



The Effect of Growth Conditions on Mycelial Run of Oyster Mushrooms spp. (*Pleurotus* spp.): Implication for Agricultural Practices

Kartik Charan Lenka^{#1}, Bandana Padhan^{#2}, Naina Pradhan³,
Truptimayee Mantry¹, Romalin Sahu⁴, S. Venkatlaxmi⁴

10.18805/BKAP470

ABSTRACT

Background: The *Pleurotus* genus is one of the most investigated white-rot fungus due to its excellent ligninolytic characteristics. It is a tasty mushroom that also has a number of biological impacts due to the presence of essential bioactive compounds. Many usual fermentation parameters affect lignocellulolytic enzymes in basidiomycete fungi, such as medium composition, carbon-to-nitrogen ratio, pH, temperature, air composition etc. The survival and multiplication of *Pleurotus* mushrooms is dependent on a number of factors, which may function independently or interactive effect on the growth of mushroom. Grasp the difficulty of treating *Pleurotus* species mushrooms necessitates a fundamental understanding of their physical, chemical and biological characteristics.

Methods: In this study, the effects of culture media, temperature, carbon sources, nitrogen sources, grain sources and agricultural waste as lignocellulosic substrate on the mycelium growth of five species of *Pleurotus* species for spawn production were studied. Several trials were designed to evaluate factors affecting mycelium growth of five oyster mushroom species. The experiments were conducted during October to February in the year 2019 to 2021. The experiments were carried out in three replications per treatment.

Result: The results of the experiment indicated that potato dextrose agar (PDA) and Malt extract agar (MEA) were the most suitable media for the mycelium growth of all oyster mushrooms. The optimal temperature for mycelium growth of all the oyster mushroom species was obtained at 25±2°C. Mycelium growth of all the studied oyster mushroom was improved by carbon sources such as dextrose and glucose. The nitrogen source such as ammonium chloride and ammonium sulfate also gave the greatest values in mycelium colony diameter of mushroom. Wheat was found to be favourable for mycelium growth of all the oyster mushroom species. In addition, paddy straw and sugarcane residue were selected as suitable lignocellulosic substrate sources for mycelium growth of all the studied oyster mushrooms.

Key words: Biological efficiency, Mycelium diameter, Nutritional condition, Oyster mushroom, *Pleurotus* spp.

INTRODUCTION

To diversify present-day agriculture as well as to search for alternative food and feed ingredients has the potential to address food insecurity (Janardhanan *et al.*, 2003). In the developed countries, mushrooms occupy a vital role in the field of horticulture due to the presence of high nutritive component including protein, minerals and vitamins as well as natural biopolymers having medicinal properties (Pushpa and Purushothama, 2010; Egwim *et al.*, 2011; Atri *et al.*, 2012). Besides their nutritional value mushrooms are good source of several bioactive compounds having pharmacological properties such as hypoglycemic, immunomodulatory, anti-inflammatory, antitumor, antiviral, antibacterial or antiparasitic activities (Wasser and Weis, 1999). More than 2000 species of fungi are reported to be edible throughout the world, out of which about 283 species are reported to be available in India (Purkayastha and Chandra, 1985). Over 200 species of mushrooms have been utilized as functional foods around the world for a long time (Bellettini *et al.*, 2019), but only about 35 have been commercially produced (Bellettini *et al.*, 2019). They are high in nutrients, including proteins, minerals and vitamins B, C and D. (Bellettini *et al.*, 2019). Mushrooms have a protein content of 20-35 per cent

¹M.S. Swaminathan Research Foundation, Jeypore-764 002, Odisha, India.

²Department of Biotechnology, School of Life Science and Biotechnology, Adamas University, Kolkata-700 126, West Bengal, India.

³Department of Zoology, Khariar Autonomous College, Khariar, Nuapada-766 107, Odisha, India.

⁴Department of Botany and Biotechnology, Khallikote Autonomous College, Berhamapur-761 008, Odisha, India.

[#]These two authors have equal contribution.

Corresponding Author: Bandana Padhan, Department of Biotechnology, School of Life Science and Biotechnology, Adamas University, Kolkata-700 126, West Bengal, India.
Email: miki.bandana@gmail.com

How to cite this article: Lenka, K.C., Padhan, B., Pradhan, N., Mantry, T., Sahu, R. and Venkatlaxmi, S. (2022). The Effect of Growth Conditions on Mycelial Run of Oyster Mushrooms spp. (*Pleurotus* spp.): Implication for Agricultural Practices. Bhartiya Krishi Anusandhan Patrika. 37(2): 137-143. DOI: 10.18805/BKAP470.

Submitted: 21-02-2022 **Accepted:** 12-05-2022 **Online:** 15-06-2022

(dry weight), are low in fats and include all nine necessary amino acids (Bellettini *et al.*, 2019). Mushrooms are delicacy food item known for its unique texture upon biting and delicious flavour. Because of their bioactive contents, they

have gotten a lot of attention from culinary and pharmaceutical experts (Bellettini *et al.*, 2019).

