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Department of Agricultural Extension
Ministry of Agriculture
Sobhanbag, Savar, Dhaka-1340
Bangladesh

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Books:

Gomez, K. A. & Gomez, A. A. 1984. Statistical Procedures of Agricultural Research, 2nd ed., John Wiley and Sons, Singapore. p. 21.

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Study of Identification, Yield Performance, Nutritional Profiles, and Determination Standard Cultural Practice of Newly Introduced Chestnut Mushroom (*Pholiota adiposa*) Strain in Bangladesh

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Abstract

This study focuses on the characterization, yield performance, nutritional profile, and optimized cultivation practices for the newly introduced Chestnut Mushroom (*Pholiota adiposa*, strain CsNt_MDI) in Bangladesh. Conducted at the Mushroom Development Institute, the research aimed to evaluate suitable adaptation techniques to enhance growth and productivity. Three treatment methods were tested: T_1 (500g packet top opening at normal culture house), T_2 (500g packet D-cut on neck portion in a normal culture house), and T_3 (packet top opening in an air-conditioned room). The study assessed their impact on yield, biological efficiency, and morphological characteristics. Results revealed significant differences in yield and biological efficiency across treatments, while morphological traits such as stalk length and pileus diameter remained statistically similar. Among the treatments, T_1 demonstrated the best performance, yielding 175.29 g per packet and achieving a biological efficiency of 58.42%, significantly surpassing T_2. Both T_1 and T_3 also produced a higher number of total and effective fruiting bodies compared to T_2. Nutritional analysis indicated that the mushroom is a valuable dietary option, containing 59.06% carbohydrates, 6.50% protein, 5.12% lipids, 8.70% ash, 1.20% fiber, and 19.42% moisture, contributing to favorable shelf life and texture. Molecular identification confirmed the genetic identity of *P. adiposa* (CsNt_MDI), showing 99.69% homology with known strains in Gen Bank. These results suggest that this strain is highly adaptable to local conditions and holds strong potential for commercial mushroom cultivation in Bangladesh due to its high yield and rich nutritional content.

Keywords: Molecular analysis, *Pholiota adiposa*, Yield performance, Nutritional profile, Standard cultural practices.

INTRODUCTION

Mushrooms are a great source of nutrients and some even have medicinal properties. Mushroom has a unique taste and aroma. They are widely used in cooking around the world (Ribeiro *et al.*, 2007). In recent years, diseases like cancer, diabetes, hypertension, and autoimmune disorders have increased. People have been looking for alternative treatments (Ergönül *et al.*, 2013).

Mushrooms contain many beneficial compounds such as carbohydrates, fiber, vitamins, minerals, proteins, fats, and secondary metabolites. These compounds have shown antimicrobial, antitumor, antifungal, and antioxidant properties (Ribeiro *et al.*, 2007; Ergönül *et al.*, 2013; Wasser and Weiss, 1999; Diyabalange *et al.*, 2008). While mushrooms are not a cure for diseases, studies suggest they may help prevent illnesses or support the body in managing them (Chang and Miles, 2004).

Popular edible mushrooms like *Ganoderma lucidum* and *Lentinula edodes* have been studied extensively (Popović *et al.*, 2013; Ferreira *et al.*, 2015; Stojković *et al.*, 2014). Recently, attention has shifted to lesser-known mushrooms and their extracts as potential sources of beneficial biomolecules (Boh *et al.*, 2007; Altobelli, 2011). Wild mushrooms are also rich in antioxidants,

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which help eliminate harmful free radicals. These radicals can damage cells and are linked to cancer, cardiovascular diseases, and diabetes (Lo and Cheung, 2005; Lindequist *et al.*, 2005).

Chestnut Mushroom are a type of mushroom with a rich, earthy flavor and a firm, meaty texture. They are good source of nutrients including protein, fiber and various vitamins and minerals. A deliciously chewy mushroom that's relatively new to mushroom cultivation.

MATERIALS AND METHODS

The experiment was conducted at Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh. In this experiment, a newly introduced mushroom strain of *Pholiota adiposa* was selected and grown in a culture house. Furthermore, identification, yield performance, adaptation procedure & nutritional profile were determined at the Mushroom Development Institute's culture house and biochemistry lab. DNA isolation, fingerprint, and genetic chromatography were examined in the Invent Technology.

MOLECULAR IDENTIFICATION

DNA extraction and purification: Genomic DNA was extracted from *P. adiposa* fruiting bodies using the Maxwell® Blood DNA Extraction Kit (Model AS1010, Promega Corp., Madison, WI, USA) according to the manufacturer's protocol. The extracted DNA was purified using the Wizard® Genomic DNA Purification Kit (A1120, Promega Corp.). DNA concentration and purity were assessed using a NanoDrop 2000c Spectrophotometer (Thermo Scientific), with a final concentration measured at 40.9 ng/µL.

PCR amplification of ITS region: The Internal Transcribed Spacer (ITS) region of the nuclear ribosomal DNA (rDNA) was amplified using the primer pair ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). PCR reactions were carried out in a 25 µL total volume using the GoTaq® Green Master Mix Kit (M7122, Promega Corp.) at a 1× final concentration. Each reaction contained 5 µM of each primer and 25 ng of template DNA. Amplification was performed in a C1000 Touch Thermal Cycler (Bio-Rad®, Germany) using the following thermal profile (Fig. 1).

Gel electrophoresis and purification: PCR products were separated by electrophoresis on a 2% agarose gel at 80 V for 40 minutes and visualized under UV light using SYBR® Gold stain (Invitrogen, Carlsbad, CA, USA). Amplicons of approximately 700 base pairs were selected and purified using ExoSAP-IT® (N/P 78200, USB Affymetrix, Inc., Cleveland, OH, USA) to remove excess primers and dNTPs.

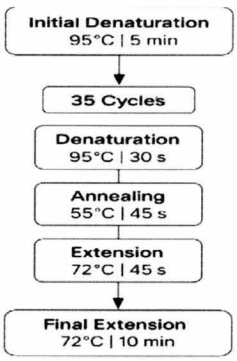

Fig. 1. The flowchart of the PCR protocol performed to amplify the DNA of chestnut mushroom.

Sequencing and data analysis: Sequencing was performed using the ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit (P/N 4336917, Applied Biosystems, Foster City, CA, USA) with both ITS5 and ITS4 primers. Sequence reactions were analyzed on an ABI 3130 Genetic Analyzer (Applied Biosystems® HITACHI, Tokyo, Japan). Raw sequences were assembled and edited using SeqMan v8 (DNASTAR®, Madison, WI, USA).

Species identification was performed by comparing the assembled ITS sequences against the GenBank database using BLASTn. The species was determined based on the highest identity match.

Yield Performance and standard cultivation practice

Treatment: Three different adaptation techniques were used as a treatment in this experiment. In total, (4 replications×8 packets per replication×3 treatments) 96 packets were used for this study. Sawdust spawn packets were tested as basic material. The sawdust spawn packet was prepared with 18cm×25cm polypropylene bags at 500 g/bag. The neck of the bag was prepared by using a resistant plastic pipe. A hole about 2/3 deep in the bag was made for space to introduce the mycelium. SS chamber sterilization technique was used for sterilization spawn packets for 4 hours at (100°C water steam temp.) The treatments were-

T_1 = 500g packet top opening at normal culture house temp.
T_2 = 500g packet D-cut in neck portion at normal culture house temp.
T_3 = 500g packet top opening at AC room temp.

After sterilization, the packets were inoculated separately with Chestnut (PA) mother at the rate of two teaspoonfuls per packet. The inoculated packets were incubated at 20±2°C. After completing mycelium in spawn packets, treatments were made and observed.

Spawn packet preparation, inoculation, and incubation: All spawn packets were kept in the culture house at 22±2°C. After the colonization of mycelium was fully completed then packets were taken to another culture house for packet opening and harvesting. Packets were cut in a "D shape" style on the shoulder of the packet both side and a "Top opening" style on the upper portion of the spawn. Relative humidity and temperature were maintained at 80-90% and 25-30°C respectively. The natural light of about 200±5 lux and proper ventilation were maintained in the culture house. The yield was obtained from the individual number of packets and calculated weight (g/packet) after removing the lower dirty portion.

Experimental design, data collection, and statistical analysis: The experiment was laid out in a Completely Randomized Design (CRD) with 4 replications. Data on yield (g/packet), number of fruiting bodies per packet, number of effective fruiting bodies per packet, length, and diameter of stalk, diameter, and thickness of pilus, and percentage of biological efficiency were collected and analyzed by Statistic 10X program. Means were separated by Duncan's Multiple Range Test (DMRT).

The biological efficiency was calculated according to the formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Total biological yield (g)}}{\text{Total dry substrate used (g)}} \times 100$$

Morphological attributes: Morphological attributes were observed by the color and shape of the mushroom. Differences of color, variation of fruiting body structure, and size of fruiting body were prioritized for this study.

Nutritional Profile

Determination of moisture & ash content: One gram of well-grained chestnut mushroom sample was taken to a Moisture analyzer (AnD MX-50) to analyze moisture content. The moisture analyzer took an average of 5-7 minutes for results by heating the sample. Then again one gram of the same sample was taken and placed into a crucible and weighed. The crucible was heated by a spirit lamp till the sample was burned and turned into black ash. Then it was taken into a muffle furnace for about 5-6 hours at 600°C. After cooling down of muffle furnace crucible was taken out and weighed. Then total ash was calculated by Raghuramulu *et al.*, (1983) as the following equation:

$$\text{Ash content} \left(\frac{\text{g}}{\text{100g sample}} \right) = \frac{\text{Wt. of ash}}{\text{Wt. of sample taken}} \times 100$$

Determination of total lipid: Total lipid was determined by using chloroform and alcohol/ methanol mixture with the sample. Five grams of grained mushroom sample was dipped into 50ml of chloroform: methanol (2:1) mixture. After 3 days the mixture was filtrated with filter paper and poured into a test tube which was pre-weighted. The test tube with the filtered mixture was placed into a dryer at 50-55°C. The upper layer of methanol was removed and chloroform was evaporated by heating. The remaining was crude lipid. Total lipid was calculated as the following equation:

$$\text{Total Lipid} = \frac{\text{2nd weight - 1st weight}}{\text{S}} \times 100$$

Determination of total protein: Five grams of grinded mushroom was taken with 50 ml of 0.1N NaOH and boiled for 30 min. The solution was cooled at room temperature. The supernatant was collected and total protein content was measured according to the method of Lowry *et al.*, (1951).

Determination of crude fiber: Ten grams of moisture and fat-free sample was taken in a beaker and 200 ml of boiling 0.255 N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue was washed with hot water till free from acid. The material was then transferred to the same beaker, and 200 ml of boiling 0.313 N NaOH was added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue was washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100^{0}C, and weighed (We) in an electric balance (Keyi: JY-2003; China). The crucible was heated in a muffle furnace (Nebertherm: Mod-L9/11/c6; Germany) at 600^{0}C for 5~6 hours, cooled and weighed again (Wa). The difference in the weights (WeWa) represents the weight of crude fiber (Raghuramulu *et al.*, 2003).

$$\text{Crude fiber} \left(\frac{\text{g}}{\text{100g sample}} \right) = [100 - (\text{Moisture} + \text{Fat}) \times (\text{We} - \text{Wa})/\text{Wt of sample}]$$

Determination of total carbohydrate: The content of the available carbohydrate was determined by the following equation (Raghuramulu *et al.*, 2003).

$$\text{Carbohydrate}\left(\frac{\text{g}}{\text{100g sample}}\right) = [100 - (\text{Moisture} + \text{Fat} + \text{Protein} + \text{Ash} + \text{Crude Fiber})]$$

RESULTS AND DISCUSSION

Study on Morphological Attributes of *Pholiota adiposa*

Cap: The caps of the chestnut mushrooms were round or slightly flattened at maturity and displayed a characteristic chestnut brown color. The surface was smooth and velvety, with a diameter ranging from 2 to 8 cm. When young and moist, the caps were slightly sticky. Distinctive white, snow-like specks appeared on the top and sides of the cap. As the mushrooms aged, the caps became progressively flatter and the color deepened to a darker brown (Fig. 2), consistent with descriptions by Ribeiro *et al.* (2007) and Stojković *et al.* (2014).

Gills: The gills were tightly packed and initially pale, gradually darkening as the mushrooms matured (Fig. 2).

Stalk: The stalks were slender, white, and smooth in texture, narrowing near the cap. They measured approximately 5 to 10 cm in length. Similar to the caps, white, snow-like dots were also observed on the surface of the stalks (Fig. 2).

Spore print: The spore print was consistently dark brown, in alignment with previously documented characteristics of the species.

Chestnut mushrooms exhibited a mild, earthy flavor with a subtle nutty note. They were deemed suitable for a variety of culinary applications, including stir-fries, pasta dishes, and soups.

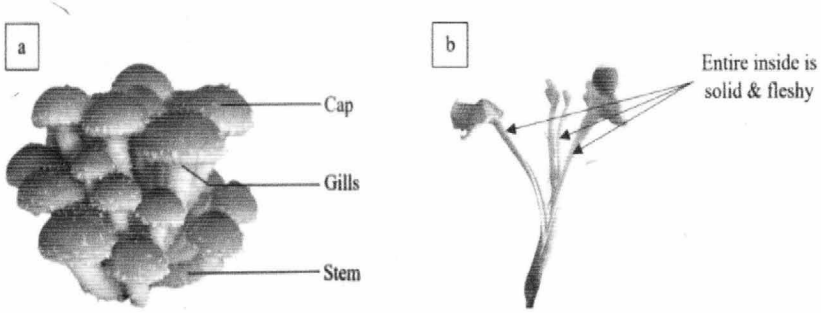

Fig. 2. Morphological appearance of Chestnut mushroom. (a) Outside appearance & (b) Inside appearance.

Study on the Molecular Identification of *P. adiposa*

The obtained FASTA sequences underwent analysis using the BLAST tool on the NCBI website. It was revealed matches with existing nucleotide sequences in the NCBI GenBank database. It has been observed that isolated *Pholiota adiposa* strain CsNt_MDI (Accession no. PV583471. 1) showed 99.69% homology to the *Pholiota adiposa* voucher HGASMF01-16470 with accession no. ON557687.1 and 99.07% homology to the *Pholiota adiposa* isolate ET37 with accession no. MN209721.1. (Table 1)

Table 1. Results of chestnut mushroom isolate's DNA sequence identification with BLAST program

LAB strain			BLAST Alignment				
Sl. no.	Identified mushroom isolates	Obtained accession no.	Species	Query coverage	E value	Percent identity	Accession no.
1.	*Pholiota adiposa* strain (CsNt-MDI)	PV583471.1	*Pholiota adiposa* voucher HGASMF01-16470	97%	0.0	99.69%	ON557687.1
			Pholiota adiposa isolate ET37	98%	0.0	99.07%	MN209721.1

Study on Yield Performance: The results of yield and yield-related attributes of the newly introduced chestnut mushroom *Pholiota adiposa* strain CsNt_MDI in Bangladesh differed significantly (Table 2).

