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Ministry of Agriculture
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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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Contents

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Bacillus velezensis: A Common Pathogen of Button Mushroom Spawn in Bangladesh and its Antimicrobial Profile

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Abstract

Through analysis of gyrA and rpoB gene sequence, *Bacillusvelezensis* is identified as a major contaminant in button mushroom mother spawn in Bangladesh. The study emphasized the crucial role of the wheat grain used in mother spawn production as the primary source of bacterial contamination. Furthermore, the identified bacterial isolate was subjected to tests involving 11 different antibiotics, revealing resistance to several including cephalosporin (Cefepime)and penicillin derivatives such Penicillin, Ampicillin, Oxacillin. Consequently, it is essential to implement strict quality control measures for wheat grain and explore alternative grain sources to minimize the risk of contamination and ensure food safety.

Keywords: *Bacillusvelezensis,* Grain spawn, Button mushroom, Sequences, antibiotics.

INTRODUCTION

The Button mushroom (*Agaricus bisporus*) is the most cultivated globally. The global market for mushrooms has grown significantly after the Covid 19 pandemic due to the changes in food habits. It was predicted that global mushroom consumption will grow from 12.74 million MT in 2018 to 20.84 million MT in 2026, reflecting a 6.41% compound annual growth rate (CAGR) (Tridge, 2021). In comparison to world production, the production of mushrooms in Bangladesh, particularly button mushrooms, is relatively limited. Usually, button mushroom production in Bangladesh has been confined to the winter months due to the specific temperature requirements (12-15°C) necessary for fruiting body formation. However, recent technological advancements have made year-round farming possible, albeit at a high cost and sophisticated endeavors. Additionally, as highlighted by Osdaghi *et al*., 2019, biotic factors, such as bacterial and fungal contaminants, significantly impact different stages of Button mushroom cultivation.

Academically, the fungal life cycle consists of two distinct phases: the vegetative stage characterized by mycelium growth, and the reproductive stage marked by fruiting body formation. Whereas, on a farm scale, a process involving three stages is employed to boost the volume of mushroom mycelium. These stages include pure culture (mycelium grown in PDA media) mother spawn (mycelium grown in grains) and developing commercial spawn (mycelium grown in commercial substrates like rice straw).

Among the stages, the production of mother spawns holds paramount importance in ensuring successful mushroom cultivation, analogous to how seeds are essential for crops. Indeed, the failure or success of mushroom production mainly depends on the timely availability of pure and high-quality spawn (Goltapeh and pujram 2003). The spawn must be devoid of any harmful elements such as fungi and bacteria.

In Bangladesh, mother spawn preparation involves immersing wheat grain overnight in normal water. 250g of wet grain are taken into polypropylene bags. Subsequently, packets are sterilized at 121°C and 15 psi for 1hours. Afterward, the packets are gradually cooled down and then inoculated with a pure culture of *A. bisporus*. Finally, the spawn is kept at 25°C and soon gets impregnated with mushroom mycelium resulting in the mother spawn being ready for use in a week.

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Interestingly, in Bangladesh, a common issue observed in Button mother spawn packets, particularly in high moisture conditions, is the presence of the wet spot or sour spot symptom (Ahlawat, 1999). This symptom is characterized by a slime texture and sour odor. *Bacillus subtills spp.* is the primary causative organism behind this problem, although the specific species of these agents have not been explored until now. Furthermore, the evolutionary processes of bacteria are influenced by natural habitats and geographic regions, leading to mutations, gene alterations, and recombination (Li *et al.*, 2014). Thus, understanding the bacterial strain involved can facilitate the implementation of strategies to reduce contamination and enhance product safety. In light of these issues, our study focuses on identifying the bacterial responsible.

Hence, we targeted the gyrA gene (protein of DNA gyrase) and the housekeeping rpoB gene (codes for the beta subunit of RNA polymerase) to identify the species of bacteria. Multiple studies support the efficacy of these genes in bacterial identification compared to the widely used 16S rRNA gene (Ogier *et al.*, 2019; Adékambi *et al.*, 2009; Liu *et al.*, 2022; Khosravi *et al.*, 2015).

Research has established a relationship between inadequate hygiene and bacterial contamination in spawn production (Pecchia and Beyer, 2013). Despite employing various sterilization techniques, including steam sterilization, alkalinization, and bleaching, the resilience of spore-forming bacteria poses challenges in eradicating contamination (Gupta *et al.*, 2020). In addition to sterilization, antibiotics are commonly used, but their impact on bacteria needs comprehensive evaluation before effective recommendations can be made.

Based on the aforementioned facts and views, the objectives of our study are to identify bacterial species responsible for contaminating button mushroom mother spawn and to develop effective management approaches including assessing their in vitro antibiotic susceptibility. Our research findings are expected to provide valuable insights into the mushroom industry in Bangladesh and beyond. Top of Form

MATERIALS AND METHODS

Sample collection: Eleven contaminated button mushroom mother spawn packets were collected from Mushroom Development Institute, Savar, Dhaka. All experimental procedures were conducted at the laboratory of the Department of Life Sciences, Independent University Bangladesh (IUB). Samples analyses were initiated within 24 hours of collection.

Isolation of the Bacteria: Ten grams of each grain sample was added to 90 ml of sterile normal saline and homogenized for 30 min using a shaking water bath at 120 rpm. Serial dilution was done up to 10^{-6} followed by a standard plating method using Nutrient agar (NA), MacConkey agar (MAC), and Mannitol salt agar (MSA). Bacterial isolates were selected based on their colony morphology and preserved using 60% glycerol for subsequent identification and characterization.

Identification of Bacterial Isolates: Presumptive identification of bacterial isolates was conducted by observing their cultural, morphological, and biochemical traits according to published protocol (Forbes *et al.*, 2007). Morphological characteristics were studied in universal growth media like NA ((liofilchem, lot 042222501), as well as selective and differential media such as MacConkey agar (Mac) (Oxoid, lot 3328884) and Mannitol Salt Agar (MSA) (Liotilchem, lot 060921502). An array of biochemical tests, including Gram Staining, Motility Indole Urea (MIU) test, Methyl Red Test, Voges-Prokauer test (MRVP), Oxidase Test, and Catalase Test (H_2O_2 3%), were employed to confirm the identity of each isolate.

Genome sequencing: Genomic DNA from bacterial samples was extracted using the Promega Wizard genomic DNA purification Kit. Subsequently, libraries with an average length of 600 bp were constructed using the Next Era DNA CD Indexes and Illumina DNA Prep Library Preparation Kit. Afterwards, the libraries were sequenced using the Illumina Miniseq Platform (Haendiges *et al.*,

2024). The raw sequence data were then processed using the NGS QC Tool Kit v2.3.3 and search tool BLAST was used for identifying the matching nucleotide sequence with the reference sequences.

Antimicrobial susceptibility test: All the isolated bacteria underwent antimicrobial susceptibility testing using the Kirbye–Bauer disk diffusion method according to the Clinical, and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). For this analysis, eleven antibiotic discs (Himedia, Mumbai) were utilized, including Ampicillin (25µg), Amoxicillin (25µg), Penicillin ((10µg), Oxacillin (1µg), Chloramphenicol (30 µg), Cefepime (30 µg), Erythromycin (15 µg), Gentamycin (10 µg), Tetracycline (13 µg), and Vancomycin (30 µg) and Trimethoprim (25 µg). After an incubation period of 18-24 hours, the zone of inhibition on the Mueller-Hinton agar is interpreted following the CLSI guidelines (2018).

RESULTS AND DISCUSSION

Isolation and Phenotypic characteristics: In the analysis of contaminated mother spawn, eleven bacterial isolates were collected based on colony morphology on the MSA and NA plates, Notably, there was no growth was observed on MAC agar plates (Fig. 1). The colonies displayed a rounded, elevated, and opaque appearance, characterized by a discrete growth pattern with rough surface texture and a somewhat uneven border on nutrient agar. The absence of growth on MAC agar indicated the presence of gram-positive bacteria which was further confirmed by gram staining. Subsequent biochemical tests (Table 1 and Fig. 2 & 3) confirmed that all isolates were presumptively identified as *Bacillus spp.*

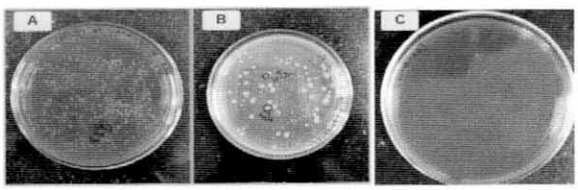

Fig. 1. Macroscopic Examination of (A) MSA (B) NA and (C) MAC plate.

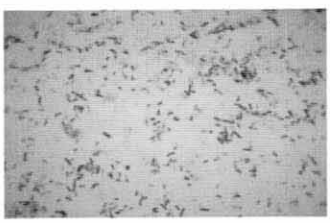

Fig. 2. Microscopic Examination (Gram Staining).

Table 1. Presumptive Results of Biochemicals Tests

Isolates Name	Citrate	KIA test						MIU test			MR	VP	Oxidase	Catalase	Identification
		Slant	Butt	Glucose	Lactose	H_2S	Gas	Motility	Indole	Urease					
S_1I_1	+	-	-	-	-	-	-	-	-	-	+	+	+	+	*Bacillus spp.*
S_1I_2	+	-	-	-	-	-	-	-	-	-	+	+	+	+	*Bacillus spp.*
S_1I_3	+	-	-	-	-	-	-	-	-	-	+	+	+	+	*Bacillus spp.*
E. coli ATCC 25922	_	+	+	+	+	-		+	+	-	+	-	+	+	*E. coli*

Here, S_1I_1= Bacteria plating method using Nutrient agar (NA), S_1I_2= Bacteria plating method using MacConkey agar (MAC), S_1I_3= Bacteria plating method using Mannitol salt agar (MSA), KIA= Kligler's Iron Agar Test, MIU= Motility-Indole-Urease Test, MR= Methyl Red Test, VP= Voges-Proskauer test. (+) denoted Positive Result, (-) denoted Negative Result.

