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Evaluation of Lignocellulosic Substrates on Productivity of Pearl Oyster Mushroom (*Pleurotus ostreatus*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Among the oyster mushrooms, Pearl oyster mushroom (*Pleurotus ostreatus*) is one of the important edible mushrooms cultivated in India. It can be grown on different lignocellulosic substrates. However, its productivity depends upon the nature and the composition of the substrates. Therefore, locally available ten different agri-wastes such as maize stalk, sugarcane bagasse, rice husk, saw dust, banana pseudo stem, groundnut haulm, paper, finger millet stalk, sesame stalk and paddy straw were collected and evaluated for their ability to produce mushroom. The trial was conducted in Centre of Tropical Mushroom Research and Training (CTMRT), OUAT, Bhubaneswar. The substrates were analysed for carbon, nitrogen, cellulose, lignin percent before mushroom production. The data on days to spawn run, pin head initiation, first harvest, biological efficiency (%), average number of fruit bodies per bag, yield (g / bag) along with morphometrics were recorded and analysed with Randomised Block Design using MS-Excel. Highest production of 1366.67 g mushroom per 2kg dry substrate with an average of 246.40 numbers of mushrooms was realised from banana pseudo stem followed by paddy straw (1246.67 g, 240.27 numbers of mushrooms). Minimum time taken, 32.60 days for mushroom production observed in case of finger millet stalk followed by sugarcane bagasse (32.67 days). From the experiment, it was also observed that a range of 40 to 60 C:N ratio and higher cellulose and lignin ratio of the substrates favoured maximum productivity of oyster mushroom.

Keywords: Pearl oyster; Pleurotus ostreatus; substrates; C:N ratio.

1. INTRODUCTION

Pleurotus spp are commonly known as oyster mushroom because of their oyster-like structure. These are the second most popular mushroom in world after button mushroom. These are known as wood fungus and in India it is popularly known as “Dhingri”. These mushrooms are grown in around 25 countries, with China, South Korea, Japan, Italy, and Taiwan being the primary producers. It is also commercially grown in the states like Chhattisgarh, Odisha, Punjab, Maharashtra, Tamil Nadu, Bihar, Gujarat and North Eastern states like Meghalaya, Tripura, Mizoram and Assam. Among the 38 species cultivated worldwide, Pearl oyster mushroom (*Pleurotus ostreatus*) is one of the important cultivated mushrooms. It belongs to family Pleurotaceae of Phylum Basidiomycota. It grows naturally in the temperate and tropical forests on dead, decaying wooden logs. It is a common edible mushroom cultivated using straw and other substrates. Different agricultural and forest by products including straw of paddy, wheat, ragi; stalk and leaves of maize, jowar, bajra, cotton, sugarcane; peanut shells; dried grasses; paper; coffee waste and synthetic compost of button mushroom with rich cellulose, hemicelluloses and lignin contents are used for cultivation of oyster mushroom in large scale. However, yield of oyster mushroom largely depends upon the nutrition and nature of the substrates. *Pleurotus ostreatus* is a potential source of protein along with vitamins and minerals (Sharma et al., 2013 and Effiong et.al., 2024). It has also got different pharmaceutical properties such as antitumor, antioxidant, anti-platelet aggregating, antimicrobial and antiviral activities. Keeping the above importance facts, efforts have been undertaken to assess the nature of substrates and their effects on the productivity of this mushroom.

2. MATERIALS AND METHODS

2.1 Oyster Mushroom Bag Preparation

All the experiments were conducted in the growing rooms of the Centre of Tropical Mushroom Research and Training (CTMRT) in the Department of Plant Pathology, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar. The test fungus (*Pleurotus ostreatus*) was procured from ICAR-Directorate of Mushroom Research, Solan (HP). The fungal culture was multiplied in Potato Dextrose Agar (PDA) medium. To evaluate the efficacy of substrates, ten different lignocellulosic substrates were collected from the locality. The dried substrates were chopped to a size of 2 inch with help of chaff cutter followed by soaking for six hours. The substrates were maintained 65% moisture for preparation of oyster mushroom bags. The bags were filled with 2kg substrates on dry weight basis in polyethene bags. About 3% of 15days old spawn were applied in layer method on the substrate. The prepared bags were incubated on different tiers of the cropping room with appropriate temperature, humidity and light for mycelia development.

