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Research Article

EFFECT OF DIFFERENT SUBSTRATES AND SUPPLEMENTS ON OYSTER MUSHROOM (*Pleurotus ostreatus*) PRODUCTION AT LAMAHI, DANG, NEPAL

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ABSTRACT. *Pleurotus ostreatus* is a commercially important edible mushroom known for its ability to decompose the complex organic material. This study was conducted to evaluate the effectiveness of different locally available substrates and supplements on oyster mushroom production. Also, it seeks to identify the most effective substrate supplement combinations for maximizing yield. The research was conducted inside a mushroom-house at Bangaun, Deukhuri, Dang. Completely Randomized Design (CRD) with four replications was employed to study the effects of nine treatments i.e. banana leaves, rice straw, banana leaves + maize flour, banana leaves + rice straw, rice straw + maize flour, banana leaves +rice straw + maize flour, banana leaves + rice bran, rice straw + rice bran and banana leaves + rice straw + rice bran. The substrates were steam sterilization, cooled, and inoculated with mushroom spawn at a rate of 3% of their weight. Data were collected over three harvests. Banana leaves were found to be the most suitable substrate with the highest biological efficiency (120.55%) (the ratio of mushroom yield to the dry weight of substrate) and yield (1.94kg). The shortest stipe length (22.55 mm) was seen in Rice straw + Maize flour while the largest cap diameter was found in Banana leaves + Rice straw + Maize flour (77.53 mm). According to the research, the greatest overall results in mushroom cultivation were obtained by using banana leaves without any supplements, although certain supplements can enhance specific growth metrics. Based on the results, farmers in Nepal are encouraged to use banana leaves as the main substrate for Pleurotus ostreatus cultivation because of their high availability and biological effectiveness.

Keywords: *Pleurotus ostreatus, Mushroom, Substrate, Biological efficiency, Yield*

INTRODUCTION

Mushrooms are a group of macro fungi that have typical fruiting bodies, unique biota and obtain nutrients by secreting exuding enzymes that breakdown complex organic matter [1]. Morphologically, they consist of a stem (stipe), cap (pileus), hymenium (lamellae), and spores on underneath the cap [2]. Among edible mushrooms, oyster mushrooms (*Pleurotus ostreatus*) are saprophytic fungi known for their nutrient rich profile which contain 25–50% protein, 2–5% fat, 17–47% carbohydrates, 7–38% mycocellulose, and 1–12% potassium, phosphorus, calcium, and sodium minerals [3]. They are also a good source of vitamins D, C, B1, B5, B6, niacin, and riboflavin [4]. In Nepal, professional mushroom cultivation has been practiced for over 30 years. In 2021/2022, Nepal produced 14,300 metric tons of fresh mushrooms [5]. Oyster mushrooms are locally known as 'kanya chyau' in Nepal and are the most suited, economical and preferred species

by farmers for cultivation [6]. Oyster mushrooms are grown using various agricultural wastes without composting. Commonly used substrates for mushroom cultivation are straw, husk, sawdust, cow dunk, wood, compost manure, banana leaves, and sunflower leaves [7].

Mushroom cultivation represents a sustainable biotechnology process that converts forests and agriculture waste residues into valuable protein-rich food products [8]. Taxonomically, *Pleurotus ostreatus* belongs to Kingdom-Fungi, Phylum-Basidiomycota, Class-Agaricomycetes, Order-Agaricales, Family-Pleurotaceae, and Genus-Pleurotus [9]. Comparatively, this strain of mushroom is less susceptible to diseases and pests, requires minimal environmental control, and has short crop cycle, making it suitable for smallholder farmers [10]. Oyster mushroom farming offers the practical solution for managing organic waste, which has become increasingly difficult to dispose of in recent years [11]. Globally, oyster mushrooms possess various industrial and medical properties, including antioxidant, antimicrobial, anti-inflammatory, antitumor, and food additive effects [12]. Most Pleurotus species generate lignocellulolytic enzymes that enable them to breakdown cellulose, hemicellulose, and lignin, influencing growth and yield [13]. Substrate composition has been shown to affect mycelia growth, pinhead formation, fruiting bodies number, and biological efficiency [14].