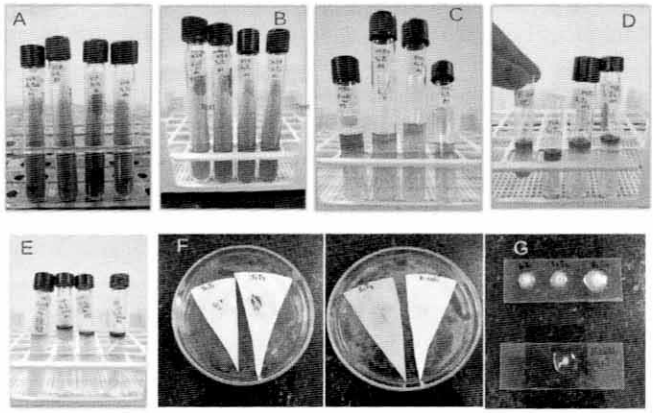

Fig. 3. Presumptive identification of the isolates using biochemical tests. (A) Simmons Citrate Agar (SCA) Test, (B) Kligler's Iron Agar (KIA) Test, (C) Motility-Indole-Urease (MIU) Test, (D) Methyl Red (MR) Test, (E) Voges-Proskauer VP, (F) Oxidase Test (G) Catalase.

Genomic Features: When NCBI nucleotide BLAST was used to search the gyrA and rpoB gene sequences, the results revealed significant matches. For the rpoB gene (>DEDKOLHF_01047 DNA-directed RNA polymerase subunit beta), there was a complete match with 100 percent query coverage and a 99.78 percent identity match to two closely related bacteria, namely *B. velezensis* and *B. amyloliquefaciens*. Similarly, the GyrA gene (>DEDKOLHF_02093 DNA gyrase subunit A) exhibited analogous results, with 100 percent query coverage and a 99.72 percent identity match to both *B. velezensis* and *B. amyloliquefaciens*. According to Huynh *et al.*, 2022, "*B. velezensis* and *B. amyloliquefaciens* belong to the "operational group *B. amyloliquefaciens*", a taxonomical unit above species level within the *B. subtilis* species complex. These bacteria share approximately 100% sequence similarity in their rpoB and GyrA genes. The study by Huynh and collagues also described the use of these two genes for the identification of *B. velezensis* and *B. amyloliquefaciens*.

Antibiotic Susceptibility: The isolated bacteria showed a diverse antibiotic resistance profile, demonstrating resistance against Cefepime, Penicillin, Ampicillin, Oxacillin, while exhibiting susceptibility to Chloramphenicol, Erythromycin, Gentamycin, Tetracycline, Trimethoprim, and Vancomycin. (Refer to Table 4 and Fig. 4).

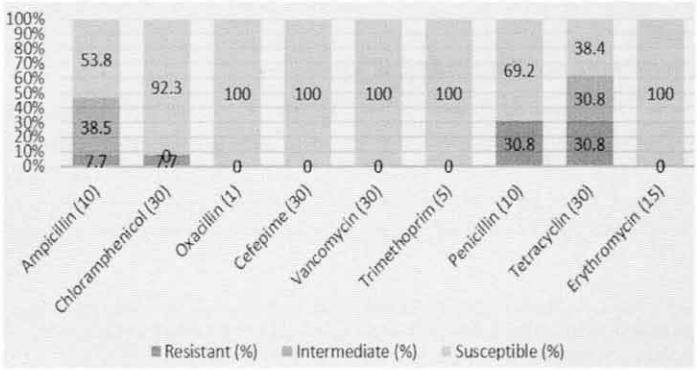

Fig. 4: Interpretation of Antibiotic Sensitivity Test.

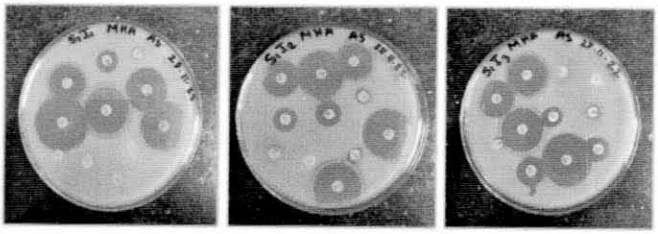

Fig. 5. Antibiotic Susceptibility Test.

In our research, the housekeeping rpoB and gyrA gene sequences from our isolate exhibited a 100% sequence similarity with *B. velezensis*.Using the available core genomes of 23 type strains, as reported by Fan *et al.*, 2017, it has been identified that the B. subtilis species complex comprises four distinct clades. Notably, clade II includes *B. siamensis, B. amyloliquefaciens, B. velezensis,* and *B. methylotrophicus*. Recent studies in the era of comparative genomics have revealed that members of the B. subtilis species complex, including *B. velezensis*and B. *amyloliquefaciens* have anti-fungal properties (Nakkeeran *et al.*, 2021; Jimenez *et al.*, 2022) meant to exhibit the capability to inhibit the growth of fungi including mushroom.

Like other *B. subtilis* spp complex, identified bacterial isolate *B. velezensis* is known to be a soil and food-born pathogen and can produce resting spores (endospore) which gives the advantage of resistance against high temperatures, acids, salt, drugs, and radiation (Setlow, 2006; Piggot and Hilbert, 2004). When exposed to favorable conditions, especially high moisture, these spores resume growth as vegetative cells (McKenney *et al.*, 2013; Zhang X. *et al.*, 2014). Moreover, *Bacillus* exhibits a high reproductive rate, making it easily mass-producible and processed. It is convenient to transport and has a high survival rate during storage (Collins and Jacobsen, 2003). Traditionally, mushroom cultivation has been conducted under sterile conditions. So, the contamination issue is likely to originate from inadequate sterile wheat grain used in mother spawn production. Wheat seeds have a relatively thin seed coat and a soft texture, bacteria including *Bacillus spp*., which can easily enter the seeds which leading to a higher rate of infection in wheat grains than other grains like paddy, maize which agreed with the findings of Gupta *et al.*, (2020).

For the management of bacterial contaminants, mushroom farmers commonly employ a range of physical and chemical treatments. However, it is noted that endospore-forming bacteria pose a challenge to eradication through these conventional methods. Consequently, on a worldwide scale, farmers often turn to antibiotics as a simple and effective tool for controlling bacterial contamination. In our study, we tested antimicrobial susceptibility in *B. velezensis* strain to 11 clinically common antibiotics. According to Liu *et al.*, 2021, all *B. amyloliquefaciens* strains possess antibiotic resistance gene Chloramphenicol-florfenicol resistance (cfr) (GenBank accession no. AM408573). However, in our study *B.velezensis* operational B group of *B. amyloliquefaciens* showed susceptibility to Chloramphenicol, Erythromycin, Gentamycine, Tetracycline, Trimethoprim, and Vancomycin. *The* results support the statement that the presence of the gene does not necessarily correlate with reduced the susceptibility, as indicated by Abdelaziz *et. al.* 2021.

It's worth noting that several scientific studies have highlighted the advantages of B. *velezensis* in the agricultural sector. For example, it is frequently used as a potential probiotic due to its strong resistance characteristics as indicated by Wang *et al.*, 2021. Additionally, B. *velezensis* demonstrates an outstanding inhibitory effect on various plant pathogenic fungi such as Fusarium, Rhizopus, Penicillium, and others, as reported by Jiao *et al.*, 2021. Furthermore, research on B. amyloliquefaciens

B-241 appears promising for the control of both compost green mold and dry bubble disease of A. bisporous fruiting bodies. Despite not stimulating yield, *strain's effectiveness in disease control was showed by* olja *et al.*, 2019.

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A Farmer Affordable Technique: Packaging with Nitrogen Gas (N_2) Enhanced Shelf-Life of Fresh Oyster Mushroom (*Pleurotus ostreatus*)

Sumaiya Khanam Onia[1], Salman Haque[1], Md. Asif Hasan Mazumder Niloy[1], Akter Jahan Kakon, Soyabur Rahman and Jebunnahar Khandakar[1]
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Abstract

The escalating production and consumption of fresh mushrooms in Bangladesh face a challenge due to their high perishability attributed to an increased respiration rate, elevated moisture content, and the absence of a protective cuticular structure, thereby limiting their commercial viability. To address this issue, various methods, including packaging updates, have been explored. Our research focuses on the use of packing gases, such as nitrogen, to extend the shelf life of Pleurotus ostreatus. Nitrogen, being an inert gas, ensures the preservation of the quality of fresh mushrooms. In our study, freshly harvested mushrooms were carefully packed in polypropylene (PP) bags both with and without nitrogen gas. These were stored both at room temperature and in the refrigerator. Throughout the storage period, we assessed seven quality parameters- total soluble sugar (TSS), browning index (BI), weight loss, pH, moisture content, odor, and texture at two-day intervals. The results revealed no significant changes in TSS or pH levels compared to the controls. Notably, mushrooms stored in PP bags with nitrogen in the refrigerator exhibited improved quality over eight days, showing advancements in BI, moisture content, and texture.

Keywords: mushroom, shelf life, nitrogen, quality parameters, packaging.

INTRODUCTION

Growing edible mushrooms offers numerous advantages. These unique fungi have the remarkable ability to extract nutrients from lignocellulosic materials, which are predominantly indigestible by humans (Isikhuemhen *et.al.,* 2009). Among these fungal species, Pleurotus ostreatus, commonly known as oyster mushrooms, stands out as an excellent source of vitamins, proteins, and healthy fats (Ofodile *et al.,* 2020) Nicholas-Okpara N.V.A., Ani E., Ikeqwu E.M., Saanu A., Ezenwa P.C., Osorine R.T. Production and nutritional composition of juice powder from oyster mushroom Pleurotus ostreatus (Jacq.. Moreover, oyster mushrooms are gaining popularity due to their therapeutic and medicinal uses *(Patel et al.,* 2012). However, the perishability of oyster mushrooms poses a significant challenge for the global mushroom industry (Datta and Das, 2021; Rosmiza *et al.,* 2016)India and also develops strategies to address the existing constraints using SWOT, TOWS, and QSPM models. SWOT analysis provides a comprehensive view of twelve internal and nine external factors present in the mushroom cultivation and marketing system. The results of Internal Factor Evaluation Matrix (2.88.

Fresh oyster mushrooms are characterized by their appealing texture, with a smooth and less resilient surface (Myronycheva *et al.,* 2017; Pathak *et al.,* 2022)Institute of Botany Kholodny, National Academy of Science, Kyiv, Ukraine (IBK. Unfortunately, their morphological characteristics start to deteriorate shortly after harvest and may completely degrade within three days. This perishability limits the shelf life of oyster mushrooms stored at ambient temperatures to just 1-3 days(Wang *et al.,* 2017; Olotu *et al.,* 2015).

One of the primary factors contributing to the high perishability of mushrooms is their respiration rate. Mushroom respiration rates surpass those of other fruits and vegetables due to their thin and porous epidermal structure(Wakchaure 2011; Ares *et al.*, 2007.).

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As mushrooms respire, they release significant amounts of water vapor, leading to shrinkage and weight loss (Das *et al.*, 2010). The released moisture can accumulate inside the packaging, promoting bacterial growth and causing the mushrooms to develop speckles and turn brown (Singh *et al.* 2010).

