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BIOCONVERSION OF LIGNOCELLULOSIC AGRI-WASTE INTO EDIBLE PROTEIN BY MUSHROOM CULTIVATION AND EVALUATION OF YIELD ON DIFFERENT AGRI-RESIDUES WITH BIOCHEMICAL COMPOSITION

Siddhant, Deepika Yadav & Rajesh Kumar*
Plant Biotechnology, Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, India
*Corresponding Author: rajitrc@gmail.com
Rajesh Kumar: https://orcid.org/0000-0003-3965-395X

ABSTRACT

Pleurotus ostreatus (Oyster mushroom), a saprophytic macroscopic fungus, can grow on several lignocellulosic substances by degradation activity supported by several enzymes secreted by them. The mushroom is widely consumed for its delicious flavour in cuisines and medicinal purposes. The present study was focused on its cultivation on three different residues viz. paddy straw (Oryza sativa), wheat straw (Triticum aestivum) and garden grass (Cynodon dactylon). The research further focused on evaluating the biochemical composition including moisture, lipids, proteins and phenolic contents. All three substrates were compared based on total yield, cropping days and biological efficiencies. Maximum yield (458.97g/5kg substrate) and biological efficiency (91.79%) were observed in paddy straw, whereas the early growth was observed on garden grass. This study concludes the presence of high proteins and low lipids content in mushrooms, which is ideal for human consumption.

Keywords: *Agricultural wastes, Mushroom, Cynodon dactylon, Oryza sativa, Pleurotus ostreatus, Triticum aestivum*

Introduction

Pleurotus ostreatus (Oyster mushroom) was initially classified under the well-defined order Agaricales and division Basidiomycota. The mushroom possesses an eccentric stalk and a caudad 'gilled' cap resembling the shape of an oyster. It is also referred as 'tree oyster mushroom'. *P. ostreatus* is an edible mushroom consumed worldwide for its high protein, fibre and carbohydrate content with low fats, sodium and calories (Cohen *et al.*, 2002). Low levels of sodium and high phosphorus and

potassium levels in *Pleurotus* are valuable health food for the hypertensive and diabetic patients (Manzi *et al.,* 1999; Chang & Wasser, 2012). It is also a good source of minerals such as calcium, magnesium, potassium, iron, selenium, zinc, manganese, copper, cobalt, chromium, molybdenum, sodium, and various vitamins such as riboflavin, biotin and thiamine (Chang & Buswell, 1996; McDowell, 2003). The presence of polysaccharide β-glucans or polysaccharide-protein complexes content in medicinal mushrooms offers a wide therapeutic application on human health due to their anti-diabetic, anti-carcinogenic, immunomodulatory, anti-inflammatory, cardiovascular, anti-microbial, hypocholesteraemia and hepatoprotective nature (Kim *et al*., 2007; Synytsya *et al*., 2009; Chaturvedi *et al*., 2018). Polysaccharides of the 1,3-β-glucans family also induce an anti-tumour effect in any part by activating certain immune cells, make it more useful in medicinal perspective (Wisbeck *et al*., 2017; Chaturvedi *et al*., 2018).

The agronomically and economically important oyster mushroom is the world's second most cultivated mushroom after *Agaricus bisporus*. It can be easily grown on various lignocellulosic substrates other than their natural habitats such as urban wastes (i.e. dry grass, pea pods, tea leaves, etc.), agricultural residues, domestic wastes, wood, straw and municipal waste etc. (Garg *et al*., 2017). Mushroom cultivation could be a profitable agribusiness in India due to the abundance of agri-waste generated in fields. The technology of artificial cultivation of mushrooms helps to improve the economic status of small farmers.

The conversion of agri-residue into highly nutritious food by mushroom cultivation increases the scope of utilization of the otherwise harmful wastes which causes air pollution if burnt. *P. ostreatus* has been successfully employed in bioremediation (mycoremediation) of contaminated soil in situ and ex-situ in Nigeria (Adenipekun & Lawal, 2012). Keeping in the mind the versatile importance of Oyster mushroom in medicinal purpose and treatment of biohazards via bioremediation, this piece of research was carried out with the objective to cultivate *P. ostreatus* on paddy straw, wheat straw and garden grass and further determine the effect of substrates on the yield, biological efficiency and their nutritional values.

