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Substrates evaluation for the quality, production and growth of oyster mushroom (*Pleurotus florida* Cetto)

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Abstract
Present study was conducted in the laboratory as well as in mushroom house to determine the effect of different agricultural wastes (wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw) on growth, production and quality of oyster mushroom (*Pleurotus florida).* The culture was maintained on Malt Extract Agar medium. Spawn was prepared on wheat grains. Spawn running took less time i.e. 20 days on wheat straw as compared to other substrates. The appearance of pinhead and their maturity also took less time i.e. 29 days and 30 days, respectively on wheat straw. Maximum yield i.e. 1360 gram was recorded on wheat straw. The first flush gave the maximum yield in all treatments and there was a progressive decrease in the yield of successive flushes. The maximum biological efficiency of 136% was observed in case of wheat straw. The maximum moisture (93.44%) and ash (1.006%) were recorded in oyster mushroom obtained from sorghum straw. Percent protein content (8.75 gram), crude fat (10%) and crude fiber (3.5%) were maximum in oyster mushroom grown on sugarcane bagasse, maize straw and wheat straw respectively.

Keywords: Substrates, growth, production, quality, oyster mushroom

1. Introduction
Oyster mushroom (*Pleurotus florida* Cetto) is an edible mushroom. It contains adequate amount of phosphorous, iron, protein, lipid, riboflavin and thiamine. Oyster mushroom fresh fruiting bodies indicates a high quantity of moisture (90.8%), where as dry as well as fresh oyster mushrooms are rich in carbohydrate (57.6%), protein (30.4%), fiber (8.7%), fat (2.2%) and ash (9.8%) with 345 kilocalories energy value on 100 g dry weight. Mushrooms are an excellent source of minerals and protein and also known as the vegetarian's meat [44]. The proteins of mushroom are considered to be intermediate between that of vegetables and animals [42]. The amino acids essential for human body are present in oyster mushroom [36].

P. florida productivity is maximum in a short time providing more protein per unit area than any other crop [35]. The high content of nitrogen and protein in oyster mushroom increases the biological efficiency and yield while it reduces the growth period. *P. florida* contains 18 essential amino acids such as methionine, isoleucine, lysine, glutamic acid, cysteine, aspartic acid phenylalanine, tyrosine, tryptophan, valine, arginine, histidine, alanine, glycine serine and proline [26]. The vitamins such as niacin, riboflavin and thiamin are found in oyster mushroom. The minerals such as ferrous sulfate, phosphorus, sodium and calcium are present in oyster mushroom [60]. It is used for both medicine and food [6]. According to Furlani and Godoy [32], mushrooms are considered as food with high nutritional value and delicious taste. Due to their low caloric value they are suitable for diets. The *P. florida* produces metabolites of medicinal and pharmacological interest, such as antimicrobials, immunostimulants, antioxidants and antitumourals [28, 56, 38].

The substrate source, spawn quality, strain and compost affect the performance and growth of oyster mushroom [77, 39]. The genus *Pleurotus* are well- known for the conversion of substrates into mushrooms. Moreover, its commercial production is easy and least expensive [9, 61].Various substrates such as wheat straw, rice straw and sawdust are used for oyster mushroom cultivation [69]. The oyster mushrooms are known for anti-inflammatory and immune-modulator effects [48]. The cultivation is easy under both temperate and tropical climatic conditions and they are cultivated and harvested all over the year [7].

The present research was carried out to evaluate different agricultural substrates for growth and production of *P. florida,* and to check quality of mushroom grown on these substrates. The objectives of this research are to evaluate different substrates for the growth and production of oyster mushroom and to find the effect of substrates on the quality of oyster mushroom.

2. Materials and Methods

The experiment was carried out in mushroom house of the Department of Plant Pathology Department. Laboratory work was conducted in the Department of Agricultural Chemistry, The University of Agriculture, Peshawar.

2.1 Preparation of pure culture

The culture was developed from the fruiting bodies growing at the mushroom House of Plant Pathology with the following method:

Malt Extract Agar (MEA) was employed as medium for the growth of pure culture of *Pleurotus florida*. For one liter of the culture medium prepared, 20 g Malt, 20 g Dextrose, 20 g Agar, 1g Peptone and distilled water were used to make the volume one liter. After autoclaving at 15 psi for 15 minutes, the pH of the medium was kept 6.5. Streptomycin was added in the sterilized medium @1 g/ L when the medium was cooled to about 40 °C to stop the bacterial contamination. The inoculation was made by cutting small pieces of the mushroom tissues from the cap to downwards with a sterile blade inside the laminar flow unit. A small piece of fruiting body was taken and inserted in to petridish. Plates were sealed with Parafilm and incubated at 25 °C. After 3 to 4 days, the white myceliums have covered the agar surface. When the mycelium fully covered the plates, then they are kept in refrigerator for the preparation of spawn. The prepared cultures were multiplied and maintained through standard methods.