In addition to texture and moisture content, browning is a crucial indicator of mushroom quality. Browning can occur due to microbial attacks, physical damage, or enzymatic reactions catalyzed by polyphenol oxidases (PPO). Breakage-induced browning can be addressed by filling and sealing the mushroom packets with air. However, browning due to the action of PPO that oxidizes phenolic compounds within the fungi in the presence of oxygen is quite difficult in its preservation (Janusz *et al.*, 2020).

Fortunately, previous research has shown that both high respiration rates and PPO activity rely on atmospheric oxygen(Stolper, *et al.*, 2010; Freeman *et al.*, 2004)but in many natural environments, its concentration is reduced to low or even undetectable levels. Although low-oxygen-adapted organisms define the ecology of low-oxygen environments, their capabilities are not fully known. These capabilities also provide a framework for reconstructing a critical period in the history of life, because low, but not negligible, atmospheric oxygen levels could have persisted before the \"Great Oxidation\" of the Earth's surface about 2.3 to 2.4 billion years ago. Here, we show that Escherichia coli K-12, chosen for its well-understood biochemistry, rapid growth rate, and low-oxygen-affinity terminal oxidase, grows at oxygen levels of $\leq$ 3 nM, two to three orders of magnitude lower than previously observed for aerobes. Our study expands both the environmental range and temporal history of aerobic organisms.","container-title":"Proceedings of the National Academy of Sciences of the United States of America","DOI":"10.1073/pnas.1013435107","ISSN":"1091-6490","issue":"44","journalAbbreviation":"Proc Natl Acad Sci U S A","language":"eng","note":"PMID: 20974919\nPMCID: PMC2973883","page":"18755-18760","source":"PubMed","title":"Aerobic growth at nanomolar oxygen concentrations","volume":"107","author":[{"family":"Stolper","given":"Daniel A."},{"family":"Revsbech","given":"Niels Peter"},{"family":"Canfield","given":"Donald E."}],"issued":{"date-parts":[["2010",11,2]]}}},{"id":836,"uris":["http://zotero.org/users/local/aANC0UAf/items/VTD2XXAF"],"itemData":{"id":836,"type":"article-journal","abstract":"Unique peatland properties, such as their ability to preserve intact ancient human remains (bog bodies. This suggests that controlling the oxygen concentration within the packaging could effectively mitigate these negative factors. While eliminating air from mushroom packets increases the risk of breakage, replacing the packaging air with inert gas nitrogen offers a plausible and convenient solution (Park *et al.*, 2019). Thus, the objective of this study was to evaluate the effects of nitrogen as a packaging gas on key quality parameters (moisture loss, weight loss, texture, browning index, pH, and total soluble sugar) of harvested oyster mushrooms. The findings from this research are expected to enhance our understanding of the post-harvest biology of mushrooms and provide valuable insights for producers and merchants seeking information on effective packaging techniques.

MATERIALS AND METHODS

In our study, we procured freshly harvested oyster mushrooms (*Pleurotus ostreatus*) from the Mushroom Development Institute (MDI) at Savar, Dhaka. The collected mushrooms underwent a meticulous sorting process based on size and appearance criteria. Specifically, mushrooms displaying a white, firm texture with a fresh and smooth appearance were selected for inclusion in the experimental samples. Any damaged, extra-large, and small mushrooms were deliberately excluded from consideration.

For packaging, we utilized polypropylene bags, with each bag containing a standardized amount of 200±5g of mushrooms. The mushroom packets were subjected to two treatment conditions: one group treated with N2 gas and another without N2 gas. After treatment, the bags were securely sealed and stored under two distinct temperature conditions, namely ambient and refrigeration. Each treatment condition consisted of five replications to enhance the robustness and reliability of the experimental design. The nitrogen gas treatment was conducted at the laboratory of the Department of Environment Sciences at Independent University, Bangladesh.

Quality attributes: The data were gathered according to the specified parameters. Top of Form.

Texture: The mushrooms' texture was analyzed using a Perten TVT 300 XP texture analyzer, with a focus on assessing the alterations in resilience and hardnessof the fruiting bodies throughout the course of the investigation. Measurements were taken at a height of 40mm, employing a test speed of 1.5 mm/s, a trigger force of 25 g, and a compression depth of 5.0 mm, with the recorded data reflecting changes in the mushrooms' physical properties.

Moisture content (%): The moisture level in the samples were analyzed using an AnD MX-50 Moisture Analyzer, which is equipped with both heating and weighing units. The mushrooms were subjected to constant heating at 110°C within the analyzer, and the resulting total moisture contents were recorded as a percentage. The quantification process utilized the expression outlined in the study by Raghuramulu *et al.,* (2003):

$$\text{Moisture content (\%)} = \frac{\text{Weight of fresh sample-Weight of dried sample}}{\text{Weight of fresh sample}} \times 100$$

Browning Index: The assessment was conducted using a color analyzer. Prior to obtaining readings, calibration of the analyzer was performed using the black and white calibrators supplied with the equipment. From each treatment, three mushrooms were selected, and reading were taken in triplicate for each sample. The color parameter values, where L* represents lightness and darkness, a* indicates redness or greenness and b* stands for yellowness or blueness, were used to calculate the browning index using the following formula.

$$BI = 100 \times \frac{X-0.31}{0.71} \text{ Where, } X = \frac{a+1.75L^*}{5.645L^*+a^*-3.012b^*}$$ -------(Bozkurt and Bayram, 2006).

Weight loss: It must be noted that to evaluate the weight loss (W_L) of the samples, the initial weights (W_0) and the final weights (W_f) of the packaged mushrooms were weighed using an electronic balance (Xpart Weighing Scale). The readings were taken in grams and the following equation was used for the investigation.

$(W_L = (W - W_f)/W) \times 100.$ ----------(Jafri *et al.,* 2013).

Results were expressed as an average of three replicates.

Odor: The distinctive aroma of oyster mushroom is widely acknowledged, and alterations in smell are often associated with the extent of mushroom spoilage. To assess the odor of mushroom samples over varying storage durations, a panel of 10 evaluators was assembled. All the panel members possessed a high level of education (above secondary education) and were well-conversant in agricultural products. Most of the evaluators were experts in mushrooms, including scientists and technicians affiliated with the National Mushroom Development Institute. The olfactory profiles of the mushrooms were documented daily from the initial day through the last day of storage, with the intensity of the odor categorized accordingly.

Score	Odor
4	It has strong fragrance
3	Normal, No peculiar smell
2	Slightly unpleasant smell
1	Serious unpleasant smell

pH Measurement: The pH is measured using a pH meter, which includes a sensing unit comprising a glass electrode and a reference electrode (commonly a calomel electrode) connected by KCl Bridge to the pH sensitive glass electrode. An indicating unit is also part of the system, providing the corresponding pH reading based on the electromotive force detected.

TSS Measurement: Total Soluble Solids (TSS) were measured by refractometer. The measurement was conducted by dripping the liquid extract of mushroom fruiting bodies onto the detector. The TSS value is expressed as % Brix, and the displayed value is derived from the ratio of the speed of light in a vacuum to the speed of light through the sample.

Statistical Analysis: Three independent replications of all experiments were conducted. Data were analyzed with the statistical analysis software MS Excel. All data were subjected to an analysis of variance and a least significant differences test to determine significant differences $P \leq 0.05$ among the treatments.

RESULTS AND DISCUSSION

Texture Analysis: From the perspective of consumer acceptance, texture stands out as a crucial quality parameter. Typically, mushrooms are susceptible to softening during storage. Our investigation aimed to understand the impact of various storage conditions on the texture of mushrooms, with a focus on firmness, hardness, and resilience. As illustrated in Table 1, we observed a gradual decline in firmness over the storage period, indicating the susceptibility of mushrooms to softening during storage. The results indicate a relationship between texture and storage temperature, highlighting that lower temperature contributes to better-preserving mushroom texture. Additionally, a significant observation in our study was the effect of adding N2 gas to the samples. This addition demonstrated a notable impact on preserving mushroom freshness, leading to prolonged firmness.

The softening or loss of hardness in mushrooms during storage has been attributed to membrane changes (AresG, *et al.*, 2007). As noted by Zivanovic *et al.*, (2000), texture alternations are also linked to protein and polysaccharide degradation, hyphae shrinkage, disruption of the central vacuole, and expansion of the intercellular space at the piles surface.

Table 1. Analysis of the Texture of Oyster Mushrooms during Storage

Treatment	1st day		2nd day		4th day		6th day		8th day	
-	Hardness	Resilience	Hardness	Resilience	Hardness	Resilience	Hardness	Resilience	Hardness	Resilience
Ambient	172.00±3.5	0.24±0.05	75.66±2.08	0.55±0.02	-	-	-	-	-	-
N2 + Ambient	176.67±3.05	0.32±0.04	154.33±4.16	0.56±0.02	84.66±6.65	0.58±0.07	-	-	-	-
Refrigeration	170.03±3.5	0.22±0.07	142±3.53	0.25±0.02	116.5±3.5	0.30±0.02	96 ±5.65	0.35±0.04	-	-
N2 + Refrigerator	172.67±3.8	0.23±0.02	156.67±8.32	0.33±0.02	101.33±1.5	0.42±0.01	81.33±4.50	0.52±0.06	75.66±2.08	0.55±0.02

Moisture Content: The moisture content of food plays a pivotal role in influencing various attributes such as taste, texture, appearance, shape, and weight. Fresh oyster mushrooms typically have a moisture content ranging from 85% to 95% (kumar *et. al.*, 2013). Upon analysis of the provided bar chart (Fig-1), a consistent decrease in moisture percentage is evident at two-day intervals. Notably, samples stored in ambient conditions exhibit more pronounced fluctuations in moisture percentage compared to their refrigerated counterparts. In contrast, samples subjected to refrigerated + N2 gas show reduced moisture loss and display a tendency towards shrinkage. The prolonged shelf life and sustained freshness observed in refrigerated+N2 samples can be attributed to the lower rates of moisture exchange and metabolic activities. The use of N2 gas, being an inert gas, contributes to creating an environment that minimizes the impact of respiration on moisture content.

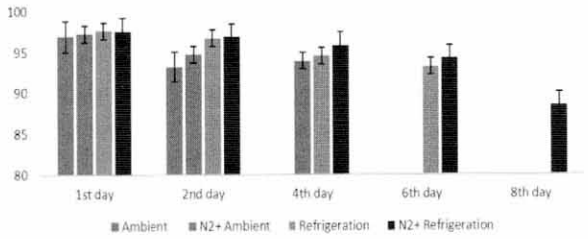

Fig. 1. Moisture Content (%) of Mushroom During Storage.

