

1 Comparative study on Performance of Different
2 Substrates on Oyster Mushroom (*Pleurotus ostreatus*)

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4 **Abstract**

Substrate type is an important factor determining growth and yield of oyster mushroom. Five different substrates namely rice straw, maize hulls, banana leaves, finger millet husk and mixture of rice straw & black gram pod shell (1:1) were evaluated for the yield and related attributes of *Pleurotus ostreatus*. Standard cultivation practice was followed with steam sterilization and spawning was done on 575 g of substrate in individual poly-bag. The data of three flushes were recorded. Our results revealed that full spawn run completed earlier (18.57 days) in finger millet husk as compared to any other tested substrates. The highest total quantity yield was obtained in finger millet husk (1024.57 g/bag) and rice straw (956.14 g/bag) with corresponding biological efficiency 178.19% and 166.29% respectively which were significantly higher than all other treatments. The cropping duration was significantly higher in maize hulls and banana leaves as compared to rest of three treatments viz. finger millet husk, rice straw and mixture of rice straw and black gram pod shell (1:1). These three treatments were not statistically different for cropping duration with each other. Considering the biological efficiency and earliness of crop the performance of finger millet husk, followed by rice straw was found to be better.

5 **Keywords**

6 Agricultural byproducts; Biological efficiency; Cropping duration; *Pleurotus*
7 *ostreatus*

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8 1. Introduction

9 Oyster mushroom (*Pleurotus species*) is an edible, saprophytic and lignocel-
10 lulolytic type of fungus belonging to the class Agaricomycetes, order Agaricales
11 and family Pleurotaceae. It is the second widely cultivated mushroom following
12 the *Agaricus bisporus* in the world (Saánchez, 2010). There are over 70 species
13 of oyster mushroom been discovered (Kong, 2004) and still there are lots to
14 explore. *Pleurotus ostreatus* is the most popular species of oyster mushroom
15 found in Nepal. The Latin word ‘Pleurotus’ means beside the ear and ‘ostreatus’
16 means oyster shaped (Cohen et al., 2002) and in Nepal it is often called “Kanya
17 chayū” due to the ear like appearance. *Pleurotus* species can efficiently degrade
18 agricultural byproducts and can grow on wide range of agricultural wastes. A
19 substrate is any material that serves as a medium of growth for a living thing in
20 which enzymes can act upon and break it to release nutrients for the growing
21 organism. There are a range of wastes that can be used for oyster mushroom
22 cultivation, but it depends on the basis of availability of the substrate and its
23 cost.

24 The availability of good substrate is an important requirement for the better
25 growth and higher yield of mushroom. An ideal substrate should contain adequate
26 amount of nitrogen and carbohydrates for rapid mushroom growth (Khare et
27 al., 2010). Total Carbon (C), total Nitrogen (N), Carbon/Nitrogen ratio (C/N)
28 are important factors that determines the mycelium colonized and development
29 of fruiting bodies in oyster mushroom. Hong et al. (1981) have shown that in
30 both *Agaricus bitorquis* and *Pleurotus ostreatus* the yield of mycelium decreases
31 under lower or higher C/N ratio. *Pleurotus* fungi mobilizes the carbohydrate
32 composed in rice straw mainly through cellulose and hemicellulose degradation
33 (Fazaeli et al., 2006). Rice straw is a popular substrate for *Pleurotus* cultivation
34 in Asia, mainly favored for its composition of slow degrading carbohydrates. Rice
35 straw has the chemical composition of (in percentage dry mass basis) 0.96% N,
36 73.01% NDF, 41.59% ADF, 31.42% Hemicellulose, 33.35% Cellulose and 4.84%
37 Acid detergent lignin when sampled over different stages of growth, amounting
38 to a generalized carbon(lignocellulosic)-nitrogen ratio of 72% (Sarnklong et al.,
39 2010). Similarly corn cob contains 47% cellulose, 25% lignin, total Carbon
40 47%, Nitrogen 0.48% and C/N ratio of 97:1 that can be used as substrate for
41 *Pleurotus* cultivation (Wha Choi, 2004). Amongst various cereal straws, paddy
42 straw was reported to be the best substrate for the cultivation of oyster mushroom
43 (Khanna and Garcha, 1982). The leaves and pseudostems of banana contain
44 high levels of lignocellulose (Reddy, 2001). These lignocellulose materials are
45 efficient substrates for white-rot fungi, which produce lignolytic and cellulolytic
46 enzymes (Pointing, 2001). Legume straws are rich in Nitrogen content and
47 are suitable as *Pleurotus* substrates (Poppe, 1995). However, selection of right
48 substrate to achieve high yield of oyster mushroom can be a challenge as there
49 seems to be a wide range of agricultural crop residues available in which the
50 oyster mushroom can be grown. The objective of this study was therefore to
51 determine the effectiveness of different agricultural wastes (i.e. rice straw, maize
52 hulls, banana leaves, finger millet husk and black gram pod shell) on the yield

53 performance and biological efficiency of *Pleurotus ostreatus* in the subtropical
54 condition of midhills of Nepal.

