

Cultural studies on mycelia of *Pleurotus ostreatus* (Oyster mushroom)

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Oyster mushroom, also known as *Dhingri*, is an edible fungus, capable of producing protein rich food on a variety of agro-wastes without the need for composting (Sardar *et al.*, 2017). *Pleurotus* has become more popular as a health promoter and environmental restorer than other mushrooms, leading to an increase in research and development (Patel *et al.*, 2012). The current study was planned to determine the optimal culture media, temperature, pH, and light intensity for *Pleurotus ostreatus* mycelial growth.

The study was conducted in Centre for Mushroom Research and Training (CMRT) unit of Department of Plant Pathology CSKHPKV, Palampur and the pure culture of *P. ostreatus* was procured from CMRT unit and maintained on PDA (Potato Dextrose Agar Medium) using standard procedure for further studies.

Ten different solid media *viz.*, Potato Dextrose Agar, Potato Dextrose Yeast Agar, Malt Extract Agar, Czapek's Dox Agar, Potato Malt Agar, Corn Meal Agar, Ashby's Mannitol Agar, Yeast Extract Agar, Yeast Malt Agar and Potato Sucrose Agar were evaluated to find out the optimal medium for mycelial growth of *P. ostreatus*. Different solid media were prepared in flasks and sterilized in an autoclave for 15 minutes at 15 psi pressure. Sterile glass Petri plates, (9 cm dia.) were used to pour autoclaved and cooled medium (20 ml/plate) and allowed to solidify at room temperature under aseptic conditions. Petri plates of each culture medium were inoculated using

7 mm bit of actively growing mycelium of pure culture of *P. ostreatus* using cork borer in the centre of the plate and incubated at 22 ± 2 °C for 9 days and the growth was recorded at different interval of 3, 6 and 9 days.

Similarly, ten liquid media *viz.*: Potato Dextrose Broth, Potato Dextrose Yeast Broth, Malt Extract Broth, Czapek's Dox Broth, Potato Malt Broth, Corn Meal Broth, Ashby's Mannitol Broth, Yeast Extract Broth, Yeast Malt Broth and Potato Sucrose Broth were evaluated. Liquid medium were inoculated using 7 mm bit of actively growing mycelium of *P. ostreatus* in 150 ml conical flasks containing 50 ml liquid medium. The inoculated flasks were incubated for 14 days at 22 ± 2 °C for mycelial mat formation. After 14 days of growth, the mycelial mat was taken out and average fresh and dry weight of mycelial mats were recorded. For dry weight measurement the mycelial mat was dried at 50°C for over 12 hours until constant weight was achieved. Best solid and liquid media supporting mycelial growth of *P. ostreatus* was used for further studies.

Five different temperatures *viz.*, 10, 15, 20, 25, and 30 °C were evaluated for the mycelial growth of *P. ostreatus*. The basal media were Yeast Malt Agar (solid medium) and Potato Dextrose Yeast broth (liquid medium). The experiment for determining the optimal pH for mycelial growth of *P. ostreatus* was carried out using same basal media, i.e. Yeast Malt Agar (solid medium) and Potato Dextrose Yeast broth (liquid medium). The pH of the basal medium was

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altered using NaOH and HCl to a range of 5.0, 5.5, 6.0, 6.5 and 7.0. Five different light intensities, i.e. 100 lux, 200 lux, 300 lux, complete light, and complete darkness were evaluated to observe the mycelial growth of *P. ostreatus* in both solid as well as liquid media. The light intensity was measured using luxmeter.

All the experiments were done in triplicate in Complete Randomized design. Of the ten solid media evaluated, Yeast Malt Agar (YMA) and Malt Extract Agar (MEA) were fully colonized on 6th day i.e. 8.50 cm and 8.43 cm, respectively and were statistically at par with each other. All the media were fully colonized after 9th day of inoculation (Table 1). These results are in conformity with many other earlier workers. Nguyen and Ranamukhaarachchi (2020) reported that *P. ostreatus* had a better mycelial growth on YMA and MEA media and did not show significant differences in the mycelial diameter and growth rate. Nasim *et al.* (2001) used three culture media for tissue culturing of oyster and paddy straw mushroom, and reported the maximum mycelial growth on MEA as compared to Potato Dextrose Agar. Mahadevan and Shanmugasundaram (2018) also reported that MEA and YMA were found to

stimulate luxuriant mycelial growth of *Pleurotus sapidus*.

