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Cultivation of *Plearotus ostreatus* on *Hordeum vulgare* (Barley) Straws on Rural Basis in District Kurram, Pakistan

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Fungi of the genus *Pleurotus* are consumed all over the world due to taste, flavor, high nutritional values and some medicinal properties. This investigation was designed to cultivate *Pleurotus ostreatus* on barley straw in a controlled and supplemented environment. We observed shorter growth period, high yield and good biological efficiency on barley straw, because barley straw contained maximum fiber and carbon to nitrogen ratio so that they increase the yield, excellent growth period, body weight and biological efficiency. We collect dry samples of about 1kg and analyzed the sample for physiochemical composition. Results showed that it contained moisture content (6%), ash (5.4%), crude protein (25%), crude fat (2.3%), crude fiber (28%), and carbohydrates (39.3%). The mushroom has been reported to have anticancer, anti- diabetic, anti-obesity, hepatoprotective, anti-aging, anti-microbial, anti-allergic and antioxidant activities. The aim of present research is to cultivate the oyster mushroom on locally easily available substrates with low cost and provide the opportunity for both man and women to start its cultivation in their home to improve their nutrition and economy.

Keywords: ***Pleurotus ostreatus, Hordeum vulgare,*** **District Kurram, Mushroom, cultivation**

INTRODUCTION

Study area

Kurram District was the part of FATA until 2017, but after 2018 merged into the Tribal district of the Khyber Pakhtunkhwa province Pakistan (Ali et al., 2019; Abbas et al., 2020; Hussain et al., 2020). The name of the Kurram comes from the River Kurram that passes through it (Muhammad et al., 2017). Parachinar is the headquarters of District Kurram. It is situated between 33°20`to 34°10` north latitudes and 69°50 to 70°50` east longitudes (Hussain et al., 2018). Nangharhar and

Puktia provinces of Afghanistan on the northern and western sides bound district Kurram respectively, on the east by Orakzai and Khyber districts and on the south by North Waziristan districts of Pakistan. Koh-e-Safaid is the major mountain in the Kurram with Sikaram Sar peak (4,728m) forming boundary with Afghanistan. Throughout the year, it remains covered with snow (Hussain et al., 2013; Badshah et al., 2016; Hussain et al., 2018).

Kurram plateau has a moderate climate. In winter, the climate is discomfort due to snowfall and heavy rainfall and cold weather (Samina et al., 2019). In summer season, the valley climate stays pleasant and comfort but during winters, the temperature goes towards negative point. Average rainfall of this district is 1239.96 mm (Ajaib et al., 2014). During rainy season mushrooms, grow naturally on the different substrates such as tree trunks, garden soil, and termite nest, decaying wood, beneath shadows of tree and rubber plantations, leaf litters, tea, coffee (Sardar et al.,2015). For the growth of mushrooms several factors such as PH, temperature, moisture content, spawn growing media, light intensity is essential (Kidiri et al.,1999; Sardar et al., 2015). Many of the around 200 mushrooms are used as as food,

but only 35 species of mushroom are cultivated (Aida et al., 2009; Xu et al., 2011). These edible mushrooms mostly contain essential elements i.e., thiamine niacin, pantothenic acid, calcium, phosphorous, iron, sodium. Biotin, folic acid, vitamin B, C, D, A and K (Gbolagade et al., 2006; Sardar et al., 2015; Ishaq et al., 2017). Mushrooms are very useful as nutrition because they have low fat and have a high amount of protein and all essential amino acids therefore it is very useful in treatment. Edible mushrooms have anticancer, antiviral, antioxidant, antidiabetic as well as they have antimicrobial activities such as antiviral, antifungal and antibacterial (Das et al., 2020). Starch, sucrose and fructose are required for the growth of mushroom and mycelia and can be enhanced by the addition of gibberellic acid (GA) and vitamin. Under the exposure to UV light cultivated mushrooms are able to produce more vitamin D as compared to normal mushrooms (Chakravarty, 2011; Dolay et al., 2020). The cultivation of edible mushrooms helps in the recycling of lignocellulose waste and help in the reduction of the pollution from the environment (Sanchez, 2010).

