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RESEARCH PAPER

TITLE:

CULTIVATION OF *PLEUROTUS OSTREATUS* (WHITE AND GREY OYSTER) ON TWO SELECTED SUBSTRATES

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ABSTRACT

A mushroom is a conspicuous umbrella-shaped fruiting body of a macrofungi. Mushrooms provide a considerable nutritional profile, encompassing around 2,000 edible species that have been discovered across various regions of the globe. Mushroom cultivation has become very popular worldwide in recent years due to its nutritional and medicinal importance. More than 16% of the world's mushroom harvest comes from the *Pleurotus* genus, which includes many popular edible mushroom varieties grown in industrial scale. In this study, wheat straw and sawdust are employed as mushroom-growing mediums. For spawn preparation, fresh mushrooms were placed on a paper overnight to collect its spores. Malt extract and agar were used as the culturing media, then, wheat grains were inoculated with the collected spores and freshly cut pieces of the different parts of the mushroom (preferably gills and stipe). Substrate pre-treatment was done by soaking it overnight and adding gypsum to maintain the pH (5 – 6.5). The substrate was kept in bags after inoculation with spawn for about 28 days at 22 ± 2 ℃. During the incubation period, the inoculated bags were exposed to light for about 8 hours daily and the humidity was maintained between 80 – 90% with the help of a humidifier. After the incubation period of 28 to 30 days, pinheads appeared and the bags were cut open with the help of a sterilized knife. The complete process took about 30 to 35 days to get a good and healthy yield. Ultimately, the biological efficiency was determined by measuring the weight of mushrooms obtained per bag. This study can help to enhance the mushroom growth rate and production yield to fulfil increasing food demands.

KEYWORDS: Spawn, sawdust, wheat straw, cultivation

1. INTRODUCTION:

Mushroom or toadstool is a spore-bearing fruiting body of a fungus. It is fleshy and typically produced in soil, above the ground, on the wood or on any organic food substrates. They have nutritional and medicinal properties and play important roles in human food (Musanze, 2013). Around 2,000 edible species of mushrooms with high nutritional value are now found around the world (Rathore et al., 2019). It has been acknowledged that approximately 160,000 out of the 1.5 million identified species of fungi exhibit sporocarps of sufficient dimensions to be classified as macro-fungi or mushrooms (Hawksworth, 2012). Among the 16,000 species of mushrooms that have been identified, about 7000 have varying degrees of edibility (Hawksworth, 2012), the primary edible species are about 3000, and approximately 700 species of the mushrooms are considered healthy therapeutic mushrooms (Li et al., 2021). According to Manzi et al., (2001), mushrooms have low fat content, a high protein content, and a mineral and vitamin content that includes vitamin B, vitamin D, vitamin K, and sometimes vitamin

A and vitamin C. According to Demirbas' (2001) research, they have a protein content of 17.54%, a carbohydrate content of 39.91%, and a fat content of 2.93% based on their dry weight. Several mushrooms contain a wide variety of metabolites that are of great interest to the food and beverage industries and pharmaceutical companies. The metabolites have anti-oxidant, immunomodulatory, anti-tumor, antigenotoxic, anti-inflammatory, hypo-cholesterolemic, antihypertensive, and antimicrobial properties (Chang, 2007).

Cultivating mushrooms is a potential technique that can recycle lignocellulosic organic waste to profitable product at a reasonable cost. The goal of this technology is to reduce environmental pollution (Sanchez, 2009). Different agro-industrial wastes such as crop waste, cotton stalks, and soybean straw can be used to grow oyster mushrooms, because of the extensive enzyme system present which degrades the complex organic compounds in organic wastes (De Silva et al., 2012). The growth of mycelium, the quality of the mushroom, and its yield are greatly affected by the nature of the substrate and its nutrient constituents (Baldrian and Valaskova, 2008). The substrate used for mushroom cultivation should be a great source of nitrogen supplements and carbohydrates for the rapid growth of mushrooms et al., 2014). Commercial cultivation of oyster mushrooms (*Pleurotus* species) occurs on a global scale. According to Adebayo et al., (2012), it is a highly sought-after item in the global mushroom market. The mushroom caps the genus *Pleurotus* are in different colors, which may be pink, white, grey, or dark brown and measured between 4 – 15 cm in diameter. However, fruiting bodies have been seen in the winter and early warm springs. The oyster mushroom is one of the easily growing species of mushroom (Mahari et al., 2020). Among the world mushroom production, oyster mushrooms hold the third position after white button and Shiitake mushrooms (Gyorfi and Hajdu, 2007). According to Daba et al., (2008), there are a lot of proteins (30.4%), carbs (49.6%), fat (2.2%), fiber (8.7%), ash (9.08%), and water (90.8%) in *Pleurotus ostreatus*, and it has a lot of energy (345 K (cal)) per 100 grams dry weight basis. On a per-100-g-dry-weight basis, it contains a plethora of essential nutrients, including vitamins B1 (thiamin) B2 (niacin), and B3 (riboflavin), as well as the minerals P (476 mg), Na (61 mg), Ca (98 mg), and Fe (8.5 mg).

