

Impact of *Pleurotus eryngii* spent mushroom substrate on yield potential and enzymatic activity in *Pleurotus florida* cultivation

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ABSTRACT

This study investigated the effects of *Pleurotus eryngii* spent substrate (PESS) supplementation on the growth performance, yield, and enzymatic activity of *Pleurotus florida*. Wheat straw (WS) as a control (Control-2) demonstrated the fastest spawn run (18.25 days) and pinhead formation (22.38 days), while PESS alone (Control-1) showed the longest durations (28 days and 33.13 days, respectively). WS also produced the highest number of sporocarps (25.50) and achieved the highest yield (321.75 g/kg) and biological efficiency (107.25%). The combination of PESS, WS, wheat bran (WB), and calcium carbonate (CaCO_3) (50:40:9.5:0.5) yielded 292 g/kg with 97.33% biological efficiency, while PESS alone resulted in the lowest yield (83.75 g/kg) and biological efficiency (27.91%). Enzymatic analysis revealed that laccase activity in WS was lowest during spawn run (0.09 ± 0.03 and 0.35 ± 0.00) but increased during pinhead initiation (0.83 ± 0.00 and 1.16 ± 0.00), peaking at fruit body maturation (2.05 ± 0.00 and 3.47 ± 0.00). A similar trend was observed in the PESS+WS+WB+ CaCO_3 combination. Xylanase activity followed a parallel pattern, with the lowest levels during the spawn run and the highest post-harvest. These findings emphasize the significant role of substrate composition and enzymatic activity in optimizing *Pleurotus florida* cultivation.

Keywords: *Pleurotus florida*, enzymatic activity, *Pleurotus eryngii* spent mushroom substrate, cultivation

The cultivation of edible mushrooms, particularly *Pleurotus florida*, commonly known as the oyster mushroom, is gaining global attention due to its high nutritional value, rapid growth, and adaptability to a wide range of substrates. Among the various agricultural residues and organic wastes utilized as substrates, the spent mushroom substrate (SMS), a by-product of mushroom cultivation, has emerged as a promising resource. SMS is rich in organic matter, cellulose, hemicellulose, and lignin, making it a valuable material for recycling in subsequent mushroom production cycles.

Reutilizing SMS as a supplement in mushroom cultivation has dual advantages: enhancing the sustainability of mushroom farming and reducing environmental waste. Studies have suggested that supplementing primary substrates with SMS not only improves the yield potential of mushrooms but also influences their enzymatic activity, which plays a critical role in substrate degradation and nutrient utilization. Enzymes such as cellulases, hemicellulases, and ligninases are pivotal for breaking down complex organic matter into simpler compounds that can be absorbed by the mushroom mycelium (Chang and

Miles, 2004). However, *Pleurotus* species play a vital role in breaking down various agricultural wastes due to the production of extracellular enzymes throughout their growth and developmental stages. These enzymes degrade the large, insoluble components of lignocellulosic materials into smaller, soluble compounds, which are subsequently absorbed and metabolized by the mushroom to meet its nutritional requirements. Consequently, these enzymes are crucial for the growth and development of mushrooms (Kuforiji and Fasidi, 2008).

Furthermore, the supplementation of substrates with SMS can significantly impact the biological efficiency and overall productivity of *P. florida*. This approach aligns with circular economy principles by integrating waste recycling into agricultural practices, thus contributing to sustainable development goals (Zied *et al.*, 2011). This study investigates the impact of *Pleurotus eryngii* spent mushroom substrate (PESS) supplemented substrate on the yield potential and enzyme activity of *Pleurotus florida*, highlighting its viability as an environmentally friendly and cost-effective approach to enhancing mushroom cultivation. The results offer valuable insights for mushroom growers and researchers, underscoring the significance of optimizing substrates to boost productivity and promote sustainable practices.