The Oyster mushroom is commonly known as “Dhingri”. The mushroom grows on the stamps of tress, on logs, forest of Azad Kashmir, Khyber Pakhtunkhwa and other plains of Sindh, Punjab at the time of monsoon (Sardar et al., 2015). The Oyster mushroom belongs to class Basidiomycetes, sub class Hollobasidomycetideae, order Agaricales, family pleurotaceae, with 40 species (Alexopolous and Mims, 1996; Jose and Janadhanan, 2000). Due to the pleasant and excellent flavor and taste, this mushroom is widely used in the different part of the world (Chang and Miles, 1992; Shah et al., 2004; Jonathan and Esho, 2010; Sardar et al., 2015; Ishaq et al., 2017), and ranks as third most widely used food mushroom and most economically important fungus worldwide (Ventura-anguilar, 2017; Oh et al., 2018).

Oyster mushroom contains minerals, fibre, maximum amount of protein and all the essential amino acids, required for human body. This mushroom is also used in pharmaceutical and in drug development due to its antioxidant, antitumor and antimicrobial activity (Wang et al., 2017; lyn et al., 2020). The shelf life depends on moisture content, respiration rate and on the epidermal structure (Wei et al., 2017). It is cultivated in Pakistan at a high ratio for the purpose of Agribusiness that might improve the economic status of farmers. The awareness of cultivation of mushrooms is increasing day by day in Pakistan, especially in past two decades. In Islamabad and Swat, the national logistic cell of Pakistan initiated the mushroom cultivation, and the annual production so far reached 48 tons. About 80% of mushroom is cultivated for the purpose of exportation to attain foreign exchange (Akhtar, 1992; Alam and Raza, 2001; Sher, 2006; Flores, 2006; Sher et al., 2010).

The Oyster mushroom ranks in the 2nd position among the world mushroom production after button mushroom (Sanchez, 2010; Ishaq et al., 2017). Due to some unique characteristics such as fast growth, short life cycle and resistance to diseases, this mushroom is widely cultivated around the globe. Similarly, this mushroom also has the ability to grow in variable climatic condition as well as have low production charges and low input requirement. Due to these reason it is cultivated more as compared to other mushrooms

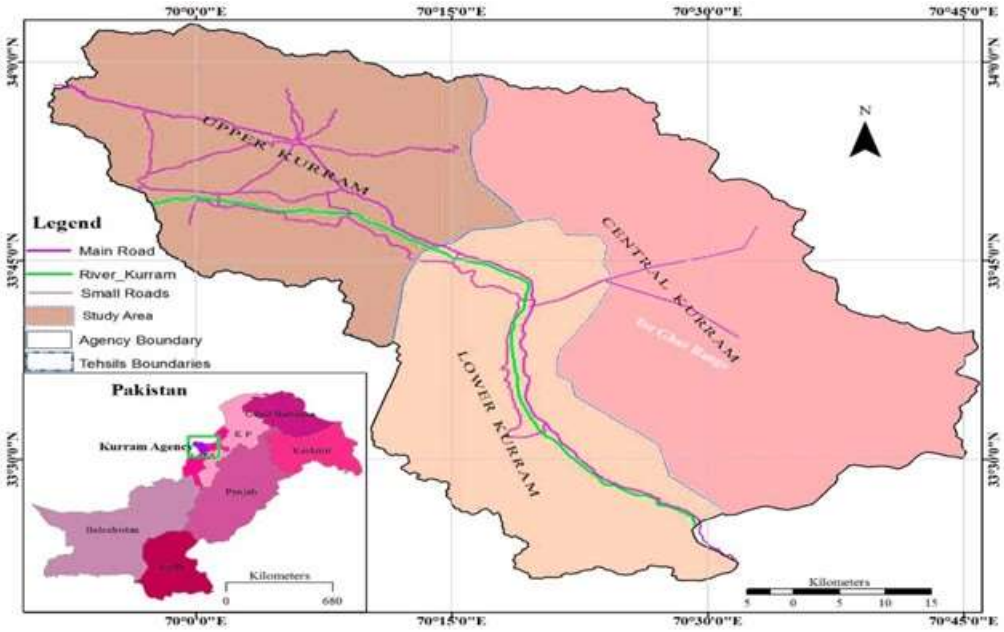

Figure1: Location map of study area.

