Research Article

Growing Substrate Influenced Production and Quality of Oyster Mushroom Strains

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Abstract | In Mushroom cultivation growing substrate is a critical determinant of biomass accumulation and secondary metabolites production. In this study, three Strains of Oyster Mushroom namely (*Pleurotus eryngii, Pleurotus pulmunarius, and Pleurotus ostreatus)* were cultivated on wheat straw and saw dust to find out best strain and growing substrate. The results revealed that fastest colonization of mycelia (48.88 days), early pinhead initiation (17.50 days), maximum number of pinheads (7.53 pinheads), minimum days to first picking (20.00 days), larger diameter of pileus (87.44 mm), higher moisture content (87.16%), better total flavonoid content (8.26 mg g^{-1} DW) and more radical scavenging activity (76.55%) were recorded in Mushroom harvested from wheat straw substrate, whereas more dry matter content (19.19 %) and total phenolic contents (34.69 mg g^{-1} DW) were recorded in Mushroom harvested from saw dust. Among the three tested strains, minimum days (48.88) to complete colonization, more number of pinheads bag^{-1} (7.78 pinheads), higher fresh weight (130.00g bag^{-1}), larger diameter of pileus (88.50 mm), maximum moisture content (90 %) were recorded in *Pleurotus ostreatus*, whereas minimum days to pinhead initiation (19.83 days), days to first picking (22.16 days), more dry matter content (19.23%), best total phenolic content (34.00 mg g^{-1} DW), total flavonoid content (8.00 mg g^{-1} DW) and maximum radical scavenging activity (70.33%) were recorded in *Pleurotus eryngii*. It was concluded that *Pleurotus ostreatus* Mushroom yielded more on wheat straw substrate, while *Pleurotus eryngii* Mushroom contained better nutritional qualities on wheat straw substrate.

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Introduction

Oyster mushroom (*Pleurotus* spp.) belongs to family Tricholomataceae (Owaid *et al.*, 2015). German has introduced its farming in 1917 on broken old trees trunk and wood logs (Upadhyay and Singh, 2011). The productions have been improved from that time and vary significantly throughout the world technologies

(Quimio *et al*., 1990). The first large- scale cultivation of Mushroom's on logs was introduced in Hungary in 1969 (Martínez, 1998). Around the world more than 200 species of mushrooms are still been used as useful food (Kalac, 2013), but only 35-40 species have been cultivated commercially (Xu *et al*., 2011). China is the leading country in mushroom production followed by USA and Netherland (Amin *et al*., 2014). In Pakistan mushrooms production is quite less as compared to other countries. In Swat and Islamabad, initiative was taken by National logistic cell to grow mushrooms and has got production capacity of 48 tons^{-1} annum (Alam and Raza, 2001).

Oyster Mushrooms cap (pileus) is shell-like and fleshy, with irregular stipe defending on varieties (Martínez, 1998). In oyster mushroom species King oyster mushroom (*Pleurotus eryngii*) has been found one of the best specie because of its brightness in cap and stem steadiness, the storage life is very long and it is also a great source of bioactive compounds. Therefore, in many countries it is used as favorite food and having high consumer demand (Oke and Aslim, 2011). *Pleurotus ostreatus*, is generally known as the oyster mushroom and is a standard edible mushroom. In Germany it was cultured for the first time as a survival measure at the time of World War I. Kaufert was the first who documented farming of Oyster mushroom (Eger *et al*., 1976).

In world about 60% of the population has limited access to safe, nutritious and sufficient food, hence from malnutrition many people are suffering especially children and women (Sher and Hussain, 2009). Animal protein is further difficult to purchase in many countries because about 86% of people are living below the poverty level (Porter *et al*., 2017). Mushroom is a food which contains high nutrient, low calories and is rich in protein and vitamins. Oyster mushroom protein has highly digestible value and contains all the essential amino acids (Ali *et al*.,2007). The fresh Oyster mushroom contains about 3% protein, 4% carbohydrates, 85-90% moisture, 1% minerals and vitamins and 0.3-0.4% fats (Thakur and Singh,2014). Mushroom protein can be used in place of animal's protein (Kurtzman,1976). Oyster mushroom contain good amount of iron, copper, phosphorous and potassium but less calcium. Mushroom also contains some secondary metabolites like steroids, terpenes, polypeptides and phenolic compounds. Mushroom is a rich source of polysaccharide-peptides, polysaccharides, polysaccharide-protein complexes and lectins. It contains better level of Niacin, pantothenic acid and biotin thus immune modulatory and carcinogenic activities (Sun and Liu, 2009).

