Comparative Analysis of Growth Media for Enhancing Yield and Development of Oyster Mushroom

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Abstract
A study on the effect of different substrates on oyster mushroom yield and growth performance was conducted at a mushroom house at the University of Agriculture, Peshawar, Pakistan, from January to May 2022. An edible macrofungus, commonly known as a mushroom, is cultivated in several countries using agricultural waste. Agro-based lignocellulosic waste and leftovers are bio-converted throughout the mushroom production process. Because mushrooms grow using agricultural waste, their gardening is similar to sustainable farming. For this purpose, the objective of the study was to produce spawns from wheat substrate and to cultivate the oyster mushroom Pleurotus ostreatus using various substrates, including paddy straw, Wheat straw, Peanut straw, Brassica straw, and sawdust. The substrates were soaked overnight (16–18 hours) and then removed and autoclaved for one hour to sterilise them. After that, it was placed in polythene bags, and the appropriate amount of spawn was added. Following packaging, spawn sacks were placed in a closed posture with a temperature of approximately 18-28 °C, and humidity was maintained at a constant level by misting twice daily. Result revealed that Paddy straw showed minimum number of days required for mycelium growth (18), pin head formation (25) and for maturation of fruiting bodies (28) also indicates maximum value for number of fruiting bodies (26.27), pileus diameter (6.04), pileus thickness (0.63), stipe girth (4.73), stipe length (2.07) and biological yield (1083), followed by wheat, sawdust, peanuts, and brassica straw.
Keywords: Oyster Mushroom, Comparative Analysis, Growth Performance.

Introduction
Oyster mushrooms (*Pleurotus ostreatus*) are edible fungi with exceptional flavour and taste, making mushroom production a lucrative agricultural industry. It is a member of the order Agaricales, class Basidiomycetes, and subclass Holobasidiomycetidea. It grows naturally on forested hillsides and is cultivated in temperate and subtropical climates worldwide. Incorporating unconventional crops into the current agricultural system can assist small farmers in improving their social and economic conditions—a relatively recent discovery. A niche alternative for

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farmers with limited land, small-scale mushroom growing offers a possibility for those interested in starting a second business. If agricultural and food processing by-products are used as the growing medium for edible fungi, mushroom farming can play a significant role in managing farm organic waste. After that, the used substrate can be composted and added directly to the ground. Since the beginning of life on Earth, fungi have been active. The primary appeal of mushrooms as a food source lies in their edibility, nutritional value, and medicinal benefits. Wild varieties of edible mushrooms have occasionally been found to be unappealing and less delicious. Nevertheless, mushrooms are recognised to have a variety of purposes in both food and medicine. (Alice & Kustudia, 2004).

In Zimbabwe, oyster mushrooms (*Pleurotus ostreatus*) serve as a valuable food source for alleviating hunger, as they can grow rapidly, utilise agricultural waste, require less land, and consume less water than other field crops (Tavarwisa et al., 2021). Due to their exceptional flavour and beneficial health and nutritional properties, oyster mushrooms are commercially cultivated worldwide (Tesfaw et al., 2015). In many nations, including Ghana and China, mushrooms are used as a relish. In Asian nations like China and Korea, they are used to create very expensive sauces and soups. In terms of non-starchy carbs, dietary fibre, proteins, most amino acids, minerals, and vitamins, mushrooms are regarded as a vibrant source (Zied *et al*., 2017; Yao *et al.*, 2019). They serve as a meat alternative in vegetarian diets and are a good source of protein. Oyster mushrooms contain 25–30% protein, 2.50% fat, 17–44% sugar, 7–38% myco-cellulose, and roughly 8–12% mineral content (potassium, phosphorus, calcium, and sodium) (Stanley *et al*., 2012). Furthermore, given their chemical composition, mushrooms exhibit immune-stimulatory and anticancer properties, in addition to containing a significant amount of dietary fibre (Cheung, 2013; Lemieszek & Rzeski, 2012). The mushrooms also have anticancer, anti-diabetic, and antioxidant effects (Meng et al., 2016; Dávila, 2020).