In liquid media, Yeast Malt broth supported best growth of *P. ostreatus* after 14 days of incubation with dry mycelial mat of weight 0.47 g followed by Malt Extract broth (0.40 g) (Table 1). No growth was observed on Corn Meal broth. Amita and Atri (2017) studied the growth of *P. cystidiosus* on fourteen liquid media and reported maximum dry weight on Yeast Glucose Medium.

Amongst five different temperatures evaluated on Yeast Malt Agar, the maximum mycelial growth of 8.53 cm was recorded at 25 °C followed by 20 °C (7.65 cm) and was statistically at par with each other. The minimum mycelial growth (1.32 cm) was recorded at 10 °C (Table 2, Fig. 1). The results are in consonance with the findings of Sardar *et al.* (2015), who reported that *Pleurotus* spp. showed fastest mycelial growth when grown at temperature of 25 °C. Zharare *et al.* (2010) studied eight strains of *Pleurotus* at different temperature and reported that *Pleurotus* had better mycelial growth at temperature 25°C. Pant *et al.* (2020) also reported the maximum mycelial growth of *Pleurotus osteratus*

Table 1. Mycelial growth of *P. ostreatus* on different solid and liquid media

S. No.	Media	Solid media (cm)		Liquid media (g) (14 days of incubation)
		Day 6	Day 9	Dry wt. of mycelia
1	Yeast Malt Medium	8.50	9.00 ^a	0.47 ^a
2	Malt Extract Medium	8.43	9.00 ^a	0.40 ^{ab}
3	Potato Sucrose Medium	7.56	9.00 ^a	0.36 ^b
4	Potato Dextrose Yeast Medium	4.60	8.74 ^{ab}	0.29 ^c
5	Czapek's Dox Medium	4.23	8.61 ^{ab}	0.19 ^d
6	Potato Malt Medium	4.03	8.34 ^b	0.13 ^e
7	Yeast Extract Medium	3.80	7.83 ^c	0.09 ^f
8	Potato Dextrose Medium	3.23	6.41 ^d	0.02 ^g
9	Ashby's Mannitol Medium	3.06	5.56 ^e	0.01 ^h
10	Corn Meal Medium	2.73	4.45 ^f	-
	CD (0.05)	0.21	0.43	0.02

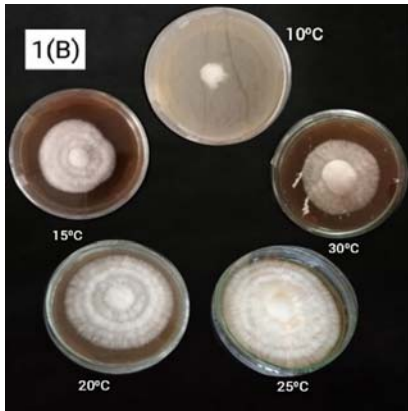


Fig. 1. Mycelial growth on Yeast Malt Agar at different temperatures after 9 days

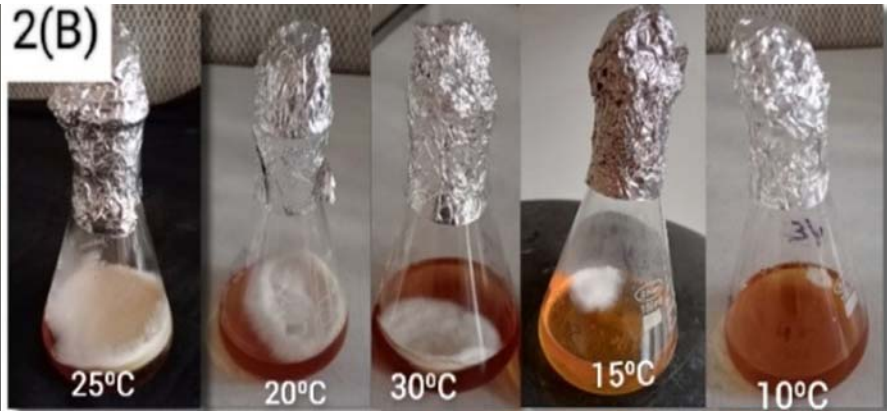


Fig. 2. Mycelial growth in Potato Dextrose Yeast Broth liquid media at different temperatures after 14 days

