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# Enhancing Pleurotus Florida (Oyster) Production through Substrate Optimization: A Study on Yield and Antibacterial Effects

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**Abstract:** Pleurotus florida is an intriguing and distinct species in the fascinating world of fungus, and it needs to be recognized and considered. This amazing fungus, commonly known as the Florida oyster mushroom, is a nutritious delight in addition to being an astonishing marvel of nature.Pure culture of P. floridagrew on potato dextrose agar media. Spawn was prepared and leaf straw, paddy straw and wheat straw were used to cultivate mushrooms.P. florida's growth and production vary greatly based on the different kinds of substrates, including wheat, paddy straw, and dried leaves. Paddy straw had the highest observed output of fruiting bodies among the three substrates.The bacterial pathogens Bacillus cereus (MTCC-1305), Escherichia coli (MTCC), Staphylococcus aureus (MTCC-3160), and Micrococcus luteus (MTCC-1809) are all significantly inhibited by the Pleurotus floridaextract.Our research demonstrated the Pleurotus floridaextract has broad-spectrum antibacterial activity against Gramnegative and Gram-positive pathogenic microorganisms.

**Keywords**: Antioxidant, Antimicrobial, Mushroom, Fungi.

## 1.1 Introduction

Mushrooms, which are categorized as macrofungi have become more and more popular as sources of functional foods and potential subjects for pharmaceutical and nutraceutical research (Gregori et al., 2007). In the fascinating world of fungi, Pleurotus florida is a species that is both interesting and unique, and it deserves recognition and consideration. This extraordinary fungus, usually referred to as the Florida oyster mushroom, is a nutritional treat in addition to being an incredible wonder of nature. Because oyster mushrooms (Pleurotus spp.) have a high protein, vitamin and mineral content along with their usage as medicinal purposes hence they are cultivated for both food and therapeutic uses (Devi and Jennifer, 2020).

However, oyster mushroom culture utilizes a wide range of agricultural residues that contain lignocellulose, making it one of the most effective technologies for recycling this waste and producing food (Chávez et al., 2019). Oyster mushrooms thus have a major commercial influence on the world mushroom market. Compared to other fungi, oyster mushrooms are easier to cultivate and require less time to attain maturity (Grabarczyk et

al., 2019). Agricultural wastes produced during farming operations, such as bread grass, soybean husks, green gram husks, lentil husks, wheat husks, etc., can be utilized as inexpensive substrates for the growth of mushrooms (Rathod, 2023).

Pleurotus species can be cultivatedat temperatures between 15$^{O}$C and 30$^{O}$C on a range of substrates, such as weeds, food waste, vitamins, enzymes, and other pharmaceuticals (Jonathan et al., 2012). Pleurotus species are attractive as therapeutic mushrooms because of their hematological, antiviral, anticancer, antibiotic, antibacterial, hypocholesterolemic, and immunomodulatory properties (Cohen et al., 2004). A threat to the entire planet currently exists from the emergence of multidrug-resistant bacteria brought on by the extensive uses of synthetic antibiotics in recent years. Thus, the search for naturally occurring antibacterial chemicals derived from plants is required.Several natural compounds with antibacterial properties from a variety of plant species have been assessed for this purpose (Karuppusamy, 2009). Therefore, the purpose of this work was to assess Pleurotus florida's ability to grow on various substrates and to evaluate its antibacterial effectiveness.

## 2. Materials and Methods

**2.1 Collection of culture:** The pure culture of Pleurotus florida was obtained from the mushroom research lab at CCSHAU Hisar (Haryana)and maintained on freshly prepared Potato dextrose agar (PDA) medium in the laboratory of the department of Biotechnology at Pt C.L.S. Govt. College, Karnal for further studies.

**2.2 Spawn Preparation:** Wheat grain spawn was generated in accordance with Thakur and Rathod's method (2021).Pure and wholesome wheat grains were employed to produce the spawn. After being rinsed three times under running water, 1 kilogram of grain and 1.5 liters of water were used to partially boil the grains for 15 minutes. After it boiled, the grain was spread out over a wire mesh to drain excess water. It was then mixed with calcium sulphate (13.5 g/kg) and carbonate (3.5 g/kg) based on dry grain weight. After that, the mixture was poured into 500 ml bottles until about two thirds of the way to the top. An autoclave was used for sterilizing the bottles. The sterilized bottles were cooled to room temperature and vigorously shook to avoid grain clumping. The bottles were inoculated with equal volumes of mycelial fragments derived from pure culture using laminar air flow. For seven days, the inoculation bottles were kept in a BOD incubator at 25°C to facilitate the growth of the fungal mycelium.