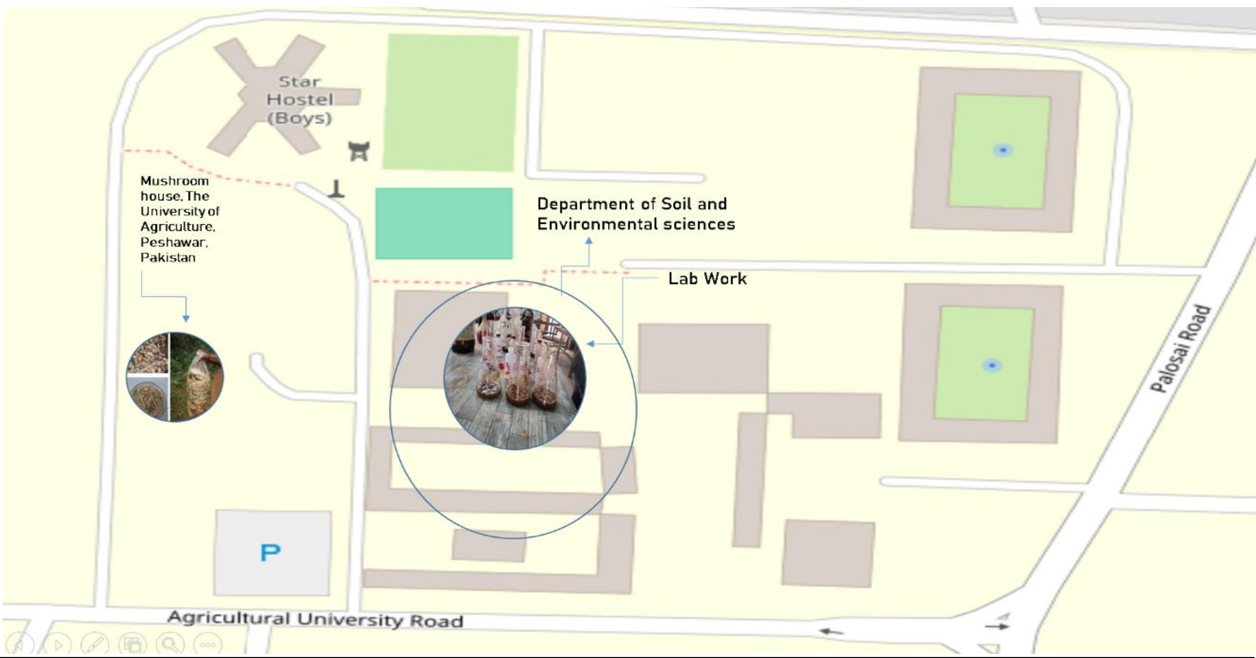
Mushroom growth and nutritional makeup are significantly impacted by substrate composition (Bellettini et al., 2019; Sebaaly et al., 2019). On certain ligno-cellulosic materials, such as sawdust (Hanafi et al., 2018), soybean straw, wheat straw (Cao et al., 2019), groundnut shells (Mane et al., 2007), newspaper and tea leaves (Jain, 2005), cotton waste and paddy straw (Ashraf et al., 2013), date palm waste (Alananbeh et al., 2014), mushrooms may be able to thrive. However, due to its effective lignin-degrading property, the optimum substrate for mushrooms should have nitrogen, carbohydrates, cellulose, and lignin for rapid growth (Das & Mukherjee, 2007). Pakistan exported approximately 201,000 kilograms (kg) of mushrooms, with a total export value of $12.930 million, in 2016. Not only was the increase in the value of mushroom exports phenomenal, but they also contributed over 25 per cent to the overall vegetable exports of more than $101 million in the same year. There is a need to develop diversified agriculture in Pakistan. Unfortunately, in Pakistan, mushrooms are not widely regarded as a significant food item, primarily due to their limited availability and low public awareness. The reason for its limited adoption is the unavailability of mushrooms at affordable prices and a lack of awareness about them. The farmer should consider cultivating edible mushrooms, such as oyster mushrooms (*Pleurotus ostreatus*), on a commercial scale to meet the requirements of a balanced diet. This study aims to investigate the cultivation of oyster mushrooms on various substrates, including paddy straw, Wheat straw, Peanut straw, Brassica straw, and sawdust.

