Cultural Studies and Yield Attributes of Pink Oyster Mushroom (*Pleurotus djamor*) in West Bengal

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The pink oyster mushroom, scientifically known as *Pleurotus djamor*, is characterized by its appealing color, positive sensory qualities, substantial nutritional content, and its possession of antioxidant, antimicrobial, and medicinal properties. Mushrooms degrade lignocellulosic substrates through lignocellulosic enzyme production and utilize the degraded products to produce their fruiting bodies, contributing to sustainable agriculture and forestry and a short-term generation of income. The present study was carried out to assess the effect of various cultural parameters *viz.* temperatures, pH, solid culture media, carbon, and nitrogen sources on mycelial growth of the fungus and to identify the suitable grain for spawn production, optimum dose of spawn and suitable substrate for obtaining the highest yield of the mushroom. All of the experiments were conducted following standard protocols after procuring the pure culture of *P. djamor* from DMR-Solan. The optimum temperature for mycelial growth of the fungus was 28 °C at pH 7.5, and the best solid culture media was oat meal agar. Starch was the best carbon source and 0.3% L-asparagine served as the best source of nitrogen. Sorghum grains promoted the fastest spawn production. Out of five different doses of spawn and two assessed substrates, 4% spawn on paddy straw promoted the highest yield.

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INTRODUCTION

Mushrooms are macrofungi that possess distinctive fruiting bodies that can be either underground or aboveground (Chang and Miles 1992). Cultivating mushrooms is a beneficial technique for converting agricultural waste materials and timber (lignocellulosic wastes) into valuable resources, contributing to sustainable agriculture and forestry. In addition to being low in fat and high in protein and vitamins, mushrooms are abundant in various minerals, trace elements, and dietary fibers. They also serve as excellent sources of polysaccharides, peptides, unsaturated fatty acids, tocopherols, ascorbic acid, terpenoids, phenolic compounds, nutraceuticals, antioxidants, and compounds with diverse health benefits, including anticancer, prebiotic, immune-modulating, anti-inflammatory, cardiovascular, antimicrobial, and antidiabetic properties. Numerous studies have highlighted mushrooms' remarkable array of beneficial compounds (Badalyan 2014; Rathore *et al*. 2017; Gupta *et al.* 2018; Kumar *et al.* 2021; Nayak *et al*. 2022).

In India, five main types of cultivated mushrooms are the white button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* spp.), paddy straw mushroom (*Volvariella volvacea*), milky mushroom (*Calocybe indica*), and shiitake mushroom (*Lentinula edodes*). Among these, the oyster mushroom holds the second position in terms of production, contributing 16% to the country's total mushroom production (Sharma *et al.* 2017). *Pleurotus*, also known as the 'Oyster' mushroom globally and 'Dhingri' in India (National Horticulture Board 2020), is cultivated extensively in Southeast Asia, India, Europe, and Africa (Sharma and Madan 1993). In India, commonly cultivated commercial varieties of oyster (*Pleurotus*) mushrooms include *Pleurotus ostreatus, P. florida, P. sajor-caju*, and *P. sapidus*.

Pleurotus varieties are highly valued for their medicinal and nutritional advantages, as well as their impressive capacity to convert lignocellulosic materials such as straw, bagasse, sawdust, crop stubbles, and compost into valuable products (Pant *et al.* 2006; Pratibha et al, 2010, Bellettini *et al.* 2019; Balan *et al.* 2022; Scholtmeijer *et al.* 2023). Paddy straw, the residual material left after rice harvesting, comprises cellulose (32 to 47%), hemicellulose (19 to 27%), lignin (5 to 24%), ash (10 to 17%), and silica content (10 to 18%) (Ramesh *et al.* 2022). On the other hand, sugarcane bagasse, a by-product of the sugarcane industry, is characterized by its cellulose (45%), hemicellulose (32%), and lignin (17%) content, along with low ash levels (Yadav *et al*. 2022).