Browning Index: Consumers generally prefer white mushrooms such as Oyster, Button, and Milky white varieties, due to their pristine white appearance. Post-harvest browning is a critical factor in determining mushroom pricing as it significantly impacts the quality of the mushrooms. This browning occurs mainly because phenolic substances convert into quinones through oxidation, primarily driven by the enzyme Polyphenol oxidase (PPO) (Huang *et al.*, 2017) The browning index gradually increases during the storage period, with mushrooms stored in all treated conditions ambient, refrigeration with N2 or without N2. Comparative analysis reveals that mushrooms stored in ambient conditions undergo noticeable browning and develop a slimy appearance by the second day of the experiment. In contrast, under refrigeration samples exhibited the least browning.

Table 2. Browning Index of oyster mushroom during storage

Treatment	1st day	2nd day	4th day	6th day	8th day
Ambient	1.08	2.81	-	-	-
N2-ambient	1.05	2.93	4.23	-	-
Refrigeration	0.96	1.31	2.01	4.23	-
N2 -Refrigeration	1.02	1.41	2.03	2.82	4.47

Weight Loss and Microbial Study: Previous research, such as that conducted by Xiao *et al.*, 2011, has established weight loss as a prevalent factor in the post-harvest loss of mushrooms. However, in our study, no significant losses in the weight of mushrooms were observed. This discrepancy may be attributed to the relatively small quantity of mushrooms used in the experiment, with only 200g per packet. Similarly, throughout the experiment, no evidence of microbial contamination was identified.

Odor: The distinctive aroma of mushrooms is a result of the intricate interplay among various compounds, including alcohols, aldehydes, ketones, acids, hydrocarbons, esters, as well as groups such as heterocyclic, aromatic, and sulfur compounds (Aisala *et al.*, 2019). These compounds not only play a crucial role in determining the post-harvest quality of mushrooms, contributing to their distinctive aroma, but also serve important functions in the growth physiology and interactions of mycelium (Ditengou, 2015). Our sensory panel observed noticeable changes in the aroma of mushrooms under different storage conditions. At ambient temperature, the mushrooms exhibited signs of spoilage by the second day with a score of 1, indicating a seriously unpleasant smell. Similarly, under ambient temperature with N2 gas, the smell had onset to change, registering a score of 2 for a slightly unpleasant smell within 4 days. In contrast, mushrooms stored under refrigeration, both with and without N2 remained unchanged, receiving a score of 3, indicating a normal, no peculiar smell up to day 6. Further assessment on day 6 focused solely on the refrigerated samples. The sample without N2 had undergone a noticeable change in odor, while the sample with N2 had also started to show alterations. These findings highlight the impact of storage conditions, particularly temperature and the presence of N2 gas, on the aroma of mushrooms.

Changes in pH: The pH of foods is a critical factor influencing their appearance, texture, flavor, nutritional content, and safety. The pH value reflects the concentration of free hydrogen ions in a food, directly impacting its acidity or alkalinity. According to Table 3, the pH is gradually decreasing in both ambient and refrigerated conditions, signaling a shift towards which indicates heading to alkaline conditions. To maintain the freshness of mushrooms post-harvest, it is recommended to keep the pH within the range of 6.0 to 7.0 (Tarlak *et al.*, 2020).

Table 3. Changes in pH among the Treatment

Treatment	pH				
-	1st day	2nd day	4th day	6th day	8th day
N2 + Ambient	6.03±0.05	7.24±0.02	7.48±0.02	-	-
Refrigeration	6.05±0.08	6.08±0.03	7.15±0.05	7.67±0.06	-
N2 + Refrigerator	6.59±0.07	6.66±0.05	7.51±0.03	7.71±0.02	7.8±0.02

Changes in Total Soluble Sugar (TSS): The concentration of total soluble sugar (TSS) was determined by assessing the cumulative content of sugar forms in the fruit, encompassing sucrose, glucose, fructose, and sorbitol. As depicted in Table 4, there is a gradual decrease in TSS level in both conditions, although no significant changes were observed. Particularly noteworthy is the observation that, after four days of storage, the TSS value remained constant in the refrigerator condition. Despite an overall decline in sugar levels in both storage conditions, it was noted that the samples subjected to refrigeration exhibited a slower rate of sugar decrease compared to those stored in ambient conditions.

Table 4. Changes in TSS among the Treatments

Treatment	Total Soluble Sugar (TSS)				
-	1st day	2nd day	4th day	6th day	8th day
N2 + Ambient	6.3±0.3	5.5±0.2	5.0±0.7	-	-
Refrigeration	5.9±0.8	5.2±0.6	5.1±0.3	4.6±0.8	-
N2 + Refrigerator	6.2± 0.6	5.7±0.6	4.9±0.4	4.9±0.5	4.8±0.4

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Minerals and Heavy Metals Uptake by Oyster Mushroom (*Pleurotus ostreatus*) from Rice Straw and Sawdust

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Abstract

In Bangladesh, the cultivation of oyster mushrooms (Pleurotus ostreatus) traditionally relies on rice straw (RS) and sawdust (SD) substrates. Interestingly, the mushroom fruiting bodies displayed reduced levels of Na and Fe, despite these elements being more concentrated in the growing substrates. This suggests that P. ostreatus may not efficiently bioaccumulate Na and Fe. Conversely, higher bioaccumulation was observed for Zn and P, surpassing substrate levels. Notably, our study revealed minimal levels of lead and arsenic in the mushroom samples, affirming their safety for consumption.

Keywords: Oyster mushroom, Mineral, Heavy Metals, Bioaccumulation.

INTRODUCTION

Oyster mushrooms, known scientifically as *Pleurotous spp* are well recognized as primary decomposers, and their ability to thrive in fruiting bodies on a diverse range of substrates. Mushroom production is one of the most effective biotechnological approaches for recycling lignocellulosic organic waste. It can secret an array of lignocellulosic enzymes for degrading environmental pollutants *(Carrasco et al., 2018; Kabel et al.*, 2017). In addition to converting environmental pollutants into protein-rich human food, mushrooms also produce notable nutraceutical compounds like biologically active proteins, antioxidants, terpenoids, and sterol- which makes mushrooms a great choice for human health (Bell *et al.*, 2022; Jana and Acharya, 2022; Kour *et al.*, 2022). Due to the saprophytic nature of mushrooms, they derive their nourishment from the growing substrate, which has an impact on the chemical, functional, and sensory properties of mushroom fruit bodies (Saba *et al.*, 2016). Additionally, mushrooms can accumulate various metals from their substrates, including essential nutrients like Cu, Fe, Mn, and Zn (Demková *et al.*, 2021; Damodaran *et al.*, 2014; Lalotra *et al.*, 2016; Seyfferth *et al.*, 2016).

Mushrooms hold significant dietary importance in Bangladesh, particularly Oyster mushrooms (*Pleurotous ostreatus*), which are predominantly cultivated using sawdust and rice straw. The capacity of mushrooms to accumulate heavy metals is well-known. These pollutants can pose adverse effects not only on environmental organisms but also on humans through the food chain. Hence, the objective of this study is to analyze the metal content of cultivated mushrooms, intending to enhance local awareness regarding the safety of their consumption.

MATERIALS AND METHODS

Materials: Rice straw (RS) and Sawdust (SD) were sourced from local farmers and sawmills, serving as substrates for mushroom cultivation. The cultivation of mushrooms took place at The Mushroom Development Institute in Savar, Dhaka.

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Minerals and toxic metals analysis

Sample preparation: The obtained mushroom samples and the substrates were oven-dried at 60±2° C until a constant weight was achieved. The dried samples were ground into a fine powder using a mortar & pestle. All mushroom samples were mineralized prior to analysis. In this regard, 0.5g of powdered samples were placed into the Teflon vessels with 5 mL 65% Nitric Acid (Analar Grade, Merck, Germany) and 2 mL 30% Hydrogen peroxide (Merck, Germany). The digestion was completed in Microwave Digester (Model: Ethos One, Milestone, USA) at 180 °C for 45 minutes (Lao *et al.*, 2023). After the completion of digestion, the digests were transferred into 50 mL volumetric flasks and made up the volume by Class I (18 MΩ) deionized water. All reagents used for heavy metal analysis were of Analar grade.

Analytical Methods: Analyses of mineral elements (Na, Mg, P, Zn, Fe, Cu) were performed using Flame Atomic Absorption Spectrophotometry (FAAS) (Brzezicha *et al.*, 2019). The toxic heavy metals (Pb, and As) were performed using Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS) (Dowlati *et al.*, 2021). Both flame and electrothermal modes were operated by the Shimadzu Instrument, Model AA-7000 (Japan origin). The analytical conditions of the AAS instrument such as detection limits, wavelength (nm), cathode lamp current (mA), slit width (0.2-0.7), and flame (air-acetylene mixture) were adjusted based on the heavy metals according to available literature. The standard recovery percentages of the analytes ranged to be from 95-105%.

Statistical analysis: One-way ANOVA was performed using SPSS software version 20 (SPSS, Inc., Chicago, Illinois, USA). DMRT test for the separation of means was applied for $p < 0.05$ when the ANOVA was significant ($p<0.05$).

RESULTS AND DISCUSSION

Mushrooms are important sources of minerals and play a vital role in the proper development of the human body. Thus, we evaluated whether the mineral contents of the substrate influenced the yield and quality of *Pleurotus ostreatus*.

Minerals and Heavy Metals Concentration in Sawdust Substrate before Inoculation and after Mushroom Fruiting bodies: Table 1 illustrates the mineral profile, based on dry weight, encompassing both macronutrients Sodium (Na), Magnesium (Mg), and Phosphorous (P) as well as micronutrients Zinc (Zn), Iron (Fe), and Copper (Cu) in both the substrate and the fruiting bodies of oyster mushrooms cultivated in the substrate.

Table 1. The Mineral Content of the Sawdust Substrates and Mushroom Fruiting Bodies

Treatments	Macronutrient (mg/100g dm)			Micronutrients (mg/100g dm)			Toxic Minerals (mg/100g dm)	
	Na	Mg	P	Zn	Fe	Cu	Pb	As
Before Substrate (SD)	2951.64^{a} ± 0.7	3177.56^{a} ± 0.5	1.78^{e} ± 0.12	1.58^{d} ± 0.4	94.71^{a} ± 0.5	1.01^{c} ±0.5	0.012^{b} ±2.9	0.03^{a} ±3.5
After Mushroom (SD)	680.99^{d} ± 2.06	1594.28^{c} ± 0.85	2.384^{b} ± 0.95	8.82^{a} ± 0.88	5.56^{c} ± 0.07	1.58^{b} ±1.01	0.0013^{d} ±0.006	BDL

Here, SD= Sawdust, Na= Sodium, Mg= Magnesium, P= Phosphorous, Zn= Zinc, Fe= Iron, Cu= Copper, Pb= Lead, As= Arsenic and BDL= Below detectable level. Values are the means of three replicates. Means followed by different letters in each column are significantly different ($P<0.05$) according to Duncan's Multiple Range Test (DMRT).