2.2 Preparation of Spawn

Spawn was prepared in jam bottles. The method of Jain (2005) was followed with some modifications. The wheat grains were boiled for 20-30 min. After boiling, excess water was drained off by spreading the grains on a wire mesh. Chalk powder (calcium carbonate) and gypsum (calcium sulphate) were added to the grains @ of 2% and 0.5%, respectively on dry weight basis. The jam bottles containing wheat grains were sterilized in an autoclave for one hour at 121 ºC. The grains were allowed to cool at room temperature and inoculated with actively growing mycelium of *Pleurotus florida* from malt extract slants inside the laminar flow unit and incubated at (27 ± 2 ºC) for mycelial growth without any light for 10-15 days until the mycelium fully covered the grains.

2.3 Preparation of substrates

Five substrates (wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw) were collected from different localities of Peshawar. These substrates were sundried and broken into small pieces. These substrates were soaked in water to maintain 65-75% moisture content. Wheat bran (10%) was added to the substrates. The soaked substrates were piled up after and covered with polythene sheet. Substrates were allowed to ferment for hours and spread on floor for evaporation of excess moisture. The experiment was laid by using Completely Randomized design having ten replications.

2.4 Sterilization

The substrates were filled in 50 glass bags (12×18 inch) and bag mouth was loosely tied with fiber thread. All the five substrates were sterilized in an air tight container at 15 psi for one hour. The sterilized bags cooled for 2-3 hours were inoculated with spawn @ 25 gm per kg bag [21]**.** The sterilized bags were then kept in mushroom house at ambient temperature.

2.5 Incubation

These bags after inoculation were then incubated for spawn running in a mushroom house under darkness at ambient temperature.

2.6 Cropping

When mycelia fully covered the substrates after 15-16 days, the bags were torn apart to open the substrates. The compact substrates were irrigated at least twice a day by sprinkling fresh water. After 7–8 days of the opening of bags small size pin heads (4-5 cm in diameter) appeared on all sides of the bags. These pinheads attained the full size in about 2-3 days and when fruiting body fully matured then they were harvested. The pin heads appearance time was also recorded.

2.7 Ventilation

The oxygen needed for the fruiting bodies development was fulfilled by running fan several times daily. The experiment was laid out in a complete randomized design (CRD) having 5 treatments and ten replications.

2.8 Recording of Data

Data were recorded on the following parameters

2.8.1 Days for completion of spawn running

Data on spawn running was recorded in days at 25, 50, 75 and 100% spawn running on different substrates.

2.8.2 Appearance of pinhead

After the completion of spawn running the pinheads appearance of *Pleurotus florida* was observed. The data was recorded in days taken from spawning to the appearance of pinheads in each substrate.

2.8.3 Maturation of pinheads

When the pinheads reached to maximum size then time period was recorded in days from appearance of pinheads to maturation of pinheads in all treatments.

2.8.4 Flush wise Yield

The data on the weight of mushroom in gram was recorded for the harvesting of mushroom in three flushes. The first and respective harvesting done at maturity and the yield of different flushes of fruiting bodies was noted.

2.8.5 Total yield

The total yields of basidiocarp were measured for each treatment. The accumulations of three flushes were noted as the total mushroom yield.

2.8.6 Biological efficiency

The biological efficiency (yield of mushroom per kg substrate on dry wt. basis) of oyster mushroom was determined by the following formula [22].

$$\text{Biological efficiency \%} = \frac{\text{Weight of fresh mushroom fruiting bodies}}{\text{Weight of dry substrate}} \times 100$$

2.9 Statistical analysis
The recorded data were analyzed by using analysis of variance and mean were separated by Least Significant Difference (LSD) test [88].