Pleurotus djamor, a common *Pleurotus* species in tropical and subtropical regions, grows well at temperatures up to 30 °C. It can produce fruiting bodies in 1 to 2 weeks on lignocellulosic substrates, making it best suited for cultivation in tropical areas (Salmones and Mata 2015). Various factors, including substrates, spawn quality, and physical conditions (such as temperature, humidity, C:O ratio, growing media, pH, and light intensity), greatly influence the growth and quality of mushrooms (Kadiri and Kehinde 1999; Bellettini *et al.* 2019). Healthy and active mycelial growth is essential for protecting mushrooms against stress factors and ensuring efficient spawn production (Herderio *et al.* 2006). It is crucial to maintain clean, viable, and stable mushroom strains for scientific and industrial purposes (Bhatt *et al.* 2010). The choice of medium is vital, as it provides essential nutrients for mycelial growth and enables year-round production (Chang 2001). Different species of *Pleurotus* have been extensively studied in Indian conditions however very little work has been done on cultural and yield aspects of *P. djamor* in Indian conditions, and the authors are not aware of any studies in West Bengal. The pink oyster mushroom, being widely adaptable, can be cultivated globally, with its mycelial growth affected by variations in pH, temperature, and media composition (Bugarski *et al.* 2002, Bellettini *et al.* 2019). This study aimed to identify the optimal solid media, pH, temperature, carbon, and nitrogen sources for achieving rapid mycelial growth of the fungus and to identify the best grain for spawn production, the optimum dose of spawn and the superior substrate that promotes minimum spawn run days, the highest yield and suitable biological efficiency.

EXPERIMENTAL

Materials and Methods

The pure culture of the pink oyster mushroom (*P. djamor*) was obtained from ICAR-Directorate of Mushroom Research (DMR), Solan (India) and stored at a temperature of 4 °C. To ensure its viability, the culture was regularly multiplied every

fortnight. All the studies were conducted in the Department of Plant Pathology Laboratory, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India, during the period 2019 to 2020.

Mycelial Growth Studies

The study investigated the effects of different parameters *viz.* temperature, pH, growth media, and C &N sources on the mycelial growth of the fungus. The impact of four different temperatures (20, 24, 28, and 32 °C) on the radial mycelia growth of the fungus was examined. Additionally, five different pH levels (6, 6.5, 7, 7.5, and 8) were tested to determine the optimum pH for the mycelia growth of *P. djamor*. The mycelia pattern was studied using three growth media (potato dextrose agar (PDA), malt extract agar (MEA), and oat meal agar (OMA) (Singh and Singh 2018), along with the influence of five different carbon sources (dextrose, sucrose, starch, maltose, and mannitol) and three different nitrogen sources (L-asparagine, ammonium chloride, and ammonium nitrate) at concentrations of 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%. The radial mycelial growth of the fungus in every experiment was recorded after three, five, and seven days of inoculation. The aim was to assess the effects of these parameters on the growth and development of the fungus.

Effect of Different Grains on Spawn Production Time

Spawns act as the main inoculum for the cultivation of mushroom. Therefore, the fastest production of quality spawns is the primary requirement in mushroom cultivation. Three different grains, *viz.* jowar (sorghum), bajra (pearl millet), and wheat grains, were used for the spawn preparation following the mycelial bit method for obtaining the fastest spawn. Good quality grains were first cleaned and washed thoroughly with water and soaked overnight. The grains on the next day were cooked for about thirty minutes with continuous monitoring to ensure that they were half-boiled (soft but not sticky) and cooled by spreading them on a polypropylene sheet. Pharmaceutical grade calcium carbonate ($CaCO_3$),@20g/kg cooked grains were mixed thoroughly. The processed grains were then filled in glass bottles @150 to 200g/bottle and sterilized in an autoclave at 121 °C (15 lb pressure/sq. inches) for about 1 to 1.5 hours. The sterilized bottled grains were then inoculated with fresh mycelial culture in a laminar air flow cabin and were incubated at 27±1 °C (Borah *et al.* 2019).