The cumulative yield of each replication was recorded which was represented as weight (g) per unit of dry substrate. The yield was expressed in terms of biological efficiency (B.E.) and calculated using following formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

2.2 Biochemical Analysis of Substrates

2.2.1 Estimation of carbon percentage

The carbon content of the substrates was determined according to report of Nelson and Sommers (1982). The percentage organic carbon content was then calculated according to the formula below;

$$C (\%) = M \times (V_{bl} - V_s) \times 0.003 \times 1.33 \times 100 / G$$

Where,

M =Molarity of $FeSO_4$

V_{bl} = ml $FeSO_4$ of blank titration

V_s = ml $FeSO_4$ of substrates titration

g= mass of substrates taken in gram

0.003= milli-equivalent weight of C in grams (12/4000)

1.33 = correction factor used to convert the wet combustion C value to the true C value since the wet combustion method is about 75 % efficient in estimating C value , (i.e. 100/75 = 1.33)

2.3 Estimation of Nitrogen

Similarly, the nitrogen content of the samples was determined according the procedure described by Bremner and Mulvaney (1982).

2.4 Cellulose Determination

The amount of cellulose was determined using procedure followed by Philip and Adrian (1998).

2.5 Lignin Determination

Klason method was used for determination of lignin content of substrates.

2.6 Statistical Analysis

The field trials were conducted with randomized block design (RBD) and analysis of data were performed by using MS-Excel. The mean values of each character were worked out by dividing the total value with number of observations.

3. RESULTS

The analysed data in Table 1 revealed that days to spawn run in the substrate varied from 17.93 days to 28.53 days. Minimum days requirement for spawn run was 17.93 days observed in sugarcane bagasse followed by paddy straw (21.47 days) and finger millet stalk (21.53 days). Moreover, sesame stalk (23.40 days), maize stalk (23.93 days), rice husk (24.00 days) and banana pseudo stem (24.07 days) were statistically at par with each other. The maximum time 28.53 days was taken by saw dust. Early pinhead initiation was observed in finger millet stalk (25.93 days) followed by paddy straw (27.40 days), sugarcane bagasse (28.33 days) and maize stalk (28.33 days) respectively. Days to first harvest of mushroom was recorded from finger millet stalk (32.60 days) which is statistically at par with that of sugarcane bagasse (32.67 days), paddy straw (33.80 days) and sesame stalk (33.80 days). The range of oyster mushroom yield varied from 1366.67 g to 383.33 g from 2 kg dry substrate. Highest yield of mushroom recorded 1366.67 g from banana pseudo stem with 246.40 number of mushrooms. The second highest yield of mushroom 1246.67 g observed from paddy straw with 240.27 numbers of mushrooms which was statistically at par with finger millet stalk (1203.33 g, 248.87 mushrooms). Minimum yield 383.33 g harvested from paper with lowest number of mushroom (69.00). Similar trend was observed in biological efficiency with a range of 19.17 percent to 68.33 percent. It was interesting that maximum fruit body weight of mushrooms 5.70 g with lower biological efficiency (43.83 %) recorded in case of saw dust as compared to the other substrates.

The data on morphological characters of mushroom harvested from different substrates are presented in Table 2. The morphology of mushroom has a great role in yield of mushroom per bag. Pileus size of mushrooms varied from 55.00 mm to 72.67 mm. Maximum pileus size (72.67 mm) was observed in mushrooms harvested from saw dust which is at par with that of banana pseudo stem (71.53 mm). Minimum pileus size (55.00 mm) observed in case of finger millet stalk and sesame stalk which were at par with that of rice husk (55.40 mm) and groundnut haulm (58.67 mm). Similarly, maximum stipe length was observed in case of saw dust (31.73 mm) followed by paper (29.67 mm). However, paddy straw (22.47 mm), banana pseudo stem (22.53 mm) and rice husk (23.07 mm) were statistically at par with each other with respect to stipe length. Stipe diameter of mushrooms harvested from different substrates varied from 5.67 mm to 7.80 mm. Maximum stipe diameter (7.80 mm) recorded in the mushroom harvested from banana pseudo stem was statistically at par with that of paddy straw (7.13 mm), groundnut haulm (7.13 mm), finger millet stalk (7.07 mm) and saw dust (7.00 mm).

