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Impact of Chickpea, Soybean and Wheat Straw on Cultivation of Oyster Mushroom (*P. membranaceus*)

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***Abstract*:** This experiment aimed to evaluate the use of soybeanstraw (SS) and chickpea straw (CS) with wheat straw (WS) in different rations like 100%: 0%, 75%: 25%, 50%: 50%, 25%: 75% and 0: 100% to grow *P. membranaceus* as an edible mushroom. Among all aspects, WS (control) was the best substrate with yield (902.10 gm) and biological efficiency (90.21%) followed by SS 75% + 25 % WS (865.08g and 86.50%) and the lowest was from CS 100% (764.30 g and 76.43%).The study corroborates that WS is one of the best substrates for oyster mushroom cultivation, while combinations with SS can enhance certain fruiting characteristics. However, CS appears less effective for growth and yield, potentially due to its slower decomposition and lower nutrient availability. Future studies could explore additional supplementation of CS to improve its performance.

***Keywords*:** Mushroom cultivation, *P. membranaceus*, WS, Chickpea straw, Biological efficiency

Oyster mushrooms (*Pleurotus* spp.) are among the most widely cultivated edible mushrooms globally due to their high nutritional value, medicinal properties and ability to grow on various lignocellulosic substrates. Traditionally, substrates such as rice straw, WS and maize have been employed for mushroom cultivation. However, the search for alternative, cost-effective substrates that can enhance yield and promote sustainable agricultural practices has intensified recently (Koutrotsios et al., 2014). Chickpea (*Cicer arietinum*) and soybean (*Glycine max*) straws are by-products of legume cultivation that are rich in lignin, cellulose and hemicelluloses-components crucial for the growth of oyster mushrooms. Utilizing these agricultural residues not only adds value to otherwise underutilized by-products but also contributes to environmental sustainability by reducing agricultural waste (Patel et al., 2012). *Pleurotus membranaceus* is widely consumed globally due to its taste, flavor, high nutritional content and medicinal properties. *P. membranaceus* has a pileus diameter of 3-10 cm, dimidiate to flabelliform, with a skinny point of attachment and attenuate base; the floor is white (after drying it seems cinnamon brown), coarsely striate and the margins are lobed and irregular. Stripe is usually absent; then again it might show up in occasion

In addition, *P. membranaceus* may offer potential advantages when integrated into the cultivation substrate. If *P. membranaceus* is a plant-derived component, it might provide additional nutrients or bioactive compounds that enhance fungal growth and yield. If it refers to a specific strain of *Pleurotus*, it could offer better adaptation to the substrates and environmental conditions, improving overall productivity (Owaid et al., 2015, Kausar et al., 2020). The study focuses on optimizing substrate formulations to maximize yield, improve mushroom quality and assess the added benefits of *P. membranaceus* in the cultivation process. By exploring these novel substrate combinations, this study contributes to the development of more sustainable and efficient mushroom production practices, while also providing an innovative use for agricultural residues.

MATERIAL AND METHODS

These steps are used in oyster mushroom production procedures pure culture preparation**,** straw preparation, spawn production, growing and harvesting at maturity stages at different intervals.

Location and treatments: The present finding was carried out at the Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.)located at 23.09° N latitude and 79.58° E longitude at an altitude of 411.78 meters above the sea level The single factor experiment consisted of five treatments, i.e., different types of substrates: SS (RS), CS (CS) mixed with WS (WS) in different combination.

Pure culture preparation: The pure culture was isolated from the fruiting body of *P. membranaceus* in the Mushroom Research Laboratory of Jabalpur and multiplied on potato dextrose agar medium for 7 days before being kept in test tubes with potato dextrose agar. After being sterilized for 30 minutes at 121°C and 1.5 *p.s.i*, clean test tubes were allowed

to harden in a slant position. To create pure culture, a small amount of soft tissue (from the original *P. membranaceus* culture) was aseptically transferred to individual PDA slants at 20±2°C. Once the mycelium had completely colonized the agar media, the culture was utilized to prepare the spawn.