Mushroom is a traditional medicine since Greek and Roman classical time. It is believed that mushroom need antibacterial compounds to survive in their natural environment (Manjunathan and Kaviyarasan, 2010). Due to its bioactive constituents, mushrooms got massive attention from medical researchers and food technologist (Sheu *et al*., 2007; Mariga *et al*., 2014). They are the source of extraordinary power and have medicinal properties like and is used in preventing diseases like hypertension, hypercholesterolemia and cancer (Bobek and Galbavy, 1999). These functions are due to dietary fiber, chitin and beta glucans (Manzi *et al*., 2001). Additionally, *Boletus pseudocalopus* (Basidiomycetes) from which Grifolin derivatives are isolated which is used as anticancer and moderate radical scavenging activities (Song *et al*., 2009). Moreover, *Pleurotus pulmunareus* and *Pleurotus ostreatus* are rich sources of immune modulatory and anti-inflammatory properties because of its chemical composition (Lavi *et al*., 2010; Selegean *et al*., 2009).

Genetic makeup and growing substrate are influential factors effecting mushroom biomass and secondary metabolites production. Mostly, for farming Oyster mushrooms (*Pleurotus* spp.) the plastic bags are used as substrate containers. The plastic bags which are used for cultivation are made up of polyethylene, polyvinyl chloride or polypropylene (Kashangura, 2004). Many crops residues are used as substrates for farming Oyster mushrooms with different substrate containers (Bisaria *et al*., 1989). Mostly rice and wheat straws, woodlogs, sawdust, banana leaves, woodchips, sugarcane. bagasse, cotton.seed hulls., corn cobs,. rice and wheat bran are used as substrates for edible mushroom cultivation (Orts *et al*., 2008; Saber *et al*., 2010). Mushrooms are grown on a variety of substrates and the choice of substrate depends on availability and cost.

This is on the background that momentous variation on biological efficiency, mushroom nutritive content and yield on different substrate weight have been reported worldwide. Different biological efficiency has been associated with different substrates supplemented with different additives resulting into

specific nutritional composition of the product. It is reasonably to suggest that in order to be able to adequately address issues related to mushroom productivity, there is need for thorough assessment of the effects of different substrates on mushroom yields. Thus, interventions that seek to improve mushroom production need to consider the trade-offs inherent in availability and suitability of substrates in mushroom cultivation. Due to importance of different species of mushroom in food and pharmaceutical industry, and role of growing substrate in its production the current study was conducted to know the influence of growing substrate on growth, yield and quality of different mushrooms strains.

Materials and Methods

Source of spawn of mushroom

Three strains of oyster mushroom (*Pleuratus eryngii, Pleuratus pulmunarius* and *Pleuratus ostreatus*) were obtained from Plant Tissue Culture Laboratory (Agriculture Institute, Tarnab, Peshawar, Pakistan). These were maintained and subcultured on Potato Dextrose Agar (PDA) media plates at 25 °C.

Culture media preparation

For media preparation, 250 g sliced potato were washed and boiled in 800 ml distill water until softness. After boiling, the pure extract was poured in 1000 ml beaker, 15g agar powder (Sigma USA) and 10g dextrose (Solarbio Life Sciences) were added. Thereafter, the final volume was made (1000 ml) by adding distil water. The prepared media was autoclaved at 121°C for 20 minutes. After autoclaving, the media was poured to petri dishes (90×90 mm) in laminar flow unit in aseptic condition and was left open up till it cool down.

