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Bioconversion of Spent Mushroom Substrate (SMS) of Button Mushroom for Cultivation of Oyster Mushroom (*Pleurotus florida*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mushrooms offer a sustainable solution to global food security by converting agricultural waste into nutritious protein. *Pleurotus* species are particularly valuable, requiring simple cultivation methods thus optimizing substrate combinations of organic wastes can significantly enhance mushroom yields and production efficiency. The present study evaluated the bioconversion potential of spent mushroom substrate (SMS) from button mushroom cultivation for enhancing the growth, yield, and

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nutritional quality of oyster mushroom (*Pleurotus florida*). Different combinations of wheat straw and SMS were tested, with the 4:1 ratio (wheat straw + SMS) (T5) emerging as the most effective treatment. Results showed that T5 significantly reduced spawn running (16 days), pinhead initiation (20 days), and harvesting periods (26, 32, and 41 days for three flushes) compared to the SMS-alone control (22, 28, and 36–55 days). The highest yield (1316.99 g/bag) and biological efficiency (87.73%) were recorded in T5, along with superior morphological traits such as maximum fruiting bodies (87.25/bag), stalk length (6.78 cm), and cap diameter (3.54 cm). Moisture content was optimized (~90%), and dry weight peaked at 135.60 g in T5. The study demonstrates that supplementing SMS with wheat straw (4:1 ratio) enhances *P. florida* productivity and offers an efficient waste-to-food solution.

Keywords: Pleurotus florida; spent mushroom substrate (SMS); wheat straw; yield.

1. INTRODUCTION

The world of mushrooms has long captivated human curiosity, largely due to their sudden emergence and their often striking and enigmatic appearance. It is estimated that approximately 12,000 mushroom species exist worldwide, of which around 2,000 are recognized as edible. However, only about 35 edible species are cultivated on a commercial scale, while nearly 200 wild mushroom species are traditionally utilized for their medicinal properties (Beulah and Margret, 2013).

Among the various edible mushrooms, Oyster mushrooms are particularly favored due to the relative simplicity and low cost of their cultivation techniques. *Pleurotus* mushrooms are valued for their comprehensive nutritional profile. Oyster mushrooms contain high levels of protein at 29% and dietary fiber at 13%, along with various vitamins and minerals, while being cholesterol-free (Chukwu et al., 2024). Their protein content is particularly notable for containing significant amounts of essential amino acids including leucine, glutamine, and valine. Research has identified various bioactive compounds in *Pleurotus* species that demonstrate therapeutic potential, including properties that may help manage chronic diseases, inhibit tumor growth, and fight bacterial infections (Khare *et al*., 2018). Their composition also includes 0.46 g nitrogen, 2.3 g protein, 0.19 g total fat, 0.73 g as hand 6.94 g carbohydrates (Manzi, 2001).

The recycling and utilization of agricultural and agro-industrial waste represent a crucial strategy for promoting sustainable resource management and enhancing agricultural productivity. *Pleurotus* species are particularly well-suited for cultivation on a wide variety of lignocellulosic agro-waste substrates (Yamauchi *et al.,* 2018; Mandal et al., 2021).

In light of the aforementioned significance and potential on advancements for cultivating *Pleurotus florida* in both rural and urban regions of the country, the study was undertaken as title “Bioconversion of Spent mushroom substrate (SMS) of button mushroom for cultivation of Oyster mushroom (*Pleurotus florida*)” in the present organization.

2. MATERIALS AND METHODS

2.1 Experimental Site

The present investigation was conducted at the Mushroom Research and Development Centre, Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur from 2023 to 2025.

2.2 Substrate Preparation and Chemical Sterilization

The wheat straw used in this study was first thoroughly washed in fresh water before undergoing chemical treatment by soaking for 18–20 hours in a formalin solution (2-4%). On the next day, all the wet wheat straw was separated from the water and the additional water was carefully drained. The substrates were kept on a concrete floor that had been sterilized with a 2-4% formalin sterilization. Thus, the substrates were prepared for use in the mushroom culture procedure.

2.3 Spawning

Fresh spawn (15-20 days old) was used in a pre-fumigated room (disinfected with 2% formaldehyde for 36 hours). Polypropylene bags (75×450 cm) were filled by layering substrate and 50 g spawn alternately, topped with a final substrate layer. Bags were sealed, perforated with 8-10 holes, and placed on racks for optimal mycelial growth.