Oyster mushroom (*Pleurotus* spp.), family of Tricholomataceae belongs to a group known as “white rot fungi” (Tsujiyama and Ueno, 2013). It is the second widely cultivated mushroom worldwide followed by the *Agaricus bisporus* (Kües and Liu, 2000) which usually grows naturally on dead trees at spring season (Lee, 1993). *Pleurotus* species are most cultivated mushroom due to their simple, low cost production technology and high biological efficiency (Mane *et al.*, 2007). *Pleurotus ostreatus* (oyster mushroom), *P. eryngii* (king oyster or Cardoncello), *P. pulmonarius* (phenix mushroom), *P. djamor* (pink oyster mushroom), *P. sajor-caju* (indian oyster), *P. cystidiosus* (abalone oyster), *P. citrinopieatus* (golden oyster mushroom) and *P. cornucopiae* are all commercially cultivated *Pleurotus* (Bellettini *et al.*, 2019). In comparison to other mushrooms, *Pleurotus* species grow quickly. Its fruiting body is resistant to diseases and pests and it can be produced in a simple and inexpensive manner, producing a high yield, a wider substrate usage, sporelessness, a wide range of temperature and chemical tolerance and environmental bioremediation (Bellettini *et al.*, 2019). *Pleurotus* species are efficient to degrade the wide range of lignocellulosic materials including agricultural wastes and other wastes which are produced in agricultural, forest and food-processing industries (Sánchez, 2010). The oyster mushrooms contain high rate of minerals including potassium to sodium, which will be an ideal food for patients suffering from hypertension and heart diseases (Dehariya *et al.*, 2013). Several species of oyster mushrooms have pharmacological properties including antioxidant activity (Li *et al.*, 2007), antitumor activity (Chorváthová *et al.*, 1993) and antidiabetic properties (Chorváthová *et al.*, 1993). These medicinal values of *Pleurotus* species are due to the chemical composition or nutrition of these mushrooms. Though, mushrooms are demonstrated as potential source of many bioactive compounds, large scale production is the major constraints in order to fulfill the huge requirement of bioactive materials. However, fungal mycelium of the mushroom are the best source for production of extracellular and intracellular bioactive compounds useful for formulation of nutraceutical drugs.

The growth of mushroom related to a number of factors including chemical composition, water activity, ratio of carbon to nitrogen, minerals, surfactant, pH, moisture, sources of nitrogen, particle size and amount of inoculum, which are linked to mushroom production (Eira, 2003). The process of oyster mushrooms cultivation include 3 mainsteps: isolation of good quality mycelium from fruiting bodies of

mushroom, preparation of spawn and cultivating mushrooms from these spawns in suitable substrate (Dung, 2003). The aims of this study are to study the effect of temperature and nutritional conditions for the mycelium growth of five oyster mushroom species and their cheapest way to produce mushroom spawn. This study will provide useful synthetic information which may help different users associated with farming and agro-food industry. An in-depth understanding of the technical features is needed for an appropriate and efficient production of *Pleurotus* spp.

MATERIALS AND METHODS

The effects of culture media, temperature, carbon sources, nitrogen sources, grain sources and agricultural waste as lignocellulosic substrate on the mycelium growth of five species of *pleurotus* species for spawn production were studied. Several trials were designed to evaluate factors affecting mycelium growth of five oyster mushroom species.

Collection of materials

The mother culture of five different *Pleurotus* species such as *P. sojarcaju*, *P. florida*, *P. ostreatus*, *P. eous* and *P. eryngii* were obtained from Centre of Tropical Mushroom Research and Training, Department of Plant Pathology, College of Agriculture, Orissa University of Agriculture and Technology in Bhubaneswar and maintained in potato dextrose agar medium (PDA). The different food grains (paddy, millet, corn and wheat), agricultural lingo cellulose substrates (paddy straw, sugarcane residues, corn cob and millet straw) were obtained from the local farmers. The experiments were conducted in mushroom spawn production laboratory of M. S. Swaminathan Research Foundation in Jeypore during October to February in the year 2019 to 2021. The experiments were carried out in three replications per treatment.

Temperature and nutritional conditions affecting the growth of mycelium

The effects of culture media, temperature, carbon sources, nitrogen sources, grain sources, lignocellulosic substrate on the mycelium growth of five species of *Pleurotus* were studied by the method of Hoa and Wang (2015) with some modifications. Several trials were designed to evaluate the factors affecting mycelium growth of *Pleurotus* species. For each test, mycelium colony diameter was measured.