Pure culture preparation

For pure culture, the prepared media was used. The selected mushroom strain fruiting body was divided into two parts. The inner sterile part was cut into small pieces (5 mm) by using sterile surgical blade and was cultured in media (Figure 1a, b, c). Cultures were kept in room temperature for a week and then were shifted to 500ml flasks containing moist wheat grains.

Wheat grain media preparation

Wheat grains were washed 4 to 5 times with tape water to clean it from dust and residues. After that it was heated upto 70 °C for 1 hrs, care was taken that grains were not burst. The heated grains were shed on newspaper for 4 hrs that excess water may drains from it and its moisture become up to 38%. After that for maintaining its pH value 8, gypsum @ 4 % was added to it.

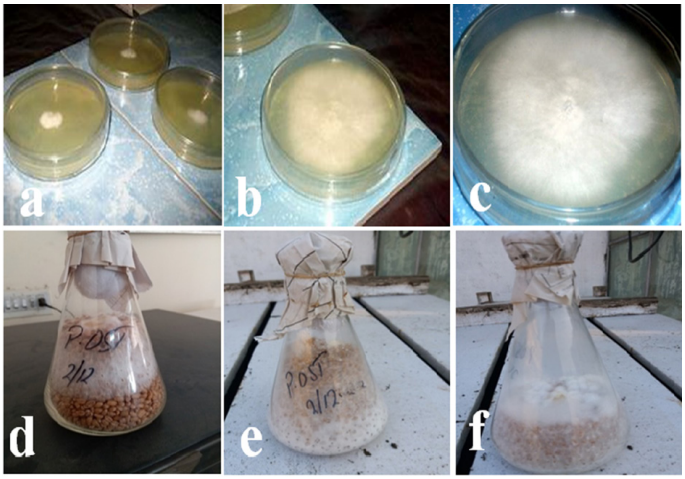

Figure 1: *Culturing of oyster mushroom strains on potato dextrose (a, b and c), and on wheat grains (d, e and f).*

Mother culture preparation

Mother culturing was carried out in 500 ml flasks. Moist wheat grains (250 g) and 4% gypsum was put into 500 ml flasks and was covered by cotton plug, and then it was autoclaved at 121°C for 30 minutes. On second day (after cooling) 2-3 pieces of spawn was inoculated in autoclaved flasks in sterilized environment i.e., in Laminar flow unit (Figure 1d, e, f).

Substrate preparation

Two substrates i.e., saw dust and wheat straw were obtained from Agriculture Research Institute, Tarnab, Peshawar. Wheat straw was soaked in water for 4 hours. After draining of excess water then it was lift for 24 hrs that moisture level may came down up to 60%. After that polypropylene bags (38 cm × 45 cm) were filled up from the prepared substrate (weighting 2 kg bag^{-1}). For saw dust, some water was added to it to maintain its moisture level up to 60%. In both 20 % wheat brain was added. After that polypropylene bags (20 cm × 30 cm) were filled from it and its mouths were closed with rubber band.

Pasteurization of substrates

To avoid germ and toxic fungal activities in substrate, the prepared substrate bags were steam posturized in an Autoclave for 2 hours at 121 °C (Nanbei Instrument limite, China). Before putting the bags in autoclave, little water was added into the tank to produce steam.

