Paddy straw: an economical substrate for oyster mushroom (*Pleurotus ostreatus*) cultivation

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Abstract. Cultivation of the *Pleurotus ostreatus*, oyster mushroom on paddy straw without supplements was investigated to follow circular economy concept to convert agricultural waste to value added products. Substrate nutrients, mushroom yield, and biological efficiency were determined. Three different extracts were used in this study (methanol, ethyl acetate, and hexane). Antioxidant and scavenging activity was determined using DPPH and H_2O_2. To find the essential compounds present in the mushrooms, GC-MS was analyzed. It was found that mushroom growth on paddy substrate was less than five days with excess mushroom yield. The biological efficiency was found between 54.5-130.9%, with the moisture of 93%. It was found that C, P, N, and K were integrated into mushrooms with these elements than in the utilized substrate. In DPPH results, the minimum concentration was 37.07 µg/ml, and the maximum was 67.2 µg/ml. IC50 value of 42.6 µg/ml were 50% for inhibition concentration. In H_2O_2, the minimum concentration was found to be 72.57 µg/ml, and the maximum was 98.02 µg/ml. This concentration indicates that the IC50 value of 84.07 µg/ml can be used in the biological process or component by 50% for inhibition concentration. The compounds include Oxirane, 2-Methyl-3-(1-Methylethyl)-, O-Methylisourea Hydrogen Sulfate, Diethyl Phthalate, 1,1,3,3-Tetrapropoxy- were found commonly in all three extracts. Hence, analysis of mushroom extracts is needed to determine the mechanisms of action of the various components for antimicrobial activity and inhibitory activity. Therefore, paddy straw could be used as an effective and economical substrate for oyster mushroom cultivation.

Keyword. *Pleurotus ostreatus*, Paddy straw, *DPPH,* Mushroom, Cultivation

1 Introduction

Oyster mushrooms are generally valued for their taste and nutritional and medicinal properties, which have been extensively studied [1]. It contains a vital nutritional source of protein, vitamins, carbohydrates, iron and calcium. In addition, these mushrooms obtain nutrients from organic materials, such as straw, deadwood, dung, manure, and decaying organic matter. Growing these mushrooms with lignocellulosic agricultural waste results in value-added food for humans. It has been cultivated widely in different parts of the world and is known as fruiting bodies of fungi. Therefore, it can grow over a wide range of temperatures using a variety of lignocelluloses [2]. In India, the cultivation of mushrooms began in the 19th century. According to the Food and Agriculture Organization (FAO) of United Nations statistics, China is the world's top leader in mushroom production, as reported in 2018 [3]. Consequently, they are widely used in the bioremediation of pollutants and decomposition of lignocellulosic residues under the action of various enzymes.

Mushrooms require inorganic compounds, carbon and nitrogen as a food source and primary nutrients, such as cellulose, hemicellulose and lignin. Paddy or rice straw as a substrate for mushroom cultivation is produced as an agricultural by-product [4]. Currently, rice fields are disposed of by burning in the open air, leading to serious environmental problems. If paddy straw can nourish the growth of oyster mushrooms, this could convert the non-edible waste into profitable and edible biomass. These fungi can be collected in the wild during the last the wettest part of the rainy season, where they meet and grows on deeply decaying organic matter. However, not all mushrooms growing in the wild are suitable for human consumption. In addition, this biomass could serve as an inexpensive substrate for mushroom growers. However, it is reported that the use of rice straw in oyster mushroom cultivation is not widespread in China due to its low biological competence and yield [3]. However, it also has medicinal benefits for people who have cancer and diabetes. In particular, *Pleurotus* species constitutes high potassium and sodium, edible food for

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patients suffering from high blood pressure and heart disease [1]. In addition, mushroom cultivation practice could improve the straw quality by reducing the lignocellulosic biomass composition. Thus, this current study focuses on using paddy straw as a basal substrate for the cultivation of oyster mushrooms.

