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Effects of Temperature and Hydrogen Peroxide (H_2O_2) on Mycelial Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*)

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ABSTRACT

Background and Objective: Oyster mushroom (*Pleurotus ostreatus*) growth and yield are influenced by environmental factors such as temperature and chemical treatments like Hydrogen Peroxide (H_2O_2), which can affect mycelial development and productivity. This study explores the influence of temperature and Hydrogen Peroxide (H_2O_2) concentrations on the mycelial growth and yield of oyster mushrooms (*Pleurotus ostreatus*). The primary objective was to identify optimal conditions for maximizing growth and fruiting body production. **Materials and Methods:** Experiments were conducted at the Plant Pathology Laboratory, Patuakhali Science and Technology University. Mycelial growth was assessed on potato dextrose agar (PDA) under four temperatures (15, 20, 25, and 30°C) and five H_2O_2 concentrations (0.1, 0.25, 0.5, 0.75, and 1%). Spawn preparation and fruiting body cultivation were carried out on a sawdust-wheat bran substrate in controlled conditions. Key parameters, including mycelial growth rate, mycelium running time, yield, and yield attributes, were statistically analyzed using ANOVA with Duncan's Multiple Range Test (DMRT) at $p<0.05$. **Results:** The optimal temperature for mycelial growth was 25°C, with complete mycelial coverage of the PDA plate within 9 days. Growth was significantly reduced at 30°C. Among the H_2O_2 treatments, 0.1% resulted in the highest mycelial growth (3.2±0.05 cm within 3 days), the shortest mycelium running time (16.12±0.94 days), and the maximum fruiting body yield (578.67±6.21 g per spawn packet). Higher H_2O_2 concentrations negatively affected both growth and yield. **Conclusion:** A temperature of 25°C and an H_2O_2 concentration of 0.1% were determined to be optimal for the growth and yield of *P. ostreatus*. These findings offer valuable guidelines for enhancing mushroom production efficiency under controlled conditions.

KEYWORDS

Oyster mushroom, *Pleurotus ostreatus*, mycelial growth, temperature, hydrogen peroxide, yield optimization

INTRODUCTION

Oyster mushrooms (*Pleurotus ostreatus*) are among the most widely cultivated edible fungi globally, valued for their high nutritional content, medicinal properties, and culinary versatility[1]. They are known for their rapid growth, adaptability to a wide range of agro-waste substrates, and relatively simple cultivation techniques, making them an important resource for sustainable food production and economic development, especially in rural areas[2].

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Approximately 0.13 million tons of mushrooms are produced in India annually. Between 2010 and 2017, India's mushroom business grew at an average annual rate of 4.3%. Following oyster mushrooms (16%), paddy straw mushrooms (7%), and milky mushrooms (3%), white button mushrooms account for 73% of the total amount of mushrooms produced. In comparison to other vegetables, mushrooms are consumed in India at a very low rate per person-less than 100 g annually, according to data[3]. Previous studies have indicated that *P. ostreatus* exhibits optimal mycelial growth within a specific temperature range, typically between 20 and 28°C, although variations may occur depending on the strain and substrate used[4,5]. Understanding the precise temperature requirements is essential for maximizing yield and ensuring consistent production across different environmental settings.

Whole fresh mushrooms were immersed in hydrogen peroxide or citric acid solutions for 10 min, then sliced, packaged, and stored at 4°C for a period of up to 19 days. Both treatments were found to reduce the levels of pseudomonad bacteria and improve storage stability compared to control samples soaked in water. Using a benchmark of 75 Hunter L units to assess shelf life, it was determined that the treatments extended the mushrooms' shelf life by approximately 50%[6]. Its application in mushroom cultivation has shown potential in reducing contamination rates and promoting healthier mycelial development. However, the concentration of H_2O_2 is critical, as excessive amounts can inhibit mycelial growth and reduce yield, while insufficient concentrations may fail to provide adequate sterilization benefits. Despite its prospective advantages, comprehensive studies investigating the optimal concentrations of H_2O_2 for *P. ostreatus* cultivation under varying temperature conditions remain limited.

