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EFFICACY OF VARIOUS ORGANIC SUBSTRATES AND THEIR COMBINATIONS ON MORPHOMETRIC CHARACTERISTICS AND YIELD PERFORMANCES OF OYSTER MUSHROOM (*PLEUROTUS FLORIDA*)

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ABSTRACT

The quality and yield of Oyster mushrooms (*Pleurotus* sp.) are greatly influenced by chemical and nutritional composition of the substrates used for cultivation. Rice straw is the most popularly known substrate. However, there are other biological waste materials that can meet the criteria of low nitrogen and high carbon, essential for mushroom growth. Thus, to explore other organic waste materials that can be used for mushroom cultivation instead of rice straw, seven types of waste materials namely rice straw (RS), newspaper (NP), coconut husk (CH), sugarcane bagasse (SB), wood residue (WR) (bark of dry timber of *Swietenia mahagoni*), Sal leaves (SL) (*Shorea robusta*) and one weed *i.e.*, Gu phool (*Lantana camara*) (GP) were investigated solely and in combinations of RS + NP, RS + SL and NP + SL in 1:3, 1:1 and 3:1 ratios for cultivation of *P. florida*. Number of days required for attaining different growth phases of the mushroom as well as total number of fruit bodies, cap diameter, stalk length and total yield was estimated. During the initial experiment, when substrates were used solely the highest (733.58g/ 1000g of dry substrate) and lowest (205.42 g/ 1000 g dry substrate) mushroom yield was obtained from RS and CH, respectively. Whereas, during the second phase of experiment using different substrate combinations the highest yield (892.36 g/ 1000g of dry substrate) was obtained from RS+ SL (3:1) and lowest (393.49 g/ 1000g of dry substrate) from RS+ NP (1:3).

Key words : Cultivation, Oyster mushroom, Substrate, Waste materials, Yield.

Introduction

Oyster mushroom (*Pleurotus* sp.) popularly known as “Dhingri” in India account for nearly one-fourth of all commercially produced mushrooms worldwide, ranking second globally next to Button mushroom (*Agaricus bisporus*) (Patel *et al.*, 2012; Hoa and Wang, 2015; Sanjel *et al.*, 2021). In Indian subcontinent four varieties of mushrooms are cultivated commercially, with Button mushroom leading the pack at 70% share, followed by Oyster mushroom at 17%, Paddy straw mushroom (*Volveriella volvacea*) at 9% and Milky mushroom (*Calocybe indica*) at 3% share. The additional 1% consists of many other types like Reishi mushroom (*Ganoderma* sp.), *Cordyceps militaris*, Shiitake mushroom (*Lentinula edodes*) and so on (Sharma *et al.*, 2017; Singh *et al.*, 2020). Oyster mushroom is gaining popularity due to its outstanding taste, flavor, maximum productivity in a short time span, supplying more protein per unit area, and its year-round cultivation through many species suitable for various climates (Hossain, 2017). Furthermore, oyster mushroom has good medicinal value. It is rich in vitamin C, D, B complex (Thiamin, Niacin, Riboflavin and Cyanocobalamin) and folic acid that help to cure anemia (Elattar *et al.*, 2019). Due to its low

sodium: potassium ratio, less fat content it is very much suitable for people suffering from diabetes, cancer and hyper tension (Randive, 2012; Nongthombam *et al*., 2021; Sanjel *et al*., 2021). The antioxidant property of oyster mushroom along with its high vitamin, protein, calcium, and iron content makes it a highly valuable bioactive food material for good human health (Venturella *et al*., 2015; Elattar *et al*., 2019).

Additionally mushroom cultivation is a good option for production of quality food without fertile land and direct sun light. Oyster mushrooms being wood decaying fungi may transform readily available, discarded compost materials more precisely lignocellulosic agricultural waste into palatable, high-value protein rich food (Agba *et al*., 2021; Ejigu *et al*., 2022). Thus, materials containing lignin, cellulose, and hemicellulose such as cotton seed hulls, rice and wheat straw, waste paper, sawdust and sugarcane residue can be utilized as mushroom substrates because they supply the nutrients that mushrooms need the most: less nitrogen and more carbon (Chang, 1989; Kirbag and Akyuz, 2008; Hultberg *et al*., 2023). All together India being an agro-based country a huge amount of ligno-cellulosic agricultural crop residues byproducts rich in organic compounds are annually generated in our country. According to the Indian Ministry of New and Renewable Energy (MNRE), India generates on an average 500 million tons (Mt) of crop residue per year (NPMCR, 2019). Majority of this crop residue is in fact used as fodder, fuel, or other domestic and industrial purposes. Yet there is a huge surplus, which is burned each year causing serious environmental pollution (Akter *et al*., 2022). Among bioconversion processes, mushroom cultivation is an appropriate technology for management of agricultural and agro-industrial residues (Chang and Miles, 1992; Agba *et al*., 2021). Several researchers have already observed that the rate of mycelial run, growth, yield, biological efficiency (% BE) (Muswati *et al*., 2021; Akter *et al*., 2022) and the quality of oyster mushroom depend on the chemical and nutritional content of substrates (Tesfaw *et al*., 2015; Markson *et al*., 2017). In addition, these substrates after final mushroom harvesting can also be used to prepare compost or organic manure with significant nutrient supplements.

Rice straw is commonly utilized in India as a substrate for mushroom cultivation; however, due to the expansion of cattle husbandry, its demand is rising regularly (Akter *et al*., 2022). Due to farm mechanization nowadays harvesting of paddy is done with combine harvester which ultimately dissipates the straw in the field. As a result, the availability of rice straw in all over the country throughout the year is uncertain. On the other hand, oyster mushrooms having the ability to make food by degrading the lignocellulosic substances with their widespread enzyme system (Kumla *et al*., 2020) can be grown on a diverse variety of substrates that are inexpensive and readily available in agricultural farms or rural areas. The use of organic waste materials as substrates for production of oyster mushrooms not only provides effective utilization of the waste materials alleviating environmental pollution but also helps in poverty impoverishment, fighting against malnutrition and most importantly growing mushroom at a very low cost (Yohannes *et al*., 2020). Hence the objective of the experiment was to evaluate the influence of different agro-wastes on growth, yield attributes and biological efficiency (% BE) of *Pleurotus florida*.