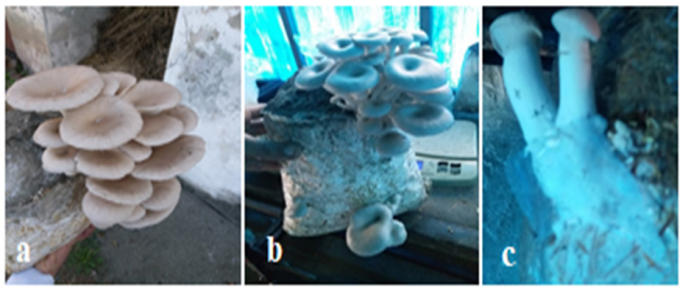

Figure 2: *Pleurotous ostreatus (a), Pulmunnarius (b), Pleurotus eryngii (c).*

Spawn inoculation

The cultured spawn (mother culture) were placed in posturized substrate bags. The spawn was placed in each bag on both the sides and on the top. The inoculated bags were closed with rubber band and for the exchange of gases in wheat straw bags four holes were made on each side through a needle while in saw dust for the exchange of gases its mouth were closed through rubber band with placing cotton plugged in it and four holes were made on each side through needle. The bags were hanged in a culture room at 25°C temperature with 65–70% relative humidity. Mycelium growth rate in each substrate was recorded after 10 days of spawn inoculation. After 100% colonization of mycelia in bags, up to one inch 3-4 cuts were made on the surface of spawn packets.

Experimental design

The experiment was consisted of two factors and was carried out according to the Completely Randomized Design (CRD). Substrates was (Factor A), while Strains was (Factor B). There were 6 treatments combination in a single pseudo replication. These combinations of treatment were replicated three times to reduce the experimental error and get accurate results.

Parameters studied

Days for complete colonization: The cultured substrates bags were kept in culture room at 25 °C temperature and up to 60% relative humidity. The number of days from inoculation of spawn to the complete colonization of mycelia was recorded.

Days to pinhead initiation: The days to first pinhead appearing in each treatment was recorded and mean was worked out.

Days to first picking: Days to first picking was recorded in each treatment and average was worked out.

Number of pinheads: This was counted directly by counting the number of pinheads on each substrate.

Diameter of the pileus (mm): The diameter of pileus was measured in millimeters with Vernier caliper.

Fresh weight of fruit bodies (g): Fresh weight of fruit for each treatment was calculated by using an electrical weight balance.

Dry matter of fruit bodies (%): The fruit bodies were kept in a dryer at 70 °C for 2 hours to evaporate all moisture content. After complete drying, the dry matter was calculated by using an electrical weight balance and was converted to percentage.

Yield bag^{-1} (g): The yield bag^{-1} in gram was recorded from the randomly selected three strains of Oyster mushroom grown on both substrates and then their average yield bag^{-1} was computed.

Moisture content (%): Mushroom sample was put in dry dish. After that it was covered and kept in dryer and was maintained at 70°C for 2 h. After cooling to room temperature, the samples were weighed soon. The process was repeated three times for each treatment, and loss in weight was reported as moisture content.

Formula used for moisture content:

$$Fresh\ weight - Dry\ weight = X$$

$$Moisture\ content\ (\%) = (X \times 100)/Fresh\ weight$$

Total phenolic content (TPC): Total Phenolic content of sample was measured according to the method of Gutfinger (1981). Briefly, the extract (1.0 ml) was mixed with 1.0 ml of 2% Na_2CO_3 and 0.2 ml of 50% Folin Ciocalteau reagent was added into the mixture. After incubation for 30 minutes at room temperature, the mixture was centrifuged at 13,400 × g for 5 min. The absorbance was measured on spectrophotometer at a 750 nm. Total phenolic content was expressed as Gallic acid equivalents.

Total flavonoid content (TFC): Total flavonoid contents in the methanolic extracts of oyster mushroom were determined by Choi *et al.* (2006). Briefly, mushroom extract (250 µl) was mixed with 1.25 ml of distilled water and 75 µl of 5% $NaNO_2$ solution. After incubation for 5 minutes, 150 µl of 10% Anhydrous Alumunium chloride ($AlCl_3 \cdot H_2O$)

was added. After 6 min, 500 µl of 1 M NaOH and 275 µl of distilled water were added to the mixture. The solution was mixed well and the intensity of pink color was measured on spectrophotometer at 510 nm.

Radical scavenging activity: The scavenging activity was determined by the DPPH (1,1.diphenyl-2-picrylhylhydrazyl) method (Blois, 1958). Briefly, 1ml of methanolic extract was mixed with 1 ml of ethanol solution containing DPPH radicals (Sigma Chemical Co), it was resulting in 0.041 mM of the final concentration of DPPH. The mixture was shaken vigorously and left to stand for 10 minutes. The absorbance was measured at 517 nm using a spectrophotometer.

