



Research Article

Alternate Substrates for the Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract | This study aims to find alternate sources (agro-based wastes) for mushroom cultivation due to the increasing demand and price of wheat straw. These alternate sources include sugarcane leaves, sugarcane bagasse, and spear grass. This study explored the effects of different agro-wastes on oyster mushroom growth, yield, and nutritional composition. The effects of various substrates on the oyster stalk length, cap diameter, weight, nitrogen value, protein contents, number of fruiting bodies, and primordial were analyzed. Among the different substrates, sugarcane leaves were found to be one of the best with maximum productivity. The protein contents of oysters were also comparatively higher, growing on sugarcane leaves (18.38%), followed by spear grass (17.85%) and wheat straw (control 13.83%). In comparison, the lowest crude proteins were obtained from the sugarcane bagasse (7.61%). Our results demonstrate that sugarcane leaves can be used as an alternative and cheap substrate for mushroom cultivation.

Received | December 12, 2024; **Accepted** | March 15, 2025; **Published** | March 26, 2025

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Citation | Faiq, M., A. Rauf, M. Qayash, M. Ikram, K. Jabbar, G. Khan, T. Barin, W. Khan, T. Ullah, M. Ullah, I. Khan, F. Bibi and M. Munir. 2025. Alternate substrates for the growth and yield of oyster mushroom (*Pleurotus ostreatus*). *Pakistan Journal of Weed Science Research*, 31(1): 66-73.

DOI | <https://dx.doi.org/10.17582/journal.PJWSR/2025/31.1.66.73>

Keywords | *Pleurotus ostreatus*, Sugarcane leaves, Speargrass, Sugarcane bagasse, Wheat straw, Oyster mushroom



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Introduction

The oyster mushroom (*Pleurotus ostreatus*) has an excellent taste and flavor and belongs to the class Basidiomycetes, subclass Hollobasbasidiomycetidae, and order Agaricales. Mushrooms have distinctive fruiting bodies, which are large enough to be seen with the naked eye and can be picked by hand. It is cultivated in temperate and sub-tropical regions of the world (Patel *et al.*, 2019).

The mushroom grown on organic substrates (lignocelluloses), supports mushroom development, and fruiting (Mondal *et al.*, 2010). Mushroom's mycelium arises from the spawn, they have a tremendous ability to use various lignocelluloses substrates with a large amount of plant waste that includes wheat straw, sawdust, paddy straw, sugarcane bagasse, corn stalk, corn cobs, waste cotton, leaves and pseudo stem of bananas, water hyacinth, duckweed, rice straw, etc. can be used to cultivate oyster

mushrooms (Chakravarty, 2011; Mahari *et al.*, 2020).

P. ostreatus is commercially produced and sold in Asia, America, Europe, and Africa. Every year about 90 tonnes of mushrooms are exported from Pakistan to Europe (Shah *et al.*, 2004). Currently, oyster mushrooms are the world's 1/3 largest, unusually placed species of cultivated mushrooms after button and shiitake mushrooms (Femandes *et al.*, 2015). Poland is the main producer of oysters in Europe as the annual production exceeds about 80,000 tonnes (Sokołowski *et al.*, 2022). The *P. ostreatus* and *P. pulmonarius* are the most economically extensive species of oyster mushrooms (Bazanella *et al.*, 2013). The farmers should come forward to cultivate edible mushrooms like *P. ostreatus* on a commercial scale to fulfill the requirement of a balanced diet. Pleurotus species are the best wood decomposers and are grown in a wider range of forests and agricultural waste than any species from other groups (Adebayo *et al.*, 2015). Several species of Pleurotus are also capable of acting as parasites of living trees, attacking nematodes or bacterial colonies (Martinez, 1998).

The proteins of oyster mushrooms are moderate between vegetable and animal proteins. The human body's amino acids are also found in oyster mushrooms. Fresh oysters have higher moisture contents (90%) than driers, while fresh oysters have carbohydrates (57.6%), protein (29.2%), fat (2.1%), fiber (8.2%), and ash (9.8%) (Elattar *et al.*, 2019). It also contains enough phosphorus, iron, protein, lipid, riboflavin, and thiamine (Khan *et al.*, 1981). Furthermore, oyster mushrooms also contain a measurable amount of Niacin, Pantothenic acid, and Biotin (Subramanian, 1986).