2.10 Laboratory study
2.10.1 Moisture content determination
Mushroom sample used in the study was oven dried at 105 ±5 °C for 24 hours till constant weight was achieved. The loss in weight after drying is known as the moisture content, which can be calculated by using the following equation [70].

$$\text{Moisture} = \frac{\text{Initial weight-Final weight}}{\text{Initial Weight of sample}} \times 100$$

2.10.2 Total ash determination
The dried mushroom samples were put in furnace at 550 °C for 6 hours. After cooling in desiccators the weight was taken**.** The total ash was calculated as following equation [70].

$$\text{Ash (g/100g)} = \frac{\text{weight of ash}}{\text{Weight of sample}} \times 100$$

2.10.3 Determination of crude fiber
The mushroom samples of weight three gram each was transferred in a 1000 ml beaker and H_2SO_4 @ 200 ml of 1.25% were added to the beaker. It was boiled for half an hour. These samples were washed 3--4 with hot water until it become free of acid. The mushroom sample was then transferred in to another beaker of 1000 ml and NaOH @ of 200 ml of 1.25% were added. The boiled residue was washed 2-3 times with hot `water until it become free of alkali. It was then dried in an oven at 100 ºC for 3-4 hour. After oven drying it was kept in a furnace at 550 ºC for 4 h. After furnace drying the colour of ash was grey. The weight was taken after cooling in desiccator. The crude fiber (%) was calculated by using the following formula

$$\text{Crude fiber (\%)} = \frac{\text{Loss of weight after ignition (g)}}{\text{Weight of sample (g)}} \times 100$$

2.10.4 Estimation of crude protein
Protein content in the mushroom samples was determined by Kjeldhal method

Procedure for Digestion
2.5 g of dried mushroom sample were digested with 5g of digestion mixture ($CuSO_4$: K_2SO_4: $FeSO_4$ 9:90:1) and 25ml concentrated H_2SO_4. After digestion transparent material was obtained and 10 ml of this digested material was1distilled with 40% NaOH in Kjeldhal apparatus. The liberated ammonia was collected in 4% boric acid solution. The methylene was used as an indicator. It was then titrated against standard N/10 H2SO4 solution taken in the burette until the appearance of golden yellow colour as end point.

Calculation

$$\frac{\text{Volume of N/10 } H_2SO_4 \text{ x 0.0014 (ml) x volume dilution Nitrogen (\%)}}{\text{Wt of sample x ml of digested material used.}} \times 100$$

Protein (%) = N% x 6.25.

2.10.5 Crude fat determination
For the determination of fat Soxhlet apparatus was used. The dried mushroom samples of weight 3g were taken in a thimble and put in an extraction tube of Soxhlet apparatus. The ether drops fell on the sample in the extraction tube petroleum ether (B.P. 40-60 °C) for was used for extraction hours. The solvent was evaporated under the fume hood and the samples were removed and dried in an oven at 105 °C for 30 minutes. After cooling in desiccators the weight of extract was recorded.

$$\text{Crude fat (\%)} = \frac{\text{(W2- W1)}}{\text{Weight of sample}} \times 100$$

Where
W1=weight of beaker
W2= weight of beaker+oil

2.10.6 Total carbohydrate estimation
The content of the available carbohydrate was determined by the following equation [24] (Raghuramulu *et al*., 2003).
Carbohydrate (g/100 g sample) = 100 − (moisture + fat + protein + ash + crude fiber) g/100 g

2.10.7 Determination of Vitamin C
The mushroom samples were grinded to obtain juice. Then one ml of the juice was taken along with 3.4% oxalic acid solution to make the volume up to 10 ml. The dye was titrated against 10 ml diluted sample until light pink color appeared.
Percent ascorbic acid content was calculated by using the equation.

$$\text{Ascorbic acid= (mg/gram)} = \frac{\text{F xT x100}}{\text{D xS`}} \times 100$$

Where
F = factor for standardization=ml of ascorbic/ml of dye.
T = (ml) used dye solution.
D = diluted sample for titration.
S = mushroom juice (g) for dilution

2.10.8. Determination of phosphorous
1. 0.5 ml of sample was taken in a 50 ml volumetric flask.
2. Few drops of NH_3HOCL mixture and 12.5 ml of baron's reagent were added to the sample and volume was made (50 ml) with distilled water.
3. Absorbance reading was taken after 10 minutes at 470 nm against a blank to determine the amount of phosphorous in the sample using standard curve.

$$P = \frac{\text{Atomic Absorbtion Spectrophotometer reading}}{\text{Weight of sample}\times 10000.} \times 100\text{ml} \times 2$$

2.10.9 Iron determination
For iron determination the solution obtained after wet digestion was passed through the atomic absorption machine to determine the iron content. The ammonium ferrous sulphate @ 10 mg/L and standard iron concentration 10 mg/L were transferred in a 100 ml flask. The HCl @ 2 ml were added to the flask and make the volume one liter with distilled water.

$$Fe = \frac{\text{Atomic Absorbtion Spectrophotometer reading}}{\text{Weight of sample}\times 10000.} \times 100\text{ml}$$