Results and Discussion

Days to complete colonization

Faster colonization of Mushroom was recorded on wheat straw substrate as compared to saw dust. Whereas, among the three different strains, minimum days (48.80) to colonization were recorded for *Pleurotus ostreatus*, followed by *Pleurotus errryngii* (56.83) days, whereas, maximum days (77.33) to colonization was taken by *Pleurotus pulmmunarius* (Figure 3a). Best interaction was found in *Pleurotus ostreatus* and wheat straw (48.88 days) for early colonization. In our study, wheat straw was effective for early colonization; this may be due to the difference in total nitrogen and carbon content of substrate. Carbon, nitrogen ratio has influential effect on the mycelium growth, formation and development of fruiting body (Hoa *et al*., 2015). The moisture contents have influential effect on mycelia growth; wheat straw has maximum moisture contents as compared to sawdust (Onyango *et al*., 2011). In this study the growth of mycilium was slower than the findings of Dahmardeh *et al*. (2010) who reported that mycelium growth in substrate was completed in three weeks and pinhead appeared after 2-3 days. Whereas, Bughio (2001) reported that in wheat straw *Pleurotus ostreatus* took (43.25) days for pinhead formation after spawn inoculation.

Days to pinheads initiation

Pinhead initiation was faster in wheat straw as compared to saw dust. Among the three different tested strains, earlier pinhead initiation (18.00 days) was recorded in *Pleurotus ostreutus*, while maximum days to pinhead initiation (22.83) were recorded for *Pleurotus pulmunarius*. Best interaction was found among *Pleurotus ostreatus* and wheat straw which took (17.50) days for pinhead initiation (Figure 3b).

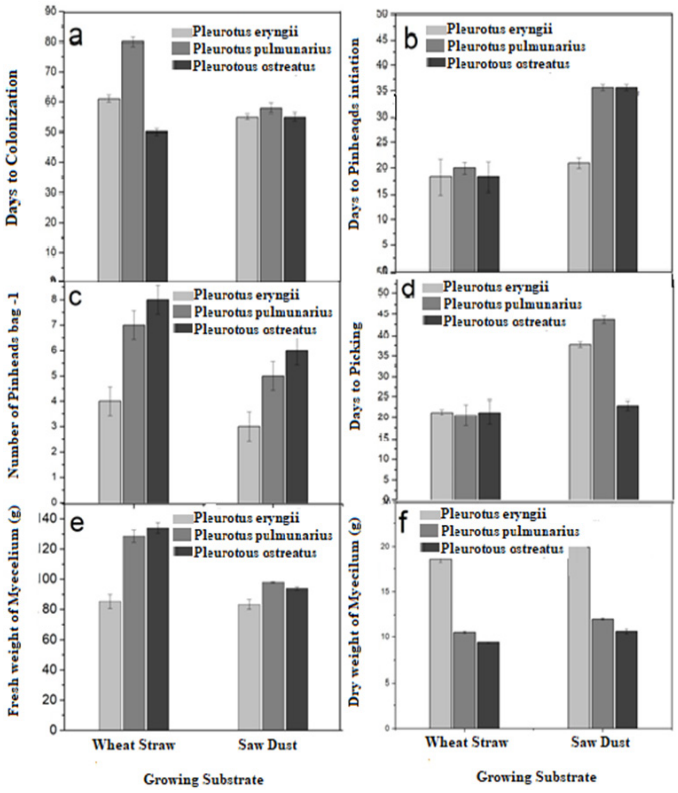

Figure 3: *Vegetative attributes of mushroom strains grown on two different substrates. (a) days to colonization, (b) days to pinhead initiation, (c) number of pinheads per bag, (d) days to picking, (e) fresh weight of mycelium (g), dry weight of mycelium (g). data are means of three replicated data.*