2 Materials and Methods

2.1. Substrate preparation

Experimental design involves four steps in mushroom cultivation production: substrate preparation, inoculation, growing, and harvesting. Substrate preparation includes spawning production, in which wheat grains were soaked in water for 7-8 h and boiled in water for 30 min in the ratio of [1:2 w/v]. Later, it was cooled, and calcium carbonate was mixed in the ratio of [50:1 w/w]. This mixture was autoclaved at 121°C for 15 min. Thereafter, it was incubated at 21°C for 15- 20 days. Lignocellulosic biomass, i.e. paddy straw, was used as the substrate [5]. The growth of mycelium was seen in the production of mushrooms. The substrate was pre-treated with hot water at 70°C for a few hours. Thereby, the substrate was soaked with benzoyl to avoid contamination of *Trichoderma*. Paddy straw was obtained from Velapadi village, Vellore. It was stored for three months after harvesting **Fig. 1**. The straw was chopped into 2.5cm for every cultivation using a hand cutter.

(a)

(b)

Fig. 1 (a) Substrate preparation **(b)** Paddy straw

2.2. Inoculation

In order to avoid contamination, 15 g formalin was mixed with 1 kg batch culture.

2.3 Cultivation of mushroom

For the cultivation of mushrooms, bedding preparation was done. Initially, 200-250 g of the substrate was taken in a polythene bag, 1st layer of the substrate was about 12 cm. 2nd layer of spawns was scattered for about 12-20 days for incubation. During the incubation period, the temperature and pH were maintained. An optimized temperature of about 20-25°C was maintained. Casein soil was used for the bedding of mushrooms [6]. The casing mixture was prepared using soil, coco-peat and sand in the ration of [3:2:1]. Earlier, this casing mixture was sterilized for 30 min at 121°C. Temperature and moisture were maintained by spraying the water between 21-23°C.

2.4 Harvesting

The mushrooms were harvested using an environmental chamber (temperature, ventilation, and humidity). Relative humidity and temperature were controlled at 70-80% and 24 ± 1°C, respectively. These conditions were determined using the preliminary test carried out using different conditions. The inoculated bags were kept in the environmental chamber to develop the fruit bodies. After harvesting, the mushrooms were gently twisted, and the end parts were cut off and later weighed. Also, the substrate in the bag was weighed. The samples of both substrate and mushrooms were analyzed for the contents of biological efficiency.

$$BE,\% = \frac{W2}{W1} \times 100 \quad (1)$$

Where, BE (Biological efficiency, %) W1- Total dry weight of the compost, W2- Total weight of fresh mushroom.

2.5 Analysis of nutrient elements of substrate and mushroom

To understand the concentration of nutritional elements such as nitrogen (N), carbon (C), phosphorus (P), and potassium (K) in the substrate were analyzed using an inductively coupled plasma atomic emission spectrometer (ICP-AES) [7].

2.6 *DPPH* radical scavenging activity

Antioxidant activity of *P. ostreatus* was determined using the purple DPPH radical solution. 1 mg/ml of extract was dissolved in methanol, ethyl acetate, and hexane using different concentrations (25, 50, 75, and 100 µg/ml). For control, DPPH and methanol were used without the extract mixture [8]. The mixture was vigorously mixed and stored in the dark for 30 min. The scavenging activity was measured at 517 nm using a UV-VIS spectrophotometer. For positive control, ascorbic acid was used.

2.7 Hydrogen peroxide scavenging activity assay

Hydrogen peroxide (H_2O_2) solution was prepared using phosphate buffer with the pH of 7.4 methods proposed by [9]. Different concentrations of extract (25, 50, 75, and 100 µg/ml) in 1 ml of phosphate buffer with addition of H_2O_2 solution 0.6 ml. The mixture was stored at room temperature for 10 min. Using a spectrophotometer, the absorbance of the mixture was determined at 230nm, and ascorbic acid was used as control. The scavenging activity was calculated using the following equation.

$$Scavenging\ effect\ (\%)/\%\ Inhibition = A0 - A1\ /A0 \times 100$$

Where A_0= the absorbance of control. A_1 absorbance of standard.

