

EFFECT OF DIFFERENT SUBSTRATES AND SUPPLEMENTS IN PRODUCTION OF *Pleurotus ostreatus* IN DANG, NEPAL

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ABSTRACT

An investigation was laid out to evaluate the production of *Pleurotus ostreatus* on different substrates and supplements in inner Terai of Nepal, Dang, Deukhuri. Ten different treatments with different substrates and supplements were tested in completely randomized design, replicated four times during the spring season with an average daily temperature of 20-27°C and relative humidity ranging from 80-95%. The parameters taken for the observation during the experiment were spawn run period, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, fresh weight of mushroom, ash content, moisture content and biological efficiency of treatments. The research revealed that there were statistically significant yield differences associated with rice straw + banana leaves + rice bran (2.46 kg/ball), followed by rice straw + chickpea flour (2.35 kg/ball). The spawn run duration (23 days) was shortest in banana leaves with highest stipe length (5.01cm). The biological efficiency of various mixtures was found between 40 to 159%, the highest being in treatment rice straw + banana leaves + rice bran and followed by rice straw + chickpea flour. Highest moisture percentage (89.4%) was recorded in rice straw + maize flour. It was accomplished that rice straw + banana leaves whenever abundantly present, can be reasonably used in mixtures with rice bran supplement for better mushroom cultivation. Thus, its use is suggested to farmers in this country where large volumes of rice bran and dry banana leaves are discarded as agricultural residue.

Keywords: Banana leaves, Chickpea flour, Maize flour, Rice bran, Rice straw

1. INTRODUCTION

Oyster mushroom (*Pleurotus* spp) is an efficient decomposer of wood with an amazing flavor and taste (Mothlamme, 2019). According to Kong (2004) there are altogether 18 species Oyster mushroom under genus *Pleurotus* that are edible as well as commercially cultivated. In Nepal, three species of oyster mushrooms are cultivated. They are *Pleurotus sajor-kaju*, *P. ostreatus* and *P. florida* (Neupane et al., 2018). Among them, *P. ostreatus* is the most economical with high productivity, promising to eat and most preferred by farmers (Kong, 2004). Also, it requires less environmental control and exhibits resistance to diseases and pests compared to other mushroom species (Sharma et al., 2013). Oyster mushrooms are also excellent lignin degraders and can be cultivated using various agro-based residues (Jandaik & Goyal, 1995). Consequently, oyster mushroom cultivation is burgeoning in Nepal and worldwide (Raut, 2019). The proper growth and development of oyster mushroom entails substrate containing lower content of nitrogen but high carbon nutrient source (Sharma et al., 2013). Therefore, many organic matters containing cellulose, hemi-cellulose and lignin like

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rice straw, wheat straw, cottonseed hulls, corncob, sugarcane bagasse, sawdust, waste paper, leaves and pseudo stem of banana, water hyacinth, duck weed, etc on simple processing can be used as a substrate for oyster mushroom (Sharma et al., 2013; Sitaula et al., 2018).

It has already been reported that for the economical cultivation of oyster mushrooms, the quality and nutrient content of the substrate are major concerns (Ibekwe et al., 2008). In Nepal, rice straw is commonly used as a substrate for mushroom cultivation, but its availability, quality, and increasing prices present challenges to farmers (Raut, 2019). There are numerous locally available organic materials that can be used as supplements to enhance the growth and production capacity of mushroom substrates. However, many farmers in Nepal are unaware of these alternatives, and the organic matter is often either burned or fed to livestock (Sanjel et al., 2021) leading to reduced profits and environmental pollution.

Developing countries like Nepal still continue to suffer from nutrient deficiency and food security. Mushroom cultivation, particularly oyster mushrooms, offers a promising solution to address issues of nutrient deficiency, food security, and rural economy improvement (Pokhrel, 2016). Oyster mushroom cultivation can be successfully cultivated using a wide range of ligno-cellulose containing plant by-products (Mondal et al., 2010). Dry banana leaves can be the best alternative to rice straw as it contains high amounts of ligno-cellulose (Reddy, 2001) and is economical than rice straw. Furthermore, use of dry banana leaves as substrate can also prevent banana plant from unwanted disease source and lower environmental problem. Supplementing substrates with organic matter such as rice bran, chickpea flour, and maize flour can further enhance mushroom growth and yield. The present study focuses on determining the most economical substrate and supplement combination for the enhanced production of *Pleurotus ostreatus* in the inner Terai region of Nepal. The objective is to improve the socio-economic status of mushroom farmers through increased production at a low input cost.

