

Recycling of Lignocellulosic Waste Materials Through Cultivation of *Pleurotus djamor* for Sustainable Food Production

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ABSTRACT

The cultivation of *Pleurotus djamor*, a pink oyster mushroom, presents a sustainable approach to recycling lignocellulosic agricultural waste while producing a nutritious food source. This study investigates the use of various substrates, including paddy straw, groundnut husk, newspaper, and cardboard, to optimise mushroom yield and quality. The methodology involves the preparation, spawning, and controlled growth conditions necessary for the successful cultivation of *P. djamor*. Key factors influencing mushroom production, such as temperature, pH, moisture, and aeration, are discussed. Nutritional analysis reveals that *P. djamor* is rich in proteins, essential amino acids, and bioactive compounds, positioning it as a valuable functional food with potential health benefits. The findings highlight the significant role of *P. djamor* in bio converting agricultural residues into high-quality food while addressing environmental waste management. This cultivation method not only contributes to sustainable food production but also supports efforts to mitigate malnutrition, particularly in tropical and subtropical regions where agricultural waste is abundant. Overall, this research underscores the potential of mushroom farming as a viable solution for enhancing food security and promoting sustainable agricultural practices.

Key words: Eco-friendly, Lignocellulosic waste, Oyster mushroom, Sustainable food.

1. INTRODUCTION

The mushroom production from Agro industrial wastes is expanding quickly to become a lucrative field apart from being a sustainable and environment friendly source of food. Modern technology allows farmers to grow mushrooms with maximum quality and yields with the least amount of expense for input resources in high-resource environments^[1]. The usage of mushrooms is followed, since decades hence it's been closest to humankind. Since they have been around for millions of years, people have gazed down on them and also valued their growth as a source of nutrition and nourishment. During the Middle Ages, when mushrooms were an integral part of the regal feasts of the Greek and Roman Emperors, the renowned Greek philosopher Theophrastus (c. 372-287 BC) wrote on the nutritional value of mushrooms. Some people in other places believed that mushrooms were the work of the devil, yet in the classic writings of the Bible and Vedas, they were described as "Food for Gods" ^[2]. Currently over 2000 edible kinds of mushrooms, which are fungi having notable nutritional value are prevalent all over the world. The most frequently grown species are oyster mushrooms (*Pleurotus spp.*), shiitake mushrooms (*Lentinula edodes*), and button mushrooms (*Agaricus bisporus*). China is the largest mushroom farmer in Asia, accounting for around 35% of the global fresh mushroom sector, with a current value of the fresh mushroom market estimated at 38 billion US dollars in 2018. Up to 76% of the world's mushrooms are produced in Asian nations, with the United States (4.9%) and Europe (17.2%) coming in second and third, respectively^[3].

The world has seen a significant increase in the cultivation of oyster mushrooms, (*Pleurotus spp.*), which are typically produced on pasteurised agricultural wastes. It may be cultivated on a wide variety of lignocellulosic substrates, enabling it to play an essential role in handling organic wastes whose disposal is troublesome. An easy, economical, and ecologically benign method for making use of agricultural and agro-industrial wastes is mushroom farming. *Pleurotus djamor* a pink oyster mushroom, is one such species that is grown on suitable substrates. This species is growing in importance, as it is an edible fungus that is simple to grow throughout the year^[4]. One of the most successfully cultivated specialised mushrooms is the oyster mushroom, *Pleurotus species*, which is now

considered as a delicacy. Numerous researchers provide insight into the increasing prevalence of different biological materials^[5]. Asia has seen an increase in oyster cultivation because of the species' low production costs, easy access to substrates, tolerance to fluctuating temperatures, and high yield potential. Oyster mushrooms are valued highly as a functional food because of its delicious taste, flavour, aroma, nutritional and therapeutic qualities. Protein, fibre, vitamins B complex, C, and D2, minerals including potassium, phosphorus, selenium, zinc, and important amino acids are all present in high concentrations in them. Due to their ability to flourish in both tropical and temperate climates without the need for intricately controlled environments, these mushrooms show high adaptability^[6].

The primary obstacles to the development of mushrooms are the lack of understanding about the nutritional value and food value of mushrooms. Therefore, encouraging technology transfer with regard to mushroom farming is an urgently needed intervention strategy. This justification led to the start of the current research project, which had the objective to determine how suitable, different lignocellulosic wastes are for oyster mushroom production. It aimed to find out the potential substrates, namely paddy straws, groundnut husk and paper waste which includes newspapers and cardboard waste (growing performance and yield) (Fig-1). This study's implications include identifying the higher yield production of mushroom cultivation in the study area for the improvement of people's lives and facilitating the technological adoption of oyster mushroom cultivation using agricultural wastes^[7].

