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Evaluation of Physiological Parameters and Antimicrobial Properties of *Pleurotus eous*

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ABSTRACT

The present investigation was carried out during September, 2021–March, 2022 at the laboratory of Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India to study physiological parameters and antimicrobial properties of *Pleurotus eous.* Out of seven different solid and liquid media under study, malt extract medium was found to be the best with maximum diametric growth (83.25 mm), growth rate (13.04 mm day^{-1}) and biomass (1591.47 mg) however, minimum diametric growth (49.57 mm), growth rate (7.43 mm day^{-1}) and biomass (89.83 mg) was recorded in yeast malt media. Among nine different pH levels under study, the maximum diametric growth (83.25 mm) and growth rate (13.04 mm day^{-1}) was recorded at 7.5 pH level while, minimum diametric growth (60.92 mm) and growth rate (9.32 mm day^{-1}) was recorded at pH level 9.0. Further, 25°C was found to be the best for the maximum diametric growth (82.25 mm) and growth rate (12.88 mm day^{-1}) of *P.eous* and minimum diametric growth (14.77 mm) and growth rate (1.55 mm day^{-1}) was recorded at 35°C among different temperature regimes under study. Among different nitrogen, carbon sources and trace elements under study, sodium nitrate (114.04 mg), starch (171.82 mg) and ferrous sulphate (115.60 mg) respectively, were found to be the best with highest biomass while minimum biomass among different nitrogen, carbon sources and trace elements was recorded in glycine (36.13 mg), maltose (82.67 mg) and ammonium molybdate (23.77 mg), respectively.

KEYWORDS: *Pleurotus eous,* cultural parameters

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Pleurotus eous which is commonly referred to pink oyster mushroom or dhingri is considered as one of the best and economically important mushrooms belonging to class Basidiomycetes, subclass Holobasdiomycetidae, order Agaricales and family Pleurotaceae. As it contains high protein, low fat and cholesterol content, high vitamins and minerals, *P. eous* is considered as functional food (Kortei and Waife-K-Wagyer, 2015). These are widely considered as a future vegetable due to their medicinal and nutritional properties and also, consumer demands are increasing day by day (Sharma et al., 2017). It is widely cultivated in Asia, America and Europe due to their low cost of production and high biological efficiency (Hoa and Wang, 2015). There are different species of this genus which are being cultivated like *P. ostreatus*, *P. florida*, *P. cornucopiae*, *P. eryngii*, *P. pulmonarius*, *P. eous* etc (Jatwa et al., 2016). At present total mushroom production, in India, is 2.68 lakhs tons (Anonymous, 2021). Oyster mushrooms are the third largest produced mushroom in the world while, ranked at second place in India (Thakur, 2014, 2020). In the present time, production of this crop in India is around 1500 tons (Anonymous, 2019). In Himachal Pradesh, total mushroom production in 2016–2017 was 9150 metric tons out of which, oyster mushroom contributed 110 metric tons (Sharma et al., 2017). The physiological and nutritional studies become more vital factor in case of mushrooms, as success or failure in their cultivation is mainly dependent on the clear and correct understanding of nutritional and environmental needs (Lilly and Barnett, 1951). Mushroom growth is highly influenced by many factors such as growing media, temperature, moisture content and light intensity as well as pH (Kadiri and Keinde, 1999). In different physiological studies, malt extract medium and potato dextrose medium was found to be the best for mycelial growth and biomass production of *Pleurotus* spp (Eswaran and Ramabadran, 2000, Singh and Kapoor, 2016, Aziz et al., 2018). A range of 6.0–7.5 pH levels was found to be the best for mycelial growth of *Pleurotus* spp. (Sardar et al., 2015 and Ahmed et al., 2019) and also different temperature regimes ranges from 20–30°C was found to be the best for its mycelial growth. The different nitrogen sources, carbon sources and trace elements also play important role in mycelial growth of *Pleurotus* spp. potassium nitrate, Peptone and ammonium chloride were the best nitrogen sources for the its growth. Among different carbon sources, glucose, starch and sucrose were found to be the best carbon sources in different investigations. Similarly, Boron and copper sulphate were the best trace elements for mycelial growth of *Pleurotus* spp. (Rana and Dahot (2017), Hoa and Wang (2015) and Debnath et al. (2021)). Natural compounds with biological activities are normally present in plants, mushrooms and other natural sources. To survive in natural habitat, mushrooms need antibacterial and antifungal compounds. Therefore, antifungal compounds with more or less strong activities could be isolated from many mushroom species (Yamac and Bilgili, 2006). Thus, mushroom exhibit antifungal activities against different phytopathogenic fungi (Smania et al., 2007). In agriculture, fungal pathogens bring about serious reduction in yield and quality of crops leading to huge economic losses. Thus, research on antifungal properties of mushrooms may help to isolate the compounds and introduce genes encoding them into the plants thus boosting their resistance (Chu et al., 2005) and (Wong et al., 2010). The different *Pleurotus* spp. shows anitimicrobial activity against different pathogenic fungi such as *Fusarium oxysporum*, *Aspergillus fumigatus*, *Botrytis cinerea*, *Bacillus* spp. and *Streptococcus* spp. (Wang and Ng, 2004, Chu et al., 2005 and Bastida et al., 2016).