Materials and Methods

The study was carried out at Plant Biotechnology, Rajiv Gandhi South Campus (RGSC), Banaras Hindu University (BHU), Barkachha, Mirzapur, Uttar Pradesh between January – June, 2019.

Experimental design

The whole experiment was planned in a completely randomized design (CRD). Mushrooms were cultivated on the three different substrates in 5 kg plastic bags, with two replicas of each substrate. Total yield was obtained in three flushes per bag.

Collection of samples

Dried agri-waste residues i.e., paddy straw, wheat straw and garden grass were

collected from wheat and paddy fields and Malviya Park, RGSC (BHU), respectively. These residues were treated with 4% formaldehyde solution and autoclaved separately.

Culture of Pleurotus ostreatus

The seeds of *P. ostreatus* were collected from the National Horticultural Research and Development Foundation, Janakpuri, New Delhi. The cultures were maintained on potato dextrose agar (PDA) slants at 24±2°C in BOD incubator. Subculturing was done in every 14-15 days.

Spawn preparation

Spawns were prepared in polythene bags. 1 kg of wheat grains were boiled (10-15 min on water bath) and mixed with 2% (w/w) of $CuSO_4$ and 4% (w/w) of $CaCO_3$. The bags were inoculated with the log phase culture of *P. ostreatus* from PDA slants. Prepared spawn bags were incubated at (27±2) °C for 12-15 days until the grains are fully covered with the thick layer of mycelium.

Cultivation of Oyster mushroom

Paddy straw, wheat straw and garden grass were used as substrates for mushroom cultivation. Dried and chopped substrates were chemically sterilized by soaking in 4% formaldehyde solution overnight. Excess water was drained off and the substrate was autoclaved. Polythene bags (5 kg) were filled with the four alternate layers of substrate and spawn. Around 200 gm of spawn was used in 5 kg substrate. Small holes were made for aeration and the mouth of the bag was sealed (**Fig. 1a**). These bags were incubated in a dark-isolated room for next 15 days. Next, the cylindrical mushroom beds were obtained by vertically cutting the bags, without damaging the bed, when a thick layer of mycelium was developed over the substrate (**Fig. 1b-c**). Watering was done thrice a day using hand sprayer. Fruiting bodies were developed after 10-12 days (**Fig. 1d-e**). Harvesting was done after 12-15 days by gently twisting them around their stipes and the mushrooms were collected in three flushes. Watering was stopped a day before harvesting of mushrooms. Harvested mushrooms were weighed and stored in a sealed polybag (**Fig. 1f**).

Biochemical profile

Biological efficiency:

Biological efficiency (BE) was calculated to compare all the three substrates used in cultivation based on its capability to support the growth of the mushrooms (average total yield was calculated). BE was calculated using the following formula:

$$\textbf{Biological effeciency} = \frac{\textbf{Fresh weight of mushroom}}{\textbf{Dry weight of substrate}} \times 100$$

Moisture content:

Moisture content was measured quantitatively by taking 5.0 gm of the mushroom sample in a crucible and transferring into hot air oven at 100-105°C until the constant weight was achieved. The moisture (%) can easily be calculated as described by Raghuramulu *et al.* (2003).

$$\textbf{Moisture content}(\%) = \frac{\textbf{Moisture content}}{\textbf{Weight of the mushroom taken}} \times 100$$

Where, Moisture content = weight of mushrooms before drying - weight of mushrooms after drying.