Effect of Different Doses of Spawn and Substrates on Spawn Run, Yield, and Biological Efficiency

Fresh spawn of the mushroom was prepared using wheat grains (Borah *et al.* 2020). Paddy straw and sugarcane bagasse were chosen as substrates. After chopping and soaking the substrates, they were pasteurized and sterilized. The substrates were then filled into polypropylene bags with different doses of spawn, *i.e.*, 10 g/kg of dry substrate (1%) and subsequent doses (2%, 3%, 4%, and 5% of dry weight of substrates), using the methodology of layer spawning. The bags were hung in a well-ventilated growing house at 26 to 29 °C. Regular monitoring and watering were conducted (Borah *et al.* 2020). Data on spawn run time, pinhead initiation time, fresh weight, and biological efficiency were collected for analysis.

RESULTS AND DISCUSSION

Mycelial Growth Studies

In this study, the mycelial growth of *P. djamor* was examined at four different temperatures (Table 1). After seven days of inoculation, the most favorable temperature range for mycelial growth was 24 to 28 °C, with the optimum temperature being 28 °C. At 28 °C, the mycelium exhibited the highest and fastest radial growth of 50.8 mm. Radial growth increased gradually as the temperature rose from 20 to 28 °C, but growth was hindered beyond 28 °C. It is worth mentioning that even though the radial growth was less at 20 °C (22.4 mm) than at 32 °C (26.8 mm), a retardation was observed at the higher temperature. These findings are consistent with earlier research on *Pleurotus sajor-caju*, which also exhibited optimal growth at 25 to 28 °C. Several other studies on *Pleurotus* spp. have also reported a suitable temperature range of 25 to 28 °C for mycelial growth (Zharare *et al.* 2010; Singh and Singh 2018)

The mycelial growth of the fungus was evaluated on three different culture media. After seven days of inoculation, the most vigorous growth was observed in OMA (64.7 mm), followed by PDA (55.5 mm), and the minimum growth was recorded in MEA (53.5 mm). Although the mycelia of *P. djamor* exhibited good growth in all three media, there was a slight but noticeable variation in growth rates (Table 2). Previous research by Singh and Singh (2018) also highlighted the superiority of oat extract agar as the most suitable medium for mycelial growth in *P. djamor*, while Ahmad *et al.* (2015) found that PDA promoted the maximum mycelial growth, followed by MEA. These findings demonstrate the influence of different culture media on the growth of *P. djamor* mycelia.

After seven days of inoculation, it was observed that the pH range of 7.0 to 8.0 was most conducive for mycelial growth. Particularly, the mycelium exhibited robust growth within the pH range of 7.0 to 7.5, with the most pronounced growth occurring at pH 7.5 (58 mm) (Table 3). These findings align with a study by Khan *et al.* (2013), who investigated various pH levels and concluded that most mushrooms, including *P. djamor*, flourish in a pH range of 7.0 (neutral) to slightly basic conditions. Deviations from this optimal pH range, either towards acidity or alkalinity, resulted in a retardation of mycelial growth, consistent with observations made by Singh and Singh (2018), who reported the highest and fastest mycelial growth at pH 7.5 after eight days of incubation. Khan *et al.* (2013) further supported these results by identifying a favorable pH range of 7.2 to 7.8 for the mycelial growth of oyster mushrooms (*Pleurotus* spp.) on cotton. These collective findings emphasize the significance of maintaining specific pH conditions for optimal mycelial growth in various *Pleurotus* species.