According to Sivrikaya et al., (2002), *Pleurotus ostreatus* has significant therapeutic value and is effective in the battle against diabetes and cancer. According to Sharma et al., (2013), the fact that it has a high ratio of potassium to sodium makes it an excellent choice for patients who suffer from hypertension and heart disease. According to Randive (2012), the treatment of anemia with oyster mushrooms is possible due to the presence of folic acid in these mushrooms. The oyster mushroom species *Pleurotus ostreatus* has been shown to have anticancer activity (Chorvathova et al., 1993), whilst the oyster mushroom species *Pleurotus cystidiosus* possesses powerful antioxidant capabilities (Li et al., 2007).

2. OBJECTIVES:

The main objectives of the research are:

- To check the growth rate of white oyster mushroom on two different substrates (wheat straw and sawdust).
- To examine the optimum humidity and temperature levels for their growth.
- To compare the yield percentage of mushrooms on the two substrates

3. MATERIALS AND METHOD:

Culture Preparation:

For the preparation of culture, 18 g agar and 25 g malt extract were dissolved in 1000 ml of distilled water with the help of an electric stirrer, and the solution was autoclaved for about 30 mins at 121 ℃ and 15 psi for sterilization. After cooling, an antibiotic (amoxil 500 mg) was added to the solution to prevent any bacterial contamination and the culture solution was poured into pre-sterilized petri plates and allowed it to solidify. The whole procedure was carried out in the laminar airflow to avoid airborne contamination. After that, different parts of the mushroom fruiting bodies (bought from a renowned seller) like pieces of pileus, gills, stipe and spores (collected overnight by placing mushroom cap on a paper) were cut with the help of a sterilized knife, added in petri plates and the plates were sealed with the help of parafilm and labeled properly. The culture plates were kept in the dark at room temperature (24 ± 2℃) for about 15 – 20 days.

Spawn Preparation:

To prepare mushroom spawn, the wheat grains were washed and soaked in water overnight to absorb a significant amount of water. The grains were removed from the water, spread on a cloth to drain excessive water and then mixed with 1.5% calcium carbonate of the dry weight of grains and were packed in polypropylene bags (2 kg per bag). The bags were autoclaved for 30 minutes at 121 ℃ and 15 psi for removal of contamination. After sterilization, the culture containing the mycelium was mixed with the sterilized grains. The bags were tightly packed with the rubber band and kept at room temperature (about 24 ± 2℃) in the dark for approximately 15 – 20 days for the invasion of mycelium.

Compost Preparation:

Wheat straw and sawdust were used as a substrate for mushroom cultivation and the compost were prepared with the help of the modified method of Sher et al., (2010). The substrates were soaked overnight in water and excess water was then carefully drained out so that the compost contained about 50% moisture. Polypropylene bags were used for packing and autoclaving because they can withstand the high temperature of an autoclave as high as 136 ℃. The bags were dipped in 70% ethyl alcohol for sterilization. The compost was then mixed with 1-1.5% gypsum, packed in bags and the bags were autoclaved for 30 minutes at 121 ℃ and 15 psi to remove impurities. After autoclaving, the bags were cooled down to room temperature before inoculation of spawn.

Spawn Inoculation:

After sterilization, the substrate was inoculated with mushroom spawn seeds. The spawn was placed between the layers of the compost such that each bag contained approximately 28 g of spawn seeds per kilogram of the substrate. After inoculation, all the bags were tightly packed and tied with a rubber band.

Incubation:

The bags were kept in the dark at room temperature. The room temperature was about 24 ± 2 ℃ and the humidity was maintained with the help of the humidifier upto about 90%. The bags were checked every 3 – 4 days to monitor the mycelial growth and water was sprinkled over the bags to maintain humidity until the appearance of pinheads.

