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Optimizing Pinhead-to-Fruiting Body Conversion in Oyster Mushrooms: Effects of Substrate Formulation, Composting Duration, and Supplementation on *Pleurotus eous* and *P. ostreatus.*

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Abstract

Mushroom cultivation offers a sustainable and environmentally friendly alternative for food production, employment generation, and economic empowerment. However, optimizing primordia conversion to mature fruiting bodies remains a critical factor influencing commercial productivity. This study evaluates the effects of substrate formulation, composting duration, and supplementation on the conversion efficiency of *Pleurotus eous* P-31 and *Pleurotus ostreatus* EM-1. The experiment was conducted using rice straw and *Triplochiton scleroxylon* sawdust as primary substrates, composted for varying durations (4, 8, and 12 days) and supplemented with different concentrations of rice bran (0%, 5%, and 10%). Mushroom pinhead formation and conversion efficiency to mature fruiting bodies were monitored over multiple flushes. Data were analyzed using ANOVA at a significance level of $p \leq 0.05$. Substrate type, composting duration, and supplementation significantly influenced pinhead formation and conversion rates ($p \leq 0.05$). *Pleurotus eous* exhibited the highest conversion rate (75.7%) on rice straw composted for 4 days and supplemented with 10% rice bran, while *P. ostreatus* achieved a maximum conversion of 74.4% on 12-day composted substrate with 5% rice bran. On *T. scleroxylon* sawdust, *P. ostreatus* demonstrated a superior conversion rate (66.0%) compared to *P. eous* (63.2%), with both species performing best on 8-day composted substrates. The findings underscore the importance of optimizing substrate formulation and composting duration to enhance mushroom yield. A 4-day composting period with 10% rice bran is recommended for *P. eous*, while *P. ostreatus* benefits from a 12-day composting period with 5% supplementation. These insights contribute to improving the efficiency of oyster mushroom cultivation, with direct implications for commercial production.

Keywords: Pinheads Formation; Substrate Formulation; Composting Duration; Conversion Efficiency; *Pleurotus eous* and *Pleurotus ostreatus*; Rice Bran Supplementation

Introduction

Mushroom cultivation is a sustainable agricultural practice with nutritional, economic, and environmental benefits. Mushrooms contain proteins, dietary fibers, vitamins, and essential minerals like copper, selenium, potassium, and zinc [1,2]. They can grow on diverse agricultural byproducts such as straw, sawdust, and coffee grounds, providing an environmentally friendly waste management solution [2-4].

Mushroom biotechnology contributes to species discovery, domestication of wild strains, nutritional enhancement, environmental restoration, and pollution mitigation. As a health-promoting food with low fat and calorie content, mushrooms integrate well into conventional agricultural systems, enhancing resource efficiency, soil health, and biodiversity conservation [5-7]. The classification of mushrooms as a health-promoting food is primarily due to their low fat and calorie content while being nutrient-dense [1-3].

A unique advantage of mushrooms is their ability to synthesize Vitamin D through ergosterol when exposed to ultraviolet light, making them a rare non-animal source of this essential vitamin. This is particularly significant in developing countries like Ghana, where malnutrition and vitamin deficiencies are prevalent. The FAO recognizes mushrooms as a valuable protein source for populations relying heavily on cereals [8-12].

While research has focused on bioconversion of agricultural waste into mushrooms and yield parameters, limited studies have assessed the conversion efficiency of pinheads (primordia) into mature fruiting bodies—a critical determinant of biological efficiency and commercial yield [13-20]. This study investigates the percentage conversion of primordia to mature fruiting bodies in *Pleurotus eous* P-31 and *Pleurotus ostreatus* EM-1 cultivated on different formulated substrates. By evaluating how substrate composition, composting duration, and supplementation affect primordia conversion rates, this research aims to optimize mushroom yield and biological efficiency, contributing to predictive models for commercial mushroom farming.

Materials and Methods

Preparation of Pure Cultures

Pure cultures of both *Pleurotus eous* and *P. ostreatus* were raised on Potato Dextrose Agar (PDA), Oxoid and Malt extract agar for 7 days. Stock cultures were grown on slants of PDA and Malt Extract Code: CM0059, Oxoid in McCartney tubes and on Petri dishes. All media used were sterilized at 1.05 kg/cm^3 pressure for 15 minutes at 121℃ and the remaining were kept in a refrigerator at 8°C. Each sterilized bottle containing grains was aseptically inoculated with one 30 mm of 7 days old culture grown on either PDA or malt extract agar. Spawns were incubated for 14 –21 days without illumination at room temperature of 28°C until complete mycelia growth. Fully complete spawns were kept in refrigerator at 8°C when not used.