This study was conducted using two locally available substrates supplemented with three types of organic matter, which are easily found in the study area as agricultural byproducts. The study examined rice straw, banana leaves, and their combination along with different types of supplements, to determine the most economical substrate and supplement combination that yields the best performance for the cultivation of *P. ostreatus*. Mushroom mycelium requires specific nutrients in the substrate for proper growth and development, so supplementing the substrate enhances mushroom yield (Sinha & Chourasia, 2021). Both organic and inorganic supplements added to the substrate can increase the yield of oyster mushroom (Kumar et al., 2020). Therefore, this study aims to identify the optimal substrate and supplement combination to improve the socio-economic status of mushroom farmers in Nepal by increasing production at a lower input cost.

2. MATERIALS AND METHODS

2.1 SITE OF STUDY

The experiment was conducted at Lamahi, Dang at the site of the Prithu Technical College during January to May 2022 for the determination of effect of different substrates and

supplements on the performance of *P. ostreatus*. The research was carried out under the poly house to provide sufficient darkness for the spawn-run.

2.2 TREATMENT DETAILS

The substrates selected for the cultivation of oyster mushroom were rice straw and banana leaves whereas the supplements used during the experiment were rice bran, chickpea flour and maize flour. The experimental design was laid in a single factorial completely randomized design. Twelve treatments were replicated four times thus making a total of forty-eight bags. A distance of 60 cm and 20 cm were kept between row-to-row distance and ball to ball distance respectively. The details of each treatment are given in the Table 1.

Table 1. Details of the treatment used in the experiment

Treatments number	Treatment details
T1	Rice straw only
T2	Rice straw + Maize flour (5%)
T3	Rice straw + Chickpea flour (5%)
T4	Rice straw + Rice bran (5%)
T5	Rice straw + Banana leaves (1:1)
T6	Rice straw + Banana leaves (1:1) + Maize flour (5%)
T7	Rice straw + Banana leaves (1:1) + Chickpea flour (5%)
T8	Rice straw + Banana leaves (1:1) + Rice bran (5%)
T9	Banana leaves only
T10	Banana leaves + Maize flour (5%)
T11	Banana leaves + Chickpea flour (5%)
T12	Banana leaves + Rice bran (5%)

2.3 SUBSTRATE COLLECTION

Paddy straw and dry banana leaves were collected from the nearest local farmers field. Similarly, chickpea and maize flour were brought from the market and sun-dried for 16 hours for milling. Rice bran was bought from the nearest rice mill.

2.4 PREPARATION OF SUBSTRATES AND SUPPLEMENT

A rough estimation of substrates was made. Paddy straw was chopped to 1.5” to 2” long pieces and dry banana leaves were chopped to 2” to 3” long pieces with the help of the knives. The paddy straw was washed and soaked in water for 12 hours to retain the appropriate moisture level whereas banana leaves were only washed but not soaked. The next day all these wet substrates were pulled out from the water tank and excess red black water was drained by spreading over a meshed sieve. The moisture level in the substrates was reduced to such a level so that the water didn’t ooze out when squeezed by hand. Similarly, for the supplement chickpea and maize flour were brought from the market and sun dried for 16 hours for milling. Rice bran was also taken from the nearest rice mill.

2.5 SANITIZING THE MUSHROOM HOUSE

The mushroom polyhouse was sterilized with formalin solution (100 ml formalin in 1 liter of water) and fumigated with potassium permanganate (15 g with 60 ml formalin in 2 different places) and was kept for 48 hours.

2.6 STERILIZATION

A stainless metal drum of 34.5" × 23" size was used for the steam sterilization of substrate and supplement. Water was poured up to a height of 6"- 7" in the bottom of the drum, above inside of which a perforated round net sieve was placed supported by a stand. Clean moist substrate after washing for 3-4 times was filled into the drum in such a way that the base of the substrate rested on the net but did not touch the water level. A polypropylene plastic filled with supplements was also placed at the side. Substrate level was maintained 2"- 3' above the actual height of the drum. Opening of the drum was sealed with a plastic sheet and tied with a rubber on the rim, so that the steam will not escape for the proper sterilization. Sterilization process continued for 30 minutes after the steam started leaking from the covered plastic sheet. After the steam sterilization completed, substrates were taken to the mushroom house and spread over a sterilized canvas for cooling.

2.7 FILLING OF SUBSTRATES IN PLASTIC BAGS

Transparent polythene bags of 16" × 24" size were used with flat-shaped-base that result in circular shape after filling them it with the substrate and supplement. The readiness of substrate for filling in the plastic bag was confirmed by squeezing the sterilized substrate with two hands and at that point it was ready to inoculate when no drops of water came out with only getting our palm wet. For the preparation of one mushroom bag 5 kg of wet substrate with 5% supplement (250g) on wet substrate basis was thoroughly mixed.