Mushrooms contain a significant amount of dietary fiber and given their chemical structure, they show immune defense and anticancer activity (Deepalakshmi and Sankaran, 2014). Oyster mushrooms are fleshy edible fungi that have acquired huge importance due to their nutritional and medicinal properties (Islam and Riaz, 2017). Other biological properties of the mushrooms are being antitumor, diabetic, and antioxidant (Elkhateeb *et al.*, 2019). The consumption of oyster mushrooms has the advantage of preventing as well as reducing diseases such as diabetes, hepatitis B, high blood cholesterol level, kidney problems, chronic fatigue syndrome, hypertension, microbial infection, liver illness, impaired immune response,

heart diseases, and gastric cancer (Yohannes *et al.*, 2020; Elahi *et al.*, 2025). Oyster mushrooms are recognized to incorporate healing elements, including nutritional fibers and numerous bioactive compounds (Deepalakshmi and Sankaran, 2014). They are saprophytes that decompose agricultural plant derivatives as they can apply cellulose, hemicelluloses, and lignin substances as a supply in their nourishment (Chang, 2008). Mushroom cultivation offers an environmentally friendly and good-value manner of changing agro-based wastes into nutritious food (Ragunathan *et al.*, 1996). On the earth's surface, around two hundred billion lots consistent per year of the natural count are produced via the photosynthetic process (Zhang, 2008).

The cultivation of oysters and other mushrooms is a common technique these days, which is easy to grow on plant products and there is no need for any skilled person. The current project aims to investigate the cultivation of oyster mushrooms on various substrates and find alternate source(s). Furthermore, to find any potential effect of different substrates on the protein content of oyster mushrooms.

Materials and Methods

Materials of this research

Spawn, Polypropylene bags (12 × 14), Substrates (agricultural wastes), Rubber bands, and Water sprayer.

Selection of different substrates

Different substrates including sugar cane leaves, sugarcane bagasse, wheat straw, and spear grass were used to examine the potential effect on the growth and yields of oyster mushrooms.

Preparation of substrates for spawn production

First, the substrates (growing media) were boiled at a temperature of 100 °C for one hour to remove the microorganism's presence on their surfaces, and then spawns were grown in all those clean substrates.

Drying the substrates

All the substrates were spread on clean and sanitized plastic bag sheets in the open air for one night. The substrates were checked after 10 to 12 hours to confirm if they were dry and ready for sowing. If the substrates contain moisture, they need to be left in the sun until they are completely dry. Fully dry substrates

were used for spawn sowing.

Filling of polypropylene bag

The polypropylene bags were filled with different substrates. Three bags of each substrate were filled and tied with rubber bands. The air was removed from the bags to enhance spawn growth. The bags were perforated by making holes with the help of a sanitized needle for enough oxygen supply.

Water sprayer machine

Watering was sprinkled twice a day i.e., morning and evening, with a sprayer. The sprinkling of water started at the beginning of mycelial growth from plastic bags. This is a very critical stage of their growth, and the presence of water is necessary for mushrooms during this period.

Incubation

The bags were kept in a dark room in which the moisture was high, and the temperature was low. The temperature was kept between 15 °C to 25 °C and moisture was in the range of 60 to 80 %.

Results

The different types of substrates were found in the growth and yield weekly mycelia growth was seen on different substrates. The long stalk length of the oyster was observed in sugarcane leaves followed by wheat straw, spear grass, and then sugarcane bagasse (Figure 3). The mycelia growth is the first step that produced suitable internal environments for fruiting. Thus, the best growth of mycelium is a major factor in mushroom cultivation and growth (Figures 1-7). The types of mushrooms like mycelia growth, useless hinges formation, and more like that body's formation are the three major developmental stages in the power better of oyster mushrooms, under proper humidity and temperature.



Figure 1: Oyster mushrooms grown on Sugarcane leaves.



Figure 2: Oyster mushrooms grown on the Speargrass.



Figure 3: Oyster mushrooms grown on Wheat straw.