2.8 GC-MS analysis

5g of dry powder sample was extracted with different solvents systems based on polar (methanol and ethyl acetate) and non-polar (hexane). The samples were kept in a shaker overnight was repeated in triplicates. Later, the extract was filtered and condensed under vacuum at 60°C using a rotary evaporator and stored at 4°C. The sample was filtered using a 0.2 µm nylon membrane and injected into Gas Chromatrography-Mass Spectrophotometry (GC-MS). The analysis was carried out on a Perkin-Elmer workstation, with model Clarus 600 GC coupled to a mass spectrometer (Perkin Elmer Technologies, Inc., Wilmington, DE) [10]. Elite-5MS (30 m x 0.25 mm width film depth of 250 µm capillary tube was used with the following conditions. Oven initial temperature of 55°C for 3 min, ramp program for 6°C/min up to 310 °C, further 3 min isothermal hold. Helium (He) gas was used with a flow rate of 10:1 ratio. 2 µl of the sample was injected into the injector was maintained at 250°C. Results were analyzed using mass spectra from the National Institute of Standards and Technology (NIST-LIB 0.5) library. The individual phytochemicals present in the crude extract were separated by GC column. The MS spectrum displays the molecular weight of individual molecules accurately.

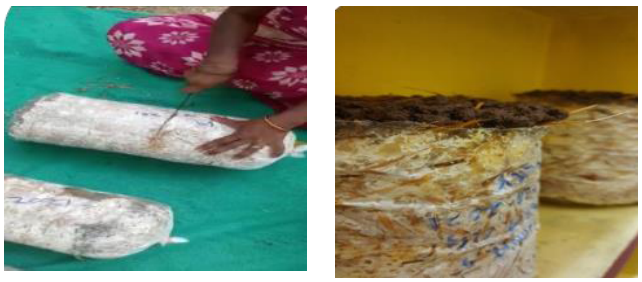
3 Results and discussion

In this work, the results discussed the mushroom yield, biological efficiency, nutrient elements of the substrate, antioxidant activity, and extracted essential compounds. The cost for the production was calculated in each step (spawn preparation, bedding supplements and packing). Mushroom bedding and fruiting are shown in **Fig. 2.** The mycelium was spread all over the mushroom bedding during incubation (10-20 days).

(a) (b)

Fig. 2 (a) Bedding preparation of mushroom **(b)** Incubation and fruiting

For harvesting the mushrooms, casein soil was used to induce the mushroom growth. This mixture was prepared as above mentioned. Instead of coco peat, farmyard manure can also be used for mushroom production due to its unique water holding capacity. After the incubation period, the beds were cut into two halves, and 3-4 cm thickness of casein soil was spread over the substrate and temperature was maintained under 21± 1°C as shown in **Fig. 3.** Similarly, the budding of mushrooms and matured mushrooms are shown in **Fig. 4.** The total cost for the production of mushrooms is listed in **Table 1.**



(a) (b)

Fig. 3 (a) Mushroom bedding was cut into 2 halves **(b)** beds with casein soil

(a) (b)

Fig. 4 (a) Budding of Mushroom **(b)** Matured species *P. ostreatus*

Table 1 Production cost of Mushrooms

S.No	Items	Cost
1	Spawn preparation	105
2	Preparation of 80 beds	270
3	Supplements	30

4	Packaging bag	30
	Total	**435/-**

In addition, to increase mushroom marketing, storage, packaging, and canning are essential sources for consumption. It has been reported that development and economic mushroom consumption are gradually generating. Hence, this method of cultivation and production could be user-friendly for farmers and consumers.

Substrate degradation and nutrient analysis determined the mushroom's yield and biological efficiency. The dry matter loss was determined by comparing mushroom yield to its biological efficiency. The fungus partially assimilated the loss of dry matter and partially lost to the atmosphere as carbon dioxide due to the respiration of the fungus. The biological efficiency was found between 54.5-130.9%, with the moisture of 93%. The nutrient elemental analysis results were determined for the chopped substrate and mushroom. The initial utilized weight was also measured. It was found that C, P, N, and K were integrated into mushrooms with these elements than in the utilized substrate. In addition, the C and N loss may be due to mushroom respiration and volatilization during the N mineralization process [11]. P and K contents were found to be well balanced among initial, utilized, and mushrooms

Table 2. Scavenging activity was determined using DPPH and H_2O_2 assay. In DPPH free radical scavenging, the mechanism for screening the antioxidant activity of the extracts (methanol, ethyl acetate, and hexane) was done, and it was found that the violet color of the DPPH solution was reduced to yellow color. The minimum concentration was 37.07 µg/ml, and the maximum was 67.2 µg/ml **Fig. 5**. IC50 was 42.6 µg/ml, where this concentration indicates can be used in the biological process or component by 50% for inhibition concentration.