2.8 INOCULATION / SPAWNING

The substrate mixtures in the bags were inoculated with grain spawn of *Pleurotus ostreatus* aseptically. Bottom layer of plastic was filled with a 2-3cm thick layer of substrate and the spawn was placed at each layer (5-6cm) of substrate along the border line in a plastic bag but the top most layers was broadcasted on the whole surface and covered with thin layer of substrate. After inoculation of the spawn the bag was tightly pressed with both hands and the mouth was secured compactly with nylon thread. For the proper aeration 30-40 small holes were made with sterilized needle throughout the bag.

2.9 INCUBATION

The packed bags were then hung on a rope in a cool, dark area to prevent direct contact with the ground. The poly-house room was well ventilated until the entire substrate was colonized by mycelium of the oyster mushroom, which typically took approximately 23-34 days. The temperature was maintained at around 20 to 27°C during the incubation period.

2.10 GROWING AND WATERING

After the spawn-run of the whole substrate was complete, the bags were cut with the help of a sterilized blade from the two opposite sides. As the first harvest was made subsequent cuts were made to other opposite part. Primordial growth of mushroom was observed within 5-7 days of plastic cutting. With the first watering, the poly-house was exposed to diffuse light by making the proper arrangement of the ventilation. Watering was done twice a day with the help of mister and to maintain the proper relative humidity the wall and the floor of the room was also irrigated. Wet jute sacks were used to cover the racks in the mushroom house so that the humidity level was maintained.

2.11 HARVESTING

This mushroom was harvested when the cap began to fold and the edges turned brown. Harvesting was done after 5-6 days of pin head formation when the cap diameter reached to 6-7 cm. Picking was carried out by twisting the mushroom gently so that it is pulled out without leaving any stub. The periodical harvests from each treatment and their replications were weighed on an electronic balance and recorded.

2.12 PEST, DISEASE AND RODENT CONTROL

Sciarid flies and green mould were the two main issues encountered during the trial. The problem of the sciarid flies was managed by keeping yellow sticky trap at four different places and later it was controlled by spraying the neem oil at a concentration of 2 ml in 1 liter of water. To control green mould and prevent infestation in other balls, rectified spirit and formalin were used. Infected balls were transferred to the next room to avoid further contamination.

2.13 DATA COLLECTION AND ANALYSIS

The relevant data on growth parameters such as time to spawn-run (days), pinhead formation (days), first harvest (days), mushroom cap (pileus) diameter (cm), mushroom stipe length (cm), total yield (kg), biological efficiency (%) and quality parameters including moisture and ash contents were collected during the experimental period. These parameters were taken from five samples of each treatment periodically during 1st harvest, 2nd harvest and 3rd harvest. The final weight of the total harvest was also recorded.

To calculate the spawn run period, time interval between incubation and full colonization of each bag in the treatment was recorded. After the full colonization, the date of pinhead formation was noted for each replication in each treatment. Stipe length, stipe diameter, pileus diameter and pileus thickness were measured by randomly selecting five samples of different sizes from each fruiting body. Vernier calliper was used to measure dimension in centimetres (cm). Average was calculated from five readings and this process was repeated for every harvest. The biological efficiency, which represents the total yield of mushrooms per kilogram of substrate on a dry weight basis, was calculated using the formula provided by Chang et al. (1981).

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushroom (kg)}}{\text{Dry weight of substrate (kg)}} = 100$$

The moisture content was calculated by drying a 15g fresh mushroom sample in an oven at 80°C for 48 hours. The loss in weight before and after drying was recorded; the moisture content was calculated using the formula (AOAC, 1990).

$$\text{Moisture content (\%)} = \frac{\text{Fresh mushroom weight (gm)}}{\text{Dried mushroom weight (gm)}} = 100$$

Ash content was calculated by keeping 2g sample from each treatment into muffle furnace at 550 - 600°C for 5 hours to convert it into ash (Das et al., 2014) and ash content was calculated by using formulae:

$$\text{Ash content (g/100 g sample)} = \text{weight of ash} \times 100 / \text{weight of sample taken (Raghuramulu et al., 2003)}.$$

The data were processed to fit into R-studio and analysis was conducted using R Studio 3.5.2. Based on the ANOVA result, Duncan's multiple range test (DMRT) was performed and the means were separated using least significant difference (LSD), at the 5% level of significance.

3 RESULTS AND DISCUSSION

Two different types of substrates along with three supplements were compared with respect to production of oyster mushroom. The substrates used in these studies exhibited variation in spawn-run period, pinhead formation, harvesting days, number of fruiting bodies, stipe length and diameter, pileus diameter and thickness, total weight, moisture content and biological efficiency.

