

Enzyme Activity Pattern During Mycelial Growth and Fruiting Body Development of *Pleurotus Florida*

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Abstract

Pleurotus florida is an economically important edible mushroom whose growth and development are governed by complex biochemical processes. This study investigated the activities of selected enzymes, catalase, laccase, and peroxidase, during mycelial growth and fruiting body development of *P. florida*. The experiment was conducted in the research laboratory of the Department of Plant and Ecological Studies, University of Calabar, Nigeria. Enzyme activities were assayed in mycelial mats at 10, 20, and 30 days of growth, as well as in mature fruiting bodies. Results revealed significant variations in catalase activity across developmental stages, with peak activity recorded at the 30-day mycelial stage. Although numerical differences were observed in laccase and peroxidase activities, they were not statistically significant. The findings indicate that enzyme activity, particularly catalase, is influenced by the developmental stage of *P. florida*. Understanding enzyme dynamics in oyster mushroom cultivation contributes to environmentally sustainable food production and efficient utilization of agro-waste substrates.

Keywords: *Pleurotus florida*, enzyme activity, catalase, laccase, peroxidase, mycelial growth**INTRODUCTION**

Mushroom cultivation has become increasingly important in agronomic and environmental research due to its contributions to food security, income generation, and sustainable waste management (Jarayaman *et al.*, 2024; Khan *et al.*, 2024). In particular, oyster mushrooms are treasured for their nutritional quality, rapid growth, and ability to degrade lignocellulosic through enzymatic activities (Bellitini *et al.*, 2019; Raman *et al.*, 2020). Their cultivation involves an environmentally friendly biotechnology that converts agro-residues into high-quality protein (Subedi *et al.*, 2023).

Fungal enzymes such as laccase, peroxidase, and catalase have been reported by several studies to play essential roles in mushroom substrate colonization, lignin degradation, and oxidative stress regulation during mushroom growth (Viswanath *et al.*, 2014; Patrick *et al.*, 2014; Kim, 2020; Aimable *et al.*, 2025). In most *Pleurotus species*, oxidative and hydrolytic enzymes fluctuate with growth phase and substrate composition, thereby affecting mycelial development and fruiting performance (Mshandete & Kivaisi, 2010; Kaur *et al.*, 2023).

In sub-Saharan Africa, including Nigeria, the abundance of agro-industrial waste, such as sawdust, has encouraged the cultivation of oyster mushrooms as a sustainable agricultural practice. However, successful production depends not only on substrate availability but also on understanding the physiological and biochemical processes key to fungal growth. Previous studies on *Pleurotus species* have mainly focused on yield and nutritional quality, with limited attention to enzyme activity pattern across developmental stages (Molobebe *et al.*, 2022; Gautam *et al.*, 2026).

Recent research has highlighted that enzyme expression in *Pleurotus* species varies across mycelial growth and fruiting stages and is closely linked to substrate utilization efficiency and oxidative balance (Aimable *et al.*, 2025). Despite these advances, data on stage-specific enzyme activity in *Pleurotus florida* under controlled cultivation conditions remain limited.

The enzymatic capacity of *Pleurotus florida* to degrade lignocellulosic materials positions it as an important organism in environmental biotechnology and sustainable waste recycling. Understanding stage-specific enzyme dynamics provides insight into how this fungus contributes to natural biomass decomposition and environmentally friendly agro-waste utilization systems.

This study therefore evaluated the activity of catalase, laccase, and peroxidase during mycelial growth and fruiting body development of *Pleurotus florida*, with implications for environmentally sustainable mushroom cultivation and agro-waste utilization in Nigeria.

MATERIALS AND METHODS

Materials and methods

Materials used

Substrate (sawdust), spawn (oyster mushrooms), wood ash, weighing scale, measuring tape, polyethylene, polyvinyl chloride (PVC), filter paper, and rubber band.

Collection of materials

The sawdust was obtained from the sawmill located in Calabar, Cross River, Nigeria. The calcium carbonate (agricultural lime) was bought from the Ministry of Agriculture, Calabar, Cross River State, while the spawn was gotten from John Collins Agro Allied Farms, Calabar.

Composting of Substrate

Hundred kilograms (100 kg) in dry weight were used for this experiment. Eighty-five percent (85 kg) of the total sawdust was soaked in water, drained to 70 % moisture, and 13 % of rice bran and 2 % of agricultural lime were added to the sawdust and composted for 3 days. After 3 days, the sawdust was divided into portions of 1kg each.