Figure 4: Oyster mushrooms grown over Sugarcane bagasse.

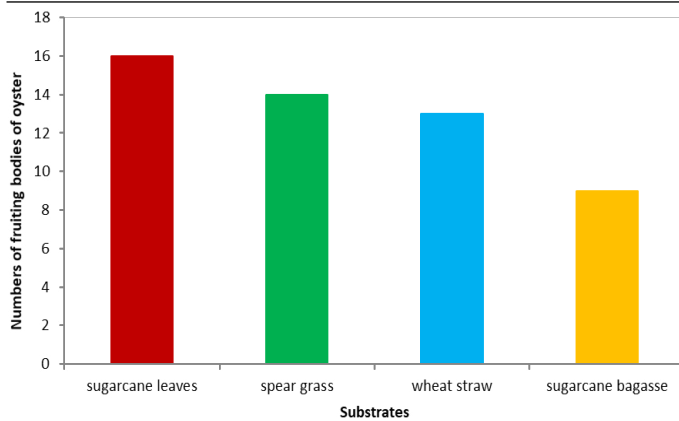


Figure 5: Graph showing numbers of fruiting of oyster mushrooms. The graph shows that mushrooms growing on sugarcane leaves have the highest number of fruiting bodies, whereas those on sugarcane bagasse have the lowest number of fruiting bodies.

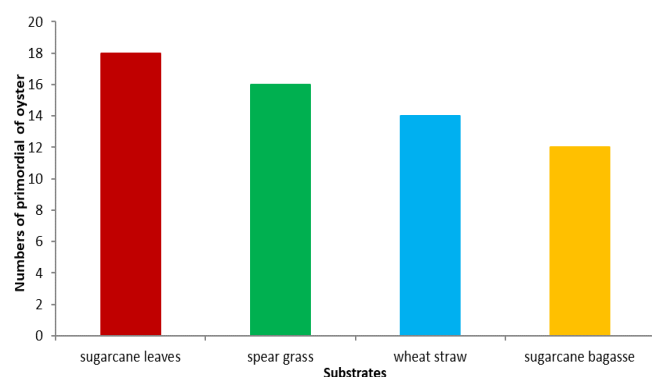


Figure 6: Graph showing the number of primordial in Oyster mushrooms. The above graph shows that the mushrooms that grow on sugarcane leaves have a maximum number of primordial, while those that grow on sugarcane bagasse have a minimum number of primordial.

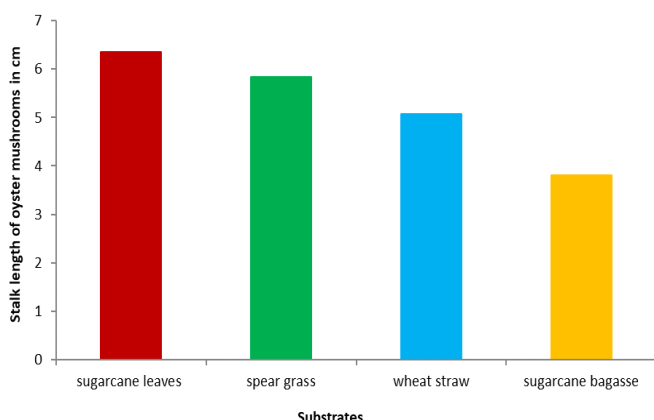


Figure7: Graph showing stalk length of oyster mushrooms. The above graph shows that the maximum stalk length was obtained by those mushrooms, which were grown on sugarcane leaves while the minimum length was obtained on sugarcane bagasse.

Spawn running (mycelia growth)

Our results showed that spawn running took 14 to 21 days after inoculation (Table 1). All substrates were inoculated on the same day.

Table 1: The table shows the times for spawn running, pinhead formation, and fruiting body formation of oyster mushrooms grown on different substrates.