Days required from inoculation to first harvest: The number of days from inoculation to first harvest did not significantly differ among treatments. at the 5% level (LSD = 3.33; CV = 2.96%), indicating uniform crop cycles across treatments.

Yield per packet: Significant differences were observed in yield per packet among treatments. T_1 produced the highest yield, which was significantly greater than T_2, while T_3 was intermediate and not significantly different from either T_1 or T_2. The high CV (42.85%) indicates notable variability in yield data.

Number of fruiting bodies and effective fruiting bodies: The number of fruiting bodies per packet was significantly higher in T_1 and T_3 compared to T_2. This suggests that T_1 and T_3 treatments were more effective in promoting fruiting body formation (LSD = 9.61; CV = 38.74%). Similarly, the number of effective fruiting bodies was significantly higher in T_1 and T_3 than in T_2. These results further support the superior performance of T_1 and T_3 treatments in producing viable fruiting structures (LSD = 6.68; CV = 46.93%).

Stalk length: No significant differences were found in stalk length among treatments. Despite numerical variation, the differences were not statistically significant (LSD = 1.42; CV = 17.58%).

Stalk diameter: All treatments had statistically similar stalk diameters. T_1 had the smallest diameter while T_2 and T_3 both recorded 0.97 cm. No significant differences were detected (LSD = 0.31; CV = 30.15%).

Pileus diameter: The diameter of the pileus did not differ significantly across treatments. T_2 had the largest mean diameter, followed by T_1 and T_3, but these differences were not statistically significant (LSD = 1.20; CV = 21.48%).

Pileus thickness: Pileus thickness also showed no significant treatment effect. Although T_3 had the thickest pileus, the variation was not significant (LSD = 0.24; CV = 25.02%).

Biological efficiency: T_1 exhibited the highest biological efficiency (58.42%), which was significantly greater than T_2. T_3 (38.62%) was intermediate and statistically similar to both T_1 and T_2. The LSD value was 20.68, with a CV of 42.85%, indicating considerable variation among treatments.

Study on the Nutritional Profile

The nutritional analysis of *Pholiota adiposa* strain CsNt_MDI revealed significant findings regarding its macronutrient composition, confirming its potential as a nutritious and commercially viable edible mushroom.

Table 2. Yield and yield contributing character of newly introduced Chestnut mushroom

Treatment	Inoculation to harvest (days)	Yield (g/ packet)	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Biological efficiency %
T_1	100.71 a	175.29 a	28.71 a	16.86 a	6.83 a	0.76 a	4.87 a	0.79 a	58.42 a
T_2	99.57 a	95.57 b	10.57 b	6.71 b	7.14 a	0.97 a	5.42 a	0.87 a	31.85 b
T_3	100.29 a	115.86 ab	27.00 a	14.43 a	7.64 a	0.97 a	4.60 a	0.93 a	38.62 ab
$LSD_{(0.05)}$	3.33	62.03	9.61	6.68	1.42	0.31	1.20	0.24	20.68
CV %	2.96	42.85	38.74	46.93	17.58	30.15	21.48	25.02	42.85
Significant Differences	NS	$T_1 > T_2$	$T_1, T_3 > T_2$	$T_1, T_3 > T_2$	NS	NS	NS	NS	$T_1 > T_2$

Here, Letters (a, b, ab) indicate significance groupings (means sharing the same letter is not significantly different), NS = Not Significant

Moisture and ash content: The moisture content of *Pholiota adiposa* was recorded at 19.42%, indicating that it retains moderate levels of water, which may contribute to its fresh texture and shelf stability. The total ash content was 8.70%, signifying a good concentration of essential minerals (Table 3). Higher ash content is indicative of the presence of bioactive minerals necessary for human health.

Lipid content: The total lipid content was found to be 5.12%, which is relatively low compared to other edible mushrooms (Table 3). This makes *Pholiota adiposa* a suitable dietary component for individuals seeking low-fat food options while still benefiting from essential fatty acids that contribute to overall health.

Protein content: The protein concentration in Pholiota adiposa was 6.50%, suggesting that this mushroom can be an alternative source of plant-based protein (Table 3). The protein content is comparable to many edible mushrooms, making it a suitable addition to vegetarian and vegan diets. The presence of bioactive proteins may also contribute to its potential health benefits, including immune system support and antioxidant activity.

Crude fiber content: The crude fiber content was 1.20%, which plays an essential role in digestive health by promoting gut motility and aiding in the prevention of constipation (Table 3). Though relatively low compared to leafy vegetables, the fiber content in *Pholiota adiposa* still contributes to dietary fiber intake.

Carbohydrate content: The carbohydrate content was the highest among all analyzed nutrients, recorded at 59.06% (Table 3). This suggests that *Pholiota adiposa* serves as an excellent energy source. Carbohydrates in mushrooms include polysaccharides, which have been reported to exhibit immunomodulatory and antioxidant properties.

Comparison with other edible mushrooms: The nutritional composition of *Pholiota adiposa* is comparable to other commercially cultivated mushrooms, such as *Agaricus bisporus* and *Lentinula edodes*. While it has lower protein content than *Lentinula edodes*, its carbohydrate and ash content make it a promising alternative for food applications.

The results of this study highlight the potential of *Pholiota adiposa* as a valuable edible mushroom with a balanced nutritional profile. Its moderate protein and lipid content, along with a high carbohydrate concentration, make it an energy-dense food source. The findings also support its

suitability for commercial cultivation in Bangladesh, offering a nutritious alternative to other commonly grown mushrooms. Further studies on its bioactive compounds and health benefits may provide additional insights into its functional food potential.

Table 3. Nutritional status of newly introduced chestnut mushroom *P. adiposa* strain CsNt_MDI

Sl. No.	Nutrient Compositions	Nutritional Values (%)
01	Total Ash	08.70
02	Moisture	19.42
03	Lipid	05.12
04	Protein	06.50
05	Fiber	01.20
06	Carbohydrate	59.06

Study on the Standard Cultural Practices

The successful cultivation of *P. adiposa* required the adoption of specific cultural practices to optimize growth, yield, and quality presented in (Table 4) and (Fig. 3). The following standard procedures had been outlined based on experimental observations and established practices for mushroom cultivation (Ergönül *et al.*, 2013).

Table 4. Standard cultural practices of *P. adiposa* strain CsNt_MDI

Substrate (100 packets)		Standard cultural practices					
Sawdust	16 kg	Temperature	Relative humidity	Light	Ventilation	Total Duration	Packet opening
Rice burn	8 kg	22±2°C	80-90%	Natural light 200±5 lux	Open air flow in culture house	95-120 days	Neck opening
Lime	100g						
Water	45%						
PP size	7inch× 10 inch						
Packet size	500 grams						

High-quality *Pholiota adiposa* mother spawn was used for inoculation.

- Sawdust-based spawn packets were prepared using **500g** of substrate per packet, packed in **18 cm × 25 cm** polypropylene bags.
- A heat-resistant plastic pipe was used as a neck for spawn introduction.
- Substrate sterilization was performed using an SS chamber at **100°C for 4 hours**.
- Inoculation was carried out by introducing **two teaspoons** of Chestnut Mushroom mother culture per spawn packet.
- **Temperature:** Maintained at **22 ± 2°C** in the culture house.
- **Humidity:** Kept at **80–90%** to support fruit body development.
- **Light Exposure:** Maintained at **200 ± 5 lux** of natural light.
- **Ventilation:** Proper airflow was ensured to prevent excessive CO_2 accumulation.

- **Packet Opening:** Top opening. Normally cultivation in winter season.
- Water misting was applied regularly to **maintain humidity** without oversaturating the substrate.
- The first harvest was obtained 90-100 days after inoculation.
- **Post-harvest:** Mushrooms were cleaned by removing lower dirty portions. Fresh mushrooms were packed in ventilated containers to prevent moisture buildup. Dried storage was recommended for long-term preservation (Boh *et al.*, 2007).

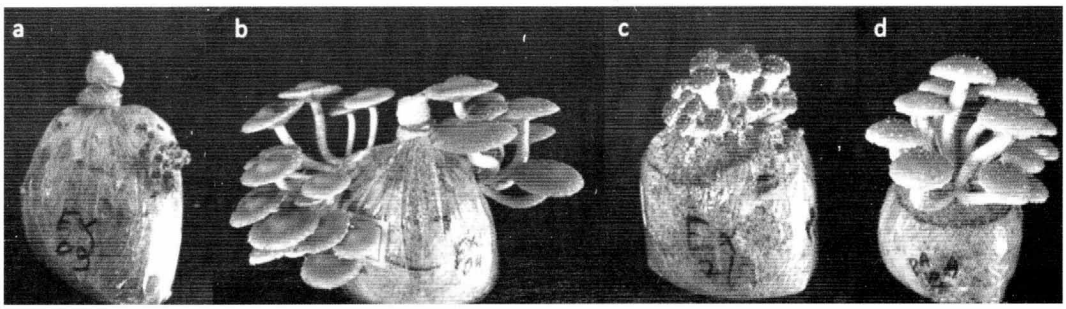

Fig. 3. Standard packet opening technique for *Pholiota adiposa* cultivation. Here, (a)= 'D' cut packet opening, (b)= mushroom from 'D' cutting packet at neck region, (c) = top opening & (d)= mushroom from top opening packet.

This study established an effective cultural practice for *Pholiota adiposa* in Bangladesh. Optimal incubation and fruiting conditions, along with the neck opening technique, yielded the best results. These guidelines provide a foundation for large-scale cultivation and commercial production of Chestnut Mushrooms.

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Performance of Different Types of Supplements and Their Combination on Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract

The present study was conducted at the Tissue Culture Laboratory and Culture House of the Mushroom Development Institute in Savar, Dhaka, from October 2022 to January 2023, with the aim of enhancing oyster mushroom production by identifying the most effective substrate supplement for promoting mycelial growth and yield. A single-factor Completely Randomized Design (CRD) was employed to assess ten treatment combinations: T1 = Wheat bran (8 kg), T2 = Wheat bran (6 kg) + Straw (2 kg), T3 = Wheat bran (6 kg) + Maize powder (2 kg), T4 = Maize powder (8 kg), T5 = Maize powder (6 kg) + Straw (2 kg), T6 = Maize powder (6 kg) + Wheat bran (2 kg), T7 = Rice bran (8 kg), T8 = Rice bran (6 kg) + Straw (2 kg), T9 = Rice bran (6 kg) + Wheat bran (2 kg), and T10 = Rice bran (2 kg) + Wheat bran (2 kg) + Maize powder (2 kg) + Straw (2 kg). The results revealed significant variation among treatments, with T10 showing the best overall performance: the shortest mycelial running time (20.70 days), the earliest pinhead initiation (3.05 days), and the quickest time to first harvest (3.14 days). T10 also recorded the highest number of total fruiting bodies (48.00), effective fruiting bodies (18.00), and superior morphological traits, including stalk length (5.07 cm) and pileus diameter (8.70 cm). Additionally, it yielded the highest production per packet (212.06 g) and biological efficiency (81.05%). In contrast, T8 consistently exhibited the poorest performance, with the longest developmental durations, lowest yield (151.33 g), and biological efficiency (57.84%). These findings emphasize the crucial role of substrate composition in mushroom cultivation and demonstrate that the combination used in T10 is the most effective for maximizing both growth rate and yield in oyster mushrooms.

Keywords: Wheat bran, Maize Powder, Rice bran, Straw, Mushroom production.

INTRODUCTION

Oyster mushrooms, belonging to the genus *Pleurotus*, are widely cultivated and consumed worldwide, valued for their delicate flavor and tender texture. The term "oyster mushroom" encompasses several species within this genus, the most common being *Pleurotus ostreatus* (Nongthombam *et al.*, 2021). Native to various regions in Europe, Asia, and North America, oyster mushrooms have been foraged and cultivated for centuries (Nongthombam *et al.*, 2021; Oloke, 2017). Oyster mushrooms are ideal for commercial cultivation, yielding high productivity from minimal substrate space (Crisan and Sands, 1978). Due to their nutritional and therapeutic qualities, edible mushrooms such as oyster mushrooms are becoming more and more common in daily diets (Mattila *et al.*, 2002; Barros *et al.*, 2007). Oyster mushrooms are highly valued for their health benefits, being low in calories but rich in proteins, vitamins, minerals, and dietary fibers (Ouzouni *et al.,* 2009; Manjunathan and Kaviyarasan, 2011). Additionally, they are rich in B vitamins (especially niacin and riboflavin) and vitamin D after sun exposure, as well as essential minerals like potassium,

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iron, zinc, and phosphorus, making them a valuable choice for health-conscious diets (Kim *et al.*, 2008).

Mushroom farming is becoming popular in Bangladesh because a lot of people want mushrooms, both in the country and abroad. In 2018-2019, Bangladesh produced about 40,000 metric tons of mushrooms (Ferdousi *et al.*, 2020). People there mostly grow Oyster, Reishi, Milky, Button, Straw, and Shiitake mushrooms, but oyster mushrooms are grown all year round due to the favorable weather and climate conditions.

To cultivate mushrooms like the Oyster mushroom, substrate preparation is indeed crucial. Mushroom cultivation faces several challenges, particularly with substrates. Substrate availability and cost can limit cultivation, as some may be expensive or difficult to obtain in large quantities (Balan *et al.*, 2022). Additionally, substrate composition and quality directly affect mushroom yield and nutritional content, making careful selection essential (Vieira and de Andrade, 2016). Substrate type significantly influences Oyster mushroom productivity and nutritional value (Ashraf *et al.*, 2013). In Bangladesh, a large quantity of agricultural by-products is generated annually, yet much remains unused. These by-products hold potential as valuable resources for food production, particularly for cultivating Oyster mushrooms (Ashraf *et al.*, 2013). Mushroom yields are highly responsive to substrate selection, with different combinations producing varied outcomes (Sitaula *et al.*, 2018). A mixed substrate of equal parts wheat bran, maize powder, and rice straw yielded the highest production for *P. ostreatus* compared to single substrates (Harun, 2021). Thus, a focused exploration into how substrate mixtures influence mushroom cultivation could greatly benefit the agricultural sector there.

MATERIALS AND METHODS

The study was conducted to evaluate the impact of different types of supplements and their combination on the growth and productivity of Oyster mushrooms. The experimental activities were done at the Mushroom Development Institute (MDI) in Savar, Dhaka. This study was spanned from October 2023 to January 2024.