2. MATERIALS AND METHODS

The experiment was conducted during 2021–2022 (September, 2021–March, 2022) at College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India.

2.1. Evaluation of basal medium

Seven different solid as well as nutrient media viz., potato dextrose medium, malt extract medium, Czapek's Dox medium, Hopkin's medium, Lily medium, yeast malt medium and mushroom complete medium were evaluated for mycelial growth and biomass production of *P. eous*. A culture bit of test fungus (5 mm dia.) was taken and place it on solidified media and flasks of 250 ml with 100 ml of broth were inoculated with culture bit (5 mm dia.) of actively growing mycelium of *P. eous* from pure culture. After inoculation, these Petri plates and flasks were incubated in a BOD incubator at 25±1°C and observations were recorded in terms of diametric growth (mm), colour of mycelium and type of growth after 6 days of incubation in solid media and dry mycelial weight of test fungus was recorded after 14 days of inoculation.

Growth rate (mm day^{-1}) was further calculated as per following formula: $r_g = d_g t_2 - d_g t_1 / t_2 - t_1$ Where, $d_g t_2$=Diametric growth (mm) at time t_2 and $d_g t_1$=Diametric growth (mm) at time t_1.

The dry weight of the test fungus was calculated by using the following formula:

Dry weight=Weight of filter paper+mycelium-Weight of filter paper+mycelium

Based on these studies, best nutrient medium was selected for further experiments.

2.2. Effect of different pH levels on mycelial growth of P. eous

To see the effect of different pH levels study viz., 5.0, 5.5,

6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 on the mycelial growth of *P. eous*, the best solid medium selected under 2.1 was adjusted to different pH levels under with the help of a pH meter and poured in the Petri plates after autoclaving. These Petri plates were then inoculated with test fungus (5 mm dia.) and incubated in the BOD incubator at 25±1°C. The data were recorded as per in 2.1.

2.3. Effect of temperature regimes on mycelial growth of P. eous

The growth of *P. eous* was studied at different temperature regimes viz., 15, 18, 22, 25, 30 and 35°C. Petri plates containing basal medium (2.1) adjusted to best pH (2.2) were inoculated with pure culture bit (5 mm dia.) of the test fungus. The Petri plates were incubated at different test temperature regimes in BOD incubator. The data were recorded as per in 2.1.

2.4. Effect of different nitrogen, carbon sources and trace element on biomass of P. eous

To determine the effect of different nitrogen sources, carbon sources and trace elements, Czapek's Dox broth was used as basal medium. Different nitrogen, carbon and trace elements were substituted with sodium nitrate, sucrose and ferrous sulphate, respectively, in the basal medium on the basis of their respective molecular weight, so as to provide equal amount of nitrogen. Hundred ml of broth was put in the Erlenmeyer flasks of 150 ml capacity and inoculated with a culture bit (5 mm dia.) of test fungus and the broths were then incubated at best temperature (2.3) in BOD incubator up to 14 days. The data were recorded in terms of average dry weight (mg) of mycelium of *P. eous* after 7 and 14 days of inoculation.

2.5. Antimicrobial properties

To see the antimicrobial properties of test fungus, interaction between *P. eous* and different phytopathogenic fungi was studied by growing these fungi in dual culture. The per cent inhibition of *P. eous* was worked out, keeping in view the growth of test pathogens towards and away from test fungus in dual culture by using the formula:

Inhibition (%)=A-T/A×100 Where: A=radial growth (mm) of *P. eous* away from test pathogen in dual culture. T=radial growth (mm) of *P. eous* towards test pathogen in dual culture.