Different parameters of oyster mushrooms	Time
Spawn running	14 to 21 days
Pinheads' formation	14 to 20 days
Fruiting body formation	21 to 42 days

Pinheads' formation

It begins with shoulder straps of mycelium built up of hyphae, the hair-like cells that make up the mycelium, that join to form hyphal knots. These hyphal bumps then develop into blooming buds or cubes which ever growers commonly sight mushroom apprehension because they often simulate pinheads. Spikes are the second stage of mycelial growth during mushroom culture. A small pinhead-shaped structure was formed between 14 and 20 days in the back and the time for pinhead formation was noted as 23 to 27 days (Table 1).

Fruiting body formation

The fruiting body is the final stage during the cultivation of mushrooms. The fruiting bodies appeared from 21 to 42 days after pinheads and took 26 to 33 days later after inoculation of spawn (Table 1).

Growth and harvest of oysters on the sugarcane leaves

The best growth was observed on sugarcane leaves in which the spawn of oysters showed the maximum yields (Figure 9). The first mycelium appeared after thirteen (13) days of spawning. Then these mycelia fully come out from the bags after twenty-three days (23). The mushroom harvest was done when they were fully developed after 31 days (Table 2).

Oyster growth on the speargrass

The first mycelial growth appeared on the speargrass after fourteen (14) days from the spawning. Mycelia come out from the bags after twenty-five (25) days of spawning. The harvest was done at the end of the 35th day after the mushrooms were fully matured (Table 2).

Oyster growth and harvest from wheat straw

The mycelia appeared on wheat straw after seventeen (17) days of spawning. The mycelia come out from the bags after twenty-seven (27) days of spawning. They were harvested at the end of the 36th day as they were fully matured. The wheat straw was taken as a control to compare the growth of oysters with all other substrates. This showed that wheat straw is not the best-growing substrate compared to sugarcane leaves and sugarcane bagasse but showed better performance compared to speargrass (Table 2).

Table 2: The table above shows the appearance of the first mycelia and their harvesting times from different substrates in different median bags.

Different sub- strates	First mycelial appearance	Mycelial appearance outside of bags	Har- vesting time
Sugarcane leaves	13 days	23 days	31 days
Speargrass	14 days	25 days	35 days
Wheat straw	17 days	27 days	36 days
Sugarcane bagasse	18 days	29 days	40 days

Oyster growth on sugarcane bagasse

The first mycelia appeared on sugarcane bagasse after eighteen (18) days of spawning. The mycelia showed good germination on these substrates and came out from the bags after twenty-nine (29) days of spawning. They were harvested after eleven (11) days when they became fully matured (Table 2).

Numbers of the fruiting body

The total number of fruiting bodies of oysters observed on different substrates were 16, 14, 13, and 9 on sugarcane leaves, spear grass, wheat straw, and sugarcane bagasse respectively (Figure 5).

Numbers of primordial

The average numbers of primordial grown on different substrates differ remarkably. The highest number of primordial per packet (18) was found in sugarcane leaves followed by the speargrass (16) and then the wheat straw (14), while the lowest number of primordial (12) was noted in sugarcane bagasse (Figure 6).

Stalk length of oyster mushrooms

The maximum stalk length noted from those mushrooms which are obtained from the sugarcane leaves (6.35 cm) on average, followed by spear grass which is (5.84 cm), and then the wheat straw (5.08 cm)

while the lowest length of the stalk is the sugarcane bagasse (3.81 cm) (Figure 7).

Cap diameter of oyster mushrooms

The cap diameter of oyster mushrooms differs on different substrates. The maximum cap diameter noted from those mushrooms which are obtained from the sugarcane leaves (12.7 cm) is an average, followed by spear grass (11.93 cm) and then the wheat straw (10.16 cm), while the lowest cap diameter was attained by sugarcane bagasse (9.08 cm) (Figure 8).

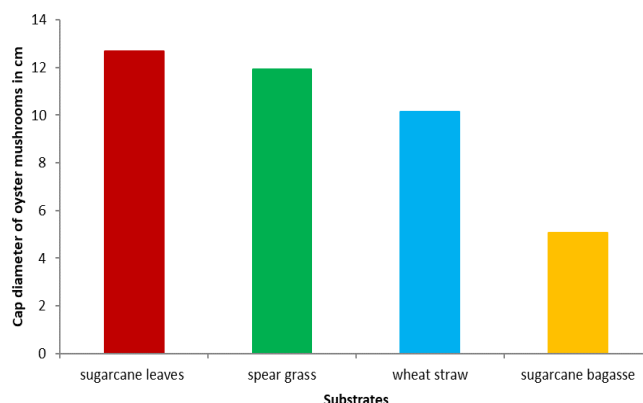


Figure 8: Graph showing the cap diameter of Oyster mushrooms. The above graph shows that those mushrooms grown on sugarcane leaves have a maximum cap diameter while those grown on sugarcane bagasse have a minimum.