2. ABBREVIATIONS

2.1. Common abbreviations:

- *P. djamor* - *Pleurotus djamor*
- spp - species
- °C - Degree Celsius
- SD - Standard Deviation
- ACE - Angiotensin converting enzyme
- FRAP - Ferric reducing ability of plasma

2.2. Units abbreviation:

- cm - centimeter
- g - gram
- mg - milligram
- ml - millilitre
- µg - microgram
- nm - nanometer

3. METHODOLOGY

3.1. Collection of spawn:

The ready to fruit mushroom spawns were collected in December, 2022 from Biotechnology centre, Hulimavu, Bengaluru (latitude and longitude 12.880505° N and 77.608192° E). The bags of spawn were packed, labelled, numbered, and the collection of data were recorded^[8].

3.2. Substrate preparation:

An assortment of agricultural waste substrates, encompassing paddy straws, newspaper, cardboard and groundnut husk have been used to grow oyster mushrooms. The most commonly used substrate for growing mushrooms is paddy straw, since the mushroom yield and growth rate is higher. Moreover, it is easily available, affordable, and can be pasteurised^[9]. The substrates which include paddy straws and newspaper were cut into small pieces and the groundnut husk was crushed.

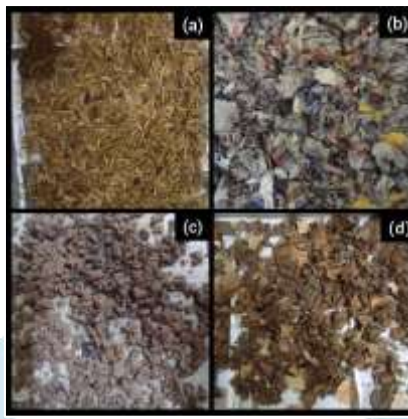


Figure-1 : Different substrates used for mushroom cultivation.
(a) Paddy straw, (b) News paper, (c) Groundnut husk and (d) Cardboard substrates.

They were soaked for 12 to 14 hours, and completely rinsed with clean water later it was drained. After that, each substrate was packed into polythene bags (Fig-2) and autoclaved at 121°C^[10].



Figure-2 : Preparation of substrates for mushroom cultivation.
(a) Cardboard, (b) Newspaper, (c) Paddy straw and (d) Ground nut husk substrates.

3.3. Spawning:

Spawning is the process of introducing a culture into a substrate^[11]. The spawning can be followed by various methods; the commonly used method for the spawning is 'Layer spawning'. In this method a 10 cm layer of straw was added into the bag and pressed down on the layer with sterile palm, compacting it to a height of about 4-5 cm. Then, 50 g of spawn was used to spawn on the paddy straws, newspaper substrate, cardboard substrate, and crushed groundnut husk by layering on to it and placing more spawn on the sides and less in the centre. Later by filling the polythene bag similarly, with a total of five layers of straw and four layers of spawn in between. After that bag has been fully filled, securing the open end by cotton plug and a tied up with a piece of jute thread (Fig-3). Name of the species, making of bags and the spawning date was mentioned on the bags for documentation analysis^[12].

3.4. Spawn running:

After the inoculation process, they were maintained in the dark room at a controlled temperature of 20–25 °C for 15 days^[13]. After the incubation period there was mycelium formation that had colonised in the polythene bags containing the substrates and which were then moved to the cropping chamber. Using autoclaved needles, holes were punched in the polythene bags, for aeration purpose (Fig-3). To maintain the moisture level in the cropping chamber and the water activity of the substrates in the polythene bags, the bags were watered twice a day and it was thoroughly monitored^[14].



Figure-3 : (a) Spawning and (b) Spawn running of *Pleurotus djamor* mushroom.

3.5. Harvesting:

Harvesting was done when the fruiting bodies were fully formed, and the caps were fully opened when they were upright and curled in appearance ^[15]. Harvesting, oyster mushrooms entails taking hold of each stalk individually and twisting the mushroom until it comes free of the substrate. It is crucial to maintain a high humidity level as the mushrooms start to fruit. As earlier, air is allowed to pass through the growth space before spraying (oyster mushrooms need a steady supply of fresh air). Now, temperatures may be higher than during the first step of pinning (Fig-4) ^[16]. Pinhead mushroom initiation begins after 10 to 12 days, and the mushroom crop is harvested after 20 to 25 days ^[17].