2.10.10. Determination of zinc
1. 10 ml nitric acid were added to one gram dried mushroom sample in digestion tubes and kept it overnight.
2. 4 ml Perchloric acid was added after 24 hours

3. The digestion tubes were put on a heater and raised the temperature to 200 °C. The heating was continue till the white dense fumes appeared.
4. The tubes were cooled at room temperature after digestion and transfer the contents to 100 ml volumetric flasks to makes volume with distilled water.
5. For the determination of zinc the samples were injected in Flame photometer and atomic absorption spectrophotometer.

$$\text{Calculation Zinc (mg/L)} = \frac{\text{Atomic Absorbtion Spectrophotometer reading} \times 100\text{ml}}{\text{Weight of sample} \times 10000}$$

3. Results

Effect of substrates on growth of oyster mushroom (*Pleurotus florida*)

3.1. Spawn running of *Pleurotus florida* on different substrates.

The experiment was carried out to find the effective substrate for rapid spawn running among wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw. The mycelium of *P. florida* covered the wheat grains are shown in Fig. 1. The result shown in Table 1 indicates that fastest spawn running took place on wheat straw. It takes 13, 18, 22 and 26 days for 25, 50, 75 and 100% spawn running, respectively. It was followed by sorghum straw that took 15, 19, 25, and 34 days for 25, 50, 75 and 100% spawn running, respectively. In case of sugarcane bagasse 25, 50, 75 and 100% spawn running completed in 17, 25,32 and 40 days, respectively. The maize straw took 16, 25, 32 and 39 days for 25, 50, 75 and 100% spawn running, respectively. In case of rice straw 25, 50, 75 and 100% spawn running completed in 16, 23, 28 and 37 days, respectively.

3.2 Days to maturity and pinhead appearance of *Pleurotus florida*

Result of the experiment about the appearance of pinheads and their maturity are presented in Table 2. It was observed that time taken for first appearance of pinhead after spawning of the substrates was fastest (27 days) in wheat straw. Sorghum straw also proved a better substrate in case of pin-head formation. It ranked second after wheat straw having 35 days for the appearance of pinheads. The rice and maize straw took 37 and 40 days for the appearance of pinheads, respectively. The longest time was taken by sugarcane bagasse which took 40 days for the appearance of pinheads. In response to pinheads appearance, the statistical analysis shows that wheat straws are highly significant. Sugarcane bagasse and maize straw are not significantly different from each other but they are significantly different from other substrates. However, the means of other substrates are significantly different from one another. The data on time taken to the maturity of pinheads are presented in Table 4.2. In case of the maturity of pinheads minimum number of days from appearance of pinheads to the maturity of pinheads was taken on the wheat straw (30 days), which proved to be the best substrate followed by sorghum straw (37 days). The rice straw and sugarcane bagasse took 39 and 41 days, respectively from appearance of pinheads to the maturity of pinheads. Maximum time period (42 days) was required for the maturity of fruiting bodies in case of maize straw. In response to maturity of pinheads, the statistical analysis shows that wheat straw is highly significant. The maize straw and sugarcane bagass are significantly same but they are significantly different from rice, maize and wheat straw. Similarly, the sorghum straw and rice straw are significantly same but they are different from other substrates.

3.3 Flush wise and total yield of *Pleurotus florida*

The data regarding number of flushes, flush wise yield, total yield and their means of different substrates such as wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw are presented in Table 3. The average yields of these substrates from first flush were 655, 620, 385, 500 and 590 gram, respectively. In the second flush these substrates had shown an average yield of 435, 375, 242, 290 and 345 gram, respectively. The third flush showed the lowest yield having an average of 270, 235, 129, 170 and 230 gram, respectively. Total mean yield of three flushes from wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw were 453.3, 410, 252, 320 and 388.33 gram, respectively. The total yield was determined by taking the weight of mushroom obtained after1st, 2nd and 3rd flush. Out of these five substrates wheat straw yield was highest (655 gram) in the first flush where as the lowest yield (129 gram) was obtained in case of sugarcane bagasse in the third flush. The highest (1360 gram) total yield was obtained in case of wheat straw followed by rice straw having the total yield of 1230 gram. The sorghum and maize straw have the total yield of 1165 and 960 gram, respectively. The lowest (756 gram) total yield was obtained in case of sugarcane bagasse.

