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Effect of Substrates on Growth and Nutritional Composition of Oyster Mushroom (*Pleurotus ostreatus*)

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ABSTRACT

Mushrooms have been utilized as food and supplements since the dawn of mankind. They're becoming more well recognized as one of the most important food components because to their important functions in human health, nutrition, and disease. If waste isn't managed, it can cause environmental concerns, but it can be beneficial if it's used as a growing media supplement for oyster mushroom production. The goal of this research is to look into and evaluate the impact of sawdust, rice straw, and paper, as well as their mixtures, on the yield and nutritional composition of Pleurotus ostreatus mushrooms.The results revealed that different substrates formulas gave a significant difference in total colonization period, characteristics of fruiting bodies, yield, biological efficiency (BE), nutritional composition of *Pleurotus ostreatus* (os) mushroom. The highest length and diameter of stock, highest diameter and thickness of pileus was observed in the treartment SD+RS+PAPER (3.32,1.24,7.32,0.88cm) and the lowest was observed in paper (2.1,0.89,5.73,0.71 cm). The highest time from inoculation to primordia initiation was observed in paper (47 days) and the lowest time from inoculation to primordia initiation was in the treatment rice straw (26 days). The highest average number of fruiting body/packet was observed in the treatment saw dust + rice straw + paper (23.00) and the lowest average number of fruiting body /packet was in the treatment paper (15.00).The highest biological yield was counted under treatment SD+RS+PAPER (253.41g) and the lowest biological yield was counted under paper (177.5g). The content of protein varied from 12.88-9.03% (w/w). The lowest lipid percentage was counted under treatment saw dust + rice straw (3.38%) and the highest lipid percentage was counted under saw dust (7.71 %). The highest percentage of crude fiber was counted under treatment SD+RS+PAPER (10.48%) and the lowest crude fiber percentage was counted under RS+SD (7.01%). The lowest percentage of carbohydrate was counted under treatment SD (51.82%) and the highest carbohydrate percentage was counted under SD+RS (56.63%). The lowest percentage of moisture was counted under treatment SD+RS (8.86%) and the highest moisture percentage was counted under RS (11.2%). The highest percentage of ash was counted under treatment SD+RS (13.5%) and the lowest ash percentage was counted under RS (9.1%).Therefore, it can be concluded that sawdust+rice straw+paper can be utilized as a better substrate for the cultivation of *Pleurotus ostreatus* oyster mushrooms, lowering costs while enhancing yield and nutritional quality.

Keywords

Biological yield
Economic yield
Mushroom
Mycology
Nutrition
Rice straw
Saw dust

INTRODUCTION

Mushrooms are increasingly being recognized as important food products for their significant role in human health, nutrition and disease [1]. *Pleurotus* species that are also known as oyster mushrooms, are edible fungi cultivated worldwide. *Pleurotus* genus is one of most extensively studied white-rot fungi due to its exceptional ligninolytic properties. Furthermore, these mushrooms have the ability to colonize and degrade a wide variety of lingo-cellulosic wastes with relatively short cycle [2-10]. Growing oyster mushrooms convert a high percentage of the lingo-cellulosic substrate to fruiting bodies increasing profitability. Of them, *Pleurotus ostreatus* demands few environmental controls, and their fruiting bodies are not often attacked by diseases and pests, and they can be cultivated in a simple and economic way [11, 12]. It requires a short growth time in comparison to other edible mushrooms. All this makes *P. ostreatus* cultivation an excellent alternative for production of mushrooms when compared to other mushrooms [13].

Mushrooms with their flavor, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries [14, 15]. *P. osteratus* are rich source of proteins, minerals & vitamins [16]. Apart from food value, its medicinal value for diabetics and in cancer therapy has been emphasized [17]. *Pleurotus* species contain high potassium to sodium ratio, which makes mushrooms an ideal food for patients suffering from hypertension & heart diseases. The practice of mushroom cultivation not only produces medicinal and nutritious food but also improves the straw quality. This takes place by reducing lignin, cellulose, hemicelluloses, tannin and crude fiber content of straw making it ideal for animal feed [18-20].