This research aims to systematically examine the effects of different temperature regimes and H_2O_2 concentrations on the mycelial growth and yield of *P. ostreatus*. By conducting controlled experiments and analyzing growth parameters across various treatments, the study seeks to identify optimal conditions that promote maximal mycelial proliferation and fruiting body production. The findings are expected to contribute valuable insights into efficient cultivation practices for oyster mushrooms, facilitating improved productivity and sustainability in mushroom farming operations.

MATERIALS AND METHODS

Study area: This study was conducted during the 2019-2020 academic year at the Plant Pathology Laboratory, Patuakhali Science and Technology University.

Collection of oyster mushrooms: The oyster mushroom species *Pleurotus ostreatus*, which is commonly cultivated, was selected for this research. Spawn packets of *P. ostreatus* were obtained from the National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh. A pure culture was prepared by isolating tissue from the fruiting bodies of the collected mushrooms, which served as the source for mother culture preparation.

Preparation of pure culture: A pure culture of *P. ostreatus* was prepared on potato dextrose agar (PDA) medium. The PDA medium was prepared by boiling 200 g of peeled and sliced potatoes in tap water until soft. The potato extract was separated, and distilled water was added to make up 1 L of solution. To this, 20 g of dextrose and 18 g of agar were added, and the mixture was autoclaved at 121°C and 1 kg/cm^2 for 15 min. After sterilization, the medium was poured into Petri dishes under aseptic conditions[1].

To initiate the culture, a young *P. ostreatus* sporophore was surface sterilized with 70% ethanol. A small piece of tissue was excised from the stalk's center and placed on the PDA medium. The inoculated Petri dishes were incubated at 27°C for 8 days, allowing the fungal colony to fully cover the plate, which was then used to inoculate the mother culture.

Evaluation of H_2O_2 on mycelial growth of *P. ostreatus*: The effect of H_2O_2 on the mycelial growth of *P. ostreatus* was tested at five concentrations: 0.1, 0.25, 0.5, 0.75, and 1%. These concentrations were prepared by adding H_2O_2 to the PDA medium before sterilization. Fifteen Petri dishes were used for each treatment. After inoculation with a mycelial disk from the pure culture, the petri dishes were incubated at 27°C. The mycelial growth was observed and measured after the incubation period[2].

Evaluation of temperature on mycelial growth of *P. ostreatus*: The impact of temperature on mycelial growth was assessed at four temperature levels: 15, 20, 25, and 30°C. The experiment was conducted using incubators set at these specific temperatures. Twelve Petri dishes containing PDA medium were inoculated with mycelial disks from the pure culture of *P. ostreatus* and incubated at the designated temperatures. The radial colony diameter was measured after two to three days of incubation[3].

Preparation of mother culture packets of *P. ostreatus*: Mother culture packets were prepared by mixing wheat bran and sawdust in a 2:1 ratio, supplemented with 0.2% calcium carbonate. The moisture content was adjusted to 65% by adding water. The mixture was then packed into 18×25 cm^2 polypropylene bags, which were sterilized at 121°C and 15 PSI for 1 hr. After cooling, the bags were inoculated with the pure culture of *P. ostreatus* and incubated at 25±2°C. The mycelium colonized the mother culture within 15-16 days, turning the substrate white and indicating readiness for spawn preparation.

Adjustment of H_2O_2 with substrate and preparation of spawn packets of *P. ostreatus*: Spawn packets were prepared by mixing wheat bran and sawdust in a 2:1 ratio with 0.2% calcium carbonate. The moisture content was adjusted to 65%. Five levels of H_2O_2 (0.1, 0.25, 0.5, 0.75, and 1%) were tested. The substrates were soaked overnight, boiled for 5-10 min dried, and adjusted for moisture content. The mixture was then packed into polypropylene bags, sterilized, cooled, and inoculated with mother culture. The spawn packets were incubated at 25±2°C until the mycelium fully colonized the substrate, turning it white.

Cultivation of fruiting bodies of *P. ostreatus*: Once the mycelium had fully colonized the spawn packets, the necks of the bags were removed, and D-shaped holes were cut into the bags. The exposed surfaces were gently scraped to remove the thin mycelial layer. The bags were then soaked in water, drained, and placed in a culture house with controlled humidity and temperature. The fruiting bodies were harvested when the margins of the mushrooms began to wave slightly. Subsequent harvests followed a similar process[4], with scraping and soaking as needed.