MATERIALS AND METHODS

The experiment was conducted in the Plant Pathology Laboratory of Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.). In this study, one-year-old spent substrate of *Pleurotus eryngii* used for substrate supplementation for cultivation of *P. florida*. The PESS was obtained from the university's mushroom laboratory.

Spawn and Substrate Preparation

The spawn was prepared using standard protocol developed by ICAR-DMR Solan (Sharma and Kumar, 2011). Basal substrate used was wheat straw (WS), wheat bran (WB), and calcium carbonate (CC). *Pleurotus eryngii* spent substrate (PESS) was used as supplements in two different combinations i.e. WS(50):WB(40): CC(9.5): PESS(0.5) and WS(60):WB(30):CC(9.5): PESS(0.5) to evaluate their effectiveness in the cultivation of *Pleurotus florida*. In the experiment *P. eryngii* SMS served as Control-1 and wheat straw as Control-2. The PESS and wheat straw were soaked in water overnight, after which the pre-wetted straw and PESS were supplemented with wheat bran and calcium carbonate in different proportions as mentioned above. The mixture was then placed in a pasteurization chamber, where steam was introduced until the temperature reached 65°C and maintained for 5-6 hours. After pasteurization, all the treatments were allowed to cool, then packed into polythene bags. The bags were inoculated with 2% *P. florida* spawn (on a wet weight basis), distributed in three layers to ensure even distribution, and bags were sealed with nylon thread. Standard package of practices was followed for the cultivation (Sharma *et al.*, 2020). The different morphological parameters i.e., spawn run days, pin head formation, number of sporocarps, yield, biological efficiency (%) were recorded.

Enzymatic activity

The enzymatic activity in the substrate was quantitatively assayed at various stages of cultivation, including the time of spawning, primordial initiation, fruit body maturation, and after crop harvest. The crude enzyme extract was prepared by soaking 20g of substrate (with active mycelia) in 50 ml of phosphate buffer, followed by agitation on an orbital shaker at 120 rpm for one hour. The mixture was then filtered through double-layered cheesecloth and centrifuged at 6000 rpm for 10 minutes to remove

residual particles. The resulting clear supernatants were collected as crude enzymes and used for subsequent enzyme activity assays.

Laccase activity

Laccase activities in the crude enzyme extract was determined by monitoring the absorbance change at 420 nm (A_{420} nm) related to the rate of oxidation of 1mM ABTS in 50mM sodium acetate buffer (Ren *et al.*, 2023). The activity of laccase assay was calculated by using this formula:

$$\text{Laccase activity} = \frac{A \times V}{t \times e \times v} \times 100$$

Where,

A = Absorbance; V = Total volume of the reaction mixture; t = Incubation time; e = Extinction coefficient of Guaiacol at 470 nm (0.6740 $\mu\text{M}/\text{cm}$); v = Volume of enzyme supernatant

Carboxymethyl cellulase (CMCase)

The activity of CMCase enzyme was determined by the methodology given by Ghose (1987). The activity was assayed by measuring the amount of glucose liberated in reaction mixtures containing 0.5 per cent carboxymethyl cellulose and 3ml enzyme supernatant and absorbance was measured at 600 nm. Enzyme activity (U) was expressed as the $1\mu\text{mole}$ of glucose released per ml per min. The activity of CMCase assay was calculated by using the formula:

$$\text{Glucose released } \text{ml}^{-1} \text{ min}^{-1} = \frac{\text{Product concentration} \times \text{Total ml} \times \text{dilution factor}}{\text{Molecular weight} \times \text{ml of enzyme} \times \text{Incubation time}}$$

Xylanase

Xylanase was estimated using the DNS method described by Miller (1959). The activity was assayed

by measuring the amount of reducing sugars (xylose) liberated in reaction mixtures containing 0.5 per cent birch wood xylan and 3 ml supernatant. Absorbance was measured at 540nm. The activity of Xylanase assay was calculated by using the formula:

$$\text{Glucose released } \text{ml}^{-1} \text{ min}^{-1} = \frac{\text{Product concentration} \times \text{Total ml} \times \text{dilution factor}}{\text{Molecular weight} \times \text{ml of enzyme} \times \text{Incubation time}}$$

