EFFECT OF DIFFERENT MEDIA, PH AND TEMPERATURE ON GROWTH OF *PLEUROTUS OSTREATUS*

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ABSTRACT

Oyster mushroom locally known as "Dhingri". *Pleurotus ostreatus* have abilities to grow at a wide range of temperature and utilizing various agro-based residues. *Pleurotus ostreatus* grows best on Potato dextrose agar media (8.38 cm) among different media used. The maximum mycelial growth was recorded at pH 7 (8.19 cm). Temperature of 25ºC was found the best temperature for its mycelial growth (8.63 cm).

***Keywords*:** *Pleurotus ostreatus,* media, pH, temperature

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INTRODUCTION

The oyster mushroom grows naturally on trees or dead woody branches of trees (Chang and Hayes., 1999) as saprophytes and primary decomposers and therefore known as "Wood Fungus". In India it is commonly name as Oyster mushroom because of their tongue shaped pileus having a lateral stipe. Many species of *Pleurotus* have worldwide distribution in nature which grows saprophytically on dead wood logs and trunk of trees (Zadrazil and Kurtzman, 1984). They have a wide range of ecological adoption also have the ability to transform natural wastes into edible biomass. Mushrooms belong to a group of organisms known as fungi. A mushroom is the visible reproductive structure of a fungus. They include a wide variety of other fungi of which some are in edible (Sanchez and Gonzalez, 1996) while some are poisonous.

Pleurotus species are a good source of vitamins, protein and minerals and are known to have a broad range of uses which includes both food and medicine. A high nutritional value of oyster mushroom has been reported with protein (25-50%), minerals (potassium, phosphorus, calcium and sodium) of about 8-12%, fat (2-5%), sugars (17-47%) and myco cellulose (7-38%). These mushrooms reported to have anticancer, antioxidant, anti hypercholesterolemic, antihypertensive, antiaging, antiallergic and antimicrobial properties (Patel *et al*., 2012).

Mushroom growth is highly influenced by several factors such as spawn, growing media, pH, temperature, moisture content and light intensity. In mushroom cultivation spawn production is one of the major limiting factors to all over the world (Stanley and Waadu, 2010). It was noted that many exhibited species have maximum mycelium growth at pH 6, however the minimum growth was recorded at pH 4 and were cultured aseptically on PDA at different temperature ranges but grows best at 25°C (Sardar *et al.*, 2015). They require high humidity 80-90% and high temperature was needed *i.e.* 25-30% for vegetative growth known as spawn run and low temperature 18-25°C for fruiting. The substrate on which *Pleurotus ostreatus* in grown, affects the mushroom production.

MATERIALS AND METHODS

The investigation was carried out in Department of Plant Pathology, College of Horticulture, VCSG Uttarakhand University of Horticulture & Forestry Bharsar, Pauri Garhwal, Uttarakhand during 2018-2019.

Multiplication of pure culture of *Pleurotus* species

The strain *Pleurotus ostreatus* was collected from Department of Plant Pathology (VCSG UUHF, Bharsar)

Maintenance of pure culture

PDA medium was prepared by dissolving 39.0 gm of PDA powder in 1000 ml of distilled water. The solution was heated until the solid components completely dissolve then it is sterilized by autoclaving. *Pleurotus osteratus* was cultured under sterilized condition in a laminar air flow and incubated at 25±1°C for 8 to 10 days till proper growth is observed. Periodically, sub culturing of the stored cultures was done.

Effect of different culture media at 25^{0}C temperature

Five different culture media namely Potato dextrose agar media, Oat meal agar, Malt extract agar, Corn meal agar and Wheat extract agar were used to find out the best medium for the growth of *Pleurotus osteratus*.

Composition of culture media:

(i) Potato Dextrose Agar (PDA) medium

Potato (peeled and sliced)	-	250 gm
Dextrose	-	20 gm
Agar	-	20 gm
Distilled water	-	1000 ml

(ii) Oat Meal Agar (OMA) medium

Rolled oats	-	70 gm
Dextrose	-	20 gm
Agar	-	20 gm
Distilled water	-	1000 ml

(iii)Malt Extract Agar (MEA) medium

Malt extract	-	25 gm
Agar	-	20 gm
Distilled water	-	1000 ml

(iv) Corn Meal Agar (CMA) medium

Corn meal	-	40 gm
Dextrose	-	20 gm
Agar	-	20 gm
Distilled water	-	1000 ml

(v) Wheat Extract Agar (WEA) medium

Wheat grains	-	100 gm
Agar	-	20 gm
Distilled water	-	1000 ml

Sterilization

Sterilization of different media were done in an autoclave at 15 psi pressure for 15 minutes and all the glassware were sterilized at 180ºC for 30 minutes in an electric oven. The inoculation needle was initially dipped in ethyl alcohol and finally flame sterilized and used after complete cooling.