Data collection: Data were collected on the following parameters[5]:

- **Number of fruiting bodies:** The total count of mushrooms that emerged from each spawn packet was recorded
- **Weight of fruiting bodies:** The harvested mushrooms were weighed to determine overall yield
- **Length of stalk:** The vertical measurement of the mushroom stalk was taken from the base to the pileus
- **Diameter of stalk:** The thickness of the mushroom stalk was measured at its widest point
- **Diameter of pileus:** The cap diameter of each mushroom was recorded to assess fruiting body size
- **Thickness of pileus:** The depth of the cap was measured to evaluate morphological characteristics

Statistical analysis: The data collected were statistically analyzed. Analysis of Variance (ANOVA) was conducted using the F-test to determine the significance of the treatments. Mean differences were determined using Duncan's Multiple Range Test (DMRT) at $p<0.05$.

RESULTS

Effect of temperature on mycelial growth of *P. ostreatus*: Temperature is a crucial environmental factor affecting the growth of *Pleurotus ostreatus* mycelium. To determine the optimal temperature for mycelial growth, the species was cultivated on potato dextrose agar (PDA) medium at four different temperatures: 15, 20, 25, and 30°C. Table 1 illustrated the impact of these temperatures on the mycelial growth of *P. ostreatus* after 3, 6, and 9 days post-inoculation.

After 3 days, the highest mycelial growth was observed at 25°C with an average growth of 3.2±0.03 cm. Growth at 20°C was 2.5±0.01 cm, followed by 1.7±0.02 cm at 15°C, and the lowest growth was recorded at 30°C with 0.6±0.01 cm. Similar trends were observed at 6 and 9 days post-inoculation, with the mycelium fully covering the Petri plate at 25°C after 9 days, while at 30°C, growth was significantly stunted. Thus, the optimal temperature for mycelial growth of *P. ostreatus* is 25°C (Fig. 1).

Effect of Hydrogen Peroxide (H_2O_2) concentrations on mycelial growth of *P. ostreatus*: Table 2 presents the impact of five different H_2O_2 concentrations (0.1, 0.25, 0.5, 0.75, and 1%) on the mycelial growth of *P. ostreatus*. After 3 days, the highest growth was observed at a 0.1% concentration, with a mean growth of 3.2±0.05 cm. Growth decreased with increasing H_2O_2 concentrations, with the lowest growth at 1% (1.1±0.03 cm). Similar trends were noted after 6 and 9 days, with the 0.1% concentration consistently showing the best growth performance (Fig. 2).

Effect of H_2O_2 concentrations on mycelium running time and fruiting body formation: The study also explored how different H_2O_2 concentrations affected the time required for mycelium running and the formation of fruiting bodies. The shortest time for the mycelium to complete running in spawn was recorded at a 0.1% H_2O_2 concentration (16.12±0.94 days). Higher concentrations required longer times, with the maximum time observed at 1% H_2O_2 (25.00±1.07 days).

Table 1: Effect of temperature on mycelial growth of *P. ostreatus*

Temperature (°C)	Mycelial growth (cm)±SE (after 3 days)
15	1.7±0.02^{c}
20	2.5±0.01^{b}
25	3.2±0.03^{a}
30	0.6±0.01^{d}

Values within a row with different letters are significantly different ($p<0.05$) by DMRT

Table 2: Effect of H_2O_2 concentrations on mycelial growth of *P. ostreatus*

H_2O_2 concentration (%)	Mycelial growth (cm)±SE (after 3 days)
1	1.1±0.03^{e}
0.75	1.8±0.02^{d}
0.5	2.2±0.02^{c}
0.25	2.7±0.03^{b}
0.1	3.2±0.05^{a}

Values within a row with different letters are significantly different ($p<0.05$) by DMRT