Among the five tested media, starch proved to be the most favorable, promoting the most vigorous growth (68.8 mm), followed by sucrose (64.0 mm), and mannitol (68.8 mm). In contrast, the minimum growth was observed in dextrose after 7 days of incubation (Table 4). Similar research by Kumar *et al.* (2018b) revealed that for *P. florida*, the maximum mycelial growth occurred in fructose, followed by starch, sucrose, and mannitol. Meanwhile, *P. sajor-caju* demonstrated the best growth on starch, sucrose, mannitol, and fructose when tested with eight different carbon sources. Chandra and Purkayastha (1977) also found that various fungi, such as *Agaricus campestris*, *Lentinus subnudus*, *Calocybe indica*, *Volvariella volvacea*, and *Termitomyces eurrhizus*, exhibited excellent growth on mannitol, dextrin, glucose, fructose, and sorbitol, respectively.

The PDA supplemented with L-asparagine demonstrated the maximum mycelial growth (70.5 mm), followed by ammonium chloride (68.7 mm) and ammonium nitrate (62.7 mm) after seven days of inoculation. Further analysis of different concentrations of each compound revealed that 0.3% L-asparagine supported the highest mycelial growth (70.5 mm), followed by 0.1% (67.1 mm), and 0.5% (47.2 mm) with a gradual decrease in growth at higher concentrations. Similar trends were observed with ammonium chloride and ammonium nitrate, with 0.1% concentration showing the best growth (68.7 mm) followed by 0.3% (56.8 mm), and 0.5% (47.1 mm), respectively (Table 5). Based on these findings, it can be concluded that 0.3% L-asparagine and 0.1% ammonium chloride are more conducive to hyphal proliferation compared to ammonium nitrate. These results align with previous studies, Hesami *et al.* (2014) observed that 0.15% asparagine significantly enhanced the mycelial growth of *Agaricus bisporus* by 117%. Chandra and Purkayastha (1977) reported that asparagine and aspartic acid increased the growth of mycelia. Upadhyay *et al.* (2002) also found that the presence of accessible amino acids or proteins can lead to increased performance in the fungal mycelium. Additionally, Kumar *et al.* (2018b) observed that ammonium chloride promoted better mycelial growth for *Pleurotus florida* and *Pleurotus sajor-caju*, followed by ammonium nitrate and ammonium phosphate.

Effect of Different Grains on Spawn Production

Spawn serves as the inoculum for the mycelial colonization on the substrate. Thus, good-quality spawn is a primary requirement of mushroom cultivation. Three cereal grains *viz.* sorghum, bajra, and wheat were assessed to identify the most suitable. Sorghum grains exhibited the shortest colonization time (12.2 days), followed by bajra (13.8 days), while wheat grains took the longest (15.6 days) for spawn production (Table 6). Despite the faster colonization in sorghum and bajra, their quality was not superior, as mycelia were loosely attached and grains were sticky. In contrast, wheat grains provided better mycelial attachment. Considering affordability, wheat grains seemed more suitable for spawn production because of their lower market price. Similar findings were reported by Baghel *et al.* (2020).

Spawn Run Days

The spawn run days vary depending on the species, substrates, meteorological factors, and spawn doses. Thus, a change in spawn dose changes the amount of inoculum and the fungus colonizes the substrate at a respective dose. The present investigation aimed to assess how substrates and spawn doses influence the spawn run days. The paddy straw exhibited the fastest mycelial colonization, thereby the minimum spawn run days (11.2 days) compared to that of sugarcane bagasse (43.4 days) (Table 7). A decrease in the spawn run days was observed in both substrates with the increase in spawn dose percentage. In paddy straw, 3%, 4%, and 5% spawn doses promoted the fastest spawn run (10 days) while the maximum time taken was at 1% (14.2 days). In contrast, in sugarcane bagasse the fastest spawn run was observed at 4% (40.8 days) followed by 5% (41.5 days), and the slowest was found at 1% (47 days). Kumar *et al.* (2018a) showed that an increase in the spawn dose of *Pleurotus florida* reduces the spawn run days on different substrates. Pal *et al.* (2017), showed similar results where they found that spawn run days are minimum at a higher rate (8%) of *P. pulmonarius* spawn.