RESULTS AND DISCUSSION

Impact of supplemented spent mushroom substrate on the sporophore yield of *Pleurotus florida*

The study revealed (Table1) significant effects of substrate supplementation on the growth performance and yield of *Pleurotus florida*. Wheat straw (WS) as control-2 showed the fastest spawn run (18.25 days) and pinhead formation (22.38 days), while PESS alone (control-1) required the longest time (28 days and 33.13 days, respectively). The number of sporocarps was also highest in the WS control (25.50), with PESS alone producing the fewest (15) sporophores. The WS substrate (control 2) achieved the highest yield (321.75 g/kg) and biological efficiency (107.25%), followed by the PESS+WS+WB+CaCO₃ combination (50:40:9.5:0.5), which recorded a yield of 292 g/kg and 97.33% biological efficiency. In contrast, SMS alone produced the lowest yield (83.75 g/kg) and biological efficiency (27.91%). These results highlight the importance of substrate supplementation, particularly with wheat straw, in enhancing mushroom yield and resource efficiency.

The current findings align closely with a series of studies that have explored the benefits of substrate supplementation in mushroom cultivation. Sharma (2021) highlighted the remarkable yield of *Pleurotus*

IMPACT OF SPENT MUSHROOM SUBSTRATE ON YIELD POTENTIAL AND ENZYMATIC ACTIVITY

Table 1. Effect of supplemented spent mushroom substrate on sporophore yield of *Pleurotus florida*

Substrates	Time taken to spawn run (days)	Time taken for pin head formation (days)	Total number of sporocarps	Average yield (g)/ kg wet substrate	Biological efficiency (%)
PESS+WS+WB+CaCO ₃ (50:40:9.5:0.5)	20.25	24.75	24.25	292.00	97.33
PESS+WS+WB+CaCO ₃ (60:30:9.5:0.5)	26.28	29.25	14.50	166.75	55.58
PESS (control 1)	28.00	33.13	15.00	83.75	27.91
WS (control 2)	18.25	22.38	25.50	321.75	107.25
C.D _(0.05)	0.36	0.86	1.59	24.61	
SE(m)	0.12	0.29	0.53	8.22	

WS= wheat straw, PESS= *Pleurotus eryngii* spent substrate, WB= wheat bran, CaCO₃= calcium carbonate

eryngii (587.58 g/1.5 kg wet substrate) grown on wheat straw, demonstrating its potential as a highly effective substrate. Royse (1992) showed that adding 12% soybean and 1% calcium carbonate to spent shiitake substrate resulted in the highest yield and an impressive biological efficiency of 79% for *Pleurotus sajor-caju*. Yoshida *et al.* (1993) further supported the role of supplementation, achieving maximum yields by enriching substrates with 45% wheat bran, rice bran, and bean curd refuse. Likewise, Siddhant and Singh (2009) demonstrated that incorporating 25% spent mushroom substrate (SMS) into the cultivation of three *Pleurotus* species (*P. sajor-caju*, *P. florida*, and *P. flabellatus*) significantly boosted both yield and biological efficiency. Ashraf *et al.* (2013) found that supplementing SMS with 60% sawdust and 20% wheat bran led to the highest yield for *P. ostreatus* and *P. florida*. Similarly, Giminez (2012) observed that combining SMS from oyster mushrooms with wheat straw in a 1:1 ratio and adding calcium sulphate (50 g/kg) and calcium carbonate (10 g/kg) dramatically enhanced production compared to un-supplemented substrates. Earlier work by Sharma and Jandaik (1985) revealed that starch, peptone, and wheat bran supplementation could significantly increase the yield and biological efficiency of *Pleurotus sajor-caju*. However, Shashirekha *et al.* (2005) highlighted that supplementing substrates not only boosted biomass but

also increased mushroom productivity. The depletion of nutrients in the substrate, caused by their continuous utilization by mushroom mycelium, rendered SMS alone insufficient for producing a good mushroom yield. Similar observations were made by Ashrafi *et al.* (2014), who reported minimal yields of *Pleurotus* species cultivated on spent mushroom substrate derived from *Pleurotus ostreatus*.