The use of agricultural residues like rice and wheat straw adds value by transforming these materials into nutritious food [15]. Several studies in Nepal have evaluated rice straw as a commonly utilized and useful substrate, with good biological efficiency and profitability for oyster mushroom growing [12], [16], [17]. However, alternatives such as banana leaves, maize cob, water hyacinth, and sawdust have also shown various results in yield and economic returns [16]. According to Thakur [17], dry banana leaves are a cost-effective alternative to rice straw due to their high lignocellulose content. Using dry banana leaves as a substrate protects banana plants from illness and reduces environmental impact. Adding organic matter to substrates like rice bran, maize flour can boost mushroom growth and yields by providing additional nutrient for mycelial development.

A systematic assessment of locally accessible substrate combinations and supplements is still lacking, despite the fact that oyster mushroom cultivation has been researched in Nepal on individual substrates such paddy straw, maize cob, and sugarcane bagasse [18]. The most widely used substrate, rice straw, is becoming more and more expensive, and substitutes with a higher lignocellulose content, such as banana leaves, are not being used as much. Combination of multiple subtracts and supplement has potential to improve yields but requires further investigation under local conditions [19]. The lack of detailed, region-specific data on substrate mixes and supplements limits farmers' capacity to optimize mushroom output and economic returns throughout Nepal's different agro-climatic zones.

Therefore, this study aims to evaluate the effects of different locally available substrates and supplements on oyster mushrooms production. Also, the study aims to identify the most effective substrate supplement combinations that maximize mushroom production and provide practical recommendations to enhance sustainable mushroom cultivation.

MATERIALS AND METHODS

Experimental site

This research was carried out inside a controlled mushroom house at College-Prithu Technical College at Bangaun, Deukhuri, Dang, Nepal. The site is located in a tropical climate zone; however, the study was carried out during winter season, which makes it quite a favorable environment for *Pleurotus ostreatus* cultivation.

Sanitizing the mushroom house

Pleurotus ostreatus, is a popular oyster mushroom species well suited for winter cultivation in Nepal. The bamboo used to support mushroom bags was cleaned using sandpaper and then sprayed with a sodium hypochloride solution to remove any dirt that could cause the spread of disease. The entire surface of the room, including the ceilings, and side plastics was sprayed with 4% formalin, followed by spraying 2gm Bavistin in 10 liters of water for sterilization. Then, fumigation of the room was carried out using potassium permanganate and formalin. During the fumigation period, the whole room was closed and then reopened 48 hours before inoculation to allow fresh air to replace the formalin gas.

Substrate collection

Locally available substrates like rice straw and banana leaves were used. The selected substrates were cleaned and made free from dirt, other free materials, and greenish tint. All these substrates collected were collected and chopped into small pieces (1-2 inches long) using a threshing machine. These substrates were washed and soaked in water 4 to 5 hours before substrate sterilization, to remove all dust and impurities. The moisture level was reduced to approximately 65-70%, using the gravimetric method to ensure consistency.

Sterilization

A metal drum was used for steam sterilization of substrates. A stand-supported perforated round net sieve was positioned inside the drum, which was filled with four to six inches of water. The drum was filled with moist substrate in such a way that the base of the substrate rested on the net but did not touch the water level. The top opening of the drum was tied with a plastic sheet and rubber around the rim to retain steam during sterilization, and the fire was set up underneath. The sterilization was carried out for about 1 hour to kill all insects and fungal contaminants.

Spawning and Incubation

After steam sterilization, substrates were cooled by spreading on a clean, sterilized plastic mat, and when the temperature lowered to about 25°C, the substrates were packed along with dry spawn in the mushroom bag. Each bag was filled with 5 kg substrates, divided into four strata of 1 kg each. In the final stratum, spawn was mixed with approximately 800 gm of substrate, and an additional 200 gm of substrate was used to cover the spawn on top. The spawn rate was maintained at 3% on a wet weight basis. The packed bags were then incubated in a cool, dark and well-ventilated room until the entire substrate was colonized by yellowish-white mycelium, typically within 20 to 35 days. During incubation, the mushroom sacks were sprayed with tap water as needed to maintain a humidity between 85 to 90%. The temperature was maintained between 20 and 24 °C during incubation, and there was little exposure to light.