Materials: The cultivation of a specific variety of Oyster mushroom, namely *Pleurotus ostreatus* (PO_2), was carried out within the control environment (20±2°C) at Mushroom Development Institute's culture house. The strains utilized for this scientific endeavor were sourced from the extensive collection maintained by the Germplasm Center at the Mushroom Development Institute, Savar, Dhaka. These specimens underwent a meticulous process of culture in the biotechnology laboratory of the institute before being transferred to the culture house for the cultivation phase. This step was critical in ensuring the growth and development of the mushrooms under optimal conditions.

Treatments: The experimental setup comprises ten treatments (T_1-T_{10}) of several supplements with different combinations for 100 spawn packets, and each treatment is replicated sixteen times. The treatments are as follows:

T_1 = Wheat bran 8kg
T_2 = Wheat bran 6kg + Straw 2kgT_3 = Wheat bran 6kg + Maize powder 2kg
T_4 = Maize powder 8kg
T_5 = Maize powder 6kg + Straw 2kg
T_6 = Maize powder 6kg + Wheat bran 2kg
T_7 = Rice bran 8kg

T_8 = Rice bran 6kg + Straw 2kg
T_9 = Rice bran 6kg + Wheat bran 2kg
T_{10} = Rice bran 2kg + Wheat bran 2kg + Maize powder 2kg + Straw 2kg

Design and layout: The structure of the experiment was based on a single-factor Completely Randomized Design (CRD) framework. This design facilitated the evaluation of 10 distinct treatments. Each of these treatments was subjected to sixteen separate instances of replication, ensuring a robust data set for analysis. Within each replication, three spawn packets were utilized. This comprehensive layout aimed to maximize the reliability and validity of the experimental results by thoroughly investigating the effects of the treatments under the study.

Preparation of mother culture: To produce a mother culture packet, several key components are utilized in the preparation process. These components include brown paper, rubber bands, cotton plugs, plastic necks, sawdust, wheat bran, $CaCO_3$, and water. Sawdust served as the primary substrate, complemented by wheat bran as a supplement with a ratio of 300 grams of each component per mother packet. This blend was meticulously mixed before being carefully packed into heat-resistant polypropylene bags measuring 6 inches by 8 inches. The packets underwent autoclaving at 121°C and 15 PSI pressure for 1 hour, after which they were allowed to cool naturally. Each mother packet was then inoculated with a pure culture at a precise rate of 5 square millimeters per packet.

Preparation of spawn packet: A precise blend of various supplements with substrates was accurately portioned and loaded into polypropylene bags according to treatments. Each measuring 7 inches by 10 inches and containing roughly 500 grams of the blend. For the preparation of spawn packets, sawdust was used as the primary substrate, and various ratios of the mixture were combined as the treatment for this experiment. A total weight of 500 grams and a moisture content of 60% was maintained. These packets were then placed in an autoclave where they underwent sterilization at a temperature of 121°C and 15 PSI pressure for 2 hours. After sterilization, the packets were allowed to cool before being moved to the inoculation chamber. They remained there for 24 hours and after that time, these packets were kept at a temperature of 22±2°C in the culture house.

Mycelium running in spawn packet: To grow mycelium running, the packets were kept at 20±2°C. temperature until the packets become white with mushroom mycelium. After completing mycelium running, the rubber band, cotton plug, and plastic neck of the mouth of the spawn packet were removed and the mouth was wrapped tightly with the rubber band. Then these spawn packets were transferred to the culture house.

Opening the spawn packet: Both ends at the top of the plastic bag were meticulously shaped into a 'D' configuration using a blade, then the cut sections were peeled away to expose the substrate. To ensure optimal growth conditions, the humidity in the growing area was regulated to remain within 80-85% relative humidity with maintained light at 300-500 lux, with ambient growing temperature was kept between 22-25°C. This condition is critical for enhancing the initiation of primordia.

Harvesting of mushroom: Oyster mushrooms reached maturity 2-3 days post-primordia formation, with the developed fruiting body being characterized by the distinctive margin

of the cap, as outlined by Amin (2002). The harvesting process involved twisting the mushrooms to detach them from their base.

Data collection: Data was collected on two parameters viz. growth parameter and yield contributing parameter. Data on days required to complete mycelium running, days required for pinhead initiation, pinhead to 1st harvest, number of fruiting body and effective fruiting body, length and diameter of stalk (cm), diameter and length of pileus (cm) and biological efficiency (%) were recorded. Biological efficiency was determined by the following formula:

$$\text{Biological efficiency} = \frac{\text{Total biological weight (g)}}{\text{Total dry weight of substrate used (g)}} \times 100$$

Statistical Analysis: The collected data on various parameters underwent statistical analysis with the use of the Statistics 10 software, applying the Analysis of Variance (ANOVA) method. To adjust mean discrepancies, Duncan's Multiple Range Test (DMRT) as described by Gomez and Gomez in 1984 was utilized. Additionally, the comparison of mean differences across treatments was conducted using the Least Significant Difference (LSD) test, with a significance threshold set at 5%.

RESULTS AND DISCUSSION

Days to complete mycelium running: Different substrate supplementation effects were shown to have a significant impact on mycelium run completion days (Table 1). The data range from 20.70-25.00 days, with T10 exhibiting the shortest duration and T8 the longest. The highest value is observed in T8 (25.00 days), where rice bran is combined with straw, which is statistically identical to T_4 (Maize powder 8kg), while the lowest value is found in T_{10} (20.70 days), which includes a combination of rice bran, wheat bran, maize powder, and straw. The results indicate that T_{10}, which incorporates a diverse substrate mixture, promotes the fastest mycelium running, suggesting that a combination of rice bran, wheat bran, maize powder, and straw provides optimal conditions for mycelial growth. Harun (2021) and Mkhize *et al.*, (2016) reported that the different substrates offer diverse nutrient compositions, allowing for a broader range of nutrients available for mushroom growth, thereby enhancing yield.

Days required from pin head initiation to 1st harvest: When different supplements were introduced to the substrates, a significant effect was seen on pin head initiation to first harvest of mushroom (Table 1). The range of days to first harvesting varies from 3.14 to 5.59 days, indicating a notable difference in the effectiveness of different treatments in facilitating earlier harvesting. The treatment T_{10} had the shortest time to first harvesting (3.14 days), while T_8 had the longest (5.59 days). Harun (2021) and Hoa *et al.*, (2015), implied that combined effect of different substrate supplements might have beneficial effects on the growth cycle.

Number of fruiting body: A significant impact was observed on number of fruiting bodies (Table 1) under various treatments, with each treatment representing a distinct substrate composition. The range of values observed in the experiment varied from 35.50 to 48.00 fruiting bodies. The highest number of fruiting bodies was recorded in T_{10}, which comprised a combination of rice bran, wheat bran, maize powder and straw. Conversely, the lowest number of fruiting bodies was observed in T_8, which is statistically identical to T_4 and T_7.

Number of effective fruiting body: Likewise, a significant impact was also observed on number of effective fruiting bodies (Table 1). The range of values observed in the experiment varied from 11.3 to 18.00 fruiting bodies. The highest number of effective fruiting bodies was recorded in T_{10}, while the lowest number of fruiting bodies was observed in T_8.

Yield of oyster mushroom: Different substrate supplementation effects were shown to have a significant impact on mushroom yield per packet (Table 1). The yield per packet ranges from 151.33 grams (T_8) to 212.06 grams (T_{10}), indicating significant variability across treatments. The highest yield is observed in T_{10}, while the lowest yield was recorded in T_8 treatment.

Table 1. Impact of various supplements on growth and yield of oyster mushroom

Treatment	Days to complete mycelium running	Days to first harvesting	Number of fruiting bodies	Number of effective fruiting bodies	Yield (gm/ packet)
T_1	22.5 a	3.57 fg	44.2 ab	15.3 ab	190.63 a
T_2	23.2 a	3.83 e	41.9 b	12 c	180.51 bc
T_3	22.3 a	3.40 g	45.3 a	15 ab	199.58 a
T_4	24.9 a	5.13 b	36.1 c	11.7 c	154.53 ef
T_5	23.7 a	4.17 d	39.1 b	12 c	174.04 c
T_6	23.1 a	3.72 ef	40.3 b	14 b	185.6 b
T_7	24.3 a	4.57 c	36.7 c	14.7 b	157.4 e
T_8	25 a	5.59 a	35.5 c	11.3 c	151.33 f
T_9	24 a	4.47 c	38.47 c	13.1 b	165.32 d
T_{10}	20.7 a	3.14 h	48 a	18 a	121.06 g
CV%	4.06	2.25	4.19	4.42	4.03
LSD (0.05)	0.1594	0.1706	0.2776	0.9438	13.925

Length of stalk: When different supplements were introduced to the substrates, a significant effect was seen on stalk length of mushroom (Table 2). The data range from a minimum of 3.14 cm for T_8 to a maximum of 5.07 cm for T_{10}, which is statistically similar to T_3, indicating substantial variability in stalk length across treatments. T_{10} exhibits the highest stalk length, while T_8 shows the lowest. The results suggest that the inclusion of wheat bran, either alone or in combination with other ingredients, tends to positively influence stalk length (Table 1).

Diameter of stalk: Different substrate supplementation effects were shown to have a significant impact on stalk diameter of mushroom (Table 2). The range of stalk diameters observed in the study varied from 0.85 cm to 1.43 cm. The lowest diameter was recorded in T_8, while the highest diameter was observed in T_{10} which is statistically identical to T_3.

Length of pileus: Significant impact was detected on pilius diameter when various supplements were added to the substrates (Table 2). The range of diameter values observed in the study varied from 6.03 cm (T_8) to 8.70 cm (T_{10}). Treatment T_{10} exhibited the highest diameter of pilius, which is statistically identical to T_3, while T_8 showed the lowest value, which is statistically identical to T_4.

Thickness of pileus: The thickness of pilus (cm) across ten different treatments presented in Table 2. The data range from a minimum thickness of 0.57 cm in Treatment T_8 to a maximum thickness of 0.95 cm in Treatment T_3. Among the treatments, Treatment T_3 exhibited the highest thickness of pilus, while Treatment T_8 showed the lowest thickness. These results indicate significant differences in the effect of feed composition on pilus thickness.

Table 2. Impact of various supplements on size of fruiting body of oyster mushroom

Treatment	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1	4.53 b	1.27 b	7.88 b	0.84 bc
T_2	4.17 cd	1.15 cd	7.23 c	0.83 bcd
T_3	4.87 a	1.37 a	8.33 a	0.95 a
T_4	3.31 gh	0.92 f	6.21 fg	0.63 g
T_5	3.97 de	1.10 de	7.03 cd	0.75 ef
T_6	4.37 bc	1.21 bc	7.43 c	0.80 cde
T_7	3.47 fg	1.03 e	6.50 ef	0.71 f
T_8	3.14 h	0.85 g	6.03 g	0.57 g
T_9	3.77 ef	1.08 de	6.68 de	0.76 def
T_{10}	5.07 a	1.43 a	8.70 a	0.89 ab
CV%	5.57	5.90	3.21	6.73
LSD (0.05)	0.3209	0.0675	0.4328	0.0707

Biological efficiency: The biological efficiency (%) of different treatments in the cultivation process is shown in Fig. 1. The biological efficiency values range from 57.84% to 81.05%, with Treatment T_{10} exhibiting the highest efficiency at 81.05%, while Treatment T_8 showed the lowest efficiency at 57.84%. The results suggest that a balanced combination of wheat bran, maize powder, rice bran, and straw (as demonstrated in Treatment T_{10}) could significantly enhance biological efficiency in the cultivation process. A similar trend was observed by Patil *et al.*, (2010), and Hasan *et al.*, (2010).

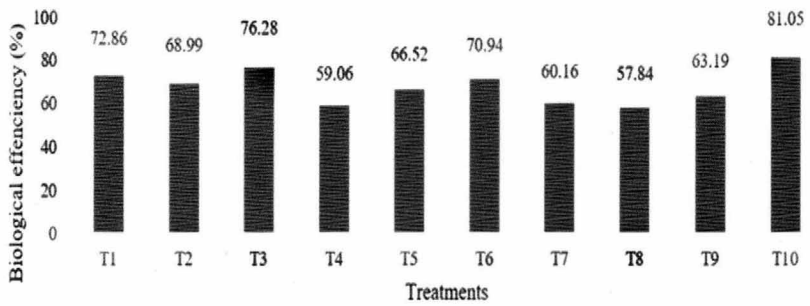

Fig. 1. Impact of various supplements on biological efficiency % of oyster mushroom cultivation.

The experiment highlights the significant influence of supplement treatments on the growth, yield, and efficiency of substrate-based oyster mushroom cultivation, with Treatment T_{10} which was a combination of all the supplements with equal ratio consistently achieving the best results across all growth and yield parameters. In contrast, Treatment T_8 showed slower growth, lower yields, and reduced biological efficiency, indicating it as the least effective supplement.

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Identification of Causal Organism of Orange Mold Contamination and Disclosed Its Pathogenicity with Oyster Mushroom

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Abstract

Mold contamination, particularly from green and orange molds, poses a significant threat to mushroom cultivation during the spawn running stage, especially in hot and humid summer conditions. While green mold has been extensively studied, orange mold remains relatively underexplored. This study investigates orange mold contamination with the aim of identifying the causal agent, evaluating its pathogenicity, and exploring potential control measures. Internal Transcribed Spacer (ITS) region sequencing confirmed *Neurospora sitophila* as the causative organism. Pathogenicity was assessed using the dual confrontation plate method, which revealed that *N. sitophila* hinders mushroom mycelium colonization by competing for nutrients and space. These findings underscore the need for targeted management strategies to mitigate orange mold outbreaks in mushroom cultivation.

Keywords: *Neurospora sitophila*, Fungal pathogen, Mushroom disease, ITS.

INTRODUCTION

Mushrooms are extensively valued for their nutraceutical and therapeutic benefits and serve as key recyclers in the natural ecosystem by decomposing organic substrate. Successful commercial cultivation of edible mushrooms relies heavily on the quality of the growing substrate, including the carbon-nitrogen (C/N) ratio, the amounts of cellulose, hemicellulose, and lignin (Suwannarach *et al.*, 2022; Balan *et al.*, 2022). Environmental conditions, particularly high humidity, and optimal temperature are crucial for the establishment of mushroom mycelium on the substrate (Zhan *et al.*, 2021). However, unwanted intrusions of foreign microorganisms such as various mold, bacteria, insects, and mites pose challenges for mushroom cultivation.

