Cultivation of *Pleurotus eous* under controlled conditions using different culture media

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Abstract: Cultivation of pink oyster mushroom (*Pleurotus eous*) is a profitable agribusiness and ranks second among the important cultivable mushrooms. In present study the pink oyster mushroom cultivable was carried out using different cultural media. The study was aimed at generating data on the influence of different cultural media upon the mycelial growth of pink oyster mushroom (*Pleurotus eous*) under controlled (*in-vitro*) conditions.

Key words: Cultural media; mycelia growth; Pink oyster mushroom; *Pleurotus eous*.

1. Introduction

Mushroom cultivation is a profitable agribusiness and a potential biotechnological process for converting lingo cellulosic wastes into protein rich food for human consumption. Out of 2,000 species of prime edible mushrooms, about 80 per cent have been grown experimentally, 20 cultivated commercially and 4 to 5 species produced on industrial scale throughout the world (Bandopadhyay and Chatterjee, 2009). Oyster mushroom (*Pleurotus* spp.) ranks second among the important cultivated mushrooms in the world. It is commonly called as *dhingri* in India because of its oyster like shape. Cultivation and popularity of oyster mushroom has been increasing during the last few years due to easy cultivation, high yield potential and high nutritive value. It is an efficient lignin degrading mushroom and can be grown on a variety of agricultural wastes.

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Different species of this mushroom genus can be grown well under variable temperature conditions; hence they are ideally suited for cultivation throughout the year in various tropical to temperate regions of the country. Oyster mushroom is known to modulate the immune system, inhibit tumor growth, reduces inflammation, has hypoglycemic activities, prevent high blood pressure and are known to possess antimicrobial activities (Crisan and Sands 1978). *Pleurotus eous* is one of the important edible species of *Pleurotus* and has attractive pink color and cherished taste, which in turn helps the growers to grasp the attraction of consumers. Its cultivation like other oyster species is easy, cost of cultivation is minimal, has high yield potential and thus has high benefit cost ratio.

2. Material and Methods

The material and methods adopted during the course of present investigation are described under:

Evaluation of different media for the mycelial growth of *Pleurotus eous*:

Five different media were evaluated for their effaces in supporting mycelia growth of *P*leurotus *eous*. The media used were Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Corn Meal Extract Agar, Sabouraud Extract Agar (SEA) and Sawdust Extract Agar. The ingredients and their quantity used in preparation of test media are listed as under:

Potato Dextrose Agar (PDA):

Peeled potatoes = 200 g

Dextrose = 20 g

Agar-agar = 20g

Method of preparation: Peeled potatoes (200g) were cut into small pieces, boiled with 500 ml distilled water and sieved through fine muslin cloth. To this lukewarm potato broth, 20 g of dextrose was added and mixed well using glass rod. Thereafter, 20g of Agar- Agar was added to it and mixed well by stirring. The final volume of the extract was made upto 1000ml and then poured into 500ml flasks. Flasks were plugged with non- absorbent cotton and cotton plugs were covered with butter paper. The flasks were then sterilized in auto-clave at 15 psi for 20 minutes.

Malt Extract Agar (MEA):

Malt = 200 g

Dextrose = 20 g

Agar-Agar = 20 g

Method of preparation: 200g malt, 20g dextrose and 20g agar-agar were taken and added to 1000ml water. This whole mixture was boiled for 10-15 minutes. The mixture was then sieved through fine muslin cloth and poured into 500ml flasks. These flasks were plugged with non- absorbent cotton plugs and the plugs were covered with butter paper and sterilized in an autoclave at 15lbpsi for 20 minutes.

Sabouraud Extract Agar (SEA):

Sourbadose = 200 g

Dextrose = 20 g

Agar-Agar = 20 g

Method of preparation: 200g sabouraud, dextrose 20g and 20g agar –agar were boiled in 1000ml water for 15 minutes. The boiled liquid media were sieved through muslin cloth and transferred into 500ml flask. These flasks were plugged with non- absorbent cotton plug and then were sterilized in an autoclave at 15psi for 20 minutes.

Corn Meal Agar (CMA):

Method of preparation: 17g of corn meal extract agar media (HI MEDIA) was suspended in 1000ml of distilled water. The mixture was heated to boiling to dissolve the medium completely and the mixture was added to 500ml flasks. The flasks were plugged with non-absorbent cotton plugs and the plugs were covered with butter paper and sterilized in an autoclave at 15 psi for 20 minutes.

Saw Dust Meal Extract Agar:

Method of preparation: 200g saw dust was cleaned and boiled with 500ml distilled water and sieved through fine muslin cloth. To this saw dust extract, 20g of dextrose was added and mixed well using glass rod. Thereafter, 20g of agar-agar was added to it and mixed well by stirring. The final volume of the extract was made up to 1000ml and then poured into 500ml flasks. Flasks were plugged with non- absorbent cotton and cotton plugs were covered with butter paper. The flasks were sterilized in autoclave at 15psi for minutes.