3.1 SPAWN-RUN PERIOD AND PINHEAD FORMATION

The fastest spawn-run period (23 days) was observed in banana leaves, banana leaves + chickpea flour, banana leaves + rice bran followed by banana leaves + maize flour (23.25 days) and slowest colonization was observed in banana leaves + rice straw (1:1) (34 days) shown in Table 2. This finding is in line with the finding of Mondal et al. (2010) outcome for colonization was comparatively similar to the findings of due to the presence of right proportion of alpha-cellulose, hemi-cellulose and lignin was the probable cause of higher rate of mycelium running in banana leaves and rice straw. Pokhrel et al. (2013) also found a minimum spawn-run period on banana leaves supplemented with rice bran and stated that pinhead formation and harvest date are positively correlated. USDA (2011) had also reported that bananas are a substantial source of many vitamins and minerals that could be important for pinhead formation.

The fastest pinhead formation (31 days) was observed in banana leaves alone substrate followed by banana leaves + chickpea flour (31.25 days), rice straw + banana leaves + rice bran (31.75 days), rice straw alone (32 days), and slowest pinhead formation was observed in banana leaves + rice straw (1:1) (66.5 days). This finding is in line with the finding of Silva et al. (2018) as they have also found the fastest pinhead formation in banana source

and concluded that it is due to the nutrient composition of banana fruits. Moreover, USDA (2011) has also reported that bananas are a substantial source of many vitamins and minerals that could be important for pinhead formation. However, this finding contradicts the findings of Mondal et al. (2010) because they have found the fastest pinhead formation in rice straw + banana leaves (1:1) than other substrate treatments.

Table 2. Effect of different substrates and supplements on spawn-run period and pinhead formation of *Pleurotus ostreatus*

Treatments	Spawn run period (days)	Pinhead Formation (days)
Rice straw only	25 ^{de}	32 ^{ef}
Rice straw + Maize flour (5%)	23.75 ^{ef}	43.75 ^b
Rice straw + Chickpea flour (5%)	27.75 ^c	35.25 ^{cdef}
Rice straw + Rice bran (5%)	28 ^c	39.75 ^{bc}
Rice straw + Banana leaves (1:1)	34 ^a	66.5 ^a
Rice straw + Banana leaves (1:1) + Maize flour (5%)	28 ^c	39 ^{bcd}
Rice straw + Banana leaves (1:1) + Chickpea flour (5%)	26 ^{cd}	33.75 ^{cdef}
Rice straw + Banana leaves (1:1) + Rice bran (5%)	30 ^b	31.75 ^{ef}
Banana leaves only	23 ^f	31 ^f
Banana leaves + Maize flour (5%)	23.25 ^{ef}	38 ^{bcde}
Banana leaves + Chickpea flour (5%)	23 ^f	31.25 ^f
Banana leaves + Rice bran (5%)	23 ^f	32.5 ^{def}
Grand Mean	26.27	37.875
SEM (±)	1.369	4.6
F-test	***	***
LSD (5%)	1.969	6.62
CV (%)	5.21	12.16

Note: Means with same letter in column are not significantly different at $p = 0.05$. LSD = Least significant difference, and CV = Coefficient of variance. *, **, *** represent significant at 5%, 1%, and 0.1% level of significance, respectively.

3.2 DAYS TO FIRST HARVEST DAYS, NUMBER OF FRUITING BODIES AND TOTAL WEIGHT

Shortest time to first harvest (36.5 days) was observed in banana leaves alone substrate followed by banana leaves + chickpea flour (37 days), rice straw + banana leaves + rice bran, banana leaves + rice bran, rice straw only (37.25 days), and longest time to first harvest (72 days) was observed in rice straw + banana leaves (1:1). This finding is in line with the finding of Silva et al. (2018) as they have also found fastest harvesting days in banana source concluded that it is due to the nutrient composition of banana fruits. Moreover, USDA (2011) has also reported that bananas are a substantial source of many vitamins and minerals that could be important for pinhead formation.

The total yield was highest from treatment rice straw + banana leaves + rice bran (2.465 kg) which was at par with the rice straw + chickpea flour (2.34 kg) followed by banana leaves only (1.795 kg), banana leaves + rice bran (1.75 kg), rice straw + banana leaves + maize flour (1.592), rice straw + rice bran (1.55 kg), rice straw + maize flour (1.242 kg), banana leaves + chickpea flour (1.177 kg), rice straw only (1.147 kg), banana leaves + maize flour (1.077 kg) and lowest was observed in rice straw + banana leaves (0.625 kg). This finding was similar with the findings of Mamiro and Mamiro (2011) as they have also found high yield of *Pleurotus ostreatus* on rice straw and banana leaves (1:1) substrate because of the high-water holding properties of banana leaves makes it beneficial nutrient substrate. Peng et al. (2000) has also found that high yield of oyster mushroom in substrate supplemented with rice bran and concluded that high yield is due to nutrient supplied by bran for fruiting bodies of mushroom species. Adenipekun and Omolaso (2015) as they have also found highest total yield in rice straw supplemented by 30% rice bran than other banana leaves supplementation and rice straw only. The study revealed that the addition of rice bran to substrates could be beneficial as a nutrient supplement and promoter to growth and yield.