Preparation of Spawns and Substrates

Spawns were prepared using a modification of methods from [14,21,22]. Sorghum (*Sorghum bicolor*) grains were washed, steeped overnight, and thoroughly rinsed to remove dust and particles. The grains were drained, placed in a wire mesh, and steamed in a pressure cooker at 105°C for 30 minutes, ensuring they remained intact to reduce contamination risk. After cooling on a wooden frame with wire mesh, 3% (w/w) calcium carbonate ($CaCO_3$) was added and manually mixed before sterilization in an autoclave at 121°C for 1 hour. For substrate preparation, sawdust was processed according to [23,24], while dry paddy rice straw compost followed [25] method. Both fermented and unfermented substrates were either supplemented with varying percentages of rice bran or used without supplementation before bagging into autoclavable bags, with composted substrates steam sterilized at 100°C for at least 3 hours. Ten replicates per treatment. The total sample size (i.e., no. of bags) for the entire experiments was 720 bags.

Inoculation and Incubation of Compost Bags

After the bags were steam sterilized and allowed to cool, approximately 2.5 g of spawn were inoculated into each bag of weight of 1 kg under sterile conditions. The bags were shaken to disperse the spawns for uniform distribution of the grains in the bag. The inoculated bags were transferred into an incubation room for complete mycelia colonization in the bag. The ambient temperature of the incubation room was maintained at 25 –31°C. During this period, the mycelial growth rate was measured every week until the entire content of the bag was filled with the mycelia. After the spawn run period, the bags were transferred into a cropping house for the formation of pinheads (primordia) and fruit bodies respectively.

Primordia Initiation (Knotting)

Data patterning time taken from spawning to first pinhead (primordia) formation were collated and recorded for each species and formulated substrates. Primordia formation is considered when there is a small outgrowth of about the size of pea or when a nodule appears on the surface of the substrate.

Harvesting of Matured Mushroom and Calculation of Biological Efficiency (BE)

Biological efficiency was calculated after the primordia formed mature fruit bodies (mushrooms). Matured mushrooms were identified by the curl margin of the cap or when the in–rolled margins of the basidiophores began to flatten [49] (after Kortei & Wiafe-Kwagyan, 2014). Mushrooms were harvested by grasping the base of the stalk and pulling them by hand from the substrate or by twisting to uproot from the base, then packaged in polypropylene bags and were taken away immediately for weighing using an electric balance to determine the fresh weight. In cases where it was difficult to uproot the fruiting bodies from the base, the mushrooms were harvested with a sharp and clean scalpel.

$$\text{B.E} = \frac{\text{weight of fresh mushroom harvested } (g)}{\text{weight of dried substrate } (g)} \times 100\%$$

NB: B.E, that is, the productivity of the conversion of substrate into fruiting body (Chang et al. 1981).

Calculation of Percentage Conversion of Primordia to Matured Fruit Bodies

The percentage conversion of primordia to matured fruit bodies was calculated per flush using the formula:

$$\%\ \text{Conversion} = \left(\frac{\text{Total number of matured fruit bodies}}{\text{Total number of primordia formed}}\right) \times 100$$

NB: the number of aborted primordia was also expressed as % abortion of primordia.

Statistical Analysis

The experimental set of all treatments were arranged in a completely randomized design (CRD), with five (5) treatments and each treatment had ten bags as replicates, while each treatment was repeated three times. Data was examined statistically using (ANOVA) analysis of variance with a probability level of p≤0.05% to achieve the significance of the research.

Results

Conversion of primordia to matured fruiting bodies of *P. eous* and *Pleurotus ostreatus* on rice straw only after three flushes

The study assessed yield characteristics for two oyster mushroom species (*P. eous* and *P. ostreatus*) grown on rice straw composted for different durations (0, 4, 8, and 12 days). *P. eous* showed consistently higher productivity, with its best performance producing 75 pinheads that developed into 56 mature fruiting bodies.

In contrast, *P. ostreatus* reached a maximum of 30 pinheads yielding 19 mature fruiting bodies. The conversion rate from pinheads to mature fruiting bodies for *P. eous* ranged from 77.0% (0-day compost) to 61.3% (12-day compost), showing a declining trend with increased composting time. Similarly, *P. ostreatus* showed conversion rates between 68.0% (4-day compost) and 60.7% (12-day compost).

After three flushes, total fruiting body production for *P. eous* was highest with 4-day composted straw (56 bodies) and lowest with 12-day composted straw (38 bodies). For *P. ostreatus*, production peaked with 8-day composted straw (19 bodies) and was lowest with raw straw (15 bodies). Overall, primordia conversion rates decreased with longer composting periods. The average abortion rate was 28.7% for *P. eous* and 36.4% for *P. ostreatus* (Tables 1 a).

Table 1a: Pinhead formation and conversion to matured fruit bodies per flush of *P. eous* P-31 on only rice straw composted for the indicated periods

Period of composting	1st Flush	No. of pinheads 2nd Flush	3rd Flush	Total No. of pinheads	% Conversion of pinheads to matured fruit bodies
0	43 (30)	13 (10)	17 (8)	61 (48)	78.7[a]
4	44 (37)	14 (9)	17 (10)	75 (56)	74.7[a]
8	38 (31)	16 (10)	18 (11)	72 (52)	72.2[a]
12	30 (21)	16 (10)	16 (7)	62 (38)	61.3[b]

NB: () indicates the number of matured fruit bodies Values in the same column followed by a different letter are statistically significant (p ≤0.05) from each other.