2.4 Experimental Details

The present investigation was conducted using a completely randomized design (CRD) with nine treatments and three replications per treatment. The substrate consisted of pure wheat straw supplemented with spent mushroom substrate (SMS), with 4.5 kg (dry weight) per bag, and spawning was carried out at 10% of the dry substrate weight to ensure optimal mycelial colonization.

2.5 Details of Various Treatments

List 1. Various treatments details

Treatments	Details
T_1	Fresh wheat straw (FWS) + SMS (1:1)
T_2	Fresh wheat straw + SMS (1:2)
T_3	Fresh wheat straw + SMS (1:3)
T_4	Fresh wheat straw + SMS (1:4)
T_5	Fresh wheat straw + SMS (4:1)
T_6	Fresh wheat straw + SMS (3:1)
T_7	Fresh wheat straw + SMS (2:1)
T_8	100% FWS without SMS
T_9	100% SMS without FWS

2.6 Observations Recorded

During the experimental period, observations were systematically recorded for various observations such as radial mycelial growth in centimeters, growth behavior (spawn run period, pinhead formation, and three harvesting phases in days), growth parameters (number, weight, stalk length, cap diameter, and stalk diameter of fruiting bodies), yield potential (per harvest and total), biological efficiency, and fresh/dry weights.

2.7 Biological Efficiency

The biological efficiency (BE) of the substrate was calculated using the following formula:

Biological Efficiency (BE) = (Fresh weight of mushrooms/Dry weight of substrate) * 100

2.8 Statistical Analysis

Each treatment was replicated thrice and values were means ± SE. The data were computed using SPSS software version 21.

3. RESULTS AND DISCUSSION

The present study was undertaken to assess the influence of various plant growth regulators on the morphological traits, yield attributes, and nutritional composition of *Pleurotus florida*. The findings of the experimental study are detailed as below.

3.1 Effect of Spent Mushroom Substrate on Duration of Growth Stage of *Pleurotus florida*

3.1.1 Spawn Running and pin head initiation

The results as observed from Table 1 revealed that substrate composition significantly impacts spawn running and pinhead initiation in *Pleurotus florida*. Treatment T5 (Wheat straw + SMS) showed the fastest spawn running (16 days) and earliest pinhead formation (20 days), followed by T6 (17 days; 21 days) and T7 (17 days; 22 days), while the SMS-alone control performed poorest (22 days; 28 days). These results demonstrate that wheat straw supplementation enhances mycelial growth efficiency and accelerates development stages.

3.1.2 Harvesting

The results as observed from Table 1 demonstrated significantly shorter harvesting times for wheat straw-supplemented substrates compared to the control. Treatment T5 (Wheat straw + SMS) showed the fastest harvest intervals at 26, 32, and 41 days for the first, second, and third flushes, respectively. Treatment T6 followed closely with harvests at 27, 34, and 43 days. In contrast, the SMS-only control exhibited the longest intervals (36, 45, and 55 days), confirming that wheat straw enrichment substantially reduces the cropping cycle of *Pleurotus florida*. These findings align with previous studies by Godse *et al*., (2021) who reported similar acceleration in harvest timing through substrate optimization and growth regulators.

3.2 Effect of Various Combination of SMS on Yield Parameter of *Pleurotus florida*

3.2.1 Yield in three harvesting

Pleurotus florida exhibited significant yield variations across three harvest flushes as observed from Table 2. Treatment T5 (Wheat straw + SMS) achieved the highest yields: 470.00 g (1st flush), 450.33 g (2nd), and 396.66 g (3rd) per bag. T6 and T7 followed closely, with T6 yielding 460.76 g, 444.00 g, and 390.33 g, and T7 producing 456.44 g, 440.00 g, and 378.00 g per bag for the respective flushes. In contrast, the SMS-only control yielded significantly less (310.00 g, 290.00 g, and 250.66 g). The results confirm that wheat straw supplementation boosts yield, with the first flush consistently outperforming subsequent harvests.

3.2.2 Total yield

The results as mentioned in Table 2 revealed substantial differences in total yield across substrate treatments. Treatment T5 (Wheat straw + SMS combination) achieved optimal productivity, yielding 1316.99 g per cultivation bag - representing a 54.8% increase over the SMS-only control (850.66 g). The progressive yield reduction in treatments T6 (1295.09 g) and T7 (1274.44 g) with decreasing wheat straw ratios further confirmed the importance of substrate composition. These findings align with established research (Kumar *et al*., 2023) demonstrating that organic supplements like wheat straw enhance yield through improved nutrient availability and mycelial colonization.

