	
Potato dextrose agar	0.31	0.45	0.60	0.76	0.81	0.58
Malt extract agar	0.28	0.35	0.35	0.56	0.69	0.45
Carrot extract agar	0.25	0.27	0.32	0.34	0.38	0.31
Czapex's Dox agar	0.26	0.32	0.47	0.53	0.56	0.43
Asthana and Hawker's agar	0.24	0.27	0.32	0.40	0.44	0.33
Overall Mean	0.27	0.33	0.41	0.52	0.58	
	Nutrient medium		Duration		Interaction	
CD (p=0.05)	0.01		0.01		0.02	

Table 3. Effect of different liquid media on biomass production and cultural characteristics of *Hypsizygus ulmarius*

Nutrient medium	Average biomass (g) after incubation duration (days)			Overall mean	Colour of mycelium	Type of growth
	5	10	15			
Potato dextrose broth	0.23	0.49	0.77	0.50	Absolutely white	Thick and Cottony
Malt extract broth	0.16	0.24	0.41	0.25	White	Cottony and fluffy
Carrot extract broth	0.24	0.52	0.79	0.52	Dull white	Thick, compact and
Czapex's Dox broth	0.04	0.09	0.16	0.09	White	Thin
Asthana and Hawker's broth	0.02	0.03	0.07	0.04	Light white translucent	Very thin and transparent
Overall Mean	0.13	0.27	0.44			
	Nutrient medium		Duration	Interaction		
CD (p=0.05)	0.03		0.04	0.06		

(Table 3). However, significantly minimum biomass was in A&HB (0.04 g) followed by CDB and MEB. Significantly mean maximum biomass was after 15 days of incubation (0.44 g) followed by 10 days and 5 days of incubation, irrespective of different liquid media investigated (Plate 3). The maximum biomass of *H. ulmarius* (0.79 g) was recorded in CEB after 15 days of inoculation which was statistically at par with the biomass in PDB (0.77 g) after same interval of inoculation followed by 10 days after inoculation in CEB (0.52 g) and PDB (0.49 g). However, mean minimum dry weight of the test fungus was recorded in A&HB (0.02 g) after 5 days of incubation. The colour of the mycelium was absolutely white to white in PDB, MEB and CDB while, it was dull white in CEB (Table 3). Light translucent colour of the mycelium was in AH&B. The growth was observed to be thick cottony in PDB, cottony and fluffy in MEB, thick, compact and cottony in CEB while, thin to very thin in CDB and AH&B respectively after 15 days of incubation was observed.

The results obtained in present study are in consonance with the results of Jatav et al (2013), Sumi and Geetha (2016) and Baghel et al (2019) where PDA as the best and most suitable medium for the growth of *H. ulmarius*. In the present investigations, maximum mean biomass of the test fungus was in CEB which was statistically at par with PDB. The present findings are also in agreement with the findings of Shendge (2018) where PDA was most suitable medium for growth and biomass production of *H. ulmarius*. PDA, MEA, CDA, A&HA, oat meal agar and yeast extract agar have also been tried by different workers (Krasnopolskaya *et al* 2008, Rawte and Diwan 2011, Sardar et al 2017) for culturing of *Pleurotus* spp. and *H. ulmarius* (Mishra et al 2015, Sumi and Geetha 2016 , Kumar and Eswaran, 2016).

CONCLUSION

Potato dextrose agar medium exhibited mean maximum diametric growth followed by malt extract agar medium. Minimum growth was observed in carrot extract agar. Growth rate was maximum in potato dextrose agar medium followed by malt extract agar medium while it was least in carrot extract agar medium after 120 h of incubation. Mean maximum biomass was observed in carrot extract broth which was statistically at par with potato dextrose broth. However, mean minimum dry weight of the test fungus was in Asthana and Hawker's broth medium after 15 days of incubation. In all the treatments colour of the mycelium varied from white to transparent white and type of growth observed as cottony, fluffy, compact, thin and transparent having ray and circinate patterns.

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