Mushrooms require carbon, nitrogen and inorganic compounds as their nutritional sources and the main nutrients are carbon sources such as cellulose, hemicellulose and lignin. Oyster mushrooms require less nitrogen and more carbon source. Thus, most organic matters containing cellulose, hemicellulose and lignin can be used as mushroom substrate i. e. rice and wheat straw, cottonseed hulls, corncob, sugarcane baggase, sawdust, waste paper, leaves, and so on. However, demanded amount of each nutritional source differs according to mushroom species and substrate used. The demand of mushroom has been mounting day by day due to population growth, market expansions, changing of consumer behavior, and developments. Rice straw is the principal substrate for oyster mushroom cultivation in Nepal. Hence, in this study, we attempt to identify the alternative substrates from various agricultural and forest residue and to assess the growth performance and yield as well as the nutrient content of *P. ostreatus* [21-29]. Thus, present study has been aimed at determining the growth pattern of oyster mushroom on various waste materials such as rice straw, paper, and sawdust; growth pattern on their different mixtures and evaluate the grown mushroom's nutritional status. Using nutritional content and a benefit-cost analysis, we would choose the best substrate for growing *P. ostreatus* spawn packets.

MATERIALS AND METHODS

Location of experiment:

The research was conducted at the Mushroom Development Institute, Savar, Dhaka from November 2021 to March 2022.

Experiments and Treatments:

The experiment was set up using a single-factor Completely Randomized Design (CRD). To get the desired results, four separate treatments with four replications (n=4) of each strain were carried out. Total 60 spawn packets had been prepared using the substrates paper, rice straw, saw dust, rice straw: saw dust (1:1) and paper : rice straw : saw dust (1:1:1).

Collection of mother spawns:

The required number of mother spawns of oyster mushroom were collected from the mother spawn culture house of Mushroom Development Institute, Savar, Dhaka, Bangladesh. Their mycelium run was fully completed.

Mixing and Sterilization of the substrates:

At first, the rice straw and water were weighted precisely by a weight balance. The ratio of rice straw and water were 10:9. Then, they were mixed thoroughly with hand and feet for 10-15 minutes. The mixing was finished when there was no more water remaining after the rice straw had absorbed all of it. The moist rice straw was then placed in a net bag. The bag's opening was lightly bound and placed in the pasteurization chamber. The chamber's gate remained shut. At a temperature of 60 °C, the pasteurization process continued for 1.5 hours. A thermometer was used to closely monitor the temperature. For the following 24 hours, the chamber's gate remained shut. The chamber was reopened the next day and the moist rice straw-filled bag had been removed from the chamber. The rice straw had been disinfected and was ready to be used in the spawn packets.

Preparation of spawn packets:

The substrate and mother spawn were layered and mixed in a precise size PP bag for the specific treatment. For every 500g of rice straw, 100 g mother spawn was added. The filled polypropylene bags were made by placing a plastic neck at the opening part, plugging it with cotton, and securing it with a rubber band. Then, using a permanent marker, the treatment number, replication number, and packet number were written on the body of the spawn packet. The body of the 1500g and 2000g spawn packets was pierced with several little pores with a pin. After that, the spawn packets were transferred to the culture house.

Cultivation of spawn packet:

After 15-20 days, when the mycelium runs were completed, the spawn packets were ready to cut. Two ends of the upper position of the plastic bag, opposite one other, were cut in a "D" form with a blade and opened by removing the plastic sheet, following which the opening surface of the substrate was scraped slightly with a tea spoon to remove the thin whitish mycelium layer. From that day the relative humidity in the culture room was kept at 80-85% by spraying water three times a day. The culture house was kept consistently lit at 300-500 lux and ventilated. The culture house's temperature was kept between 22 and 25 °c. Depending on the packet size, the first pinhead appeared 3-7 days after scribing. Harvesting time was also affected by the amount of substrate used.

Collection of produced mushrooms:

After pinhead formation, oyster mushrooms matured in 4-6 days. The curial border of the capwas used to identify the ripened fruiting body. Mushrooms were gathered by twisting the base of the mushroom to uproot it. Then the lower hard and dirty area of the fruiting body were removed with a scissors & put the mushrooms in marked packets.