Table 2. Mycelial growth of *P. ostreatus* at different temperatures on solid and liquid medium

S. No.	Temperature	Mycelial growth		
		Solid medium (cm)		Liquid medium (g) (14 days of incubation)
		Day 6	Day 9	Dry mycelial weight (g)
1	10 °C	0.00	1.32	0.00
2	15 °C	3.09	6.22	0.014
3	20 °C	7.65	9.00	0.192
4	25 °C	8.53	9.00	0.408
5	30 °C	4.40	6.81	0.079
	CD (0.05)	0.432	0.11	0.021

at temperature of 25°C and least at 35°C. Temperature optima were also studied in liquid culture medium. Potato dextrose yeast broth was used as basal media. The maximum dry mycelial weight 0.408 g was recorded at 25 °C followed by temperature 20 °C (0.192 g). No growth was observed at 10°C (Table 2, Fig 2). The results are in conformity with the findings of Sardar *et al.* (2015), Zharare *et al.* (2010), Gorai and Sharma (2018) and Pant *et al.* (2020).

The effect of pH levels ranging between 5.0-7.0 were evaluated for mycelial growth of *P. ostreatus* on Yeast Malt Agar. The maximum mycelial growth (8.19 cm) was recorded at pH 6.5 followed by 6.0 (8.01 cm) and were statistically at par with each other.

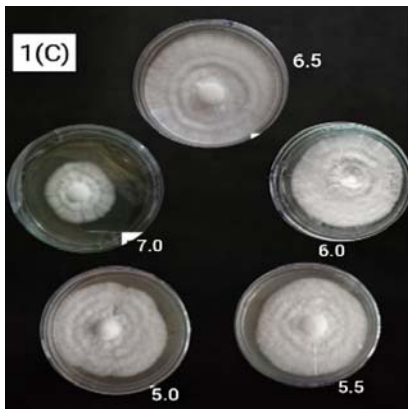


Fig. 3. Mycelial growth on Yeast Malt Agar at different pH after 9 days

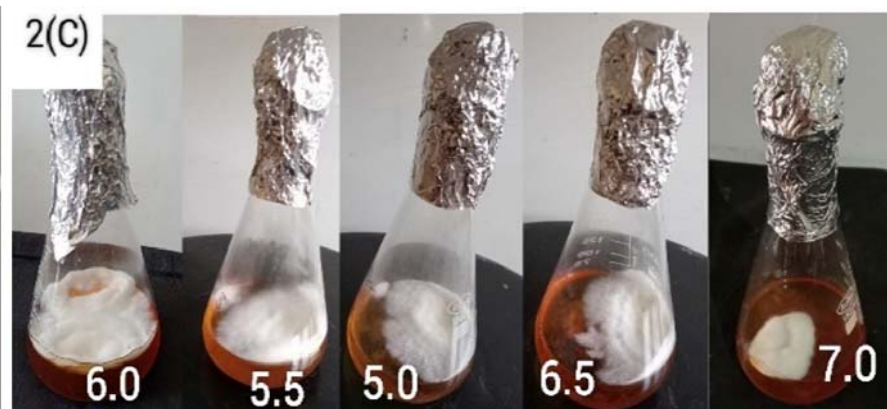


Fig. 4. Mycelial growth in Potato Dextrose Yeast Broth liquid media at different pH after 14 days

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Table 3. Mycelial growth of *P. ostreatus* at different pH levels on solid and liquid medium

S. No.	pH	Mycelial growth		
		Solid medium (cm)		Liquid medium (g) (14 days of incubation)
		Day 6	Day 9	Dry mycelial weight (g)
1	5.0	5.36	7.85	0.23
2	5.5	6.15	8.94	0.30
3	6.0	8.01	9.00	0.42
4	6.5	8.19	9.00	0.39
5	7.0	4.92	7.12	0.09
	CD (0.05)	0.17	0.08	0.02