Table 3. Effect of different substrates and supplements on days to first harvest days, number of fruiting bodies and total weight of *Pleurotus ostreatus*

Treatments	Days to first harvest (days)	Number of fruiting bodies	Total Weight (kg)
Rice straw only	37.25 ^{de}	122.25 ^{cd}	1.15 ^{bcd}
Rice straw + Maize flour (5%)	46.25 ^b	171 ^{bcd}	1.24 ^{bcd}
Rice straw + Chickpea flour (5%)	41.25 ^{bcd}	345 ^a	2.35 ^a
Rice straw + Rice bran (5%)	44 ^{bc}	202.5 ^{bcd}	1.55 ^{bc}
Rice straw + Banana leaves (1:1)	72 ^a	115.5 ^d	0.625 ^d
Rice straw + Banana leaves (1:1) + Maize flour (5%)	44.25 ^{bc}	231.75 ^b	1.59 ^{bc}
Rice straw + Banana leaves (1:1) + Chickpea flour (5%)	39.5 ^{cde}	233.25 ^b	1.86 ^{ab}
Rice straw + Banana leaves (1:1) + Rice bran (5%)	37.25 ^{de}	370 ^a	2.46 ^a
Banana leaves only	36.5 ^e	215.25 ^{bc}	1.79 ^{abc}
Banana leaves + Maize flour (5%)	43.5 ^{bcd}	190.25 ^{bcd}	1.07 ^{cd}
Banana leaves + Chickpea flour (5%)	37 ^{de}	244.5 ^b	1.77 ^{bcd}
Banana leaves + Rice bran (5%)	37.25 ^{de}	242.75 ^b	1.75 ^{abc}
Grand Mean	43	223.67	1.55
SEM (±)	4.525461	65.75	0.51
F-test	***	***	***
LSD (5%)	6.510417	94.59	0.73
CV (%)	10.52433	29.4	32.4

Note: Means with same letter in column are not significantly different at $p = 0.05$. LSD = Least significant difference, and CV = Coefficient of variance. *, ** and *** represent significant at 5%, 1%, and 0.1% level of significance, respectively.

3.3 STRIPE DIAMETER, STRIPE LENGTH, PILEUS DIAMETER AND PILEUS THICKNESS

Although there was slightly high stipe diameter of mushroom from rice straw + banana leaves + maize flour (0.79 cm) followed by rice straw + banana leaves + chickpea flour (0.73 cm) and lowest in rice straw + banana leaves + chickpea flour (0.65 cm). Analysis of variance of stipe length shows that there was no significant difference obtained among the tested substrates ($p \leq 0.05$). The highest stipe length (5.01 cm) was observed in banana leaves substrate followed by rice Straw + banana leaves + maize flour (4.62 cm), rice straw + banana leaves + rice bran (4.31 cm), banana leaves + rice bran (4.28 cm) and lowest stipe length on rice straw + banana leaves (1:1) substrate (3.46 cm). Mondel et al. (2010) have also reported lowest stipe length in rice straw + banana leaves (1:1) substrates which is statically similar to our findings. Furthermore, it has been also reported that the quality of mushroom decreases with the increase in oyster mushroom stipe length (Zadrazil, 1978). The quality of oyster mushroom *P. florida* depends on the length of stalk, the higher the length of stalk, the poorer the quality of mushroom (Zadrazil, 1978).

The highest pileus thickness was observed in rice straw + banana leaves + maize flour (0.425 cm) followed by rice straw + chickpea flour (5%) (0.42 cm), rice straw + banana leaves + chickpea flour (0.38 cm), rice straw + banana leaves + chickpea flour (0.38 cm) and lowest in rice straw + banana leaves (1:1) (0.28 cm). This finding is in line with the finding of Mondal et al. (2010) as they have also found lowest pileus thickness in rice straw + banana leaves (1:1) substrate. It can be also concluded that the pileus thickness can be high due to presence of adequate nutrients in the substrates and it is a yield attributing character in which the thickness of pileus increases with increase in yield (Mondal et al., 2010). Analysis of variance of pileus diameter shows that there was no significant difference obtained among the tested substrates ($p \leq 0.05$). The highest pileus diameter was observed in rice straw + banana leaves + rice bran (7.03cm) and lowest on rice straw + banana leaves + chickpea flour (5.12cm).