Table 1. Effect of Different Temperatures on Radial Growth (mm) of *P. djamor*

Temperature	DAY 3	DAY 5	DAY 7	Mean (Temp.)
20°C	10.00	16.43	22.37	16.27
24°C	18.07	29.18	45.10	30.78
28°C	20.35	31.78	50.85	34.33
32°C	13.10	19.42	26.80	19.77
Mean (Day)	15.38	24.20	36.28	
	Temperature	Days	Temperature X Day	
Sem	0.21	0.18	0.36	
CD	0.62	0.54	1.07	

Table 2. Effect of Different Culture Media on Radial Growth (mm) of *P. djamor*

Media	DAY 3	DAY 5	DAY 7	Mean (Media)
PDA	17.76	35.16	55.5	36.14
MEA	16.66	32.63	53.46	34.25
OEA	18.45	39.15	64.72	40.77
Mean (Days)	17.63	35.65	57.89	
	Media	Days	Media X Days	
Sem	0.22	0.22	0.38	
CD	0.70	0.70	1.22	

Table 3. Effect of Different Ph on Radial Growth (mm) of *P. djamor*

pH	DAY 3	DAY 5	DAY 7	Mean (pH)
6	10.00	22.03	35.00	22.34
6.5	14.00	25.00	35.16	24.72
7	16.33	27.36	39.00	27.56
7.5	21.00	31.50	58.00	36.83
8	19.50	28.06	43.1.	30.22
Mean (Days)	16.16	26.79	42.05	
	pH	DAYS	pH X Days	
Sem	0.33	0.25	0.58	
CD	0.97	0.75	1.68	

Fresh Weight and Biological Efficiency

Upon reaching maturity, the sporophores/basidiocarps/fruit bodies of the mushroom were harvested. Pinheads emerged within 6 to 8 days after the spawn run was completed, and these pinheads matured within 3 to 4 days. Tables 8 and 9 present the findings, indicating that paddy straw yielded the highest average (297 g/kg dry substrate), followed by sugarcane bagasse (268 g/kg dry substrate), with respective average biological efficiencies of 29.7% and 26.8%. Among the different spawn doses tested, the most productive results were observed with paddy straw at 4% (324 g), followed by 5% (306 g) and 3% (302 g), yielding biological efficiencies of 32.4%, 30.6%, and 30.2%, respectively. In sugarcane bagasse, the highest yield was obtained at 4% (290), followed by 5% (272 g) and 3% (270 g), yielding biological efficiencies of 29.0%, 27.2%, and 27.0% respectively.

Overall, paddy straw at 4%, 5%, and 3% spawn dose exhibited satisfactory yield and biological efficiency, making it the preferable substrate for cultivation.

Table 4. Effect of Different Carbon Sources on Radial Growth (mm) *of P. djamor*

Carbon Source	DAY 3	DAY 5	DAY 7	Mean (C-sources)
Dextrose	15.00	33.13	53.00	33.71
Sucrose	18.00	36.10	63.96	39.35
Starch	19.00	37.66	68.76	41.81
Maltose	16.16	33.13	60.50	36.6
Mannitol	17.16	34.66	64.90	38.91
Mean (Days)	17.06	34.94	62.22	
	C-sources	Days	C-sources X Days	
Sem	0.53	0.41	0.92	
CD	1.54	1.20	2.68	

Table 5. Effect of Different Nitrogen Sources on Radial Growth (mm) of *P. djamor*