Spawn preparation: Wheat (*Triticum aestivum*) grain was used in the spawn preparation process.Wheat grain was partially boiled for 20–25 minutes, rinsed and left to cool to room temperature. The pH of the grain was adjusted to 9.0 and 1% CaCo3 (calcium carbonate) was added as a food supplement (Romero 2007). The boiled grain was filled in glass bottles into two-thirds portions, sealed and pasteurized for one hour at 121 °C and 1.5 *p.s.i* to sterilize them. Sterilized glass bottles were aseptically inoculated with mycelia culture bits (5mm). The bottles were kept at 20±2 °C for 14 days to allow the mycelia to completely colonize the grains. and after 15 days, the grain spawn was ready for utilisation.

Substrates preparation and spawning: Chickpea and soybean straw collected from Agronomy farms of college and was cut into small pieces (3-5 am length). The substrates were chemically sanitized by adding water containing 750 ppm formaldehyde, then left outdoors for 18 hours at temperatures ranging from 40°C to 45°C. Excess water was removed by spreading the straw on a flat, inclined surface covered with a polypropylene sheet, or by placing it on a 150-mesh iron frame. Once the substrates cooled to room temperature, they were mixed with WS in different ratios: 100: 0, 75: 25, 50: 50, 25: 75 and 0: 100%. (Agro-waste: WS). After mixing inoculated with oyster mushroom spawn at a rate of 4-6% (w/w) of the wet weight of the substrate. The inoculated substrates were mixed thoroughly and packed into sterilized polypropylene bags. The bags were tied at the top and small holes were poked for air exchange. The inoculated bags were incubated in a dark room at 25–28°C with 70–80% relative humidity for 15–20 days until complete colonization by the mycelium. Once fully colonized, the bags were transferred to a fruiting chamber with light (12-hour light/dark cycles).The fruiting room was maintained at 22–26°C with a relative humidity of 85–90%.The plastic bags were cut open to expose the substrate and regular misting was done to maintain humidity and after 7–10 days, mushroom pinheads began to form, followed by mature fruiting bodies.

Harvesting: Mushrooms were harvested when the caps were fully developed but before they began to curl upwards. The yield of oyster mushrooms was recorded by weighing the fresh mushrooms from each substrate.

Statistical analysis: The variance of the mean values was analyzed using the Duncan Multiple Range Test (DMRT) with SAS software (The SAS System for Windows, Version 8.1, 1999).

RESULTS AND DISCUSSION

Growth and development of mycelium and fruiting bodies: The shortest spawn run period was observed in WS (16.88 days), followed by the mixture of CS 25% + 75% WS (18.05 days) (Table 2). However, CS 100% took a longer time (20.58 days) for mycelial growth.The WS100% required significantly fewer days (18.98) for pinhead initiation followed by SS 25% + 75% WS (20.93 days) (Table 1). In contrast, theCS 100% took a longer time (23.48 days) to pin head initiation. The longest length of stalk was observed in the SS 100% (3.80 cm) followed by SS 75% + 25 % WS (3.60 cm). In all cases, the lowest length of stalk was in CS 100 % (2.86 cm) followed by CS 75% + 25% WS (3.05 cm).The maximum width of the stalk was observed same in the CS 100% and CS 25% + 75% WS(0.85 cm) and minimum in WS 100% (0.17

Physical Parameter

Parameter	Procedure of measurement
Days required to spawn run	This refers to the number of days needed from inoculation to the mycelium has fully colonized the substrate. The process is considered complete when the entire spawn packets turns white due to the growth of the mycelium, which serves as the indicator of successful mycelium colonization.
Days required to pin head initiation	The number of days from cutting the spawn packet to the initiation of primordia was recorded.
Average length and width of stalk (cm)s	The width and length of the stalk of each fruiting body were measured from the top to the base of the stalk.
The average diameter of the cap (cm)	The cap diameter over one gram (wt.) was recorded
Number of fruiting bodies	Only well-developed fruiting bodies were considered effective and were counted, with the total number expressed per packet. Tiny fruiting bodies were excluded from the count.
Harvest flush	It is the period when a cluster of mushrooms grows and matures together. During this stage, the mushrooms are harvested together as a group.
Harvested yield	The yield refers to the number of mushrooms produced from the substrate.
Biological efficiency	The following formula is used to calculate biological efficiency $\text{Biological Efficiency (BE)} = \frac{\text{Fresh weight of mushroom}}{\text{y weight of substrate}} \times 100$

cm) followed by CS 25% + 75% WS (0.28 cm). The highest cap diameter was inthe SS 100% substrate (10.63 cm), while the lowest was in WS100% (9.03 cm) in the first flush (Table 1). The number of fruiting bodies per packet (NFBP) varied from 16.10 to 21.13 among the substrates (Table 1,2). The maximum NFBP was counted in WS100% (21.13), followed by SS 75% + 25 % WS (18.08), while the minimum number was in CS 100% (16.10).