In recent years, the threat of mold contamination in the mushroom industry has intensified due to climate change and rising temperatures. Competitor molds, like green and orange mold significantly hinder the colonization of mushroom mycelium on the substrate. Green mold outbreaks, caused primarily by Trichoderma, and sporadically by other fungi such as Penicillium and Aspergillus, have been a persistent issue in oyster mushroom cultivation across the globe (Allaga *et al.*, 2021; Ahedo *et al.*, 2024; Šašić Zorić *et al.*, 2023; Cao *et al.*, 2024). Similarly, orange mold, while a more recent concern in Bangladesh, poses a serious threat to the mushroom industry. This issue has been notably problematic during the spawn running stage, where distinctive orange color contamination rapidly overwhelms the farms, often within a week. The problem is exacerbated during the moist summer months, with temperatures exceeding 32°C and relative humidity reaching 70 - 80%. Moreau, 1956 asserted that orange mold on mushroom

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beds is caused by the obligatory aerobes Neurospora spp., which naturally thrive in moist tropical or subtropical climates, as their latent ascospores are activated by high temperatures. Despite being a contaminant in the mushroom industry, Neurospora spp. has been utilized in the food industry, particularly as a pigment producer in the traditional Indonesian dish oncom merah (Nout and Aidoo, 2011). Interestingly, no evidence has been obtained that Neurospora is the causal agent of any disease or infection in humans and animals (Perkins and Davis, 2000). The research focused on identifying and characterizing the organisms responsible for orange mold and assessing their pathogenicity concerning *Pleurotus ostreatus*.

MATERIALS AND METHODS

Pure culture preparation and microscopic observation of orange mold: Orange mold-contaminated sawdust spawn packets were gathered from the local mushroom farm in Savar, Dhaka. A 10 gm of sawdust sample was taken into a 100 mL conical flask containing 90 mL of sterile distilled water, and then shaken briefly to ensure thorough mixing. A 10^{-3} dilution was prepared from this mixture, and 1mL aliquots were spread onto a Petri dish containing Potato Dextrose Agar (PDA). After incubating the dishes at room temperature for 24 hours, a pure culture of the orange mold was obtained. This pure culture was then subjected to microscopic examination to observe its morphological characteristics. A loopful of the culture was placed on a slide, stained with lactophenol cotton blue, and observed under a light microscope at 40x magnification.

Molecular identification of the mold and phylogenetic tree: Molecular identification of the fungus was carried out using PCR amplification and sequencing of the Internal Transcribed Spacer (ITS) region of rDNA with universal fungal primers. The Internal Transcribed Spacer (ITS) region is a highly variable genetic marker found in all fungal species, making it an essential tool for distinguishing closely related species (White *et al.*, 1990). The ITS region comprises two variable segments, ITS1 and ITS2, which are separated by the more conserved 5.8S rRNA gene (Schoch *et al.*, 2012)the second largest kingdom of eukaryotic life, by a multinational, multilaboratory consortium. The region of the mitochondrial cytochrome c oxidase subunit 1 used as the animal barcode was excluded as a potential marker, because it is difficult to amplify in fungi, often includes large introns, and can be insufficiently variable. Three subunits from the nuclear ribosomal RNA cistron were compared together with regions of three representative protein-coding genes (largest subunit of RNA polymerase II, second largest subunit of RNA polymerase II, and minichromosome maintenance protein. Typically, ITS4 refers to the region that includes ITS2 and occasionally parts of the flanking regions (Gardes and Bruns 1993). DNA extraction and quantification of the DNA followed the protocol provided with the Maxwell Blood DNA extraction kits (Model AS1010, Promega Corp, Madison, WI, USA). The mold DNA was further purified using the Wizard® Genomic DNA purification Kit (A1120, Promega Corp., Madison, WI, USA). The extracted DNA was quantified at 40.9ng/ µL using a NanoDrop 2000c Spectrophotometer. PCR reactions were carried out in a final volume of 25µL using the GoTaq ® Green Master Mix Kit (M7122, Promega Corp, Madison, WI, USA), with a concentration of 1x. The reaction mix included 5µM of the ITS1-Forward primer, 5µM of ITS4-Reverse primer, and 25 ng of genomic DNA. Amplification was performed in a C1000 thermal cycler (Bio-Rad® Germany) following the protocol shown in (Fig. 1). The PCR products were separated by agarose gel electrophoresis (2%) at 80V for 40 minutes and visualized using SYBR Gold® (Invitrogen, Carlsbad, CA, USA). DNA fragments of approximately 700

base pairs were selected for purification using ExoSAP-IT (N/P 78200, USB Affymetrix, Inc., Cleveland, OH, USA). The purified fragments were sequenced with the ABI PRISM BigDye® Terminator sequencing kit v3.1 (P/N4336917, Applied Biosystems, Foster City, CA, USA) using the ITS1 forward primer (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4-Reverse primer (5′-TCCTCCGCTTATTGATATGC-3′). Fragment analysis was conducted on a Genetic Analyzer 3130 sequencer (Applied Biosystems® HITACHI Tokyo, Japan). Sequences were assembled using SeqMan software 8 (LaserGene) (DNASTAR®. Madison, WI, USA) and analyzed using the GenBank database ("National Center for Biotechnology Information," n.d.).The top hit from the BLAST analysis in GenBank was used to identify the fungal species.

Dual confrontation assays: A dual culture technique was employed for assessing the aggressiveness of *Neurospora sitophila* isolates on *Pleurotus ostreatus*. Initially, 6 mm diameter agar plugs of *P. ostreatus* mycelium were positioned 1.5 cm from the edge of PDA Petri dishes and incubated at room temperature for 5 days to allow mycelial growth. Notably, *P. ostreatus* demonstrated a significantly slower growth rate, taking approximately 12 to 15 days to achieve full mycelial coverage. Afterward, similarly prepared mycelial plugs of *N. sitophila*, were placed on the opposite side of the same plate, also 1.5 cm away from the edge. The plates were then incubated for an additional 24 hours at ambient temperature as *N. sitophila* exhibited rapid mycelium expansion. The interaction between the *N. sitophila* and *P. ostreatus* mycelia was meticulously observed and documented.

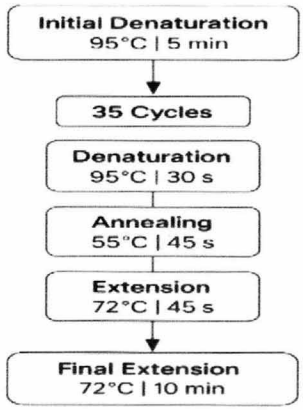

Fig. 1. Flowchart of the PCR protocol performed to amplify the DNA of orange mold.

RESULTS AND DISCUSSION

Isolation, morphological characterization, and pathogenicity of orange mold: To investigate the pathogen responsible for orange mold contamination, fungi were isolated from the contaminated spawn packet (Fig. 2A) and cultured on PDA media. The results showed that the young colony of fungi appeared off-white with a fluffy or cottony texture (Fig. 2B). As the colony matured, its color transitioned to orange (Fig. 2C). Microscopic examination of the isolates revealed distinctive features characteristic of *Neurospora sitophila,* including branched, septate hyphae, abundant conidiophores that are simple or slightly branched, and the production of conidia in chains. The conidia were unicellular and oval to elliptical in shape. These morphological characteristics supported the identification

of the isolate as *Neurospora* spp. (Fig. 2D). According to Koch's postulate, the pure culture was inoculated onto a sawdust substrate to verify whether isolated fungi were responsible for orange mold (Byrd and Segre, 2016) Robert Koch established his famous postulates as stringent guidelines to evaluate causation in infectious disease. The characteristic orange color symptoms subsequently developed in the newly inoculated sawdust packets, indicating that the isolated fungus is a causal organism of orange mold. The fungus starts as an orange-white wisp but rapidly transforms into a bright orange, powdery patch. If allowed to progress, these patches develop into round, lumpy formations. Considering the above characteristics, the selected isolated fungi presumed *Neurospora* spp.

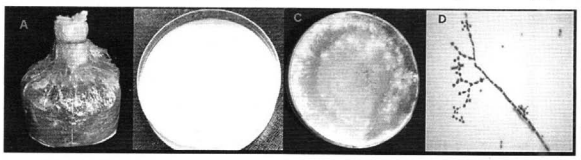

Fig. 2. Morphological study of the causal organism of orange mold contamination: A. contaminated spawn packet, B. initial colony of the isolate, C. matured colony of the isolate, and D. microscopic observation of the isolate under a compound microscope at 40X.

Study of DNA sequencing and phylogenetic analysis: Identification based on cultural features was confirmed by DNA sequence analysis of the isolates. In this study, the ITS region of a presumptive *Neurospora* species was amplified, and the resultant sequence, designated as Neurospora_ITS_4, was analyzed. Basic Logical Alignment Search Tool (BLAST) results of ITS region in the National Centre for Biotechnology Information (NCBI) database revealed relationships and similarities with reference sequences in GenBank. DNA sequencing of the ITS 4 region showed the highest similarity to *Neurospora sitophila* (Accession No. ON712132.1) with 100% query coverage and 100% identity match, followed by *Neurospora crassa* with 100% query coverage and 98% identity match (Accession No. MH790467.1). A phylogenetic tree was constructed using BLAST Tree View provided by the National Center for Biotechnology Information (NCBI) BLAST tool, incorporating Neurospora_ITS_4 and other highly similar sequences identified in the BLAST analysis. The phylogenetic tree revealed that Neurospora_ITS_4 clusters closely with *Neurospora crassa* (MH790549.1) and *Neurospora sitophila* (OW982620.1), indicating a close evolutionary relationship with these species. This placement suggests that Neurospora_ITS_4 is the *Neurospora* genus fungal species shown in Fig. 3.

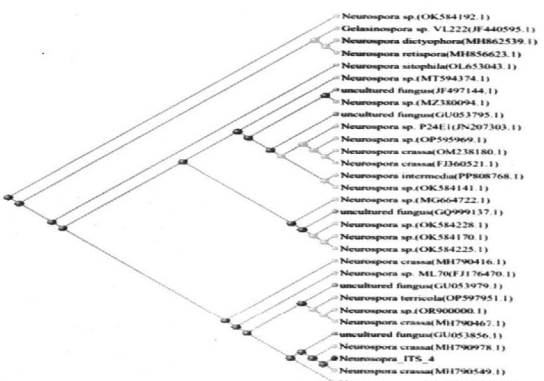

Fig. 3. Phylogenetic tree based on ITS gene using Blast Tree View. Branch length indicates evolutionary distance.

Study of dual confrontation assays: The plate dual culture experiments were conducted to assess the impact of *N. sitophila* on *P. ostreatus*. The results revealed that *N. sitophila* neither inhibited nor exhibited any antagonistic effect on the growth of *P. ostreatus* (Fig. 4). However, within 24 hours, *Neurospora* mycelium had completely overrun the mushroom mycelium. The mycelium growth of *Neurospora* was notably faster than that of *P. ostreatus*, eventually producing an irregular cluster of orange-red conidial as it expanded, the same as shown in Fig. 2C.

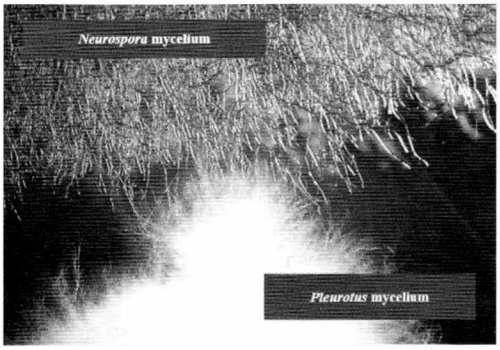

Fig. 4. Dual cultivation of *Neurospora* and *Pleurotus* mycelium on PDA media.

This study intended to identify the organism responsible for orange mold, understand its pathogenic effect on *Pleurotus ostreatus*, and determine effective control measures. Following Koch's postulates, the isolated pure culture of mold displayed the characteristic orange color when reintroduced to new sawdust packets, confirming its role in causing orange mold. The colony morphology and the presence of macroconidia observed under a bright light microscope suggested that this fungus is likely *Neurospora spp.* (Kuo *et al.*, 2014). It is noted, however, that identifying fungal species solely through morphological and microscopic studies can be challenging without concurrent DNA analysis. To further confirm the identity, ITS-4 region was sequenced followed by the BLAST search against ITS-4 region showed a 100% query coverage and 100% identity match with the *Neurospora sitophila* (Accession No. ON712132.1) and 100% query coverage and 98% identity match, with *Neurospora crassa* (Accession No. MH790467.1). Furthermore, molecular phylogenetic tree analysis revealed that Neurospora_ITS_4 clusters closely with *Neurospora crassa* (MH790549.1) and *Neurospora sitophila* (OW982620.1), indicating a close evolutionary relationship with these species. Thus, this study concluded that the causal entity of orange mold is *Neurospora sitophila.*

To examine the nature of *Neurospora sitophila* pathogenicity with the oyster mushroom *Pleurotus ostreatus*, an in vitro confrontation assay was meticulously carried out. According to several studies, fungal pathogenicity mechanisms can be broadly categorized into antagonism, inhibition, and competition (Asad, 2022; Zeilinger *et al.*, 2016). Antagonism is characterized by the mycelium growing in the opposite direction, leading to the formation of mycelial cords. Inhibition is marked by the presence of a distinct clear zone, primarily caused by the release of enzymes or metabolites. Competition, however, arises from the rivalry for space and nutrition. The results revealed that the rapidly growing *Neurospora* mycelium covered the entire Petri dish within 24 hours, forming irregular orange-red conidial clusters over time. These findings indicate that *Neurospora* significantly impedes mushroom

mycelium colonization on lignocellulose substrate by aggressively competing for space and nutrients rather than through antagonism or inhibition. This aggressive competition resulted in the demise of mushroom mycelium growth and the eventual destruction of the mushroom spawn packets. A similar pattern of pathogenicity has also been extensively documented in *Trichoderma* spp. (Lombardi *et al.*, 2023; Allaga *et al.*, 2021).

CONCLUSION

This study successfully identified *Neurospora sitophila* as the causal agent of orange mold contamination in mushroom cultivation, specifically affecting *Pleurotus ostreatus*. Morphological characterization, microscopic examination, and molecular identification through ITS region sequencing and phylogenetic analysis confirmed the identity of the pathogen. Pathogenicity tests following Koch's postulates validated the fungus's role in producing characteristic orange mold symptoms on inoculated substrates. The dual confrontation assay revealed that *N. sitophila* does not inhibit or antagonize mushroom mycelium directly, but instead exerts its pathogenic effect through rapid growth and competitive exclusion for space and nutrients. This competitive mechanism significantly hinders the colonization and development of *P. ostreatus*, ultimately leading to the failure of spawn packets. These findings highlight the importance of early detection and targeted control strategies to mitigate *N. sitophila* contamination and ensure healthy mushroom production.