Treatment and treatment details

Nine treatments with four substrates were used for the experiment, i.e., banana leaves, rice straw, maize flour, and rice bran. Banana leaves and rice straw were used individually as a treatment, whereas maize flour and rice bran were mixed in various proportions to create different treatment combinations. Details of each treatment, including the exact proportions of the substrates used are listed in **Table 1**.

Table 1. *Treatment and treatment details.*

Treatment	Treatment Details	Short form
T1	Banana leaves(47.5%) + Rice straw (47.5%) + Maize flour (5%)	BL + RS +MF
T2	Banana leaves (100%)	BL
T3	Rice straw (95%) +Maize flour (5%)	RS+MF
T4	Rice straw (100%)	RS
T5	Banana leaves (95%) + Rice straw (5%)	BL+RS
T6	Rice straw (95%) + Rice bran (5%)	RS+RB
T7	Banana leaves (47.5%) + Rice straw (47.5%) + Rice bran (5%)	BL+RS+RB
T8	Banana leaves (95%) + Rice bran (5%)	BL+RB
T9	Banana leaves (95%) + Maize flour (5%)	BL+MF

BL: Banana leaves, RS: Rice straw, MF: Maize flour, RB: Rice bran.

Pest, disease and rodent control

Flies, rodents, and green mould were the major issues faced during this research. To control flies, yellow sticky traps were installed in the mushroom house. The problem of rodents was managed by keeping traps in the corner of the mushroom house. Rectified spirit and cotton were used to clean the bags which were infested by green mould. To prevent further infestation, infected bags were transferred to the next room.

Experimental design and data collection

The experimental design was carried out in a completely randomized design (CRD), with nine treatments and four replications, resulting in a total of 36 bags. Data on growth parameters were collected throughout the experimental period, including colonization period (days), pinhead formation (days), first harvest (days), second harvest (days), third harvest (days), pileus diameter (mm), stipe length (mm), stipe diameter (mm), total yield (kg), and biological efficiency. These parameters were measured from five samples of each treatment during 1st, 2nd, and 3rd harvest from different fruiting bodies but the same mycelial body in each harvest. The total yield was calculated as the weight (kg) of fresh mushrooms. The Biological Efficiency (B.E) was calculated by following the standard formula [20].

$$Biological\ Efficiency\ (B.E) = \frac{Total\ weight\ of\ fresh\ mushrooms}{dry\ weight\ of\ substrate} \times 100$$

Eqn. 1

Statistical analysis

For statistical analysis, data were collected and charted using Microsoft Excel. Thereafter, the data was processed for analysis in R-studio. From the ANOVA result, Duncan's multiple range

test (DMRT) was run, and the means were differentiated by the Least Significant Difference (LSD) at the 5% level of significance. Before doing an ANOVA, the Shapiro-Wilk and Levene tests were used to verify the normality and homogeneity of variance.

RESULTS

A total of nine treatments were prepared using different combinations of substrates for the production of oyster mushrooms. The substrates used in studies exhibited variation in colonization period, pinhead formation, number of fruiting bodies, stipe length and diameter, pileus diameter, and total yield as determined by analysis of variance (ANOVA) and least significant difference (LSD) tests.

Effect of different substrates and supplements in colonization period and pinhead formation.

The longest colonization period was recorded in four treatments i.e, BL (Banana leaves (100%)), RS+MF (Rice straw +Maize flour), BL+RS+RB (Banana leaves + rice straw + rice bran), and BL+RB (banana leaves + rice bran). In contrast, RS (rice straw) showed the shortest colonization period. These findings are visually presented in Fig. 1.