Materials and Methods

Details of different steps of the experiment are given in Fig. 1.

Site of experiment

The entire experiment and mushroom cultivation process were conducted throughout the period of 2018 to 2020 at the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia - 741 252, West Bengal, India (22°40 N latitude, 88°18 E longitude, 7 m above mean sea level).

Materials and equipment used

Materials required for the experiment were fresh culture of *Pleurotus florida*, spawn (mushroom seed material), substrates like rice straw (RS), newspaper (NP), coconut husk (CH), sal leaves (SL) (*Shorea robusta*), sugarcane bagasse (SB) (*Saccharum officinarum*), wood residue (WR) (bark of dry timber of *Swietenia mahagoni*), Gu Phool (GP) (*Lantana camara*), plastic drum, electronic balance, hot air oven, laminar air flow cabinet, micro woven, BOD Incubator, autoclave, refrigerator, petri plates, spirit lamp, inoculation needle, beakers, aluminum box, forceps, cork-borer, tags, polythene bags of different size, rubber bands, bamboo basket, lime and fungicide (Carbendazim 50% WP).

Collection of mushroom cultures

The pure culture of *Pleurotus florida* was collected from Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia - 741 252, West Bengal, India and cultures were preserved on Potato Dextrose Agar (PDA) media at 4°C. Sub culturing was done in every 15 days.

Collection of substrates

Rice straw and *Lantana camara* were collected from Jaguly Instructional Farm, B.C.K.V. Remaining substrates such as newspaper, coconut husk, sal leaves,

Fig. 1 : Different steps of mushroom cultivation.

Table 1 : List of substrates used in two different phases of mushroom cultivation.

First phase of cultivation	**Second phase of cultivation**	
Rice straw (RS)	Substrate Combination	Ratio
Newspaper (NP)	Rice straw+ Newspaper	3:1
Coconut husk (CH)	Rice straw+ Newspaper	1:1
Sal leaves (SL) (*Shorea robusta*)	Rice straw+ Newspaper	1:3
Sugarcane bagasse (SB) (*Saccharum officinarum*)	Rice straw+ Sal leaves	3:1
Wood residue (WR) (bark of dry timber of *Swietenia mahagoni*)	Rice straw+ Sal leaves	1:1
Gu Phool (GP) (*Lantana camara*)	Rice straw+ Sal leaves	1:3
	Newspaper+ Sal leaves	3:1
	Newspaper+ Sal leaves	1:1
	Newspaper+ Sal leaves	1:3

sugarcane bagasse and wood residue were brought from local market.

Spawn production

300g of wheat grains were boiled in 1L of water for 15-20 minutes. After draining the additional water, the boiled grains were spread out on a fresh polythene sheet and placed in the shade. When the grains were cooled enough gypsum and calcium carbonate (1.5% weight of each) were mixed with the wheat grains thoroughly. 200g of the treated grains was then taken into a 500 ml conical flask and plugged with nonabsorbent cotton, covered with brown paper cap with rubber bands and autoclaved at 15 psi for 25-30 min. The grains were then cooled, inoculated with 1 cm disc of actively growing *Pleurotus florida* and incubated for two weeks at 25°C.

Preparation of the substrates for cultivation

At first all the substrates were dried under sunlight. Then chopped in 2-3 cm size in length by the paddy chaff cutter machine. After thoroughly cleaning the materials two or three times with tap water, they were submerged in water for 12 hours. After soaking substrates were then cleaned again in tap water and placed in bamboo basket to drain out the excess water. Water-soaked substrates were kept in the bamboo basket till all the excess water drained off and then steam sterilization was done in autoclave for 30 minutes. After sterilization substrates were spread in thin layer on a piece of polythene sheet for cooling and removal of excess moisture. Squeezing the substrates between the palms to check that water droplets do not trickle out from the substrate allowed us to determine that the substrate had enough moisture.

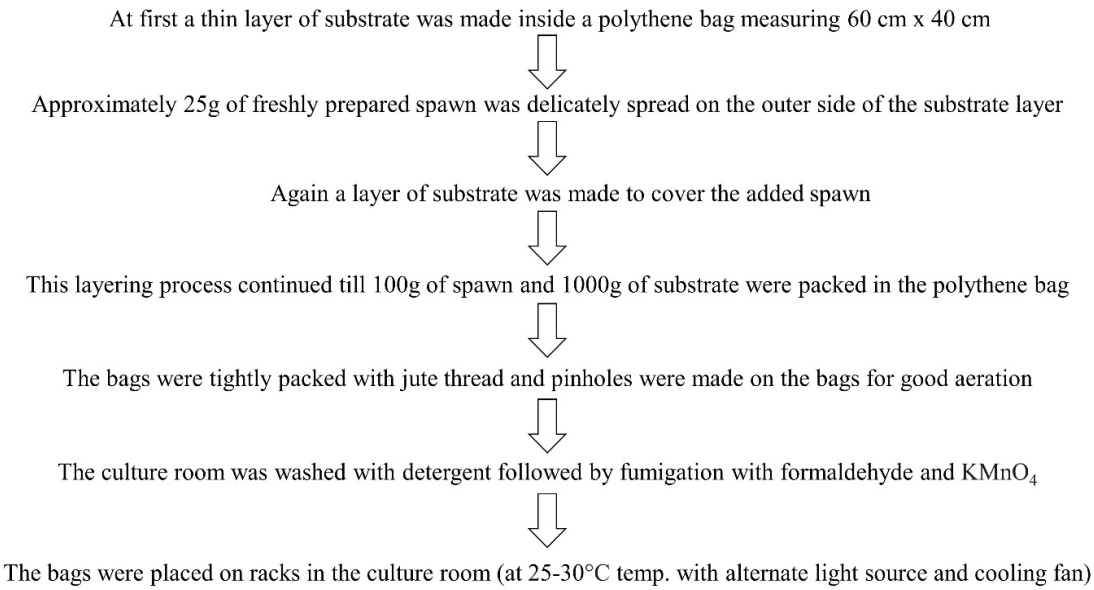

Fig. 2 : Flow chart of preparation of spawn packets.