Figure-4 : Harvesting of *Pleurotus djamor* mushroom using different substrates.

(a) During 3rd week (b) During 4th week and (c) During 5th week.

4. FACTORS AFFECTING THE MUSHROOM PRODUCTION:

Fundamental understanding is being created through investigations of the different elements influencing the growth of mycelium and fruit bodies in the field of mushroom physiology ^[18].

Mushroom survival and reproduction are influenced by a number of factors that may work independently or in conjunction with one another. It has been determined that a basic grasp of the temperature, air humidity, pH, aeration, CO₂ concentration, and light requirements of *Pleurotus species* mushrooms is necessary to comprehend the issues associated with treating them as shown in the below ^[19].

4.1. Temperature:

Depending on the species and strain, different temperatures are ideal for fruiting. Usually, fruiting occurs between 13° and 24°C, depending on the species, although vegetative development typically varies from 5° to 33°C. Mycelia and spores are destroyed by high temperatures, which lowers the output. Warm, humid weather is typically favourable for mushroom production ^[20].

4.2. pH:

The ideal pH ranges for fruiting and growth may be different. The internal pH of the fungus is impacted by the pH of the external media. As a result, the organism's development, enzymatic activity, cell membrane permeability, and absorptive capacity are also impacted. The pH range of 4.0 to 7.0 is ideal for mycelial colonization. While the water used for spraying should not have an excessively acidic or alkaline pH and should be devoid of hazardous salts ^[20].

4.3. Moisture:

In order to allow *Pleurotus spp.* to grow to a reasonable level, the proper moisture in the substrate should include a range between 50% and 75%. This is because the use of high moisture content leads to the spoilage of mushrooms ^[21].

5. APPLICATIONS:

5.1. Nutritional characteristics

Pleurotus mushrooms are considered rich in several nutritional compounds like dietary fibre, proteins, carbohydrates, essential amino acids, water-soluble vitamins, and minerals as well as many functional bioactive molecules including polyphenols, polysaccharides,

lipids, terpenoids also vitamin B range , lutein, zeaxanthin, fibre and beta-glucan are present with low fat hence they are considered as healthy food.

After harvesting the first and second flushes, the cultivation residue's mean (SD) protein content increased from 18.2% (0.3) in the initial substrate to 20.8% (0.2) and 23.3% (0.1), respectively, according to the protein analyses using Kjeldahl crude protein. **Table 1** displays the amino acid composition of the initial substrate and cultivation residue following harvesting the second flush. The original substrate included 13.7% of total amino acids and 5.2% of total essential amino acids. Total amino acid content and total essential amino acid content in the culture residue were 10.8% and 4.2%, respectively. In both samples, glutamic acid and aspartic acid were the two amino acids that were most prevalent. After oyster mushroom culture, the initial substrate's amino acids decreased, with the exception of arginine and threonine and highest reduction was observed in glutamic acid.

Amino acids		Substrate (g/ 100g)	Cultivation Residue after 2nd flush (g / 100g
1.	Tryptophane	0.26	0.12
2.	Alanine	0.82	0.74
3.	Arginine	0.88	0.91
4.	Aspartic acid	1.17	1.16
5.	Glutamic acid	2.54	1.34
6.	Glycine	0.77	0.72
7.	Histidine	0.37	0.30
8.	Hydroxyproline	<0.20	<0.20
9.	Isoleucine	0.51	0.46
10.	Leucine	1.00	0.78
11.	Lysine	0.72	0.51
12.	Ornithine	0.05	0.05
13.	Phenylalanine	<0.71	<0.56
14.	Proline	0.94	0.60
15.	Serin	0.63	0.57
16.	Threonine	0.58	0.63
17.	Tyrosine	0.45	0.28
18.	Valine	0.79	0.68
19.	Cysteine + Cystine	0.34	0.31
20.	Methionine	0.27	0.18

Table:1 - The amino acid profile of initial substrate and cultivation residue after harvesting the second flush of oyster mushrooms ^[22].