In the same way, the maximum number of days for pinhead formation was observed in BL+RS (Banana leaves + rice straw) and BL+RS+RB (Banana leaves + rice straw + rice bran), both of which included banana leaves and rice straw. The shortest duration to pinhead formation was observed in BL+RS+MF (banana leaves + rice straw + maize flour). This trend is illustrated in Fig. 2.

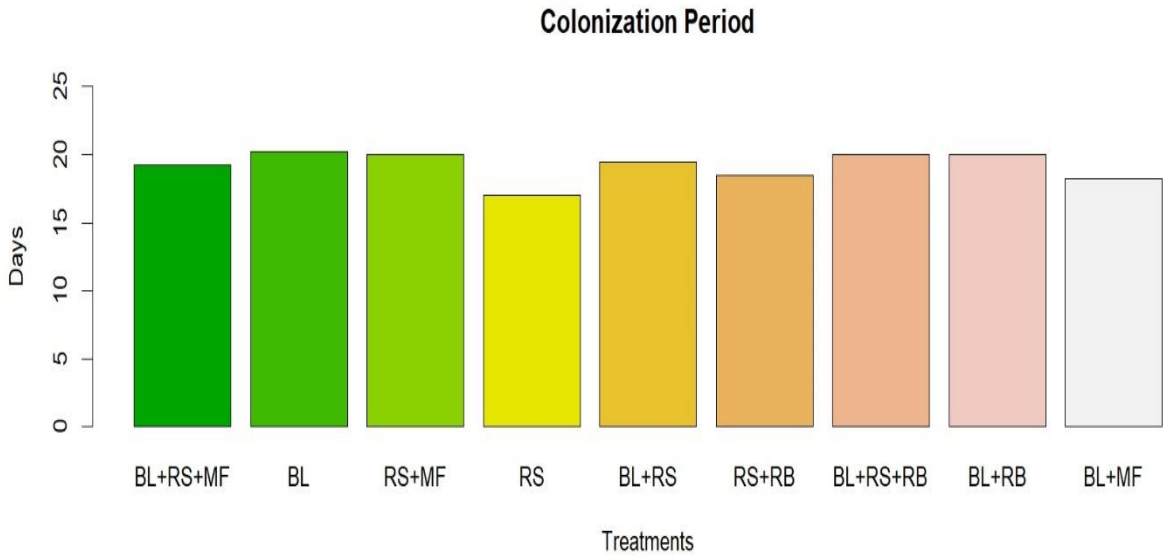

Fig. 1. *Effect of different substrates and supplements on colonization period.*

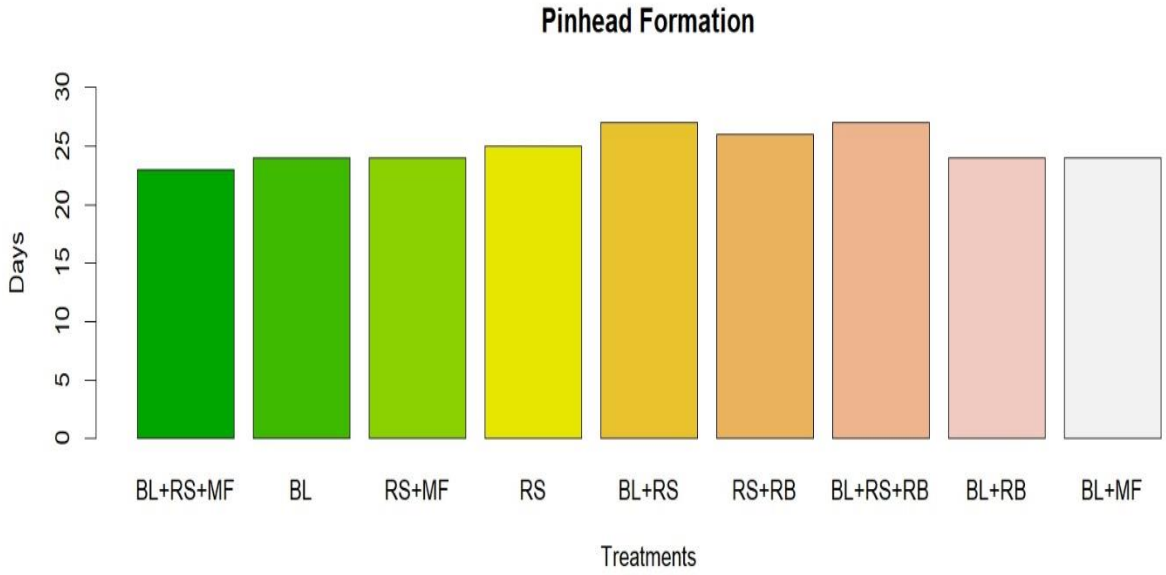

Figure 2. *Effect of different substrates and supplements on pinhead formation.*

Effect of different substrates and supplements in fruiting bodies (no), stipe length (mm), stipe diameter (mm), and cap diameter (mm) of mushrooms at First Harvest.

The highest number of fruiting bodies at first harvest (36.75) were observed in the treatment with the mixture of rice straw (95%) and rice bran (5%) followed by the mixture of banana leaves (47.5%), rice straw (47.5%) and rice bran (5%) (35.0) (Table 2). Whereas, banana leaves (100%) produced the lowest number of fruiting bunches (14.00). Here, banana leaves seem to be the most effective treatment for stipe length (69.92mm) and stipe diameter (10.97mm). It indicates banana leaves along support better vegetative growth. Whereas, the combination of banana leaves (95%) + rice straw (5%) expressed the shortest stipe length (39.40mm). While the mixture of banana leaves (95%) + rice bran (5%) produced the narrowest stipe diameter (5.92mm). For cap diameter, the treatment containing banana leaves (47.5%), rice straw (47.5%), and maize flour (5%), yielded as best treatment (77.53), followed closely by banana leaves (100%) (76.16). The smallest cap diameter was measured in the rice straw (100%) (54.24mm).