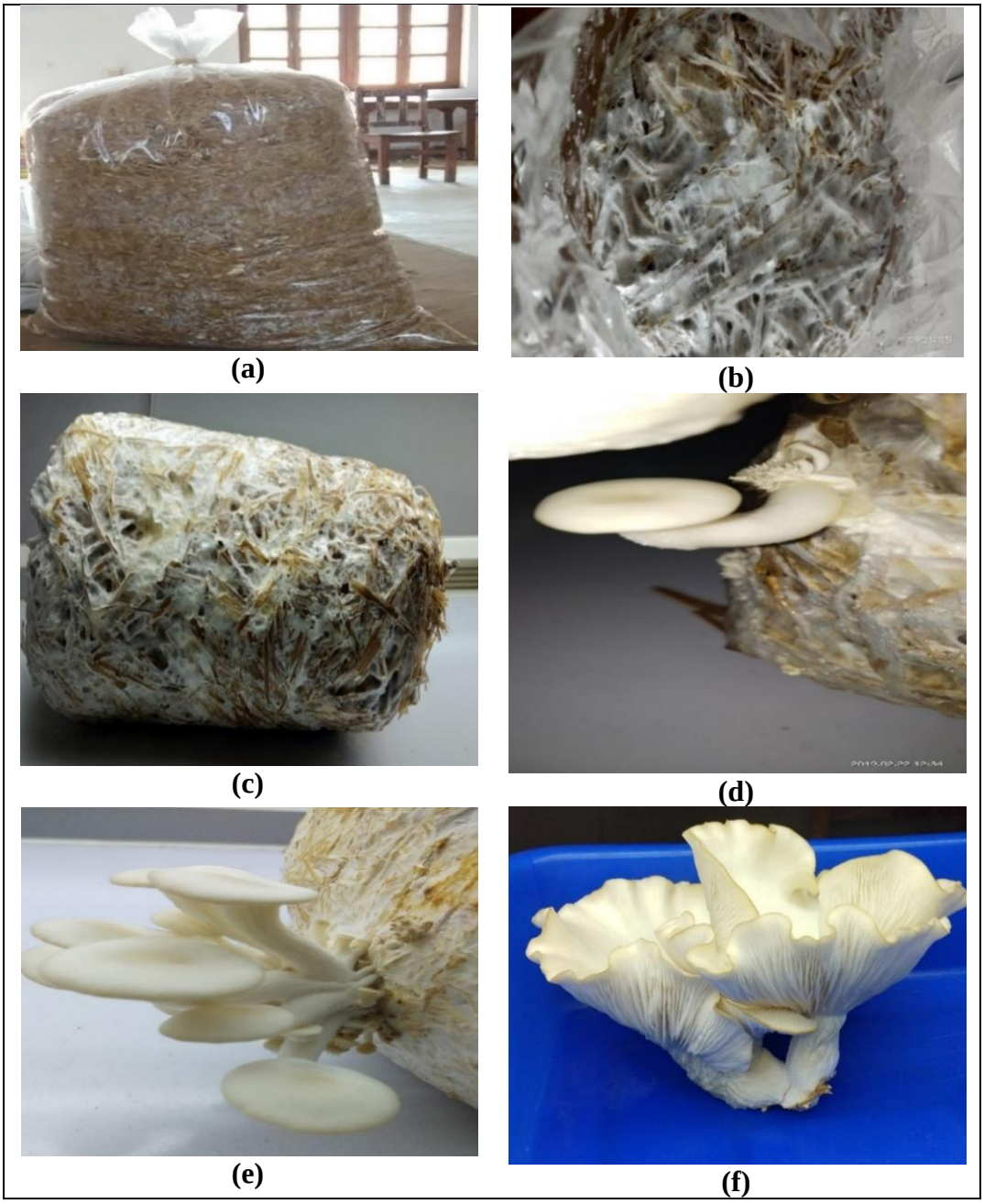

Figure 1: (a) spawning stage, (b) mycelium stage, (c) hyphae stage, (d) budding fruiting body, (e) mature fruiting bodies, (f) harvesting

Phenolic content:

The methanolic extract was prepared by crushing 5 gm sample in 50 ml of 80% methanol into a homogeniser and then filtering through a muslin cloth. About 0.5 ml of this methanol extract was taken into a fresh test tube and 125µl Folin-ciocalteau reagent was added and the mixture was then kept for 10 min at room temperature. Then, 125µl of 20% (w/v) sodium carbonate was added and mixed gently by stirring and left