Enzymatic activity of the substrate during different stages of *Pleurotus florida* cultivation

Enzymes are essential for the breakdown and conversion of different organic materials in mushroom substrates, which are widely used by mushroom mycelium. In the present study, substrates yielding the highest mushroom production were selected for enzymatic analysis. Among the substrates, wheat straw exhibited the maximum yield, followed closely by a combination of *Pleurotus eryngii* spent mushroom substrate, wheat straw, wheat bran, and calcium carbonate (50:40:9.5:0.5).

The enzymatic activity patterns observed during the cultivation process were noteworthy and are presented in Table 2. Lower levels of laccase activity in wheat straw were recorded during the spawn run period. However, as the cultivation progressed to the pinhead initiation phase, laccase activity increased

Table 2. Enzyme activity of the substrate at different stages of cultivation of *Pleurotus florida*

Treatments	Laccase activity				Carboxymethyl cellulase (CMCase) activity				Xylanase activity			
	Spawn run (U/ml)	Pinhead initiation (U/ml)	Mature fruit body (U/ml)	After harvest (U/ml)	Spawn run (U/ml)	Primordial initiation (U/ml)	Mature fruit body (U/ml)	After harvest (U/ml)	Spawn run (U/ml)	Primordial initiation (U/ml)	Mature fruit body (U/ml)	After harvest (U/ml)
Wheat straw	0.09±0.03	0.83±0.00	2.05±0.00	1.03±0.00	4.40 ±0.01	6.26±0.01	2.48±0.00	3.46±0.00	5.03±0.00	5.09±0.00	5.89±0.01	6.42±0.01
PESS+WS+WB+ CaCO ₃ (50:40:9.5:0.5)	0.35±0.00	1.16±0.00	3.47±0.00	1.87±0.00	6.56±0.01	8.74±0.01	1.85±0.00	2.18±0.01	8.38±0.00	8.46±0.00	10.23±0.01	13.42±0.01
C.D _(0.05)	0.00	0.06	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.06	0.02	0.02
S.E(m)	0.00	0.02	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.01

WS= wheat straw, PESS= *Pleurotus eryngii* spent substrate, WB= wheat bran, CaCO₃= calcium carbonate

substantially (Fig. 1) and reached its peak during fruit body maturation. A significant reduction in laccase activity was observed following the harvesting of the fruit bodies. A similar trend in laccase activity was noted for the substrate combination of *Pleurotus eryngii* spent mushroom substrate, wheat straw, wheat bran, and calcium carbonate (50:40:9.5:0.5). Regarding xylanase activity, irrespective of the substrate used, the lowest levels were recorded during the spawn run period (Fig. 2). However, as the cultivation progressed to pinhead formation and fruit body maturation, xylanase activity steadily increased, reaching its highest levels post-harvest. These findings indicate the dynamic nature of enzymatic activity throughout the cultivation cycle and underscore the significant role of enzymatic processes in the growth and development of *Pleurotus florida*.