***Table 2**. Effect of different substrates and supplements in fruiting bodies (no), stipe length (mm), stipe diameter (mm), and cap diameter (mm) of mushrooms at First Harvest.*

Treatment	FB1	Stipe length (mm)	Cap diameter(mm)	Stipe diameter(mm)
Banana leaves (47.5%) +Rice straw (47.5%) +Maize flour (5%)	25.50^{bc}	64.50^{a}	77.53^{a}	9.95^{ab}
Banana leaves (100%)	14.00^{d}	69.92^{a}	76.16^{a}	10.97^{a}
Rice straw (95%) + Maize flour (5%)	18.00^{cd}	47.14^{bc}	62.61^{bc}	9.51^{ab}
Rice straw (100%)	24.75^{bc}	47.75^{bc}	54.24^{c}	9.99^{ab}
Banana leaves (95%) + Rice straw (5%)	32.25^{ab}	39.40^{c}	56.61^{c}	6.98^{bc}
Rice straw (95%) +Rice bran (5%)	36.75^{a}	51.41^{b}	54.78^{c}	6.83^{bc}
Banana leaves(47.5%)+Rice straw(47.5%)+Rice bran(5%)	35.00^{a}	51.10^{b}	59.42^{bc}	7.80^{bc}
Banana leaves (95%) + Rice Bran (5%)	21.50^{cd}	51.40^{b}	61.43^{bc}	5.92^{c}
Banana leaves (95%) + Maize flour (5%)	19.25^{cd}	48.16^{bc}	68.25^{ab}	7.52^{bc}
Grand Mean	25.22	52.3	63.45	8.38
LSD	7.54	11.35	11.27	3.33
CV (%)	20.62	14.96	12.24	27.36
F test	***	***	***	*

***: Significant at 0.1% level of significance, *: Significant at 5% level of significance. FB1: fruiting bodies at first harvest, LSD: least significant difference, CV: coefficient of variation. The letters a-d indicate the least significant difference (LSD) test rankings; 'a' represents the highest followed by b to d. The similar letters represent that the means are significantly at par with each other.