(Bonatti, 2004; Synytya et al., 2009; Silveria, 2014; Sulistiany et al., 2016).

Cultivation of edible mushrooms is very important, because it can convert waste material into valuable human food (Ishaq et al., 2018; Zhang et al., 2020) and is also important for the clearance of environmental pollution (Boswell and Chang, 1993). *Pleurotus* spp, are very famous in different parts of the world and the farmer build up millions of dollar (Jonathan et al., 2008). The *Pleurotus ostreatous* is cultivated in tropical, sub-tropical and temperate regions of the world (Jonathan, 2012).

The estimated annual world production of mushroom cultivation reached up to over 1.2 million metric tons. In 1986, the production was about 2.2 million metric tons, with 56% production of button mushroom (*Agaricus bisporous*). The other species include shiitake (*Lentinula adodes*), straw mushroom (*Volvariella volvaceae*), Oyster mushroom (*Pleurotus* spp) and wood ear mushroom (*Auricularia* spp), enokitake (*Flammulina valutipes*) and other spp. China is the major producer of Straw mushroom and *Pleurotus* spp, in 1986 with 56% and 59% respectively (Breene et al.,1990). In India more than 85%, the total production is the white button mushrooms followed by the oyster mushrooms (Dey et al., 2020). Now for the first time it is also cultivated in different areas of district Kurram. The people of the Kurram cultivate the mushroom at their own home to reach the daily basis diet and as a source of income for the poor people. This research aimed to investigate to find out the cheap method for cultivation of Oyster mushroom on barley straw, nutritional properties of Oyster mushroom and provide alternate protein source for local people.

MATERIALS AND METHODS

The agro-wastes namely *Hordeum vulgare* waste was collected from barely field of district Kurum, province Khyber Pakhtunkhwa, Pakistan. National Institute of Food and Agriculture, Peshawar, Pakistan provided the fungus culture. The culture further used for spawn preparation on wheat grains in this laboratory.

Sterilization of the rooms of mushrooms

For sterilization of rooms, all windows of the emptied room were closed, after mixing the chemicals to produced smoke and were opened again after three days. Before entering the room, a layer of lime was sprayed outside the room and hands were washed after taking of the shoes.

Spawning

For sterilization, the drum was placed on heater above eight bricks. The drum was filled with water up-to four inches, and 20-25 plastic bags were also put inside the drum on the bricks. The lid of the drum was tightly covered with a cloth like porous sheet. After one hour the drum

was lowered down from the fire and after removing the plastic bags spores were mixed (Randive et al., 2012). The porous plastic bags were kept in the room and were sprinkled after every two days. Spores sprouted from the holes after 45 days and were fully grown in 65 days. Three crops grew in each plastic bag which means until three seasons nutritional and minerals compositions maintained.

Proximate Analysis

The present research work done at the department of Applied Agricultural Chemistry, Agricultural University of Peshawar, and Khyber Pakhtunkhwa. In this study, we investigated moisture, crude protein, ash, crude lipid/fat, crude fiber and carbohydrate of Oyster mushroom by using the standard Association of Official Analytical Chemists (AOAC) method 2000. The sample were cleaned and allowed to stand in room temperature away from sunlight and were air dried for several days. Dry samples were shaped and grinded with a clean grinder and kept under room temperature i.e. (25-30 C^o) and store in paper bags. These were analyzed for their proximate composition.

Moisture determination

In order to determine the percentage of moisture content the samples were dried in oven [PRECISION *Thelco,* model 17] at 105C^o following the AOAC method (2000) (Alam et al., 2008; Chiring and Intaraphechet, 2009; Ali et al., 2010; Randive et al., 2012). One-gram sample taken in a dried and clean petri dish and was weighed (W1). The petri dishes were partially covered and then placed in the oven and heated at 105 C^o. Then after 4-6 hours they were shifted to desiccators where they were kept for 20 minutes for cooling. They were again weighed (W2). The formula for this process is:

Moisture (%) = Wa1-Wa2 / weight of sample ×100

Where Wa1 (g) represents the early weight of petri dish with sample and Wa2 (g) represents later weight of petri dish after taking out of the oven with sample.