Table 1: Days to spawn running of *Pleurotus florida* on different substrates

Days to Spawn running						
S. No	**Substrates**	**25%**	**50%**	**75%**	**100%**	**Means**
1	Wheat straw	13 b	18 b	22 d	26 d	19.75 a
2	Rice straw	16 a	23 a	28 b	37 b	26 c
3	Sugarcane bagasse	17 a	25 a	32 a	40 a	28.5 d
4	Maize straw	16 a	25 a	32 a	39.1a	28 cd
5	Sorghum straw	15 a	19 b	25 c	34 c	23. 25 b

LSD for 25% spawn running = 1.824
LSD for 50% spawn running = 1.656
LSD for 75% spawn running = 1.553
LSD for 100% spawn running = 1.867

Table 2: Days to maturity and pinhead appearance of *Pleurotus florida*

S. No	Treatments	Days of pinheads	Days of maturity
1	Wheat straw	27 d	30 c
2	Rice straw	37 b	39 b
3	Sugarcane bagasse	40 a	41 a
4	Maize straw	40 a	42 a
5	Sorghum straw	35 c	37 b

LSD for pinheads appearance = 1.830
LSD for maturity of pinheads = 1.900

Table 3: Flush wise yield (g) and total yield (g) of *Pleurotus florida*)

S. No,	Treatments	Flush 1	Flush 2	Flush 3	Total yield	Means
1	Wheat straw	655	435	270	1360	453.3
2	Rice straw	620	375	235	1230	410
3	Sugarcane bagasse	385	242	129	756	252
4	Maize straw	500	290	170	960	320
5	Sorghum straw	590	345	230	1165	388.33

3.4 Biological efficiency (BE) of oyster mushroom (*Pleurotus florida*)

The biological efficiency of wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw are presented in Table 4. The accumulations of three flushes yield were noted as the total mushroom yield. The biological efficiency was calculated by the formula Chang *et al*., (1981). The biological efficiency of different substrates was calculated to be 136, 123, 75.6, 96 and 116.5 percent, respectively.

Table 4. Biological efficiency of *Pleurotus florida*.

S. No.	Treatments	Biological efficiency (%)
1	Wheat straw	136
2	Rice straw	123
3	Sugarcane bagasse	75.6
4	Maize straw	96
5	Sorghum straw	116.5

3.5 Moisture, Ash, Protein, Crude fat and Crude fiber in oyster mushroom (*Pleurotus florida*)

The moisture content of mature fruiting bodies of *Pleurotus florida* cultivated on different substrates (wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw) are shown in Table 5. The Table indicates that higher moisture contents (93.44%) were observed in case of sorghum straw followed by sugarcane bagases and maize straw having the same moisture content of 90.14%. The wheat straw has the moisture content of 90.00%. The lowest (88.20%) moisture content was observed in case of rice straw. The total Ash content of mature fruiting bodies of *Pleurotus florida* grown on different agricultural wastes are shown in table 5. The result indicates that the ash content of sorghum straw (1.006%) was higher from all other substrates. It was followed by wheat straw having the ash content of 0.863%. The rice straw and maize straw have the same ash content of 0.48%. The lowest (0.33%) ash content was observed in case of sugarcane bagasse. The protein content of mature fruiting bodies of *P. florida* cultivated on different substrates (wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw) are presented in table 5. The result indicates that sugarcane bagasse have high protein contents (8.75 gram) from all other substrates. It was followed by sorghum straw have the protein content of 7.87 gram. The rice straw and wheat straw have the protein content of 7.0 and 6.125 gram, respectively. The lowest protein content was 5.25 gram which was observed in case of maizfce straw. The amount of crude fat determined in different agricultural wastes (wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw) are presented in table 5. From the Table it was observed that the maize straw have the high fat content (10%) from all other substrates. It was followed by sorghum straw have the fat content of 8%. The wheat straw and rice straw have the same fat content of 5%. The lowest (4%) fat content was observed in case of sugarcane bagasse. The amount of crude fiber in mature fruiting bodies of *P. florida* grown on wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw are shown in Table 3.5. The result indicate that wheat straw have the high fiber content (3.5 gram). It was followed by rice straw have the fiber content of 2.75 gram. The sugarcane bagasse and maize straw have the same amount of fiber content of 2.5 gram. The lowest fiber content of 2.25 gram was observed in case of sorghum straw.