Culture media

Four types of culture media were used such as, potato dextrose medium (PDA), yeast malt extract medium (YMA), malt extract Agar medium (MEA) and Sabouraud dextrose medium (SDA). The ingredients for the culture media are

Table 1: Details of the different culture media used for the growth study of oyster mushrooms.

Culture media	Media composition (per ltr.)
PDA (Potato dextrose agar medium)	Potato-200 g, Dextrose-20 g, Agar-20 g
MEA (Malt extract agar medium)	Malt extract-30 g, Agar-20 g
YMA (Yeast malt agar medium)	Malt-20 g, Yeast-2 g, Agar-20 g
SDA (Sabouraud's dextrose agar medium)	Dextrose-40 g, Agar-20 g, Peptone-10 g

presented in Table 1. Media and petri dishes (10-cm diameter) were autoclaved at 121°C (at 15 psi pressure) for 20 min. The inoculated petri dishes with each *Pleurotus* species were incubated under aseptic condition at 28±2°C temperature in the darkness. The diameter of the mycelium expansion was measured after 8-10 days by using a transparent scale.

Temperature

The petri dishes containing sterilized PDA medium (20 ml) were inoculated with the mycelium discs (1-cm diameter) of each *Pleurotus* spp. under aseptic condition and incubated in the darkness at four different temperature (25°C, 27°C, 30°C and 35°C) in bacteriological incubation. The diameter of the mycelium expansion was measured.

Carbon sources

Potato agar (PA) medium containing different carbon sources (glucose, dextrose, maltose and sucrose) at 2% concentration was used in the experiment in order to identify the appropriate carbon source for the *Pleurotus* species. Influences of different concentrations of carbon source on mycelium growth were investigated.

Nitrogen sources

Potato dextrose agar (PDA) medium containing different nitrogen sources such as 0.05% ammonium chloride (NH₄Cl), 0.05% ammonium sulfate (N₂H₈SO₄), 1% peptone, 1% malt extract and 1% yeast extract were tested. Influences of different nitrogen sources on mycelium growth of the *Pleurotus* species were investigated.

Different grain sources for spawn production

Four different grains such as corn, millet, paddy and wheat grain were used for production of spawn of *Pleurotus* species. The grains were washed in clean water three times to remove chaff and dust. The grains were then soaked in water for 8 hr for maximum absorption of water. Soaked grains were again washed and cooked until the grains became soft. The cooked grains were cooled in air for excess removal of water. The grains were mixed with chalk powder (20 g per kg grain) and filled the grains in cylindrical (25 cm height) bottles and closed the mouth with cotton plug. Then the bottles were autoclaved for 2 hr at 15 psi pressure. After cooling, each bottle was inoculated with 1 cm mycelium disc of each *Pleurotus* species under aseptic condition and was incubated at 28°C under dark condition. The diameter of the mycelium expansion was measured after one week.

Different lignocellulosic substrate sources for spawn run

Four different agricultural wastes such as sugarcane residue, corn cob, paddy straw and millet straw were investigated. The agricultural wastes were copped into pieces and soaked in water for overnight. Then it was packed in autoclavable polybags and autoclaved for 3-4 h at 15 psi pressure. After cooling, each polybags was inoculated with mycelium disc (1 cm) of each *Pleurotus* species under aseptic condition and was incubated at 28°C under dark condition. The mycelium run was measured after 8 days.

Yield of mushroom

The five different oyster mushroom was cultivated using paddy straw as the substrate in a poly bag methods of ICAR manual for cultivation technology of oyster mushroom. The yield per bag was evaluated after harvesting the mushroom in one month and pooled data were presented.

Statistical analysis

All parameters were analyzed by two-way analysis of variance (ANOVA) with the variety and different treatment levels. Differences between various parameters were compared by ANOVA with Duncan's multiple range tests to compare the mean significant differences ($p < 0.05$) among treatments by using SPSS (16.0) software.

RESULTS AND DISCUSSION

Effect of different culture media on the mycelium growth

The growth of the mushroom depends on different composition of nutrient medium which plays an important role for mycelia expansion and reproduction. In order to culture the oyster mushrooms *in vitro*, it is necessary to provide such compounds in the media which are requires for its growth and other life process. In this present study, five *Pleurotus* species (*P. sojarcaju*, *P. florida*, *P. ostreatus*, *P. eous* and *P. eryngii*) were cultured successfully in an aseptic condition. During the present investigation, different solid culture media, i.e., potato dextrose agar, malt extract agar, yeast malt extract and sabouraud dextrose medium were used to identify their effect on mycelium growth. There was significant difference found in relation to the mycelium diameter of these five mushrooms on four different media.