Table 4. Effect of different substrates and supplements on stripe diameter, stripe length, pileus diameter and pileus thickness of *Pleurotus ostreatus*

Treatments	Stripe Diameter (cm)	Stripe Length (cm)	Pileus Diameter (cm)	Pileus Thickness (cm)
Rice straw only	0.66	3.63 ^{cd}	5.77	0.32 ^{cd}
Rice straw + Maize flour (5%)	0.69	4.22 ^{bc}	6.475	0.34 ^{cd}
Rice straw + Chickpea flour (5%)	0.72	3.98 ^{bcd}	6.18	0.42 ^{ab}
Rice straw + Rice bran (5%)	0.68	4.31 ^{abc}	5.86	0.36 ^{bc}
Rice straw + Banana leaves (1:1)	0.65	3.4575 ^d	5.37	0.28 ^d
Rice straw + Banana leaves (1:1) + Maize flour (5%)	0.79	4.62 ^{ab}	6.36	0.425 ^a
Rice straw + Banana leaves (1:1) + Chickpea flour (5%)	0.73	3.88 ^{bcd}	5.12	0.38 ^{abc}
Rice straw + Banana leaves (1:1) + Rice bran (5%)	0.72	4.49 ^{ab}	7.03	0.38 ^{abc}
Banana leaves only	0.72	5.01 ^a	6.51	0.35 ^c

Treatments	Stripe Diameter (cm)	Stripe Length (cm)	Pileus Diameter (cm)	Pileus Thickness (cm)
Banana leaves + Maize flour (5%)	0.69	3.95 ^{cd}	5.57	0.37 ^{abc}
Banana leaves + Chickpea flour (5%)	0.66	3.91 ^{cd}	5.9	0.35 ^{bc}
Banana leaves + Rice bran (5%)	0.72	4.28 ^{abc}	6.31	0.345 ^c
Grand Mean	0.71	4.145	6.04	0.36
SEM (±)	0.06	0.52	0.82	0.043
F-test	NS	*	NS	**
LSD (5%)	NS	0.74	NS	0.06
CV (%)	7.89	12.51	13.73	12.08

Note: Means with same letter in column are not significantly different at $p = 0.05$. LSD = Least significant difference, and CV = Coefficient of variance. *, **, and *** represent significant at 5%, 1%, and 0.1% level of significance respectively and NS = Non-significant.

3.4 BIOLOGICAL EFFICIENCY, MOISTURE CONTENT AND ASH CONTENT

Highest biological efficiency was found in rice straw + banana leaves + rice bran (159.03%), followed by rice straw + chickpea flour (156.33%), rice straw + banana leaves + chickpea flour (120.32%), banana leaves only (115.81%) and lowest was found in rice straw + banana leaves (40.32) as shown in figure 2. When 5% supplement is added, biological efficiency increases at the peak than with other different levels of supplement and without supplement (Mamiro & Mamiro, 2011).

Highest moisture content was found on rice straw + chickpea flour (89.49%) followed by rice straw + banana leaves + maize flour (89.3%), banana leaves + rice bran (89.1%), and lowest was found on banana leaves + chickpea flour (83.68%). Mushrooms are highly perishable because of their moisture content, fragile cell structure and susceptible to enzymatic browning (Kumar et al., 2013). So, high moisture content mushrooms are not desirable.

Highest ash content was found on rice straw + chickpea flour (13.41%) followed by rice straw + maize flour (13.26%), and lowest was found on banana leaves + chickpea flour (4.44%). The ash content denotes the minerals and inorganic substances that remain after subjecting the sample to intense heat, which eliminates moisture, volatile components, and organic matter. The primary minerals and inorganics typically found are calcium, magnesium, sodium, and potassium, while smaller amounts of manganese, zinc, iron, and other elements may also be detected (Monti et al., 2008).