The present findings align with those reported by Chen *et al.* (2004), who observed that in *Volvariella volvacea*, laccase activity increased immediately following the vegetative growth phase at the onset of fruiting body initiation. These elevated levels persisted throughout the fruiting body development and declined at harvest. Similarly, Wood and Goodenough (1997) reported that in *Agaricus bisporus*, laccase and

peroxidase activities rose from the vegetative growth phase to the early stages of fruiting body development, but decreased drastically during fruit body maturation. Sherief *et al.* (2010) also noted an early increase in xylanase production in *Pleurotus ostreatus*, with the highest activity recorded post-harvest, a pattern consistent with the current study. Matsumoto (1998), while studying *Lentinus edodes* cultivation on eucalyptus sawdust, observed that xylanase activity increased during fruiting body development, peaking at mushroom maturation. The increase in enzymatic activities during fruiting has been attributed to the fungus's need to mobilize substantial amounts of carbon for mushroom formation (Mata and Savoie, 1998). Our results also corroborate those of Isikhuemhen and Mikiashvili (2009) and Rajarathnam *et al.* (1987), who reported that laccase activity peaked at sporophore maturation. Additionally, Yildirim *et al.* (2015) investigated the effect of agricultural waste and growth periods on laccase activity in *Pleurotus eryngii*, finding the highest laccase activity in mature mushrooms grown on wheat straw and cotton stalks.

Regarding CMCase activity, the present study showed an increase during primordial initiation,

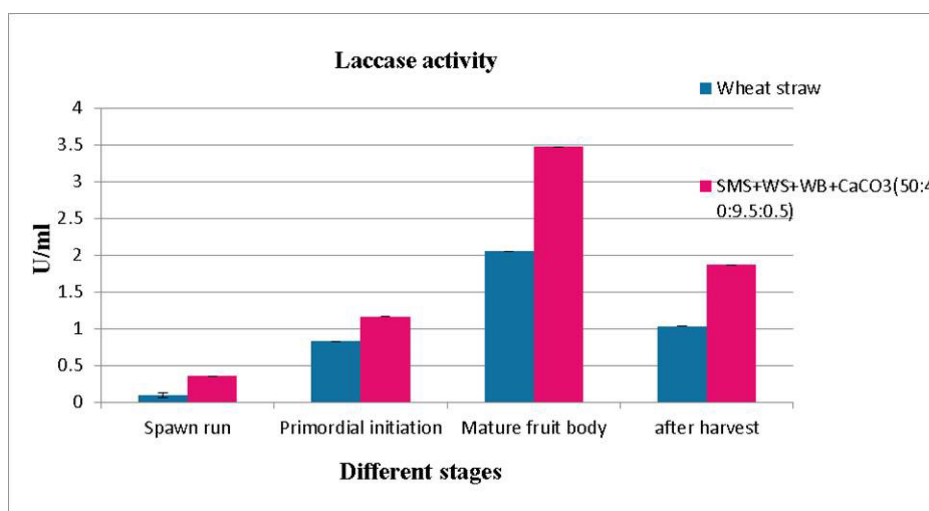


Fig. 1. Laccase activity in the substrate during various stages of *Pleurotus florida* cultivation

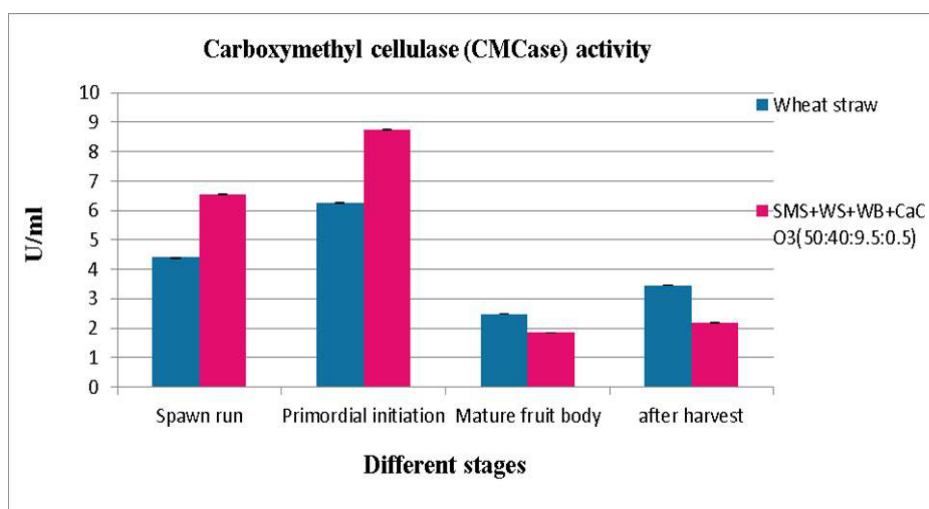


Fig. 2. Carboxymethyl cellulase (CMCase) activity in the substrate during various stages of *Pleurotus florida* cultivation