3. RESULTS AND DISCUSSION

3.1. Effect of different nutrient media on myceilal growth of Pleurous eous

Growth media provides important nutrients for the mycelial growth of test fungus. Among the different nutrient media studied viz., PDA (Potato dextrose agar), MEA (Malt extract agar), CDA (Czapek's Dox agar), HA (Hopkin's agar), LA (Lily Agar), YMA (Yeast Malt Agar) and MCM (Mushroom complete agar) the maximum diametric growth (83.25 mm) and growth rate (13.04 mm day^{-1}) was recorded on MEA (malt extract agar) while minimum mycelial growth (49.57 mm) as well as growth rate (7.43 mm day^{-1}) was recorded in YMA (yeast malt agar) after 6 days of incubation (Table 1, Figure 1). As far as its mycelial colour and type of growth were concerned, the colour of mycelium varied from transparent to white and type of growth varied from sparse, cottony and dense to cottony and fluffy. The transparent mycelium was observed in HA and YMA and in rest of the media the colour of mycelium was white. Further, sparse growth was recorded in HA, YMA and MCM, cottony and dense were recorded in MEA and in rest of the media cottony and fluffy growth was recorded (Table 1, Figure 1). As far as biomass production was concerned the maximum biomass was recorded in malt extract broth (1591.47 mg) and minimum was recorded in yeast malt broth (89.83 mg)

Table 1: Effect of different solid and liquid media on various growth parameters of *Pleurotus eous*

Nutrient medium	Diametric growth (mm) after 6 days of incubation	Growth rate (mm day^{-1})	Type of growth	Colour of mycelium	Biomass after 14 days of inoculation
Potato dextrose agar	81.83	12.81	Cottony and fluffy	White	1323.40
Malt extract agar	83.25	13.04	Cottony and dense	White	1591.47
Czapek's Dox agar	66.00	10.17	Cottony and fluffy	White	115.80
Hopkin's agar	65.03	10.03	Sparse	Transparent	954.17
Lily agar	81.50	12.75	Cottony and fluffy	White	1222.73
Yeast malt agar	49.57	7.43	Sparse	Transparent	89.83
Mushroom complete agar	68.08	10.51	Sparse	White	358.57
CD ($p \geq 0.05$)	2.05	0.70			2.08
SEd±	0.95	0.32			0.96

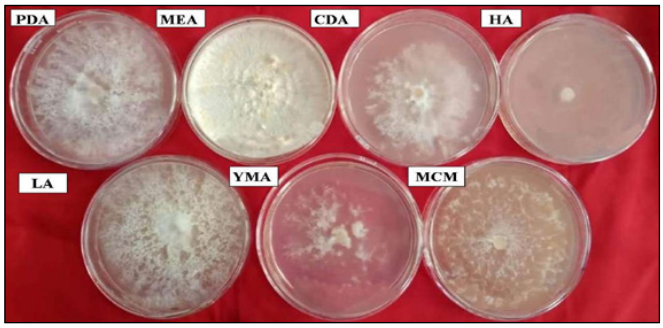

Figure 1: Comparative mycelial growth of *Pleurotus eous* on different media

after 14 days of incubation. This study revealed that MEA is the best nutrient media for the mycelial growth of *P. eous*. The present results are in conformity with findings of Nasim et al. (2001), Asghar et al. (2007), Dhiman (2009), Aziz et al. (2018) and Maurya et al. (2019) who also reported malt extract agar to be the best for mycelial growth of *Pleurotus* spp.

3.2. Effect of different pH levels on mycelial growth of Pleurotus eous

pH plays a major role for the growth of mycelium. After 6 days of incubation the maximum mycelial growth (83.25 mm) and growth rate (13.04 mm day^{-1}) was recorded at pH level 7.5 and minimum mycelial growth (60.92 mm) and growth rate (9.32 mm day^{-1}) was recorded at pH level 9.0 after 6 days of incubation (Table 2, Figure 2). As far as the colour and type of growth of mycelium were concerned the colour of mycelium was white in all the treatments. The type of growth was fluffy with irregular margins at pH level 5.0, 6.0, 6.5, 7.0, 7.5 and 8.0 and Normal with irregular margins at pH levels 5.5, 8.0 and 9.0 (Table 2, Figure 2). This study revealed that pH level 7.5 is optimum for mycelial growth of *P. eous*. The present findings are supported by the findings of Rawte and Diwan (2011) and Khan et al. (2013) who also reported pH 7.5 to be the optimum for the growth of *Pleurotus spp*.