Evaluation of Media: Each prepared media was poured under aseptic condition in 90 mm petri-plates and kept for solidification. Bits (5mm) from fourteen days old pure culture of *Pleurotus eous* were cut with cork-borer and inoculated in the centre of solidified petri plates under aseptic conditions in a laminar flow. Each treatment was replicated four times and petri plates were kept in BOD incubator at 28±1°C. The vegetative growth was measured by recording radial growth of the fungus at different time intervals till full growth was achieved in control plates.

Evaluation of different pH for the growth of *Pleurotus eous*:

Malt Extract Agar (media evaluated best for the growth of *Pleurotus* eous) media was poured under aseptic condition in 90 mm petri- plates and kept for solidification. After that, bits (5.0 mm) from fourteen days old culture of *Pleurotus eous* were cut with cork-borer and inoculated in the centre of solidified petri plates aseptically under laminar flow. Each treatment was replicated four times. pH of media was adjusted to 6.0, 6.5, 7.0, 7.5 and 8.0 by addition of NaOH/HCl.

Evaluation of different temperatures for the mycelial growth of *Pleurotus eous*:

Malt Extract Agar (media evaluated best for the growth of *Pleurotus eous*) was poured under aseptic condition in 90.0 mm petri- plates and kept for solidification. Bits (5mm) from fourteen days old culture of *Pleurotus eous* were cut with cork-borer and inoculated in the centre of

solidified petri plates aseptically under laminar flow. Each treatment was replicated four times. These petri- plates were placed in different BOD incubators adjusted at different temperatures *viz*., 16,20,24,28 and 32°C.

3. Results

The results of present investigation "Studies on evaluation of agro wastes for cultivation and nutritional quality of *Pleurotus eous*" are described here under the following heads:

In vitro evaluation of media for growth of *Pleurotus eous*

Solid media evaluation

The data presented in Table No.1 reveals that the test media significantly influenced the mycelial growth of *Pleurotus eous*. The growth of *Pleurotus eous* was highest (90.0 mm) in Malt Extract Agar (MEA) and significantly superior to other media on the 14th day of inoculation followed by Potato Dextrose Agar (PDA) with radial growth of 80.6 mm. However, the mycelial growth on Corn Meal Extract Agar and Sabouraud Extract Agar was 74.5 mm and 71.3 mm respectively. All the treatments were significantly different from each other. However, saw dust extract agar did not elicit any growth even after 14 days of inoculation.

It was observed that the media had also some influence on the colony characteristics of *Pleurotus eous*. On Malt Extract Agar (MEA), the colony was white, strandy with concentric rings and smooth margins. On the contrary, Potato Dextrose Agar (PDA) showed white, fluffy mycelial growth with irregular margins. Corn meal extract medium showed white mycelial growth raised at centre and in concentric rings towards periphery. Sabouraud Extract Agar medium showed white mycelial growth, thin at centre and fluffy towards the periphery. However, no growth was observed in saw dust.

Table 1. Effect of different solid media on growth and colony characteristics of *Pleurotus eous*

Media	Diameter of colony (mm) in days							Colony characters
	2	4	6	8	10	12	14	
Malt Extract Agar (MEA)	6.0	12.1	28.5	43.5	60.8	80.6	90.0	White uniform mycelial growth with smooth margins; strand; in concentric rings.
Potato Dextrose Agar(PDA)	4.0	11.6	26.4	37.1	53.0	75.5	80.6	White fluffy mycelial growth; raised at the periphery than in the centre with irregular margin.
Sabouraud Agar(SA)	2.6	7.8	28.5	31.7	46.1	66.0	71.3	White mycelial growth, thin at centre and fluffy towards the periphery, irregular margin.
Corn meal Extract Agar(CMA)	4.0	11.6	24.5	34.1	50.8	69.0	74.5	White mycelial growth raised at centre and in concentric rings towards periphery.
Sawdust* Extract Agar	0.0	0.0	0.0	0.0	0.0	0.0	0.0	The substance did not registered any growth of the fungus
C.D(p=0.05)	**2.91**	**N.S**	**0.78**	**N.S**	**8.9**	**N.S**	**10.3**	-

*The observations were not taken into consideration for statistical analysis.

Effect of temperature

Observations with regard to the influence of different temperature regimes on mycelial growth of *Pleurotus eous* on Malt Extract Agar has been presented in the Table No.2. The overall view of the Table suggests that the most suitable temperature, observed on 14th day of incubation was 28 °C. At this temperature the radial mycelial growth was 90.0 mm on MEA which was significantly superior to growth observed at other temperatures. The next best temperature observed was 32°C which gave radial mycelial growth of 70.9 mm and was significantly different from other treatments.