Material and Methods

A study on the effect of different substrates on oyster mushroom yield and growth performance was conducted at a mushroom house at the University of Agriculture, Peshawar, Pakistan, from

January to May 2022. For seven days, a mushroom culture was cultivated on Malt Extract Agar medium (MEA). The media were prepared, sterilised, and given time to set up slantwise. A piece of *Pleurotus ostreatus* soft tissue was solidified and then aseptically transferred to individual MEA slants in the laminar airflow chamber. The cultures were grown at 25 °C after inoculation until enough growth was achieved. The slant culture was moved to Petri plates with MEA medium and cultured for seven days at 25 °C. After seven days, the mycelia growth covered the agar medium, and the culture was used for spawn preparation (Girmay et al., 2016). Wheat is used as the source material for spawn. The grains were generated by following the procedures outlined by Mondal (2010) and Ayodele (2007).

Figure 1: Site of Mushroom house and Lab analysis



Different substrates (paddy straw, wheat straw, peanut straw, sawdust, brassica straw) were taken. The substrates were soaked for 16 to 18 hours at night. Then removed and autoclaved for a full hour. They were dried in the shade after sterilization. Following that, spawns were added according to the amount of substrates that had been bagged in polypropylene. The bags were then sealed for spawn development after being pressed gently. After that, tiny holes were made in the bags for the mycelium to breathe, and cotton was inserted into these holes to filter contaminated air. The temperature of the spawned bags was set between 18–28°C, and the humidity was maintained by twice-daily water spraying (Oei, 2006). Maintaining a suitable moisture level encourages the growth of mushrooms (Abdul, 2011). After inoculation, the mycelia start to develop.

Data Collection and Analysis

Every day, the mushrooms' growth and development were observed. It was noted how many days it took from inoculation to mycelium formation and how long it took till the first round of harvesting. Before each harvest, slide calipers were used to measure growth metrics, including stipe length (cm), stipe diameter (cm), pileus diameter (cm), and pileus thickness (cm). At the time of harvest, other yield indicators were also noted, including the number of fruiting bodies per bunch and the overall fresh weight (g) of the mushrooms. Matured fruiting bodies (white, with up-curved pileus) were harvested with a sharp blade. Throughout the trial, there were three rounds of mushroom harvests across all substrate types. Yield and biological efficiency were calculated to evaluate the growth performance of mushrooms on different substrates. Accordingly, biological yield (g) was determined by weighing the whole cluster of fruiting bodies without removing the base of stalks, and economic yield (g) was determined by weighing all the fruiting bodies on a substrate after removing the base of stalks. Finally, Biological efficiency was calculated by

$\%BE = (FWm/DWs) \times 100$

Where BE refers to Biological efficiency, FWm is the total fresh weight (g), and DWs is the dry weight of substrate (g).

Results

The results from mycelia running, pinhead formation, and the maturation of fruit bodies are illustrated in the following lines. Five types of substrates as paddy straw, peanut straw, brassica straw, and sawdust, were compared with each other for the cultivation of mushrooms.

Mycelium Growth

The appearance of fungal growth/whitish growth in different substrates took 16 to a maximum 21 days; however, the effect of substrate remained non-significant (Table 1). The highest mean days were, however, taken by wheat straw (21), while the lowest mean values (18) were recorded in the paddy straw. The non-significant variation among substrates reveals that all of the tested substrates could be used. Khan et al. (1991) reported that various available agricultural wastes and substrates containing wheat straw give an optimum linear growth of mycelia.

Pinhead Formation

Required days for pin head formation range from 24-30 (Table 1). However, due to maximum variation among the replicates, it did significantly vary among substrates. 29 days is the highest value recorded in peanut straw, while 25 days is recorded lowest in paddy straw. The days for pin head formation by each substrate are non-significant, but they could be changed by increasing the spawning rate. By increasing the spawning rate, the time required for spawning reduces (Biswas et al. 1997).