Figure 2: Comparative mycelial growth of *Pleurotus eous* on different pH levels

Table 2: Effect of different pH levels on various growth parameters of *Pleurotus eous*

pH levels	Average diametric growth (mm) after 6 days of incubation	Growth rate (mm day^{-1})	Type of growth	Colour of mycelium
5.0	68.17	10.53	Fluffy mycelial growth with irregular margins	White
5.5	71.58	11.10	Normal mycelial growth with irregular margins	White
6.0	69.92	10.82	Fluffy mycelial growth with irregular margins	White
6.5	67.42	10.40	Fluffy mycelial growth with irregular margins	White
7.0	69.75	10.79	Fluffy mycelial growth with irregular margins	White
7.5	83.25	13.04	Fluffy mycelial growth with irregular margins	White
8.0	74.08	11.51	Normal mycelial growth and irregular margins	White
8.5	74.83	11.64	Fluffy mycelial growth with irregular margins	White
9.0	60.92	9.32	Normal mycelial growth with irregular margins	White
CD ($p \geq 0.05$)	1.35	0.76		
SEd±	0.64	0.36		

3.3. Effect of different temperature regimes levels on mycelial growth of Pleurotus eous.

Six different temperature regimes were evaluated for the mycelial growth of *P. eous* and maximum mycelial growth (82.25 mm) and growth rate (4.16 mm day^{-1}) was recorded on 25°C however, the minimum mycelial growth (14.77 mm) as well as growth rate (1.55 mm day^{-1}) was recorded on 35°C after 6 days of incubation (Table 3, Figure 3). Further, the type of growth and colour of mycelium was observed. The type of growth varied from ring to ray pattern with regular and irregular margins. Circular pattern was observed at 15°C however ray pattern was observed at 18°C, 22°C, 30°C and 35°C. The colour of mycelium was snow white at 15°C and 35°C and it was white at 18°C, 22°C, 25°C and 30°C (Table 3, Figure 3). This study revealed that 25°C was optimum temperature for the mycelial growth of *P. eous*. These findings are further strengthened by the work of Sardar et al. (2015), Soylu et al. (2014), Aziz et al. (2018) and Ahmed et al. (2019) who also reported 25°C to be the best temperature for mycelial growth of different *Pleurotus* spp.

Table 3: Effect of different temperature regimes on various growth parameters of *Pleurotus eous*

Temperature (°C)	Average diametric growth (mm) after 6 days of incubation	Growth rate (mm day^{-1})	Type of growth	Colour of mycelium
15	19.69	2.45	Irregular growth with circular rings pattern	Snow white
18	29.94	4.16	Thin, regular growth with ray pattern	White
22	28.31	3.89	Regular growth with the ray pattern	White
25	82.25	12.88	Regular growth with ray pattern	White
30	32.31	4.55	Thin, regular growth with ray pattern	White
35	14.77	1.55	Regular growth with ray pattern	Snow white
CD ($p \geq 0.05$)	1.24	0.83		
SEd±	0.56	0.38		

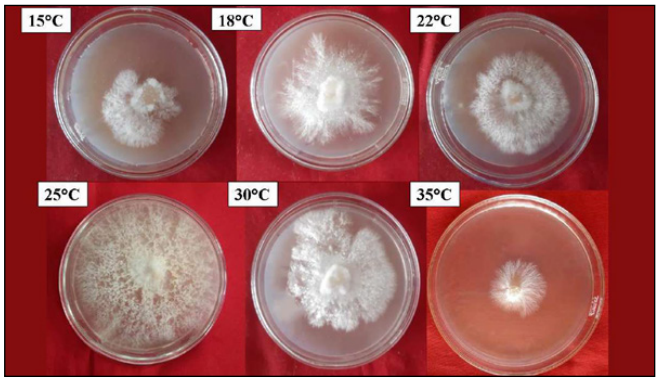

Figure 3: Comparative mycelial growth of *Pleurotus eous* at different temperature regimes