Bagging and sterilization

Polypropylene bags measuring 12 by 8 cm were used to bag the substrates. The substrate-filled bags were put inside an aluminum drum having a wooden platform to prevent the bag from being fully submerged in water and constantly heated for a minimum of 3 hours

Inoculation and incubation

After sterilization, the substrate was allowed to cool for 12 hours at room temperature. The mushroom species, *Pleurotus florida*, was identified and authenticated by Professor A.A. Markson, an applied mycologist, and voucher specimens were deposited at the herbarium of the Department of Botany, University of Calabar, with voucher number BOT/herb/UCC/003. Thereafter, oyster mushroom spawn was inoculated through a one-inch polyvinyl chloride (PVC) pipe of at least 3 inches in diameter. The opening was then covered with cotton wool and secured with a rubber band.

Cropping and harvesting

The bags were open for fruiting after the mycelium had fully covered the bags. The opened bags were stacked on wooden shelves and sprayed with clean water at 2-days intervals. The humidity in the cropping room was maintained at 85 % to the support proper mushroom growth.

Enzyme Extraction

The activity of polyphenol oxidase, peroxidase, and catalase was assayed using the methods described by Nkang *et al.* (2000) and Ngele *et al.* (2024)

One gram (1g) of sample was macerated, and the enzyme was extracted by agitation for 15 minutes using 5ml of deionized water, 5ml of 20 mM potassium phosphate buffer (pH 7.5) containing 1% of the phenolic absorbent- polyvinyl pyrrolidone (PVPP). This was centrifuged at 4000 rpm (rotation per minute) for 5 minutes. The supernatant was stored in an ice chamber and used as a crude enzyme extract.

Enzyme Assay

The enzyme assay was carried out in a mixed phosphate buffer (20mM monobasic and dibasic potassium phosphate salts).

The laccase assay was performed using guaiacol as the substrate, and the reaction mixture was prepared as follows. 1ml of 2 mM guaiacol, 3ml of assay buffer, and 1ml of enzyme extract were added to a test tube; in a second test tube, water was added instead of enzyme to serve as a blank. The mixture was incubated at room temperature for 15 minutes, and absorbance was read at 450 nm using a UV-VIS spectrophotometer.

The peroxidase assay was carried out by adding 2 mL of assay buffer to a cuvette, 0.6 mL of enzyme extract, and 0.1 mL of 10 mM guaiacol. These were mixed, and the reaction was initiated by adding 0.1 mL of 10 mM H₂O₂; the absorbance was read at 436nm after 30 seconds.

To assay for catalase, crude enzyme was further purified using ammonium sulphate (NH₄)₂ SO₄ precipitation at 80% saturation. In a cuvette containing 2 mL of assay buffer, 0.6 mL of purified enzyme was added, and the reaction was initiated by adding 0.2 mL of 10 mM H₂O₂. The absorbance was measured at 240 nm after 10 seconds.

Absorbance readings were used in calculating enzyme activity as:

$$E.A = A \times V / t \times e \times v$$

Where E. A (µg/ mL) = enzyme activity, A = absorbance, V = total mixture volume (mL), v = enzyme volume (mL), t = incubation time (mins.), e = extinction coefficient: 0.6740 µM/ cm for guaiacol and 43.6 µM/cm for hydrogen peroxide (Ngele *et al.*, 2024).

Experimental design and data analysis

The Experiment was laid out in a Complete Randomized Design (CRD) with three replications. Data collected was subjected to analysis of variance (ANOVA) using SPSS version 23.0, and means were separated using Duncan multiple range test (DMRT).

Data Collection

Mycelial samples were collected at 10, 20, and 30 days of incubation, while fruiting body samples were collected at maturity prior to spore release.

RESULTS

The enzyme activities (catalase, laccase, and peroxidase) were assessed at various developmental stages of *P. florida*. This includes the 10-day, 20-day, and 30-day mycelial mats, as well as mature fruiting bodies. Results are shown as graphs in Figures 1-3

Catalase activity

There was significant variation in catalase activity across all developmental stages ($P \leq 0.05$). The 30-day mycelial mat (0.24 ± 0.00^a) showed the highest activity, significantly higher than at all other stages. There was no significant difference in the fruit body (0.18 ± 0.00^b) and the 10-day mycelial mat (0.17 ± 0.00^c); however, both were significantly higher than the 20-day mycelial mat (0.02 ± 0.00), which showed the lowest catalase activity.

Laccase Activity

No significant difference in laccase activity was observed across developmental stages ($P \leq 0.05$). Although there was variation in the numerical values, the 30-day mycelial mat recorded the highest mean value (0.34 ± 0.16), and the 20-day mycelial mat recorded the lowest. The fruiting body (0.12 ± 0.03) and the 10-day mycelial mat (0.15 ± 0.04) also showed comparable enzyme activity.