Proximate analyses

Proximate analyses had been performed following established methods in biochemistry.

- **Determination of total protein**: Total protein content was measured according to the Biuret method **Determination of total lipid**: Total lipid was determined by slight modified method of Folch *et al.* (1957) [30].
- **Determination of crude fiber**: Crude fiber was determined by gravimetric method [31].
- **Determination of total ash**: Ash was determined by following the standard Association of Official Agricultural Chemists (AOAC) method [32].
- **Determination of total carbohydrate**: The content of the available carbohydrate was determined by the following equation [33]:

Carbohydrate (g/100g sample) = [100 – (Moisture + Fat + Protein + Ash + Crude Fiber)].

RESULTS AND DISCUSSION

Effect of substrates on the yield, and biological efficiency of *Pleurotus ostreatus* (os).

Number of fruiting body: The number of fruit bodies was between 15 to 23, whereas the number of active fruiting was between 10 to 18. The maximum number of fruit bodies was found on the combined substrates (Sawdust + rice straw+ paper (1:1:1) grown mushroom (**Table 1**).

Effect of substrate on the physiological appearances, yield, and harvesting time of *Pleurotus ostreatus*.

Length of Stock (cm): The length of the stock ranges from 2.1 to 3.13 cm and the thickness was between 0.68 to 0.88 cm. The mushroom growing on the combined substrates (Sawdust + rice straw+ paper) had a higher thickness and length than others (**Table 2**).

Harvesting Days: The time from inoculation of mushrooms spore in different substrates to the first harvest and total harvesting time (from the first to the last harvest) were observed and recorded. At every flush, the harvested fruiting bodies were pick up carefully with fresh hand and ensured the fruit body remain intake, weighed and mushroom size was measured. The harvesting time ranges from 34 to 45 days (Table 2).

The total yield of the *Pleurotus Ostreatus* (OS) mushrooms grown on different substrate

Pleurotus Ostreatus (OS) mushrooms were grown on different substrates, and the total yield of mushrooms was different. The total yield of the mushrooms grown on sawdust was 194.74 g/bag; rice straw was 201.35 g/bag; paper was 177.5 g/bag; sawdust + rice straw (1:1) was 205.39; and sawdust + rice

Table 1: Effect of substrate on the yield, and biological efficiency of *Pleurotus ostreatus*.

Substrate Name	Completion Days	Pinhead days	No. of Fruiting body	No. of effective Fruiting Body
Sawdust	25	31	20	15
Rice straw	20	26	19	14
Paper	33	47	15	10
Sawdust + Rice straw (1;1)	26	35	21	16
Sawdust + rice straw+ paper (1:1:1)	26	34	23	18

Table 2: Effect of substrate on the physiological appearances, yield, and harvesting time of *Pleurotus ostreatus*.					
Substrate Name	**Length of Stock (cm)**	**Diameter of Stock (cm)**	**Diameter of Pileus (cm)**	**Thickness of Pileus (cm)**	**Harvesting Days**
Sawdust	2.2	0.99	6.31	0.79	38
Rice straw	2.41	1.3	6.21	0.82	34
Paper	2.1	0.89	5.73	0.71	40
Sawdust + Rice straw (1:1)	2.6	0.91	5.81	0.68	45
Sawdust + rice straw+ paper	3.13	1.09	7.32	0.88	44
CV (%)	0.164	0.161	0.101	0.105	

straw + paper (1:1:1) was 253.41g/bag. From this study, it is clear that the combined substrates (Sawdust + rice straw + paper (1:1:1) has the potentiality to increase the yield of the *Pleurotus ostreatus* (OS) mushrooms effectively (**Table 3**).

Biological Efficiency, BE (%): The biological efficiency shows in table 4.3. In this study the highest (84.7%) BE was found in Sawdust + rice straw + paper (1:1:1) grown mushrooms while the lowest was 59.17% in paper grown mushrooms. In one study it was found that the mean BE was 63.7% when they use cotton and different combination of cotton and straw as mushroom grown substrate (Muswati *et al.*, 2021).