**2.3 Preparation of Substrate:** In the current study, leaf straw, paddy straw and wheat straw were used to cultivate mushrooms. Firstly, cleaned and uncontaminated straws were made. The straws were chopped into small pieces.

**2.4 Sterilization:** A container with a 50-liter capacity was filled with 30 liters of tap water. A stock solution was formed by mixing these 30 liters of water with 25 milliliters of formaldehyde and three grams of Bavistin. Now, this chemical solution had been thoroughly infused with 5 kg of dry straw substrate for 18 hours. The straw was removed from the chemical solution after 18 hours and put on a wire sieve to drain any excess solution.

**2.5 Bagging and Spawning:** The substrate was placed into the bags once it had cooled. Next, the substrate was inoculated with mushroom mycelium. The mycelium of the mushroom grows on the entire substrate by propagating via the spawn. Placing the spawn on top of the substrate was the most effective way to inoculate it. A 1.5 kg substrate bag was filled with 50 g of spawn. The bags had many holes put in them to allow air flow.

**2.6 Handling after spawning**

The spawned bags were kept at room temperature. Spawn colonization was observed in these bags at interval of 24 hr. To maintain the humidity in cropping chamber, water was sprayed onto the walls, roof, floor and beds using a sprayer. To allow the spawn to proliferate and colonize the substrate, the bags were kept in dark conditions. Bags were incubated at the ideal temperature and humidity,required for the growth of mushrooms. Temperature and humidity should be maintained in the incubation rooms.

**2.7 Harvesting and extract preparation**

P. florida fruiting bodies were freshly picked and then coarsely crushed after drying in the shade. Twenty grams of crushed mushroom were extracted using different batches with 200 ml of 95% methanol as the solvent. Vacuum distillation was utilized to filter and evaporate the residual extract, and the filtrate that resulted was used to create mushroom extract (Jayakumar et al., 2009).

**2.8 Antibacterial Activity**

The antibacterial activity of P. florida extract against pathogenic microorganisms was evaluated using the agar well diffusion technique. The test cultures used in every experiment were provided by the Microbial Type Culture Collection Center (MTCC), which is situated in Chandigarh, India. The test cultures, which included Micrococcus luteus (MTCC-1809), Staphylococcus aureus (MTCC-3160), Escherichia coli (MTCC), and Bacillus cereus (MTCC-1305), were maintained on nutrient agar at 4°C until they were needed. Following that, P. florida extract was added to each well on each plate using a micropipette.Following a 24-hour incubation period at 37°C while the plates were in upright position, the zone of inhibition was assessed.

**Results and Discussion**

Pleurotus florida colonies appeared white, filamentous, and circular (Fig. 1). The spawns of Pleurotus floridaprepared in a mushroom cultivation laboratory are shown in Fig. 2.


**Fig.1: Pleurotus florida colonies**

**Fig.2: Spawns of Pleurotus florida**

Three different types of substrates wereinvestigated to determine the growth and yield of oyster mushroom Pleurotus florida. Theresults in Table 1 showed that, there were significant differences in growth of Pleurotus florida mushroom grown on three substrate formulas. Maturation of Pleurotus florida was completed in 15 days on wheat straw after incubation whereasin other substrate it varied from 22 days for paddy straw and about 24 days for leaves straw.

**Table 1: Significant differences in growth of Pleurotus florida mushroom grown on three substrate formulas**

| **Progress in days** | **Wheat Straw** | **Paddy Straw** | **Leaves Straw** |
|---|---|---|---|
| Preparation of substrate. | 1st day | 1st day | 1st day |
| Spawn Addition in substrate | 2nd day | 2nd day | 2nd day |
| Bagging and Incubation | 3rd day | 3rd day | 3rd day |
| Appearance of white colored mycelia | 7th day | 8th day | 10th day |
| Enormous mycelia observed | 11th day | 14th day | 16th day |
| Hyphae observed. | 13th day | 16th day | 18th day |
| Cap appeared on the hyphae. | 14th day | 18th day | 20th day |
| Mushroom got matured. | 15th day | 22th day | 24th day |