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Molecular Profiling of Novel Mushroom Germplasm from Different Strains of King Oyster and Button Mushrooms Available at Mushroom Development Institute for Conservation Purposes

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Abstract

This study was undertaken to assess the genetic diversity and molecular identity of newly introduced strains of *Pleurotus eryngii* (King Oyster Mushroom) and *Agaricus bisporus* (Button Mushroom) maintained at the Mushroom Development Institute (MDI), Bangladesh, with a focus on supporting germplasm conservation. Molecular characterization was performed using ITS4 and ITS5 primers targeting the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. Genomic DNA was extracted, amplified, and sequenced from five isolates: Pe-2 and Pe-T (*P. eryngii*), and Ab-in, Apex-2, and Apex-3 (*A. bisporus*). The resulting amplicons ranged from 627 to 699 base pairs. Sequence alignment and BLAST analysis revealed over 97% similarity with reference sequences in GenBank. Evolutionary divergence, calculated using the Tamura-Nei model, indicated low intra-species variation between the *P. eryngii* strains (0.0079) and between *A. bisporus* strains Apex-2 and Apex-3 (0.0051), while Ab-in exhibited greater divergence from both. Phylogenetic analysis using the Neighbor-Joining method showed clearly separated clades for the two genera, with closely related strains clustering together. These results confirm that ITS-based molecular profiling is an effective and reliable tool for distinguishing mushroom strains, offering valuable insights for conservation strategies and future breeding programs at MDI.

Keywords: *Pleurotus eryngii*, *Agaricus bisporus*, ITS sequencing, Molecular identification, Mushroom germplasm.

INTRODUCTION

Mushrooms represent a diverse and economically important group of fungi that serve as a source of food, medicine, and ecological services. Among edible fungi, oyster mushrooms (*Pleurotus* spp.) and button mushrooms (*Agaricus bisporus*) are among the most widely cultivated species worldwide, attributed to their rich nutritional profile, low production cost, and relatively simple cultivation techniques (Chang & Miles, 2004). In addition to their culinary value, these mushrooms are known for their bioactive compounds with potential antimicrobial, antioxidant, and immunomodulatory properties (Heleno *et al.,* 2015).

In Bangladesh, mushroom cultivation has seen significant growth over the past two decades, particularly among small-scale farmers and rural entrepreneurs. This expansion is supported by favorable climatic conditions, increased public awareness, and governmental initiatives aimed at improving nutrition and income generation. The Mushroom Development Institute (MDI), the country's principal research and development center for mushrooms under the Department of Agricultural Extension (DAE), plays a crucial role in strain development, extension services, and the dissemination of cultivation technologies. MDI maintains a collection of both local and exotic mushroom germplasm, with a strong focus on species adaptation, yield improvement, and conservation.

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Despite the increased popularity of mushroom cultivation in Bangladesh, systematic molecular characterization of the available strains remains limited. Traditional methods of morphological and phenotypic identification are often insufficient for distinguishing between closely related strains or identifying cryptic diversity. Therefore, molecular profiling techniques offer a more reliable and reproducible approach to assess genetic variation, identify novel or elite germplasm, and inform breeding and conservation strategies.

The Internal Transcribed Spacer (ITS) region of ribosomal DNA has been widely adopted as a universal DNA barcode for fungi due to its high interspecific variability and ease of amplification (Schoch *et al.*, 2012). Phylogenetic analysis based on ITS sequences enables researchers to resolve evolutionary relationships among mushroom strains and supports accurate taxonomic identification. This study aims to conduct a molecular profiling of selected strains of *Pleurotus eryngii* and *Agaricus bisporus* mushrooms preserved at the Mushroom Development Institute (MDI).

MATERIALS AND METHODS

The experiment was conducted at Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh in 2024. In this experiment, newly introduced mushroom varieties Pe-2 and Pe-T of *Pleurotus eryngii* and Ab-in, Apex-2, and Apex-3 of *Agaricus bisporus* were selected and grown at the Mushroom Development Institute's culture house. DNA isolation, fingerprint, and genetic chromatography were examined in the Invent Technology.

Molecular Identification

DNA extraction and purification: Genomic DNA was extracted from the mushrooms fruiting bodies using the Maxwell® Blood DNA Extraction Kit (Model AS1010, Promega Corp., Madison, WI, USA) according to the manufacturer's protocol. The extracted DNA was purified using the Wizard® Genomic DNA Purification Kit (A1120, Promega Corp.). DNA concentration and purity were assessed using a NanoDrop 2000c Spectrophotometer (Thermo Scientific), with a final concentration measured at 40.9 ng/μL.

PCR amplification of ITS region: The Internal Transcribed Spacer (ITS) region of the nuclear ribosomal DNA (rDNA) was amplified using the primer pair ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). PCR reactions were carried out in a 25 μL total volume using the GoTaq® Green Master Mix Kit (M7122, Promega Corp.) at a 1× final concentration. Each reaction contained 5 μM of each primer and 25 ng of template DNA. Amplification was performed in a C1000 Touch Thermal Cycler (Bio-Rad®, Germany) using the following thermal profile (Fig. 1).

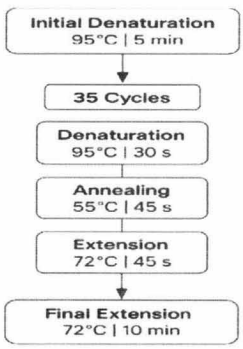

Fig. 1. The flowchart of the PCR protocol performed to amplify the DNA of the King oyster and the Button mushroom varieties.

Gel electrophoresis and purification: PCR products were separated by electrophoresis on a 2% agarose gel at 80 V for 40 minutes and visualized under UV light using SYBR® Gold stain (Invitrogen, Carlsbad, CA, USA). Amplicons of approximately 700 base pairs were selected and purified using ExoSAP-IT® (N/P 78200, USB Affymetrix, Inc., Cleveland, OH, USA) to remove excess primers and dNTPs.

DNA sequencing: Sequencing was performed using the ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit (P/N 4336917, Applied Biosystems, Foster City, CA, USA) with both ITS5 and ITS4 primers. Sequence reactions were analyzed on an ABI 3130 Genetic Analyzer (Applied Biosystems® HITACHI, Tokyo, Japan). Raw sequences were assembled and edited using SeqMan v8 (DNASTAR®, Madison, WI, USA). The resulting Sanger sequences were processed using Chromas 2.6. software to generate a FASTA file containing the partial sequence. Species identification was performed by comparing the assembled ITS sequences against the GenBank database using BLASTn. The species was determined based on the highest identity match.

Sequence analysis: The FASTA files of all the obtained sequences were analyzed through nucleotide BLAST by submitting the sequences in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to match with existing sequences in the GenBank and obtained accession numbers. The estimation of evolutionary divergence between sequences was analyzed using the Tamura-Nei model. After that, the evolutionary history was inferred using the Maximum Likelihood method and the Tamura-Nei model (Tamura and Nei, 1993). The bootstrap consensus tree inferred from 1000 replicates was constructed to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model and then selecting the topology with superior log likelihood value. This analysis involved 5 nucleotide sequences. Codon positions included were 1st+ 2nd+ 3rd+ Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were performed in MEGA11 software (Tamura *et. al.,* 2021).

RESULTS AND DISCUSSION

Molecular Identification of Oyster and Button Mushroom Varieties

A molecular approach was performed and two King Oyster and three Button mushroom varieties were identified. The identified mushroom varieties are shown in (Table 1).

Table 1. Identification of newly introduced oyster and button mushroom varieties by molecular analysis at Mushroom Development Institute

Sl. No.	Name of the mushroom	Scientific name	Variety name
01.	King Oyster Mushroom	*Pleurotus eryngii*	➢ Pe-T ➢ Pe-2
02.	Button Mushroom	*Agaricus bisporus*	➢ Ab-in ➢ Apex-2 ➢ Apex-3

Study on PCR products: The Gel-Doc system visualized PCR products of mushroom isolates generated by ITS4 and ITS5 primers, which resulted in the DNA bands typically ranging between 500-750 base pairs (bp). Variability in amplicon sizes ranging from 627 (isolate

Pe-T) to 699 (isolate Apex-3) bp was observed in the GenBank database. The generated DNA bands indicated the successful PCR amplification, which were extremely similar in size to the target band. The size of the amplified DNA fragment was verified using a Bench Top 1 kb DNA ladder, which acts as a size marker. Fig. 2. (Lanes 1-5) shows the successful amplification of DNA bands obtained from several mushroom isolates. These confirm the specificity and successful amplification of the desired DNA fragments under the utilized primer and PCR conditions, as demonstrated by the Gel-Doc system.

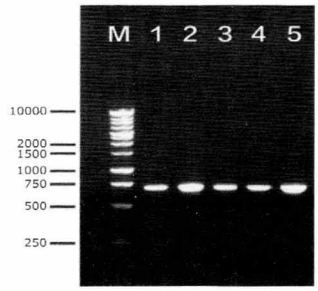

Fig. 2. PCR amplified products of mushroom obtained from 1. *Pleurotus eryngii* isolate Pe-T, 2. *Pleurotus eryngii* isolate Pe-2, 3. *Agaricus bisporus* isolate Ab-in, 4. *Agaricus bisporus* isolate Apex-2, and 5. *Agaricus bisporus* isolate Apex-3. M: denotes 1kb DNA ladder (Marker).

Analysis of DNA Sequences: The obtained FASTA sequences were analyzed using the BLAST tool on the NCBI website. These sequences were found to match existing nucleotide sequences in the NCBI GenBank database. All the sequences exceeded 97% (97.02-99.20%) homology with their corresponding sequence. Subsequently, unique accession numbers were assigned to the partial sequences for five mushroom isolates (Table 2).

Estimation of Evolutionary Divergence Between Sequences: Estimates of evolutionary

Table 2: Results of the mushroom's gene identification with the BLAST Program

Lab Strain			BLAST Alignment				
Sl. no.	Identified Species	Obtained accession no.	Species	Query coverage	E value	Percent identity	Accession no.
1.	Pleurotus eryngii isolate Pe-T	PV643228.1	Pleurotus eryngii isolate tw1	99%	0.0	97.02%	AY589047.1
			Pleurotus eryngii strain NAAS00290	99%	0.0	97.02%	JN043320.1
2.	Pleurotus eryngii isolate Pe-2	PV643227.1	Pleurotus eryngii isolate PET1	99%	0.0	97.52%	HM561985.1
			Pleurotus eryngii strain ATCC	99%	0.0	97.21%	OL687127.1
3.	Agaricus bisporus isolate Ab-in	PV643229.1	Agaricus bisporus strain Korean	98%	0.0	99.20%	OQ699263.1
			Agaricus bisporus voucher HGASMF01-16467	99%	0.0	99.20%	ON557695.1
4.	Agaricus bisporus isolate Apex-2	PV643230.1	Agaricus bisporus isolate S7	98%	0.0	97.72%	MH394711.1
			Agaricus bisporus strain JUFD101	99%	0.0	97.44%	OR484862.1
5.	Agaricus bisporus isolate Apex-3	PV643231.1	Agaricus bisporus strain AB37	99%	0.0	98.21%	JN222415.1
			Agaricus bisporus strain KME59003	99%	0.0	98.06%	AF188033.1

divergence between the ITS sequences of the five mushroom isolates revealed varying degrees of genetic distance. The closest genetic relationship was observed between *Pleurotus eryngii* isolates Pe-2 and Pe-T, with a minimal evolutionary distance of 0.0079, indicating very low intra-species variation. This close similarity was also supported by a low standard error value of 0.0346.

Among *Agaricus bisporus* strains, isolates Apex-2 and Apex-3 showed a low divergence of 0.0051, suggesting a high genetic similarity between these two strains. In contrast, the Ab-in isolate of *A. bisporus* showed substantially higher divergence from both Apex-2 (0.2977) and Apex-3 (0.3031), as well as from *Pleurotus isolates* (ranging from 0.7608 to 0.7655), indicating greater genetic differentiation (Table 3).

Overall, the evolutionary divergence analysis clearly differentiates *Pleurotus* from *Agaricus*, and also highlights significant intra-species diversity among *A. bisporus* isolates.

Table 3. Pairwise evolutionary distances among *Pleurotus eryngii* and *Agaricus bisporus* isolates based on ITS sequences

	Isolate Pe-2	Isolate Pe-T	Isolate Ab-in	Isolate Apex-2	Isolate Apex-3
Isolate Pe-2		0.0079	0.0747	0.0264	0.0281
Isolate Pe-T	0.0346		0.0745	0.0274	0.0281
Isolate Ab-in	0.7608	0.7655		0.0888	0.0813
Isolate Apex-2	0.2867	0.2977	0.8070		0.0051
Isolate Apex-3	0.3115	0.3031	0.7839	0.0188	

Here, Values below the diagonal represent base substitutions per site; values above the diagonal represent standard error estimates obtained by 1000 bootstrap replicates under the Tamura-Nei model.

Analysis of Phylogenetic Tree: The phylogenetic trees were analyzed using the King Oyster and Button mushroom sequences, where the highest log likelihood was -2452.09. There was a total of 831 positions in the final dataset obtained from mushroom sequences. The phylogenetic analysis based on the internal transcribed spacer (ITS) region sequences revealed a clear separation between the two mushroom genera, *Pleurotus* and *Agaricus*. The dendrogram constructed using the Maximum Likelihood method in MEGA 11 shows that the *Pleurotus eryngii* isolates (Pe-2 and Pe-T) formed a highly supported monophyletic clade with a bootstrap value of 100%, indicating a close genetic relationship and low intra-species divergence (Fig. 3). The genetic distance between these two isolates was minimal, suggesting they are closely related variants or sub-strains.

Conversely, the *Agaricus bisporus* isolates showed more genetic variation. The Apex-2 and Apex-3 isolates grouped together in a distinct clade with a bootstrap support of 98%, reflecting high sequence similarity and potential common origin. However, the Ab-in isolate of *A. bisporus* formed a separate branch, exhibiting a notably larger genetic distance from both Apex-2&3 isolates (Fig. 3). This indicates significant genetic divergence, suggesting that the Ab-in isolate may represent a genetically distinct lineage within *A. bisporus*.

Overall, the phylogenetic tree successfully distinguished the mushroom strains by species and strain-level variation, validating the molecular profiling approach and highlighting the utility of ITS-based analysis for germplasm conservation and strain identification.