The average mycelium diameter of five oyster mushroom species ranged from 6.60 to 9.53 cm at 8 days after inoculation. The mycelium growth of all the *Pleurotus* species on PDA followed by SDA and MEA media showed better growth than the other media. The result indicated that out of these four cultural media, PDA and SDA media more suitable for the mycelium growth of oyster mushroom. This may be due to the availability of required nutrients for the growth and development of the mushroom. Earlier researchers has been reported that potato dextrose agar was suitable media for culture of *Pleurotus* species to achieve high level mycelium biomass, exopolysaccharides and mycelium protein (Mshandete and Mgonja, 2009; Mansur *et al.*, 2012; Hoa and Wang, 2015). In Odisha, potato is cheaper than other raw materials of other culture media, so PDA medium was more suitable and efficient to use as alternatives for culture of these *Pleurotus* species.

Effect of temperature on mycelium growth

Environmental temperature plays a very important role for the mycelium growth of fungi (Hoa and Wang, 2015). The five species of *Pleurotus* were cultivated in PDA medium at various range of temperatures (25°C, 27°C, 30°C and 35°C) in order to determine the optimal temperature for mycelium growth. The optimum temperature for all the oyster

mushroom species was found to be 25°C to 27°C (Table 2). The mycelium growth of mushroom *P. ostreatus* and *P. florida* was significantly faster than the other species of mushroom at 25°C where as at 27°C the growth of *P. eous* was more. It was observed that the growth of mycelium of all the mushroom was decrease with the increase of environmental temperature. The finding of the present study was quite similar to the earlier reports by Kashangura, 2008, Choi *et al.*, 2003; Neelam *et al.*, 2013; Hoa and Wang, 2015 where they indicated that the optimum temperature for mycelium growth of different oyster mushroom is 25-30°C. This result indicated that all the species of oyster mushroom can able to grow better during winter and autumn season in Odisha which shows potential opportunity to develop oyster mushroom production in the country.

Effect of carbon sources on mycelium growth

Carbon source in the form of carbohydrates, plays key role as structural and storage compounds in cell. Many

mushrooms grow in wide range of carbon sources. In the present study, the suitability of various carbon sources on mycelium growth of five oyster mushrooms was studied (Table 2). Different types of carbon sources (2% concentration) including glucose, dextrose, maltose and sucrose were added into the PDA basal medium. The result indicated that there was a significant difference in mycelium growth of all the oyster mushroom grown on various carbon sources except dextrose and glucose. Among four carbon sources tested, dextrose, glucose and sucrose were favorable to the mycelium growth of oyster mushroom (Table 2). The highest mycelium diameters of all the oyster mushroom were obtained from the media containing dextrose followed by glucose. This finding was contrary with the observation of Fu *et al.*, 2013 where sucrose was suitable for the fungal growth of *Villosiclava virens*. Neelam *et al.*, (2013) and Mao *et al.*, (2005) reported that dextrose and glucose was the best carbon source for mycelium growth of *P. florida* and *Cordyceps militaris* respectively. The preference of dextrose,

Table 2: Effect of different culture conditions on the mycelium growth of five oyster mushrooms.

Species	Culture medium				Temperature (°C)			
	PDA	MEA	YMA	SDA	25	27	30	35
<i>P. sojarcaju</i>	9.31 ^b	8.52 ^{a*}	6.99 ^{b*}	8.52 ^{a*}	8.29 ^a	8.05 ^a	7.18 ^{b*}	6.27 ^{a*}
<i>P. florida</i>	9.52 ^a	7.16 ^{b*}	6.53 ^{b*}	9.58 ^{a*}	8.40 ^a	8.07 ^{a*}	7.16 ^{b*}	6.12 ^{b*}
<i>P. ostreatus</i>	9.06 ^a	7.53 ^{b*}	6.05 ^{c*}	9.49 ^{a*}	8.47 ^a	8.31 ^a	7.08 ^{b*}	6.06 ^{b*}
<i>P. eous</i>	9.08 ^a	9.53 ^{a*}	6.52 ^{b*}	9.06 ^{a*}	8.06 ^a	8.16 ^{a*}	6.64 ^{c*}	6.47 ^{b*}
<i>P. eryngii</i>	9.30 ^b	9.20 ^{a*}	7.68 ^{a*}	9.33 ^{a*}	8.42 ^a	8.05 ^{a*}	7.61 ^{a*}	6.11 ^{b*}
Mean	9.25	8.39	6.75	9.20	8.33	8.25	7.13	6.21
LSD (P<0.05)	0.56				0.31			