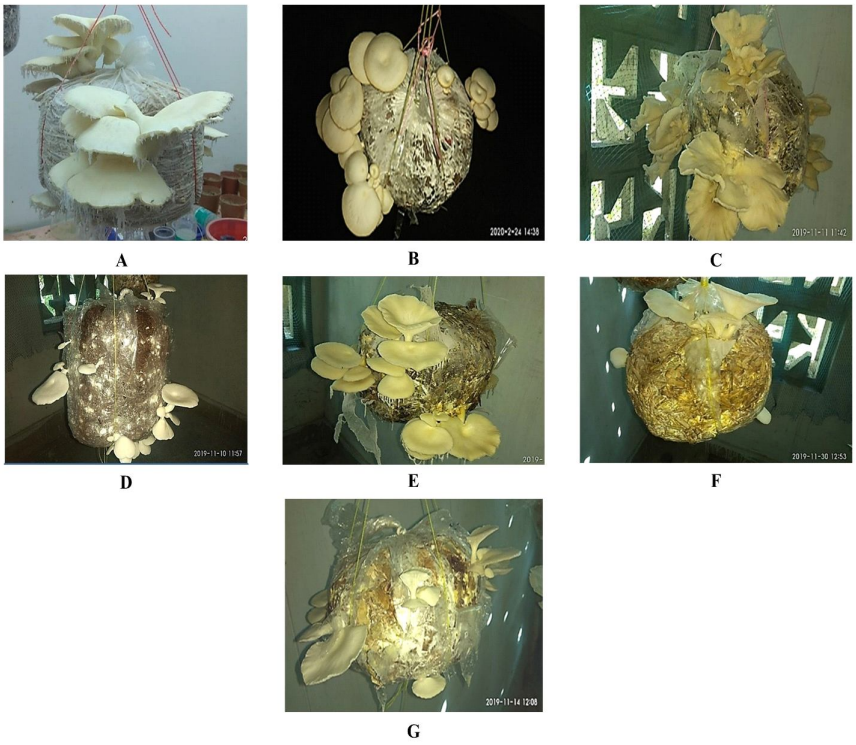

Fig. 3 : Fruit body of mushroom (*Pleurotus florida*) on different substrates (A. rice straw, B. newspaper, C. *Lantana camara*, D. coconut husk, E. sal leaves, F. sugarcane bagasse and G. wood residue).

Thus, the substrates could be used to grow mushrooms.

List of substrates used

Details of substrates and substrate combinations used in two phases of cultivation are listed in Table 1.

Spawn packet prepation

Details of preparation of spawn packets are presented in Fig. 2.

Collection of morphological data

Observations were made on the following parameters such as time taken for initiation of mycelial run, time taken for pin head formation, time required for first, second and third harvesting. After harvesting (Figs. 3 and 4) of all the three flushes total number of fruit bodies, average length of the mushroom stalks, average diameter of the cap and total yield of mushrooms from three harvesting were calculated. Lastly biological efficiency (BE) was estimated as the ratio of fresh weight of harvested mushroom and dry weight of the substrate with the following formula (Wang *et al.*, 2001).

%BE = (Total weight of fresh mushroom /Dry weight of substrate) × 100

Statistical analysis

The experiment was designed as a complete randomized design (CRD), with seven treatments at first phase and nine treatments at second phase with each treatment having three replications. Finally, all the data were analyzed using Microsoft Excel and the SPSS version 25 software. The significance of variation among the yield characteristics of oyster mushrooms in the different treatments was evaluated using a one-way analysis of variance (ANOVA) at 5% level of significance. The results are presented as mean ± standard deviation (SD) in tables.

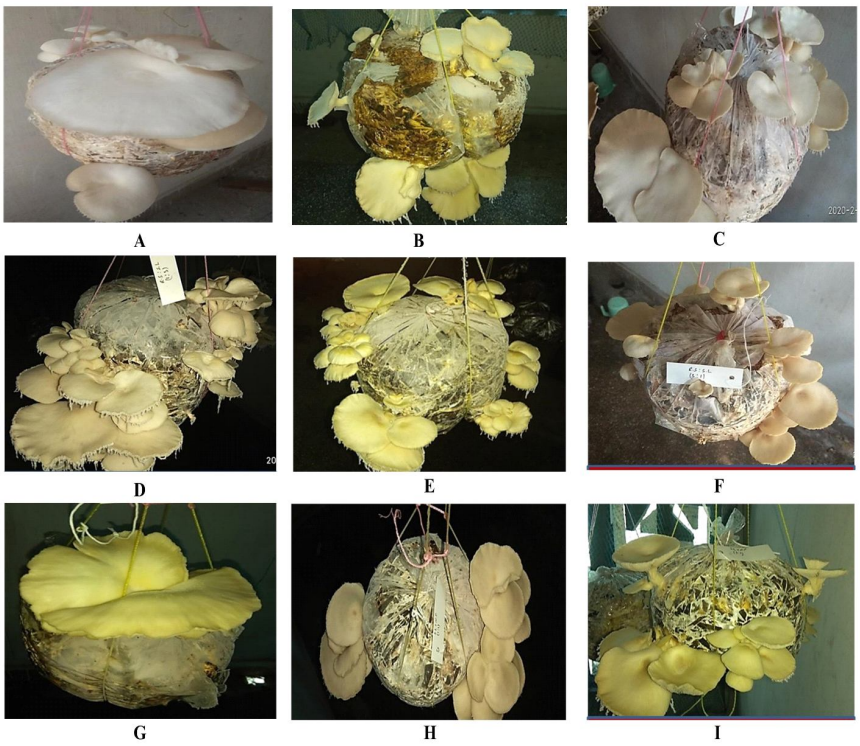

Fig. 4 : Fruit body of mushroom (*Pleurotus florida*) on different substrate combinations {A. rice straw+ newspaper (3:1), B. rice straw+ newspaper (1:1), C. rice straw+ newspaper (1:3), D. rice straw+ sal leaves (3:1), E. rice straw+ sal leaves (1:1), F. rice straw+ sal leaves (1:3), G. newspaper+ sal leaves (3:1), H. newspaper+ sal leaves (1:1) and I. newspaper+ sal leaves (1:3)}.