In the case of the SD substrate, there were distinct and similar concentration patterns of macro-nutrients observed in both the substrate and fruiting bodies, with the pattern being Mg > Na > P. Specifically, in the SD substrate, the concentration were Mg (3177.56 mg/100gm), Na (2951.64 mg/100gm), and P (1.78 mg/100gm). This concentration pattern was mirrored in mushroom fruiting bodies grown in the SD substrate, with Mg (1594.3mg/100gm), Na (680.95 mg/100gm) and P concentration 2.39mg/ 100gm.

Minerals and Heavy Metals Concentration in Rice Straw Substrate before Inoculation and after Mushroom Fruiting bodies: In contrast, when considering the Rice Straw (RS) substrate, the macronutrient content exhibited a different order, with Na (4862.34mg/100gm) being the highest, followed by Mg (1453.60mg/100gm) and then P (3.89mg/100gm). Interestingly, the mushrooms cultivated in the RS substrate displayed a distinct pattern, with Mg (2120.09mg/100gm) being the highest, followed by Na (1196.33mg/100gm), and then P (2.77mg/100gm) (Table 2).

Table 2. The Mineral Content of the Rice Straw Substrates and Mushroom Fruiting Bodies

Treatments	Macronutrient (mg/100g dm)			Micronutrients (mg/100g dm)			Toxic Minerals (mg/100g dm)	
-	Na	Mg	P	Zn	Fe	Cu	Pb	As
Before Rice Straw (RS)	4862.34^{a} ± 4.1	1453.60^{a}± 2.1	3.89^{a}± 0.09	7.62^{b}± 2	23.84^{b}±2	1.24^{b}±1.1	0.026^{a}±2.8	BDL
After Mushroom (RS)	1196.33^{c}±2.51	2120.09^{b}± 1.0	2.77^{b}± 0.03	5.41^{c}±2.02	1.78^{d}±1.01	0.72^{d}±0.03	0.0091^{c}±0.05	BDL

Here, RS= Rice straw, Na= Sodium, Mg= Magnesium, P= Phosphorous, Zn= Zinc, Fe= Iron, Cu= Copper, Pb= Lead, As= Arsenic and BDL= Below detectable level. Values are the means of three replicates. Means followed by different letters in each column are significantly different (P<0.05) according to Duncan's Multiple Range Test (DMRT).

Furthermore, trace elements, including Fe, Zn, and Cu were detected in both the substrate and the mushroom (Table 1&2), revealing significant differences, and a clear trend of Fe > Zn > Cu in the substrate. However, in mushroom fruiting bodies, the concentration pattern for these trace elements shifted to Zn > Fe > Cu in both substrate SD and RS (Table 1&2). Our observation aligns with the findings of Falandysz and Borovička, 2013 who also asserted higher Zn concentration compared to Cu in mushrooms.

In our study, the levels of lead and Arsenic in the mushroom samples were found to be very low, indicating that these mushrooms are safe for consumption.

The analysis of minerals elements (Na, Mg, P, Zn, Fe, and Cu) in three substrates used in the study revealed macronutrient Na and Mg concentrations were high while P concentration was significantly lower (Table 1&2). Similar trends were also observed in mushroom fruiting bodies. Furthermore, our result established that mushrooms possess the ability to accumulate minerals in substantial amounts, often exceeding those present in the substrate which is supported by the line findings Florczak *et al.*, 2014.; Mleczek *et al.*, 2016. Surprisingly, the P concentration in the SD substrate was significantly lower than RS substrates. However, this difference was not reflected in the elemental composition of the fungal fruiting bodies, as a significantly higher P value was observed in the fruiting bodies produced on SD.

Our result also showed that Na content in the substrate is higher than that found in the mushroom fruiting bodies. This suggested that mushrooms lack the capacity for bioaccumulation of Na. In contrast, multiple investigations have shown that mushrooms bioconcentrate Mg and P in their fruiting bodies (Frankowska *et al.*, 2010; Hultberg *et al.*, 2023; Kojta and Falandysz, 2016; Li *et al.*, 2017; Malinowski *et al.*, 2021)including toxicologically important Cd and Hg, in popular and prized wild King Bolete mushrooms. We investigated the importance of soil substratum as a source of these metals. ICP–OES and CV-AAS were applied to determine the profile of Al, Ba, Ca, Cd, Cu, Fe, Hg, K, Mg, Mn, Na, Sr and Zn in caps and stipes of King Bolete mushroom and in the surface layer of soil (0–10 cm. The biological conversion factor (BCF) of Mg ranged from 1.5 to 7.2, and P ranged from 10 to 50 times (Falandysz and Borovička, 2013; Frankowska *et al.*, 2010; Rudawska and Leski, 2005). Our research finding confirmed that *Pleurotus ostreatus* exhibits the ability to bioconcentrate Mg and P but not Na.

Consistent with other studies, we found that in mushroom fruiting bodies, the amount of Zn is higher than that of Cu irrespective of the substrate. Furthermore, it is noteworthy to note that the substrate's Fe content was significantly higher than that of mushroom fruiting bodies. Our findings contradict those made by (Almeida et al., 2015), who claimed that P. ostreatus was capable of bioaccumulation of Fe. It is well known that Fe uptake by fungus is a complex process, and it depends on the speciation

of Fe (II) and Fe (III) forms (Philpott, 2006). More importantly, our results for trace elements falling within acceptable nutritional levels for human consumption are shown in Table 3.

Table 3. Recommended Daily Intake (RDI) of trace elements

Element	RDI	Reference
Cu	2mg/day	WHO 1996
Fe	18mg/day	WHO 1996
Zn	15mg/day	WHO 1996

Here, Cu= Copper, Fe= Iron, Zn= Zinc and RDI= Recommended Daily Intake.

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Utilization of Tea Waste as Substrate for the Production of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract

Tea waste, an abundant and economical lignocellulosic leftover from the tea industry, has the potential to serve as an alternative substrate for cultivating oyster mushrooms (*Pleurotus ostreatus*). This study addresses the dual concerns of environmental impact associated with tea waste disposal and the viability of using it for mushroom cultivation. A variety of substrate formulations were prepared by combining waste tea leaves (WTL) with sawdust (SD) and rice straws (RS). The resulting mushroom yield in terms of biological efficiency was analyzed. The finding of our study showed that tea trash alone is not an optimal substrate for mushroom growth. However, when blended with sawdust in the proportion of up to 50%, it significantly enhances mushroom yield and biological efficiency. Our study has proved waste tea leaves as a profitable and sustainable supplement with sawdust not with rice straw for mushroom cultivation in terms of biological efficiency and cost-benefit ratio.

Keywords: Tea wastes, Sawdust, Biological Efficiency, Cost-Benefit Ratio, Substrate.

INTRODUCTION

Oyster mushrooms (*Pleurotous* spp) are well-recognized as primary decomposers, and ability to produce fruiting bodies on a wide array of substrates (Fernandes *et al.*, 2015). According to kabel *et al.*, (2017) and Carrasco *et al.*, (2018), mushroom production is one of the most effective biotechnological approaches for recycling lignocellulosic organic waste since it can secret an array of lignocellulosic enzymes for degrading the environmental pollutant. In addition to converting environmental pollutants into protein-rich human food, it also can produce noteworthy nutraceutical compounds like biologically active proteins, antioxidants, terpenoids, and sterol which makes mushrooms a great choice for human health (Jana & Acharya, 2022; Bell *et al.*, 2022; Kour *et al.*, 2022).

In Bangladesh, Oyster mushrooms are primarily produced on sawdust and rice straw. Currently, commercial mushroom farmers are finding it more difficult and expensive to obtain due to the rising demand in the livestock sector and mushroom farming. In light of this problem, finding substitute substrates or reducing the use of sawdust and rice straw for mushroom growing are now beginning to receive much-deserved attention.

Like other nations, tea is one of the most consumed beverages in Bangladesh, and its consumer markets are currently expanding and growing a variety of items. However, after extracting water-soluble components from tea leaves a massive amount of tea waste is left. As a result, managing waste tea is a challenge for many enterprises that produce tea. The growing amount of tea waste not only poses a threat to the environment but also requires a lot of labor and space to be disposed of. Thus, it is urgent to review the options for solving this issue. Although several measures, such as using it as livestock food or turning it into fertilizer, have been suggested, very few are practical enough to meet the demands of the tea processing businesses.

Numerous studies have looked at how to best combine tea waste with various substrates like sugarcane bagasse and tea leaves (Chukowry *et al.*, 2009), cottonseed hull and tea waste (Doudou *et al.*, 2015), tea waste and bran (Baktemur *et al.*, 2018), among others. Notably, Bangladesh does not use cottonseed hull, or sugarcane bagasse for commercial mushroom growing. Therefore, it is important to consider tea

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waste might be used in place of sawdust and rice straw in the production of mushrooms, either whole or in part. In addition, a detailed substrate formula should be developed and optimized for practical large-scale production. Thus, based on the aforesaid facts, our project objective is the assessment of different formulas of tea waste combined with rice straw and sawdust.

MATERIALS AND METHODS

Materials: Black tea residue left after the water-soluble contents of tea had been extracted in hot water (above 100°C) was used as tea waste. We collected tea waste from the local tea stall. Rice straw and Sawdust were obtained from local farmers and sawmills. Mushroom (*Pleurotus ostreatus*) inoculum was obtained from Mushroom Development Institute Savar, Dhaka.

Treatment: Different ratios of waste tea leaves (WTL), sawdust (SD), and Rice straw (RS) (shown in Table 1) were used as substrate for mushroom cultivation. In the experiment, sawdust supplemented with wheat bran and rice straw without any supplement was used as a control.

Table 1: Different substrate combinations used as a treatment

Treatment	Composition
Control	90%Sawdust + 10% wheat bran
$T_{1.1}$	75% of SD + 25% of WTL
$T_{1.2}$	50% of SD +50% of WTL
$T_{1.3}$	25% of SD +75% of WTL
Control	100% Rice straw
$T_{2.1}$	75% of RS + 25% of WTL
$T_{2.2}$	50% of RS +50% of WTL
$T_{2.3}$	25% of RS + 75% of WTL

Here, SD= Sawdust, WTL= Waste tea leaves.