Conversion of primordia to matured fruiting bodies of *P. eous* and *Pleurotus ostreatus* on rice straw supplemented with 1% $CaCO_3$ and 10% rice bran

Previous research has shown that nitrogen and carbohydrate supplements significantly affect mushroom yield and quality. Cereal straw, commonly used for oyster mushroom cultivation, contains limited nitrogen (0.5-0.8%), which becomes insufficient during fruiting after being depleted during mycelial growth. This study used rice bran to supplement rice straw to increase nitrogen content. The study evaluated pinhead formation and maturation into fruiting bodies for both *P. eous* and *P. ostreatus* grown on supplemented rice straw composted for various durations (0, 4, 8, and 12 days). *P. eous* demonstrated a better minimum conversion rate (62.2%) compared to *P. ostreatus*

(58.2%), resulting in lower pinhead abortion rates for *P. eous* (37.8%) against *P. ostreatus* (41.8%). For both species, the conversion rate from primordia to mature fruiting bodies decreased as composting duration increased.

The results showed that *P. eous* consistently produced more fruiting bodies than *P. ostreatus,* though their conversion rates from pinheads to mature fruiting bodies were comparable. On average, *P. ostreatus* had a slightly higher primordia abortion rate (34.9%) than *P. eous* (33.7%), though this difference was not statistically significant (p>0.05) (Tables 2a & b).

Table 1b: Number of pinhead formation and conversion to matured fruit bodies per flush of *P. ostreatus* EM-1 on rice straw substrate composted for the indicated periods

Period of composting	No. of pinheads			Total No. of pinheads	% Conversion of pinheads to matured fruit bodies
	1st Flush	**2nd Flush**	**3rd Flush**		
0	12 (8)	6 (4)	6 (3)	24 (15)	62.5 [a]
4	13 (7)	7 (6)	5 (4)	21 (17)	68.0 [a]
8	17(11)	5 (3)	8 (5)	30 (19)	63.3 [a]
12	11 (8)	13 (6)	4 (3)	28 (17)	60.7 [a]

NB: () indicates the number of matured fruit bodies Values in the same column followed by the same letter are statistically not significant (p ≥0.05) from each other.

Table 2a: Total number of pinheads and matured fruit bodies per flush of *P. eous* P-31 grown on supplemented rice straw with 1% $CaCO_3$ and 10% rice

Period of composting / day(s)	1st Flush	Total no. of fruit bodies2nd Flush	3rd Flush	Total No. of fruit bodies	% Conversion of pinheads to matured fruit bodies
0	45 (32)	14(7)	12 (6)	71 (45) [a]	63.4 [a]
4	48 (39)	27 (17)	14 (7)	89 (63) [b]	70.8 [b]
8	37 (23)	22 (14)	15 (9)	74 (46) [a]	62.2 [a]
12	44 (30)	21 (14)	15 (11)	80 (55) [b]	68.8 [b]

NB: () indicates the number of matured fruit bodies Values in the same column followed by a different letter are statistically significant (p ≤0.05) from each other.

Table 2b: Total number of pinheads and matured fruit bodies per flush of *P. ostreatus* EM- on rice straw supplemented with 1% $CaCO_3$ and 10% rice

Period of composting		No. of pinheads		Total No. of pinheads
	1st Flush	**2nd Flush**	**3rd Flush**	
0	18 (13)	10 (8)	8 (6)	37 (27)[a]
4	17 (12)	12 (8)	10 (6)	39 (26)[a]
8	22 (14)	17 (11)	13 (7)	52 (32)[b]
12	22 (13)	19 (10)	14 (9)	55 (32)[b]

NB: () indicates the number of matured fruit bodiesValues in the same column followed by a different letter are statistically significant ($p \leq 0.05$) from each other

Percentage conversion of primordia to matured fruiting bodies of *P. eous* and *Pleurotus ostreatus* on supplemented rice straw with 1% $CaCO_3$ and 10% rice bran and additional of varying proportions of rice bran 5, 10 and 15%

Research has confirmed that adding specific amounts of inorganic substrate increases biological efficiency in mushroom cultivation. This study evaluated how supplementary rice bran affects the conversion of primordia to mature fruiting bodies in two oyster mushroom species grown on composted rice straw. Results showed that both mushroom species maintained over 50% conversion rates from pinheads to mature fruiting bodies across all substrate conditions, though with notable variations.

For *P. eous*, the highest conversion rate (75.7%) occurred on 4-day composted straw supplemented with 10% rice bran, followed by 8-day composted straw (73.6%). The lowest conversion (56.2%) was observed with 4-day compost containing 5% rice bran. Interestingly, although the highest number of pinheads developed on raw straw with 5% rice bran, this did not result in proportionally high mature fruiting body production. The 12-day compost with 15% rice bran showed significantly better conversion (66.7%) than the same compost with lower rice bran concentrations (Table 3b).