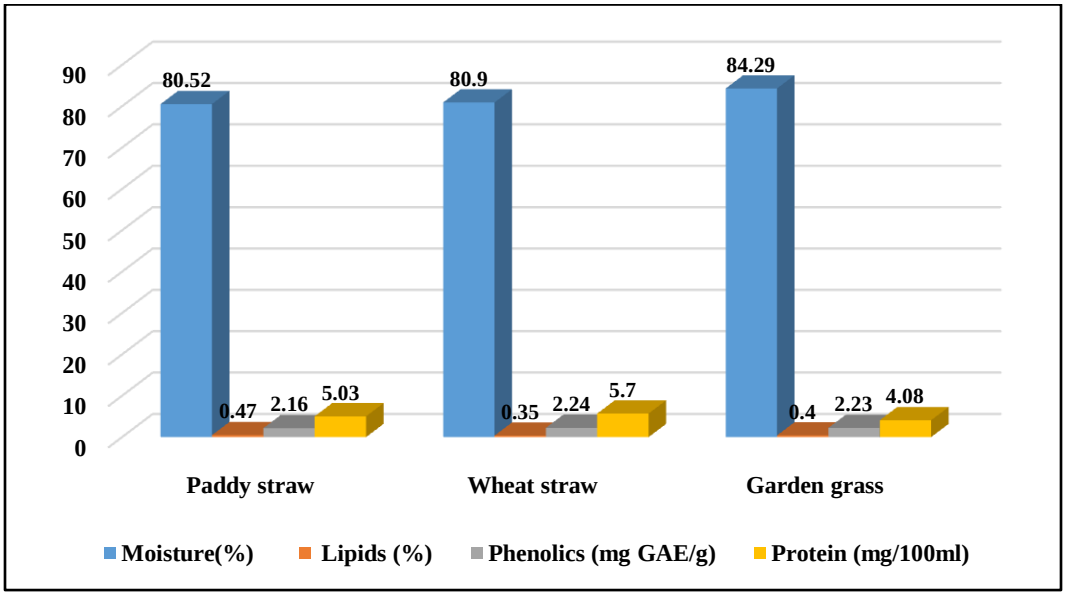

Figure 2: Nutritional values of harvested fruiting bodies of *P. ostreatus* cultivated on different substrates

for another 60 min at room temperature. Finally, the absorbance was recorded at 670 nm using UV-Vis spectrophotometer. The phenolics content was calculated from a standard calibration curve of Gallic acid (Amin *et al.*, 2006).

Lipids:

Lipid profile was determined using Folch *et al.* (1957) method with slight modifications. About 5.0 gm sample was crushed and mixed with 2:1 ratio (v/v) of 50 ml of chloroform:methanol mixture and kept it undisturbed for 3 days and then centrifuge the solution at 1000 rpm after filtering through a muslin cloth. The upper layer of methanol was separated with Pasteur pipette and the second layer of chloroform was evaporated on water bath at 70°C; the last turbid layer containing lipid was weighed on weighing balance. The lipid content was calculated by the following formula:

$$\text{Lipids } (\%) = \frac{\text{Lipid content}}{\text{Weight of mushroom taken}} \times 100$$

Protein estimation:

About 2 gm of the sample was minced with 10 ml phosphate buffer extract and the mixture was homogenised and filtered. Protein estimation was done using the standard bovine serum albumin (BSA) curve method using spectrophotometry (Lowry *et al.*, 1951). Different dilutions of BSA protein solutions were prepared by mixing BSA (1 mg/ml) and water. The final volume of each test tube was made up to 2 ml. Then, 2 ml of alkaline copper sulphate was added, mixed thoroughly and incubated the solution at room temperature for 10 min. Then, added 0.2 ml of the Folin-ciocalteau reagent to each test tube and incubated at room temperature for the next 30 min. Readings were taken at 660 nm in UV-Vis spectrophotometer.