The modification of environmental factors, such as suitable temperature, relative humidity, and moisture content, are extremely significant for the generation of oyster mushroom fruiting bodies (Fahad Alkoaik et al., 2015; Karuppuraj et al., 2014). The current research indicated that the growth and yield of P. florida varied widely depending on the different kind of substrates such as wheat, paddy straw and dried leaves. The maximum production of fruiting bodies observed for paddy straw among the three different substrates. The time taken for the mycelium growth differs for each substrate. In paddy straw, maximum growth was observed on 15th day and in case of wheat straw, it took 18-22 days for maximum growth whereas in case of leaf straw it took 20-24 days (Table 2).P. florida's growth and production vary greatly based on the different kinds of substrates, including wheat, paddy straw, and dried leaves. Paddy straw had the highest observed output of fruiting bodies among the three substrates. Each substrate has a different growing period for the

mycelium. Maximum growth observed in paddy straw was on the fifteenth day and in wheat straw in between 18 and 22 days, and in leaf husk between 20 and 24 days.

**Table 2: Harvesting of Pleurotus florida in gm**

| **Spawning Substrate** | **1st harvesting (gm)** | **2nd harvesting (gm)** | **3rd harvesting (gm)** | **Total yield (gm)** |
|---|---|---|---|---|
| Paddy straw | 823 | 642 | 438 | 1903 |
| Wheat straw | 716 | 545 | 262 | 1523 |
| Leaves husk | 536 | 482 | 234 | 1252 |

Almost all types of agricultural wastes are beneficial for growing mushrooms, and they offer the best source of ingredients for substrate composition (Diana et al. 2012). Nitrogenous compounds, cellulose, and hemicellulose are the main macromolecules that oyster mushrooms require for development and fructification, despite the fact that different agricultural wastes provide a range of nutrients and minerals. Consequently, in the present investigation, Pleurotus florida thrived and fructified successfully in consortiums that had substrates made of wheat straw.

**Determination of antibacterial efficacy**

The bacterial pathogens Bacillus cereus (MTCC-1305), Escherichia coli (NCDC249), Staphylococcusaureus (MTCC-3160), and Micrococcusluteus (MTCC-1809), all were significantly inhibited by the Pleurotus floridaextract (Table 3, Fig 3). The antibacterial activity of Pleurotus floridaextract was best against B. cereus, and E. coli, having 20 mm and 22 mm zone of inhibition (Table 3). Our research demonstrated the Pleurotus floridaextract has broad-spectrum antibacterial activity against

Gramnegative and Gram-positive pathogenic microorganisms. Zones of inhibition was developed when the Pleurotus floridaextract was exposed to various test microorganisms, but no such zones were observed when Milli-Q water was usedalone.

**Table 3: Antimicrobial Activity of Pleurotus Floridaextract Representing Zone of Inhibition (In Mm)**

| **Bacteria** | **Zone of inhibition in mm** |
|---|---|
| Bacillus cereus | 19 ± 0.5 |
| Micrococcus luteus | 15 ± 0.5 |
| Escherichia coli | 22 ± 1.0 |
| Staphylococcus aureus | 17 ± 1.1 |