Effect of substrates on the yield, Biological efficiency, and cost-benefit ratio of *Pleurotus Ostreatus* (OS) mushrooms.

Biological Yield (g): The supplementation of different substrates had a great effect on biological yield. The highest biological yield was 253.41g while *Pleurotus ostreatus* mushrooms were grown on Sawdust + rice straw+ paper (1:1:1), and the lowest biological yield was 177.5 when grown on paper. The other biological yield was 194.74g (sawdust), 201.35g (rice straw), 205.39g (sawdust + rice straw (1:1) (**Table 4**).

Economic Yield (g): The highest economic yield was recorded at 253.3g when mushrooms were grown on sawdust + rice straw+ paper (1:1:1), and the lowest economic yield was counted 177.45g when gowning on paper. The other economic yield was 194.69g (sawdust), 201.29g (rice straw), 205.31g (sawdust + Rice straw (1:1), respectively (Table 4).

Dry yield: The dry yield of mushrooms was maximum when mushrooms were grown on sawdust + rice straw+ paper (1:1:1) (25.23g), and the lowest dry yield was counted at 18.01g when mushrooms were grown on paper. The others dry yield was 20.21g (sawdust), 20.86g (rice straw), 21.32g (sawdust + Rice straw (1:1) (**Table 4).**

Table 3: Total yield of the *Pleurotus Ostreatus* (OS) mushrooms grown on different substrates.					
Substrates formula	1st flush (g/bag)	2nd flush (g/bag)	3rd flush (g/bag)	Total yield (g/bag)	BE (%)
Sawdust	70.31	65.01	59.42	194.74	64.91
Rice straw	74.46	68.01	58.88	201.35	67.12
Paper	64.89	58.67	53.94	177.5	59.17
Sawdust + rice straw (1:1)	76.81	68.19	60.39	205.39	68.46
Sawdust + rice straw + paper (1:1:1)	90.96	83.46	78.99	253.41	84.7

Table 4: Effect of substrate on the yield, Biological efficiency, and cost-benefit ratio of *Pleurotus Ostreatus* (OS) mushrooms.					
Treatments	Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)	Cost-benefit ratio
Sawdust	194.74	194.69	20.21	64.91	7.29
Rice straw	201.35	201.29	20.86	67.12	7.58
Paper	177.5	177.45	18.01	59.17	6.34
Sawdust + Rice straw (1:1)	205.39	205.31	21.32	68.46	7.32
Sawdust + rice straw+ paper (1:1:1)	253.41	253.3	25.23	84.7	6.67
CV (%)	0.134	0.137		0.136	0.45
LSD (0.05)	2.25	2.65		0.091	0.089

Biological efficiency: The highest biological efficiency was 84.7 % when mushrooms were grown on combined substrates (sawdust + rice straw+ paper (1:1:1), and the lowest biological efficiency was 59.17% when they grow on paper. The others biological efficiencies were 64.91%, 67.12%, and 68.46% when they grow on sawdust, rice straw, sawdust + Rice straw (1:1), respectively (**Table 4).**

Cost-benefit ratio: The highest cost-benefit ratio was 7.58a when mushrooms were grown on rice straw, and the lowest cost-benefit ratio was 6.34b when mushrooms were grown on paper. Table 4 shows the other cost-benefit ratio of mushrooms grown on different substrates.

Nutrient contents of *Pleurotus ostreatus* (OS) mushrooms grown on different substrates (Sawdust, Rice straw, Paper)

In this study mushrooms (*Pleurotus ostreatus*) grown on sawdust formulas are rich in protein (12.88gm/100gm), lipid 7.71gm, and carbohydrate was about 51.82 gm/100gm, respectively (**Figure 1**). But the protein content in *Pleurotus ostreatus* mushrooms grown in rice straw was relatively low, about 9.03±1.16gm/100gm than mushrooms grown in sawdust (**Figure 2**). But the fiber content in rice straw grown mushrooms was higher (9.3±0.141gm/100gm) than in sawdust grown mushrooms (7.16±0.23gm/100gm), as well as the Carbohydrate content and Ash was significantly higher in rice straw grown mushrooms than in sawdust grown mushrooms, respectively. Besides, the protein content in *Pleurotus ostreatus* (OS) mushrooms grown on Paper was about 11.53±1.30 gm/100gm but most significantly the lipid content was very low about 4.67±1.01gm/100gm (**Figure 3**).