Species	Carbon source				Nitrogen source			
	Dextrose	Maltose	Sucrose	Glucose	PDA (control)	Peptone	Ammonium chloride	Ammonium sulfate
<i>P. sojarcaju</i>	9.78 ^a	6.79 ^{a*}	9.21 ^a	9.64 ^a	6.54 ^b	7.40 ^{a*}	8.29 ^{b*}	8.33 ^{b*}
<i>P. florida</i>	9.91 ^a	6.99 ^{a*}	9.34 ^a	9.61 ^a	6.17 ^b	7.81 ^{a*}	9.26 ^{a*}	8.95 ^{a*}
<i>P. ostreatus</i>	9.65 ^a	6.94 ^{a*}	9.13 ^a	9.44 ^a	6.49 ^b	6.55 ^{b*}	8.62 ^{b*}	8.79 ^{a*}
<i>P. eous</i>	9.56 ^b	6.86 ^{a*}	9.33 ^a	9.43 ^a	6.27 ^b	6.23 ^{b*}	7.50 ^{c*}	7.69 ^{c*}
<i>P. eryngii</i>	9.75 ^a	7.08 ^{a*}	9.36 ^a	9.58 ^a	6.55 ^a	6.29 ^{b*}	9.39 ^{a*}	8.72 ^{a*}
Mean	9.73	6.93	9.27	9.54	6.40	6.86	8.61	8.50
LSD (P<0.05)	0.29				0.45			

Species	Grain				Agricultural waste			
	Wheat	Corn	Paddy	Millet	Paddy straw	Corn cob	Millet straw	Sugarcane residue
<i>P. sojarcaju</i>	8.79 ^a	8.12 ^a	5.58 ^{b*}	5.98 ^{a*}	5.96 ^a	4.59 ^{c*}	3.97 ^{c*}	5.25 ^{c*}
<i>P. florida</i>	7.39 ^{a*}	4.33 ^d	3.46 ^a	5.27 ^{a*}	5.59 ^b	5.62 ^{b*}	4.28 ^{b*}	5.73 ^{a*}
<i>P. ostreatus</i>	8.89 ^{a*}	8.87 ^a	4.25 ^{b*}	5.35 ^{a*}	6.13 ^a	6.63 ^{a*}	5.45 ^{a*}	5.50 ^{b*}
<i>P. eous</i>	7.93 ^{a*}	5.39 ^c	3.15 ^{c*}	4.62 ^{a*}	5.65 ^b	4.66 ^{c*}	3.21 ^{d*}	4.23 ^{d*}
<i>P. eryngii</i>	8.02 ^{a*}	6.57 ^b	3.68 ^{b*}	4.35 ^{a*}	5.94 ^a	5.53 ^{b*}	3.96 ^{c*}	5.89 ^{a*}
Mean	8.20	6.65	4.02	5.11	5.85	5.41	4.17	5.32
LSD (P<0.05)	0.88				0.21			

Data are the mean of three replications \pm SD.

Means followed by a common letter in the same column are not significantly different at the 5% level by Fisher's least significance difference (LSD) test. *Represents the significantly different from the control at P<0.05.

glucose and sucrose by oyster mushrooms maybe due to the ease to metabolized and production of cellular energy for the growth. The ability of *Pleurotus* species to use different carbon sources may be due to the expression of the physiological differences within the species (Kurtzman and Zadražil, 1989). Among the four carbon sources tested (glucose, dextrose, sucrose and maltose) for the different oyster mushroom, sucrose is the cheapest sources and there is no significance differences found among the use of dextrose, glucose and sucrose in relation to mycelium growth. Hence, sucrose was more suitable and effective than other carbon source for the large scale production of oyster mushroom.

Effect of nitrogen sources and concentration on mycelium growth

Nitrogen plays an essential role for the synthesis of nitrogenous base of DNA, protein and it is an essential component of cell wall of all fungi (Miles and Chang, 1997). In this investigation, both organic nitrogen and inorganic nitrogen sources were used to evaluate their effects on mycelium growth of oyster mushrooms. There was significant difference found in mycelium growth of the studied oyster species among nitrogen sources (Table 2). Significantly highest mycelium colony diameters of *P. eryngii* (9.39) followed by *P. florida* (9.26) were obtained in media containing ammonium chloride (NH_4Cl) as nitrogen source. Ammonium sulfate ($\text{N}_2\text{H}_8\text{SO}_4$) is the second best nitrogen source for mycelium growth of oyster mushroom. The mycelium diameter of five oyster mushroom was significantly different as compared to control medium. There was an increase of mycelium expansion in all treatments added different nitrogen sources. Mycelium expansion occurred in the medium containing inorganic nitrogen was more compared to the organic nitrogen. The result is in agreement with the finding of another study by Cheng *et al.* (2013); Neelam *et al.* (2013); Fu *et al.* (2013) where ammonium chloride and ammonium sulfate were suitable nitrogen sources for mycelium growth.