Sawdust packet preparation: Sawdust and tea leaves were taken into a mixing machine and gradually added tap water to adjust the moisture at 65-70%. A 500g of wet substrate was taken in a polypropylene bag and autoclaved at 121°C for 1h. After cooling, each bag was spawned with fungal seed and incubated at 25±2°C, and the humidity was 50–60% at which point the substrates were densely colonized with mycelium. After those packets were transferred to the culture room for fructification.

Rice Straw Preparation: Prior to usage, the dried rice straw (RS) was chopped into small pieces (3-5cm long) using chaff cutter tools. The pieced RS was then completely moistened by soaking it in tap water for 24 hours, and it was pasteurized using a clean steel drum. On the other hand, waste tea leaves (WTL) are collected and placed in a plastic bag before being pasteurized in the same drum. Pasteurization process, in brief, the water was heated at 60°C initially. The substrate was then added and left in warm water for 30 min. Both substrates were pasteurized before being wrapped in a sterile plastic sheet (2m×4m) and placed on a steep concrete floor to drain excessive water and achieve a moisture level of 65-75%. Layer spawning was employed to mix fungal seed with the RS and WTL before being tightly sealed in polypropylene bags (35×17 cm2) and allowed to packet in the dark room (light intensity<50 lux), where the temperature was 25±2°C, and the humidity was 50–60%. The plastic bags containing the substrates were moved to the cropping room after the mycelia had colonized them.

For fruiting body formation, the temperature was 25 to 30°C, the humidity was 80–90%, and the illumination was 150–200 lux. The bags were opened when spawning was complete and placed in a

fruiting body formation atmosphere.

Data Collection for Yield Performance: The growth and development of mushrooms were monitored daily. The time (numbers of days) required from inoculation to completion of mycelium running, and the time elapsed between opening the plastic bags to pinhead formation were recorded. Mushrooms were harvested when the mushroom cap surface was flat to slightly uprolled at the cap margins. For the collection of data for yield parameters and biological efficiency, the harvested fruiting bodies in each bag were then counted and weighed. A total of two flushes were recorded. Accordingly, biological yield (g) was determined by weighing the whole cluster of fruiting bodies without removing the base of stalks. Finally, the biological efficiency (%) was evaluated according to the reported method and calculated as follows: BE=(wf/wd) ×100, where E is the biological efficiency, wf is the weight of the fresh harvested fruiting body, and wd is the weight of dry matter of the substrate.

Statistical analysis: One-way analysis of variance (ANOVA) was used to analyze significant differences and mean values at the 5% level of significance. One-way ANOVA was performed using SPSS software version 20 (SPSS, Inc., Chicago, Illinois, USA). Duncan's HSD test for the separation of means was applied for $p < 0.05$ when the ANOVA was significant ($p<0.05$).

RESULTS AND DISCUSSION

Time elapsed for mycelium running and pin-head formation: The total colonization time and days from bag opening to primordia formation of oyster mushroom cultivation on different substrates are presented in Fig. 1a and 1b. Intriguingly, T1.3 (75% WTL + 25% SD) and T2.3 (75% WTL + 25% RS) showed weak mycelial growth that eventually ceased, which prevented colonization. So, these packets were thrown away. Furthermore, no discernible changes were found in the number of days needed for mycelium completion (MCD) among the various substrate formulae. The time needed for completing spawn running ranged from 27 to 30 days for SD + WTL-based substrate formulas and 30 to 31 days for RS + WTL-based substrate formulas, showing that the presence of tea waste did not slow down or speed up the mycelial growth.

Fig. 1a and 1b also showed the data patterning time taken for the appearance of the pinhead after the opening of the complete spawn packet. Time taken for pinhead initiation (TPI) varied significantly ($p<0.05$) among the treatments. In SD+ WTL preparation, T1.2 (50% WTL + 50% SD) showed the lowest time ranged between 6 to 8 days. In RS + WTL, the lowest TPI (ranging from 4 to 5 days) was observed at control (100% RS).

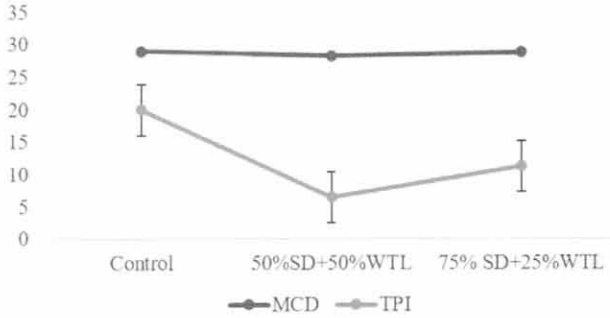

Fig. 1a. Time elapsed for mycelium completion and Pinhead initiation of oyster mushroom on Sawdust (SD) and different combination with Waste tea leaves (WTL).

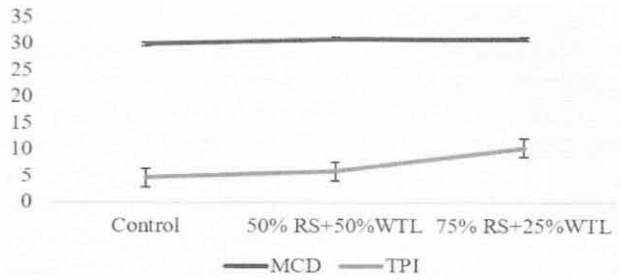

Fig. 1b. Time elapsed for mycelium completion and Pinhead initiation of oyster mushroom on Rice straw (RS) and different combinations with Waste tea leaves (WTL).

Yield and Biological efficiency: The yield of two flushes was recorded and analyzed as shown in Fig. 2. The yield significantly ($p<0.05$) varied among the different tea waste ratios irrespective of sawdust and rice straw. In sawdust, adding 50% tea leaves ($T_{1.2}$) showed a significant yield increase of 189g fresh mushroom/packets and BE (79%) whereas control (only sawdust) showed the lowest yield of 130g /packets and BE (62%). Unlikely, rice straw control (only rice straw) showed the highest yield 357.4g/packet and BE (89%) while the lowest yield was observed at $T_{2.2}$ and BE (64%).

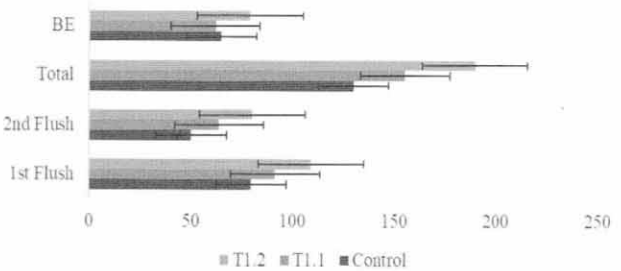

Fig. 2a. Yield performance of oyster mushroom on Sawdust (SD) and different combinations with Waste tea leaves (WTL).

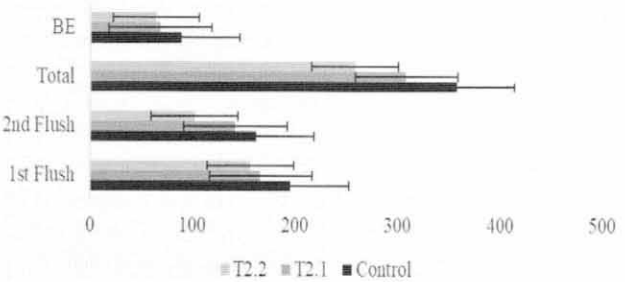

Fig. 2b. Yield performance of oyster mushroom on Rice straw (RS) and different combinations with Waste tea leaves (WTL).

Biological efficiency (BE) is used to assess how effectively substrate is converted in the mushroom cultivation process and is calculated as the ratio of the fresh weight of harvested mushrooms to the dry weight of the cultivation substrate. A greater BE score ensures a high likelihood of substrate utilization for mushroom growth. To know, whether using a substrate formulated for mushroom farming whether profitable the BE value must be higher than 50% (Wakchaure, 2011). Additionally, the BE of the substrate is related to the mushroom species, substrate, and the pertinent environmental conditions. (Grimm and Wösten, 2018; Sadh *et al.*, 2018).

Economic Analysis: The economic analysis presented in Table 2 utilized the benefit-cost (B: C) ratio, as defined by Falahzadah et al., 2023. The ratio is calculated by dividing gross sales revenue from mushroom cultivation by all related expenses. B: C ratio greater than 1 indicates project acceptance with higher values signifying increased project viability from an economic standpoint. In our study, the B: C ratio ranged from 2.45 to 3.95 for each 20kg of substrates, reflecting the income-to-substrate cost ratio. With a mushroom value of 250tk/kg, these results demonstrate the overall economic advantage of all substrate formulations for mushroom production. For each 20kg of the substrates, the income-to-substrate cost ratio was computed. The mushroom worth is 250tk/kg. In our study B: C ranged from 2.45 to 3.95 demonstrating that all substrate formulations for mushroom production are advantageous from a business standpoint.

Table 2. The benefit - cost ratios of the substrates used in the experiment

Substrate	Cost of spawning 20kg substrate (BDT)	Variable cost/20kg (BDT)	Yield/kg of substrate (g)	Income/20kg of substrate (BDT)	Benefit-Cost ratio
100% SD	250	600	520	2600	3.06
75% SD + 25% WTL	192	600	622	3110	3.93
50% SD + 50% WTL	360	600	758	3790	3.95
100% RS	300	600	615	3075	3.42
75% RS + 25% WTL	290	600	616	3080	3.46
50% RS + 50% WTL	250	600	416	2080	2.45

Here, SD= Sawdust, WTL= Waste tea leaves.

In our study, the rice straw control group outperformed the sawdust control group in terms of mycelium growth, biological yield, and biological efficiency. Noteworthy, no mushroom mycelium growth was observed in a 75% WTL combination, regardless of substrate, and comparable findings were made by Doudou *et al.*, 2015. The possible reasons are WTL content less amount of cellulose, hemicelluloses, low C/N, and greater amount of lignin shown in Table 3. Notably, the differences in yield of *Pleurotus spp* were caused by variations in the physical and chemical composition of substrate formulations, such as cellulose, hemicellulose, lignin, mineral content, and C/N ratio (Suwannarach *et al.*, 2022). Additionally, Mintesnot *et al.*, 2014 asserted that substrate having a greater C/N ratio favored mycelial growth and was less favorable for fruiting body formation. Moreover, the substrate with a high lignin concentration is less favorable for mushroom growth because makes it less accessible to enzymes. (Kaya *et al.*, 2022). The yield and biological efficiency are increased remarkably when WTL and SD are combined, showing that WTL may aid in reducing the substrate's C/N ratio.