3.2.3 Biological efficiency (BE %)

The results as observed from Table 2 shows that the biological efficiency (BE) of *P. florida* varied significantly (56.66–87.73%) across treatments, with wheat straw-enriched substrates outperforming the control. T5 (Wheat straw + SMS) achieved maximum BE (87.73%), followed by T6 (86.33%) and T7 (84.93%), while the SMS-only control showed lowest efficiency (56.66%). These results demonstrate wheat straw's crucial role in enhancing BE. Similar findings were reported by Upadhyay *et al*., (2002), where substrate supplementation yielded 30–90% BE in *Pleurotus* species.

3.3 Effect of Different Combinations of Spent Mushroom Substrate on Morphological Parameters of *Pleurotus florida*

The parameters assessed included the number of fruiting bodies, maximum individual fruiting body weight, stalk length, and cap diameter.

3.3.1 Number of fruiting bodies

The results as mentioned in Table 3 revealed significant variation in fruiting body numbers across treatments. T5 (Wheat straw + SMS) produced the highest count (87.25/bag), followed by T6 (85.41) and T7 (84.90), while the SMS-only control yielded fewest (75.42). These results confirm wheat straw's positive effect on fruiting body formation in *P. florida*. The findings align with Bhatti *et al*., (2007), who reported similar substrate-dependent improvements in mushroom growth parameters.

3.3.2 Stalk length

The results as observed from Table 3 revealed significant variations in stalk length across various treatments, with T5 (Wheat straw + SMS) showing maximum stalk length elongation of 6.78 cm. T6 and T7 showed stalk length of 6.53 cm and 6.10 cm respectively, while the SMS-only control had the shortest stalks length (4.10 cm). These results demonstrate wheat straw's positive effect on stalk development, consistent with findings by Nirdesh Kumar *et al*., (2019) regarding substrate optimization.

3.3.3 Cap diameter

The data presented in the Table 3, showed that there was a significant difference in average cap diameter were observed across all treatments, with values ranging from 1.61 cm to 3.54 cm. The largest cap diameter was observed in treatment T5 (Wheat straw + Spent by Mushroom Substrate), reaching 3.54 cm. The smallest cap diameter was recorded in the control treatment (SMS alone), measuring 1.61 cm. This was followed by treatments T6 (Fresh wheat straw + SMS (3:1)) and T7 (Fresh wheat straw + SMS (2:1)) both exhibiting per data exhibiting cap diameters of 3.34 cm. Similar enhancements were reported by Sheesh Pal *et al*., (2022) using enriched substrates.

3.3.4 Diameter of pileus

The data presented in the Table 3, showed that there was a significant difference in average pileus diameter across various treatments. T5 (Wheat straw + SMS) showed the largest diameter (10.68 cm), followed by T6 (10.54 cm) and T7 (10.21 cm), while the SMS-only control had the smallest (8.43 cm). These results indicate wheat straw supplementation enhances pileus development. Similar improvements were reported by Onokpise *et al*., (2008) and Zerihun (2015) using enriched substrates.

Table 1. Effect of Spent Mushroom Substrate on duration of growth stage of *Pleurotus florida*

Treatments	Days of spawn run	Days of pinhead formation	First harvesting (Days)	Second harvesting (Days)	Third Harvesting (Days)	Total cropping period
T1	17.00	22.00	28.00	36.00	45.00	51.00
T2	18.00	23.00	29.00	36.00	45.00	52.00
T3	18.00	23.00	29.00	37.00	46.00	53.00
T4	19.00	24.00	31.00	39.00	49.00	55.00
T5	16.00	20.00	26.00	32.00	41.00	47.00
T6	16.00	21.00	27.00	34.00	43.00	48.00
T7	17.00	22.00	28.00	34.00	44.00	50.00
T8	19.00	24.00	33.00	42.00	51.00	58.00
T9	22.00	28.00	36.00	45.00	55.00	59.00
C.D. at 5%	1.88	2.39	3.10	2.72	9.35	
SE(m)	0.62	0.79	1.03	0.90	3.12	

Table 2. Effect of various combination of Spent Mushroom Substrate on yield parameter of *Pleurotus florida*