Table 1. Evaluation of ligno-cellulosic substrates for production of oyster mushroom (*Pleurotus ostreatus*)

Sl. No.	ligno-cellulosic substrates	Days to spawn run	Days to pin head initiation	Days to 1st harvest	BE (%)	Average numbers of fruit bodies/ bag	Yield (g)/ 2 kg dry substrate
1	Paddy straw	21.47 b	27.40 b	33.80 a b	62.33 g	240.27 e	1246.67 f g
2	Sugarcane bagasse	17.93a	28.33 b	32.67 a	53.33 d e	218.33 d	1066.67 d
3	Maize stalk	23.93 c	28.33 b	34.47 b	49.67 d	197.27 e	993.33 d
4	Paper	25.27 d	31.60 d	41.13 d	19.17 a	69.00 a	383.33 a
5	Sesame stalk	23.40 c	29.73 c	33.80 a b	55.83 e f	240.93 e	1116.67 d e
6	Groundnut haulm	24.73 cd	32.00 d	36.07 c	38.17 b	184.73 d	763.33 b
7	Rice husk	24.00 c	33.13 e	39.73 d	42.50 b c	174.93 c	850.00 b
8	Banana pseudo stem	24.07 c	30.67 c d	35.20 b c	68.33 h	246.40 e f	1366.67 g
9	Saw dust	28.53 e	36.93 f	43.20 e	43.83 c	153.93 b	876.67 c
10	Finger millet stalk	21.53 b	25.93 a	32.60 a	60.17 fg	248.87 f	1203.33 e f
	SE(m)±	**0.23**	**0.34**	**0.49**	**1.62**	**2.03**	**32.36**
	CD(0.05)	**0.67**	**1.02**	**1.45**	**4.81**	**6.04**	**96.14**

Table 2. Morphometrics of oyster mushroom (*Pleurotus ostreatus*) as influenced by ligno-cellulosic substrates

Sl. No.	ligno-cellulosic substrates	Pileus size (mm)	Stipe length (mm)	Stipe diameter (mm)
1	Paddy straw	61.07 b c	22.47 b	7.13 b c
2	Sugarcane bagasse	64.53 c d	27.47 d	6.80 b
3	Maize stalk	63.07 c	28.67 d e	5.67 a
4	Paper	67.60 d e	29.67 e	6.00 a
5	Sesame stalk	55.00 a	24.07 c	6.53 a b
6	Groundnut haulm	58.67 a b	18.53 a	7.13 b
7	Rice husk	55.40 a	23.07 b c	6.47 a
8	Banana pseudo stem	71.53 e	22.53 b	7.80 c
9	Saw dust	72.67 f	31.73 f	7.00 b c
10	Finger millet stalk	55.00 a	23.80 c	7.07 b c
	SE(m)±	**1.32**	**0.50**	**0.32**
	CD(0.05)	**3.91**	**1.49**	**0.96**

Table 3. Chemical composition of ligno-cellulosic substrates

Sl. No.	Ligno-cellulosic substrates	Carbon (%)	Nitrogen (%)	Carbon nitrogen ratio	Cellulose (%)	Lignin (%)	Cellulose: Lignin
1	Paddy straw	63.6	1.19	53.45	32.61	14.52	2.25
2	Sugarcane bagasse	56.34	0.74	76.14	41.30	22.41	1.84
3	Maize stalk	62.63	0.84	74.56	39.20	31.37	1.25
4	Paper	42.51	0.40	106.28	89.14	8.46	10.54
5	Sesame stalk	64.34	0.84	76.60	43.20	12.18	3.55
6	Groundnut haulm	56.66	2.02	28.05	32.60	25.65	1.27
7	Rice husk	67.22	0.56	120.04	29.20	17.52	1.67
8	Banana pseudo stem	59.35	1.41	42.09	53.32	13.75	3.88
9	Saw dust	53.27	0.40	133.18	38.60	27.3	1.41
10	Finger millet stalk	57.23	0.92	62.21	37.30	13.83	2.70