P. ostreatus showed similar conversion patterns but produced fewer primordia and mature fruiting bodies overall. Its highest conversion rate (74.4%) occurred on 12--day compost with 5% rice bran, followed by 4-day compost with 10% rice bran (73.0%). The lowest conversion (63.4%) was recorded on 8-day compost with 5% rice bran (Table 3b). The average primordia abortion rates were 35.0% for *P. eous* and 30.5% for *P. ostreatus* (Tables 3a & b).

Table 3a: Total number of pinheads and fruit bodies after three flushes by *P. eous* P-31on rice straw amended with 1% $CaCO_3$ and 10% rice bran at composting and supplemented with additional proportions of 5, 10 and 15% rice bran at bagging before sterilization

Period of composting/ day(s)	Treatments	1st Flush / fruiting bodies	No. of pinheads / fruit bodies per flush2nd Flush / fruiting bodies	3rd Flush / fruiting bodies	Total number of pinheads / fruiting bodies	% Conversion to fruiting bodies
0	A_{15} A_{10} A_5	42 (33) 38 (25) 51 (35)	21 (13) 20 (12) 15 (10)	11 (6) 14 (6) 19 (9)	74 (52) 72 (43) 85 (54)	70.3[a] 59.7[b] 63.5[c]

4	B_{15} B_{10} B_{5}	49 (30) 42 (23) 41(29)	20 (14) 13 (10) 18 (13)	15 (9) 15 (8) 14 (8)	84 (53) 70 (53) 73 (41)	63.1^{c} 75.7^{a} 56.2^{b}
8	C_{15} C_{10} C_{5}	42 (34) 35 (27) 34 (21)	15 (10) 17 (8) 18 (13)	15 (9) 17 (9) 20 (12)	72 (53) 69 (44) 72 (46)	73.6^{a} 63.8^{c} 63.9^{c}
12	D_{15} D_{10} D_{5}	31 (22) 39 (27) 34 (22)	17 (11) 15 (8) 17 (11)	12 (7) 13 (6) 17 (9)	60 (40) 67 (41) 68 (42)	66.7^{c} 61.2^{c} 61.8^{c}

Keys:

Value of fruit bodies are in parenthesis ()

Values in the same column followed by a different letter are statistically significant ($p \leq 0.05$) from each other

A_{15}, A_{10}, A_{5} represent uncomposted (0 day) rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

B_{15}, B_{10}, B_{5} represent 4 days composted rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

C_{15}, C_{10} C_{5} represent 8 days composted rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

D_{15}, D_{10}, D_{5} represent 12 days composted rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

Table 3b: Total number of pinheads and fruiting bodies from three flushes by *P. ostreatus* EM-1 raised on rice straw amended with 1% $CaCO_3$ and 10% rice bran during composting (0-12 days) and supplemented with different proportions (5, 10 and 15%) rice bran at bagging before sterilization

Period of composting/ day(s)	Treatments	1st Flush / fruiting bodies	No. of pinheads / fruit bodies per flush 2nd Flush / fruiting bodies	3rd Flush / fruiting bodies	Total number of pinheads / fruiting bodies	% Conversion to fruiting bodies
0	A_{15} A_{10} A_{5}	13 (09) 15 (10) 16 (10)	9 (06) 9 (07) 6 (04)	8 (6) 8 (6) 6 (4)	30 (21) 32 (23) 28 (18)	65.6^{a} 71.9^{b} 75.0^{b}
4	B_{15} B_{10} B_{5}	18 (12) 18 (12) 19 (12)	16 (11) 12 (09) 10 (08)	8 (6) 7 (6) 7 (6)	42 (28) 37 (27) 36 (26)	66.6^{a} 73.0^{b} 72.2^{b}
8	C_{15} C_{10} C_{5}	20 (13) 20 (12) 20 (11)	17 (12) 11 (10) 13 (10)	12 (9) 11(7) 8 (5)	49 (34) 42 (29) 41(26)	69.3^{a} 69.0^{a} 63.4^{a}

12	D_{15} D_{10} D_5	10 (08) 25 (17) 22 (15)	10 (06) 11(08) 11(09)	9 (6) 9 (7) 10 (8)	29 (20) 45 (32) 43 (32)	68.9[a] 71.1[b] 74.4[b]

Keys: Value of fruit bodies are in parenthesis ()

Values in the same column followed by a different letter are statistically significant ($p \leq 0.05$) from each other

A_{15}, A_{10}, A_5 represent uncomposted (0 day) rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

B_{15}, B_{10}, B_5 represent 4 days composted rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

C_{15}, C_{10} C_5 represent 8 days composted rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

D_{15}, D_{10}, D_5 represent 12 days composted rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

Assessment of total number of primordia conversion to matured fruit bodies of *Pleurotus eous* and *P. ostreatus* on rice straw and rice husk mixture substrate supplemented with additional 5, 10 and 15% of rice bran

The nitrogen content in mycelium typically ranges from 3% to 6%, depending on the nutrient composition of the substrate, which leads to variations in mushroom yields. When rice straw and rice husk mixtures were supplemented with 5%, 10%, and 15% rice bran, *P. eous* showed the highest primordia-to-fruiting body conversion of 74.1%. This was observed in the 0-day compost mixture with 15% rice bran. The next highest conversion rate (72.5%) occurred in the 8-day compost mixture with 10% rice bran, while the lowest conversion rate (58.5%) was seen in the 12-day compost with 15% rice bran (Table 4a).