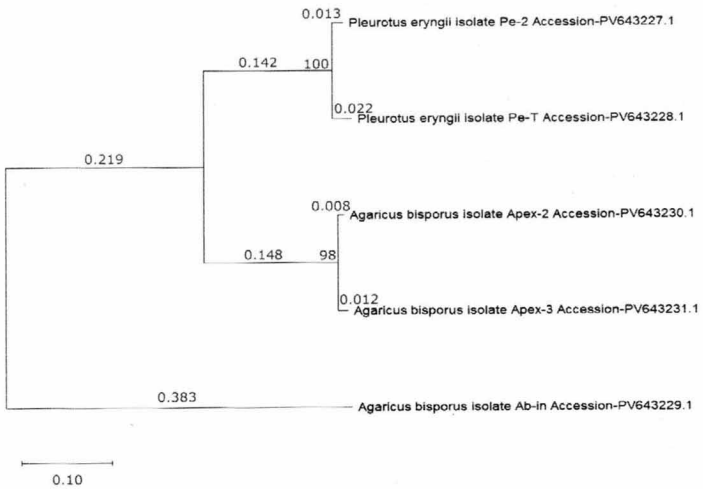

Fig. 3. Phylogenetic tree based on ITS region sequences of *Pleurotus eryngii* and *Agaricus bisporus* isolates using the Maximum-Likelihood method. Bootstrap values from 1,000 replicates are shown at branch nodes. The tree clearly separates the two genera into distinct clades.

CONCLUSION

The molecular characterization of *Pleurotus eryngii* and *Agaricus bisporus* strains at the Mushroom Development Institute (MDI) successfully demonstrated the effectiveness of ITS-based profiling for species identification and genetic differentiation. High sequence similarity with GenBank references confirmed the taxonomic identity of all five isolates, while evolutionary divergence analysis highlighted both intra- and inter-species variability. The close clustering of Pe-2 and Pe-T, as well as Apex-2 and Apex-3, reflects their genetic similarity, whereas the distinct divergence of the Ab-in isolate underscores the presence of potential strain-level variation within *A. bisporus*. These results provide a valuable genetic baseline for the conservation and selective breeding of mushroom germplasm in Bangladesh, reinforcing the utility of ITS markers in fungal genetic resource management.

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Effect of Harvesting Age on Texture, Colour, and Nutritional Composition of Black Mushroom (*Auricularia auricula*)

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Abstract

Mushroom quality is significantly influenced by the stage of maturity at harvest. Optimal harvesting time ensures desirable colour, texture, and nutritional value, which are key factors preferred by consumers. In the case of black mushroom (Auricularia auricula) cultivation, farmers often face uncertainty regarding the ideal harvesting stage, as delayed harvesting can lead to deterioration in both colour and texture. This study evaluated black mushrooms harvested at various maturity stages, assessing their sensory qualities—such as colour, texture, and overall quality—along with detailed nutritional profiling. Among the seven treatments tested, T3 (harvested 12 days after pinhead emergence) and T4 (harvested after 15 days) showed superior results. The T3 group exhibited higher levels of lipids (1.74%), fiber (17.22%), protein (3.6%), and moisture content (17.17%), while T4 mushrooms were notably richer in carbohydrates (61.65%). Conversely, mushrooms harvested after 16 days showed marked quality decline and are not recommended. Based on these findings, the optimal harvesting window for black mushrooms lies between 10 to 14 days post pinhead emergence to ensure both superior quality and nutritional benefits.

Keywords: Texture, Quality, Nutrition, Black mushroom, *Auricularia auricula.*

INTRODUCTION

Ear mushrooms, scientifically known as *Auricularia* species, are a group of edible fungi commonly found in tropical and temperate regions worldwide. They are named for their ear-like shape and gelatinous texture (Priya *et al.*, 2016). Among them, *Auricularia auricula-judae*, also known as wood ear or jelly ear, is one of the most well-known species. These mushrooms typically grow on decaying wood, particularly on the elder trees (Nieuwenhuijzen & Oei, 2005). They have been widely used in Asian cuisine and traditional medicine.

Among 14000 described species of the millions of fungi, approximately 200 species have been successfully grown at laboratory scale (Kirk *et al.,* 2008). In terms of edibility, only more than 10 species of mushrooms are considered for safe farming on an industrial scale among 7000 species of mushrooms (Chang, 2006; Chang and Miles, 2004; and Martins, 2016). Ear mushrooms are valued for their nutritional benefits, including high fiber content, antioxidants, and polysaccharides that may support immune function and cardiovascular health (Cheung, 2008). They are also popular for their unique texture, adding a crunchy yet gelatinous consistency to soups, stir-fries, and salads.

Due to their medicinal properties, they have been utilized in traditional Chinese medicine for centuries to promote circulation and respiratory health. Additionally, they have a long history in traditional Chinese medicine, where they have been used to improve blood circulation, reduce cholesterol levels, and support respiratory health (Chang & Miles, 2004).

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Besides, it has high dietary fiber, vitamin D, and beneficial bioactive ingredients, such as antioxidants and polysaccharides ((MEXT, 2015., El-Ramady *et al.*, 2022).

The demand for black mushrooms (Auricularia spp.), has increased, especially in Asia, and their production volume is ranked third in the world after that of shiitake and oyster mushrooms (Royse et al., 2017). Due to their easy cultivation and nutritional benefits, ear mushrooms have gained global recognition as a functional food. They are often dried for storage and rehydrated before cooking, retaining their characteristic texture and mild flavor. Variability studies on cultural, morphological, and nutritional features of the mushroom are of immense use in understanding the nature of the mushroom. As research continues, their potential medicinal applications in modern health science are being further explored. Though a little ample information on these aspects is available in the literature, little is known about the harvesting time requirements of *Auricularia.* In this experiment, a brief study was conducted about the perfect harvesting age of black mushroom, their texture, and their nutritional properties.

MATERIALS AND METHODS

Fresh black mushroom was cultivated and harvested from the culture house at Mushroom Development Institute, Savar, Dhaka, Bangladesh from July 2024 to October 2024. Mushroom was carried out for nutritional analysis in the "Quality Control and Quality Assurance" laboratory of Mushroom Development Institute.

Treatments: Seven different harvesting times (T_1-T_7) of the black mushroom fruiting body were selected for this study. All packets of black mushroom varieties were cultivated on sawdust subtracts mother culture and spawn packets. The harvested mushroom was checked for quality and then dried and grained for analysis of protein, lipid, fiber, ash, moisture, and carbohydrate.

Determination of Quality: Mushrooms were harvested and checked by a Texture analyzer machine for quality. Human sensible organs i.e. visual and olfactory organs were used for the determination of quality, texture, and preference of mushrooms.

Determination of moisture content: One gram of well-grained black mushroom sample was taken to a Moisture analyzer (AnD MX-50) to analyze moisture content. The moisture analyzer took an average of 5-7 minutes to results by heating the sample.

Determination of total ash content: One gram of each sample was taken placed into a crucible and weighed. The crucible was heated by a sprit lamp till the samples were burned and turned into black ash. Then it was taken into a muffle furnace for about 5-6 hours at 600°C. After cooling down of the muffle furnace crucible was taken out and weighed. Then total ash was calculated by Raghuramulu *et al.,* (2003), as following the equation:

$$\text{Ash content}\left(\frac{\text{g}}{\text{100g sample}}\right) = \frac{\text{Wt of ash}}{\text{Wt. of sample taken}} \times 100$$

Determination of total lipid: The total lipid was determined by using chloroform and alcohol/methanol mixture with sample. A 5gm of grained mushroom sample was dipped into 50ml of chloroform: methanol (2:1) mixture. After 3 days, the mixture was filtrated with filter paper and poured into a test tube which was pre weighted. The test tube with the filtered mixture was placed into a dryer at 50-55°C. The upper layer of methanol was removed and chloroform was evaporated by heating. The remaining was crude lipid. Total lipid was calculated as the following equation:

$$\text{Total Lipid} \quad \frac{\text{2nd weight - 1st weight}}{\text{S}} \times 100$$

Determination of total protein: Five grams of grinded mushroom was taken with 50 ml of 0.1N NaOH and boiled for 30 min. The solution was cooled in room temperature. The supernatant was collected and total protein content was measured according to the method of Lowry *et al.*, (1951).

Determination of crude fiber: Ten grams of moisture and fat-free sample was taken into a beaker and 200 ml of boiling 0.255 N H2SO4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker, and 200 ml of boiling 0.313 N NaOH added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by a washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80~100° C and weighed (We) in an electric balance (Keyi: JY-2003; China). The crucible was heated in a muffle furnace (Nebertherm: Mod-L9/11/c6; Germany) at 600°C for 5~6 hours, cooled and weighed again (Wa). The difference in the weights (WeWa) represents the weight of crude fiber.

Crude fiber (g/100 g sample) = [100 − (moisture + fat)] × (We-Wa) / Wt of sample (Raghuramulu *et al.*, 2003).

Determination of total carbohydrate: The content of the available carbohydrate was determined by the following equation (Raghuramulu *et al.*, 2003).

$$\text{Carbohydrate}\left(\frac{\text{g}}{\text{100g sample}}\right) = \text{[100-(Moisture + Fat+Protein+Ash+Crude Fiber)]}$$

RESULTS AND DISCUSSION

Study on the Effect of Harvest Maturity of Black Mushroom: Good quality mushroom requires perfect shape and color which are determined by the right harvesting time. Different harvesting time of black mushroom showed significantly different results on mushroom texture, color, and nutritional status. Fig. 1. and Table 1. showed the variation of texture and colour of ear mushrooms which had an effect on harvesting time.

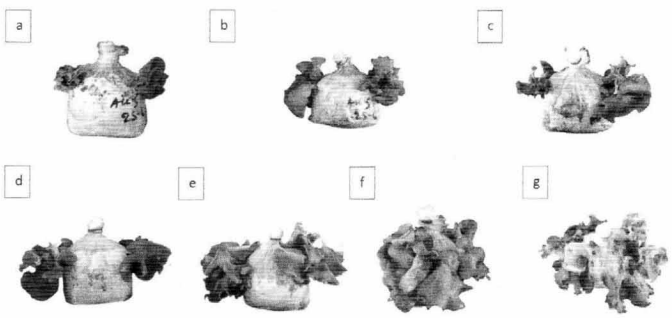

Fig. 1. Effect of harvesting age on texture & colour of Black Mushroom (*Auricularia auricula*). Here, the Pinhead appearance (a) after 8 days, (b) after 10 days, (c) after 12 days, (d) after 15 days, (e) after 18 days, (f) after 21 days & (g) after 24 days.

Table 1. Quality and Morphological effect of harvesting age on texture & colour of Black Mushroom (*Auricularia auricula*)

Treatment	Days of Harvest	Morphological status	Quality of mushroom	Colour stage	Market priority
T_1	8 days	No odor, natural	Initial stage	Medium purple	Medium
T_2	10 days	No odor, natural deep color	Good quality	Medium purple	Medium to High
T_3	12 days	No odor, perfect color	Perfect quality for harvest	Medium to dark purple	High
T_4	15 days	No odor, perfect color	Yellow spot appears in maturity	Dark purple	High
T_5	18 days	Slightly odor and color decline	Over mature. Yellow colour appeared	Dark to medium purple	Low
T_6	21 days	Bad odor and melt-down	Quality decline, feel sticky substance in hand	Medium to light purple	Low
T_7	24 days	Bad odor and melt-down	Bad quality. Mushroom was not consumable.	Light purple	None

Quality of mushroom significantly depends on harvesting age. In black mushroom, the best quality was appeared form 11-14 days after pinhead initiation with great colour and quality. Before 11 days, mushroom could be harvested but mushroom might be smaller than previous mentioned time.

The Fig. 1. and Table 1. demonstrate that harvesting after 18 days of black mushroom from pinhead initiation, reduced the quality, mushrooms appeared over mature and odor came out from the mushrooms. Day after 18 days mushroom quality declined in significant amount and started melting. After 24 days of pinhead initiation, the mushroom was fully destroyed, the bad smell was all over the body with fully melted and sticky in hand.

NUTRITIONAL STATUS

Total ash content: Total ash content was varied in different treatments of black mushrooms from 3.2%-4.5% per 100g of dried mushroom. Total ash content was found highest in T_5 treatment (4.5%) followed by T_1 (4.0%) and T_4 (3.8%). (Fig. 2a).

Lipid content: Different treatments of black mushrooms contain different lipid content ranges of about 1.32-1.88 g per 100 g of dried sample. The highest lipid content (1.88g/100g) was estimated in the T_1 treatment (harvest after 8 days of pinhead initiation) of black mushrooms. The lowest lipid content was found in the T_6 treatment (harvest after 21 days of pinhead initiation) (Fig. 2b).

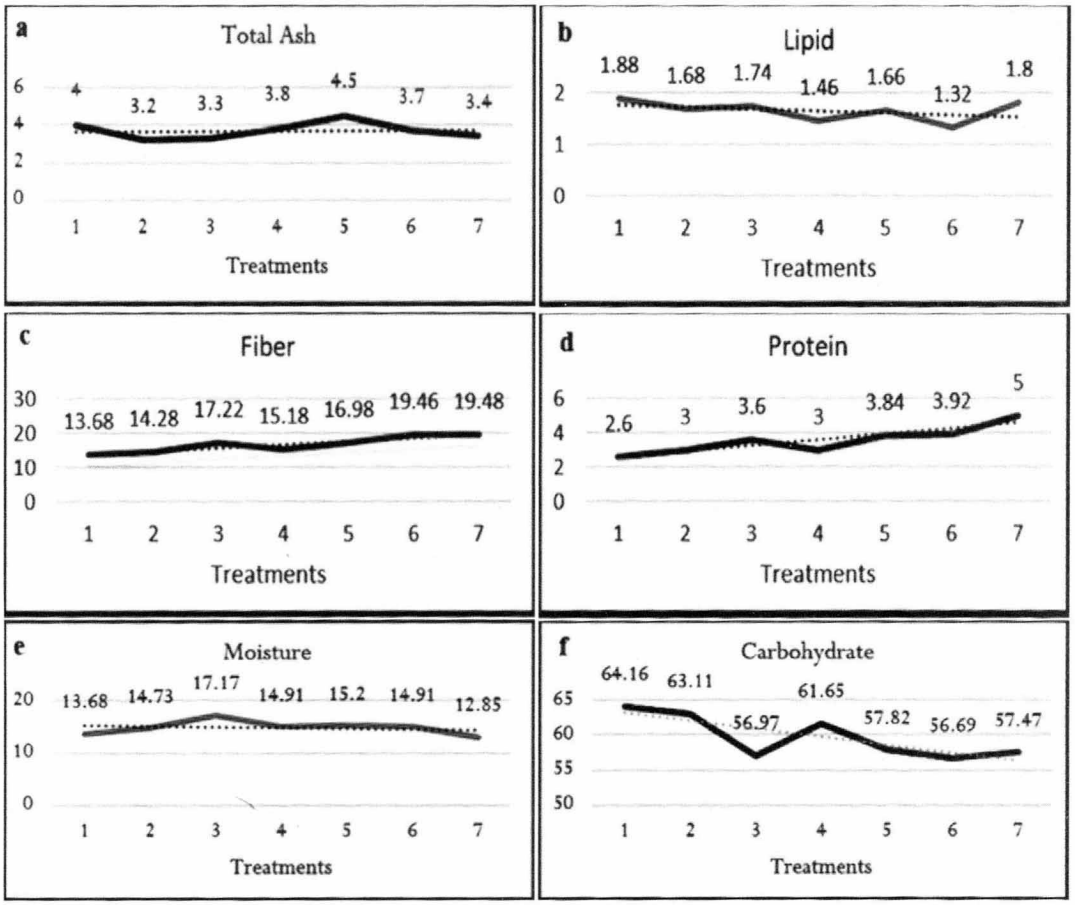

Fig. 2. Nutritional effect of harvesting age on texture & colour of Black Mushroom (*Auricularia auricula*). Here, 1,2,3,4,5,6 & 7 refer to different Treatments (T_1-T_7).