3.6. Vitamin C, Phosphorous, Iron, Zinc and Nitrogen free extract in oyster mushroom (*Pleurotus florida*)

The vitamin C contents of mature fruiting bodies of *P. florida* cultivated on different substrates (wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw) are presented in table 6. The result indicates that rice straw, maize straw and sorghum straw have the same vitamin C content of 11.35mg/ 100gram while the sugarcane bagasse and wheat straw have the same vitamin C content of 11.11mg/100gram. The amount of phosphorous determined in different agricultural wastes (wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw) are shown in table 6. From the Table it was evident that sugarcane bagasse have the highest phosphorous amount of 1.40 mg/100 gram. It was followed by wheat straw have the phosphorous amount of 0.68 mg/100 gram. The sorghum straw and maize straw have the phosphorous content of 0.53mg/100 gram and 0.44 mg/100gram, respectively. The lowest phosphorous amount of 0.38 mg/ 100 gram was observed in case of rice straw. The iron contents of mature fruiting bodies of *P. florida* cultivated on different substrates (wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw are shown in table 6. From the result it was observed that maize straw have the highest iron content of 0.013 mg/ 100 gram. It was followed by sorghum straw have the iron content of 0.012 mg/ 100 gram. The rice straw and wheat straw have the iron content of 0.008 mg/ 100 gram and 0.007 mg/ 100 gram, respectively. The amount of zinc found in mature fruiting bodies of *P. florida* cultivated on different substrates(wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw) are shown in Table 6. From the result it was evident that maize straw have the maximum phosphorous content of 0.014 mg/100 gram. It was followed by wheat straw have the zinc content of 0.006 mg/100 gram. The sorghum straw and rice straw have the phosphorous content of 0.004 mg/100gram and 0.002 mg/100gram, respectively. Sugarcane bagasse has the lowest phosphorous content of 0.001mg/100gram. The nitrogen free extract of mature fruiting bodies of *P. florida* cultivated on different substrates(wheat straw, rice straw, sugarcane bagasse, maize straw and sorghum straw) are shown in table 6. From the result it was observed that sorghum straw have the high nitrogen free extract of 12.56 gram/100 gram. It was followed by maize straw have the nitrogen free extract of 8.37gram/100 gram. The sugarcane bagasse and wheat straw have the nitrogen free extract of 5.72gram/100gram and 5.48gram/100 gram, respectively. Nitrogen free extract was lowest in rice straw (3.43gram/100gram) from all other substrates.

Table 5: % Moisture, Ash, Protein, Crude fat and crude fiber in oyster mushroom (*Pleurotus florida*) grown on different substrates.

S. No.	Treatments	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)
1	Wheat straw	90.00	0.863	6.125	5	3.5
2	Rice straw	88.20	0.48	7	5	2.75
3	Sugarcane bagasse	90.14	0.33	8.75	4	2.5
4	Maize Straw	90.14	0.48	5.25	10	2.5
5	Sorghum straw	93.44	1.006%	7.87	8	2.25

Table 6: Vitamin C, Phosphorous, Iron, Zinc and Nitrogen free extract in *Pleurotus florida* (mg/100 gram) grown on different substrates.

S. No.	Treatments	Vitamins C mg/100 gram	Phosphorous mg/100 gram	Iron mg/100 gram	Zinc mg/100 gram	Nitrogen free extract mg/100 gram
1	Wheat straw	11.11	0.68	0.007	0.006	5.48
2	Rice straw	11.35	0.38	0.008	0.002	3.43
3	Sugarcane bagasse	11.11	1.40	0.001	0.001	5.72
4	Maize Straw	11.35	0.44	0.013	0.014	8.37
5	Sorghum straw	11.35	0.53	0.012	0.004	12.56