Pinhead Formation:

After the incubation period of almost 28 to 30 days, pinheads appeared in the inoculated compost bags. The bags were then cut open with the help of a sterilized knife and hung with the help of a rope. The light was provided for 6 – 8 hours daily because it is a necessary condition for the growth of the mushrooms. The humidity was maintained at about 85-90% by spraying water.

Harvesting:

After 4 – 5 days of the cut opening of the bags, the mushrooms started appearing from those cuts. The bunches of the fully mature mushroom appeared after 3 to 4 days. The mature mushrooms were harvested within 3 – 4 days. Two flushes were obtained from each bag and the harvested mushrooms were weighed and preserved.

Preservation:

The mushrooms were preserved by drying and dipping methods. In drying method, the mushrooms were dried in the open air and kept in the refrigerator at 4 ℃. During chemical preservation, the mushrooms were dipped in a solution made by adding 2 g citric acid and 3 g salt were dissolved in 1000 ml of distilled water (Brennan et al., 2000).

4. RESULTS AND DISCUSSION:

Morphological Features:

White Oyster: The cap of the white oyster mushroom was bright white to creamy white in colour. The diameter of the pileus ranges from 3 – 8 cm. The stipe was 1 – 5 cm long and 0.5 to 1 cm in diameter. It is stem-like part of the mushroom, which supports the pileus. The gills are white in colour and extend down the stem (decurrent). The edges of the fresh mushroom were smooth and enrolled. These became scaly and wrinkled on drying.

Grey Oyster: The pileus colour of the grey oyster mushroom was creamy white but the edges were light grey or silver. The diameter of the pileus varies from 5 – 10 cm and the stipe of the mushroom was 2 – 5 cm long. Decurrent arrangement of gills was observed and the mushroom does not contain volva or annulus.

Experimental procedure:

Spawn Running: The 1st batch on the substrate, wheat straw, included total 20 bags (10 bags containing white oyster and 10 of grey oyster spawn) and 1st batch on saw dust included total 10 bags (5 bags containing white oyster and 5 bags of grey oyster spawn) that were incubated on 16th November 2022. The bags were checked weekly to monitor the growth of mycelium in the substrate. There was no mycelial growth in the bags even after 20 days of incubation (Fig. 4.1 a). After one month (15th December) mycelium appeared in just 6 bags of white oyster mushroom out of 10 (Fig. 4.1 b) and the grey oyster showed no mycelial growth till then (Fig. 4.1 d). The mycelium fully covered the bags of white oyster in approximately 35 days after the appearance of the mycelium (in other words, 56 days after inoculation of bags) (Fig. 4.1 c) and it took 45 days for the grey oyster to

develop complete mycelium in 6 bags (Fig. 4.1 f). The 2nd batch included total 20 bags (10 of white oyster and 10 bags of grey oyster) was incubated on 21st February 2023. This batch showed faster growth of mycelium as compared to the bags of 1st batch due to low temperature in February as compared to the temperature in November. An incredible growth of mycelium was observed in the bags on 6th March (after 14 days). The bags were fully covered with the mycelium after 20 days (Fig. 4.2 a, b, c).