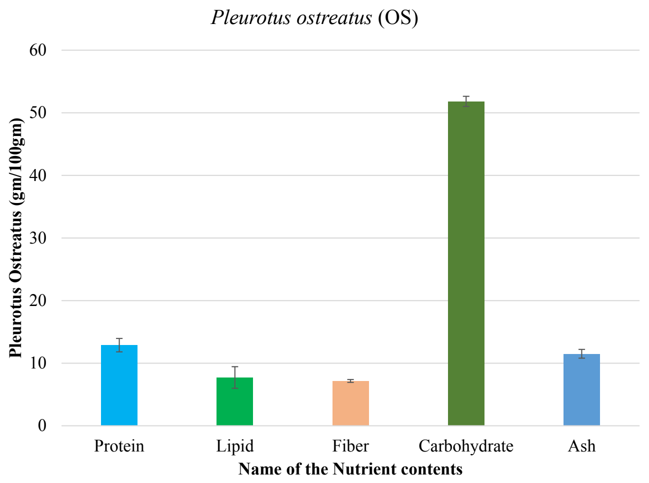

Figure 1: Nutrient contents of *Pleurotus Ostreatus* (OS) mushrooms grown on Sawdust (gm/100gm of Fresh sample).

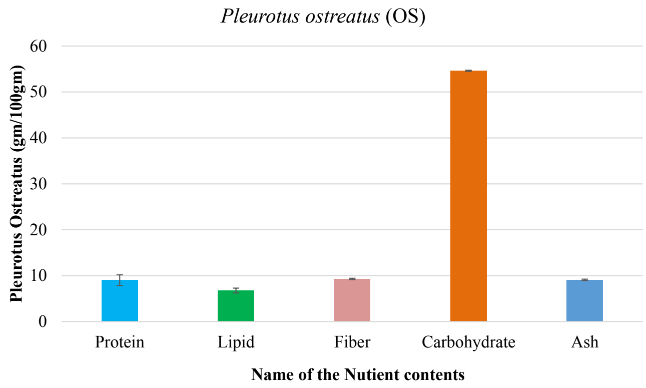

Figure 2: Nutrient contents of *Pleurotus Ostreatus* (OS) mushrooms grown on Rice Straw (gm/100gm of Fresh sample).

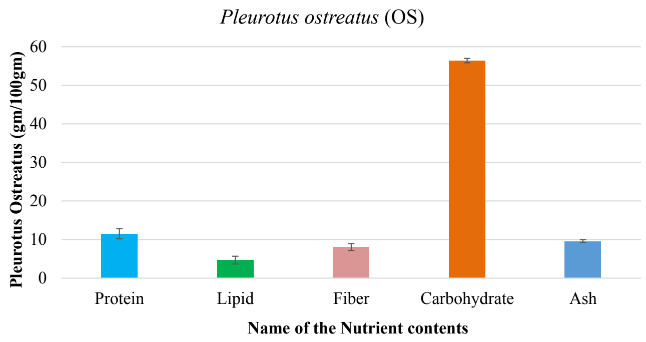

Figure 3: Nutrient contents of *Pleurotus ostreatus* (OS) mushrooms grown on Paper (gm/100gm of Fresh sample).

A study has shown that *Pleurotus sajorcaju* mushrooms grow using cotton waste as substrates. Different kinds of substrates and their combination such as straw, cotton waste, cotton waste plus straw, and cotton waste plus used tea-leaves were used to determine the maximum efficiency of mushrooms growth. In their study, they found that straw substrate-grown mushrooms have the lowest protein content (26.6 % of dry weight), and the mushrooms grown in cotton waste have higher protein content, respectively. At the same time when cotton waste was mixed with used tea-leaves used as a substrate, the protein content was 35.6%. [47].

Nutrient contents of *Pleurotus ostreatus* (OS) mushrooms grown on different combination of the substrates (Rice Straw + Sawdust (1:1), Rice Straw + Sawdust + Paper (1:1:1).