Table 1: Yield of *P. ostreatus* on different substrates viz. paddy, wheat and garden grass

Sl. No.	Substrate	Yield (I flush) g	Yield (II flush) g*	Yield (III flush) g*	Total yield (g)*
1.	Paddy straw	282.355	135.495	41.12	458.97
2.	Wheat straw	135.49	72.8450	33.78	242.12
3.	Garden grass	296.03	99.10	37.35	432.49

**** Mean for 2 trials; expressed in g/ 5kg substrate***

Table 2: Harvesting total yield, days of spawn run and biological efficiency of *P. ostreatus* on different substrates viz. paddy, wheat and garden grass

Sl. No.	Substrate	Spawn run (days)	Total Yield (g/5kg substrate)	B.E (%)
1.	Paddy straw	15	458.97	91.79
2.	Wheat straw	18	242.12	52.66
3.	Garden grass	14	432.49	89.19

Table 3: Biochemical profile of *P. ostreatus* on different substrates viz. paddy, wheat and garden grass

Sl. No.	Substrate	Moisture (%)	Phenolics (mg GAE/g)	Proteins (mg/ 100ml)	Lipids (%)
1.	Paddy straw	80.52±0.23	2.16±0.02	5.03±0.05	0.47±0.01
2.	Wheat straw	80.90±0.08	2.24±0.03	5.70±0.02	0.35±0.05
3.	Garden grass	84.29±0.02	2.23±0.03	4.08±0.005	0.40±0.02

Mean of 3 tests taken; represented in value ±S.E.

Results and Discussion

Evaluation of different substrates for the cultivation of mushroom

While comparing the three substrates, various parameters such as yield, cropping time and biological efficiencies were taken into consideration. The highest yield of *Pleurotus* was recorded on paddy straw as shown in **Table 1**. Our findings are supported by the published work of Porselvi & Vijayakumar (2019) and Mamiro & Mamiro (2011), who recommended the use of paddy straw for higher yield. Nasreen *et al.* (2016) suggested the use of 1.25% acid - or base along with the substrates for better yield. Zhang *et al.* (2011) reported approximately 10% more yield on paddy straw than that on wheat straw.

The cropping time was affected by different substrates. The fastest growth was observed when garden grass was used as the substrate. The BE or simply the effectiveness of the mushroom strain was obtained to be highest (91.79%) on paddy straw (**Table 2**). Sharma *et al.* (2013) reported the highest yield and BE values (381.85 gm & 95.46% respectively) of mushrooms cultivated on paddy straw.

Evaluation of biochemical composition of mushrooms grown on three different substrates (Figure 2, Table 3)

Estimation of moisture and lipids content:

The highest moisture content (84.29%) in the present study was observed in mushrooms cultivated on garden grass (**Table 3**). The moisture content of fresh mushroom was reported in other studies ranging between 85-88% (Alam *et al.*, 2008; Deepalakshmi & Mirunalini, 2014). Whereas, typically high moisture content up to 89.69% was reported by Kajendran *et al.* (2018).

The amount of lipids is comparatively low in mushrooms. Kwon & Uhm (1984) and Paul *et al*. (2016) reported 0.5% and 4.2% lipids in *P. ostreatus* mushroom. A significant decrease in the concentration of TLC (total lipid content) from 0.36% in the first flush to 0.07% in the third flush was detected by Abeer *et al.* (2013). The amount of lipid contained is enumerated in the **Table 3**. *P. ostreatus* cultivated on paddy straw was found to have the highest lipid content (0.47%) whereas the lowest lipid content (0.33%) was observed in mushrooms cultivated on wheat straw.

Estimation of phenolics and protein content:

The total phenolics and protein content were comparable in all substrates. Total phenolic content (TPC) in mushroom cultivated on garden grass (2.225 mg GAE/g[*]) were notably higher than that in wheat straw (2.243mg GAE/g[*]) and paddy straw (2.162mg GAE/g[*]). On the other hand, the estimated total protein contents were significantly higher in mushrooms grown on wheat straw (5.70 mg/100ml[**]) than that in paddy straw (5.034 mg/100ml[**]) and garden grass (4.082 mg/100ml[**]) (**Table 3**).

The results obtained in the present study exhibits similarity with the findings of Yilmaz *et al.* (2017) who quantified the value of TPC=2.67 mg GAE/g in *P. ostreatus* cultivated on walnut tree saw dust. Whereas, the substrates were found to have significant effect on total phenolic contents which are evident from previous studies. Yildiz *et al.* (2017) reported TPC= 1.77 mg GAE/g in *P. ostreatus* cultivated on 100% chestnut (*Castanea sativa*) saw dust. Yilmaz *et al.* (2016) reported TPC= 1.51 mg GAE g^{-1} in *P. ostreatus* cultivated on *Tilia tomentosa* leaves. Yim *et al.* (2010) quantified the TPC in commercially cultivated mushrooms found 7.98 mg GAE g^{-1}.