Harvesting time, yield and biological efficiency: The first flush harvesting time ranged from 23.78 to 28.53 days, with the shortest time in WS 100%) and the longest in CS 100% (28.53 days). Similar patterns were observed for the second and third flushes, with the minimum time taken by WS 100% and the maximum time by chickpea straw 100%. The total weight of first flush fruiting bodies per beg ranged from 323.73 to 351.57 g.In the first flush, the highest yield per beg was harvested from WS 100% (351.57 g), while the lowest was from CS 100% (323.73 g). In the second and third flushes, the highest yield per beg was observed in WS 100% (302.68 and 247.85 g) but the lowest yield per beg was observed in SS 100% (257.60 g) (Table 1) in the second flush and in third flush CS 100% produce minimum yield per beg (174.98 g) (Table 2).In all cases, maximum average total yield and biological efficiency were obtained from WS 100% (902.10 g and 90.21%) followed by SS 75% + 25 % WS (865.08 g and 86.51%) and the lowest was from chickpea straw100% (764.30 g and 76.43%).

The observed variations in mushroom growth and yield across different substrate ratios can be linked to the differences in the chemical composition and physical properties of the substrates. The higher mycelial growth and faster spawn running observed in WS 100% may be due to its high cellulose and lignin content, which provides an ideal structure and nutrient base for oyster mushroom growth. Previous studies have confirmed that WS supports robust mycelial colonization and consistent yields in oyster mushrooms (Royse et al., 2017). It provides a balanced carbon-to-nitrogen ratio and an optimal lignocellulosic structure, which facilitates the breakdown of complex compounds by mushroom enzymes (Ritota and Manzi 2019). The favorable nutrient composition and physical structure in WS 100% may have supported rapid colonization (Wan-Mahari et al., 2020). Similarly, the faster pinhead initiation with WS and SS mixtures is supported by findings from other studies that confirm WS's superiority in inducing quicker fruiting (Fernandes et al., 2015). The substrates like CS took longer to achieve full colonization and pinhead formation, which could be due to differences in their lignocellulosic

Table 1. Effect of soybean straw on yield contributing characters of *P. membranaceus*

Sr. No.	Treatments	Spawn run (days)	Pinhead initiation (days)	Stipe length (cm)	Stipe width (cm)	Cap diameter (cm)	No. of fruiting bodies	Flush time (days)			Flush yield (g)			Average total yield (g)	Biological efficiency (%)
								1st	2nd	3rd	1st	2nd	3rd		
T_1	SS 100 %	20.10^{a}	22.70^{a}	3.80^{a}	0.75^{d}	10.63^{a}	16.40^{d}	27.05^{a}	42.88^{a}	57.40^{a}	334.70^{e}	257.60^{e}	193.13^{e}	785.43^{e}	78.54^{e}
T_2	SS 75% + 25 % WS	19.70^{ab}	22.30^{ab}	3.60^{ab}	0.77^{c}	10.33^{a}	18.08^{b}	26.58^{ab}	41.88^{b}	56.58^{b}	339.33^{b}	288.08^{b}	237.68^{b}	865.08^{b}	86.51^{b}
T_3	SS 50% + 50 % WS	19.15^{b}	21.75^{b}	3.48^{abc}	0.82^{b}	9.85^{b}	17.63^{bc}	26.03^{b}	42.18^{b}	56.03^{b}	337.63^{c}	286.98^{c}	220.85^{c}	845.45^{c}	84.55^{c}
T_4	SS 25% + 75 % WS	18.33^{c}	20.93^{c}	3.38^{bc}	0.85^{a}	9.35^{c}	17.03^{cd}	24.93^{c}	40.18^{c}	55.15^{c}	335.68^{d}	269.93^{d}	206.75^{d}	812.35^{d}	81.24^{d}
T_5	WS 100 %	16.88^{d}	18.98^{d}	3.18^{c}	0.17^{e}	9.03^{c}	21.13^{a}	23.78^{d}	37.95^{d}	53.40^{d}	351.57^{a}	302.68^{a}	247.85^{a}	902.10^{a}	90.21^{a}