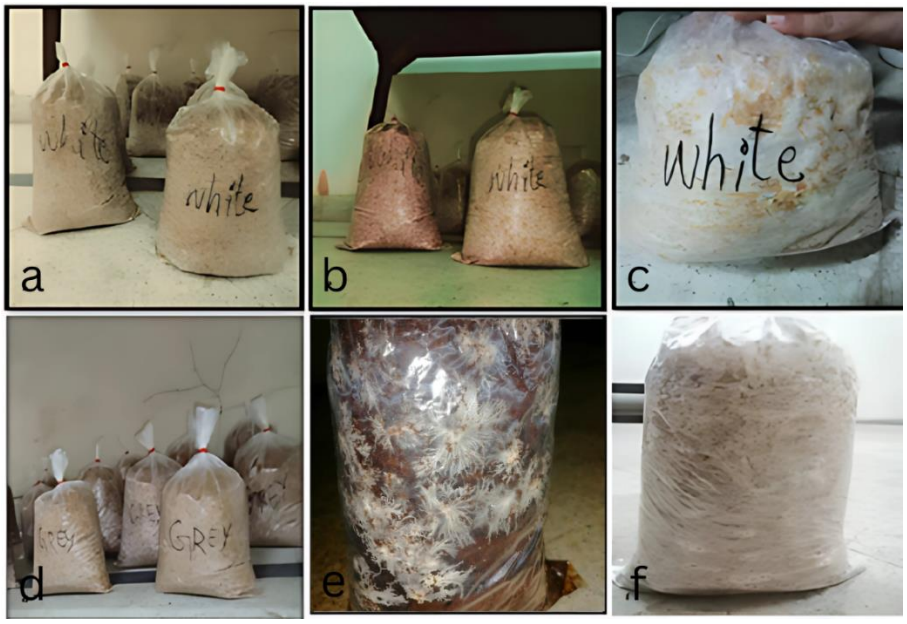


Fig. 1: (a, b, c) Spawn running in white oyster of 1st batch (d, e, f) Spawn running in grey oyster of 1st batch

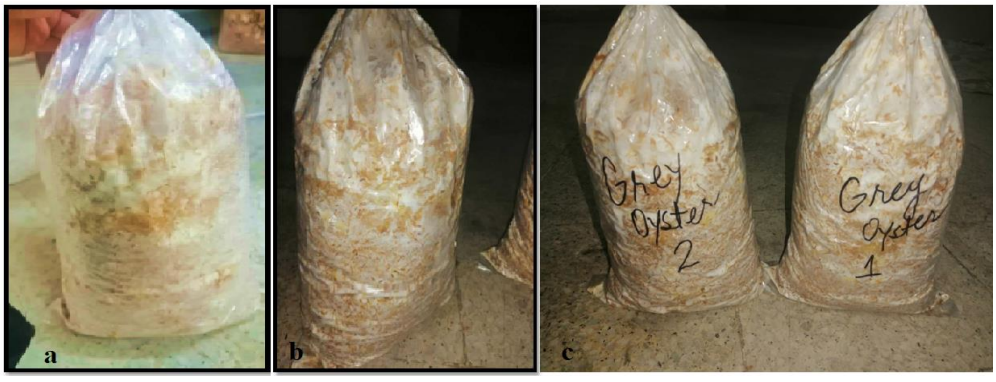


Fig. 2: (a, b) Spawn running in white oyster 2nd batch (c) Spawn running in grey oyster 2nd batch

Pinhead Formation: Pinheads started appearing after 58 days of inoculation in the bags of white oyster and 68 days of grey oyster bags of 1st batch. Pinheads started

forming after only 17 days in the bags of 2nd batch. The second batch showed better growth compared to the first batch. The bags were then cut opened with a sterilized knife and hanged with the help of the rope. The light was provided for 6 – 8 hours daily because it is necessary condition for the growth of the mushrooms. The humidity was maintained to about 85 – 90 % by spraying water. The mushrooms started sprouting after 2 – 3 days of the opening of the bags.

Harvesting of Mushrooms: The mushrooms became fully mature after 3 – 4 days of sprouting. These were harvested after 4 days with the help of a sterilized knife or blade. Two flushes were obtained from the 1st batch of both of the mushrooms, whereas the 2nd batch of white oyster gave three flushes. Mushroom yield was calculated by weighing fresh mushrooms with an electric weight machine. The yield of both of the batches is expressed in the form of a table. The biological efficiency can be calculated of each bag by the following formula:

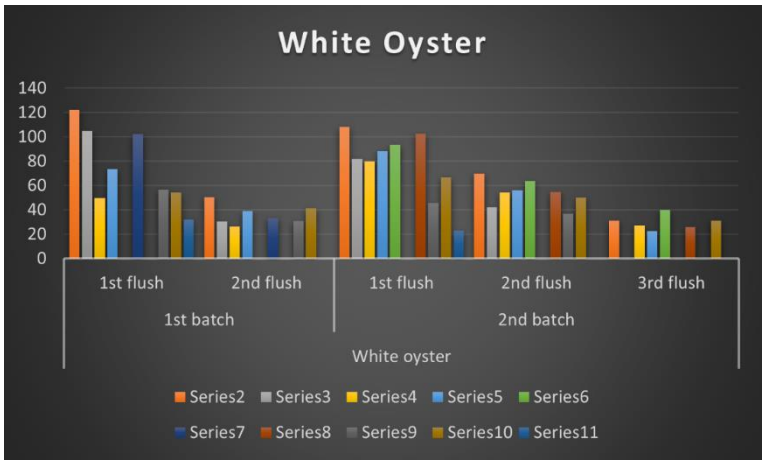
$$\text{Biological Efficiency (BE)} = \frac{\text{Weight of fresh mushrooms}}{\text{Weight of dry substrate}} \times 100$$