The results obtained for total protein content present in mushrooms cultivated on paddy straw (5.034 mg/100ml) were quite closer to the results (5.27 mg/100ml) attained by Kajendran *et al.* (2018). Whereas, protein content reported in our study was much higher than content 3.4 mg /100 ml obtained by Alam *et al.* (2008).

Conclusion

In conclusion, three substrates were found to have significant effects on the growth pattern and nutritional value of the mushrooms cultivated on them. Good

[*] Results were expressed in mg GAE/g of extract

[**] Results are expressed in mg/100ml of extract

amounts of protein, phenolics, moisture and low lipid levels were found in *P. ostreatus*, which also indicates the good nutritive values for the human being. The data reported above reveals the implementation of paddy straw as a substrate for high yield and biological efficiency and *C. dactylon* (garden grass) for early growth. Garden grass was able to retain relatively high percentage of moisture, which was an important intrinsic factor for optimum growth. High protein content in mushrooms indicates its potential use as a protein supplement. From an economic point of view, the cultivation of protein-rich mushroom species *P. ostreatus* enables the farmer to get extra income and more protein harvest from his agri-waste.

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References

Abeer, H., Alqarawi, A., Al-Huqail, A., & Abd Allah, E. (2013). Biochemical composition of *Pleurotus ostreatus* (Jacq.) P. Kumm. grown on sawdust of *Leucanena leucocephala* (Lam.) de Wit. *Pakistan Journal of Botany*, 45(4), 1197-1201.

Alam, N., Amin, R., Khan A., Ara, I., Shim, M. J., Lee, M. W. , & Lee, T. S. (2008). Nutritional analysis of cultivated mushrooms in Bangladesh - *Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus florida* and *Calocybe indica*. *Mycobiology*, 36(4), 228-232.

Amin, I., Norazaidah, Y., & Hainida, E. K. I. (2006). Antioxidant activity and phenolic content of raw and blanched *Ameranthus* species. *Food Chemistry*, 94(1), 47–52

Adenipekun, C. O., & Lawal, R. (2012). Uses of mushrooms in bioremediation: A Review. *Biotechnology and Molecular Biology Reviews*, 7(3), 62-68.

Chang, S. T. & Wasser, S. P. (2012). The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. *International Journal of Medicinal Mushrooms*, 14(2), 95–134.

Chang. S. T., & Buswell. J. A. (1996). Mushroom Nutriceuticals. *World Journal of Microbiology & Biotechnology*, 12, 473-476.

Chaturvedi, V., Agrawal, S., Gupta, K., Ramteke, P & Singh, M. P. (2018). Medicinal mushroom: boon for therapeutic applications. *3 Biotech*, 8(8), 334.

Cohen, R., Persky, L., & Hadar, Y. (2002). Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. *Applied Microbiology and Biotechnology*, 58(5), 582–594.

Deepalakshmi, K., & Mirunalini, S. (2014). *Pleurotus ostreatus*: an oyster mushroom with nutritional and medicinal properties. *Journal*

of Biochemical Technology, 5(2), 718-726.

Folch, J., Lees., M., & Sloane Stanlet, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226(1), 497-509.

Garg, P., Saxena, G., Kanodia, S., Gupta, S., Goel, V. K., Rajan, Gaurav, Siddhant, Mohini, Nidhi, Mohit, Ankush, Juned, Akanksha, & Priyanka. (2017). Conversion of urban waste into highly nutritious food by *Pleurotus* cultivation. *IOSR Journal of Biotechnology and Biochemistry*, 3(4), 15-18.

Kajendran, M., Balaji, S. S., & Sathya, S. (2018). Cultivation and determination of nutritional value on edible mushroom *Pleurotus ostreatus. European Journal of Biotechnology and Bioscience*, 6(3), 40-44.

Kim, S. W., Hwang, H. J., Lee, B. C., & Yun, J. W. (2007). Submerged production and characterization of *Grifola frondosa* Polysaccharides- A new application to cosmeceuticals. *Food Technology and Biotechnology*, 45(3), 295–305.