Fiber content: The fiber content varied from 13.68g to 19.48g per 100g of dried sample. The fiber content was found highest in the T_7 treatment (19.48g/100g dry sample) followed by T_6 (19.46g/100g) & T_3 (17.22g/100g). And lowest was found in T_1 (13.68g/100g) (Fig. 2c). It is clearly seen that fiber content was increasing time of mushroom age.

Protein content: The protein content was found in the T_7 treatment (5g/100g dry sample) which was the highest among all the treatments. Experimental data showed that the higher the age of the mushroom, the higher the amount of protein (Fig. 2d).

Moisture content: Considering moisture content varied from 12.85% - 17.17% per 100g of dried mushroom. T_3 treatment (17.17g/100g dry sample) was found the highest moisture content. Where lowest moisture content was found in T_7 (Fig. 2e).

Carbohydrate content: The carbohydrate content was drastically changed due to the age of mushroom harvesting time. The highest carbohydrate was found in T_1 (64.16g/100g) followed by T_2 (63.11g/100g) and T_4 (61.65g/100g). Carbohydrate content includes fiber, such as the structural polysaccharides beta-glucans, chitin, hemicelluloses, and pectin substances (Fig. 2f).

CONCLUSION

It is clear that black mushroom quality is highly dependable with harvesting age and nutrition also varies due to different harvesting times. Harvesting the black mushroom after 16 days is highly discouraged. For good quality and nutritional benefits, mushroom age within 10-14 days is highly recommended for harvesting of black mushrooms. The colour of the ear mushroom is varied due to climate change will need further discussion.

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Review article on: Bioactive Compounds and Health Benefits of Oyster Mushroom (*Pleurotus* spp.)

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Abstract

Oyster mushrooms (*Pleurotus* species) are widely recognized as functional foods owing to their rich content of nutraceutical compounds. They are highly valued for their nutritional profile, characterized by high protein levels, low fat, and low caloric content. These mushrooms are excellent sources of essential minerals like iron and phosphorus, as well as vitamins including riboflavin, thiamine, niacin, ergosterol, and ascorbic acid. Additionally, they are abundant in bioactive compounds such as secondary metabolites (e.g., terpenoids, organic acids, alkaloids, sesquiterpenes, polyphenols, lactones, sterols, nucleotide analogues, and metal-chelating agents), and polysaccharides primarily β-glucans and glycoproteins. Due to these bioactive constituents, *Pleurotus* mushrooms exhibit a wide range of therapeutic properties, including immunostimulatory, antineoplastic, antidiabetic, anticancer, antiatherosclerotic, anti-inflammatory, antibacterial, antiviral, and hypocholesterolemic effects. Their low fat and high fiber content, along with strong antioxidant capacity, make them especially promising for the prevention of cardiovascular diseases and for combating oxidative stress. Despite this impressive profile, mushrooms remain underutilized as a nutritional and therapeutic resource. Extracts from individual *Pleurotus* species offer great potential for use in dietary supplements aimed at enhancing immunity, and they can also be incorporated into functional foods as probiotics or used in specialized diets for individuals with specific health conditions. This review aims to explore the bioactive constituents of oyster mushrooms and highlight their nutraceutical potential, therapeutic applications, and overall health benefits.

Keywords: Hypocholesterolemic agent, Nutraceutical components, Health-promoting activities, Functional food.

INTRODUCTION

The health-promoting properties of mushrooms have long been recognized in some countries, especially in China. Application of modern analytical techniques has identified various mushroom-derived compounds, polysaccharides and tri-terpenoids for example, which exhibit a wide range of medicinal properties including immuno-enhancing, anti-tumor, antiviral and hypocholesterolemic activities (Wasser, 2010). It has been proved that mushroom nutraceuticals help to boost immunity. Mushrooms produce many bioactive proteins and peptides, primarily including lectins, fungal immunomodulatory proteins, ribosome-inactivating proteins, antimicrobial/antifungal proteins, ribonucleases and laccases (Xu *et al.*, 2011). The metabolic diversity of mushrooms is integral to bioremediation and biocontrol functions (Petre, 2016). *Ganoderma lucidum* and *Lentinula edodes* are the best-known species with therapeutic properties. An increasing number of studies from different centers confirm the fact that mushroom species of the *Pleurotus* genus exhibit multidirectional health-promoting effects (Khan and Tania, 2012; Correa *et al.*, 2016). Many authors indicate that oyster mushrooms could be classified as a functional food due to their positive effect on the human organism (Synytsya *et al.,* 2008; Patel *et al.*, 2012).

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Nowadays, oyster mushrooms are the world's third most common species of cultivated mushrooms after button mushrooms and shiitake mushrooms (Fernandes *et al.*, 2015). There are about 40 species in the Pleurotus genus (Kues and Liu, 2000). These mushrooms grow on various lignocellulosic substrates and form fruiting bodies of high nutritional value as they are rich in proteins, vitamins and minerals. Oyster mushrooms are a good source of vitamin C and B complex. Niacin content is very high in oyster mushrooms. The crude protein content of oyster mushrooms is lower than animal meats but higher than milk. Pleurotus species have been recognized as edible mushrooms with dual functions for humans; both as food, and medicine. It contains mineral salts which are helpful to the human body (Adebayo and Oloke, 2017). Mushroom is a good source of folic acid which can fulfill its daily requirement in our body. The inclusion of mushrooms in the diet of people suffering from hypertension, obesity, and diabetes has given promising results. Moreover, dietary supplements of mushrooms has given satisfactory results in patients suffering by acidity and constipation problems. Pleurotin is an aromatic compound found in *Pleurotus griseus* which exhibited antibiotic properties (Naraian *et al.*, 2016). *Pleurotus eryngii* is known as king oyster mushroom (KOM) and popular for its rigid structure, delicious taste, savory flavor and nutrient content. Oyster mushroom waste (OMW) is a by-product that can be used as an additive in poultry nutrition (Hassan *et al.*, 2020). Oyster mushrooms are used in traditional Chinese medicines to stimulate both innate and adaptive immunity.

The multidirectional health-promoting and therapeutic effects of mushrooms of the *Pleurotus* genus result from the presence of secondary metabolites, which have been isolated from fruiting bodies and mycelia of oyster mushrooms (Morris *et al.*, 2017). The bioactive compounds identified in *Pleurotus* mushrooms can be divided into those with a high and those with a low molecular weight. High-molecular weight bioactive compounds chiefly encompass polysaccharides, including β-glucans, peptides and proteins. Low-molecular-weight bioactive compounds include terpenes, fatty acid esters and polyphenols (Patel and Goyal, 2012). Bioactive substances exhibit immunostimulatory, anti-neoplastic, anti-diabetic, anti-atherosclerotic, anti-inflammatory, hepatoprotective and antioxidative properties (Lindequist *et al.*, 2005; Alam *et al.*, 2009; Jayakumar *et al.*, 2011; Wasser, 2014).

BIOACTIVE COMPOUNDS PRESENT IN OYSTER MUSHROOM

Polysaccharides: The polysaccharides found in the mushroom cell wall include β-glucans and α-glucans (Table 1). These compounds are composed of glucopyranose molecules linked with glycosidic bonds of the type (1→3)-β, (1→6)-β- or (1→3)-α. β-glucans are a group of polysaccharides which has been researched well. β-glucans acquired from mushrooms differ in their structure, water solubility, size of the molecule and molecular weight. They exhibit a very wide spectrum of health-promoting effects (Zhu *et al.*, 2015; Friedman, 2016). β-glucans of higher molecular weight are more effective (Wasser, 2002). The effectiveness of β-glucans also depends on their solubility (Wasser, 2011). The physicochemical modification of polysaccharides by changing the degree of their branching or by adding substituent groups (sulfates, selenates) influences their bioactivity (Li and Shah, 2015; Witkowska, 2014). According to Rop *et al.* (2009), oyster mushrooms are one of the most important sources of β-glucans. Individual oyster mushroom species differ in the concentration of total glucans, α and β glucans. According to Sari *et al.* (2017), total glucans range from 18.260 g 100 g-1 D.M. (dry mass) in *P. citrinopileatus* to 25.636 g 100 g-1 D.M. in *P. ostreatus*. The concentration of β-glucans ranges from 15.321 g 100

g-1 D.M. in *P. eryngii* to 24.230 g 100 g-1 D.M. in *P. ostreatus*. β-glucans can also be found in the mycelium of oyster mushrooms. The total concentration of β-glucans in *P. ostreatus* fruiting bodies reaches 9 g 100 g-1 D.M. The concentration of β-glucans in the oyster mushroom mycelium ranges from 2.5 g 100 g-1 D.M. in *P. pulmonarius* to 4.6 g 100 g-1 D.M. in *P. ostreatus* (Nitschke *et al.*, 2011). According to Synytsya *et al.*, (2009), the concentration of β-glucans in the stem of *P. ostreatus* fruiting bodies ranges 32.5-50% D.M. and is higher than in the cap (27.4-39.2% D.M.). The concentration of β-glucans in the fruiting bodies of *P. eryngii* amounts to 39.1 D.M. in the stem and 20.4% D.M. in the cap. Pleuran is the best known β-glucan extracted from oyster mushrooms. It is composed of D-glucose molecules linked with bonds of the type (1→3)-β and (1→6)-β. Extraction with hot water is the most common method of acquiring pleuran from fruiting bodies. It can also be obtained from liquid cultures (Maftoun *et al.*, 2013). A group of polysaccharides which has not been investigated so well is that of α-(1→3) glucans. They can be found in the deepest layer of the mushroom cell wall. The total concentration of α-(1→3)-glucans in the fruiting bodies of various oyster mushroom species ranges from 2.0% D.M. in *P. eryngii* to 4.0% D.M. in *P. citrinopileatus* (Sari *et al.*, 2017).

Proteins, peptides and lectins: Proteins, peptides and lectins are other high molecular weight substances acquired from mushrooms of the Pleurotus genus which exhibit medicinal properties (Table 1). Nebrodeolysin is a haemolytic protein isolated from P. nebrodensis (Lv et al., 2009). A protein derived from P. ostreatus has a structure similar to ubiquitin and inhibits HIV-1 reverse transcriptase (Wang and Ng, 2000). In addition, eryngin and pleurostrin – the proteins isolated from the P. eryngii and *P. ostreatus* species (Erjavec *et al.*, 2012). The *P. cornucopiae* species has provided two oligopeptides (Jang *et al.*, 2011). Moreover, the mycelium and fruiting bodies of *P. citrinopileatus* contain large amounts of ergothioneine, a water-soluble amino acid, (Lin *et al.*, 2016). Ribonuclease has been isolated from *P. djamor* (Wu *et al.*, 2010), laccase isolated from *P. ostreatus* (El-Fakharana *et al.*, 2010).

Lectins are another group of mushroom compounds with multidirectional health-promoting effects (Wang and Ng, 2000; Hassan *et al.*, 2015). Lectins include polysaccharide-protein and polysaccharide-peptide complexes. The polysaccharide-peptide complex has been isolated from *P. abalonus* (Chen *et al.*, 2015). Lectins has been isolated from *Pleurotus citrinopileatus* (Li *et al.*, 2008).

Other compounds: Mono and sesquiterpenoids, ergosterol and fatty acid esters are low-molecular-weight bioactive compounds identified in oyster mushrooms. Terpenoids have been isolated from the *P. cornucopiae* mycelium (Wang *et al.*, 2013). Menikpurage *et al.* (2009) has been isolated different fractions containing ergosterol – 3β, 5 α, 6 β-trihydoxyergosta- 7, 22-diene from *P. cystidiosus*. Fatty acid esters have been found in an extract from *P. eous* (Suseem and Saral, 2013). The fruiting bodies of mushrooms of the *Pleurotus* genus contain lovastatin, according to Alarcon *et al.*, (2003), the average lovastatin content in the dry matter of oyster mushrooms amounts to 0.7-2.8%. The concentration of lovastatin in oyster mushroom species varies, ranging from 101 mg kg-1 D.M. in *P. cystidiosus* to 216 mg kg-1 D.M. in *P. ostreatus* fruiting bodies (Chen *et al.*, 2012a). Many authors have indicated that oyster mushrooms contain phenolic compounds (Palacios *et al.*, 2011; Muszyńska *et al.*, 2013; Piska *et al.*, 2017). Aqueous and ethanol extracts have been isolated from *P. citrinopileatus* fruiting bodies and mycelium. The highest activity has been shown by ethanol extracts from the fruiting bodies of this species due to the high

total concentration of phenolic compounds (Lee *et al.*, 2007). Investigations conducted by Jaworska *et al.* (2015) found that the total phenolic content in *P. ostreatus* fruiting bodies amounted to 708 mg 100 g-1 D.M., in which the flavonoid content amounted to 170 mg 100 g-1 D.M. According to Gąsecka *et al.* (2016), ferulic acid and *p*-coumaric acid are the chief phenolic acids in oyster mushrooms. Their concentrations in the fruiting bodies amount to, respectively, 30.00 and 10.54 μg g-1 D.M. in *P. ostreatus* and to 29.00 and 13.49 μg g-1 D.M. in *P. eryngii*. The bioactive substances found in some *Pleurotus* species are presented in Table 1.