Effect of different grain sources on the mycelium growth

Different grains are used as substrate for mycelia growth to produce spawn of edible mushrooms. In the present study, four different grains including paddy, corn, wheat and millet

were used to determine their effects on spawn production of oyster mushroom (Table 2). The result indicated that, mycelium run of oyster mushroom were significantly slower than that of control (wheat) which is universally used for mushroom spawn production. The mycelium extension of *P. ostreatus* and *P. sojarcaju* mycelium was higher than the other species in all the grain substrate. Wheat and corn was found to be the most favorable to the mycelium growth of the oyster mushroom in the present findings. On the 8th day of incubation, the highest mycelium diameters of *P. ostreatus* and *P. sojarcaju* obtained in wheat grain were 8.89 and 8.79 respectively. Corn and millet were next to wheat with the mean values for mycelium diameter of all the oyster species was 6.65 and 5.11 cm respectively. All grains supported the mycelium growth of studied *Pleurotus* species but in varying proportion. The least support for all the species studied was shown in paddy. This may be due to the hard cover layer of paddy grain when compared to other grain. The larger surface area and pore of substrates are suitable for mycelium growth rate (Tinoco *et al.*, 2011). This could account be the reason for the results recorded by wheat and corn.

Effect of different agricultural lignocellulosic wastes

In the present study, some agricultural wastes such as sugarcane residue, paddy straw, corn cob and millet straw were used as substrates to evaluate their effect on the mycelia run of oyster mushrooms. The results of the study indicated that the mycelium growth of oyster mushroom all cases was significantly lower than that in control substrate (Paddy straw). The mycelium growth trend of all oyster mushroom species in response to lignocellulosic substrates was very similar. Highest growth of mycelium of all the oyster mushroom species were recorded at paddy straw and sugar cane residues substrate (6.13 and 5.73 cm respectively). The lowest mycelium run were obtained in millet straw for all the mushroom. From the result it showed that the substrates of paddy straw and sugarcane residue followed by corncob were more suitable for the mycelium growth for all the mushrooms. This may be due to rich carbon and nitrogen composition in the substrates which support good mycelium growth (Hoa and Wang, 2015). Furthermore, the saprophytic potential of the fungi produce cellulose

Table 3: Sum square is the absolute value and percentage of total (in bracket) of main effect resulting from analysis of variance of studied parameters in oyster mushroom species subjected to different treatments.

Parameters	Source of variation		
	Species (S, <i>df</i> =4)	Treatment (T, <i>df</i> =3)	Species × Treatment (S×T, <i>df</i> =12)
Culture medium	32.00** (46.9)	15.14** (22.2)	20.22** (29.6)
Temperature	10.12** (71.0)	3.59* (25.0)	0.43 ns (3.0)
Carbon source	11.73** (63.0)	4.70** (25.0)	2.09* (11.0)
Nitrogen source	116.79** (41.0)	110.88** (38.0)	55.25** (19.0)
Grain	517.13** (33.0)	332.22** (21.2)	711.62** (45.4)
Agricultural waste	74.81** (68.5)	15.10 ** (13.8)	19.01** (17.4)

df: Degrees of freedom; Total *df*=23; The *P* of overall ANOVA for species, treatment and species × treatment interaction for each parameters.

P*<0.05, *P*<0.01, ns: not significant.

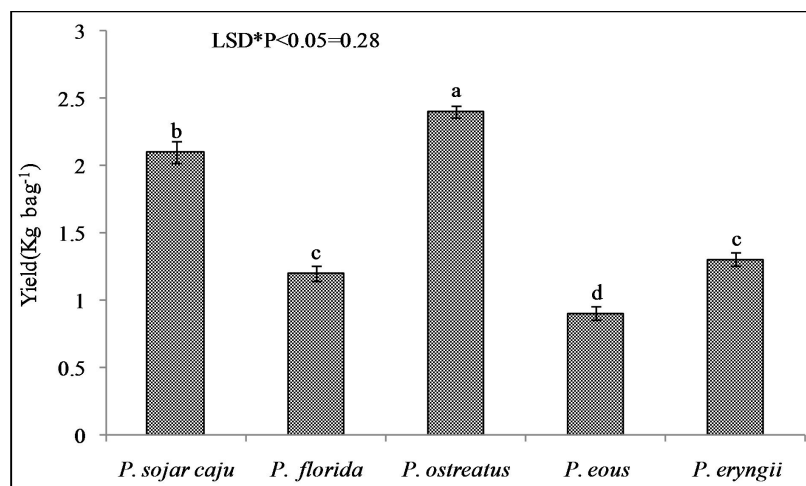


Fig 1: Fresh mushroom yield (kg bag⁻¹) in different species of oyster mushroom.

Data are the mean of three replications and bars indicate the standard deviation of the means (n=3).

Means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

hydrolyzing enzymes to hydrolyze cellulose, lignin and hemicelluloses present in these substrates to simple sugars for their growth (Hoa and Wang, 2015). Similarly, Kuforiji and Fasidi (2007) also reported that substrates such as corn cob, paddy straw and sawdust supported the growth of *Pleurotus* species. Based on the present results, paddy straw, sugarcane residue and corn cob were selected as the most suitable lignocellulosic substrate sources to produce oyster mushroom spawn besides spawn source produced from grains. Moreover, these lignocellulosic substrates were also suggested to cultivate oyster mushrooms of the studied species.