For *P. ostreatus* strain EM-1, the conversion of primordia to matured fruit bodies was similar to that of *P. eous* strain P-31, though the differences were not statistically significant ($p \geq 0.05$). However, *P. ostreatus* generally showed slightly lower conversion rates compared to *P. eous*. The highest conversion for *P. ostreatus* was 71.1%, recorded in the 12-day compost with 10% rice bran, followed by 68.9% in the 0-day substrate with 15% rice bran. On average, *P. eous* had a conversion rate of 67.3%, while *P. ostreatus* had a conversion rate of 64.0% (Tables 4a & b).

Table 4a: Total number of pinheads and fruiting bodies from three flushes by *P. ostreatus* EM-1 on rice straw and rice husk mixture (1:1w/w) amended with 1% $CaCO_3$ and 10% rice bran during composting (0-12 days) and supplemented with different proportions (5, 10 and 15% of rice bran at bagging before sterilization

Period of composting/ day(s)	Treatments	1st Flush / fruiting bodies	No. of pinheads / fruit bodies per flush2nd Flush / fruiting bodies	3rd Flush / fruiting bodies	Total number of pinheads / fruiting bodies	% Conversion to fruiting bodies
0	A_{15} A_{10} A_5	20 (15) 18 (10) 24 (16)	14 (9) 14 (10) 14 (9)	11(7) 11 (6) 11 (5)	45 (31) 43 (26) 49 (30)	68.9[a] 60.5[a] 61.2[a]
4	B_{15} B_{10} B_5	18 (10) 14 (8) 18 (13)	15 (9) 13 (7) 08 (5)	12 (7) 11 (7) 11 (6)	45 (26) 38 (22) 37 (24)	57.8[b] 57.9[b] 64.9[a]

8	C_{15} C_{10} C_{5}	19 (13) 19 (12) 11 (9)	15 (9) 11 (7) 12 (7)	15 (8) 08 (4) 10 (6)	49 (30) 38 (23) 33 (22)	61.2^{a} 60.5^{a} 66.7^{a}
12	D_{15} D_{10} D_{5}	23 (17) 19 (15) 23 (16)	12 (8) 14 (10) 30 (20)	13 (8) 12 (7) 10 (7)	48 (33) 45 (32) 63 (43)	68.8^{a} 71.1^{c} 68.3^{a}

Keys: Values of fruiting bodies are in parenthesis ()

Values in the same column followed by a different letter are statistically significant ($p \leq 0.05$) from each other

A_{15}, A_{10}, A_{5} represent uncomposted (0 day) rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

B_{15}, B_{10}, B_{5} represent 4 days composted rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

C_{15}, C_{10} C_{5} represent 8 days composted rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

D_{15}, D_{10}, D_{5} represent 12 days composted rice straw supplemented with additional 5, 10 and 15% rice bran respectively.

Table 4b: Total pinheads and fruiting bodies produced by *P. eous* P-31 on a rice straw and rice husk mixture (1:1 w/w) with 1% $CaCO_3$ and 10% rice bran during composting (0-12 Days), supplemented with varying proportions of rice bran (5%, 10%, and 15%) at bagging

Period of composting/ day(s)	Treatments	1st Flush / fruiting bodies	No. of pinheads / fruit bodies per flush2nd Flush / fruiting bodies	3rd Flush / fruiting bodies	Total number of pinheads / fruiting bodies	% Conversion to fruiting bodies
0	A_{15} A_{10} A_{5}	50 (38) 34 (23) 37 (24)	27 (20) 21 (14) 25 (15)	12 (8) 16 (9) 16 (12)	89 (66) 71 (46) 78 (51)	74.1^{a} 64.8^{b} 65.4^{b}
4	B_{15} B_{10} B_{5}	24 (14) 25 (18) 26 (15)	14 (10) 16 (13) 14 (10)	12 (8) 12 (7) 10 (8)	50 (34) 53 (38) 50 (33)	68.0^{b} 71.7^{a} 66.0^{b}
8	C_{15} C_{10} C_{5}	26 (17) 21 (14) 22 (16)	16 (11) 10 (10) 14 (10)	9 (6) 9 (5) 11 (6)	51 (34) 40 (29) 47 (32)	66.7^{b} 72.5^{a} 68.1^{b}
12	D_{15} D_{10} D_{5}	30 (20) 27 (18) 27 (18)	13 (8) 14 (10) 15 (10)	10 (8) 10 (7) 11 (6)	53 (30) 51 (35) 53 (34)	58.5^{c} 68.2^{b} 64.1^{b}

Keys: Values of fruiting bodies are in parenthesis ()

Values in the same column followed by a different letter are statistically significant ($p \leq 0.05$) from each other

Total primordia and fruiting bodies produced by *P. eous* and *P. ostreatus* on sawdust amended with 1% $CaCO_3$ and 10% rice bran before composting and bagging

Mushroom substrate composition particularly carbon-to-nitrogen ratio, moisture content, and nutrient availability significantly impacts yield. This study examined primordia conversion rates on hardwood sawdust (*Triplochiton scleroxylon*) for two oyster mushroom species.