Vitamins		Substrate (mg/100 g)	Cultivation Residue after 2nd flush (mg/100g)
1.	Thiamine	0.47	0.04
2.	Riboflavin	0.14	0.57
3.	Niacin	3.01	42.4
5.	Pyridoxine	0.47	0.24
6.	Lutein	<0.02	<0.02
7.	Zeaxanthin	<0.02	<0.02

Table:2 - Selected vitamin Bs, lutein and zeaxanthin in the initial substrate and cultivation residue after harvesting the second flush of oyster mushrooms ^[22].

The vitamin B range, lutein and zeaxanthin content of the initial substrate and cultivation residue (after harvesting the second flush) are presented in Table 2. The thiamine and pyridoxine content of the initial substrate reduced after cultivation, whereas its riboflavin and niacin content improved. The niacin content of cultivation residue increased from 3 mg/100 g to 42 mg/100 g after cultivation. The quantity of lutein and zeaxanthin was insignificant in both samples ^[22].

- This mushroom maintains a great attraction from consumers due to its desirable flavour and aroma.
- Mushrooms have great antifungal, anti-inflammatory, antibacterial, antiviral, antidiabetic, hepatoprotective, hypolipidemic, hypotensive and cytotoxic attributes.
- Pleurotus species has been successfully applied as a food supplement for cardiac patients to decrease the blood cholesterol level due to their content of nicotinic acid, mevinolin and higher levels of β -glucan ^[23].
- During the growth period, the organic components of the growth substrate would break down into smaller molecules that could be taken for the formation and growth of the fruit bodies. As a result, it is determined that the mushroom is a good option with the ability to naturally break down, alter, and use organic waste components as nutrients for the construction of its fruit body ^[24].
- The genus Pleurotus is a cosmopolitan group of mushrooms with high nutritional value and therapeutic properties, besides a wide array of biotechnological and environmental applications ^[25].
- In oyster mushrooms, Polyphenol oxidant extracts have a potential for removal of phenolic compounds in bioremediation and in the food and drug industries ^[26].
- Demand is increasing in the food industry for unique functional ingredients or bioactive compounds from biological sources, as they are widely applied in the formulation of functional foods. This is encouraging, especially in the recent years, an increasing interest is noticed in extracting ingredients from foods such as mushrooms and in formulating functional foods ^[25].
- Pleurotus spp. has proven to have beneficial medicinal attributes. Hence, they have been documented among “mushroom nutraceuticals” and categorised as both functional foods and medicinal mushrooms. Pleurotus spp. is utilised for the prevention and treatment of atherosclerosis via reduction of oxidative stress, hypertension, and hypercholesterolemia in terms of the therapeutic compounds responsible
- Pleurotus spp. have been reported to have anti-atherogenic capabilities, with six of them possessing high levels of anti-atherogenic compounds such as ACE inhibitor peptide, ergothioneine, chrysin, and lovastatin. Hence, it has been indicated that Pleurotus spp. has tremendous potential use as food or extracts from fruiting bodies or mycelium in an alternative therapy for atherosclerosis, through prevention and treatment of oxidative stress, hypertension, and hypercholesterolemia ^[27].
- The FRAP mean value demonstrates the ability of Pleurotus ostreatus extract to reduce Iron (III) to Iron (II) indicates its redox potential which illustrates mushroom as a good antioxidant source, suitable for health benefits when consumed.
- Cultivation of edible mushrooms is a biotechnological procedure for lignocellulosic organic waste recycling. It might be the only existing process that combines the production of protein-rich food with the reduction of environmental pollution ^[28].

6. RESULTS AND DISCUSSION:

The *Pleurotus djamor* (pink oyster mushroom) is pink, It's cap is fan-shaped, moderately convex to plane, and ranges 2.5 - 5 cm in width and 3 - 7 cm in length. The edge is inrolled. The gills are 0.5 - 0.7 μm wide and vary in colour from bright pink to cream. The white, hair-matted stem is usually very short or nonexistent - these are the macroscopic characteristics of *Pleurotus djamor* mushroom (Fig-5).

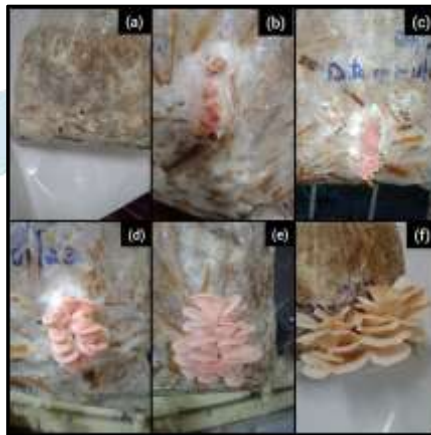


Figure - 5 : Macroscopic view of different stages of *Pleurotus djamor* mushroom cultivation.