The mycelial growth of 7.12 cm was recorded at pH 7.0 (Table 3, Fig 3). In liquid media studies, Potato dextrose yeast broth prepared with different pH levels ranging from pH 5.0-7.0, the maximum dry mycelial weight of 0.42 g was recorded at pH 6.0 followed by pH 6.5 (0.39 g) (Table 3, Fig 4). Gorai and Sharma (2018) reported maximum growth of *Pleurotus* spp. at pH ranges of 6.5 to 7.5. Amita and Atri (2017) also reported that *P. cystidiosus* had better mycelial growth at pH 6.5. Similar results were reported by Sardar *et al.*, 2015 while studying effect of different pH levels on *Pleurotus* species.

The light intensity of 200 lux supported the maximum mycelial growth in solid medium 8.26 cm followed by 100 lux (8.17 cm) and the growth was moderate in complete darkness 7.94 cm. However, minimum mycelial growth (6.87 cm) was recorded in complete light (Table 4, Fig 5). These results are in conformity with Rout *et al.*, 2015 who reported that low intensity (200 lux) near darkness or darkness is suitable for mycelial growth of Oyster species.

The maximum dry mycelial weight of 0.43 g was recorded at light intensity of 200 lux followed by 100 lux (0.13 g) and the minimum myelial growth (0.07g) was recorded in 300 lux and complete light (Table 4).

Table 4. Mycelial growth of *P. ostreatus* at different light intensities on solid and liquid medium

S. No.	Light intensity	Mycelial growth		
		Solid medium (cm)		Liquid medium (g) (14 days of incubation)
		Day 6	Day 9	Dry mycelial weight (g)
1	Complete darkness	7.94	9.00	0.12
2	Complete light	4.91	6.87	0.08
3	100 lux	8.17	9.00	0.13
4	200 lux	8.26	9.00	0.43
5	300 lux	6.68	8.58	0.07
	CD (0.05)	0.172	0.061	0.04

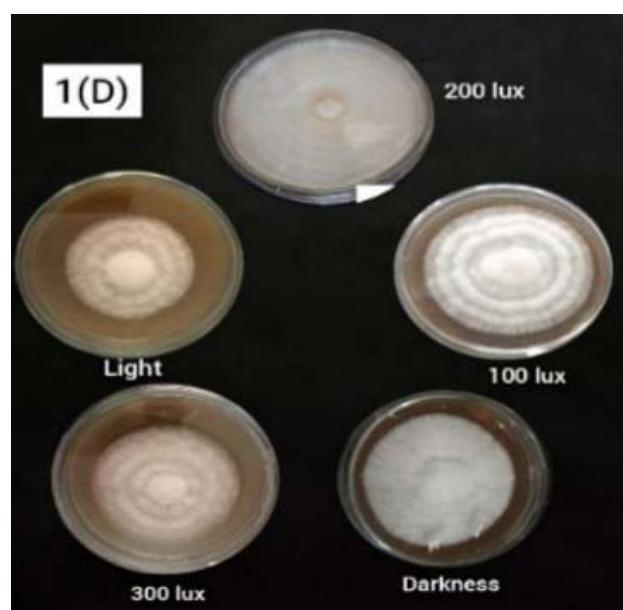


Fig 5. Mycelial growth on on Yeast Malt Agar at different light intensities after 9 days

These results are in conformity with Rout *et al.* (2015). Amita and Atri (2017) also studied the effect of light and darkness on *P. cystidiosus* and maximum mycelial growth was observed under dark conditions both in solid and liquid media.

From the above findings, it could be inferred that best mycelial growth of *P. ostreatus* on solid medium was supported by Yeast malt agar followed by Malt

extract agar and that on liquid medium on Potato dextrose yeast broth followed by Yeast malt broth. Corn meal agar and Corn meal broth supported minimum growth. The optimum temperature for the mycelial growth of *P. ostreatus* both in solid as well as in liquid medium was 25°C. pH 6.0-6.5 supported maximum mycelial yield. Low light intensity up to 200 lux gave better mycelial growth both in solid as well as liquid media.

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