Both species showed different conversion patterns on sawdust substrate. While *P. eous* produced more pinheads overall, it demonstrated lower conversion rates to mature fruiting bodies compared to *P. ostreatus*. The average conversion rates were 63.2% and 66.0% for *P. eous* and *P. ostreatus* respectively.

Interestingly, both species achieved their highest conversion rates on 8-day composted sawdust, with *P. eous* reaching 67.6% and *P. ostreatus* achieving 75.4%. Similarly, both recorded their lowest conversion rates on uncomposted sawdust (0-day), with *P. eous* at 57.9% and *P. ostreatus* at 62.8%. Despite these differences in conversion performance, statistical analysis showed no significant difference ($p \geq 0.05$) in primordia formation and maturation between the various composting durations (0-12 days) for either species (Tables 5a and 5b).

Table 5a: Total number of pinheads and fruit bodies per three flushes of *P. eous* P-31 on sawdust amended with 1% $CaCO_3$ and 10% rice bran

Period of composting		No. of pinheads / fruit bodies per flush		Total No. of pinheads / fruiting bodies	% Conversion to fruit bodies
	1st Flush	2nd Flush	3rd Flush		
0	40 (23)	24 (15)	12 (6)	76 (44)	57.9[a]
4	38 (25)	21 (13)	14 (8)	73 (46)	63.0[b]
8	37 (23)	19 (14)	15 (11)	71 (41)	67.6[b]
12	38 (27)	21 (10)	14 (10)	73 (47)	64.4[b]

Keys *No. of fruiting bodies are in parenthesis ()

Values in the same column followed by a different letter are statistically significant ($p \leq 0.05$) from each other

Table 5b: Total number of pinheads and fruit bodies per three flushes of *P. ostreatus* EM-1 on sawdust amended with 1% $CaCO_3$ and 10% rice bran

Period of composting		No. of pinheads / fruit bodies per flush		Total No. of pinheads / fruiting bodies	% Conversion to fruit bodies
	1st Flush	2nd Flush	3rd Flush		
0	29 (16)	30 (19)	19 (14)	78 (49)	62.8[a]
4	24 (15)	17 (12)	21 (12)	62 (39)	62.9[a]
8	26 (21)	17 (13)	22 (15)	65 (49)	75.4[b]
12	24 (16)	18 (12)	15 (9)	57 (37)	64.9[a]

Keys

*Fruiting bodies are in parenthesis ()

Values in the same column followed by a different letter are statistically significant ($p \leq 0.05$) from each other

Discussion

Mushroom cultivation plays a crucial role in enhancing food security through its nutritional contributions while supporting sustainability by utilizing waste materials efficiently and minimizing environmental impact. Mushrooms have therefore become an attractive crop to cultivate in both developing and developed countries for many reasons. Oyster mushroom (*Pleurotus* spp.) cultivation has increased tremendously throughout the world. There are varieties of agricultural waste used as substrates for mushroom cultivation. These agricultural residues are rich in lignin, cellulose and hemicellulose which are required for mushroom growth and development [26]. However, different substrates have varying nutrients, and different strains of mushrooms yield variably. This study investigated % conversion of primordia to matured fruiting bodies by *Pleurotus eous* and *P. ostreatus* on different formulated rice wastes and 'wawa' sawdust of *Triplochiton scleroxylon* supplemented with varying proportions of rice bran.

It has been established that the effects of physical and chemical properties of substrates influence the yield and biological efficiency (BE) on mushrooms [18,27-29]. The present results demonstrate significant variations in the conversion rates of primordia to matured fruiting bodies for both *Pleurotus eous* strain P-31 and *Pleurotus ostreatus* EM-1 across different substrate composition and composting periods. These findings align with and extend previous research on oyster mushroom cultivation techniques.

Rice Straw Substrate Findings

The higher conversion rates observed for *P. eous* (77.0%) compared to *P. ostreatus* (68.0%) on uncomplemented rice straw substrates corroborate findings by [24], who reported species-specific responses to cultivation substrates. The decline in conversion rates with increasing composting duration contradicts expectations based on [25] work, which suggested that composting should improve substrate digestibility. This unexpected result may be attributed to the loss of essential nutrients during extended composting periods, as hypothesized by [30].