More numbers of pinhead (8) per bag were observed in Pleurotous ostreatus on wheat straw substrate (Figure 3c). The time to pinhead initiation of mushrooms is highly dependent on nutritional status, air circulation and moisture content of the substrate (Philippoussis *et al*., 2001). Onyango *et al*. (2011) reported that wheat straw contains higher nutritional material which makes mycelia growth faster and rapid resulting in vigorous growth and early pinning. Oyster mushroom has been cultured on many agricultural products such as wheat straw, olive cake, tomato tuff and pine needles (Alananbeh *et al*., 2014). However, wheat straw is widely used in mushroom culturing for the early initiation of pinheads (Mintesnot *et al*., 2014). In our study, wheat straw was effective for early initiation of pinheads from mother culture of mushrooms, while among the strains minimum time was taken by *Pleurotus ostreatus*. According to Hoe *et al*. (2015) wheat straw contains more carbohydrates which are effective for early pinhead initiation of Mushroom culture.

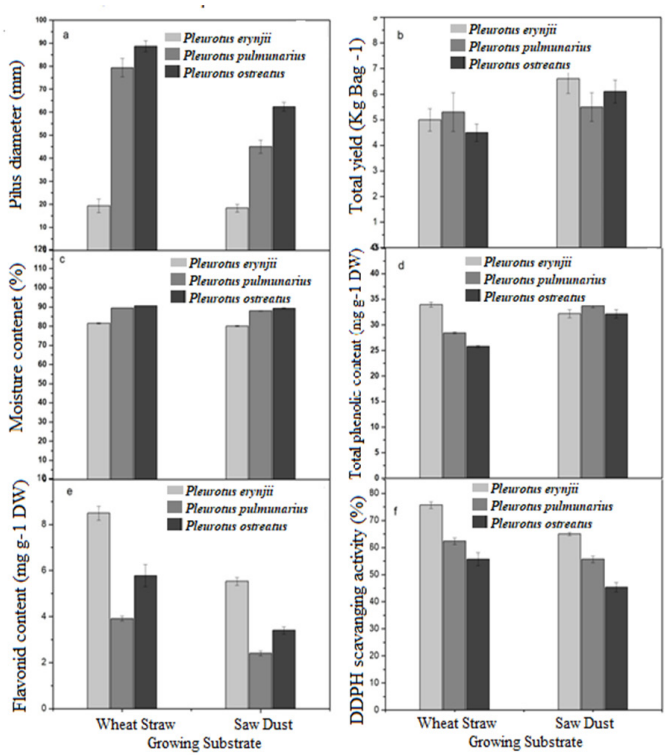

Figure 4: *Mushroom strains grown on two different substrates. (a) Pilus diameter (mm), (b) Total yield (Kg Bag^{-1}), (C) Moisture content (%), (d) Total phenolic content (mg g^{-1} DW) (e), Flavonid content (mg g^{-1} DW), (f) DPPH scavenging activity (%). Data are means of three replicated data.*

Days to first picking

Minimum time for first picking of mycelia (21.12 days) was recorded for *Pleurotus pulmunarius*. While maximum days to picking (46 days) were recorded for same strain on saw dust (Figure 3d). Wheat straw contains high carbon and cellulose content, high nutritional material makes mycelia to grow faster and provide earlier mycliea for harvest (Onyango *et al.*, 2011). Naraian *et al.* (2008) harvested earlier mycelia on substrate containing wheat straw and wheat bran as compared to saw dust and sugarcane Bagass. In contrast, Hoa *et al.* (2015) harvested *Pleurotus ostreatus earlier* (46.02 days) grown on Corncob substrate, whereas *Pleurotus cystidiosus* took the longest time (64.24 days) for the first harvest. This shows that genotype has a determinant rule in time of harvest.

Fresh weight of mycelium (g)

Among mushroom strains *Pleurotus ostreatus produced m*aximum fresh weight of mycelium (138g) on wheat straw (Figure 3e). Over all, wheat straw substrate was beneficial for maximum biomass production whereas, minimum biomass production was recorded on saw dust substrate. Wheat straw substrate consists of higher amount of cellulose, which is beneficial for Mushroom yield (Onyango *et al.*, 2010). Iqbal *et al.* (2005) recorded higher biomass accumulation of *Pleurotus ostreatus* and *Pleurotus sajarcaju* on wheat straw substrate. In a study carried out by Gupta and Vijay (1991) growing substrate supplemented with wheat bran produced maximum mycelium biomass as compared to mycelium grown on other substrate.