55 2. Materials and Method

56 The research was conducted under a condition in Institute of Agriculture and
57 Animal Sciences, Lamjung, from December-2016 to March-2017. The experiment
58 was designed in a Completely Randomized Design (CRD) with five treatments
59 (i.e. Rice straw, maize hulls, banana leaves, finger millet husk, mixture of rice
60 straw and black gram pod shell (1:1)) and seven replications per treatment.

61 The substrates were chopped to about 3-5 cm in length and soaked overnight
62 in the tank filled with water. Steam sterilization method was followed for at
63 least 15-20 minutes in a Metallic drum to reach temperature up to 90 °C. Then,
64 the substrates were spread over sterilized clean plastic sheet for air cooling below
65 25 °C. Transparent poly-bags were taken for filling of substrate in clean and
66 sterile condition. The moisture content of the substrates while filling was around
67 60%. The substrates were filled on dry weight basis i.e. 575 g per poly-bag.
68 The spawning was done at the rate of 10% on dry weight basis. Three layer of
69 spawning was done, one at bottom, another at the mid-section and lastly at top,
70 starting from the bottom layer. After spawning the bag was tightly knocked
71 with rope. The spawn in the substrate should be clearly seen from outside. The
72 bags after spawning were weight and their respective weights maintained. The
73 bags were then perforated in 8-10 numbers with sterilized needle to permit air
74 circulation.

75 The bags were moved to a production room after spawning and were hanged
76 randomly. The room was made completely dark using black poly ethylene sheets.
77 The temperature was around 17-20 °C and relative humidity around 90%. For
78 the first 15 days of spawn run the artificial lighting was not provided. After the
79 proper development of white mycelium, the polythene covers were removed. At
80 the end of the spawn run, for pinning dim light along with sufficient fresh air
81 was introduced in to the room through ventilation and CO₂ concentration was
82 lowered. The temperature and relative humidity was maintained by spraying
83 water twice a day on the ball of mushroom and on the floor of the room. The
84 insecticide Nuvan was sprayed in substrate to avoid the appearance of several
85 insects. The application of Nuvan was done only before pin head appearance
86 or after harvesting of crop. The harvesting of mushroom was done when the
87 cap began to fold. The picking was done by twisting the mushroom gently and
88 pulling out, leaving any stub. Cropping was done up to three flushes.

89 The Parameters taken were days taken for full spawn run (days), Quantity
90 harvest in three flushes (g), Days taken for first and second harvest, cropping
91 duration (days) and total quantity harvest (g). The fruiting bodies after harvested
92 are weighted and measurement of mushroom was taken. The biological efficiency
93 of mushroom per gram of substrate on dry wt. basis was calculated by using the
94 following formula:

$$\text{B.E.}\% = \frac{\text{TQH from each bag}}{\text{DW of substrate on each bag}} \times 100$$

Where,

TQH/bag: Total quantity harvest from each bag

DW: dry weight of substrate on each bag

The difference of mean was compared, using Tukey's test at the level of 5% significance.

3. Results and Discussion

The results obtained from the studies on use of different substrates for the cultivation of *Pleurotus ostreatus* in the growth performance and yield is presented in the tables and discussed below.

3.1. Days taken for full spawn run

The number of days taken for full spawn run ranged from 18.57 days to 23.29 days on different substrates (Table 1). Significantly lowest number of days for full spawn run was recorded on finger millet husk (18.57 days) and the mixture of rice straw and black gram pod shell (1:1) (19.0 days). Banana leaves (23.29 days) and rice straw (22.14 days) took the highest number of days for completion of full spawn run followed by maize hulls (20.86 days). Our results were almost similar to the findings of Shah et al. (2004) who reported that the spawn running took in 16-25 days after inoculation. This variation in number of days taken for full spawn run in different substrates could be due to the variations in chemical composition and Carbon to Nitrogen ratio (C: N) of the substrates used (Bhatti et al., 2007).