Peroxidase Activity

Similarly, peroxidase activity did not differ significantly across developmental stages. The 20-day (6.41 ± 0.45) mycelial mat recorded the highest activity, followed by the 30-day mycelial mat (5.97 ± 0.75) and the 10-day mycelial mat (5.57 ± 0.98). The fruiting body had the lowest peroxidase activity (5.20 ± 1.77); however, this difference was not statistically significant.

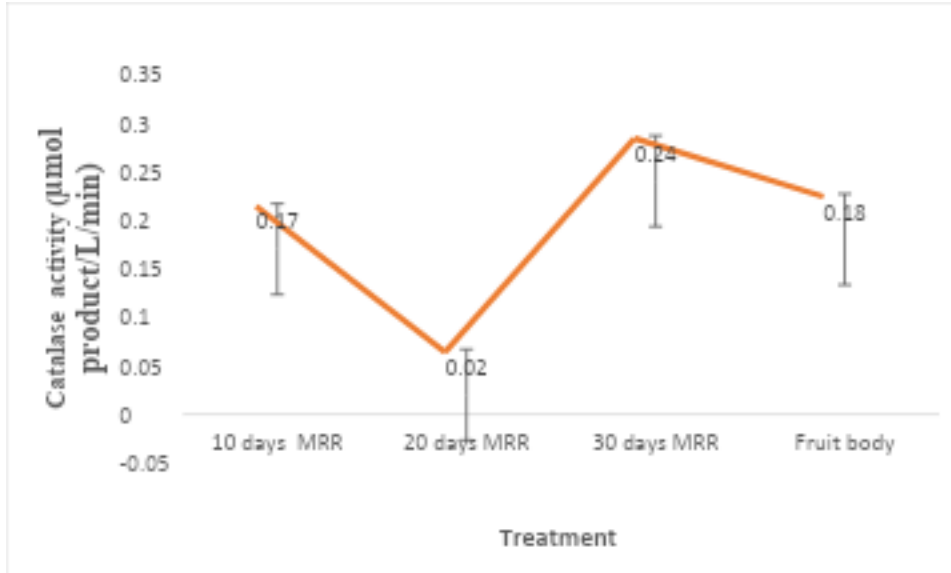


Fig 1: Catalase activity ($\mu\text{mol product/L/min}$) of *Pleurotus florida* during MRR and fruit body formation

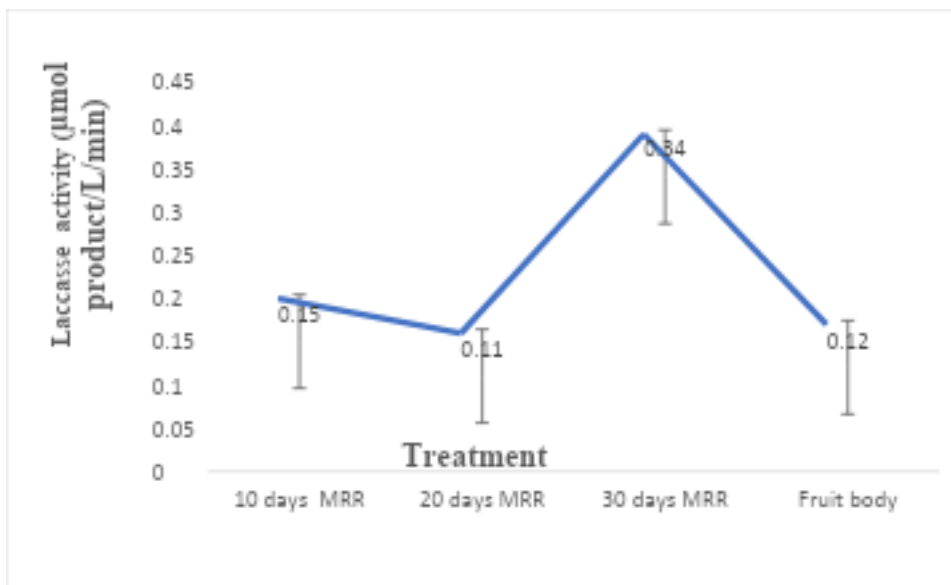


Fig 2: Laccase activity ($\mu\text{mol product/L/min}$) of *Pleurotus florida* during MRR and fruit body formation