Test of significance

In the present study, we compared the effect of different culture conditions on the mycelium growth of different species of oyster mushrooms. All parameters were analyzed by two-way analysis of variance (ANOVA) with species and different treatments as the main factors. The test of significance of different mushrooms subjected to different treatments is presented in Table 3. The differences were non-significant in the case of replication (R) in all the parameters but in case of species (S), treatment (T) and species×treatment (V×T) interactions were highly significant.

Variation in mushroom yield

In the present study some of the oyster mushroom such as *P. ostreatus* and *P. sojarcaju* showed significantly ($P<0.05$) higher mushroom yield compared to the other species (Fig 1). The species *P. eous* showed very low yield. The ability to form mushrooms is dependent on the substrate utilization potential and is affected by environmental factors such as, temperature and cultivation practices (Hoa and Wang, 2015).

CONCLUSION

In the present study, the mycelium growth of the studied oyster mushrooms were affected by different temperature

conditions and nutritional sources. It was concluded that potato dextrose agar (PDA) and Malt extract medium were the most favourable media for the mycelium growth of mushroom. Maximum mycelium growth of oyster mushrooms were observed by cultivating them at the optimum temperature of $25\pm 2^{\circ}\text{C}$. In relation to carbon source the mushrooms growth was identified to be the most suitable to PA medium with 2% of dextrose and glucose concentration, while 2% sucrose also gave the good mycelium growth of oyster mushroom. This study also determined that nitrogen sources such as ammonium chloride and ammonium sulfate gave the greatest values in mycelium colony diameter of oyster mushrooms. Regarding to grain and agricultural lignocellulosic substrate sources to produce mushroom spawn, wheat followed by corn and millet was found to be favourable grains, while paddy straw and sugarcane residue were suitable lignocellulosic substrate sources for the mycelium. The yield of all the mushrooms also evaluated and it was found that *P. ostreatus* gave highest yield than other species. In all studied cases, mycelium growth of oyster mushroom *P. ostreatus* and *P. eryngii* was significantly better than that the other oyster mushrooms.

ACKNOWLEDGEMENT

The author would like to thank the Director of M.S. Swaminathan Research Foundation, Jeypore for his periodic guidance and advice.

Author contributions

Bandana Padhan designed the research, analyzed the data and prepared the manuscript. Kartik Charan Lenka performed the experiments and revised the manuscript. Naina Pradhan gave her valuable suggestions and correction in writing the manuscript. All the authors read and approved the final manuscript.