3.2. Days taken for first and second harvest

The days taken for first harvest of mushroom (Table 1) were significantly affected by substrates used. The average numbers of days taken for first harvest were between 38-46 days. The earliest harvest was noted on finger millet husk (38.71 days) and mixture of rice straw: black gram pod (1:1) (38.71 days) followed by maize hulls (40.14 days) and rice straw (40.86 days). Banana leaves took significantly the highest number of days (46.86 days) for first harvest. Contrary to our study Quimio et al. (1990) reported that good harvest of *P. ostreatus* was obtained 3-4 weeks after spawn inoculation.

Similarly, the average numbers of days taken for second harvest were between 59-60 days. Finger millet husk (51.29 days), rice straw (53.14 days) and mixture of rice straw and black gram pod shell (53.14 days) took minimum days for second harvest. Whereas banana leaves (69.29 days) and maize hulls (67.86 days) took the maximum number of days to give second harvest. The early harvest of mushroom in finger millet husk, rice straw might be due to the availability of nutrients required for the mushroom growth particularly for its spawn run and pin head development was supplied by substrates which decomposed quicker compared to other tested substrates.

Table 1: Effect of different substrates on days taken for full spawn run, days taken for first and second harvest and cropping duration of *Pleurotus ostreatus*.

Substrates	Days taken for full spawn run	Days taken for first harvest	Days taken for second harvest	Cropping duration
Rice straw	22.14cd	40.86c	53.14a	72.43a
Maize hulls	20.86bc	40.14bc	67.86b	96.86b
Banana leaves	23.29d	46.86d	69.29b	94.43b
Rice straw: black gram pod shell (1:1)	19.00ab	38.71ab	53.14a	71.00a
Finger millet husk	18.57a	38.00a	51.29a	69.57a
Significance at 5%	**	**	*	*

^a The symbols * denotes significant and ** denotes highly significant

3.3. Cropping duration

The data recorded for cropping duration of oyster mushroom 1 indicates highly significant difference between tested substrates. The cropping duration was significantly longest on maize hulls (96.86 days) and banana leaves (94.43 days). Whereas the shortest cropping duration was recorded in finger millet husk (69.57 days), rice straw (72.43 days) and mixture of rice straw and black gram pod shell (1:1) (71 days) which were not significantly different with each other. The cropping duration in our study ranged from 69 days to 96 days which is similar to findings of Khanna and Garcha (1982) that it may take up-to 104 days to harvest yield from oyster mushroom grown on paddy straw. The variation in cropping period among different substrates could emanate from variations in the time elapsed in formation of pinheads, maturation of fruiting bodies, interval between flushes, number of flushes and yield, which in turn is affected by the nature of the substrates (Chang et al., 1981).

3.4. Quantity harvest (three flushes) and Total quantity harvest

The quantity harvest varied significantly on the different substrates in all of the three flushes (Table 2). In the first flush, significantly highest quantity harvest (571.43 g) was obtained on finger millet husk followed by rice straw (453.57 g) and mixture of rice straw and black gram pod shell (1:1) (426.86 g). The lowest yield was obtained on banana leaves (256.71 g) and maize hulls (258.86 g).

In the second flush, rice straw (369 g) and finger millet husk (328.29 g) produced the highest yield followed by the mixture of rice straw and black gram pod shell (1:1) (241.43 g). In the same flush, the lowest yield was obtained on maize hulls (151.43 g) and banana leaves (218.71 g).

In the third flush highest yield was obtained on rice straw (133.57 g), mixture of rice straw and black gram pod shell (1:1) (127.71 g) and finger millet husk

161 (124.86 g) respectively. In the same flush, the lowest yield was recorded on maize
162 hulls (45.27 g) and banana leaves (53.43 g).