Results and Discussion

Effect of different substrates on time requirement for different growth phases of oyster mushroom (*Pleurotus florida*)

The experiment was conducted to record the time requirement for initial mycelial run, pin head formation and different harvest phases of oyster mushroom grown on different substrates. The data presented in Table 2 indicated that time requirement for initial spawn run, pin head development and different harvest phases for oyster mushroom was significantly different ($p \leq 0.05$) between the treatments. Minimum time for initiation of mycelial run or mycelial growth was recorded in sal leaves (3 days), which was significantly different from all other substrates except coconut husk (4.33 days), they were followed by rice straw (5.33 days), *Lantana camara* (8 days) and wood residue (8.33 days) respectively while maximum time was observed in newspaper (12.67 days) and it was also significantly different from all other treatments. The second highest time for initiation of mycelial run was recorded in sugarcane bagasse (9.67 days) with significantly difference from all other substrates except wood residue. Ejigu *et al.* (2022) have reported that the average days required for completion of mycelial run varies from 21.50 to 31.25 days, which is relatively higher than the present observation. This variation may be due to the use of different oyster mushroom species, different substrate composition and weather factor. Biswas and Biswas in 2015 also reported that *P. florida* takes about 14 days for completion of spawn run in wheat straw which is closely related to this finding.

The interim period of mushroom pin head formation (Table 2) was significantly different ($p \leq 0.05$) with the substrates, ranging from 16 to 24.33 days after spawning. Pin head formation was found quickly in *Lantana camara* (16 days) having significant difference from all other treatments. It was followed by rice straw (17.67 days) which was statistically different from all substrates except sugarcane bagasse (18 days) and coconut husk (18.33 days). It took slightly longer time in newspaper (24.33 days) with significant difference from all the treatments. The observation on the density of mycelia reveals that Rice straw, sal leaves and *Lantana camara* showed higher density of mycelial run as compared to other substrates. Almost similar findings were reported by Hossain (2017) who reported that pin head formation of oyster mushroom (*P. sajorcaju*) on different substrates required 21-30 days. The study of Tirkey *et al.* (2017) revealed that average number of days required for pin head formation of *P. florida* varies from 17.20 to 26.20 days.

Time required for first flush of harvesting (Table 2) was significantly different ($p \leq 0.05$) between the substrates. Among the substrates *Lantana camara* took minimum time (19 days) for first harvest and it was statistically different from all other substrates. It was followed by coconut husk (20.67 days) with significant difference from all substrates except sugarcane bagasse (21 days) and rice straw (21.33 days). Wood residue (23.33 days) and sal leaves (23.67 days) had no significant difference with each other, but they were different from other substrates. Newspaper however took relatively longer period (27.67 days) for first harvest having significant difference from all the treatments. Similar result was observed by Neupane *et al.* (2018) that fastest harvesting of *P. florida* was done in case of banana

Table 2 : Effect of different substrates on time requirement for initial mycelial run, pin head development and different harvest phases from the day of spawning for oyster mushroom.

	Mean ± S. D				
Substrate	**Initial mycelial run (days)**	**Pin head development (days)**	**First harvest (days)**	**Second harvest (days)**	**Third harvest (days)**
Rice straw	5.33 ± 0.58^{d}	17.67 ± 1.15^{d}	21.33 ± 1.15^{c}	36.33 ± 0.58^{e}	52.33 ± 0.58^{d}
Newspaper	12.67 ± 1.53^{a}	24.33 ± 0.58^{a}	27.67 ± 1.53^{a}	38.67 ± 0.58^{d}	51.67 ± 0.58^{d}
Gu phool (*Lantana camara*)	8.00 ± 1.00^{c}	16.00 ± 0.00^{e}	19.00 ± 1.00^{d}	36.00 ± 0.00^{e}	56.33 ± 0.58^{c}
Coconut husk	4.33 ± 0.58^{de}	18.33 ± 0.58^{d}	20.67 ± 1.53^{c}	48.33 ± 0.58^{b}	71.00 ± 1.00^{a}
Sal leaves	3.00 ± 1.00^{e}	21.67 ± 0.58^{b}	23.67 ± 1.15^{b}	39.67 ± 0.58^{c}	56.67 ± 0.58^{c}
Sugarcane bagasse	9.67 ± 0.58^{b}	18.00 ± 0.00^{d}	21.00 ± 1.73^{c}	36.67 ± 0.58^{e}	70.33 ± 0.58^{a}
Wood residue	8.33 ± 0.58^{bc}	19.67 ± 1.15^{c}	23.33 ± 2.00^{b}	55.00 ± 0.58^{a}	68.67 ± 0.58^{b}
CD*	1.59	1.28	2.65	0.95	1.16
SEM	0.52	0.42	0.86	0.31	0.38

Treatment mean values in the same column followed by a different superscript letter are separated by Duncan's Multiple Range Test (DMRT).
* At 5% level of significance.

leaves (23 days) and highest harvesting duration was reported in case of saw dust *i.e.*, 50.25 days.

Time requirement for second and third harvesting was also statistically different ($p \leq 0.05$) among the treatments. Minimum time for second harvesting was observed in *Lantana camara* (36 days) that has significant difference with all the substrates except rice straw (36.33) and sugarcane bagasse (36.67). In case of third harvesting minimum time was recorded in newspaper (51.67 days), which was statistically different with all other substrates except rice straw (52.33). Highest time for second harvesting was observed in wood residue (55 days) with statistical difference from all other treatments. Maximum time for third harvesting was recorded in coconut husk (71 days), which was significantly different from all the substrates except sugarcane bagasse (70.33). It was observed that mycelial run was started in coconut husk after 4.33 days of spawn cultivation which was second lowest, but third harvesting from coconut husk took maximum days (71 days). Elsewhere in newspaper mycelial run was started at last (12.67 days) but during third harvesting it took minimum days (51.67 days). The reason behind this may be the chemical composition and water holding capacity of the two substrates. Coconut husk being direct plant product can initially provide all the necessary materials required for mushroom growth. Thus, mycelial run started early. But due to its high lignin content and less water holding capacity up to third harvest it takes highest time. In addition, newspaper, produced from plant parts contain very selective growth substances resulting delay in initiation of mycelial run. But due to its high water holding capacity and high biomass up to third harvesting it takes minimum time.