Effect of different substrates and supplements in fruiting bodies (no.), stipe length (mm), and cap diameter (mm) of mushrooms at the second harvest.

The effect of different substrate treatments was significant across all measured parameters (Table 3). According to the results, the combination of banana leaves (47.5%) + rice straw (47.5%) + maize flour (5%), showed the highest number of fruiting bodies (37.50), followed by banana leaves (95%) + maize flour (5%) (36.0) at the second harvest. Whereas rice straw (95%) + rice bran (5%) was observed to have the lowest number (19.75). Additionally, the combination of banana leaves (95%) + maize flour (5%) produced the longest stipe length (36.76 mm), while rice straw (100%) had the shortest stipe (24.71 mm). Rice straw (100%) produced the largest cap diameter (70.83mm). Whereas the smallest cap diameter (52.75 mm) was observed in rice straw + rice bran treatment. In terms of stipe diameter, the widest diameter was produced by banana leaves (10.33 mm). The narrowest stipe diameter (5.50mm) was observed in rice straw (100%). In general, treatments with maize flour and banana leaves tended to do better across the majority of morphological characteristics.

Table 3. *Effect of different substrates and supplements in fruiting bodies (no), stipe length (mm), and cap diameter (mm) of mushrooms at the second harvest.*

Treatment	FB2	Stipe length (mm)	Cap diameter(mm)	Stipe diameter(mm)
Banana leaves (47.5%) +Rice straw (47.5%) +Maize flour (5%)	37.50^{a}	26.83^{cd}	53.31^{d}	9.94^{ab}
Banana leaves (100%)	27.00^{d}	28.03^{c}	52.95^{d}	10.33^{a}
Rice straw (95%) + Maize flour (5%)	23.25^{e}	32.33^{b}	59.40^{bc}	8.95^{ab}
Rice straw (100%)	31.25^{bc}	24.71^{d}	65.98^{a}	5.50^{e}
Banana leaves (95%) + Rice straw (5%)	29.75^{c}	32.40^{b}	63.60^{ab}	5.80^{de}
Rice straw (95%) + Rice bran (5%)	19.75^{f}	33.87^{ab}	52.75^{d}	6.15^{cde}
Banana leaves(47.5%)+Rice straw(47.5%)+Rice bran(5%)	32.00^{b}	33.81^{ab}	57.53^{cd}	7.95^{bcd}
Banana leaves (95%) + Rice Bran (5%)	27.75^{d}	33.79^{ab}	56.17^{cd}	8.11^{bc}
Banana leaves (95%) + Maize flour (5%)	36.00^{a}	36.76^{a}	62.90^{ab}	8.20^{abc}
Grand Mean	29.36	31.39	58.29	7.88
LSD	2	3.06	5.36	2.19
CV (%)	4.66	6.73	6.34	19.16
F test	***	***	***	***

*** = Significant at 0.1% level of significance, FB2: fruiting bodies at second harvest, LSD: least significant difference, CV: coefficient of variation, the letters a-f indicate the least significant difference (LSD) test rankings; 'a' represents the highest followed by b to f. The similar letters represent that the means are significantly at par with each other.

Effect of different substrates and supplements in fruiting bodies (no), stipe length (mm), stipe diameter (mm), and cap diameter (mm) of mushrooms at third Harvest.

According to the results, the combination of rice straw (5%) and banana leaves (95%) produced the highest number of fruiting bodies (28.50) (Table 4). In contrast, Banana leaves (95%) + maize flour (5%) showed the lowest number of fruiting bunches in the third harvest (14.75). The longest stipe length (32.62mm) and largest cap diameter (70.83mm) was recorded from a mixture of rice straw (95%) and rice bran (5%). Similarly, a blend of rice straw (95%), and rice bran (5%) worked well in stipe diameter at approximately 8.08mm. Overall, the most successful treatment was rice straw (95%) + rice bran (5%), which performed well in terms of stipe diameter, length, and cap diameter but not on fruiting bodies. Mixture of rice straw (95%) +maize flour (5%) showed the shortest stipe length (22.55mm). Rice straw (100%) exhibited the lowest result for cap diameter (49.56mm). Stipe diameter was seen lowest in banana leaves (952%) +rice straw (5%) as shown by measurement 4.84mm.