Effect of Supplementation

The supplementation of rice straw with 1% $CaCO_3$ and 10% rice bran demonstrated the importance of nitrogen enrichment for mushroom cultivation, consistent with [18,31], who emphasized that cereal straws low nitrogen content (0.5-0.8%) can limit mushroom yield. However, despite supplementation, the primordia abortion rates remained relatively high (33.7% for *P. eous* and 34.9% for *P. ostreatus*), suggesting that factors beyond nutrition might influence fruiting body development.

When varying proportions of rice bran (5%, 10%, and 15%) were tested, *P. eous* achieved its highest conversion rate (75.7%) on 4-day composted straw with an additional 10% rice bran. This aligns with [23], who reported optimal results with moderate supplementation levels rather than the highest levels. For *P. ostreatus*, the highest conversion (74.4%) occurred on 12-day composted straw with only 5% additional rice bran, suggesting different nutritional requirements between the two species, as previously noted by [21].

Rice Straw and Rice Husk Mixture Performance

The mixed substrate of rice straw and rice husk produced generally favourable results, with *P. eous* achieving a 74.1% conversion rate on uncomposted substrate with 15% rice bran. This supports [22] observations that diverse substrate compositions can enhance cultivation outcomes. *P. ostreatus* performed best (71.1%) on 12-day composted mixture with 10% rice bran, again highlighting species-specific preferences.

Sawdust Substrate Performance

On *Triplochiton scleroxylon* sawdust substrate, both species showed moderate conversion rates, with *P. ostreatus* (66.0%) on the average outperforming *P. eous* (63.2%) average. The highest conversion for both species on 8-day composted sawdust (67.6% for *P. eous* and 75.4% for *P. ostreatus*) corresponds with findings from Royse (2002), who demonstrated that partial decomposition of lignocellulosic materials enhances mushroom yield. The lower performance on uncomposted sawdust (57.9% for *P. eous* and 62.8% for *P. ostreatus*) underscores the importance of substrate preparation methods, as emphasized by [32].

Comparative Analysis

Overall, the results demonstrate that substrate type, composting duration, and supplementation levels significantly influence the conversion of primordia to matured fruiting bodies in both *Pleurotus* species. The species-specific responses observed is in parallel with previous research by [33], who noted that different *Pleurotus* species exhibit varied efficiency in utilizing substrate components. The generally higher performance of *P. eous* across most substrates extends our understanding of species selection for optimal cultivation outcomes.

These findings contribute valuable insights for mushroom cultivators, suggesting that substrate choice and preparation should be tailored to the specific *Pleurotus* species being cultivated, with shorter composting periods generally yielding better results for *P. eous* and moderate supplementation levels optimizing fruiting body development for both species.

The results of the study on the conversion of primordia to matured fruiting bodies of *Pleurotus eous* and *Pleurotus ostreatus* across different substrates and composting regimes provide valuable insights into the factors influencing mushroom yield, particularly in the context of using rice straw, rice bran supplementation, and other substrate combinations.

Influence of Composting Duration on Primordia Conversion

The present data indicates a clear trend: as the duration of rice straw composting increased, the conversion rate of primordia to matured fruiting bodies decreased for both species. This is consistent with previous studies that have highlighted the negative effects of prolonged composting on substrate nutrient availability, especially nitrogen, which is crucial for fruiting body formation. The reduced nitrogen content in the substrate after extended composting may limit the ability of the mycelium to fully develop into fruiting bodies, thus leading to lower conversion rates. For instance, *P. eous* had a higher overall conversion rate compared to *P. ostreatus*, particularly when composting was done for shorter periods, which might be attributed to its greater adaptability to substrate nutrient profile, as observed in similar studies by [13,34].

The progressive decline in the primordia conversion rate with increasing composting days (0, 4, 8, and 12) also correlates with findings by [29], who observed a similar trend when *P. ostreatus* was cultivated on wheat straw, where extended composting periods led to nutrient depletion, negatively impacting the formation of fruiting bodies. The average abortion rates of 28.7% for *P. eous* and 36.4% for *P. ostreatus* are in line with the results by [35], who reported that composted substrates, when aged for extended periods, lead to a significant increase in the abortion of primordia.

Supplementation with Rice Bran and Calcium Carbonate

Supplementing rice straw with rice bran and calcium carbonate ($CaCO_3$) resulted in improved primordia conversion rates for both *P. eous* and *P. ostreatus*. This can be attributed to the enhanced nitrogen content from the rice bran and the buffering capacity of calcium carbonate, which helps in maintaining an optimal pH for fungal growth. Supplementation with rice bran, as demonstrated in previous studies [36,37], has been shown to improve the biological efficiency of oyster mushrooms by providing additional nutrients that promote better mycelial growth and fruiting body formation. [18] reported that the performance and productivity of *Pleurotus ostreatus* mushroom were highly influenced by the substrate from which it was grown. This finding is also in accordance with the findings of [38-40], these authors also observed that the Biological Efficiencies of cultivated mushrooms always vary between fungal species, type or size of substrate used, supplement used and other varying growth factors.