Effect of different substrates on yield and yield attributes of oyster mushroom (*Pleurotus florida*) after three harvesting

Average number of fruit bodies, average stalk length, average cap diameter, yield and % BE of oyster mushroom were significantly different ($p \leq 0.05$) among the substrates evaluated. After three harvesting all the records were collectively analyzed and results presented in Table 3 indicated that substrate *Lantana camara* produced maximum number of fruit bodies (64), which was significantly different from other treatments except rice straw (59) and sugarcane bagasse (49). Lowest number of fruit bodies was produced from coconut husk (38) followed by wood residue (41) with no significant difference between them. These two substrates have statistical difference with only rice straw and *Lantana camara*.

Stalk length of mushroom was observed longest from sal leaves (4.48 cm), which has significant difference with all other substrates except rice straw (3.98 cm). Shortest stalk length was recorded from wood residue (2.06 cm) having statistical difference with all other substrates. Mushroom stalk length from *Lantana camara* (3.32 cm) was significantly different from other treatments except newspaper (2.80 cm), coconut husk (3.30 cm) and sugarcane bagasse (2.98 cm) (Table 3). According to Dubey *et al.* (2019), oyster mushroom grown on rice straw had the longest stalk length, measuring 4.86 cm, followed by banana leaves, wheat straw and

Table 3 : Effect of different substrates on total number of fruit bodies, length of stalk, diameter of cap, yield, and biological efficacy (BE) of mushroom.

	Mean ± S. D				
Substrate	**Number of fruit body**	**Stalk length (cm)**	**Cap diameter (cm)**	**Cumulative yield (g/1000g of dry substrate)**	**%BE**
Rice straw	59 ± 7.55^{ab}	3.98 ± 0.07^{a}	7.97 ± 1.11^{b}	733.58 ± 30.03^{a}	73.36 ± 3.00^{a}
Newspaper	44 ± 4.58^{bc}	2.80 ± 0.26^{b}	5.85 ± 0.62^{c}	416.55 ± 99.56^{b}	41.66 ± 9.96^{b}
Gu phool (*Lantana camara*)	64 ± 7.94^{a}	3.32 ± 0.14^{b}	7.94 ± 0.16^{b}	502.47 ± 92.94^{b}	50.25 ± 9.29^{b}
Coconut husk	38 ± 7.00^{c}	3.30 ± 0.44^{b}	3.78 ± 0.61^{d}	205.42 ± 38.21^{c}	20.54 ± 3.82^{c}
Sal leaves	46 ± 6.08^{bc}	4.48 ± 0.38^{a}	9.31 ± 0.32^{a}	456.26 ± 96.95^{b}	45.63 ± 9.69^{b}
Sugarcane bagasse	49 ± 15.72^{abc}	2.98 ± 0.37^{b}	7.20 ± 0.38^{b}	498.68 ± 48.01^{b}	49.87 ± 4.80^{b}
Wood residue	41 ± 7.55^{c}	2.06 ± 0.09^{c}	5.85 ± 0.13^{c}	286.33 ± 79.37^{c}	28.63 ± 7.94^{c}
CD*	15.40	0.51	1.01	131.84	13.19
SEM	5.03	0.17	0.33	43.05	4.31

Treatment mean values in the same column followed by a different superscript letter are separated by Duncan's Multiple Range Test (DMRT).
* At 5% level of significance

sugarcane bagasse, each measuring 4.34 cm, 3.67 cm and 3.27 cm, respectively. Neupane *et al.* (2018) also reported stipe length of *P. florida* varied from 4.6 to 6.76 cm, which is slightly higher than the present findings. This may be because of substrates and domestic environmental condition for mushroom growth.

Cap diameter was recorded maximum in sal leaves (9.31 cm), which was significantly different from all other treatments. It was followed by rice straw (7.97 cm) having significant difference with other substrates except *Lantana camara* (7.94 cm) and sugarcane bagasse (7.20 cm). However, minimum cap diameter was obtained from coconut husk (3.78 cm) that was statistically different from all other substrates. Average cap diameter of mushroom was 5.85 cm harvested both from wood residue and newspaper having significant difference with other substrates (Table 3). Average cap diameter of *Pleurotus ostreatus* as reported by Ejigu *et al.* (2022) was varied from 1.65 cm to 7.14 cm, which significantly matches with the recent findings. The observations of Dubey *et al.* (2019) that cap diameter of *P. sajor caju* was highest in rice straw (5.14 cm) followed by wheat straw, banana leaves and sugarcane bagasse with a diameter of 4.11 cm, 3.48 cm and 3.25 cm respectively also closely signify present observations.

The average total yield was ranged from 205.42 ± 38.21 g to 733.58 ± 30.03 g from 1000 g dry substrates. Maximum yield (Table 3) was obtained from rice straw (733.58 ± 30.03 g/ 1000 g dry substrate) that was significantly different from all other substrates. *Lantana camara*, sugarcane bagasse, sal leaves and Newspaper gave 502.47 ± 92.94 g, 498.68 ± 48.01 g, 456.26 ± 96.95 g and 416.55 ± 99.56 g yield per 1000 g dry substrate respectively with no significant difference among them. Lowest yield was recorded from coconut husk (205.42 ± 38.21 g/ 1000 g dry substrate) having statistical difference with all treatments except wood residue (286.33 ± 79.37 g/1000 g dry substrate). Dubey *et al.* (2019) reported that maximum yield (1515 g) was recorded in paddy straw followed by banana leaves (517.5 g), wheat straw (480 g) and sugarcane bagasse (98.75 g), respectively. Hossain (2017) also obtained 260 g to 803 g fresh weight of mushroom yield / kg of dry substrate in *Pleurotus sajorcaju* on different substrates. This observation is also very close proximity to the present findings on yield (205.42 g to 733.56 g fresh weight/ Kg of dry substrate) component of *Pleurotus florida* on different substrates.