Data in the Table 3 revealed that maximum and minimum percent of carbon was observed in rice husk (67.22 %) and paper (42.51%) respectively. It was observed, that all except paper, possess more than 50 percent carbon. Nitrogen percentage among the substrates varied from 0.40 % to 2.02 %. The nitrogen content of seven substrates out of ten recorded less than one per cent except groundnut haulm (2.02 %), banana pseudo stem (1.41 %) and paddy straw (1.19 %). Similarly, carbon nitrogen ratio (C:N ratio) of the substrates varied from 28.05 to 133.18. The C:N ratio of saw dust, rice husk and paper recorded more than 100. The cellulose content of the substrates varied from 29.20 % (rice husk) to 89.14 % (paper). Similarly, lignin content varied from 8.46 % to 31.37 %. However, maximum and minimum cellulose lignin ratio was recorded from paper (10.54 %) and maize stalk (1.25 %), respectively.

4. DISCUSSION

Oyster mushrooms can grow on a wide range of agricultural wastes with variable yield potential. Hence, ten ligno-cellulosic wastes of different crops evaluated to ascertain the variability with respect to mycelia growth as well as productivity. All the field experiments were conducted in the growing room of CTMRT, OUAT, Bhubaneswar where as chemical composition of ligno-cellulosic substrates were analysed in the Central laboratory of OUAT . On the basis of days to spawn run in different substrates evaluated varied from 17.93 days (sugarcane bagasse) to 28.53 days (saw dust). As compared with other workers, spawn run in different substrates varied 13 to 20 days (Patra and Pani, 1995; Ahmed, 1998 and Jiskani, 1999). Higher cellulose content in substrates enhance the early growth mushroom (Boadu et al., 2023). Quicker spawn run in sugarcane bagasse than paddy straw reported by Iqbal et al., 2005 and Mosisa et al., 2015 corroborated the present result. Apart from mycelia growth in substrates, there was wide variation of yield potential among the substrates. Realization of higher yield of mushroom from banana pseudo stem supported the findings of Iqbal et al., 2005. However, the yield potential of sugarcane bagasse as compared with paddy straw did not agree with the result of Khalaphallah et al., 2020. Low yield of mushroom from saw dust and paper may be due to the lack of porosity that allows the mycelium for respiration and ease access in the substrate in order to get nutrition (Osunde et al., 2019). Shah et al., 2004 recorded maximum biological efficiency of oyster mushroom (*P. ostreatus*) from saw dust in comparison with wheat straw but Shauket, 2012 reported highest biological efficiency from wheat straw followed by paddy straw. Higher cellulose and lignin ratio enhanced the yield of mushroom suggested by Badu et al., 2011 supported the present findings. The variation in yield of oyster mushrooms on different substrates were because of physical and chemical characteristics such as pH, water holding capacity, porosity, carbon nitrogen ratio, cellulose lignin ratio etc. The present result of higher C:N ratio of sugarcane bagasse facilitated quicker mycelia growth than paddy straw. Similarly, the present result with respect to C:N ratio and morphology in case of banana pseudo stem and paddy straw agreed with that of lower C:N ratio favours the growth of mushroom as reported by Yang, 2000.

5. CONCLUSION

From the present experiments it was observed that a range of 40-60 carbon nitrogen ratio preferred by *P. ostreatus* for mushroom production. High cellulose content of substrate enhance the early growth of the mushroom. Similarly, higher cellulose and lignin ratio of the substrates was preferred by the fungus to produce mushroom. Banana pseudostem could be able to produce maximum fruiting body of mushroom as compared to paddy straw. Moreover, paddy straw may be recommended for mushroom production with early harvest of mushroom as well as easy management of the wastes produced from the paddy fields.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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