Dry weight of mycelium (%)

Pleurotus eryngii produced maximum dry matter (19.19%) on saw dust substrate. Among the three strains maximum dry matter content was recorded in *Pleurotus eryngii* followed by *Pleurotus pulmunarus*, whereas, minimum dry matter was produced by *Pleurotus ostreatus*. More dry matermter of mushroom was obtained on saw dust as compared to wheat straw substrate (Figure 3f). Mandeel *et al.* (2005) reported in his study that the production and dry matter content of mushroom was increased by adding wheat bran supplementation in woodchip.

Pileus diameter (mm)

Maximum pileus diameter (90 mm) was recorded for *Pleurotus ostreatus* on wheat straw substrate. Among the three different strains largest diameter of pileus was recorded in *Pleurotus ostreatus* followed by *Pleurotus pulmunarius*, whereas smallest pileus diameter (20 mm) on both substrates was recorded in *Pleurotus eryngii* (Figure 4a). Moreover, higher yield (6.7 kg bag -1) was found on Saw dust substrate by Pleurotus eranjii (Figure 4b). More cellulose content in substrate is beneficial for larger size of oyster mushroom (Shen and Royse, 2001). Similarly, the findings of Hao *et al.* (2015) recorded maximum pileus diameter (86.74 mm) *Pleurotus ostreatus* on substrate containing corncob. In a study by Yang *et al.* (2013) oyster mushroom give better pileus on substrate 45% corncob + 45% rice straw + 10% wheat bran as compared to other substrates.

Moisture contents (%)

Maximum moisture contents (90.00 %) were recorded in *Pleurotus ostreatus* on wheat straw, followed by *Pleurotus pulmunarius* (88.72%) whereas, minimum moisture contents (80.76) was recorded in *Pleurotus eryngii* (Figure 4c). Moisture content is greatly influenced by growing environment, mushroom age, mushroom strains, moisture concentration of substrate and postharvest operations (Kurtzman, 2005). According to Onyango *et al.* (2011) mushroom harvested from wheat straw exhibited higher moisture content as compare to mushroom grown on saw dust. Other researchers demonstrated significant effects

of substrate combination on moisture content of *Pleurotus ostreatus* (Ahmad *et al*., 2009; Kurtzman, 2005). Similarly, Hao *et al*. (2015) found (89.71-91.56%) moisture content in *Pleurotus ostreatus*. In a study by Ahmad *et al*. (2009) maximum moisture content (92.45%) was recorded in *Pleurotus cystidiosus* cultivated on substrates containing 100% wheatstraw.

Total phenolic contents (mg g^{-1} DW)

Maximum total phenolic content was recorded in strains grown on saw dust. Among the strains maximum total phenolic content (32.89) was produced by *Pleurotus eryngii* followed by *Pleurotus pulmunarius* (31.08) whereas, minimum total phenolic content (28.96) were obtained by *Pleurotus ostreatus*. Best interaction was found between *Pleurotus pulmunarius* and Saw dust which gives (33.74 mg/g) followed by *Pleurotus eryngii* and wheat straw which give (33.59 mg/g) total phenolic contents ((Figure 4d). Previously many researchers reported that oyster mushroom contains phenolic compounds with anti-oxidative effects (Palacios *et al*., 2011; Muszyńska *et al*., 2013; Piska *et al*., 2017). In our study maximum phenolic contents (32.89) were observed in *Pleurotus eryngii* and the minimum total phenolic contents (28.96) were observed in *Pleurotus ostreatus*. Similarly, the findings of (Kim *et al*., 2009) shows that total phenolic contents of the white oyster mushroom were in the order of *Pleurotus eryngii* (39.30 mg/g) followed by *Pleurotus ostreatus* (30.10 mg/g) and *Pleurotus cystradiatus* (21.20 mg/g). These total phenolic contents results could show the differences of the antioxidant activities of the three oyster mushrooms in terms of radical scavenging, reducing power and chelating effects.