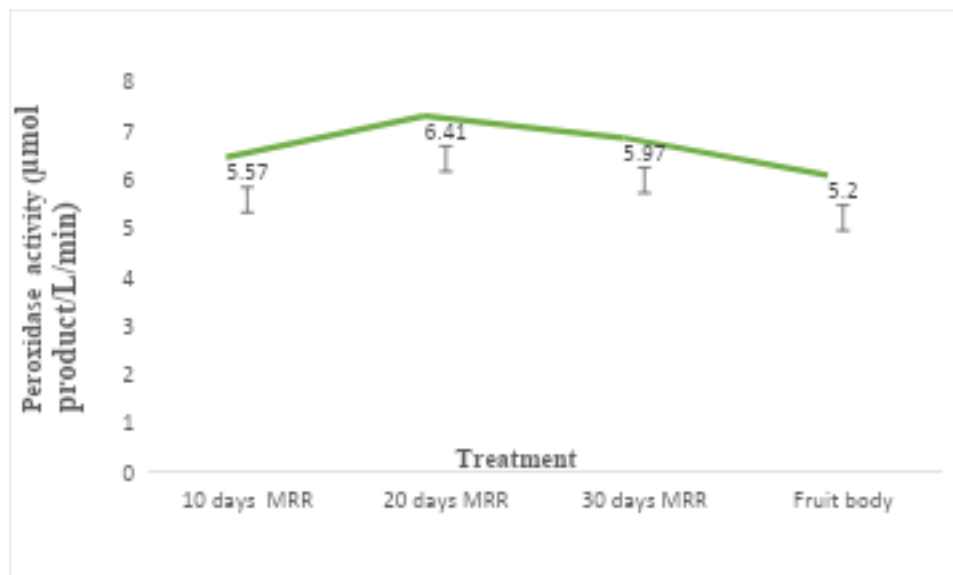


Fig 3: Peroxidase activity ($\mu\text{mol product/L/min}$) of *Pleurotus florida* during MRR and fruit body formation

DISCUSSION

The current study investigated the stage-specific enzyme activities (catalase, laccase, and peroxidase) during mycelial growth and fruit-body formation in *P. florida*. These enzymes form the key components of fungal oxidative metabolism, involved in both intracellular detoxification and extracellular substrate degradation.

Catalase activity and regulation of oxidative stress

The findings of this study indicate that there was stage-dependent variation in catalase activity, with peak expression during advanced mycelial development. Catalase is a universal antioxidant enzyme that catalyses the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen, thereby reducing stress in aerobic organisms. One function of its function is to help maintain cellular redox balance under elevated metabolic activity, where reactive oxygen species are generated during lignin modification processes (Anwar *et al.*, 2024; Rasheed, 2024; Alhumaydhi *et al.*, 2025). The peak catalase activity observed in the later mycelial stage reflects increased intracellular oxidative stress associated with extensive biomass expansion and metabolic activity. This also suggests a physiological adaptation to neutralise reactive oxygen species (ROS) during vegetative growth (Kiviniemi *et al.*, 2023). Multiple fungal studies report elevated catalase activity during later or mature mycelial stages, concurrent with increased intracellular oxidant signals (Deng *et al.*, 2016; Lovtsova *et al.*, 2023; Yan *et al.*, 2023). These stage-specific increases are consistent with an adaptive ROS-detoxification response during active vegetative growth.

Laccase and peroxidase activity in lignolytic metabolism

Laccase activity remained stable throughout the developmental stages despite numerical differences; it did not vary significantly. The relatively constant activity observed across stages suggests a sustained need for lignin modification during both mycelial growth and fruiting body phases. This pattern aligns with that of other lignocellulose-degrading fungi, where ligninolytic enzymes are maintained rather than limited to a specific developmental stage (Andlar, 2018; Suryadi *et al.*, 2022; Chen *et al.*, 2025). Laccases facilitate the biodegradation of complex lignocellulosic substrates by oxidizing phenolic compounds and lignin components (Jeon *et al.*, 2012; Tiwari *et al.*, 2023). Members of the white-rot fungi are known for degrading lignin by secreting laccase and peroxidase (Benavides *et al.*, 2024).

Peroxidase activity also demonstrated similar stability throughout different stages, suggesting that peroxidative reactions involved in lignin degradation and oxidative processes may be constitutively expressed to support continuous substrate conversion. While specific peroxidase classes, such as manganese peroxidase (MnP), assist in lignin breakdown, the absence of significant change in overall

peroxidase activity may indicate a broad role in extracellular oxidative metabolism rather than regulation tied to specific stages.

Conclusion

The study assessed the activities of lignocellulolytic enzymes (catalase, Lacasse, and peroxidase) during mycelial growth and fruit-body development of *Pleurotus florida*. Catalase activity varied across stages, with the 30-day mycelial stage exhibiting the highest activity, indicating increased activity during advanced mycelial growth. Lacasse and peroxidase activities, on the other hand, did not differ significantly. These results showed that enzyme activities in *P. florida* change with developmental stage, especially for catalase, and also highlight the importance of these enzymes in the growth and development of the mushroom

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