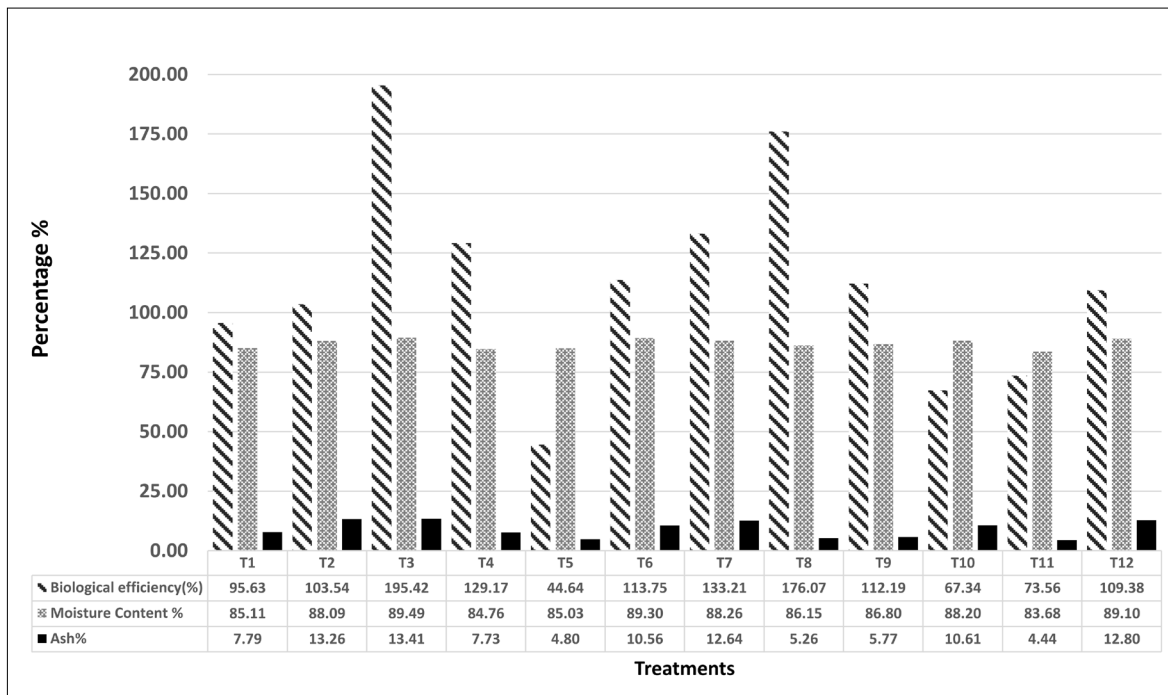


Figure 1. Effect of different substrates and supplements on biological efficiency, moisture content, and ash content

CONCLUSION

From the study it is concluded that the combination of rice straw, banana leaves, and rice bran exhibited the highest yield of 2.46 kg per ball, followed closely by rice straw supplemented with chickpea flour at 2.35 kg per ball. Banana leaves contributed to the shortest spawn run duration of 23 days, with mushrooms reaching a remarkable stipe length of 5.01 cm. Biological efficiency ranged from 40% to 159%, with the highest efficiency observed in the rice straw + banana leaves + rice bran treatment, followed by rice straw + chickpea flour. Additionally, rice straw supplemented with maize flour recorded the highest moisture content at 89.4%. The findings suggest that utilizing rice straw and banana leaves, in combination with supplements like rice bran, could enhance mushroom cultivation in Nepal. These findings underscore the potential of utilizing locally available agricultural byproducts for oyster mushroom cultivation, offering economic benefits to farmers and contributing to food security. Further research could explore optimized combinations to enhance productivity and quality while addressing nutrient deficiency challenges in developing regions like Nepal. Overall, mushroom cultivation presents a viable avenue for sustainable agriculture and rural livelihood improvement in such contexts.

DECLARATION

The authors declare no conflict of interest.