The Microscopic characteristics includes - the spores are ellipsoid and amyloid, with a pink spore print. Each of the basidia contains approximately four spores and also they have sterigmata along with clamp connectors available (Fig-6) [29,30,31].

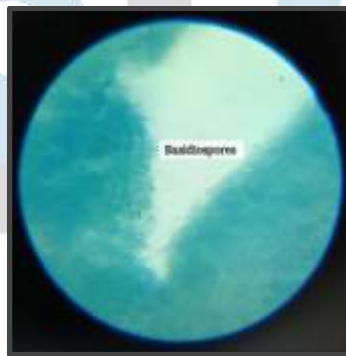


Figure - 6 : Microscopic view of *Pleurotus djamor* mushroom.

The Approximate composition of the harvested *Pleurotus djamor* mushrooms from different substrates has been shown in the below. (Fig-7)



Figure : 7 - *Pleurotus djamor* yield rate by using different substrates.

From the cultivated *Pleurotus djamor* mushroom the protein was estimated by Lowry's method (Fig -8 and 9). In each test tube, 1 mL of protein samples were collected. Before collecting the protein sample, 1 millilitre of distilled water was obtained in order to stop the protein from degrading. An alkaline copper solution (4 mL) was added to these solutions. The mixes were then thoroughly blended with a vortex shaker. Ten minutes were spent incubating the solutions at room temperature. These solutions were then thoroughly mixed using a vortex shaker after 0.5 mL of Folin-Ciocalteu phenol was added. Following that, these solutions were incubated for half an hour at ambient temperature and in a dark room. For every sample, two test tubes were made. The absorbance of unknown specimens was measured at 670 nm. [32]

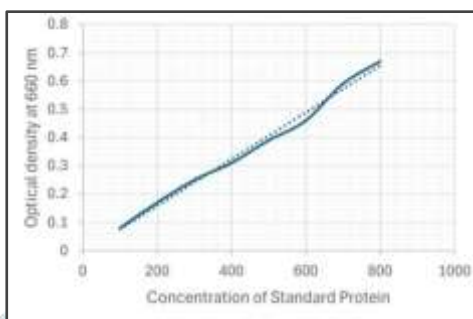


Figure : 8 - Bovine serum albumin standard curve (absorbance was plotted against protein concentration) using the Lowry method.

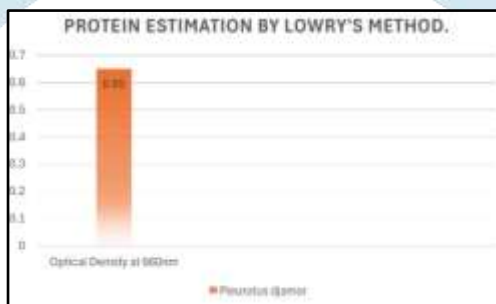


Figure : 9 - Protein estimation by Lowry's method in *Pleurotus djamor* mushroom.

<i>Pleurotus djamor</i> mushroom	Results
Protein concentration	790 µg/mL

Table : 3 - Protein Concentration of *Pleurotus djamor* from the standard curve against the concentration of mushroom.

7. CONCLUSION

This review provides valuable insights into the cultivation of *Pleurotus djamor*. Their cultivation functions as a successful bioconversion technique, transforming agricultural waste materials into possibly useful resources. This approach is very promising globally as well as in India, for sustainable agriculture and in the forest industry. *P. djamor* has given the mushroom industry a greater spectrum in terms of quantity and organic mushroom production. In nations like India, where agricultural waste is readily available throughout the entire year, this mushroom has a very wide growing area and has garnered international recognition. The second factor is that 80–90% of Indian locations have the tropical or subtropical climates needed for *P. djamor* cultivation. Therefore, *P. djamor* mushrooms may be identified and consumed as supplements, as this has high nutrient values, hence it helps to encounter malnutrition.

ACKNOWLEDGEMENT:

The authors gratefully extend their sincere gratitude to Associate Professor Praveen T. (Head of Microbiology, Vijaya college, Jayanagar, Bengaluru - 560011) and Assistant Professor KavyaSree D S (Department of Microbiology, Vijaya college, Jayanagar, Bengaluru - 560011) for their expert guidance and invaluable support throughout the course of this research.

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