Table 2: Effect of different temperature ranges on mycelial growth of *Pleurotus eous*

Temperature (°C)	Diameter of colony (mm) in days						
	2	**4**	**6**	**8**	**10**	**12**	**14**
16	4.6	5.9	13.7	20.8	24.8	29.2	32.5
20	4.6	7.9	15.1	20.8	25.8	29.9	40.6
24	4.6	9.4	18.6	27.1	35.1	52.3	70.3
28	6.0	13.3	21.2	34.4	46.4	67.5	90.0
32	4.2	9.0	18.6	25.8	33.9	52.6	70.0
CD (p=0.05)	0.56	1.13	1.12	2.11	2.09	3.42	4.89

Effect of pH

Data on the effect of different pH levels on mycelial growth of *Pleurotus eous* has been presented in Table No.3 which suggests that there is significant effect of the media pH on the mycelial growth of *Pleurotus eous*. The data reveals that maximum growth of *Pleurotus eous* (90.0 mm) on Malt Extract Agar was obtained at pH 7.5 followed by 8.0 which showed radial mycelial growth of 74.8 mm. Among the treatments evaluated minimum growth of *P. eous* (62.3 mm) was observed at pH 6.0.

Table 3. Effect of different pH levels on mycelial growth of *Pleurotus eous*

pH	Growth of mycelium (mm) in Days						
	2	**4**	**6**	**8**	**10**	**12**	**14**
6.0	6.3	9.4	14.0	26.7	32.3	47.4	58.3
6.5	6.9	11.4	18.7	27.6	35.7	49.2	65.4
7.0	7.3	14.4	26.8	32.3	41.0	54.4	69.5
7.5	8.4	21.9	30.9	46.0	59.1	75.9	90.0
8.0	7.0	16.8	24.5	29.9	39.6	46.7	66.8
CD=(0.05)	**0.98**	**1.24**	**2.38**	**2.43**	**3.51**	**3.11**	**3.42**

4. Discussions

The results of the present investigation "Studies on Evaluation of agro-wastes for cultivation and nutritional quality of *Pleurotus eous*" are discussed here under:

In vitro* evaluation for mycelia growth of *Pleurotus eous

Solid media evaluation

The effect of nutrient media on the mycelial growth of *Pleurotus eous* studied under *in vitro* condition and the results presented in (Table 1) reflect that the different media composition had significant effect on the overall radial mycelial growth of *Pleurotus eous*. Among the five solid media tested, Malt Extract Agar (MEA) registered the maximum radial mycelial growth (90.0 mm). Saw dust extract proved to be ineffective as no growth was observed on it. The above findings are in agreement to the findings of Bilay *et al*., (2000) who reported malt extract agar as the best media for attaining maximum mycelial growth in *Ganoderma lucidum*. Earlier Shukula and Uniyal (1989) and Adaskaveg and Gilbertson (1987) were also of the similar opinion. The variation in the mycelial growth could have been due to preference of mushroom fungus for some specific nutrients present in the malt extract medium. The Saw dust extract agar medium not registering any growth in the present study, could be due to non- selectivity of the medium for the growth of *Pleurotus eous* or presence of some chemicals at a concentration inhibitory to the growth of mushroom fungus.

Effect of temperature

The result (Table 2) obtained during present study with regard to the effect of temperature on myceclial growth of *Pleurotus eous* suggest that

the temperature of 28 °C showed the best mycelial growth while the lowest was at 16 °C. Shan-Hong-Tao *et al.* (2005) were of the same view that best mycelial growth was observed between temperature ranges of 22- 28°C. Lovkesh *et al.* (2006) observed a decreasing trend in the percentage growth inhibition of the *Pleurotus* species with increase or decrease in temperature from 30 °C, maximum inhibition was observed at 30 °C.

Effect of pH

The data on the effect of different pH levels (Table 3) on the mycelia growth of *Pleurotus eous* shows that the different pH levels of 7.5 showed best mycelial growth while lowest was 6.0 pH. Manj *et al.* (1997) was of same view while working on the physiological studies on *Ganoderma lucidum* reported a high pH of 7.5 as suitable pH- level for the growth of the fungus on malt extract agar medium. Singh *et al.* (2000) while working on physico-chemical preferences for efficient mycelial colonization in edible mushroom observed that pH range of 7.0 to 8.0 for *Agaricus* spp., pH of 5.0 to 8.0 for *Pleurotus* spp., pH 6.0 for *A. polytricha* and *V. volvacea*, pH 5.0 to 7.0 for *L. edodes* and pH 6.0 to 7.0 for *M. esculenta*.

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