Flavonoid contents (mg g^{-1} DW)

Maximum flavonoid content (8.5mg g^{-1} DW) was recorded in *Pleurotus eryngii* on wheat straw whereas minimum total flavonoid content (2.57 mg/g) were recorded for *Pleurotus pulmunarius* on saw dust. For total flavonid production, best interaction was found between *Pleurotus eryngii* and wheat straw, followed by *Pleurotus ostreatus* and wheat straw (Figure 4e). Flavonoids are usually glycosylated and can be classified as anthocyanidins, flavanols (catechins), flavones, flavanones, and flavonols, which are responsible for the orange, red and blue color in fruits and vegetables (Lin and Tang, 2007). In current study it was found that *Pleurotus eryngii* contains the highest flavonoid content. Similar results were founded by Kim *et al*. (2009) which shows that total flavonoid contents in oyster mushrooms were in the order of *Pleurotus eryngii* (3.16 mg g^{-1}) followed by *Pleurotus ostreatus* (2.96 mg g^{-1}) while the lowest value of TFC were found in *Pleurotus cystradiatus* (2.21 mg g^{-1}).

DPPH radical scavenging activity (%)

Among the two growing substrate maximum radical scavenging activity (75.55%) was obtained by wheat straw whereas minimum radical scavenging activity (45.33%) were obtained in saw dust. Among the three different strains maximum radical scavenging activity (75.33%) was achieved by *Pleurotus eryngii* followed by *Pleurotus pulmunarius* which have (58.99%) whereas, minimum radical scavenging activity (50.49%) were obtained by *Pleurotus ostreatus* (Figure 4f). Oyster mushrooms are essential in diet because of their free radical scavenging activity and flavonoid contents (Shahidi and Wanasundara, 1992). Radical scavenging activities are may be different from strain to strain, like in a study by Kim *et al*. (2009). In a study by Yang *et al*. (2002) it was found that winter mushroom (*Pleurotus eryngii*) was more effective in scavenging radicals than white strain (summer mushroom or *Pleurotus pulmunarius*). Thus, it can be concluded that the radical scavenging activities of oyster mushroom varied by fruit body colors, cultivation season and different strains. Similarly, in our study maximum radical scavenging activity (75.53%) was achieved by *Pleurotus eryngii* and minimum radical scavenging activity (50.49%) were recorded in *Pleurotus ostreatus*.

Conclusions and Recommendations

Growing substrate has influential effect on Mushroom production, in current study the growing substrates significantly affected different growth, yield and quality parameters of tested oyster mushroom strains. Among the growing substrates wheat straw was better for most of growth and quality attributes, whereas among the three tested strains, growth was better in *Pleurotus ostreatus*, whereas best quality attributes were recorded for *Pleurotus eryngii*. Best interaction was found in *Pleurotus eryngii* and wheat straw for quality production. Further research on growing substrate with amendment is needed for quality mushroom production.

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Novelty Statement

This study presents a comprehensive evaluation of how different growing substrates influence the yield, biological efficiency, and nutritional composition of various oyster mushroom (*Pleurotus* spp.) strains. Unlike previous studies that focus on a single substrate or strain, this research systematically compares multiple substrate combinations to identify the most effective medium for optimizing both production and quality. The findings offer valuable insights for sustainable mushroom cultivation by utilizing locally available agro-wastes, thereby promoting eco-friendly agricultural practices and enhancing food security.

Author's Contribution

Muhammad Irshad and Hafiz Muhammad Rizwan: Conducted the experiments
Haris Khan and Faisal Khan: Helped in conducting the experiments
Muhammad Noman Khan: Helped in writing the manuscript
Abubakar Sadeeq: Helped in graphics presentation
Muhammad Israr: Reviewed manuscript
Mohammad Sohil: Helped in statistical analysis

Conflict of interest
The authors have declared no conflict of interest.

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