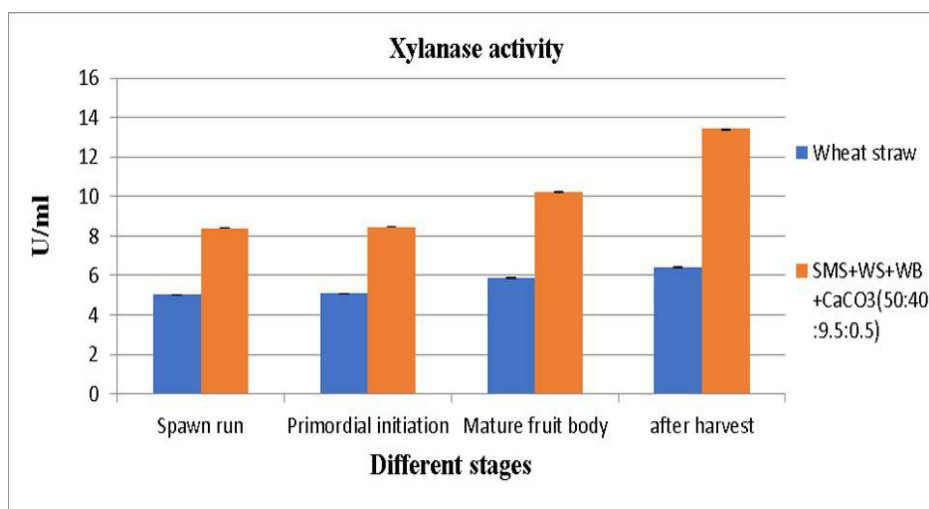


Fig. 3. Xylanase activity in the substrate during various stages of *Pleurotus florida* cultivation

followed by a decrease at fruit body maturation (Fig. 3). Similar results were reported by Wang *et al.* (2000), who detected cellulase activity throughout fungal growth, with an increase at primordial initiation and a decline post-maturation. These findings indicate that fluctuations in lignocellulolytic enzyme activity are closely linked to the stages of fruit body development and the physiological state of the mushrooms. However, enzyme profiles may vary depending on the mushroom species and likely the type of growth substrate employed.

CONCLUSION

In conclusion, the present study underscores the significant impact of substrate supplementation on the growth performance, yield, and enzymatic activity of *Pleurotus florida*. The results demonstrated that wheat straw (WS) alone achieved the fastest spawn run, pinhead formation, highest yield and biological efficiency. Supplementation with *Pleurotus eryngii* spent substrate (PESS), wheat straw, wheat bran, and calcium carbonate (50:40:9.5:0.5) also enhanced yield and biological efficiency, though not as significantly as wheat straw. In contrast, PESS alone showed the lowest yield and biological efficiency, indicating that additional supplementation is crucial for optimal mushroom production. Enzymatic analysis revealed dynamic changes in laccase and xylanase activities during the cultivation process, with both enzymes showing a marked increase during fruit body maturation. These findings emphasize the critical role of enzymatic activity in the breakdown of lignocellulosic materials and the overall development of *Pleurotus florida*. The study highlights that appropriate substrate composition is essential for maximizing yield and biological efficiency, offering valuable insights for improving mushroom cultivation practices. Further exploration of enzyme dynamics across different substrates can lead to optimized strategies for sustainable and high-yield mushroom production.

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