In this study, we used rice straw + sawdust (1:1), and rice straw + sawdust + paper (1:1:1) as the combined form of substrates for *Pleurotus ostreatus* (OS) mushrooms to grow. The nutrition value like protein, carbohydrate, fiber, and ash was high when the mushrooms were grown on combined substrates rather than the individual substrate (**Figure 4** and **5**). But the lipid content was significantly low (3.38±0.45gm/100gm) in mushrooms when they were grown on Rice Straw + Sawdust (1:1) combined substrate (**Figure 4** and **5**). Another study was evaluated the effect of mixed substrates on the growth and yield of *Pleurotus ostreatu* mushrooms. Their study revealed that substrates like cotton waste, wheat straw, crushed baobab fruit shells, and different combinational substrates like cotton husk + wheat straw + crushed baobab fruit shells, 1 : 1 : 1), baobab fruit shells + cotton husks, (1 : 1), baobab fruit shells + wheat straw, (1 : 1), and cotton waste + wheat straw, (1 : 1) can be used for the growth of *Pleurotus ostreatus* (OS) mushrooms [47].

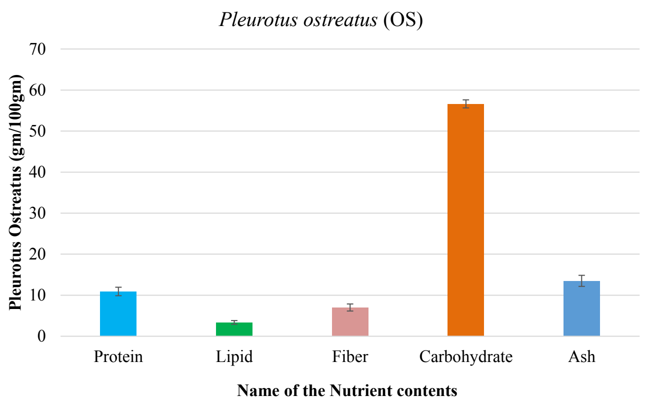

Figure 4: Nutrient contents of *Pleurotus ostreatus* (OS) mushrooms grown on Rice Straw + Sawdust (1:1) (gm/100gm of Fresh sample).

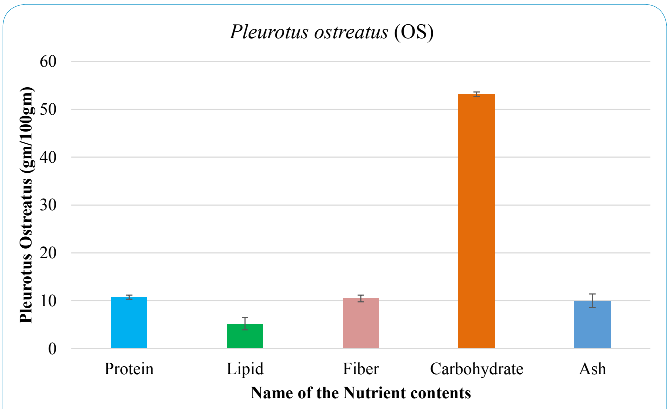

Figure 5: Nutrient contents of *Pleurotus ostreatus* (OS) mushrooms grown on Rice Straw + Sawdust + Paper (gm/100gm of Fresh sample).

CONCLUSION

This study looked into the effect of different substrates on yield and proximate analysis of different nutrients in *Pleurotus ostreatus* mushrooms. As previously stated, the yield of *Pleurotus ostreatus* mushrooms varied greatly depending on the substrate. The treatment SD+RS+PAPER outperformed the other treatments in terms of growth, yield, and nutrient content of the *Pleurotus ostreatus* mushroom. Sawdust is an industrial byproduct, rice straw and paper are waste products, and they are less expensive than gram flour, corn flour, and other flours. In this experiment, SD+RS+PAPER outperformed SD+RS+PAPER in terms of *P. ostreatus* mushroom growth, yield, and nutrient composition. As a result, the SD+RS+PAPER substrate is the most suitable one that can be recommended for wide range of cultivation of oyster mushroom.

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