3.4. Effect of different nutritional sources on biomass production of Pleurotus eous

It is evident from the data presented in Table 4. that among different nitrogen sources under study, the highest biomass production (114.04 mg) was recorded when sodium nitrate was used as nitrogen sources and minimum was recorded in glycine (36.13 mg). Out of seven carbon sources under investigation, the highest biomass (171.82 mg) was recorded when starch was used as carbon source and minimum was recorded in maltose (82.67 mg). Further, ferrous sulphate was found to be the best trace element among five different trace elements with maximum biomass production (115.60 mg) while, minimum was recorded in ammonium molybdate (23.77 mg). This study revealed that sodium nitrate, glycine and starch were found to be the best nitrogen, carbon and trace elements, respectively. Additionally, these findings are further strengthened by the work of Eswaran and Ramabadran (2000), Hoa and Wang (2015) and Debnath et al. (2021).

3.5. Antimicrobial activities of Pleurotus eous against different pathogenic fungi

A perusal of the data presented in Table 5 reveals that the growth of all test pathogens except *Rhizopus* spp. was inhibited by *P. eous*. Among all the pathogens, growth of *F. solani* was inhibited upto significantly maximum extent (66.96%) followed by that of *P. psidii* (59.95%), *C. fructicola* (57.90%) and *A. flavus* (49.55%) by *P. eous*. However, no inhibition was found against *Rhizopus* spp. Our findings are more and less similar with Ngai and Ng (2004), Wang and Ng (2004), Chu et al. (2005) and Adedeji and Aduramigbo (2016) who reported antifungal properties of different *Pleurotus* spp. against different phytopathogenic fungi (Figure 4).

Table 4: Effect of different nutritional sources on biomass production of *Pleurotus eous*

Nitrogen Sources	Biomass production after 14 days of incubation	Carbon Sources	Biomass production after 14 days of incubation	Trace elements	Biomass production after 14 days of incubation
Ammonium sulphate	90.19	Dextrose	127.89	Ammonium molybdate	23.77
Asparagine	62.53	Fructose	136.10	Cupric sulphate	47.43
Potassium nitrate	49.03	Glucose	144.33	Ferrous sulphate	115.60
Sodium nitrate	114.04	Maltose	82.67	Magnesium sulphate	44.52
Urea	67.83	Mannitol	150.83	Zinc sulphate	48.29
Glycine	36.13	Sucrose	117.27		
		Starch	171.82		
CD ($p \geq 0.05$)	0.887	CD ($p \geq 0.05$)	0.90	CD ($p \geq 0.05$)	1.55
SEd±	0.403	SEd±	0.42	SEd±	0.69

Table 5: Antimicrobial activities of *Pleurotus eous* against different pathogenic fungi

Pathogenic fungi	(%) Inhibition
Aspergillus flavus	49.55(44.72)
Colletotrichum fructicola	57.90 (49.53)
Fusarium solani	66.96 (54.89)
Pestalotiopsis psidii	59.95 (50.72)
Rhizopus spp.	0.00 (0.00)*
CD ($p \geq 0.05$)	1.37
SEd±	0.59

Figures in parenthesis represents angular transformed value; *Not considered in analysis

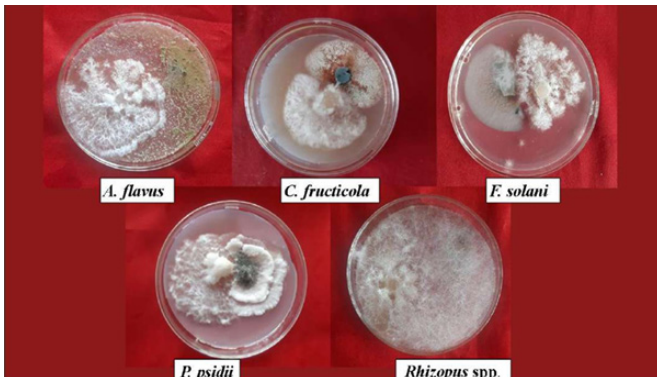

Figure 4: Antimicrobial activities of *Pleurotus eous* against different pathogenic fungi

4. CONCLUSION

The physiological studies of *Pleurotus eous* revealed that malt extract medium was best for the mycelial growth and biomass production. The temperature of 25°C and pH level of 7.5 was found to be the best for its growth. Further, sodium nitrate, glycine and starch were found to be the best nitrogen, carbon and trace elements, respectively. As far as its antimicrobial properties were concerned it showed highest inhibition against *F. solani*, however no inhibition was recorded against *Rhizopus* spp.

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