As indicated in Table 3, the average biological efficiency varied from 20.54 ± 3.82 to 73.36 ± 3.00. Highest mean total biological efficiency was recorded from rice straw (73.36 ± 3.00) and it was significantly different from other substrates. Lowest mean total biological efficiency was observed from coconut husk (20.54 ± 3.82) followed by wood residue (28.63 ± 7.94) with less difference.

Effect of different substrate combinations on time requirement for different growth phases of oyster mushroom (*Pleurotus florida*)

This time mushroom was grown on different substrate combinations containing rice straw, newspaper and sal leaves in three different ratios *i.e.*, 3:1, 1:1 and 1:3. All the treatments were replicated three times. Time required

Table 4 : Effect of different substrate combinations on time requirement for initial mycelial run, pin head development and different harvest phases from the day of spawning for oyster mushroom.

	Mean ± S. D				
Substrate	**Initial mycelial run (Days)**	**Pin head development (days)**	**First harvest (days)**	**Second harvest (days)**	**Third harvest (days)**
Rice straw+ Newspaper (3:1)	12.33 ± 0.58^{cde}	22.33 ± 0.58^{de}	25.67 ± 0.58^{cd}	44.67 ± 0.58^{d}	61.67 ± 1.15^{f}
Rice straw+ Newspaper (1:1)	8.67 ± 0.58^{f}	20.67 ± 0.58^{f}	25.33 ± 0.58^{d}	40.33 ± 1.15^{e}	59.33 ± 0.58^{g}
Rice straw+ Newspaper (1:3)	13.67 ± 1.15^{bc}	23.33 ± 0.58^{cd}	28.33 ± 1.15^{b}	47.00 ± 0.00^{c}	67.67 ± 0.58^{c}
Rice straw+ Sal leaves (3:1)	15.33 ± 0.58^{a}	23.00 ± 1.00^{d}	24.67 ± 0.58^{de}	35.67 ± 0.58^{f}	53.00 ± 0.00^{h}
Rice straw+ Sal leaves (1:1)	11.67 ± 1.15^{e}	22.67 ± 0.58^{d}	27.00 ± 0.00^{bc}	45.33 ± 1.15^{d}	63.33 ± 0.58^{e}
Rice straw+ Sal leaves (1:3)	14.67 ± 0.58^{ab}	24.33 ± 1.53^{bc}	27.33 ± 1.53^{b}	45.67 ± 0.58^{d}	64.67 ± 0.58^{d}
New paper+ Sal leaves (3:1)	12.00 ± 1.00^{de}	21.00 ± 1.00^{ef}	23.67 ± 0.58^{e}	47.33 ± 0.58^{c}	70.33 ± 1.15^{b}
New paper+ Sal leaves (1:1)	12.67 ± 0.58^{cde}	26.67 ± 0.58^{a}	31.33 ± 1.15^{a}	49.33 ± 0.58^{b}	68.00 ± 0.00^{c}
New paper+ Sal leaves (1:3)	13.33 ± 0.58^{bcd}	25.67 ± 0.58^{ab}	30.67 ± 0.58^{a}	53.67 ± 0.58^{a}	75.33 ± 0.58^{a}
CD*	1.37	1.45	1.49	1.25	1.20
SEM	0.46	0.48	0.50	0.42	0.40

Treatment mean values in the same column followed by a different superscript letter are separated by Duncan's Multiple Range Test (DMRT).
* At 5% level of significance.

for initiation of mycelial run, pin head formation and three harvesting were recorded and presented in Table 4. Data presented in Table 4 indicated that average number of days required for initiation of mycelial run, pin head development and first, second and third harvesting was significantly different ($p \leq 0.05$) among the substrate combinations. Average number of days required for initiation of mycelial run of mushroom was varied from 8.67 to 15.33 days depending on the substrate combinations. Minimum time (8.67 days) for mycelial run was recorded in RS+ NP (1:1) that was significantly different from all other treatments. It was maximum (15.33 days) in treatment RS+ SL (3:1) with significant difference against all the substrate combinations except RS+ SL (1:3) having 14.67 days. Other substrate combinations like RS + NP (1:3) took 13.67 days and NP+ SL (1:3) took 13.33 days for initiation of mycelial run. Time taken by RS+ NP (3:1), RS+ SL (1:1), NP+ SL (3:1) and NP+ SL (1:1) were statistically same having significant difference with other treatments. Jeznabadi *et al.* (2017) reported that shortest (11.25 days) and longest (18.25 days) time for mycelial run of *P. eryngii* was observed in sugar beet pulp + wheat bran and wood chips + soybean powder + rice bran + wheat bran substrates, respectively.

Time requirement for pin head development (Table 4) was varied with substrates mixture ranging from 20.67 to 26.67 days after spawning. Fruit body formation started first in RS+ NP (1:1) after 20.67 days of spawning which was significantly different from other treatments except NP+ SL (3:1). Maximum time (26.67 days) for fruit body development was recorded in NP+ SL (1:1) having statistical difference with all the treatments except only NP+ SL (1:3). Time requirement of fruit body development on RS+ NP (3:1), RS+ NP (1:3), RS+ SL (3:1) and RS+ SL (1:1) had no significant difference between each other but they were statistically different from other treatments. According to Hossain (2017), *Pleurotus sajorcaju* fruit body production took 24-35 days, which was like the pattern observed in the current research for *Pleurotus florida*, which required 20.67-26.67 days on a different substrate mixture. Sitaula *et al.* (2018) illustrated that the sugarcane bagasses + paddy straw combination was shown to require the longest time for pin head formation (23.25 days), while paddy straw alone required the shortest time (21.75 days) in case of *Pleurotus ostreatus*.