Treatments	Yield of first flush (g)	Yield of second flush (g)	Yield of third flush (g)	Total yield (g)	Biological efficiency (%)
T1	445.88	436.88	364.66	1242.42	82.80
T2	440.00	427.33	360.33	1227.66	81.80
T3	440.66	410.33	355.33	1206.32	80.40
T4	436.00	400.66	343.00	1179.66	78.60
T5	470.00	450.33	396.66	1316.99	87.73
T6	460.76	444.00	390.33	1295.09	86.33
T7	456.44	440.00	378.00	1274.44	84.93
T8	390.00	360.66	282.67	1033.33	68.86
T9	310.00	290.00	250.66	850.66	56.66
C.D. at 5%	39.941	37.637	31.992	109.496	
SE(m)	13.340	12.570	10.685	36.570	

Table 3. Effect of different combinations of SMS on morphological parameter of *Pleurotus florida*

Treatment	Average no of fruiting bodies (g)	Average stipe length(cm)	Average stipe width (cm)	Average pileus diameter (cm)
T_1	84.72	5.75	2.66	10.13
T2	83.69	5.40	2.38	9.96
T3	83.55	4.60	2.10	9.69
T4	80.72	4.35	1.77	9.32
T5	87.25	6.78	3.54	10.68
T6	85.41	6.53	3.34	10.54
T7	84.90	6.10	3.32	10.21
T8	78.88	4.21	1.68	8.97
T9	75.42	4.10	1.61	8.43
C.D. at 5%	5.357	0.323	0.152	0.626
SE(m)	1.789	0.108	0.051	0.209

Table 4. Effect of different combination of SMS on the fresh and dry weight of Oyster mushroom *(Pleurotus florida)*

Treatment	Total fresh Weight (g)	Total fresh weight increased over control (%)	Total fresh increased over control SMS (%)	Total dry weight (g)	Total dry weight increased over control (%)	Total dry weight increase over control SMS (%)
T1	1242.42	18.78	46.05	126.55	32.65	42.83
T2	1227.66	18.40	44.31	123.98	29.95	39.93
T3	1206.32	16.74	41.80	120.66	26.47	36.81
T4	1179.66	14.16	38.67	111.46	16.83	25.80
T5	1316.99	27.45	54.81	135.60	42.13	52.59
T6	1295.09	25.33	52.24	130.20	36.47	46.95
T7	1274.44	23.33	49.81	129.40	35.63	46.04
T8	1033.33			95.40		
T9	850.66			88.60		
C.D. at 5%	109.496			10.850		
SE(m)	36.570			3.624		

3.4 Effect of Different Combinations of SMS on the Fresh and Dry Weight of *Pleurotus florida*

3.4.1 Fresh weight (g)

The data as presented in Table 4 shows that treatment T5 (Wheat straw + SMS) produced the highest fresh weight (1,316.99 g), followed by T6 (1,295.09 g) and T7 (1,274.44 g), while the SMS-only control yielded least (850.66 g). These results confirm SMS integration significantly boosts yield. Similar findings were reported by Behera *et al*., (2024) showing substrate enrichment enhances mushroom productivity.

3.4.2 Moisture content

Moisture content plays a critical role in determining the post-harvest quality and shelf life of mushrooms, as higher water content often leads to rapid deterioration. As data presented in the Table 4, showed that the moisture content of *P. florida* varied across treatments, generally ranging within 85% and slightly above 90%. The control treatment (SMS alone) exhibited the highest moisture content at approximately 95%, which was notably higher than all treated samples. These results suggested that substrate supplementation with SMS contributes to a moderate reduction in moisture content, thereby potentially improving the shelf stability of the harvested mushrooms. These findings align with previous reports by Tolera *et al.,* (2017).

3.4.3 Dry weight

The dry weight of *P. florida* varied significantly across all treatments as observed from Table 4. The highest dry weight was observed in treatment T5 (Wheat straw+ Spent Mushroom Substrate), with a value of 135.60 g. This followed by T6 (130.20 g) and T7 (129.40 g), demonstrating SMS supplementation's positive effect. These findings align with findings of Behera *et al*., (2024) with confirming substrate enrichment enhances mushroom biomass production.

4. CONCLUSION

It may be concluded from the above findings that among all the treatments, T5 (wheat straw + SMS 4:1) have ability to reduce the number of days of spawn running, pinhead formation and harvesting days and change the morphological parameter like stalk length, cap diameter, pileus diameter, number of fruiting body and yield parameter on *P. florida*. Therefore, T_5 (wheat straw + SMS 4:1) are advised in commercial cultivation of *Pleurotus florida.*

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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