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Enhancing oyster mushroom cultivation by chickpea and wheat straw substrate for sustainable agriculture

Archana Kumawat[1*], Gayatri Kumawat[2], A.R. Wasnikar[1], Himanshu Mahawar[3], Surendra Bajiya[1], Hansa Kumawat[4], Mayank Bishnoi[1] and Manish Paroda[1]

[1]Department of Plant Pathology, Jawaharlal Nehru Krishi Vishwa Vidhalya, College of Agriculture, Jabalpur, Madhya Pradesh 482 004, India

ABSTRACT

This study aimed to explore the potential utilization of agro-lignocellulosic waste materials for oyster mushroom cultivation, addressing the challenge of managing waste associated with these materials. Various combinations of Chickpea Straw (CS) and Wheat Straw (WS) at different ratios-100% CS, 75% CS + 25% WS, 50% CS + 50% WS, 25% CS + 75% WS, and 100% WS-were investigated as substrates for mushroom cultivation. The experiment followed a ccompletely rrandomised design with four replications, monitoring developmental phases, yield, and biological efficiency (BE). Results indicated that using 100% WS as a substrate resulted in the fastest mycelium growth (spawn run), completing in an average of 14.50 days, 17.20 days from spawning to pinhead formation, and 20.60 days to first harvest, with the highest number of fruiting bodies produced. Chickpea straw contributed to the highest stipe width (1.52 cm), while WS 100% had the highest average yield (997.28 g) and BE (99.73%). A mixture of 75% CS and 25% WS showed promising results in terms of fruiting bodies, yield, and BE after 100% WS. Consequently, the study recommends the use of these substrates to optimize *Pleurotus ostreatus* cultivation yield.

Key words: Spawn, biological efficiency, agricultural waste, fruiting bodies.

INTRODUCTION

Mushroom cultivation presents a sustainable and viable option for individuals living in poverty, both in rural and urban areas, owing to its lower financial and labor requirements (FAO, 7). In India, the mushroom industry is predominantly dominated by white button mushrooms (*Agaricus bisporus*), comprising 85% of total production, followed by Oyster mushrooms (*Pleurotus* spp.) at 7%, paddy straw mushroom (*Volvariella volvacea*) at 6%, and milky mushrooms (*Calocybe indica*). These fungi, characterized by their fleshy stalks, caps, and spore-producing reproductive structures, have been esteemed for their nutritional and therapeutic benefits for centuries (Bhattacharjya *et al.*, 3; Shaffer, 15; Deshmukh and Deshmukh, 5; Sharma *et al.*, 17).

Cultivating edible mushrooms using agro-residues as a substrate proves to be an efficient and economically viable technology, effectively converting agricultural waste materials into valuable, protein-rich food, and establishing mushrooms as a lucrative cash crop with commercial appeal. A key component in this process is the lignocellulosic-rich mushroom substrate, vital for supporting the growth, development, and fruiting bodies of mushrooms (Gupta *et al.*, 9). Oyster mushrooms particularly depend on specific nutrients such as carbon, nitrogen, and various inorganic compounds to flourish in this setup (Royse and Schisler, 14; Dehariya and Vyas, 4).

The application of microbial technology offers a scalable solution for recycling agricultural waste on a large scale in India, contributing not only to waste reduction but also aligning with sustainable and eco-friendly practices, addressing challenges posed by climate change. Additionally, incorporating organic materials into mushroom production from agricultural residues and wastes not only addresses waste management issues but also contributes to mushroom cultivation (Khare *et al.*, 11). Therefore, the primary objective of this research is to cultivate *Pleurotus ostreatus* using agro-based by-products as growth substrates and evaluate their biological efficiency.

In Madhya Pradesh, chickpea straw (CS) has traditionally served as a growing medium for mushrooms. However, with rising consumption for cow farming expansion, questions arise about the year-round availability of CS in every region. Hence, evaluating alternative agro-wastes such as wheat straw, rice straw, maize cob, sugarcane bagasse,

*Corresponding author: archukumawat8@gmail.com

[2]Livestock Feed Resource Management and Technology Centre, Rajasthan University of Veterinary and Animal Sciences, Bikaner, India-334 001

[3]ICAR-Directorate of Weed Research, Jabalpur, Madhya Pradesh, India- 482 002

[4]Department of Soil Science and Agricultural Chemistry, Rajasthan College of Agriculture, Udaipur – 313 001, Rajasthan, India

among others, becomes crucial to finding affordable solutions that can enhance mushroom output and quality. Utilizing these agro-wastes as mushroom culture substrates offers several advantages, including improving farmers' economic status, addressing dietary issues, and reducing environmental pollution levels.