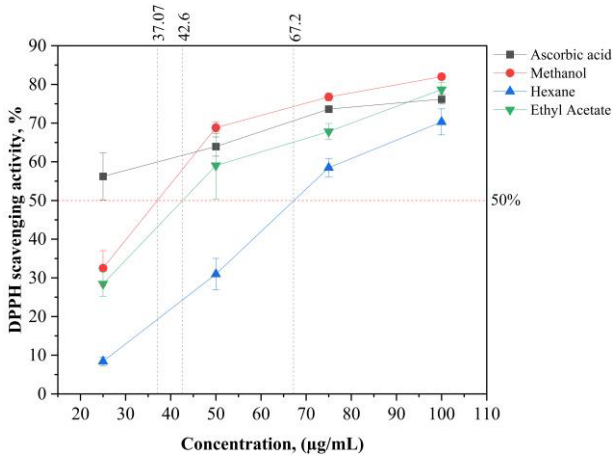


Fig. 5 DPPH radical scavenging assay

Similarly, H_2O_2 scavenging activity was determined to know the reactive oxygen metabolic, which is the key regulator of oxidative stress-related states. It was found that the quantitative determination of hydrogen peroxide in extracts (methanol, ethyl acetate and hexane) were disappeared at the wavelength of 230nm. The minimum concentration was found to be 72.57 µg/ml, and the maximum was 98.02 µg/ml **Fig. 6**. IC50 was 84.07 µg/ml, indicating that this concentration can be used in the biological process or component by 50% for inhibition concentration. This method is convenient, highly accurate, and suitable for quickly quantifying the ability of standard and natural antioxidants present in plant extracts to absorb H_2O_2.

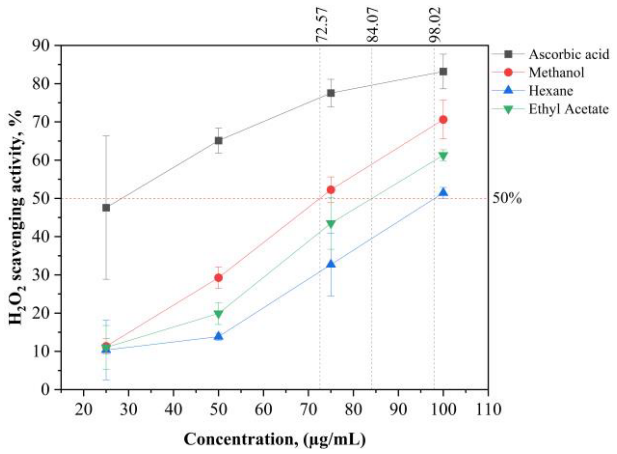


Fig. 6 H_2O_2 scavenging activity

Table 2 Nutrients elements of both substrate and mushroom

Paddy straw	Materials	Dry weight (g)	Nutrient Content C	P	N	K
Chopped	Initial substrate	100	34.8	0.10	0.80	1.15
	Utilized substrate	70.3	32.9	0.05	0.63	1.25
	mushroom	6.8	40.5	0.95	4.32	3.57

GC-MS chromatograms of the crude extracts were determined by ruling the similar compounds present in all three extracts (methanol, ethyl acetate, and hexane) using the presence of a peak at retention time [12]. On comparison of the mass spectra of the compound with the NIST library, the compound was matched. (NIST

library; Molecular weight (m/z). The common compounds present in extracts were given in Error! Reference source not found.. The compounds include Oxirane, 2-Methyl-3-(1-Methylethyl)-, O-Methylisourea Hydrogen Sulfate, Diethyl Phthalate, 1 Methyl-2-Phenoxyethylamine, 1,3 Bis(Hydroxymethyl) Urea, 2-Propanone, 1,1,3,3-Tetrapropoxy- were found commonly in all three extracts and methanol extract showed the accurate results than other extracts **Fig. 7**.