Table 3. Physico-chemical properties of Rice Straw, Sawdust, and Tea leaves

Substrate	Cellulose	Hemicellulose	Lignin	C/N ratio	Ref.
Rice straw	32-39	23-24	18-36	35-72/1	Tayeb *et al.*, 2012; Limayem *et al.*, 2012
Sawdust (Hardwood)	40-55	24-40	18-25	150-450/1	Sun *et al.*, 2002
Tea waste	28.13	10.53	28.56	28.46/1	Mahmoud *et al.*, 2020

From the economic point of view, the ratio of input (cost of substrate) and output (benefit of mushroom) calculated in Table 2 was the most effective. Although the benefit taken from waste tea leaves is less compared to control, it can be considered a profitable option.

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Effect of Spawn Age on Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract

An experiment was conducted at the Mushroom Development Institute (MDI) in Savar, Dhaka, Bangladesh, from November 2022 to March 2023, to evaluate the impact of spawn age on the growth and productivity of oyster mushrooms (*Pleurotus ostreatus*). The study employed a single- factor Completely Randomized Design with eleven treatments. Where T1=(0 days old spawn), T2=(3 days old spawn), T3=(6 days old spawn), T4=(9 days old spawn), T5=(12 days old spawn), T6=(15 days old spawn) T7=(18 days old spawn). T8=(21 days old spawn). T9=(24 days old spawn). T10=(27 days old spawn). T11=(30 days old spawn). The findings revealed that spawn age did not significantly affect the number of days required for complete mycelium running, which consistently took 26 days across all treatments. However, spawn age significantly influenced the days to pinhead initiation, days to first harvest, number of fruiting bodies, effective fruiting bodies, and various morphological traits such as stalk length, stalk diameter, pileus length, and pileus diameter. Notably, a 9-days-old spawn (T4) resulted in the shortest time to pinhead initiation (3.03 days) and first harvest (2.90 days), the highest number of fruiting bodies (69.47), and the highest yield per packet (209.20 gm). Conversely, a 30-days-old spawn (T11) exhibited the longest time to pinhead initiation (7.27 days) and first harvest (5.10 days), the lowest number of fruiting bodies (38.70), and the lowest yield per packet (114.60 gm). In conclusion, the optimal spawn age for maximizing the yield and productivity of oyster mushrooms is 9 days, whereas the least productive spawn age is 30 days.

Keyword: Spawn age, Growth and yield, Oyster, Mushroom.

INTRODUCTION

The oyster mushroom, belonging to the genus Pleurotus and family Pleurotaceae, is recognized as an edible species (Randive, 2012). Generally, mushrooms are identified by their distinct structural features: a stem (stipe), cap (pileus), gill-like structures (lamellae), and spore-bearing surface underneath the cap (Masarirambi *et al.*, 2011). Edible mushrooms are characterized by their complete edibility and absence of toxicity. Of the numerous edible mushroom species, approximately 80 have undergone experimental cultivation, with 20 being commercially farmed and 4-5 produced on an industrial scale worldwide (Thongnaitham, 2012). Oyster mushrooms are notable for their nutritional value, taste, medicinal benefits, and economic potential. The optimal cultivation periods vary by elevation: in higher altitudes, the best growing season is from March/April to September/October, whereas in lower regions, it is from September/October to March/April (Masarirambi *et al.*, 2011).

Pleurotus ostreatus, commonly known as the oyster mushroom, holds significant importance both in culinary and medicinal realms. This versatile fungus not only enriches dishes with its delicate flavor and meaty texture but also offers a plethora of health benefits. Oyster mushrooms typically comprise approximately 86-96% water, 3.15% protein, 4.2%

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carbohydrates, 0.105% fats, 1.05% minerals, and vitamins (Mostak *et al.*, 2013). On a dry weight basis, they contain 19.95- 36.75% protein, surpassing rice (8.65%), wheat (13.86%), and even milk (26.475%) (Randive, 2012). Moreover, *Pleurotus ostreatus* possesses medicinal properties that contribute to its significance in traditional and alternative medicine practices (Wasser, 2010 and Jayakumar *et al.*, 2009).

The quality of mushroom production is contingent upon the quality of spawn, thereby necessitating consideration of spawn age. This is crucial because mushroom quality is intrinsically linked to spawn quality (Sabri et al., 2020). According to Soko et al. (2019), significant variations in biological efficiency are observed across various spawn ages, emphasizing the pivotal role of spawn age in determining both yield and nutritional quality of oyster mushrooms cultivated within the unique environmental and agricultural setting of Côte d'Ivoire.

An experiment was conducted in Mushroom Development Institute to highlight a significant link between the age of spawn and the number of mushrooms produced, determined the optimum age of oyster mushroom spawn and identified the best quality spawn for maximum yield. Essentially, the age of the spawn can greatly affect how many mushrooms are yielded. Exploring this aspect further could provide valuable insights into optimizing mushroom yields in Bangladesh.

MATERIALS AND METHODS

The study was conducted to evaluate the impact of spawn age on the growth and productivity of oyster mushroom (*Pleurotus ostreatus*). The experimental activities were done at the Mushroom Development Institute (MDI) in Savar, Dhaka. These activities spanned from October 2023 to January 2024.

Materials: The cultivation of a specific variety of oyster mushroom, namely *Pleurotus ostreatus* PO2, was carried out within the controlled environment of the 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, located in 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.

Treatment: The experimental setup comprises eleven distinct spawn age categories, each replicated ten times. The study encompasses various treatments, which are as follows:

T1 = (0 days old)
T2 = (3 days old)
T3 = (6 days old)
T4 = (9 days old)
T5 = (12 days old)
T6 = (15 days old)
T7 = (18 days old)
T8 = (21 days old)
T9 = (24 days old)
T10 = (27 days old)
T11 = (30 days old)

The packets were inoculated separately with oyster mother (PO_2). The inoculated packets were incubated at 20±2^{0}C.

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 11 distinct treatments. Each of these treatments was subjected to ten separate instances of replication, ensuring a robust dataset 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 study.

Mycelium running in spawn packet: The packets were kept at 20±2^{0}C. temperature until the packets become white with mushroom mycelium. After completion of mycelium running the rubber band, cotton plug and plastic neck of the mouth of spawn packet were removed and the mouth was wrapped tightly with rubber band. Then these spawn packets were transferred to the culture house.

Opening the packet: Two end, opposite to each other of upper placed position i.e. on shoulder of plastic bag were cut in 'D' shaped with a blade and opened by removing the plastic sheet.

Cultivation of spawn packet: The moisture of the culture house was maintained 80-85% relative humidity. Regularly spraying water were done. Illumination was maintained at approximately 300-500 lux, providing adequate light for growth. Additionally, the culture house was ventilated evenly to ensure proper air circulation. Temperature control was essential for successful cultivation, with the culture house maintained at a steady 22°C to 25°C.

Data Collection: 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, diameter and length of pileus and yield (g/packet) were recorded. Weight of fruiting body was recorded after removing the lower hard and dirty portion of stipe.

Statistical Analysis: The investigation employed a Complete Randomized Design (CRD), encompassing 11 treatments with 10 replications each. Data pertaining to the examined characteristics underwent statistical analysis through Complete Randomized Design (CRD) utilizing Statistics 10 software. Analysis of variance was executed, and subsequent to this, means were juxtaposed, with the significance of differences assessed via Duncan's Multiple Range Test (DMRT) or Least Significant Difference (LSD) as outlined by Gomez and Gomez (1984), with interpretation conducted at a 5% probability level.

RESULTS AND DISCUSSION

Days required to complete mycelium running: There was no significant variation observed among the various ages of the spawn when it came to the number of days required for complete mycelium running (DCMR), which consistently took 26 days (Table 1). The treatments included T1 (0 days old), T2 (3 days old), T3 (6 days old), T4 (9 days old), T5 (12 days old), T6 (15 days old), T7 (18 days old), T8 (21 days old), T9 (24 days old), T10 (27 days old), and T11 (30 days old), and these were statistically similar in terms of DCMR. This finding aligns with Shah *et al.* (2004), who observed that the mycelial growth of *Pleurotus ostreatus* required between 16.67 and 26.00 days post-inoculation.

Days required for pinhead initiation: The highest days were required in T11 (30 days old

spawn) (7.27 days) for DRPI whereas, the lowest value (3.03 days) was found in T4 (9 days old spawn), which was statistically identical to T3 (6 days old spawn) (Fig. 1.) The observed trend can be attributed to the physiological and metabolic state of the spawn. Younger spawns are likely more vigorous and have a higher metabolic rate, which facilitates quicker colonization and subsequent pin head initiation. As spawn ages, the vigor and metabolic activity decrease, leading to delayed initiation times. This is reflected in the progressively increasing days to pin head initiation from the younger to the older spawn treatments (Obodai *et al.*, 2003).

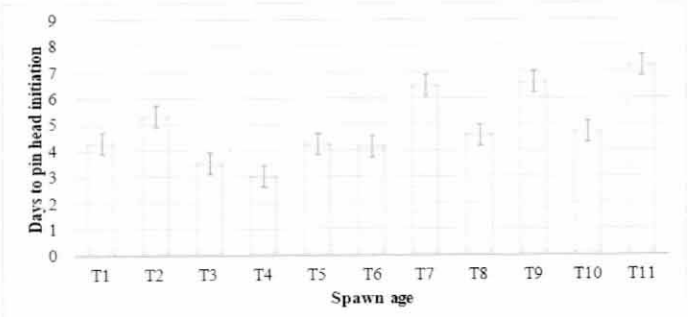

Fig. 1. Effect of age of spawn on the days required for complete mycelium running of oyster mushroom. Where T1=(0 days old spawn), T2=(3 days old spawn), T3=(6 days old spawn), T4=(9 days old spawn), T5=(12 days old spawn), T6=(15 days old spawn) T7=(18 days old spawn). T8=(21 days old spawn). T9=(24 days old spawn). T10=(27 days old spawn). T11=(30 days old spawn).

Days required from pinhead initiation to 1st harvest: The age of spawn significantly influenced the days required to first harvest (DFH) of oyster mushroom (Fig. 2) The values range from 2.90 to 5.10 days. The lowest value is observed in T4 (9 days old spawn) with 2.90 days which was statistically similar to T3 (6 days old spawn)., indicating the fastest harvesting time. This indicates that the use of a 9-day-old spawn is most effective for early. harvesting. The highest value is recorded in T11 (30 days old spawn) with 5.10 days, indicating the slowest harvesting time which was statistically identical to T9 (24 days old spawn). This suggests that older spawns delay the initial harvesting period. The outcomes of our current experiment align with the discoveries made by Foroughi and Bussler (2012), demonstrating that the duration for the first harvest varies based on the age of the spawn.