MATERIALS AND METHODS

The study was conducted at a specific location positioned at 411.78 m above sea level, with coordinates of 23.21° N latitude and 79.96° E longitude. This region experiences a subtropical climate characterized by hot summers and cool winters, with typical maximum and minimum temperature ranges of 20° to 45°C and 3° to 27°C, respectively. The relative humidity in the area typically ranges from 60 to 70%. The average annual rainfall in the region is 1300 mm. The study took place at the Department of Plant Pathology, College of Agriculture, JNKVV, Jabalpur, from November to December 2021.

To establish a pure culture of *Pleurotus ostreatus*, an initial culture was obtained from the Mushroom Research Laboratory in Jabalpur. This culture was allowed to proliferate on a potato dextrose agar (PDA) medium for a period of 7 days. Aseptic techniques were employed to transfer a small amount of soft tissue from the original *P. ostreatus* culture onto pre-sterilized individual PDA slants. These slants were then incubated at a controlled temperature of 20 ± 2°C until the mycelium had fully colonized the agar medium. Once successful colonization occurred, the pure culture was considered ready for use in subsequent steps, including spawn preparation and research on *P. ostreatus*. In the process of preparing spawn, wheat grains were utilized. These grains were partially cooked until softened, thoroughly rinsed, and allowed to cool to room temperature. The pH of the grain was adjusted to 9.0 by introducing a 1% calcium carbonate solution. The prepared mixture was then divided and filled about two-thirds of the way into glass bottles, which were sealed and subjected to sterilization for one hour at a temperature of 121°C and a pressure of 1.5 psi to ensure sterilization. Following sterilization, the glass bottles were aseptically inoculated with mycelial culture from 14-day-old samples that had been previously chilled. Subsequently, the bottles were maintained at a temperature of 20 ± 2°C for a period of 14 days, allowing the mycelia to fully colonize the grain substrate. After a total of 15 days, the wheat grain spawn was assessed for use in further stages of cultivation.

Agricultural residues such as chickpea and wheat straw were sourced from local farms affiliated with JNKVV, Jabalpur. These agricultural waste materials, including chickpea straw, were combined with wheat straw (WS) in various proportions. The agro-waste and WS were blended at five distinct ratios: 100% chickpea straw, 75% chickpea straw and 25% wheat straw, 50% chickpea straw and 50% wheat straw, 25% chickpea straw and 75% wheat straw, and 100% wheat straw (control). To ensure the mixture's hygiene, it was chemically sanitized by adding water containing 750 ppm formaldehyde, following the procedure outlined by Hoa *et al.* (10). Subsequently, this mixture was left exposed for 18 hours within a temperature range of 40 to 45°C. To remove excess moisture from the straw mixture, it was either spread out on a flat, sloping surface covered with a polypropylene sheet or poured onto a 150-mesh iron frame.

The cultivation of *P. ostreatus* was carried out using the conventional polybag technique. This involved using a spawn rate equivalent to 3% of the wet weight of the substrate, with the wet weight of the substrate itself being maintained at 3 kg (equivalent to 1 kg of dry weight). Data were collected for each treatment throughout three complete flushes, encompassing various parameters such as the time taken for spawn run completion (days), initiation of pinheads (days), stipe length (cm), stipe width (cm), cap diameter (cm), the number of fruiting bodies, and yield (g/ kg) over a period of 50-55 days. Yield was calculated using a specific formula and reported as biological efficiency (BE) in percentage terms.

$$\text{Biological Efficiency (BE)} = \left(\frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}}\right) \times 100$$

The data analysis involved four replicates and was conducted using a completely randomized design (CRD). To determine statistically significant differences, the critical difference (CD) was assessed at a 5% probability level (Gomez and Gomez, 8). Additionally, we calculated the percentage increase in yield compared to the control (WS 100%) using Abbott's formula (Gomez and Gomez, 8).