Table 3 GC-MS analysis of mushroom crude extracts

S.No	Retention Time (min)	Name of Component	Molecular weight	Area%	Molecular Formula
1.	3.31	OXIRANE, 2-METHYL-3-(1-METHYLETHYL)-	100	2.84	$C_6H_{12}O$
2.	3.92	O-METHYLISOUREA HYDROGEN SULFATE	74	0.70	$C_2H_6ON_2$
3.	4.60	DIETHYL PHTHALATE	222	1.5	$C_{12}H_{14}O_4$
4.	3.18	1-METHYL-2-PHENOXYETHYLAMINE	151	3.65	$C_9H_{13}ON$
5.	13.296	1,3-BIS(HYDROXYMETHYL)UREA	120	1.17	$C_3H_8O_3N_2$
6.	17.104	CYCLOBUTANONE, 2-METHYL-4-HYDROXY-	100	2.59	$C_5H_{12}N_2$
7.	19.30	2-PROPANONE, 1,1,3,3-TETRAPROPOXY-	290	7.05	$C_{15}H_{30}O_5$
8.	27.153	9-1-OCTADECADIENOIC ACID, METHYL ESTER	280	2.84	$C_{18}H_{32}O_2$
9.	30.769	HEPTANOIC ACID, 2-METHYL-2-BUTYL ESTER	200	6.43	$C_{12}H_{24}O_2$

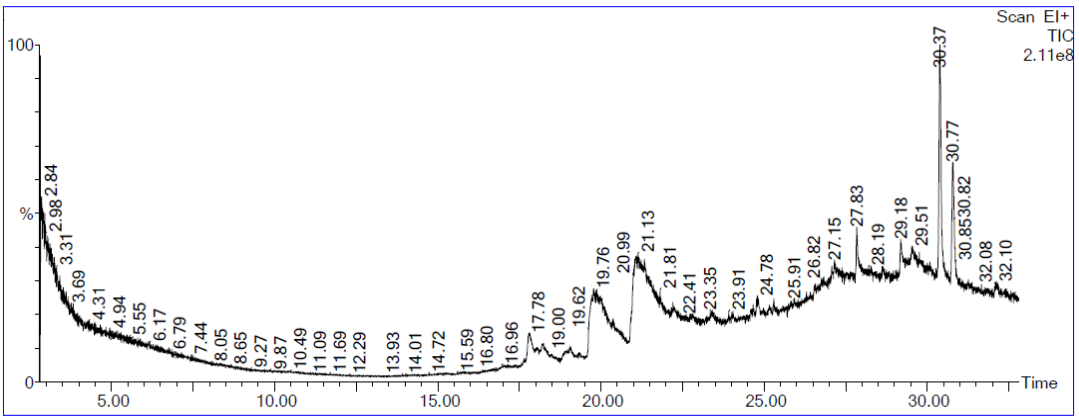


Fig. 7 Chromatogram profile methanol extract

4 Conclusion

The major finding of this study is the paddy substrate showed a better yield of mushroom production in a cheap and faster way. The mushrooms grew faster on the substrate with less than five days of growth cycles. There are several types of edible mushrooms that, however, have not been researched for their potential use in foods.

a variety of cheap agricultural or forest waste, such as rice straw, corncobs, and sawdust. Anti-fungal

These new fungal species may have different organic processes, sensory organs, and environmental effects than those exhibited by the species used so far. Therefore, there is a need to analyze these different species. In addition, any analysis is needed to determine the mechanisms of action of the various components of the fungus, for example, their antimicrobial activity, inhibitory activity, structure, textural characteristics and taste characteristics. The benefits of mushrooms are relatively economical because mushrooms are grown on

inoculants can be manufactured in the factory using today's simple techniques that usually do not produce

fungi. When looking for economic and environmentally friendly strategies for environmental restoration, the use of mushrooms can be an excellent approach and solution.

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