Weight of oyster mushrooms

The weight of oyster mushrooms is different on different substrates. The maximum fresh weight of oyster mushrooms was obtained from the sugarcane leaves (55 gm), followed by speargrass (44 gm) and then the wheat straw (40 gm), while the lowest weight was reported in the mushrooms grown on the sugarcane bagasse (37 gm) (Figure 9).

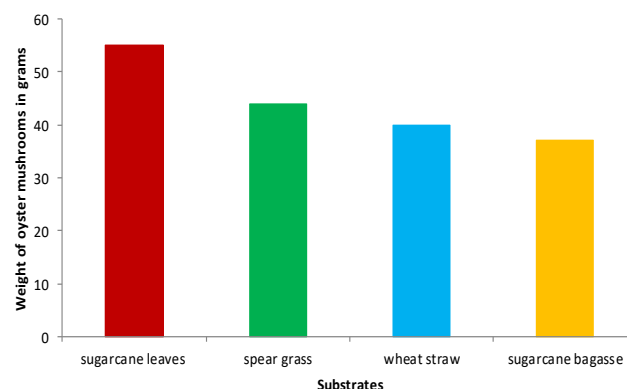


Figure 9: Graph showing the weight of oyster mushrooms. The above graph shows that mushrooms grown on sugarcane leaves have the maximum weight while those grown on sugarcane bagasse have the minimum weight.

Nitrogen value of oyster mushrooms on various substrates
The available protein contents of mushrooms depend on the growing substrate. Therefore, oyster mushrooms were sown on different growth media, and it was expected that they might have different protein contents upon obtaining them from the media. The measurement of nitrogen contents showed that oyster mushrooms have different protein contents grown over diverse substrates. The given formula was used to find the percentage of nitrogen for the samples.

$$\text{Percentage of nitrogen} = 1.4 \times N \times V / W$$

Where; N= Normality of acids, V= volume of acids which are obtained from the titration process, W= Weight of the substance.

The maximum nitrogen value was reported for mushrooms grown on sugarcane leaves (2.94 %), followed by spear grass (2.85 %), wheat straw (2.21 %), and the lowest on sugarcane bagasse (1.23 %) (Figure 10).

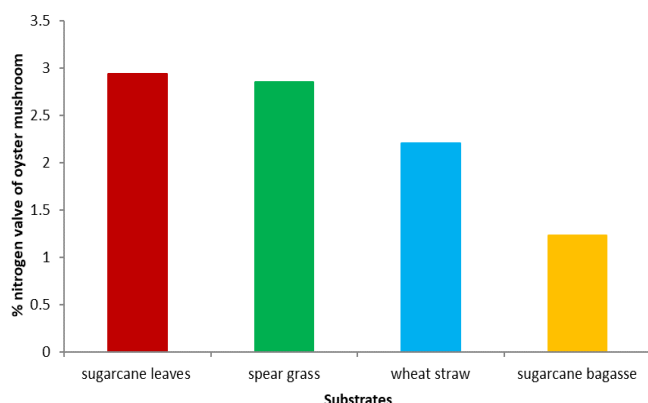


Figure 10: Graph showing nitrogen contents of oyster mushrooms. The above graph shows that the mushrooms obtained from the sugarcane leaves have a maximum nitrogen value, while those obtained from the sugarcane bagasse have a minimum nitrogen percent value.

Protein contents of oyster mushrooms on different substrates

The maximum protein value obtained from those mushrooms that were collected from the sugarcane leaves (Crude proteins= $2.94 \times 6.25 = 18.38\%$), followed by spear grass (17.85 %) and then wheat straw (13.83 %) while the lowest crude proteins have those mushrooms which are obtained from the sugarcane bagasse (7.61 %) (Figure 11). The given formula was used to find crude proteins. Crude proteins = percentage of nitrogen * 6.25.