Preparation of inoculum

In solid media studies, in presterilized petri plates 20 ml of sterilized solid media were poured under aseptic conditions. After solidification of media in each petri plate bits of test culture was then inoculated aseptically about 5 mm diameter discs of mycelial pieces was cut with the pre-sterilized needle and sufficient care was taken to avoid the use of thick agar block with mycelial discs. After cooling of media it poured into sterilized petri plates and allowed to solidify. A small block (5mm) of mycelium from an actively growing pure culture will placed at the center of the media. The inoculation petri plates incubated at 25±1^0 C for 10 days.

Effect of different pH levels at 25^{0}C temperature on PDA

After optimization of temperature, these species were taken for their pH requirement. Therefore, a different level of pH 5.0, 6.0, 7.0, 8.0 and 9.0 on the PDA media was maintained by adding 1M solutions of HCl and NaOH. The plates were incubated at temperature at 25^{0}C for 10 days. The data were analyzed statistically. In case of different pH the growth was recorded at different intervals such as 4, 6, 8 and 10th days depending the rate of growth of the fungus. The growth was measured by taking average of linear growth of the colony in the two directions.

Effect of temperature levels on the growth of *Pleurotus osteratus* on PDA.

Petri plates containing basal medium along with inoculation of *Pleurotus osteratus* were incubated at various temperature viz. 15ºC, 20ºC, 25ºC, 30ºC and 35ºC in different incubators. In case of different temperature, the growth was recorded at different intervals such as 4, 6, 8 and 10th days depending the rate of growth of the fungus. The growth was measured by taking average of linear growth of the colony in the two directions.

EXPERIMENTAL RESULTS

Mycelial growth of *Pleurotus ostreatus* on different cultural media

Different solid media *i.e.* potato dextrose agar, oat meal agar, malt extract agar, corn meal agar and wheat extract agar were used to find out the best medium for the growth of *Pleurotus osteratus*. The test fungus was studied at 25ºC. The data presented in Table 1 showed that the factor A (media) exhibited maximum mycelial growth in PDA (5.63 cm) and minimum mycelial growth was seen in corn meal agar is 4.56 cm. In case of factor B (days), the maximum mycelial growth was seen on 10th day (7.45 cm) while the minimum growth was recorded on 4th day (2.23 cm). Among interactions (media × days) the maximum growth was recorded in PDA on 10th day (8.38 cm) while least growth in corn meal agar on 4th day (1.99 cm) and it was found statistically at par with oat meal agar on 4th day (1.96 cm).

Table 1. Mycelial growth (cm) of *Pleurotus ostreatus* on different media and days at 25^{0}C

S. No.	Media	DAYS			
		DAY 4	DAY 6	DAY 8	DAY 10
1	PDA	2.64	4.83	6.70	8.38
2	OMA	1.96	4.08	5.94	7.00
3	MEA	2.24	4.77	6.19	7.72
4	CMA	1.99	3.97	5.49	6.81
5	WEA	2.35	4.31	6.12	7.33

Mycelial growth of *Pleurotus ostreatus* on different pH

Basal medium of PDA was adjusted at different pH levels of 5, 6, 7, 8 and 9 incubated at 25ºC and the growth was recorded on 4th, 6th, 8th and 10th day. The result presented in Table 2 revealed that the factor A (pH), the maximum mycelial growth was seen in pH 7 (5.05 cm) while the minimum growth was recorded in pH 9 (3.48 cm) which was statistically at par with pH 8 (3.51 cm). In factor B (days) had maximum mycelial growth that was recorded on 10th day (6.84 cm) and minimum on day 4 (1.88 cm). In case of interactions (pH × days), the maximum growth was recorded in pH 7 on 10th day (8.19 cm) and the minimum growth in pH 9 on 4th day (1.49 cm) which is statistically at par with pH 5 on 4th day (1.76 cm) and pH 8 on 4th day (1.81 cm).