4. Discussion

The study indicated that *P. florida* can be successfully grown on almost all agricultural wastes but wheat straw gave better results. Wheat, rice, maize, sugarcane bagasse and sorghum straw supported good growth and fast mycelia extension of the mushroom. The structure of substrates is also important because it help in the penetration of mycelium. Wheat spawn is commonly used for mushroom cultivation because the growth of mycelium is faster on wheat grains. Present study revealed that the growth rate of mycelium is faster in wheat straw as compared to other substrates because the carbohydrates present in the substrates were effectively utilized by the particular strain of *P. florida.* Rice straw has been used for the cultivation of oyster mushroom since the beginning of 19th century and it has been cultivated in many countries under natural condition [69]. Rice straw is the natural substrate on which oyster mushrooms are cultivated leading to name the mushroom as delicious straw reported by Fasidi (1996) [31]. According to Balasubramanya and Kathe (1996) [8], the fungus *Penicillium spp.* and *Trichoderma spp* competed with *Pleurotus spp.* after pasteurisation with hot water (80 °C for 2h) probably due to the partial breakdown of hemicellulose, cellulose and thus making them available to competitors. The successful mushroom cultivation depends on the purity and quality of spawn. Nita Bahl (1984) [58] reported that grain spawn is now almost universally used. Present study revealed that fastest spawn running took place in wheat straw followed by sorghum straw while the lowest spawn running was observed in case of sugarcane bagasse. Such results were also observed by Zadrazil *et al.* (1978) [95]. The difference in days for full mycelial running on different substrates might be due to variation in their chemical composition and C: N ratio as reported by Bhatti *et al.* (1987) [11]. The results recorded on spawn running on different substrates were almost similar to the findings of Shah *et al*. (2004). They reported that the spawn running took16-25 days after inoculation. Similar results were also reported by Tan (1981) [90] who reported 21 days for complete spawn running on cotton waste. Patra and Pani (1995) [64] recorded 13-16 days on paddy straw. Similar findings were also reported by Jiskani *et al.* (1999) [40]. The pinheads appeared earlier in wheat straw than other substrates. On sugarcane bagasse pinheads appearance took long time. There is difference in the appearance of pinheads of different substrates. The difference in time period was observed by many workers for pinheads appearance of different mushroom species. Khan *et al*. (1981) [44] observed the appearance of 30 pin-heads of *P. ostreatus* (Strain,-467) in 36 days, the same of *P. sajarcaju* and *P. ostreatus* in 40 and 46 days, after spawning. Tan (1981) [90] recorded 23-26 days for the appearance of pinheads. Ramzan (1982) [73] observed 20-40 days of five *P. ostreatus* strains on wheat and rice straw. Patra and Pani (1995) [64] recorded 20-24 days on paddy straw. The result indicated that minimum number of days from appearance of pinheads to the maturity of pinheads was taken on the wheat straw followed by rice straw. The difference in time period for the maturity of pin heads was reported by many investigators. Khan *et al.* (1981) [44] recorded 21-28 days for the maturity of pinheads in case of cotton boll locules. Khanna and Garcha (1981) [45] recorded 20-24 days for the maturity of pinheads on paddy straw and Tan (1981) [90] observed a month for the maturity of pinheads on cotton waste. There is difference in yield obtained from different substrates. The present finding indicates that highest yield was obtained in wheat straw followed by rice straw. Bhatti *et al.* 1987 [13] observed the highest yields with the shortest incubation period in case of wheat straw. It was generalized from the data that first flush yield was highest in all treatments followed by second and third flush. Other scientists also recorded similar results. Zadrazil (1973) [95] got 2 flushes, Tan (1981) [90] got three flushes, Ramzan (1982) [73] obtained 3-5 flushes from wheat and paddy straw and Bhatti (1984) [12] got 4-6 flushes from different substrates.

Kausar and Iqbal (1994) [43] investigated that B.E varied from 18.6 to 83.5% on the basis of different nitrogen supplements amended with straw. Jiskani *et al*., (1999) [40] obtained 24 and 7.6% fresh and dry yield on the basis of substrate dry weight by using wheat straw. Jiskani (1999) [40] reported that one kg of dry substrate can produce one kg of fresh mushroom which is the 100% substrate dry weight. According to Bughio (2001) [16] the maximum dry and fresh (wet) yield percentage on substrate dry weight basis (29.61 to 77.91 and 5.91 to 21.70) were obtained from wheat straw using in combination with sugarcane bagasse, paddy straw, cotton boll locules and sorghum leaves. The difference in results between our findings and other workers may be due to environmental factors, physiological requirements, controlled, semi controlled conditions e.g. constant humidity, light, temperature etc. The difference in time was observed for the formation of pinheads, maturation of fruiting bodies, period between flushes, number of flushes and yield. Present study revealed that biological efficiency (BE) was maximum on wheat straw followed by rice straw. Similar by-products have variable levels of BE on different substrates. These variations may be due to fungal species, spawn strain, spawn rate and the use of supplement added to the substrates [50]. The B.E of the *Pleurotus sp* on commonly used substrates was 85.5% (rice straw) [53], leguminous plants 103.8% [81].

Graham and Clyde (1985) [33] recorded 80-120 percent biological efficiency of *P. sajor-caju* on cotton waste. Moonmoon *et al*., (2010) [55] studied *P. eryngii* King Oyster mushroom on rice straw and saw dust in Bangladesh and found that saw dust. Nunez and Mendoza (2002) [59] reported that the biological efficiency of the studied substrates varied from 106.2 to 50.8% of *P. ostreatus*. The moisture content of the studied mushroom ranged from 88.20% to 93.44%. The moisture content of *P. ostreatus* ranged from 88.51% to 89.88% indicating high moisture content of the oyster mushroom fruiting bodies [51]. Moisture percentage in mushroom depends on the maturity of fruiting bodies, species and storage conditions during packaging or processing