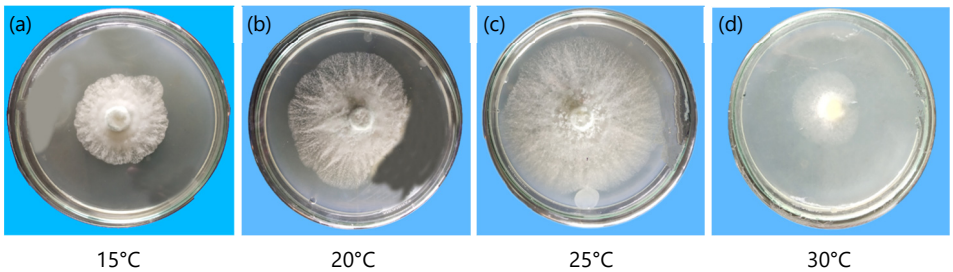

Fig. 1(a-d): Radial growth of *P. ostreatus* at different temperatures

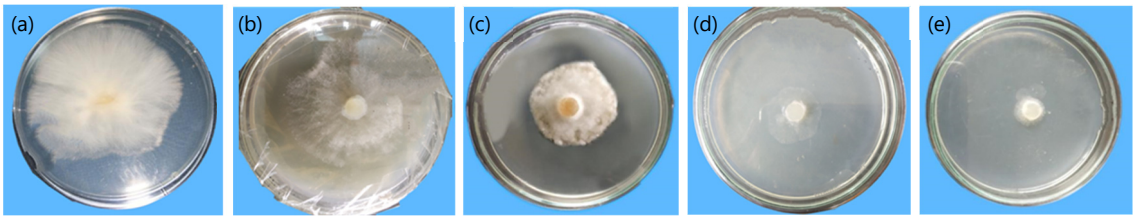

Fig. 2(a-e): Radial growth of *P. ostreatus* at different concentrations of H_2O_2

Table 3: Effect of H_2O_2 concentrations on mycelium running and fruiting body formation

H_2O_2 concentration (%)	Mycelium running (days)±SE	Primordia initiation (days)±SE	First flush (days)±SE	Second flush (days)±SE	Third flush (days)±SE	Total flush (days)±SE
1	25.00±1.07^a	6.20±0.78^a	5.67±0.93^a	10.33±0.98^a	9.67±0.93^a	32.00±1.79^a
0.75	21.67±1.21^b	5.50±0.97^b	4.20±0.86^b	8.67±0.79^b	8.33±0.75^b	25.67±1.26^b
0.5	18.23±1.02^c	4.21±0.57^c	4.33±0.75^b	7.33±0.76^c	6.67±0.81^c	21.33±1.63^c
0.25	17.00±1.05^d	3.33±0.73^d	3.87±0.59^c	6.67±0.62^d	6.33±0.79^c	19.67±1.31^d
0.1	16.12±0.94^d	3.00±0.52^d	3.23±0.83^c	6.03±0.58^e	6.00±0.82^d	18.67±1.08^d

Values within a row with different letters are significantly different ($p<0.05$) by DMRT

Table 4: Effect of H_2O_2 on yield attributes and total yield of *P. ostreatus*

H_2O_2 concentration (%)	Number of fruiting bodies±SE	Weight of fruiting bodies (g)±SE	Stalk length (cm)±SE	Stalk diameter (cm)±SE	Pileus diameter (cm)±SE	Pileus thickness (cm)±SE	Total yield (g)±SE
1	93.67±1.56^d	380.67±5.39^e	7.67±0.91^a	1.07±0.17^e	8.07±0.89^e	0.33±0.04^e	512.33±4.85^e
0.75	110.67±1.52^c	411.67±4.87^d	7.67±0.89^a	1.43±0.12^d	8.43±0.91^d	0.67±0.06^d	521.00±4.95^d
0.5	120.00±1.68^b	435.67±6.54^c	7.67±0.78^a	1.77±0.14^c	8.77±0.89^c	0.80±0.07^c	536.33±5.02^c
0.25	139.67±1.92^a	470.67±5.24^b	8.00±0.87^a	2.00±0.19^b	9.00±0.93^b	1.00±0.10^b	550.67±5.15^b
0.1	127.67±1.75^b	478.00±5.32^a	8.00±0.82^a	2.43±0.21^a	9.67±0.89^a	1.10±0.13^a	578.67±6.21^a

Values within a row with different letters are significantly different ($p<0.05$) by DMRT

Additionally, the initiation of primordia was fastest at 0.1% H_2O_2 (3.23±0.83 days), with progressively slower initiation times as H_2O_2 concentrations increased. The first flush was also quickest at 0.1% H_2O_2, taking 18.67±1.08 days, compared to 32.00±1.79 days at 1% H_2O_2 shown in Table 3.