Fig. 3. White and grey oyster (*Pleurotus ostreatus)*

Table 1. Yield of white and grey oyster mushrooms

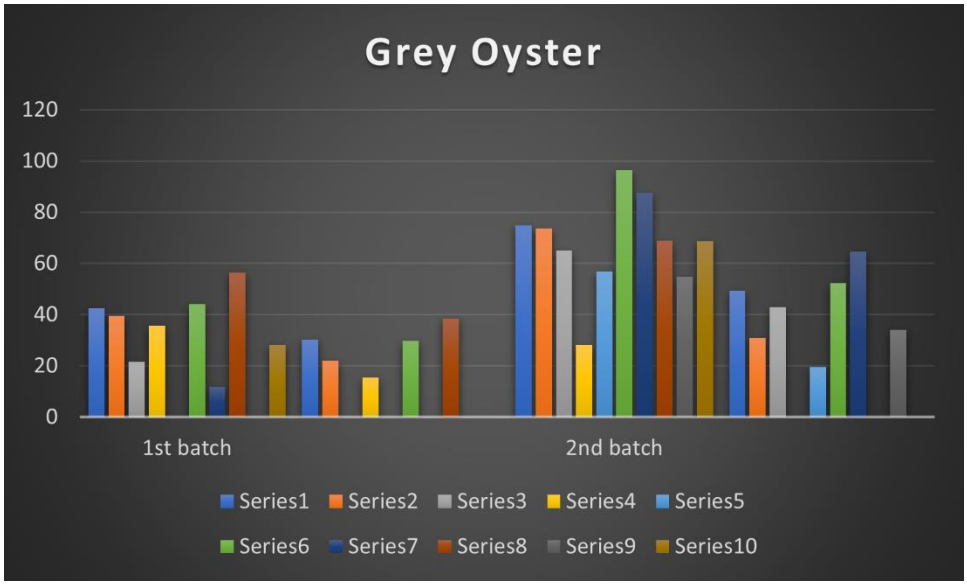
Bag no.	Yield of mushrooms (g)								
	White oyster					Grey oyster			
	1st batch		2nd batch			1st batch		2nd batch	
	1st flush	2nd flush	1st flush	2nd flush	3rd flush	1st flush	2nd flush	1st flush	2nd flush
1.	122.27	50.38	108.25	69.68	31.3	42.39	30.2	74.8	49.3
2.	105	30.62	81.95	42	_	39.6	21.9	73.7	30.8
3.	49.83	26.2	80	54.37	26.89	21.45	_	65.08	43
4.	73.65	39.02	88.39	56.02	22.36	35.7	15.36	28.19	_
5.	_	_	93.68	63.49	39.88	_	_	56.8	19.5
6.	102.3	33.09			_	44	29.8	96.45	52.3
7.	_	_	102.6	54.76	25.85	11.63	_	87.5	64.6
8.	56.34	30.78	45.64	36.78	_	56.4	38.41	68.97	_
9.	54.23	41.42	66.74	50.02	31.04	_	_	54.64	34.06
10.	32.09	_	23.00	_	_	28.08	_	68.74	_

Graph no. 1 Yield of white oyster mushrooms



The graph provides yield data for White Oyster mushrooms, divided into two batches with multiple flushes (harvests). The first batch includes data for the first and second flushes, while the second batch includes data for the first, second, and third flushes. The numbers represent yield quantities, likely measured in grams or another unit, for each flush. The yields vary significantly across flushes and batches, suggesting differences in growing conditions, timing, or other factors. This data could be used to analyze yield patterns, compare the performance of different batches, or identify trends in mushroom production.

Graph no. 2 Yield of grey oyster mushrooms



The table contains yield data for Grey Oyster mushrooms, organized into two batches with multiple flushes (harvests). The first batch includes data for the 1st and 2nd flushes, while the second batch includes data for the 1st and 2nd flushes as well. The numbers represent yield quantities, likely measured in grams or another unit, for each flush. The yields vary across flushes and batches, suggesting differences in growing conditions, timing, or other factors. This data could be used to analyze yield patterns, compare batch performance, or identify trends in mushroom production.

Experimentation on Saw Dust: Same methodology was done with sawdust. The mycelium run rate was slower in sawdust as compared to wheat straw. The mycelium grew for only some days and after that the mycelial growth was stopped (Fig. 4).