$$\text{Per cent yield increased} = \frac{(T - C)}{C} \times 100$$

'C' Yield from control bags
'T' Yield from treated bags

RESULTS AND DISCUSSION

The impact of different substrates on the growth of *Pleurotus* mushroom was demonstrated in Table 1, revealing significant variations in mycelium growth among the substrates as time progressed after

Table 1. Effect of different substrates (CS with WS) on yield contributing characters of *Pleurotus ostreatus.*

S. No.	Treatment	Spawn run (days)	Pin head initiation (days)	Stipe length (cm)	Stipe width (cm)	Cap diameter (cm)	No. of fruiting bodies
T_1	CS 100%	18.10	21.10	4.05	1.52	8.9	17.13
T_2	CS 75% + 25 % WS	17.45	20.20	4.10	1.47	8.7	20.15
T_3	CS 50 % + 50 % WS	16.30	19.30	4.16	1.09	8.6	19.10
T_4	CS 25% + 75 % WS	15.20	18.20	4.19	0.88	8.4	18.25
T_5	WS 100%	14.50	17.20	4.20	1.17	7.9	23.20
	SE (m) ±	0.42	0.40	0.03	0.02	0.20	0.19
	CD	1.27	1.23	0.10	0.07	0.61	0.59

SE ± : Standard error of mean, $CD_{0.05}$: critical difference, p = level of significance, CS: chickpea straw, WS: wheat straw.

inoculation ($p \leq 0.05$). The control substrate (WS 100%) emerged as the fastest, with the shortest initiation time (17.20 days) and spawn run period (14.50 days), while CS 100% required the maximum period for spawn run and pinhead initiation (18.10 and 21.10 days, respectively). WS 100% produced the tallest stems (4.20 cm), while CS 100% exhibited the widest stems (1.52 cm) and largest cap diameter (8.9 cm) among the mushrooms. The highest number of fruiting bodies were observed in WS 100% (23.20), followed by CS 75% + WS 25% with 20.15, while the lowest number (17.13) was recorded in CS 100%. These findings underscore the significant influence of different substrates on *P. ostreatus* mushroom growth.

The differences in mycelial growth and fruiting body development across various substrates can be attributed to the distinct compositions of these substrates. *P. ostreatus* effectively colonized diverse substrates within a period of 15.3 to 23.2 days during the spawn run. Similarly, Sharma *et al.* (16) revealed a spawn run duration for *P. ostreatus* spanning 22.40 to 26.00 days, whereas Obodai *et al.* (13) corroborated that this period exhibited variation across distinct substrates, ranged between 15 to 34 days. The study found that the faster growth of oyster mushrooms on WS compared to other substrates combinations, can be attributed to the notably higher content of cellulose (35-39%), hemicellulose (22-30%), and lignin (12-16%) in WS (Fanadzo *et al*., 6), in contrast to CS contains lower proportions of cellulose (34.8%), hemicellulose (18.5%), and lignin (11.4%) (Atila *et al*., 2). Substrates combinations with WS (CS 75% + 25% WS, CS 50% + 50% WS and CS 25% + 75% WS) recorded a longer duration for mycelial growth rate. This was probably due to high nitrogen content which is known to inhibit mushroom growth if it is in an excessive amount within the substrate (Yang *et al*., 19). These components serve as the primary nutrient source for the mycelium's growth. Both cellulose and hemicellulose are classified as carbohydrates, and their chemical bonds can be cleaved through the action of acids or enzymatic activity of *P. ostreatus*. This enzymatic breakdown results in the production of fruiting bodies that are enriched with essential amino acids, vitamins, minerals, and low-energy carbohydrates. These nutritional factors positively contribute to mushroom yield. Similar findings have been reported in previous studies by (Akter *et al*., 1).

The results are displayed in Table 2, showing the average total yield and characteristics of oyster mushrooms cultivated on diverse substrates. WS 100% exhibited the shortest growing season (50.60 days), while the combination of CS 25% and WS 75% required 51.88 days to collect fruiting bodies. Conversely, 100% CS required the longest cultivation time, taking 55.33 days. Production assessment considered the weight of the entire cluster of fruiting bodies, including their stalk bases, without discarding any components. WS 100% yielded the highest average total yield (997.28 g) and biological efficiency (BE) (99.72%), followed by a blend of 75% CS and 25% WS. These findings underscore the superior performance of WS as a substrate for cultivating *P. ostreatus* mushrooms, with potential implications for sustainable agriculture and waste management.