Determination of ash /Fixing up of Ash

For fixing up the ash, ignition method AACC 2000 was used (Chiring and Intaraphechet, 2009; Randive et al., 2012). The oven dried, crucible cooled in a desiccator, and then it was weighed. One gram of the powdered sample, which was completely dried, was taken in the crucible and measured as its original weighed (W1). It burned with light fire. Then it was put into a stifled furnace [VULCANE 3-130]. The temperature gradually reached to 550-600 C^o. The sample burned for 6 hours when it changed into gray whitish (ash) color. It was then taken out from the furnace and cooled in the desiccator and was measured for the second time as (W2). The ash was calculated through the following formula.

$$\text{Ash}\,(\%) = \frac{\text{Wt of crucible with sample} - \text{Wt of crucible with Ash}}{\text{sample weight}} \times 100$$

Determination of crude protein

Kjeldhal apparatus was used for determination of crude protein (Chiring and Intaraphechet, 2009; Ali et al., 2010). One gram of dried powder of the sample, that carefully weighed was taken into a digestive flask. To this, 8 gram of digestive mixture K_2SO_4:$CuSO_4$ (7:1) and 12 ml of concentrated Sulphuric acid (H_2SO_4) was added. The flask was spinned in order to mix up the contents and avoid crystal formation. The flask steamed heated and Kjeldhal apparatus was used for the distillation of the digest. 10 ml of the digest moved into the distillation tube. During the distillation, 10 ml of the NaOH (40%) was added constantly for 10 minutes and ultimately the flask produced NH_3, which was collected in the form of NH_4OH. Then 20 ml solution of 4% Boric acid (H_3BO_3) and few drops of methyl red indicator was added to the flask. In the process of distillation, the pink color was gradually transferred into yellowish due to the presence of NH_4OH. On the appearance of color, the distillate was titrated against standard 0.01 N HCL. After completion of the process, it calculated thus:

Crude protein (%) = 6.25 x %N (6.25 is protein factor for plants)

$$\%\ N = \frac{(S-B) \times 0.01 \times 0.014 \times 100}{\text{Wt. of sample} \times V} \times 100$$

where S represents the sample titration reading; B represents black titration reading; 0.01 is the normality of HCI; 100 is the dilution of the sample after digestion; V is the volume taken for distillation; 0.014 is the mili equivalent weight of nitrogen; and 6.25 represents protein conversion factor for plants.

Determination of crude lipids/fat

For fixing upon the crude fat, Soxhelt's apparatus was used (Chiring and Intaraphechet, 2009; Randive et al., 2012) by using petroleum ether as a solvent. In this process, one gram of

dried powder of the sample was taken in filter papers and was rivaled. They were placed in some small metal containers. After that were put into Soxhelt's extraction tubes. One third of the beaker was filled with (100ml) of petroleum ether boiling point range between (40-60C^{o}). Then it heated for five hours to be regressed. The metal containers were removed after drying up the solvent. After cooling, the beakers were carefully weighed.

Percentage of Crude fat was determined by using following formula:

Crude lipid (%) = weight of flask with fat – weight of empty flask/ weight of sample ×100

Determination of % Crude fiber

In order to fix up crude fiber an acid and alkali method was adopted. Accurately (200ml) of 2% HCL was taken in a goblet and after that 2 gram of the sample was put into it. It was boiled and whirled continuously for half an hour. It was then filtered through muslin cloth. Next, the dregs were treated with 2% NaOH solution. Washing it with hot water, it along with the crucible was measured in weighed. The crucible was then placed in oven at 105 C^{o} for 4 hours and lately it was cooled in desiccator. Next, it was put in a furnace [PRECISION Thelco, model 17] at 550 C^{o} for 4 hours. It was then again cooled in desiccator and weighed once more (Alam et al., 2008). As a result, the percentage of crude fiber was fixed up as:

Crude fiber (%) = weighed of oven dried residue (Wf1) - weight after ignition (Wf2) / weight of sample × 100

Nitrogen Free Extract (NFE)

Nitrogen free extract was adopted to show the edible carbohydrate that was sum of the percentages of crude fat, crude protein, crude fiber and ash subtracting from 100 (Iqtidar and Saleem, 2004; Chiring and Intaraphechet, 2009).