Effect of H_2O_2 on yield attributes and total yield of oyster mushroom: The impact of varying H_2O_2 concentrations on yield attributes and the total yield of *P. ostreatus* was also evaluated. The results in Table 4 demonstrate that the number of fruiting bodies, their weight, stalk length, and diameter, as well as the pileus diameter and thickness, were all significantly influenced by H_2O_2 concentration.

The highest number of fruiting bodies was recorded in the spawn treated with 0.25% H_2O_2 (139.67±1.92 per packet). However, the highest total yield was observed at a 0.1% H_2O_2 concentration (578.67±6.21 g per spawn packet).

DISCUSSION

This study highlights the significant influence of temperature and Hydrogen Peroxide (H_2O_2) concentrations on the mycelial growth, fruiting body formation, and yield of *Pleurotus ostreatus*. The optimal temperature for mycelial growth was determined to be 25°C, consistent with previous studies on *Pleurotus* species[6,7] and supported by Zharare *et al.*[8], who found maximal growth at 25-30°C, with higher temperatures (35°C) being detrimental. Temperature was shown to have a strong influence on the growth of *P. ostreatus* in sand, where growth was delayed at low temperatures (e.g., 5°C) and completely inhibited at high temperatures (e.g., 35°C)[9]. This underscores the importance of maintaining optimal temperature conditions for successful mushroom cultivation.

Similarly, H_2O_2 concentrations significantly impacted growth and yield. Lower concentrations (0.1 and 0.25%) enhanced mycelium running, primordia initiation, and yield, while higher concentrations (1%) inhibited growth due to oxidative stress. These findings align with Zharare *et al*.[8], who reported increased growth rates at low H_2O_2 levels (up to 0.001%) but reduced growth at higher concentrations, with significant variability in tolerance among strains. The study also found that 0.1% H_2O_2 resulted in the shortest mycelium running time (16.12±0.94 days), fastest primordia initiation (3.23±0.83 days), and highest yield (578.67±6.21 g per spawn packet), emphasizing the benefits of controlled oxidative conditions. However, contradictions with other studies suggest species-specific differences in oxidative tolerance and the influence of strain variations and environmental factors.

This study was limited to evaluating the effects of temperature and hydrogen peroxide on mycelial growth and yield under controlled conditions. Future research should explore long-term impacts, optimize H_2O_2 concentrations for different growth stages, and assess its effects on other *Pleurotus* species.

CONCLUSION

The study demonstrated that both temperature and Hydrogen Peroxide (H_2O_2) significantly impact the growth and yield of *P. ostreatus*. The optimal temperature for mycelial growth was determined to be 25°C. Hydrogen peroxide concentrations of 0.1% yielded the best results in terms of growth rate, mycelium running time, fruiting body formation, and overall yield. These findings provide valuable insights for optimizing oyster mushroom cultivation, particularly under controlled environmental conditions.

SIGNIFICANCE STATEMENT

Oyster mushroom (*Pleurotus ostreatus*) cultivation is highly dependent on environmental conditions, particularly temperature and substrate sterilization techniques. However, optimizing these factors remains a challenge for maximizing yield and efficiency. This study identifies 25°C as the optimal temperature for mycelial growth and demonstrates that a 0.1% Hydrogen Peroxide (H_2O_2) concentration enhances both growth rate and yield. These findings provide valuable insights into improving oyster mushroom production with minimal chemical intervention. The results are significant for commercial mushroom farming, as they offer a cost-effective and eco-friendly approach in enhancing yield and reducing contamination risks. This research contributes to sustainable agricultural practices and can be applied across diverse cultivation environments to optimize production efficiency.

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