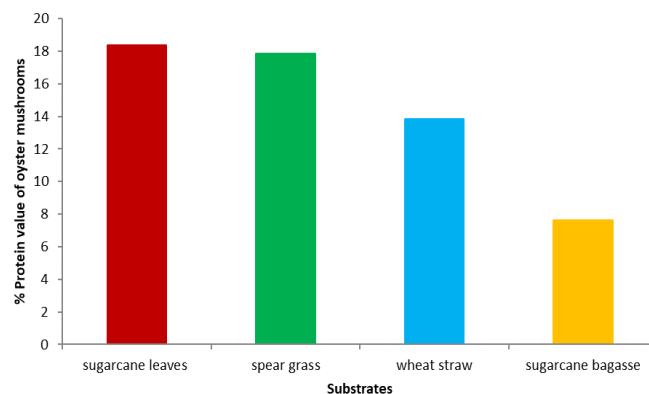


Figure 11: Graph showing protein content of oyster mushrooms. The above graph shows that the mushrooms obtained from the sugarcane leaves have a maximum protein value, while those obtained from the sugarcane bagasse have a minimum percentage of protein.

Discussion

Four different substrates were compared and explored to determine which one of these would be suitable for the cultivation and production of oyster mushrooms (*P. ostreatus*). Our analysis showed that the maximum number of fruiting bodies and cap diameter were observed in the oyster mushroom grown on the Sugarcane leaves (Figure 5 and 8). Our analysis determined the same findings as that by Shah *et al.* (2004), who stated that fruit bodies emerged after 16 days of spawning. It has been shown that sugarcane leaves are resistant to bacterial and fungal attacks (Shah *et al.*, 2004), therefore a better option for mushroom cultivation. Furthermore, sugarcane leaves are available throughout the year, are cost-effective, and mostly go to waste. The sugarcane bagasse was found to be an unsuitable substrate, as it had the highest bacterial and fungal contamination levels due to its susceptibility to bacterial and fungal attacks compared to all substrates. This may be due to improper sterilization of sugarcane bagasse as well as the other substrates used in the current project.

Mushrooms are high in protein, vitamins, and essential elements, including calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, copper, manganese, and selenium, making them a good source of, low in calories (Selvi *et al.*, 2007). The highest protein content was obtained from mushrooms grown on sugarcane leaves (18.38 %) followed by spear grass (17.85 %) and wheat straw (13.83 %), while the lowest protein content was present in mushrooms grown on sugarcane bagasse

(7.61 %) (Figure 11).

Conclusion

The effects of different agro-industrial and agricultural substrates revealed that the growth and yield of *P. ostreatus* varied based on the substrate used. It was noticed that both growth and protein contents were comparatively higher on sugarcane leaves than on other substrates. Furthermore, the cultivation of *P. ostreatus* on sugarcane leaves demonstrated excellent mycelial growth, longer stalk length, and bigger cap diameter. By contrast, wheat straw had a smaller effect on growth and protein content (Figures 5-11). Therefore, it is recommended to utilize the easily available and cost-effective sugarcane leaves for oyster cultivation as an alternate substrate.

Acknowledgement

We are pleased to thank the department of Botany, Abdul Wali Khan University, Mardan, for providing the research materials and facilitating this project.

Novelty Statement

Our analysis determined that sugarcane leaves are a better option for mushroom cultivation. Furthermore, the sugarcane leaves are available throughout the year and are cost-effective.

Author's Contribution

Muhammad Faiq: Research Experiment Executed/ MS drafting

Abdur Rauf: Research Supervision/MS drafting and proofread-ing

Muhammad Qayash, Muhammad Ikram and Mun-taha Munir: Proofreading.

Kashmala Jabbar, Wisal Khan, Tahseen Ullah, Ma-jeed Ullah and Farkhanda Bibi: Data analysis.

Guleena Khan and Tasneem Barin: Data Collection. Ikramullah Khan: Proofreading/Data analysis.

Conflict of interest

The authors have declared no conflict of interest.

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