Table 1: Days required for mycelium growth, pinhead formation, and maturation of fruiting bodies in different substrates

Treatment	Days req. for mycelium growth	Days req. for pin head formation	Days req. for maturation of fruiting bodies
Paddy straw	18	25	28
Wheat straw	21	27	30
Peanut straw	18	29	33
Saw dust	20	27	29
Brassica straw	18	25	28

Maturation of Fruiting Bodies

Analysis of variance shows that there is a significant difference between the number of days required for maturation of fruiting bodies ($p=0.02$). The highest mean value (33.33) was recorded in peanut straw, while the lowest mean value (28.33) was recorded in the paddy straw in all three replications (Table 1). This meant that paddy straw took less time than even the commonly used wheat straw for 30 days. However, there was no growth in the brassica substrate, and hence it could not be used for mushroom cultivation in the present scenario.

Number of Fruiting Bodies

Paddy straw shows a significantly higher number of fruiting bodies, while the lowest was shown by peanut straw (Table 2). The fruiting body in any substrate ranges from 20.6 to 28.5, with the mean being highest in paddy straw and a minimum of 21.97 in peanut straw. The data in the table shows that paddy straw had a higher number of fruiting bodies than commonly used wheat straw in the present situation.

Pileus Diameter

The mean pileus diameter ranged from 4.01 to 6.06 cm, considering the whole data (Table 2), and there were significant variations among the substrates. The lowest mean pileus diameter of 4.01cm was recorded in peanut straw, followed by 4.43 cm in sawdust substrate, while the mean highest pileus diameter of 6.04 cm was recorded in paddy straw, which was even higher than the commonly used wheat straw substrate. It was reported that under similar environments and cultural practices, cap diameter was also found to be highest from rice straw, i.e., 5.14cm, followed by wheat straw, banana leaves, and lastly sugarcane bagasse, which were 4.1 cm, 3.47cm, and 3.25 cm, respectively.

Table 2: The number of fruiting bodies, pileus diameter (cm), and Pileus thickness (cm) are influenced by different substrates

Treatment	No. of fruiting bodies	Pileus diameter Cm	Pileus Thickness
Paddy straw	26.27	6.04	0.63
Wheat straw	24.93	5.61	0.54
Peanut straw	21.97	4.01	0.42
Saw dust	23.38	4.43	0.46
Brassica straw	-	-	-

Pileus Thickness

Analysis of variance shows that pileus thickness significantly varied among substrates (Table 2). The highest mean value is 0.63 cm was recorded in paddy straw, while the lowest mean value is 0.42 cm was recorded in peanut straw. The values of pileus thickness are in the report ranges.

Stipe Girth

In different substrate analyses, variance showed significant variations in the stipe diameter of mushrooms grown in different substrates. The highest stipe diameter, 4.77 cm, was recorded in paddy straw, which was significantly higher than peanut straw and sawdust substrate but similar to wheat substrate (Table 3). The wheat straw produced mushrooms that had a mean stipe diameter of 4.69 cm, which was statistically superior to peanut straw and sawdust substrate. Habib (2005) found that on different substrates, the length of oyster mushroom stipes varies from 1.93 cm to 2.97 cm.

Stipe Length

Paddy straw produced significantly taller stipes of mushrooms than all the tested substrates under the given conditions (Table 3). The mean stipe lengths range from 1.02 cm to 2.08 cm, with the highest mean value of 2.07 cm in paddy straw and the lowest at 1.02 cm in sawdust. The stipe length being 10% higher than that of wheat straw indicates its suitability for cultivation in these experimental conditions. Various strains of oyster mushrooms were investigated, revealing larger mushrooms with longer stipe lengths on substrates inoculated with grain spawn (Ziaullah, 1996).