REFERENCES

- Atri, N.S., Upadhyay, R.C., Kumari, B. (2012). Comparative account of vitamin C content in *Termitophilous* and *Lepiotoid* mushrooms of Northwest India. *African Journal of Basic and Applied Science*. 4(4): 124-127.
- Bellettini, M.B., Fiorda, F.A., Maievas, H.A., Teixeira, G.L., Avila, S., Hornung, P.S., Ju'nior A.M., Ribani, R.H. (2019). Factors affecting mushroom *Pleurotus* spp. *Saudi Journal of Biological Science*. 26: 633-646.
- Cheng, Z., Wu, Q., Huang, J.B., Hu, C.G., Wang, Z.L. (2013). Effects of carbon sources, nitrogen sources and minerals on mycelia growth of *Cryphonectria parasitica*. *African Journal of Agricultural Research*. 8: 4390-4395.
- Choi, I.Y., Joung, G.T., Ryu, J., Choi, J.S., Choi, Y.G. (2003). Physiological characteristics of green mold (*Trichoderma* spp.) isolated from oyster mushroom (*Pleurotus* spp.). *Mycobiology*. 31: 139-144.
- Chorváthová, V., Bobek, P., Ginterand, E., Klavanová, J. (1993). Effect of the oyster fungus on glycemia and cholesterolemia in rats with insulin-dependent diabetes. *Physiological Research*. 42: 175-179.
- Dehariya, P., Vyas, D., Kashaw, S.K. (2013). Mushroom nutraceuticals on different substrates. *International Journal of Pharmacy and Pharmaceutical Sciences*. 5: 88-90.
- Dung, L.B. (2003). *Mushrooms in Tay Nguyen*. Ha Noi: Science and Technique.
- Egwin, E.C., Ellen, R.C., Egwuiche, R.U. (2011). Proximate composition, phytochemical screening and antioxidant activity of ten selected edible mushrooms. *American Journal of Food and Nutrition*. 1(2): 89-94.
- Eira, A.F. (2003). *Cultivo do Cogumelo Medicinal*. Editora Aprenda Fa'cil, Vic,osa (in Portuguese).
- Fu, R., Yin, C., Liu, Y., Ding, L., Zhu, J., Zheng, A., Li, P. (2013). The influence of nutrient and environmental factors on mycelium growth and conidium of false smut *Villosiclava virens*. *African Journal of Microbial Research*. 7: 825-33.
- Hoa, H.T., Wang, C. (2015). The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology*. 43: 14-23.
- ICAR-Agricultural Technology Application Research Institute (ATARI) Indian Council of Agricultural Research JNKVV, Jabalpur-482004 (M.P.). *Training Manual on Tropical Mushroom Production and Value Addition*. Pp 28.
- Janardhanan, K., Vadivel, V. and Pugalenti (2003). Biodiversity in Indian under Exploited/tribal Pulses. In: *Improvement Strategies for Leguminosae Biotechnology*, [P.K. Jaiwal and R.P. Singh (Eds.)]. Kluwer Academic Publishers. Printed in Great Britain. pp: 353-405.
- Kashangura, C. (2008). *Optimisation of the growth conditions and genetic characterization of Pleurotus species* [dissertation]. Harare: Department of Biological Sciences, Faculty of Science, University of Zimbabwe.
- Kües, U., Liu Y. (2000). Fruiting body production in Basidiomycetes. *Applied Microbiology and Biotechnology*. 54: 141-52.
- Kuforiji, O.O., Fasidi, I.O. (2007). Factor affecting the yield of fruit body and sclerotia in *Pleurotus tuber-regium*. *Advance Food Science*. 29: 211-215.
- Kurtzman, R.H., Zadražil, F. (1989). Physiological and Taxonomical Considerations for Cultivation of *Pleurotus* Mushrooms. In: *Tropical Mushrooms Biological Nature and Cultivation Methods*. [Chang, S.T., Quimio, T.H., editors]. Hong Kong: Chinese University Press. p. 299-348.
- Lee, J.Y. (1993). *Coloured Korean Mushrooms*. Seoul: Academy Press.
- Li, L., Ng, T.B., Song, M., Yuan, F., Liu, Z.K., Wang, C.L., Jiang Y., Fu, M., Liu, F.A. (2007). Polysaccharide-peptide complex from abalone mushroom (*Pleurotusa balonus*) fruiting bodies increases activities and gene expression of antioxidant enzymes and reduces lipid peroxidation in senescence-accelerated mice. *Applied Microbiology and Biotechnology*. 75: 863-869.
- Mane, V.P., Patil, S.S., Syed, A.A., Baig, M.M. (2007). Bioconversion of low quality lignocellulosic agricultural waste into edible protein by *Pleurotussajor-caju* (Fr.) Singer. *Journal of Zhejiang University Science B*. 8: 745-751.
- Mao, X.B., Eksriwong, T., Chauvatcharin, S., Zhong J.J. (2005). Optimization of carbon source and carbon/nitrogen ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. *Process Biochemistry*. 40: 1667-1672.
- Miles, P.G., Chang, S.T. (1997). *Mushroom Biology: Concise Basics and Current Developments*. Singapore: World Scientific, p. 40-4.
- Mshandete, A.M., Mgonja, J.R. (2009). Submerged liquid fermentation of some Tanzanian Basidiomycetes for the production of mycelial biomass, exopolysaccharides and mycelium protein using wastes peels media. *Journal of Agriculture Biological Science*. 4: 1-13.
- Munsur, M.A., Miah, A., Rahman, M.H., Rahman, M.M., Yahia, A.S. (2012). Effect of varieties and media on mycelial growth and substrate on spawn production of oyster mushroom. *Bangladesh Research Publications Journal*. 7: 361-366.
- Neelam, S., Chennupati, S., Singh, S. (2013). Comparative studies on growth parameters and physio-chemical analysis of *Pleurotus ostreatus* and *Pleurotus florida*. *Asian Journal of Plant Science Research*. 3: 163-169.
- Purkayastha, R.P.A. (1985). Chandra, *Manual of Indian Edible Mushrooms*, (Today and Tomorrow's Publication, New Delhi). 267-270.
- Pushpa, H., Purushothama, K.B. (2010). Nutritional analysis of wild and cultivated edible medicinal mushrooms. *World Journal of Dairy Food Science*. 5(2): 140-144.
- Sánchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology and Biotechnology*. 85: 1321-1337.
- Tinoco, R., Pickard, M.A., Vazquet-Duhalt, R. (2011). Kinetic differences of purified laccases from six *Pleurotus ostreatus* strains. *Letter in Applied Microbiology*. 32: 331-335.
- Tsujiyama, S., Ueno, H. (2013). Performance of wood-rotting fungibased enzymes on enzymic saccharification of rice straw. *Journal of Science Food Agriculture*. 93: 2841-2848.
- Wasser, S.P., Weis, A.L. (1999). Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: A modern perspective. *Critical Review in Immunology*. 19: 65-96.