SS, Soybean straw; WS, Wheat straw

Table 2. Effect of chickpea straw on yield contributing characters of *P. membranaceus*

Sr. No.	Treatments	Spawn run (days)	Pinhead initiation (days)	Stipe length (cm)	Stipe width (cm)	Cap diameter (cm)	No. of fruiting bodies	Flush time (days)			Flush yield (g)			Average total yield (g)	Biological efficiency (%)
								1st	2nd	3rd	1st	2nd	3rd		
T_1	CS 100%	20.58^{a}	23.48^{a}	2.86^{c}	0.85^{a}	10.40^{a}	16.10^{c}	28.53^{a}	44.18^{a}	58.03^{a}	323.73^{e}	262.93^{d}	174.98^{e}	764.30^{e}	76.43^{e}
T_2	CS 75% + 25 % WS	20.20^{a}	23.13^{a}	3.05^{b}	0.65^{b}	9.70^{b}	17.83^{b}	27.05^{b}	42.90^{b}	57.18^{a}	328.33^{b}	286.98^{b}	229.75^{b}	845.05^{b}	84.51^{b}
T_3	CS 50 % + 50 % WS	19.20^{b}	22.10^{b}	3.13^{ab}	0.45^{c}	9.55^{b}	16.35^{c}	25.95^{c}	41.40^{c}	55.90^{b}	326.30^{c}	274.63^{c}	220.90^{c}	821.83^{c}	82.18^{c}
T_4	CS 25% + 75 % WS	18.05^{c}	20.95^{c}	3.18^{a}	0.28^{d}	9.45^{bc}	16.68^{c}	24.80^{d}	40.35^{d}	54.70^{c}	325.70^{d}	263.63^{d}	206.20^{d}	795.53^{d}	79.29^{d}
T_5	WS 100%	16.88^{d}	18.98^{d}	3.18^{a}	0.17^{e}	9.03^{c}	21.13^{a}	23.78^{e}	37.95^{e}	53.40^{d}	351.57^{a}	302.68^{a}	247.85^{a}	902.10^{a}	90.21^{a}

CS, Chickpea straw; WS, Wheat straw

structure. CS is known to have a denser composition, which may slow down the breakdown by fungal enzymes, extending the time for mycelial spread and fruiting (Fufa et al., 2021).

The SS produced the longest stalks and largest cap diameters also aligns with literature suggesting that soybean residues provide more nitrogen, contributing to the larger fruiting bodies (Ramos et al., 2011). The lower stalk lengths in chickpea substrates may result from their lower nitrogen and nutrient availability, leading to smaller fruit bodies (Fufa et al., 2021). The highest number of fruiting bodies per packet (NFBP) in WS can be explained by its balanced nutrient profile, which supports higher biological efficiency and yield (Dubey et al., 2019). Conversely, the lower NFBP in CS points to substrate limitations in supporting extensive fruiting, a pattern also observed in other substrate experiments (Maheshwari et al., 2021).

The substrates composed entirely of WS 100% resulted in higher yields, indicating that WS plays a crucial role in supporting mushroom growth. This may be due to the structural characteristics of WS, which provide better aeration and water retention properties, as well as its favorable carbon-to-nitrogen ratio (Zhang et al., 2014). Substrates with imbalanced ratios may impede efficient mycelial colonization and fruiting body formation., SS 75% + 25 % WS mixtures produced particularly favorable results after the WS 100%, Aske et al. (2020) suggesting that a balanced substrate mixture can optimize mushroom growth. Singh et al. (2019) also observed that combining multiple agro-wastes could enhance substrate utilization efficiency. This might be attributed to a synergistic effect that improves enzyme activity, resulting in better breakdown of cellulose and hemicellulose.

CONCLUSION

This study confirms that substrate choice significantly impacts the growth and yield of *P. membranaceus*. WS, either alone or in combination with soybean straw, proved to be the most effective substrate, offering faster mycelial colonization, early pinhead initiation and higher fruit body yields. In contrast, chickpea straw, though high in protein, showed slower mycelial growth and lower yields, indicating it is less suitable for oyster mushroom cultivation without further optimization. These findings suggest that WS remains the most viable substrate for commercial cultivation, while SS can enhance specific growth parameters such as cap size and stalk length. Further research is recommended to explore alternative substrate combinations to improve sustainability and yield efficiency.

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