Table 1. Bioactive compounds of Oyster (*Pleurotus* spp.) mushrooms

Bioactive compounds	Species	References
β-glucans	*P. ostreatus, P. sajor-caju, P. eous, P. pulmonarius*	Jedinak *et al.*, 2010; Iwalokun *et al.*, 2007; Kanagasabapathy *et al.*, 2012; Suseem *et al.*, 2011; Baggio *et al.*, 2010, 2012; Jedinak and Sliva, 2008
α-glucan	*P. ostreatus*	Lavi *et al.*, 2006; Wu *et al.*, 2011;
Proteins	*P. ostreatus, P. nebrodensis*	Wang and Ng, 2000; Lv *et al.*, 2009
Polysaccharides	*P. ostreatus, P. abalones, P. florida, P. cornucopiae*	Tong *et al.*, 2009; Shamtsyan *et al.*, 2004; Ghazanfari *et al.*, 2010
Proteoglycans	*P. ostreatus*	Sarangi *et al.*, 2006
Heteroglycan	*P. cornucopiae, P. ostreatus*	Devi *et al.*, 2013
Lectin	*P. ostreatus, P. citrinopileatus, P. florida, P. citrinopileatus*	Wang *et al.*, 2000; Li *et al.*, 2008 ; Bera *et al.*, 2011; Hassan *et al.*, 2015
Lovastatin	*P. ostreatus, P. sajor-caju , P. florida*	Alam *et al.*, 2009; Khan *et al.*, 2011
Ergosterol	*P. ostreatus, P. cystidiosus*	Dissanayake *et al.*, 2009; Menikpurage *et al.*, 2009
Ergothioneine	*P. eryngii*	Abidin *et al.*, 2017
Phenols	*P. citrinopileatus*	Lee *et al.*, 2007
Pleuran	*P. ostreatus*	Park *et al.*, 2016
Peptides	*P. cornucopiae, P. abalonus*	Jang *et al.*, 2011; Chen *et al.*, 2015
Fatty acids esters	*P. eous*	Karaman *et al.*, 2010; Mirunalini *et al.*, 2012; Suseem and Saral, 2013
D-mannitol	*P. cornucopiae*	Hagiwara *et al.*, 2005
Terpenoids	*P. cornucopiae*	Wang *et al.*, 2013

HEALTH BENEFITS OF BIOACTIVE COMPONENTS

Edible oyster mushrooms have numerous therapeutic properties due to the presence of a large amount of bioactive and nutraceutical components. These are considered highly effective against various lifestyle diseases such as liver diseases, cancer, diabetes, and cardiovascular diseases (Kumar *et al.*, 2014). Furthermore, antioxidants and anti-microbial agents make them able to have immuno-modulatory, anti-ageing, and anti-microbial

effects (Fig. 1). Various health benefits of edible oyster mushroom are discussed under the following subheadings.

Anti-carcinogenic properties: Oyster mushrooms contain numerous bioactive substances with potential anticancer properties. These compounds comprise dietary fiber, polysaccharides, complexes of polysaccharides and proteins, steroids, terpenoids, phenolics, and certain types of proteins. A protein extract obtained from *P. ostreatus* exhibited a therapeutic effect towards the colorectal cancer cell line SW 480 and monocytic leukaemia THP-1 by inducing their apoptosis (Wu *et al.*, 2011). Patel and Goyal (2012) reported that the mushrooms possessing anti-carcinogenic characteristics are from the genus *Pleurotus*. Ribonuclease from *P. djamor* inhibits the proliferation of hepatic cancer and breast cancer cells (Wu *et al.*, 2010). Terpenoids exhibiting cytotoxicity towards HeLa and HepG2 cancer cells have been isolated from the *P. cornucopiae* mycelium (Wang *et al.*, 2013). Pleuran exhibits anti-neoplastic properties against various cells, including colorectal cancer cells HT-29 (Lavi *et al.*, 2006), prostate cancer cells PC-3 (Gu and Sivan, 2006) and breast cancer cells MCF-7 (Martin and Brophy, 2010). Investigations have proved that the fraction of glucans from *P. ostreatus* fruiting bodies exhibit an antineoplastic effect against colorectal cancer cell lines (Augustín *et al.*, 2007). The carboxymethylated α-(1→3) glucan isolated from *P. citrinopileatus* exhibits cytotoxic activity to cervical cancer cells but it is not toxic to normal cells (Wiater *et al.*, 2011). Polysaccharide (PAP) has been acquired from *P. abalones* that exhibit antiproliferative properties against human colorectal cancer cells LoVo (Ren *et al.*, 2015).

Antioxidative property: Antioxidant components present in different food types have the competence to entrap free radicals and inhibit the oxidative changes responsible for causing different types of degenerative diseases (Mehra *et al.*, 2020). Mushrooms, owing to phenolic components and other polysaccharides, have also been described as a rich source of antioxidant components (Dubost *et al.*, 2007). Dietary supplementation of edible mushrooms can reduce oxidative stress by increasing antioxidant defences. Mushrooms, either cultivated or wild, have substantial antioxidant characteristics, mainly due to bioactive components such as polyphenolic compounds, carotenoids, polysaccharides, and vitamins. Owing to the occurrence of antioxidants and other health-promoting components, edible mushrooms are used as prevalent delicacy foods (Kozarski *et al.*, 2015). The mycelium and fruiting bodies of *P. citrinopileatus* contain large amounts of ergothioneine, which is considered an excellent antioxidant (Lin *et al.*, 2016). Liu *et al.*, (1997) demonstrated the in vitro free radical scavenging activity of mushroom cell wall polysaccharides, and stated that these have great antioxidant potential. Pleuran has anti-oxidative and antiviral properties (Selegean *et al.*, 2009). α-glucans have been found to exhibit antioxidative properties (Wiater *et al.*, 2012). Zhang *et al.* (2012) isolated two fractions of polysaccharides from *P. ostreatus* and proved their strong anti-oxidative properties. Polysaccharides obtained from *P. eryngii* cells had stronger anti-oxidative and antibacterial properties (Li and Shah, 2015).

Hypo-Cholesterolemic Agents: Cardiovascular disorders are linked with hypercholesterolemia, low-density lipophilic oxidation and atherosclerosis. Therefore, blood cholesterol level needs to be regulated for the prevention as well as treatment of this disease. The low fat and high fibre contents of edible oyster mushrooms make them the best food for the prevention of cardiac ailments. The fruiting bodies of mushrooms of the Pleurotus genus contain lovastatin, which belongs to the group of statins affecting the metabolism of cholesterol. These compounds inhibit Low-Density Lipoprotein (LDL)

cholesterol oxidation and positively affect the coagulation system and fibrinolysis. They have anti-inflammatory, anticoagulation and antioxidative properties. Alam *et al.* (2009) conducted a study on animals and proved that the lovastatin contained in powdered fruiting bodies of P. osteratus and P. sajor-caju positively affected the lipid profile as well as the hepatic and renal functions. The total cholesterol and triglyceride levels in the rats' blood decreased.

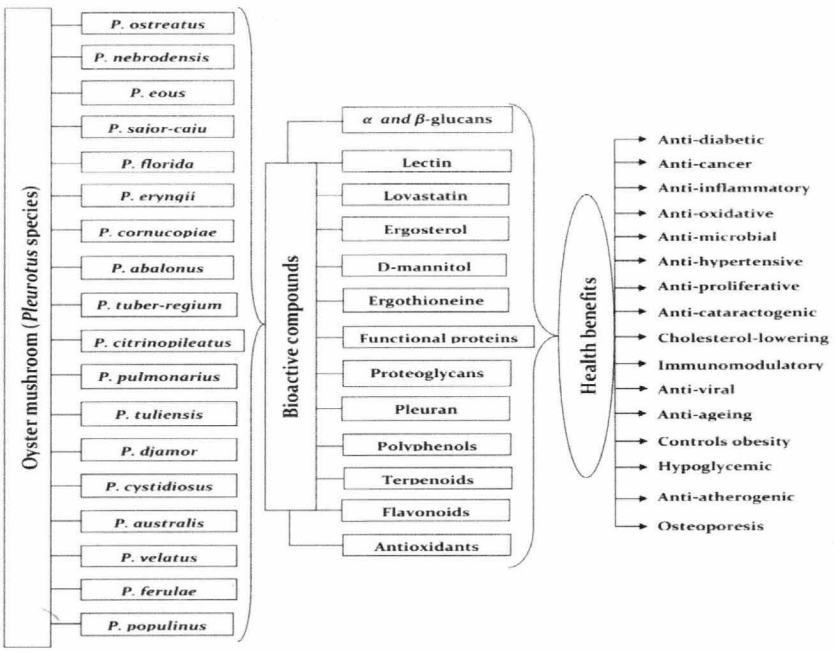

Fig. 1. Bioactive components present in Oyster mushrooms and their health benefits.

Hepatoprotective Effects: Damage to the liver is caused mainly by oxidative stress and is characterized by fibrosis, chronic hepatitis, hepatocellular carcinoma, and cirrhosis (Kodavanti *et al.*, 1989). An injury produced in the liver with diminished liver function is known as hepatotoxicity, and can be caused by the intake of any drug or different non-infectious agents (Navarro and Senior, 2006). Sumy *et al.*, (2014) studied the hepatoprotective effects of *Pleurotus florida* against the injury caused due to the intake of paracetamol in albino rats. Substances separated from *Pleurotus sp*, such as tri-terpenoids, polysaccharides, ergo-sterols, proteins, peptides, fatty acids, and trace elements, have hepatoprotective effects. Out of these, triterpenoid and polysaccharides were found as potential bioactive constituents, with a significant protective effect against liver injury caused by various toxins (Zhou *et al.*, 2002). The polysaccharide-rich extract of *Pleurotus eryngii* has been discovered to have hepatoprotective as well as hypolipidemic effects and can be utilized as an indispensable functional food additive (Chen *et al.*, 2012b).

Anti-Diabetic Effects: Diabetes mellitus is a metabolic problem that can be controlled with an improved standard of living, exercise and a suitable diet. Mushrooms can serve as functional foods in controlling diabetes. These are excellent sources of bioactive components with anti-diabetic properties. Many species of mushrooms are highly effective in controlling blood glucose levels and diabetic difficulties. Lectins include polysaccharide-protein and

polysaccharide-peptide complexes. Research has proved that the polysaccharide-peptide complex from P. abalonus reduces the blood glucose level in mice (Chen *et al.*, 2015). Pleurotus sp. are reported in various studies to exert hypoglycemic effects (De Silva et al., 2012). The edible mushrooms contain a very low amount of fat, cholesterol, carbohydrates, and are rich in protein, vitamin, and mineral contents, and thus are considered as low-calorie foods for diabetic patients (De Silva *et al.*, 2012; Cui *et al.*, 2009). Rushita *et al.*, (2013) studied the hypoglycemic characteristics of methanolic extract from Pleurotus citrinopileatus against streptozotocin-induced diabetes mellitus (type-2) in rats. There was a substantial decline in the fasting level of blood glucose, as well as the activity of serum catalase, but a significant surge in level of serum insulin was observed in groups treated with high dose of mushroom extract as compared to the untreated group. Glucan, an important polysaccharide, is widely present in mushrooms. It has been found to repair the activities of pancreatic tissues by enhancing the secretion of insulin by cells, leading to decreased levels of blood glucose. The ethanolic extract of *Pleurotus ostreatus* caused a significant reduction in the serum glucose level of alloxan-induced diabetic mice. The level of urea and creatinine in serum decreased significantly in the post-treated groups. It was found that *Pleurotus ostreatus* can be utilized in medicinal preparations against diabetes mellitus (Ravi *et al.*, 2013).

Anti-Microbial Effects: Mushrooms are considered as the best nutritional supplements, with outstanding medicinal values. Certain edible mushrooms have antimicrobial properties and can control various human diseases. These were found to have anti-fungal and anti-bacterial activities against resilient disease-causing microbes (Sharma *et al.*, 2014). The presence of phenolic compounds and ergosterol peroxide in numerous mushrooms was found to exert in vitro anti-viral effects against influenza viruses (Ali *et al.*, 2003). Chowdhury *et al.*, (2015) discovered anti-microbial activities in some varieties of edible mushrooms in Bangladesh. *Pleurotus aeruginosa* was moderately resistant and Saccharomyces cerevisiae was more sensitive as compared to other microbial isolates. Menaga *et al.*, (2012) reported that bioactive components extracted from *Pleurotus florida* can be employed as alternative therapeutics such as antibiotics. Nebrodeolysin is a haemolytic protein isolated from *P. nebrodensis*, exhibits an antiviral effect against HIV (Lv *et al.*, 2009). Eryngin and pleurostrin – the proteins isolated from the *P. eryngii* and *P. ostreatus* species exhibit antifungal and antibacterial properties (Erjavec *et al.*, 2012). The *P. cornucopiae* species has provided two oligopeptides which exhibit antihypertensive properties (Jang *et al.*, 2011). Research has also confirmed the antiviral effect of laccase isolated from *P. ostreatus* against the hepatitis C virus (El-Fakharana *et al.*, 2010). Lectins from *Pleurotus citrinopileatus* exhibit antiviral effects (Li *et al.*, 2008). Menikpurage *et al.* (2009) researched the antifungal activity of different fractions isolated from *P. cystidiosus*. They found that the fraction containing ergosterol – 3β, 5α, 6β-trihydoxyergosta -7, 22-diene was the most effective against *Colletotrichum gloeosporioides* fungi that cause anthracnose. Fatty acid esters in an extract from *P. eous* have been found to exhibit a strong antibacterial effect to inhibit the growth of Gram-positive and Gram-negative bacteria (Suseem and Saral, 2013). It concluded that mushrooms can also be used for pharmaceutical purposes in the treatment of several diseases. Shen *et al.*, (2017) reported that mushroom extracts can be utilized as food additives with antioxidant and antimicrobial activity to encounter the growing demands for food quality and safety, thereby preventing the spoilage of food products.

Mushrooms as Natural Resources of Immunotherapy: Mushrooms are well-known as significant natural sources of immunotherapeutic components. These can be utilized as

immune-stimulating and immune-modulating agents in treating certain immunodeficiency maladies such as cancer, tumour, HIV, and tuberculosis. Bioactive components extracted from *Pleurotus* mushroom are capable of enhancing or balancing an immune response in the human body. Such bioactive components include polysaccharide-proteins, polysaccharide-peptides, functional proteins (ubiquitin-like peptide, ubiquinone-9, glycoprotein, and nebrodeolysin), proteoglycans and glucans (Oloke and Adebayo, 2015).

CONCLUSIONS

Oyster mushrooms have extensive potential to be used in the diet for taking advantage of the nutraceutical properties of the bioactive compounds. Edible oyster mushrooms, due to lower fat and higher protein contents, need to be used for the preparation of low-calorie and high protein diets. Besides, their use as therapeutic foods can be helpful in the preclusion of lifestyle diseases, such as diabetes, hypertension, cancer, hypercholesterolemia, and cardiovascular diseases. Functional characteristics of the edible oyster mushrooms are primarily due to the occurrence of antioxidants, dietary fibres, lectins, anti-microbial agents, and other bioactive components. Due to their richness in immune-modulating polysaccharides, these can be incorporated into health-promoting supplements. The valuable bioactive compounds and their further incorporation for the preparation of value-added functional foods and their use in prevention, as well as the treatment of various lifestyle diseases, will continue to be the major attention of research into future prospects.

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