Table 4. *Effect of different substrates and supplements in fruiting bodies (no), stipe length (mm), stipe diameter (mm) and cap diameter (mm) of mushrooms at third harvest.*

Treatment	FB3	Stipe length (mm)	Cap diameter(mm)	Stipe diameter(mm)
Banana leaves (47.5%) +Rice straw (47.5%) +Maize flour (5%)	21.00^{bcd}	27.05^{abcd}	56.80^{bc}	7.84^{a}
Banana leaves (100%)	23.50^{ab}	28.82^{abc}	56.01^{bc}	7.56^{a}
Rice straw (95%) + Maize flour (5%)	24.75^{ab}	22.55^{d}	68.73^{a}	5.52^{b}
Rice straw (100%)	$19.00b^{cd}$	24.91^{cd}	49.56^{c}	5.13^{b}
Banana leaves (95%) + Rice straw (5%)	28.50^{a}	23.56^{cd}	50.38^{c}	4.84^{b}
Rice straw (95%) + Rice bran (5%)	16.25^{cd}	32.62^{a}	70.83^{a}	8.08^{a}
Banana leaves(47.5%)+Rice straw(47.5%)+Rice bran(5%)	19.75^{bcd}	26.21^{bcd}	61.38^{ab}	7.46^{a}
Banana leaves (95%) + Rice Bran (5%)	22.50^{abc}	31.12^{ab}	54.74^{bc}	5.58^{b}
Banana leaves (95%) + Maize flour (5%)	14.75^{d}	25.92^{bcd}	49.91^{c}	8.01^{a}
Grand Mean	21.11	27.07	57.59	6.67
LSD	7.2	5.97	9.91	1.85
CV (%)	23.52	15.31	11.84	19.14
F test	*	*	***	**

***: Significant at 0.1% level of significance, **: Significant at 1% level of significance, *: Significant at 5% level of significance. FB2: fruiting bodies at third harvest, LSD: least significant difference, CV: coefficient of variation. The letters a-f indicate the least significant difference (LSD) test rankings; ‘a represents the highest, followed by b to f. The similar letters represent that the means are significantly at par with each other.

Effect of different substrates and supplements on Total Yield and Biological Efficiency.

There were notable significant differences ($p<0.001$) between treatments in terms of total yield and biological efficiency (Table 5). Several treatments were examined for both biological efficiency and total yield. The treatment that produced the highest total yield (1.94 kg) and biological efficiency (120.5%) was banana leaves (100%). This indicates the superior performance of banana leaves in both productivity and resource utilization. Among all treatments, the lowest yield was obtained in banana leaves (47.5%) + rice straw (47.5%) + rice bran (5%) (1.34kg), whereas the lowest biological efficiency was observed in rice straw + rice bran (52.28), under similar environment and cultural practices among other substrates.

Table 5. *Effect of different substrates and supplements on Total Yield and Biological Efficiency.*

Treatment	Total yield (kg)	Biological efficiency
Banana leaves (47.5%) +Rice straw (47.5%) +Maize flour (5%)	1.612^{de}	72.3^{cd}
Banana leaves (100%)	1.94^{a}	120.5^{a}
Rice straw (95%) + Maize flour (5%)	1.64^{cde}	62.5^{d}
Rice straw (100%)	1.89^{ab}	75.6^{c}
Banana leaves (95%) + Rice straw (5%)	1.66^{bcd}	97.6^{b}
Rice straw (95%) + Rice bran (5%)	1.37^{f}	52.2^{e}
Banana leaves(47.5%)+Rice straw(47.5%)+Rice bran(5%)	1.34^{f}	60.3^{e}
Banana leaves (95%) + Rice Bran (5%)	1.42^{ef}	77.7^{c}
Banana leaves (95%) + Maize flour (5%)	1.86^{abc}	100.4^{b}
Grand Mean	1.64	79.8
LSD	0.24	10.8
CV (%)	10.02	9.3
F test	***	***

***: Significant at 0.1% level of significance, LSD: least significant difference, CV: coefficient of variation, The letters a-f indicate the least significant difference (LSD) test rankings; 'a' represents the highest followed by b to f. The similar letters represent that the means are significantly at par with each other.