The highest yield and BE were achieved when WS 100% was used as a substrate, yielding an average of 997.28 g of mushrooms with a BE of 99.72%. The next best results were obtained from a mixture of 75% CS and 25% WS, which produced 961.38 g of mushrooms with a BE of 96.13%. Conversely, the least favorable results were observed when solely employing chickpea straw, yielding

Table 2. Effect of various substrate combinations (CS with WS) on yield and biological efficiency (BE) of *Pleurotus ostreatus*.

S. No.	Treatment	1st Harvest (days)	1st Harvest yield (g)	2nd Harvest (days)	2nd Harvest yield (g)	3rd Harvest (days)	3rd Harvest yield (g)	Average total yield (g)	Biological efficiency (%)	Percentage increase in yield over control
T_1	CS 100%	25.30	352.45	41.30	290.40	55.33	201.63	844.48	84.45	-15.32
T_2	CS 75% + 25% WS	24.20	367.60	40.20	325.33	54.20	268.45	961.38	96.14	-3.60
T_3	CS 50% + 50 % WS	22.75	362.33	38.65	310.75	52.65	256.48	929.55	92.96	-6.79
T_4	CS 25% + 75 % WS	21.88	358.63	37.88	297.53	51.88	240.63	896.78	89.68	-10.08
T_5	WS 100%	20.60	383.43	35.10	334.53	50.60	279.33	997.28	99.73	0.00
	SE $_{(m)}$ ±	0.07	0.12	0.07	0.25	0.07	0.11	0.32	0.03	
	$CD_{p\leq 0.05}$	0.22	0.38	0.21	0.76	0.23	0.35	0.99	0.09	

SE ± : Standard error of mean, $CD_{p\leq 0.05}$: critical difference, p = level of significance, CS: chickpea straw, WS: wheat straw.

an average total of 844.48 g of mushrooms with a BE of 84.45%. Notably, the marginal yield increase compared to the control was minimal when using WS exclusively. These findings strongly suggest that WS stands out as the most favorable substrate for cultivating oyster mushrooms, surpassing other substrate combinations.

The present study unveils that the cultivation outcomes were distinctly influenced by various substrate treatments. Moreover, these differences in yield had discernible repercussions on the BE associated with each substrate treatment. The study revealed that the highest average total yield and BE were achieved when utilizing 100% WS as the substrate for cultivating *P. ostreatus* mushrooms. Furthermore, positive outcomes were also observed when employing a substrate blend consisting of 75% CS and 25% WS. The superior performance of WS can be attributed to its higher fiber content, which not only reduced the overall growth period and the time required for pinhead initiation but also enhanced the average total yield and BE in *P. ostreatus* cultivation. Another contributing factor is the positive correlation between BE and degradation of cellulose and hemicellulose, whereas a negative relationship between BE and lignin degradation was observed. Wheat, characterized by its elevated protein content (9.06%) and nitrogen source (1.45%), has been shown to have a significant impact on increasing production and bio-efficiency in this context. Similar findings have been reported in previous studies.

In this research study, we conducted an investigation on enhancing oyster mushroom (*P. ostreatus*) cultivation efficiency using CS and WS substrates for sustainable agriculture and waste management reduction. Among the different substrate combinations tested, it was evident that WS emerged as the most effective, closely followed by chickpea straw. Given the suitability of WS and the substrate combinations of 75% CS and 25% WS for the prevailing climatic conditions in Madhya Pradesh, these substrates are recommended for mushroom cultivation. In regions where WS may not be readily accessible, farmers have the option of turning to alternative substrates for growing *P. ostreatus* like chickpea straw, which can be conveniently sourced from their own farms and prepared by cutting it into small pieces. This approach not only reduces the cost associated with mushroom cultivation but also offers a valuable source of protein-rich nutrition. Furthermore, utilizing agricultural waste as a substrate for oyster mushroom production presents an opportunity for agribusinesses to explore new

avenues by converting by-products into high-protein mushroom products.

AUTHORS' CONTRIBUTION

All the authors involved in this study contributed to the study design, fieldwork, and cartography works. Data analysis and interpretation of the data (AK, GK, ARW, HM, SB, HK, MB and MP); Writing manuscript (AK).

DECLARATION

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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