NFE = 100 - % (crude fat + crude protein +crude fiber + ash)

RESULTS

Cultivation of mushroom on barley straw

In this study, barley straw has been used as a substrate for the growth of *Plearotus ostreatus*. It has been observed that on barley straw there is shorter growth period, high yield and good biological efficiency as compared to the wheat and other culture media, because barley straw contains maximum fiber and carbon to nitrogen ratio so therefore increases the yield, excellent growth period, body weight and biological efficiency.

Spawn Running

After 20 to 40 days under 20-25^{o}C temperature and 60-90% humidity the spawn was spread all over the substrate in the form of white thread like structures called mycelium that provide nutrients to the mushroom. Temperature was measured with Thermometer and humidity with Hygrometer. It is very important to keep the good hygiene dealing with mushroom in production system. If sometimes-blackish spots appeared in polythene bags with white mycelium, then the whole bag was discarded to avoid the contaminations of harmful fungi. After 40 days, the spawn threads were spread in the bags and all bags become white as shown in fig-2.

Pinhead formation or initiation of primordia

Pinheads or primordial appeared after the formation of mycelium. These are the small fruiting bodies of mushroom size (>0.01mm) as shown in fig-2.

Fruiting body formation

The formation of healthful mushroom relies upon temperature and humidity. After the pinhead's formation, fruiting body of mushroom were fully developed after 4-5 days and was cut by a clean knife as shown in fig-2. After 2-3 days fruiting bodies were again formed and this process continued up to 3 months. After 3 months the bags were discarded because the fruiting bodies were not formed, the mycelium lost and their color changed. Now for the new mushroom production new bags were prepared.

Proximate composition

About 1kg of dry samples of mushroom were collected and the samples were then analyzed for physiochemical composition. The work was done at the department of Applied Agricultural Chemistry Agricultural University Peshawar, Khyber Pakhtunkhwa, Pakistan. Different instruments were used for the determination of moisture, ash, protein, fat and fiber (figure-3).

Figure 2: Cultivation of *Pleurotus ostreatus*: (a, b, c), mycelial growth inside bags in form of white threads; (d, e, f), pinhead formation; (g, h, i), growth of mycelium; (j, k, l), cutting of fruiting body with clean knife

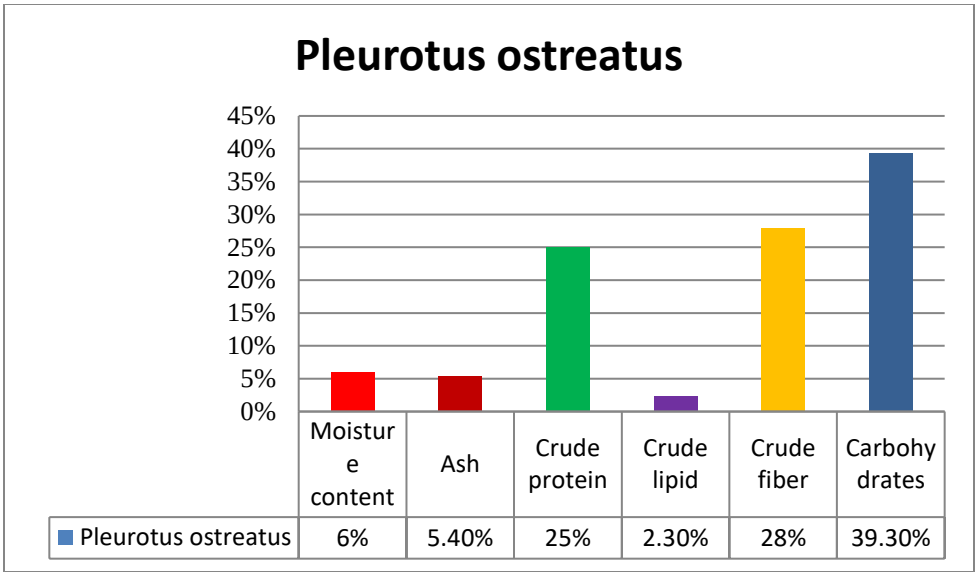

Figure 3: Showing proximate composition of *Pleurotus ostreatus*

Table 1: Proximate analysis of *Pleurotus Ostreatus*

Scientific name	Family	Local name	Moisture content (%)	Ash (%dry matter)	Crude protein (%dry matter)	Crude lipid/ Fat (%dry matter)	Crude fiber (%dry matter)	Carbohydrates (% dry matter)
Pleurotus ostreatus	Pleurotaceae	Speen- sheshte	6%	5.4%	25%	2.3%	28%	39.3%

After performing all the experimental work, we concluded the following results based on conducted research work.