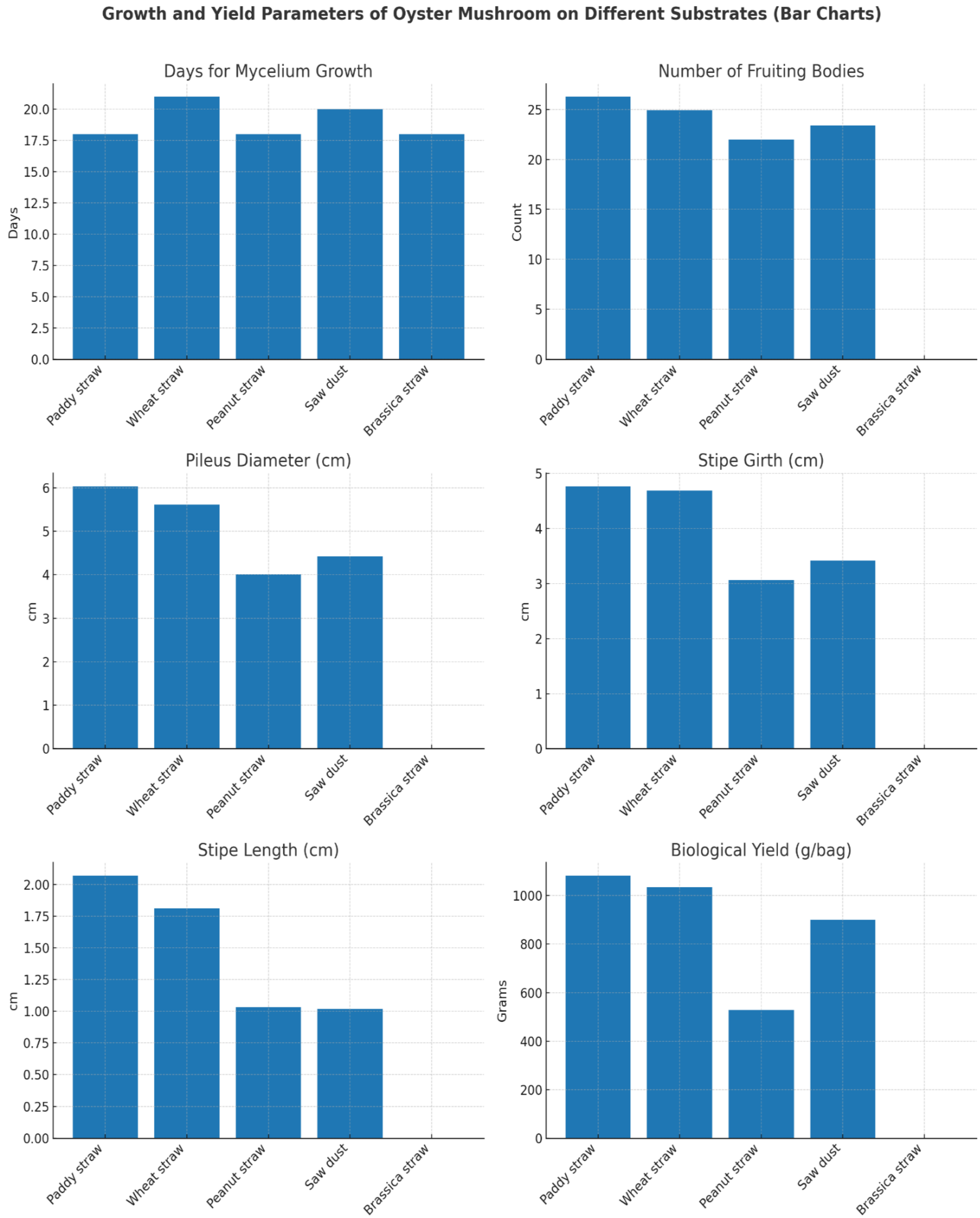
Table 3. Stipe girth (cm), Stipe length (cm), and biological yield (g) are influenced by different substrates

Treatments	Stipe girth	Stipe length	Biological Yield
	cm		g
Paddy straw	4.77	2.07	1083
Wheat straw	4.69	1.81	1035
Peanut straw	3.06	1.03	528
Saw dust	3.42	1.02	900
Brassica straw	-	-	-

Biological Yield (g bag^{-1})

The fresh weight of mushrooms was recorded in grams/bag after making three flushes from each bag of a single treatment. Data in Table 3 shows the sum of three pluckings that ranged from 535 to 1080 g/bag. It is to be mentioned here that one pot each in peanut straw and sawdust did not produce mature mushrooms, whereas all three bags (replicates) of brassica substrate failed to produce any mushrooms. The paddy straw and wheat straw produced the highest mean yield of 1083 and 1035 g bag$^{-1,}$ respectively, which indicated their suitability for mushroom cultivation. Sawdust substrate produced a mushroom of 900 g/bag, which was significantly lower than paddy straw and wheat straw but still very much higher than the 528 g/bag produced in peanut straw substrate. Mushrooms grown in the rice agricultural waste substrate have more efficiency in the absorption of available nutrients than the substrate composed of other agricultural wastes (Dubey et al, 2019).