DISCUSSION

Oyster mushrooms can be produced in various crop residues, either as a main substrate or in combination with supplements [21]. While Peng et al. [22] reported a higher number of fruiting bodies in supplemented substrates due to additional nutrients, but our findings were quite different. Although, supplementation accelerated pinhead formation in some combinations of substrate. Overall biological efficiency (BE) was observed highest when banana leaves were used alone, suggesting that substrate quality, rather that supplementation alone, is important. A good harvest of *P. ostreatus* was observed 3-4 weeks after incubation [10]. In our results, harvest times are not consistent with their results, which might be related to environmental differences. However, the colonization period (2-3weeks) was similar to Shah et al. [23], suggesting that the substrate is suitable for rapid mycelial growth. Pinhead formation took place between 23-27 days. This result is in agreement with Abid [24] who observed the pin head formation time as 23-27 days.

White-rot fungi *(Pleurotus ostreatus)* prefer substrates with higher cellulose/lignin ratios. A study by Neupane et al. [25] also reported that banana plant fibers are high in cellulose, making their breakdown by mushrooms easier. We discovered that banana residues (leaves and stalks) had much greater cellulose levels and a higher total breakdown rate, which is regarded as vital proof of Pleurotus ostreatus sensitivity to banana waste. It can transform low-quality biomass into higher-quality human food. Mondal et al. [26] found similar results according to him, the presence of the right proportion of alpha-cellulose, hemicellulose, and lignin is responsible for longer stipe length, stipe diameter, and cap diameter in banana leaves. Our study also confirms that banana leaves are effective substrates.

Tesfay et al. [27] found higher yield in banana leaves which may be due to the high-water holding capacity of banana leaves making it a beneficial nutrient substrate. In our result, this attribute most likely led to highest total yield and biological efficiency when banana leaves were

utilized as the only substrate. Ashraf et al. [21] came to similar conclusions as our findings that substrates rich in cellulose are good materials for mushroom growing which could explain the tendency toward higher productivity shown when Banana Residues were used alone. The report by Hamed et al. [28] also concluded that banana leaves have the potential to convert low-quality biomass into higher-quality human food. It provided *Pleurotus spp.* with the optimal nutrients required, resulting in a high yield with combined high BE and good quality mushrooms. Our results for overall yield and biological efficiency are highly consistent to theirs.

Overall, the results obtained confirm that cellulose rich substrates, like banana leaves are excellent for Pleurotus production. The ability of Pleurotus ostreatus to convert banana residues into high-quality edible biomass highlights its importance for sustainable agriculture and food security.

CONCLUSION

The best treatment is banana leaves; they consistently perform well in terms of biological efficiency, total yield, stipe diameter, and length. While rice straw + rice bran performs well in terms of cap diameter and stipe diameter, yield and efficiency are lacking. The combination of banana leaves + rice straw + maize flour exhibits good stipe diameter and great performance in FB2 and cap diameter. The least successful treatment, according to experiment, was a mixture of 95% rice straw and 5% rice bran, which showed poor values for measures. Even while certain supplements might enhance specific development factors, the most efficient way to achieve the greatest overall results in mushroom growing is to use only 100% banana leaves without any additives. Stipe length, stipe diameter, total yield, and biological efficiency were all exceeded by banana leaves. Rice bran is effective in certain metrics; however, it shows low performance in yield and biological efficiency. Whereas maize flour performs moderately well, but shows low values in different measurements. Based on the results, farmers in Nepal are encouraged to use banana leaves as the main substrate for *Pleurotus ostreatus* cultivation because of their high availability and biological effectiveness. Also, policymakers may support their use through local training and agro-waste utilization programs.

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