Test-tube Experiment:

An experiment was conducted in test tubes to determine the growth run-rate of the mycelium by following the same procedure. Five conditions were maintained in preparation of test tubes.

Test tube 1: Control (Culture box, Temp. 24 ± 2 ℃)

Test tube 2: Control (Room temperature 27 ± 2 ℃)

Test tube 3: Wheat straw without $CaCO_3$ but autoclaved

Test tube 4: Wheat straw without $CaCO_3$ and autoclaving

Test tube 5: Wheat straw without autoclaving but with $CaCO_3$

The test tubes were monitored weekly to check the spawn run rate. The mycelium started growing after 7 days of the inoculation and it grew at a run-rate of 2 – 3 cm per day. The test tubes were completely covered with the mycelium after 21 days. After 3 days of the spawn running, the pinheads appeared in the test tubes.

Fig. 4. Retarded mycelial growth in sawdust

Fig. 5. Test tube experiment

Fig. 6. (a) Test tubes after 1 week (b) Test tubes after 2 weeks (c) Test tubes after 3 weeks (d) Pinhead formation after 25 days of inoculation

5. DISCUSSIONS:

The present research study focused on the cultivation of white and grey oyster mushrooms (*Pleurotus ostreatus*) by using two agricultural wastes (wheat straw and sawdust) as a substrate. Highly significant results were obtained with wheat straw as compared to sawdust, even though there was a great difference in the results of 1st and 2nd batches of mushrooms cultivated on wheat straw. The mushrooms did not grow on sawdust substrate and mycelium grew to a very small extent. The spawn run rate was slower in sawdust as compared to wheat straw. After the 3 weeks of inoculation, the mycelium started to grow slowly but the after growing a few centimeters the mycelial growth was retarded in all the bags. The results were supported by the study of Girmay et al., (2016) and Mandeel et al., (2005) who reported that as compared to other substrates, sawdust resulted in the slowest mycelial run rate and lowest yield. This might be a result of extremely low protein content in sawdust, which is inadequate for mycelial growth and mushroom production (Obodai et al., 2000).

Temperature and relative humidity were the two parameters that were supposed to play a role in the variation in the results of the two batches. The temperature was lower in February at the time of the inoculation of the 2nd batch than in November at the time of the inoculation of the 1st batch. The temperature ranged from 23 – 28 ± 2 ℃ in November when the 1st batch was inoculated and in

February, the temperature ranged from 17 – 25 ± 2 ℃. The spawn run rate was faster in February than in November. The spawn running time took longer time in November (56 to 66 days) as compared to February (14 days). The results of the current study were similar to the study of Sher et al., (2011) who cultivated the oyster mushroom in two different agro-ecological zones of Pakistan where spawn running took more time in Sawat than in Peshawar because of the temperature. The relative humidity was lower in November (at the time of inoculation of 1st batch) as compared to February (at the time of 2nd batch). The mycelium run rate was faster in 2nd batch than in the 1st batch. Another reason for the slow run-rate of the mycelium was unavailability of humidifier in 1st batch. During 2nd batch, the humidity was also controlled by a humidifier. During the incubation period of 1st batch, the mycelium took 56 days to completely colonize the bags and in 2nd batch, the bags were completely covered with mycelium in just 17 days. According to Sher et al., (2011), the relative humidity remained consistently lower in Sawat than that in Peshawar, and spawn running took more time in Sawat than in Peshawar. Our results conform with the earlier studies which explained that environmental factors affect the growth of the mushroom (Alexander et al., 2002). A higher yield was obtained from the 1st batch as compared to the second in case of white oyster, but the grey oyster yielded higher in the 2nd batch as compared to the 1st. Two flushes were obtained from the bags of the 1st batch however, three flushes were obtained from the 2nd batch in white oyster. Depending on the yield obtained, the biological efficiency was higher in the case of 1st batch.

CONCLUSION:

It was concluded from the study that wheat straw is a more favorable substrate compared to sawdust for the cultivation of oyster mushrooms (*Pleurotus ostreatus*). This is attributed to the increased concentration of cellulose, hemicellulose, and lignin-rich lignocellulosic material present in wheat straw. This substrate effectively provides the necessary nutrients for the growth and development of mushroom mycelium, as well as the subsequent formation of fruiting bodies. It is also concluded that temperature and relative humidity have a direct impact on the spawn run rate, primordial initiation, and mature fruiting bodies. In addition, it is reported that the production of fruiting bodies of mushrooms is enhanced by favorable temperature and humidity.

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