[34]. Present study revealed that the ash content of studied mushroom ranged between 0.33% - 1.006%. The amount of ash was higher in sorghum than other substrates. The amount of ash depends on salt content in substrates [68]. (Bonatti *et al*. (2004) [15] reported 0.5-0.6% of ash in dried *P. sajor-caju* whereas Alam *et al.* (2008) [5] recorded 1.1 and 8.28 g/100g in fresh and dried *P. sajor-caju*, respectively. El–Kattan *et al*., (1991) [27] reported 8.00% and 6.60% ash content of *P. florida* on soybean and paddy straw, respectively. The analysis of mushroom composition indicated that sugarcane bagasse gave high amount of proteins and amino acids from other substrates. The soluble sugar from the wastes is absorbed by the mushrooms which are used for their growth and other metabolic process. The excess sugar passed through secondary metabolism (Nataraja *et al*., 2005) [57]. These results were confirmed with the findings of Kadlag *et al*., (1998) [41] and Mandhare (2000) [49]. The protein content usually ranges between 20–30% on a dry weight basis. The nitrogen present in substrate after spawn running enhances the mushroom yield and quality, in addition it help in bioconversion and bioaccumulation efficiency [63]. The fat content of *P. florida* was 10% grown on maize straw being the highest followed by sorghum straw (2.50%). The percent content were similar as reported in earlier studies [63, 62]. The fat content ranged between 2.56% to 2.82% on dry weight basis. The fat content of dried *P. sajor-caju* was 5.26 and 4.99% on rice straw and banana straw, respectively [15]. The highest crude fiber (3.5%) was obtained from mushroom on wheat straw followed by rice straw (2.75%). Other agro wastes also yielded appreciable level of crude fiber. These results were confirmed with the findings of Singh *et al.* (2003) [83], Bonatti *et al.* (2004) [15], Kadlag *et al*., (1998) [41] and Sharma & Madan (1993) [81]. The amount of protein of *P. florida* found in this study is near about similar to the results of Rai and Sohi (1988) [71] and Alam *et al.* (2007) [4]. But fiber and ash content are different from the report of Rai and Sohi (1988) [71], however relevant to Alam *et al.* (2007) [4]. Protein content in *P. florida* were also similar to the findings of Banik and Nandi (2004) [9] as well as fat value of *P. florida* is relevant to the findings of Shashirekha *et al*. (2005) [82].The results indicated that not only the protein content of the substrate but also nature of protein in the substrate influences the protein content of the fruiting bodies [92]. The amount of vitamins C were maximum (11.35mg/100 gram) in rice, maize and sorghum straw. Bano (1976) recorded 13.0 to 14.70 mg/100 g ascorbic acid in various mushroom species. Present study revealed that sugarcane bagasse have high phosphorous amount as compare to other substrates. Similar results were observed by Chang *et al*. (1981) [20] and Alam *et al*. (2007) [4], but differ from [79] who recorded 0.97% phosphorus in oyster mushrooms grown on sawdust. The amount of phosphorous was maximum on soybean straw (920mg/100gm) whereas least was found on soybean straw and wheat straw (800 mg/100gm) [17]. Phosphorus content of *P. ostreatus* ranged from 790 – 1000 mg/100g [65]. The amount of iron found in this study was highest in maize straw followed by sorghum straw. The combination of soybean straw and paddy straw showed the high iron content of 13.06 mg/100 gm where least (11.87 mg/100gm) was found on soybean straw plus wheat straw [47, 74].

The amount of Fe found in this study was similar with the findings of earlier reports [17, 47]. The amount of carbohydrate found in this study was maximum in sorghum straw followed by maize straw. The maximum Carbohydrate content of *P. florida* was 57.80% in fruiting bodies cultivated on soybean straw whereas least was 53.87% cultivated on wheat strawand paddy straw [62].

5. Conclusion

Wheat straw was found most suitable substrate for mushroom cultivation. The spawn running, appearance and maturity of pinheads were fastest in wheat straw. Wheat straw also showed the highest flush wise yield, total yield and biological efficiency. Moisture, ash, nitrogen free extract and vitamin C were maximum in sorghum straw. The highest protein content was obtained from sugarcane bagasse. Meanwhile the percentage of crude fat and iron were highest in maize straw. Crude fiber and zinc were maximum in wheat straw. The maximum amount of phosphorous was obtained from sugarcane bagasse. Farmers are advised to use wheat straw for *Pleurotus florida* cultivation for bumpy production. However as more nutrients have been found in mushroom grown on sugarcane bagasses, so they can also use sugarcane bagasse as substrate for the production of high quality mushrooms.

6. References

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