Interestingly, the highest conversion rate for *P. eous* was recorded when rice straw was composted for 4 days and supplemented with 10% rice bran, which supports the findings of other researchers who have found that a moderate supplementation of rice bran can significantly improve the yield and quality of mushrooms [41]. On the other hand, *P. ostreatus* exhibited a relatively lower conversion rate even with supplementation, a phenomenon that might be related to the species inherent differences in nutrient requirements and substrate preference, as previously discussed by [18,36,37].

Effect of Additional Rice Bran Proportions

When the substrate was further amended with varying proportions of rice bran (5%, 10%, and 15%), the conversion rates for both species fluctuated, with the highest being 75.7% for *P. eous* on 4-day composted rice straw supplemented with 10% rice bran. This supports earlier work by [7], who observed that adding rice bran to mushroom cultivation substrates increases the nitrogen content, promoting better mycelial colonization and fruiting body formation. However, the results also show that excessive supplementation (e.g., 15% rice bran) did not consistently improve the conversion rate, especially for *P. ostreatus*, where the conversion rate seemed to plateau or even decrease slightly with higher rice bran concentrations. This suggests that there is an optimal level of supplementation for each species, beyond which further additions do not yield significant benefits and may even lead to nutrient imbalances detrimental to fruiting body development, as suggested by [42,43].

Comparison of Substrate Types: Rice Straw, Rice Husk, and Sawdust

The study also examined the effects of different substrate types, such as rice straw mixed with rice husk and supplemented with rice bran, on primordia conversion. *P. eous* and *P. ostreatus* demonstrated similar trends, with rice straw and rice husk mixtures showing significant variability in conversion rates, which conciliates with earlier studies on the effects of substrate composition on mushroom yield [44]. The higher nitrogen content of the rice husk mixture likely provided better conditions for mycelial growth and fruiting body formation compared to rice straw alone, as demonstrated by the improved conversion rates in the presence of higher rice bran supplementation.

Further reports by [18,27,29,45] have shown that using diverse organic materials like coffee husk mixed with wheat bran can significantly enhance oyster mushroom yield by improving nutrient availability. Similarly, adding nitrogen-rich supplements like legume straws or cotton-sized hulls can boost yields depending on the specific mushroom variety. The present data corroborated with [41] who reported that coffee husk alone or together with different proportions of wheat bran resulted in highest growth, yield, yield related parameters and biological efficiency of the oyster mushroom.

General Observations and Species Comparison

According to [41,46,47], certain species of Pleurotus outperform others under specific conditions. Overall, *P. eous* consistently showed higher primordia conversion to matured fruit bodies than *P. ostreatus* across most substrate and composting length. This finding is supported by previous studies, who reported that *P. eous* tends to perform better in certain substrate conditions due to its higher adaptability and more efficient nutrient uptake compared to *P. ostreatus*. Furthermore, the average primordia abortion rate for *P. ostreatus* was slightly higher than that of *P. eous*, indicating that the former may be more sensitive to changes in substrate composition and composting duration.

However, [48] reported on critical environmental factors such as nutrient, temperature and light influencing fruiting body induction, development and maturation. For instance, he reported that higher nitrogen and carbon sources in the media will suppress fruiting body induction in many mushroom-forming fungi, whereas induction is being triggered by lower nitrogen and carbon concentrations. [48] further stated that some species like *Flammulina velutipes* and *Coprinopsis cinerea* can form fruiting bodies in the dark; however, light accelerates fruiting body induction in some mushroom-forming fungi. Interestingly fruiting bodies formed in the dark have tiny or no pileus on heads (i.e., a dark stipe, pinhead fruiting body, or etiolated stipe). Light is essential for pileus differentiation in many, except for *Agaricus bisporus* [48]. Carbon dioxide concentrations also affect fruiting body development; pileus differentiation is also suppressed at a high concentration of carbon dioxide [48]. Therefore, it can be conjectured that the percentage abortion of primordia recorded in this paper by both oyster mushrooms may not only be attributed to the type of substrates, the composting period but also the prevailing environmental conditions both oyster mushrooms were exposed to.

Conclusion

This study corroborates previous research on the impact of composting duration, substrate composition, and supplementation on the yield and quality of oyster mushrooms. The results emphasize the importance of optimizing composting time and supplementing rice straw with nitrogen-rich materials like rice bran to enhance the conversion of primordia to matured fruit bodies. The abortion rates of primordia could have been influenced by multiple factors beyond substrate composition, including environmental conditions such as light, carbon dioxide concentration, and temperature. This suggests that optimal cultivation conditions must consider both substrate parameters and environmental factors to maximize yield.

These findings have important implications for commercial mushroom cultivation, indicating that substrate choice and preparation methods should be species-specific to optimize yield. The study also highlights the importance of proper supplementation with nitrogen-rich materials like rice bran to enhance biological efficiency and improve primordia conversion rates in *Pleurotus eous* and *P. ostreatus* cultivation.

Future studies could explore other supplementation options and further investigate the physiological responses or genetic factors influencing primordia abortion rates of *P. eous* and *P. ostreatus* to varying substrate conditions to fine-tune cultivation practices and improve mushroom yields in commercial settings.

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