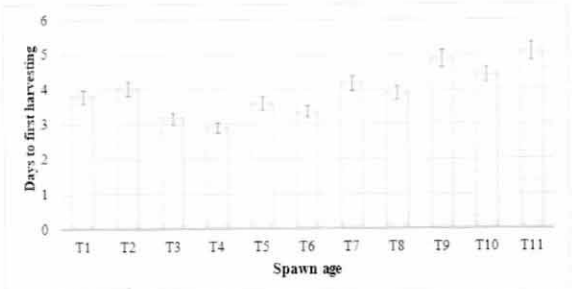

Fig. 2. Effect of age of spawn on the days required to first harvest of oyster mushroom. Where T1=(0 days old spawn), T2=(3 days old spawn), T3=(6 days old spawn), T4=(9 days old spawn), T5=(12 days old spawn), T6=(15 days old spawn) T7=(18 days old spawn). T8=(21 days old spawn). T9=(24 days old spawn). T10=(27 days old spawn). T11=(30 days old spawn).

Total number of fruiting body: The number of fruiting bodies varied significantly across treatments with different ages of spawn, ranging from 38.70 to 69.47 (Fig. 3). The highest number of fruiting bodies was observed in Treatment T4 (9 days old spawn), with an average of 69.47 fruiting bodies. This was followed closely by T5 (12 days old spawn) with 67.67 fruiting bodies and T3 (6 days old spawn) with 66.67 fruiting bodies. Conversely, the lowest number of fruiting bodies was recorded in Treatment T11 (30 days old spawn) with an average of 38.70 fruiting bodies. The data indicates that the age of the spawn has a significant impact on the number of fruiting bodies produced. Subramanian *et al.* (2015) and Foroughi and Bussler (2012) observed that as the spawn age increased, the number of fruiting bodies decreased.

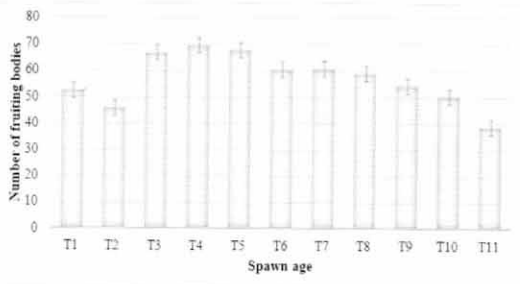

Fig. 3. Effect of different treatment on number of fruiting body per packet. Where T1=(0 days old spawn), T2=(3 days old spawn), T3=(6 days old spawn), T4=(9 days old spawn), T5=(12 days old spawn), T6=(15 days old spawn) T7=(18 days old spawn). T8=(21 days old spawn). T9=(24 days old spawn). T10=(27 days old spawn). T11=(30 days old spawn).

Number of effective fruiting body: Fig. 4 presents the number of effective fruiting bodies observed across different spawn ages (T1-T11). The data indicates a range of effective fruiting body counts, with the highest value observed in T4 (30.50) and the lowest in T11 (15.00). The trend in fruiting body formation appears to follow a pattern where the number of effective fruiting bodies initially increases, peaks around the middle stages (T4-T7), and then gradually decreases towards the later stages (T8- T11). This pattern suggests an optimal window for fruiting body formation, likely influenced by factors such as spawn age, substrate composition, and environmental conditions. Alam and Singha (2020), also underscored the pivotal role that spawn quality plays in the successful cultivation of mushrooms.

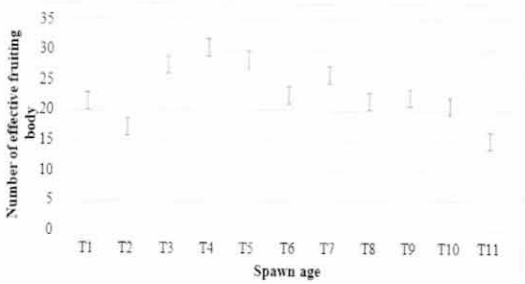

Fig. 4. Effect of age of spawn on the number of effective fruiting bodies of oyster mushroom. Where T1=(0 days old spawn), T2=(3 days old spawn), T3=(6 days old spawn), T4=(9 days old spawn), T5=(12 days old spawn), T6=(15 days old spawn) T7=(18 days old spawn). T8=(21 days old spawn). T9=(24 days old spawn). T10=(27 days old spawn). T11=(30 days old spawn).

Length of stalk (cm): A significant variation in length of stalk was found among different treatments. Stalk lengths varied from 3.10 cm (T3, 6 days old spawn) to 3.97 cm (T10, 27 days old spawn). The lowest stalk length was recorded for T3 (6 days old spawn) which was statistically identical to T5 (12 days old spawn), while the highest stalk length was observed for T10 (27 days old spawn) which was statistically similar to T4 (9 days old spawn) (Table 1). These findings imply that spawn age plays a crucial role in determining the length of stalks, with older spawn ages generally favoring longer stalks.

Diameter of stalk (cm): Table 1. presents the stalk diameter observed across different spawn ages (T1-T11). The spawn ages range from 0 days old (T1) to 30 days old (T11). The stalk diameter varied significantly across treatments with different ages of spawn. The data exhibits a range of diameters, with the smallest diameter recorded at 0.76 cm (T11) and the largest at 1.37 cm (T4) which was statistically similar to T6 (15 days old spawn). Here, the spawn age at 9 days (T4) resulted in the highest stalk diameter, while the 12-day-old spawn (T5) produced the smallest diameter. This observed pattern suggests an optimal age range for spawn development.

Length of pileus (cm): The length of pileus varied significantly across the different treatments, indicating a dynamic growth process influenced by the age of the spawn. The range of values observed in Table 1 spans from a minimum of 12.40 cm (T11, 30 days old spawn) to a maximum of 15.47 cm (T6, 15 days old spawn) which was statistically similar to T4 (9 days old spawn). This range signifies notable variability in pileus length throughout the observed age intervals. Among the treatments, T6 (15 days old spawn) exhibited the highest length of pileus at 15.47 cm, while T11 (30 days old spawn) displayed the lowest length at 12.40 cm. This suggests that the pileus tends to elongate significantly between the 12th and 15th days of spawn age (Table 1).

Table 1. Effect of age of spawn on the Days to complete mycelium running, Length of stalk, Diameter of stalk (cm), Length of pileus (cm) and Diameter of pileus (cm) of oyster mushroom

Treatment	Days to complete mycelium running	Length of stalk (cm)	Diameter of stalk (cm)	Length of pileus (cm)	Diameter of pileus (cm)
T1	26	3.45 c	1.03 d	14.68 b	0.77 bc
T2	26	3.33 c	1.15 c	13.22 e	0.63 d
T3	26	3.10 d	1.14 cd	13.97 c	0.72 c
T4	26	3.79 ab	1.37 a	15.15 a	0.96 a
T5	26	3.11 d	1.14 cd	13.44 de	0.75 bc
T6	26	3.75 b	1.32 ab	15.47 a	1.03 a
T7	26	3.47 c	1.22 bc	13.10 e	0.83 b
T8	26	3.72 b	1.15 c	13.13 e	0.81 b
T9	26	3.74 b	1.22 bc	13.95 c	0.76 bc
T10	26	3.97 a	1.11 cd	13.72 cd	0.77 bc
T11	26	3.36 c	0.76 e	12.40 f	0.81 b
CV%	3.94	8.44	7.90	5.42	5.47
LSD(0.05)	NS	0.2053	0.1164	0.463	0.0824

Here T1=(0 days old spawn), T2=(3 days old spawn), T3=(6 days old spawn), T4=(9 days old spawn), T5=(12 days old spawn), T6=(15 days old spawn) T7=(18 days old spawn). T8=(21 days old spawn). T9=(24 days old spawn). T10=(27 days old spawn). T11=(30 days old spawn).

Diameter of Pileus (cm): Table 1 presents the diameter of pileus (in centimeters) for different spawn ages, ranging from 0 to 30 days old. The diameter of pileus ranges from 0.63 cm to 1.03 cm across the various spawn ages. The lowest diameter is observed in T2 (3 days old spawn), while the highest diameter is recorded in T6 (15 days old spawn). This observed trend may be attributed to the initial establishment and proliferation of the organism in the substrate during the early stages of spawn growth, leading to a gradual increase in pileus diameter. The findings of Sharma *et al.* (1998) were in alignment with this outcome.

Yield (g/packet): Fig. 5 presents the yield per packet (in grams) for different treatments, each associated with spawn of varying ages. The yield per packet ranged from 114.60 grams to 209.20 grams, indicating a considerable diversity in productivity among the different spawn age treatments. Treatment T4, representing spawn aged 9 days, demonstrated the highest yield of 209.20 grams, while Treatment T11, representing spawn aged 30 days, exhibited the lowest yield of 114.60 grams. The yield generally showed an increasing trend from younger to older spawn age treatments up to a certain point, suggesting that as the spawn age increased, the yield tended to increase as well.

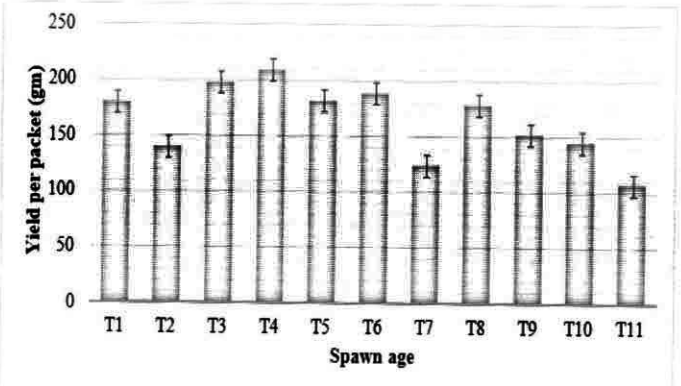

Fig. 5. Effect of age of spawn on the yield per packet of oyster mushroom. Where T1=(0 days old spawn), T2=(3 days old spawn), T3=(6 days old spawn), T4=(9 days old spawn), T5=(12 days old spawn), T6=(15 days old spawn) T7=(18 days old spawn). T8=(21 days old spawn). T9=(24 days old spawn). T10=(27 days old spawn). T11=(30 days old spawn).

Subramanian *et al.* (2015), Foroughi and Bussler (2012) and Sharma *et al.* (1998) observed that the yield of mushrooms diminished as the age of the spawn increased. This finding is consistent with the results of the current research, which also indicates a negative correlation between spawn age and mushroom production after a certain spawn age of 9 days.

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