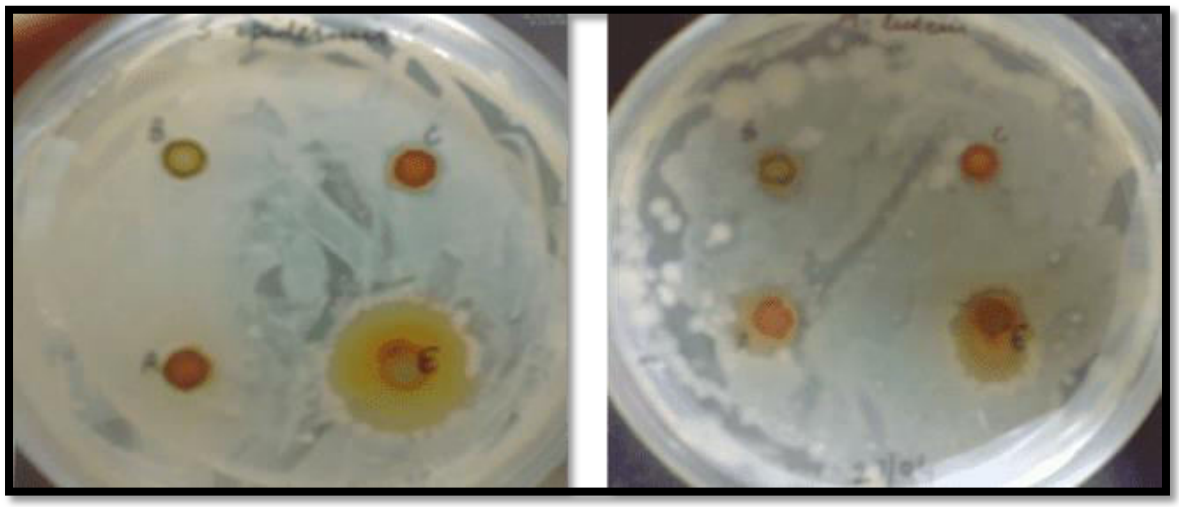

**Fig.3:** Antimicrobial activity of different extracts of P.floridausingagar well diffusion assay.

Foodborne pathogens are a class of microorganisms that can lead to food deterioration, possibly resulting in lytic enzymes, toxic tastes, and rotting, (Kitzberger et al., 2007).Some of the bacteria that need to be taken seriously are Salmonella typhimurium, Micrococcus luteus, Proteus vulgaris, Staphylococcus aureus, Bacillus cereus, and Enterobacter aerogenes. These substances produce toxins that can make people and animals sick, mainly as a result of eating these contaminated food sources. Using the paper disc diffusion method, the impact of oyster mushroom extract on the growth of harmful bacteria was evaluated. In particular, it was shown that a 95% ethanol extract from oyster mushrooms has the power to prevent the growth of B. cereus, E. cloacae, P. aeruginosa, S. marcescens, and S. typhi, resulting in inhibition zones measuring 12.33 mm, 9.56 mm, 9.65 mm, 10.11 mm, and 9.22 mm, respectively. However, the growth of E. coli, P. mirabilis, and S. aureus was unaffected by this extract. On the other hand, an inhibition zone of 12.00 mm

was detected for the oyster mushroom ethyl acetate extract, which demonstrated inhibitory efficacy against E. coli.

Han et al. in 2015 found the conflicting results about the effectiveness of 95% ethanolic oyster mushroom extract in preventing the development of harmful bacteria, which are in contrast to the findings of this study. This disagreement may be caused by variances in the extract concentrations used in the studies, which may have affected the pathogens' ability to be inhibited. The ethyl acetate extract from oyster mushrooms was shown to have an 8.33 mm inhibition zone, which significantly suppressed the growth of E. coli (Han et al., 2015). Several bacterial species, such as B. cereus, E. cloacae, P. mirabilis, P. aeruginosa, S. aureus, S. marcescens, and S. typhi, were significantly inhibited by the 95% ethyl acetate extract of oyster mushrooms. On the other hand, the 95% ethanol extract showed little promise as an inhibitor because it did not work against S. aureus, P. mirabilis, or E. coli.

Menaga et al. (2012), examined the antimicrobial activity of P. florida's ethanolic extract and found that it was most active against Pseudomonas species and Campylobacter species, while the activity of the methanol extract was higher against E. coli, Salmonella typhi, Staphylococcus aureus,Camphylobacter species, and Vibrio species. An aqueous extract showed the high zone formation against Vibrio sp. was 241.5 mm. The most effective antibacterial substances against Pseudomonas species and Staphylococcusaureus were hexane extract and ethyl acetate, respectively.

**Conclusion:**

The pure culture of P. florida obtained from mushroom research lab at CCSHAU Hisar (Haryana).Pure culture of P. Florida tested on potato dextrose agar media. The maximum production of P. floridaproduced on paddy straw proved to be the best substrate with maximum yield of about1.5 to 2.0 kg straw respectively. Three sterilization techniques of wheat straw substrates were tried to know their effect on the yield of the P. florida under the present study. The chemical sterilization method (Bavistin 10g + Formalin 125ml) gave significantly higher yields than other treatments viz. hot water and steam sterilization methods during study. The bacterial pathogens Bacillus cereus (MTCC-1305), Escherichia coli (MTCC), Staphylococcus aureus (MTCC-3160), and Micrococcus luteus (MTCC-1809) are all significantly inhibited by the Pleurotus floridaextract.

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