Moisture

The moisture content in *Plearotus ostreatus* was calculated (6%) and thought that 20% of the total water must come from food moisture because it was the main source of water.

Ash

The ash content (5.4%) was calculated in tested species as shown in Table-1. The ash content was of great importance from a biochemical point of view. They represented both micro and macro minerals which helps in the physiological function of the body.

Crude protein

The protein content ranged up to 25% in tested species. Protein is one of the main ingredients of diet. It supports growth and helps to maintain and repair body tissues. They function as enzymes, hormones and as carriers in membranes. Therefore, based on proteins values, we can say that Oyster mushroom is more important in our diet.

Crude fat

Plearotus ostreatus contains very low amount of crude fat (2.3%). The lipid is a main source of storing energy, fundamental fatty acids, and source of fat-soluble vitamins and precursors of vitamins.

Crude fibers

The crude fibers were noted as 28% in *Plearotus ostreatus*. Fiber in meal helps to minimize serum cholesterol level, risk of cardiovascular diseases, colon and breast cancer. The high intake of fiber in diet can cause intestinal irritation, low digestibility, difficulty in the absorption of minerals in plants and decrease utilization of nutrients utilization. The Dietetic Association recommends 20-30gm of fiber intakes in diet are important for food digestion and elimination of wastes.

Carbohydrates

In *Plearotus ostreatus* carbohydrates amount was recorded about 39.3%. Carbohydrate is the potential source of energy. The RDA for carbohydrate is 130g (FAO, 1998), while in Pakistan the intake of carbohydrate is 349g reported (Ministry of Health and Nutrition, 1994).

DISCUSSION

Present research was designed to cultivate *Plearotus ostreatus* mushroom on barley straw and to document their nutritional values to promote the awareness among the people of the District Kurram. According to Jafarpour and Eghbalsaeed, (Jafarpour and Iqbalsaeed, (2012) barley straw contained maximum fiber and carbon to nitrogen ratio so that they increase the yield, excellent growth period, body weight and biological efficiency. Tasfew *et al.,* (2015) used different substrates for the cultivation of *Plearotus ostreatus* and found that *Plearotus* can grow best on wheat and barley straw because both wheat and barley straw contain high Carbon to Nitrogen ratio among other substrate and also analyzed that mixture of barley straw and soya bean powder give maximum yield and biological efficiency.

The present research showed that mushroom is not only fresh, tasty and safe for consumption but also nutritionally rich source of proteins, moisture minerals etc. and essential as food supplements for local communities and can be cultivated easily on low cost. The cultivation and production of mushroom has low expenditure and interior occupation. It is very simple technique and does not require substructure potential. We have started cultivation of mushrooms at village Shalowzan, Parachinar, an awareness activity aims to share the growing process of mushrooms to local female staff so they can learn and share the experience. In this they can start growing their own mushrooms, earn their own livelihood, and overcome nutritional deficiency which is common in rural areas. Theoretically, crop takes about 60-65 days. If required temperature and humidity maintained, we can get about 2kg fresh mushroom per bag in one season. According to our experiments average cost per bag is about PK Rs.40-50, and beside May, June and July months mushroom can be cultivated in other months in Peshawar and its surrounding.

Sher *et al.,* (2010) worked on the cultivation of Oyster mushroom in two different areas in Peshawar and Swat and concluded that the yield and growth of mushroom was high in Peshawar than the Swat valley because of high relative humidity and temperature. Mycelium spread in bags in 20-40 days under the temperature of 24-25C^{o} and 60-90% humidity. After the spawn running, the pinheads formed under the same temperature and humidity and 4-5 days later fruiting bodies were formed. Tasfew *et al.,* (2015) cultivated *Plearotus ostreatus* on locally available substrate in Debri Burhan, Ethopia, and found that spawn was grown best at 25C^{o}. Our results were strongly supported by Sher *et al.,* (2010) and it was found that mycelium grew best in 45 days and fruiting body were formed after 4 days under the 22C^{o} temperature and 70% humidity in Peshawar. Rahman *et al.,* (2012) suggested that 80-85% humidity and 22-25C^{o} temperature were required for the cultivation of *Plearotus ostreatus.*