163 The total quantity harvest result showed the significant difference between
164 the tested substrates 2. Fingermillet husk (1024.57 g) and rice straw (956.14 g)
165 gave significantly higher total quantity harvest followed by mixture of rice straw
166 and black gram pod shell (1:1) (796 g). Maize hulls and banana leaves gave the
167 lowest total quantity harvest with corresponding yield of 455.56 g and 528.86
168 g respectively and had no significant difference with each other. Fingermillet
169 husk, rice straw followed by the mixture of rice straw and black gram pod shell
170 gave higher quantity harvest in all of the three flushes and resulted in higher
171 total quantity harvest and were considered as best substrates. Similar results
172 were obtained by (Khanna and Garcha, 1982) who reported paddy straw as the
173 best substrate for the cultivation of oyster mushroom amongst various cereal
174 straws. The higher yield of mushroom in rice straw is due to easier way of getting
175 nutrients from the cellulosic substances (Ponmurugan et al., 2007). The higher
176 yield of oyster mushroom in fingermillet husk and mixture of rice straw and
177 black gram pod shell (1:1) could be due to the better availability of nutrients
178 from them. Whereas the poor yield under maize hulls and banana leaves might
179 be due to their lower availability of nutrients and low water holding capacity
180 which is due to higher lignin content on them.

181 3.5. Biological efficiency

182 Biological efficiency is used to evaluate the efficiency of conversion of substrate
183 in to mushrooms. It was determined as ratio of the Total Quantity Harvest to
184 the dry weight of each substrate. In our experiment we took 0.575 kg of dry
185 substrates for each bag. Results of the biological efficiency varied significantly
186 among the substrates used 2. The significantly highest percentage of biological
187 efficiency was observed on fingermillet husk and rice straw with corresponding
188 biological efficiency of 178.19% and 166.29% respectively followed by the mixture
189 of rice straw and black gram pod (1:1) (138.43%). Whereas the least biological
190 efficiency of 79.23% and 91.98% was observed in maize hulls and banana leaves
191 respectively and they were not significantly different with each other. Núñez and
192 Mendoza (2002) reported that the biological efficiency of the studied substrates
193 varied from 50.8% to 106.2% while working with *P. ostreatus* however our results
194 are not consistent with theirs. In our study we got biological efficiency greater
195 than 100% which is supported by Contreras et al. (2004) and Mandeel et al.
196 (2005) who reported that in the case of *P. ostreatus*, it is possible to reach a
197 biological efficiency greater than 100%. Similarly, Jiskani (1999) also reported
198 that one kg of dry substrate can produce one kg of fresh mushroom which is the
199 100% dry weight of substrate used. However, these values can vary substantially,
200 depending on the type of substrate and cultivation strategy. The variation in
201 biological efficiency of oyster mushroom is mostly due to the Physiochemical
202 properties of substrate used. Fingermillet husk and rice straw contain adequate
203 C/N ratio for the better production of *Pleurotus ostreatus* compared to the rest
204 substrates. Since, the yield of *Pleurotus ostreatus* mycelium decreases under
205 lower or higher C/N ratio (Warcup, 1951).

Table 2: Effect of different substrates on quantity harvests of *Pleurotus ostreatus* during the three flushes

Substrates	First Quantity harvest QH1	Second Quantity harvest QH2	Third Quantity harvest QH3	Total Quantity Harvest (gm/bag)	Biological efficiency (%)
Rice straw	453.57b	369.00a	133.57a	956.14a	166
Maize hulls	258.86c	151.43c	45.27b	455.56c	79
Banana Leaves	256.71c	218.71c	53.43b	528.86c	92
Mixture of rice straw and black gram pod shell (1:1)	426.86b	241.43bc	127.71a	796.00b	138
Fingermillet husk	571.43a	328.29ab	124.86a	1024.57a	178
Significance at 5%	**	**	*	**	

^a The symbols * denotes significant and ** denotes highly significant

4. Conclusion

From the present study it is confirmed that oyster mushroom (*Pleurotus ostreatus*) can be cultivated on rice straw, maize hulls, banana leaves, fingermillet husk and mixture of rice straw & black gram pod (1:1) with varying growth performances. Fingermillet husk and rice straw were identified as the most suitable substrates for oyster mushroom cultivation. Fingermillet husk and rice straw followed by mixture of rice straw and black gram pod shell (1:1) produced a significantly higher yield and biological efficiency in shorter cropping duration. Fingermillet husk and mixture of rice straw and black gram pod shell (1:1) also proved to be better in terms of days taken for full spawn run. Therefore, fingermillet husk and rice straw can be recommended as the preferred substrate for oyster mushroom cultivation. In addition, mixture of rice straw & black gram pod (1:1) can be used as alternative substrate given that the growth performance, yield and cropping duration of oyster mushroom was better in it next to fingermillet husk and rice straw. The utilization of fingermillet husk and rice straw as substrates for oyster mushroom cultivation can be a solution to the huge agricultural by products available. And yet, further studies need to be conducted on the potentials of various agricultural wastes on oyster mushroom cultivation.

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