Table 2. Mycelial growth (cm) of *Pleurotus ostreatus* on different pH and days on PDA at 25ºC

S.No.	pH (A)	DAY (B)			
		DAY 4	DAY 6	DAY 8	DAY 10
1	pH 5	1.76	2.65	4.42	7.27
2	pH 6	1.87	3.75	5.77	7.81
3	pH 7	2.45	3.91	5.65	8.19
4	pH 8	1.81	2.68	3.88	5.69
5	pH 9	1.49	2.90	4.28	5.25

Mycelial growth of *Pleurotus ostreatus* on different temperature

The test fungus was incubated at different temperature ranging between 15 to 35ºC on 4th, 6th, 8th and 10th day. The data presented in Table 3. indicate that Factor A (temperature) had maximum mycelial growth at 25ºC (5.84 cm) and minimum growth was seen at 35ºC (2.69 cm). In factor B (days), the maximum mycelial growth was recorded on 10th day (6.42 cm) whereas minimum was seen on 4th day (1.68 cm). Data showed that among interactions (temperature × days) the maximum growth was recorded at 25ºC on 10th day (8.63 cm) and minimum growth was found at 35ºC on day 4th (0.64 cm).

Table 3. Mycelial growth (cm) of *Pleurotus ostreatus* on different temperature and days on PDA

S.No.	Temperature (°C)	DAY			
		DAY 4	DAY 6	DAY 8	DAY 10
1	Temp 15	1.20	1.78	4.26	5.37
2	Temp 20	1.96	2.63	4.79	6.36
3	Temp 25	2.72	5.16	6.86	8.63
4	Temp 30	1.87	2.71	4.39	6.37
5	Temp 35	0.64	1.33	3.42	5.36

DISCUSSION

Potato dextrose agar was used as the medium for maintaining the pure mycelial culture as described under Materials and Methods. Gibriel *et al*. (1996) also found that PDA was the best media tested for both rate and amount of fungal growth of *Pleurotus* strains. Sardar *et al*. (2015) also saw the same results that PDA was best media for mycelial growth of *Pleurotus* species. Various solid media were used to evaluate the effect different media *viz* CMA, MEA, PDA, OMA. Among them PDA has maximum mycelial growth (8.38 cm) while minimum in CMA (6.81 cm) at 25ºC. Same results were founded by Nasium *et al*. (2001) that *Pleurotus osteratus* have maximum growth on MEA. Sadar *et al*. (2015) also reported that PDA was found to be the best medium. Kumar and Kushwaha (2014) also saw that PDA was best media for different *Pleurotus* species.

Pleurotus ostreatus was tested for its tolerance at acidic and basic pH ranging between 5.0- 9.0 and it was founded that pH 7 (8.19 cm) have maximum mycelial growth followed by pH 6 (7.81 cm) and minimum growth in pH 9 (5.25 cm). Sardar *et al*., 2015 observed that *Pleurotus* species exhibited maximum mycelial growth at pH 6. Gorai and Sharma (2018) reveled that that maximum growth was seen in pH ranging between 6.5 to 7.5. Temperature is an important aspect in selection of mushroom, five different temperature ranging between 15-35ºC were tested on PDA to observe the optimum temperature for mycelial growth of tested fungus. The maximum growth was founded at temperature of 25ºC (8.63 cm) and least in 35ºC (5.36 cm). Previously, optimum temperature within this range was reported by Zharare *et al*. (2010) by observing

maximum growth of *Pleurotus* strains at 20 to 25ºC. Sardar *et al*. (2015) also reported that *Pleurotus* species were grows best at 25ºC. Similarly, Gorai and Sharma (2018) revealed that a temperature of 25ºC was optimum for most of the *Pleurotus* species.

CONCLUSION

Potato dextrose agar was found to be the best suitable media for mycelial growth of *Pleurotus ostreatus* followed by malt extract agar media. The pH 7 was found to be the best for mycelial growth (8.19 cm) of *Pleurotus ostreatus* on the 10th day. The maximum mycelial growth (8.63 cm) was recorded at the temperature of 25ºC and minimum growth (5.36 cm) was seen at 35ºC on the 10th day.

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