Figure 2: Growth and yield parameters of Oyster Mushroom



Discussion

The current study suggests that the growth of *P. ostreatus* mycelia was relatively faster on paddy straw as compared to the other substrates used (Wheat, Peanuts, Sawdust, and Brassica straw). On average, it took about 22 days for the mycelia to run on each substrate. This is comparable with the study of (Onuoha et al., 2009), who reported the completion of spawn running on paddy straw waste to be 15 days, while others reported it to be between 13 and 16 days using a similar substrate (Patra and Pani, 1995; Jiskani, 1999). The variation in the number of days taken for a spawn to complete colonization of a given substrate is a function of the fungal strain, growth conditions, and substrate type (Chang & Miles 2004). According to (Oei 1996), mushroom mycelia require specific nutrients for their growth; the addition of supplements can, thus, increase mushroom yield through the provision of these specific nutrients. Pin-head formation (primordium initiation) was observed following the invasion of substrates by mycelial growth. The time required for the formation of pin-heads is comparable with other studies, i.e, (Ahmed, 1998) reported pin-head formation of oyster mushrooms cultivated in different substrates to be between 23 and 27 days from spawning, while (Fan et al., 2000) reported it to be 20–23 days. It was observed from this study that the overall cropping period for oyster mushrooms varied for each of the different substrates used. It is reported that high-quality and highly rated mushrooms are those with large-sized fruit bodies (Onyango *et al*. 2011). However, excessively large fruit bodies may be considered inferior for commercial packaging due to their fragility. Sarker et al. (2007) and Habib (2005) reported that the length and diameter of oyster mushroom stipes on various substrates varied from 1.93-2.97 cm and 0.74-1.05 cm, respectively. Habib et al. (2005) discovered that due to the variety of substrates, pileus diameter varied from 4.85-8.95 cm, and thickness varied from 0.45-0.70 cm. The 20% rice husk in weight substrate produced the best production of P. ostreatus, according to Baysal et al. The maximum biological yield was found by Ruhul Amin et al. (2007) at 247.3g/packet. He also discovered that the trend in economic yield was related to various additives at various levels. According to (Khanna & Garcha, 1981), it may take up to 104 days to harvest the yield from oyster mushrooms grown on paddy straw. These variations in cropping periods were due to the variations in the growing environment and physiological requirements for mushroom cultivation, like the constant temperature, humidity, and light arrangements. It was observed that the yield components of P. Ostreatus were found to be affected by the use of different substrates. Paddy straw resulted in a relatively better growth in terms of diameter and thickness of pileus, and diameter and length of stipe. The study confirmed that the use of different substrates brought about a significant effect on the yield (biological and economic yield) of oyster mushrooms. The largest yield was harvested from Paddy straw, followed by Wheat straw, while the least was obtained from Sawdust. Similarly, the biological efficiency also varied among the different substrates. Variable ranges of BE have been reported when different lignocellulosic materials were used as substrates for the cultivation of oyster mushrooms (Liang et al. 2009).

Conclusion

The oyster mushroom is an edible mushroom that can be grown on various substrates like Paddy straw, wheat straw, Brassica straw, peanut straw, sawdust, etc. This study confirmed that the Oyster mushroom, *Pleurotus ostreatus*, grew well in Paddy straw when compared with other substrates, and also it is confirmed that the spawn produced from Wheat grain has more potential than other spawns. Further studies need to be conducted on the potential of various agricultural and industrial wastes on oyster mushroom cultivation, their economic feasibility, and other related issues of mushroom (particularly oyster mushrooms) to fully realize the multiple socio-economic and environmental significances of the mushroom

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