The proximate analysis showed that moisture content was recorded 6% in *Plearotus ostreatus* and moisture is required to maintain the turgidity of cells and their freshness and helps to determine stability of storing food material (Khan et al., 2013; Mironeasa et al., 2016; Ullah et al., 2017; Mih et al., 2017). Ash calculated in *Plearotus ostreatus* is 5.4%. This value indicates that these mushrooms are excellent source of minerals like Ca, K, Na, Fe, Zn etc. and help in physiological functions in body (Khan et al., 2013; Ullah et al., 2017; Rahman and Adnan, 2018). The crude protein was recorded maximum in *Plearotus ostreatus* i.e. 25%. After the proximate analysis of mushroom, it concluded that *Plearotus ostreatus* were rich source of protein and their consumption provide energy to overcome the malnutrition common in rural areas. Current results showed that *Plearotus ostreatus* contained decent amount of crude fat (2.3%). It means that tested species is the main source of energy and different vitamins. The crude fibers were recorded highest (28%) in *Plearotus ostreatus.* Fiber helps in the prevention of constipation and overweight (Khan et al., 2013; Ullah et al., 2017; Mih et al., 2017). We have recorded standard value of carbohydrates i.e. 39.3% and considered the chief source of energy (Khan et al., 2013; Ullah et al., 2017).

Dundar et al*.,* (2009) worked on cultivation of *Plearotus ostreatus* on different substrates and their chemical and nutritional value, and recorded average moisture about 7% on different substrate slightly high than our results. Ash was 4%, High protein (22.15g) soya beanstalk, and dietary fiber 31.32 g on millet substrate and 39.94% carbohydrate was calculated in cotton stalk. Chirinang and intarapichet, 2009; calculated moisture (4%), protein (20%), ash (5.8%), fat (0%), fiber (45%), and carbohydrate 68.35% in *Pleurotus ostreatus* as compared to *Pleurotus ostreatus* and *Pleurotus sajor-caju* for amino acids and antioxidant properties.

Ali et al., (2010); worked on proximate analysis of *Pleurotus ostreatus* and the effects of

different quantity of wheat bran on yield when combine with sugarcane bagasse, the highest content of protein 30.31%, ash 9.15%, crude fiber 24.07%, lipid 3.90%, carbohydrate 32.57% were recorded in 30% wheat bran. Rehman et al*.,* (2012); Carried out experiment on effect of different percentages of wheat bran on *Pleurotus ostreatus and* cultivation on rice straw and their proximate composition. The highest moisture was recorded 91.08% on 20% wheat bran, the protein 27.78% and the lowest amount of lipid 3.24% and carbohydrate 39.44% were observed on 30% wheat bran, crude fiber 23.38% and ash 8.12% were observed on 10% wheat bran with rice straw.

CONCLUSION

Cultivation of Pleurotus *ostreatus* on barley straw had better support the growth of *Pleurotus* as compare to wheat, maize straw etc. Barley straw (*Hordeum vulgare*) is the finest substrate for growth of the *Pleurotus ostreatus.* After their proximate analysis, It is also conducted that *Pleurotus ostreatus* contains high amount of protein, ash, fiber, fat, moisture and carbohydrate and can be used as alternate for meat and fish, which is mostly unaffordable by most of the local people because of poverty. In addition, the technique used for the production of mushrooms must be transferred from laboratories to outside world where people can be educated on how to cultivate mushrooms at homes and one of the limitation is that the lack of mushroom cultivation technology. As the climate of District Kuram is very suitable like its temperature and moisture contents, therefore low cost will come on its cultivation in order for providing of ideal condition for their growth. Aim of this study was to cultivate the Oyster on locally easily available substrate on low cost and convey such knowledge to male and female, so they that can cultivate it in their houses. This will make them able to earn livelihood and will help in improving nutrition.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

BS. MA conducted the primary research work of this study and wrote the initial draft. AA, LB, MF , ZW helped in the revision of a manuscript. DH and HH helped in analysis of data. WH supervised the project. All the authors approved the final manuscript after revision.

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