Results presented in Table 4 showed that time requirement of first harvesting of *Pleurotus florida* varied depending on the ratio and kind of substrate mixture used. Average time of first harvesting was found ranged from 23.67 to 31.33 days. Minimum time (23.67 days) for first harvest was recorded in NP+ SL (3:1) which had significant difference with all other treatments except RS+ SL (3:1). It was maximum (31.33 days) in NP+ SL (1:1) having significant difference with all the substrate combinations except NP+ SL (1:3), which took 30.67 days. Other substrate mixtures showed significantly different time requirement for first harvest *viz.* 28.33 days in RS+ NP (1:3); 27.33 days in RS+ SL (1:3); 27 days in RS+ SL

(1:1); 25.67 days in RS+ NP (3:1); 25.33 days in RS+ NP (1:1) and 24.67 days in RS+ SL (3:1). Present findings closely match with the observation of Tirkey *et al.* (2017) who reported that the time of first harvesting of *P. florida* varies from 21.20 to 35 days.

Time requirement for second and third harvesting was also statistically different ($p \leq 0.05$) among the treatments. Minimum time for second harvesting was observed in RS + SL (3:1) (35.67 days) that had significant difference with all the substrates combinations. In case of third harvesting also minimum time was recorded in RS + SL (3:1) (53 days), which was statistically different from all other treatments. Highest time for second and third harvesting was recorded in NP+ SL (1:3) *i.e.,* 53.67 days and 75.33 days respectively, which were significantly different from all other treatments. It was evident that for long term mushroom cultivation rice straw is best substrate for early harvesting of mushroom fruit bodies.

Effect of different substrate combinations on yield and yield attributes of oyster mushroom (*Pleurotus florida*)

Overall average length of mushroom stalk, cap diameter, total number of fruit bodies, yield from three harvesting of different substrate combinations and biological efficiency were analyzed and results presented in Table 5 indicated that all the yield parameters were significantly different ($p \leq 0.05$) among the substrate combinations. Highest number of fruit bodies (79) was obtained from substrate combination of RS+ SL (1:3) which was significantly different from other substrate combinations except NP+ SL (1:3) and RS+ SL (1:1). RS+ SL (3:1) and NP+ SL (1:1) had no significant difference. Lowest number (32) of fruit bodies was harvested from substrate combination of RS+ NP (1:1) having statistical difference with treatment RS+ SL (1:3), NP+ SL (1:3) and RS+ SL (1:1). Average number of fruit bodies harvested from NP+ SL (3:1); RS+ NP (1:3) and RS+ NP (3:1) were 49, 48 and 36 respectively. Pokhrel (2016) reported that highest number of fruit body of *Pleurotus florida* was produced from corn cob with chicken manure supplement (37) followed by paper waste with rice bran (34), paper waste with chicken manure (29) and corn cob with rice bran (27).

Individual substrate combinations however showed varied length of stalk ranging from 2.69 -4.02 cm as presented in table 5. Minimum stalk length (2.69 cm) was obtained from RS+ NP (1:3), which was significantly different only from RS+ NP (1:1) that produced maximum stalk length *i.e.,* 4.02 cm. Otherwise, no such significant difference was observed among other substrates. Ejigu *et al.,* (2022) indicated stalk length of *Pleurotus ostreatus* varies from 1.29 to 3.69 cm which was same as present observations. Slight variation was observed in the experiment of Pokhrel in 2016. He reported highest stipe length of *Pleurotus florida* was recorded from corn cob with chicken manure (5.29 cm) followed by paper waste with chicken manure (4.76 cm), paper waste with rice bran (4.53 cm) and corn cob with rice bran (3.96 cm).

Cap diameter also varied significantly when grown in different substrate combinations. Cap diameter was recorded maximum in RS+ NP (3:1) (10.05 cm), which was significantly different from all other treatments except RS+ NP (1:1) and RS+ SL (3:1). Second largest cap diameter (9.20 cm) was obtained from substrate combination of RS +NP (1:1) followed by 8.98 cm from RS +SL (3:1) and 8.29 cm from RS +NP (1:3). However, minimum cap diameter was obtained from NP+ SL (3:1) (6.58 cm) that was statistically different from all other substrates except RS+ SL (1:1), RS+ SL (1:3), NP+ SL (1:1) and NP+ SL (1:3) (Table 5). Present observations showed proximity with the findings of Yohannes *et al.,* (2020) who indicated that cap diameter of *Pleurotus ostreatus* was highest (17.88 cm) in Cotton + teff straw whereas, it was lowest (7.69 cm) in Teff straw + enset waste.

Wide range of variation in yield was observed among the different substrate combinations. Maximum yield (892.36 ± 85.86 g) was recorded from substrate combination of RS+ SL (3:1), which was statistically different from other treatments except RS+ NP (3:1) and RS+ SL (1:3) that produced 874.43 ± 146.96 g and 773.47 ± 78.54 g of mushroom, respectively. Treatment RS+ SL (1:1) produced 705.27 ± 72.84 g of mushroom having significant difference with all other treatments except RS+ SL (1:3). Minimum yield (393.49 ± 92.27 g) of mushroom was obtained from RS+ NP (1:3) that showed significant difference with other substrate combinations except RS+ NP (1:1), NP+ SL (3:1) and NP+ SL (1:1) (table 5). Present observation was very much similar with the observation of Ejigu *et al.* (2022), where it was reported that total yield of *Pleurotus ostreatus* ranges from 350.09 g to 957.84 g. Pokhrel (2016) also reported that total yield of *Pleurotus florida* was highest (602.50 g) in corn cob with chicken manure followed by corn cob with rice bran (545g), paper waste with rice bran (473g) and paper waste with chicken manure (438g). The present results were almost accordance with the observation of Pokhrel (2016).

Biological efficiency was also significantly varied among the different substrate combinations. Range of

Table 5 : Effect of different substrate mixtures on total number of fruit bodies, length of stalk, diameter of cap, yield, and biological efficacy (BE) of mushroom.