	L-Asparagine					Ammonium Chloride					Ammonium Nitrate				
	C_1	C_2	C_3	C_4	C_5	C_1	C_2	C_3	C_4	C_5	C_1	C_2	C_3	C_4	C_5
DAY 3	13.00	14.90	12.33	12.00	9.66	14.83	13.00	11.33	11.00	9.33	17.63	17.00	15.16	12.83	10.00
DAY 5	44.20	46.66	38.16	29.00	25.00	45.93	42.00	31.16	25.33	16.83	44.00	40.33	32.00	21.66	18.50
DAY 7	67.12	70.50	47.16	45.60	31.06	68.74	56.77	47.13	35.72	23.66	62.73	59.48	41.83	32.68	17.66
	N-source		Concen-tration		N-source X Concen-tration	Days			N-source X Days		Concentra-tion X Days		N-source X Concentra-tion X Days		
Sem	0.31		0.40		0.70	0.31			0.54		0.70		1.22		
CD	0.88		0.88		0.88	0.88			0.88		0.88		0.88		
C1=0.1%; C2=0.3%; C3=0.5%; C4=0.7%; C5=0.9%)															

Table 6. Effect of Different Grains on Spawn Production Time (Days)

Grains	R1	R2	R3	R4	R5	Mean (Days)
Jowar (Sorghum)	12	12	12	12	13	12.2
Bajra (Pearl millet)	13	14	14	14	14	13.8
Wheat	16	16	16	15	15	15.6
SE(m)						0.216
C.D.						0.673

Table 7. Spawn Run Days on Different Substrates and at Different Spawn Dose Percentages

Substrates	1%	2%	3%	4%	5%	Mean (Substrate)
Paddy straw	14.25	11.50	10.00	10.00	10.25	11.20
Sugarcane bagasse	47.00	44.75	43.25	40.75	41.50	43.45
Mean (spawn rate)	30.62	28.12	26.62	25.37	25.87	
	substrate	Spawn rate	Substrate X spawn rate			
SEm	0.127	0.202	0.285			
CD	0.370	0.585	0.827			

Table 8. Fresh Weight (g) on Different Substrates and at Different Spawn Dose Percentages

Substrates	1%	2%	3%	4%	5%	Mean (substrate)
Paddy straw	272.00	283.50	301.75	323.75	306.25	297.45
Sugarcane bagasse	262.00	247.50	270.25	289.50	272.50	268.35
Mean (spawn rate)	267.00	265.50	286.00	306.62	289.37	
	Substrate	Spawn rate	Substrate X spawn rate			
SEm	1.85	2.93	4.14			
CD	5.38	8.50	12.03			

Table 9. Biological Efficiency (%) on Different Substrate at Different Spawn Dose Percentages

Substrates	1%	2%	3%	4%	5%	Mean (Substrate)
Paddy straw	27.20	28.35	30.17	32.37	30.62	29.74
Sugarcane bagasse	26.20	24.75	27.02	28.95	27.25	26.83
Mean (spawn rate)	26.70	26.55	28.60	30.66	28.93	
	Substrate	Spawn rate	Substrate X spawn rate			
SEm	0.18	0.29	0.41			
CD	0.53	0.85	1.20			

CONCLUSIONS

1. The optimal temperature range was found to be 24 to 28 °C, with the most favorable temperature being 28 °C. Oat meal agar was the most suitable culture medium, and a pH range of 7.0 to 8.0 supported robust mycelial growth in *P. djamor*.
2. Among the carbon sources tested, starch was the most favorable, while L-asparagine and ammonium chloride were the most effective nitrogen supplements. Sorghum grains

though exhibited the fastest spawn production overall economically and good quality spawn can be obtained on wheat grains.

3. In terms of spawn run days, paddy straw exhibited the fastest colonization, requiring only 11.2 days, whereas sugarcane bagasse took considerably longer (43.4 days). Increasing the spawn dose percentage reduced the spawn run days in both substrates.
4. Regarding fruiting performance, paddy straw yielded higher average fruit body production (297.45g/Kg dry substrate) compared to sugarcane bagasse (268.35g/Kg dry substrate). The most productive results were obtained with paddy straw at 4% spawn dose percentage (324 g), followed by 5% and 3% spawn doses. In sugarcane bagasse, the highest yield was obtained at 4% spawn rate (290 g).

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