REFERENCES

- Adenipekun, C. O., & Omolaso, P. O. (2015). Comparative study on cultivation, yield performance and proximate composition of *Pleurotus pulmonarius* Fries (Quelet) on rice straw and banana leaves. *World J. Agric. Sci*, 11(3), 151-158.
- AOAC. (1990). *Official methods of analysis. 15th. ed. Association of Official Analytical Chemists*. Virginia, USA.
- Chang, S. T., Lau, O. W., & Cho, K. Y. (1981). The cultivation and nutritional value of *Pleurotus sajor-caju*. *European Journal of Applied Microbiology and Biotechnology*, 12, 58-62. <https://doi.org/10.1007/BF00508120>
- Das, A. R., Das, P., Bhattacharjee, S., & Saha, A. K. (2014). Chemical analysis of a wild edible mushroom: *Pleurotus djamor* (Rumph. ex Fr.) Boedijn. *Mushroom Research*, 23(2), 161-166.
- Ibekwe, V. I., Azubuike, P. I., Ezeji, E. U., & Chinakwe, E. C. (2008). Effects of nutrient sources and environmental factors on the cultivation and yield of oyster mushroom (*Pleurotus ostreatus*). *Pakistan Journal of Nutrition*, 7(2), 349-351.
- Jandaik, C. L., & Goyal, S. P. (1995). Farm and farming of oyster mushroom (*Pleurotus* spp.). *Mushroom Production Technology* (Eds. Singh, RP and Chaube, HS). GB Pant Univ. Agril. And Tech., Pantnagar India, 72-78.
- Kong, W. S. (2004). Descriptions of commercially important *Pleurotus* species. *Oyster mushroom cultivation. Part II. Oyster mushrooms. Seoul: Heineart Incorporation*, 54, 61.
- Kumar, A., Singh, M., & Singh, G. (2013). Effect of different pretreatments on the quality of mushrooms during solar drying. *Journal of Food Science and Technology*, 50, 165-170.
- Kumar, S., Chand, G., & Patel, D. K. (2020). Evaluation of different substrate supplements on growth and yield of oyster mushroom (*Pleurotus florida*). *Indian Phytopathology*, 73, 731-736. <https://doi.org/10.1007/s42360-020-00252-9>
- Mamiro, D. P., & Mamiro, P. S. (2011). Yield and mushroom size of *Pleurotus ostreatus* grown on rice straw basal substrate mixed and supplemented with various crop residues.
- Mondal, S. R., Rehana, J., Noman, M. S., & Adhikary, S. K. (2010). Comparative study on growth and yield performance of oyster mushroom (*Pleurotus florida*) on different substrates. *Journal of the Bangladesh Agricultural University*, 8(2), 213-220. doi: 10.3329/jbau.v8i2.7928
- Monti, A., Di Virgilio, N., & Venturi, G. (2008). Mineral composition and ash content of six major energy crops. *Biomass and Bioenergy*, 32(3), 216-223.
- Mothhalamme, T. (2019). Value-addition of cereal crop residues using low technology oyster mushroom (*Pleurotus* spp.) production to improve small-scale farmers' income and nutrition in Botswana. <http://moodle.buan.ac.bw:80/handle/123456789/301>

- Neupane, S., Thakur, V., Bhatta, B., Pathak, P., Gautam, B. B., & Aryal, L. (2018). Performance of different substrates on the production of oyster mushroom (*Pleurotus florida*) at Gokuleshwor, Darchula. *International Journal of Scientific and Research Publications*, 8(6), 231-240.
- Peng, J. T., Lee, C. M., & Tsai, Y. F. (2000). Effect of rice bran on the production of different king oyster mushroom strains during bottle cultivation.
- Pokhrel, C. P. (2016). Cultivation of oyster mushroom: a sustainable approach of rural development in Nepal. *Journal of Institute of Science and Technology*, 21(1), 56-60. DOI: <https://doi.org/10.3126/jist.v21i1.16050>
- Pokhrel, C. P., Kalyan, N., Budathoki, U., & Yadav, R. K. P. (2013). Cultivation of *Pleurotus sajor-caju* using different agricultural residues.
- Raghuramulu, N., N.K. Madhavan and S. Kalyanasundaram. (2003). *A Manual of Laboratory Techniques*. pp. 56-58, National Institute of Nutrition. Indian Council of Medical Research, Hyderabad, India.
- Raut, J. K. (2019). Current status, challenges and prospects of mushroom industry in Nepal. *International Journal of Agricultural Economics*, 4(4), 154-160.
- Reddy, G. V. (2001). Bioconversion of banana waste into protein by two pleurotus species *P. ostreatus* and *P. sajor caju* biotechnological approach. (Thesis), Sardar Patel University, Department of Bio Science, Gujrat, India.
- Sanjel, P., Shrestha, R. K., & Shrestha, J. (2021). Performance of oyster mushroom (*Pleurotus ostreatus*) grown on different finger millet husk substrates. *Journal of Agriculture and Natural Resources*, 4(1), 291-300. DOI: <https://doi.org/10.3126/janr.v4i1.33370>
- Sharma, S., Yadav, R. K. P., & Pokhrel, C. P. (2013). Growth and yield of oyster mushroom (*Pleurotus ostreatus*) on different substrates. *Journal on New Biological Reports*, 2(1), 03-08.
- Sinha, N., & Chourasia, S. 2021. Effect of different substrates with supplementation on the growth and yield of oyster mushroom species *Pleurotus florida*. *International Journal of Recent Scientific Research*, 12, 41480-41483.
- Sitaula, H. P., Dhakal, R., Geetesh, D. C., & Kalauni, D. (2018). Effect of various substrates on growth and yield performance of oyster mushroom (*Pleurotus ostreatus*) in Chitwan, Nepal. *International Journal of Applied Sciences and Biotechnology*, 6(3), 215-219. DOI: <https://doi.org/10.3126/ijasbt.v6i3.20859>
- USDA. (2011). United States Department of Agriculture Agricultural Research Service National Nutrient Database for Standard Reference Release 28
- Zadražil, F. (1978). Cultivation of *Pleurotus*. In: *The biology and cultivation of edible mushrooms*. Academic Press. USA. pp. 521-557