Kwon, Y. J. & Uhm, T. B. (1984). A study on the lipid components in Oyster mushroom. *Journal of the Korean Society of Food Science and Nutrition*, 13(2), 175-180.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265-275.

Mamiro, D. P., & Mamiro, P. S. (2011). Yield and mushroom size of *Pleurotus ostreatus* grown on rice straw basal substrate mixed and supplemented with various crop residues. *Journal of Animal and Plant Sciences*, 10(1), 1211-1218.

Manzi, P., Gambelli, L., Marconi, S., Vivanti, V., & Pizzoferrato, L. (1999). Nutrients in edible mushrooms: An inter-species comparatives study. *Food Chemistry*, 65(4), 477-696.

McDowell, L.R. (2003). Minerals in Animal and Human Nutrition, 2nd ed. Amsterdam, The Netherlands, Elsevier.

Nasreen, Z., Ali, S., Usman, S., Nazir, S., & Yasmeen A. (2016). Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on lignocellulosic by-products. *International Journal of Advanced Research in Botany*, 2(1), 42-49.

Paul, M., Sarma, T. C., & Deka, D. C. (2016). A comparative analysis of lipid contents of six important macrofungi of Assam. *International Journal of Interdisciplinary and Multidisciplinary Studies*, 3(3), 11-13.

Porselvi, A. & Vijayakumar, R. (2019). Evaluation of paddy straw varieties on the cultivation and nutritional value of two oyster mushroom species. *International Journal of Research in Advent Technology*, 7(5), 2321-9637.

Raghuramulu, N., Madhavan, N. K., & Kalyanasundaram, S. (2003). A manual of laboratory techniques, pp. 56-58. National Institute of Nutrition. Indian Council of Medical Research. Hyderabad, India.

Sharma, S., Yadav, R. K. P., & Pokhrel, C. P. (2013). Growth and yield of Oyster mushroom (*Pleurotus ostreatus*) on different substrates. *Journal on New Biological Reports*, 2(1), 3–8.

Synytsya, A., Míčková, K., Synytsya, A., Jablonský, I., Spěváček, J., Erban, V., Kováříková E., & Čopíková, J. (2009). Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: Structure and potential prebiotic activity. *Carbohydrate Polymers*, 76(4), 548-556.

Wisbeck, E., Facchini, J. M., Alves, E. P., Silveira, M. L., Gern, R. M., Ninow, J. L., & Furlan, S. A. (2017). A polysaccharide fraction extracted from *Pleurotus ostreatus* mycelial biomass inhibit Sarcoma 180 tumor. *Anais da Academia Brasileira de Ciencias*, 89 (3) Suppl, 2013-2020.

Yıldız, S., Yılmaz, A., Can, Z., Kılıç, C., & Yıldız, Ü. C. (2017). Total phenolic, flavonoid, tannin contents andantioxidant properties of *Pleurotus ostreatus* and *Pleurotus citrinopileatus* cultivated on various sawdust. *GIDA*, 42(3), 315-323

Yılmaz, A., Yıldız, S., Kılıç, C., & Can, Z. (2017). Total phenolics, flavonoids, tannin contents and antioxidant properties of *Pleurotus ostreatus* cultivated on different wastes and sawdust. *International Journal of Secondary Metabolite*, 4(1), 1-9.

Yılmaz, A., Yıldız, S., Tabbouche, S., Kılıç, A. O., & Can, Z. (2016). Total phenolic content, antioxidant and antimicrobial properties of *Pleurotus ostreatus* grown on lime (*Tilia tomentosa*) leaves. *Hacettepe Journal of Biology and Chemistry*, 44(2), 119-124.

Yim, H. S. Jr., Chye, F. Y., Tan, C. T., Ng, Y. C., & Ho, C. W. (2010). Antioxidant activities and total phenolic content of aqueous extract of *Pleurotus ostreatus* (Cultivated Oyster Mushroom). *Malaysian Journal of nutrition*, 16(2), 281-291.

Zhang, R., Li, X., & Fadel, J. G. (2001). Oyster mushroom cultivation with rice and wheat straw. *Bioresource Technology*, 82(3), 277-284.