	Mean ± S. D				
Substrate	**Number of fruit body**	**Stalk length (cm)**	**Cap diameter (cm)**	**Yield (g/1000g of dry substrate)**	**%BE**
Rice straw+ Newspaper (3:1)	36 ± 10.82^{c}	3.17 ± 0.78^{ab}	10.05 ± 1.11^{a}	874.43 ± 146.96^{a}	87.44 ± 14.70^{a}
Rice straw+ Newspaper (1:1)	32 ± 7.00^{c}	4.02 ± 0.14^{a}	9.20 ± 0.69^{ab}	470.35 ± 70.98^{cd}	47.04 ± 7.10^{cd}
Rice straw+ Newspaper (1:3)	48 ± 11.79^{bc}	2.69 ± 0.14^{b}	8.29 ± 0.05^{bc}	393.49 ± 92.27^{d}	39.35 ± 9.23^{d}
Rice straw+ Sal leaves (3:1)	52 ± 12.29^{bc}	3.42 ± 0.84^{ab}	8.98 ± 1.29^{ab}	892.36 ± 85.86^{a}	89.24 ± 8.59^{a}
Rice straw+ Sal leaves (1:1)	62 ± 14.73^{ab}	3.13 ± 0.27^{ab}	7.00 ± 0.61^{cd}	705.27 ± 72.84^{b}	70.53 ± 7.28^{b}
Rice straw+ Sal leaves (1:3)	79 ± 13.75^{a}	2.95 ± 0.92^{ab}	7.17 ± 0.44^{cd}	773.47 ± 78.54^{ab}	77.35 ± 7.85^{ab}
New paper+ Sal leaves (3:1)	49 ± 7.94^{bc}	3.05 ± 0.35^{ab}	6.58 ± 0.88^{d}	446.77 ± 62.15^{cd}	44.68 ± 6.21^{cd}
New paper+ Sal leaves (1:1)	51 ± 15.13^{bc}	3.01 ± 1.08^{ab}	7.82 ± 0.81^{bcd}	530.51 ± 58.78^{cd}	53.05 ± 5.88^{cd}
New paper+ Sal leaves (1:3)	71 ± 7.21^{ab}	3.43 ± 0.57^{ab}	6.72 ± 0.57^{cd}	548.66 ± 68.91^{c}	54.87 ± 6.98^{c}
CD*	20.01	NA	1.37	148.11	14.81
SEM	6.68	0.38	0.46	49.47	4.95

Treatment mean values in the same column followed by a different superscript letter are separated by Duncan's Multiple Range Test (DMRT).
* At 5% level of significance.

biological efficiency (%) in the present study *i.e.*, from 39.35 ± 9.23 to 89.24 ± 8.59 resembled the experiment of Ejigu *et al.* (2022), where range of biological efficiency (%) was mentioned as varied from 35.01 ± 0.81 to 95.78 ± 0.51.

Comparative analysis in time required for mycelial run, pin head development, harvest and different yield attributing factors between rice straw and rice straw + sal leaves (3:1) as substrates

One of the primary goals of the experiment was to determine the most suitable substrate or substrate combination that might produce a significant yield of *Pleurotus florida*, while also being affordable and readily available in the surrounding areas. It was noted that during the initial phase of production, the maximum yield was recorded from rice straw (733.58g/1000g of dry substrate), while in the second phase of production, during which different combinations of substrates were employed, the maximum yield was provided by rice straw+ sal leaves (3:1) (892.36g/1000g of dry substrate combination). Since, yield is the most sought quality for all mushroom growers, various yield characteristics of oyster mushrooms were compared between rice straw and rice straw + sal leaves (3:1).

The study (Fig. 5) revealed that time required for initiation of mycelial run, pin head development and first harvest was increased by 2.88, 1.30 and 1.16 folds respectively in case of rice straw + sal leaves (3:1) as compared to rice straw substrate. Similarly in rice straw

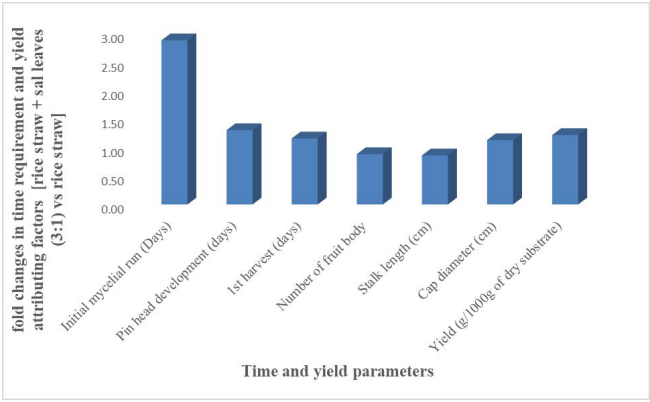

Fig. 5 : Comparative analysis in time required for mycelial run, pin head development, harvest and different yield attributing factors between rice straw and rice straw + sal leaves (3:1) as substrates.

+ sal leaves (3:1) the number of fruit bodies and mushroom stalk length was changed by 0.88 and 0.86 folds gradually while, cap diameter and cumulative yield was improved by 1.13 and 1.22 folds respectively in comparison with rice straw. Thus, rice straw + sal leaves in 3:1 ratio produced larger sized mushroom with better yield as compared to only rice straw as substrate.

Conclusion

Increasing yield or more specifically biological efficiency (% BE) was the prime objective of the present study. It was observed that among all the seven substrates rice straw exhibited highest biological efficiency (%) *i.e.*, 73.36 ± 3.00. But when combinations of substrates were used, several treatments outgrew rice straw. Rice straw+

newspaper (3:1) gave much higher biological efficiency (87.44 ± 14.70). Mixture of rice straw and sal leaves in 3:1 and 1:3 ratios also displayed higher %BE than rice straw. Rice straw+ Sal leaves (3:1) demonstrated highest %BE (89.24 ± 8.59) followed by Rice straw+ Sal leaves (1:3) (77.35 ± 7.85) among all the treatments in both the phage of cultivation. Thus, based on the present investigation it can be recommended that for production of oyster mushroom instead of only rice straw, rice straw + sal leaves (3:1) and rice straw + sal leaves (1:3) can also be used as substrate with double advantages -waste reduction and yield enhancement.

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Conflict of interest

The authors have no conflicts of interest.

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