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ORIGINAL RESEARCH ARTICLE

Evaluation of local substrates as rice straw alternatives for oyster mushroom (*Pleurotus ostreatus*) cultivation in resource-constrained Darchula, Nepal

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ABSTRACT

Oyster mushroom cultivation, though cost-effective, faces constraints due to seasonality and substrate availability. An experimental study was conducted in the resource-constrained Darchula district of Nepal from February to May 2022. The aim was to identify and recommend economically sustainable alternatives to rice straw for oyster mushroom production using local substrates in regions with limited resources. Six treatments; rice straw (T1), banana leaves and pseudostem (T2), maize cob (T3), sawdust (T4), grass (*Eulaliopsis* sp.) (T5), and spent mushroom substrates (T6); were employed in a completely randomized design with four replications. Statistical analysis of growth and yield parameters revealed significant results (P values ranging from P>0.001 to P>0.05) across all parameters. The maize cob treatment exhibited a shorter spawn run period (20.50 days) and the earliest pinhead formation (25 days). The highest total yield (3.14 kg) across three flushes was obtained from paddy straw, followed by T2 and T5, yielding 2.05 kg and 1.43 kg, respectively. Sawdust, despite its larger stalk (1.23 cm) and pileus diameter (7.72 cm), had the lowest production (0.63 kg). Maximum biological efficiency was recorded for T1 (139.63%), followed by T2, T5, and T3, respectively. Economically, T1 resulted in the highest gross margin per 10 kg of substrate (NRs.1845.22) and the highest B:C ratio (2.51), followed by T5 and T2. These findings highlight the promise of locally abundant substrates such as banana leaves, pseudo stems, *Eulaliopsis*, and maize cobs as economically viable alternatives to rice straw in regions with limited straw availability or unsuitable climates for rice cultivation.

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INTRODUCTION

Mushrooms, classified under the Basidiomycetes or Ascomycetes class, are macro fungi with distinctive epigeous or hypogeous fruiting bodies (Miles and Chang, 1997). Edible mushrooms are macro fungi with unique, toxin-free fruiting bodies (Rabiya, 2019). Due to their appealing flavor and rich nutrient content, mushrooms are recognized as an excellent source of food (Chang, 2018). Mushroom production is considered a highly profitable agricultural enterprise, thereby holding significant economic potential in many emerging and industrialized nations. Asia dominates global mushroom production, contributing approximately 92.9% of the total output, with China leading as the world's largest mushroom-producing country, accounting for an annual production of 41,126,850 tons (Shahbandeh, 2023). Oyster mushrooms (*Pleurotus ostreatus*), a significant member of the Basidiomycetes class, Hollobasidiomycetidae subclass, and Agricals order, are celebrated for their exceptional taste and flavor. Originating in China, these mushrooms are now cultivated worldwide, except in the northwest Pacific region (Shah *et al.*, 2004; Piska *et al.*, 2017). Oyster mushrooms stand out due to their resilience against biotic agents compared to other mushrooms, shorter production cycles, reduced raw material requirements, and remarkable medicinal properties. They possess strong anti-inflammatory and immune-modulatory attributes, coupled with abundant nutrient content. As a result of these characteristics, oyster mushrooms have gained global popularity for cultivation, offering a solution to the urgent malnutrition issue (Pathmashini *et al.*, 2008; Tavarwisa *et al.*, 2021). Furthermore, a recent study by Lee and Cho (2021) in Korea highlights the preference for oyster mushrooms as a superior nutrient source with minimal impact on greenhouse ecosystems. Their findings support the notion that oyster mushrooms provide valuable nutrients with a minimum impact on the environment. Oyster mushrooms play a crucial role in promoting medical and health benefits as they are a significant source of protein, vitamins, minerals, fiber, vitamin B, essential amino acids, and antioxidants like selenium. These components not only boost the immune system but also safeguard body cells from oxidative stress, mitigating the risk of chronic diseases (Sadler, 2003). Additionally, these mushrooms also contain both high-and low-molecular-weight carbohydrates (Zhou *et al.*, 2016).

The nutrient requirements for mushroom growth, including carbon and nitrogen sources and various inorganic substances, vary depending on the mushroom type and the substrate used. Substrates, which are raw materials undergoing microbial decomposition to produce essential nutrients for fungal growth, are typically organic materials like lignocellulosic farm wastes such as sawdust, rice bran, wheat bran, and wheat straw (Jongman *et al.*, 2018). Increased temperature and microbial activity have been shown to enhance the enzymatic activity and digestibility of the substrate by up to 77%. This process also reduces the cellulose and hemicellulose to lignin ratios by 9% and 19%, respectively (Vajna *et al.*, 2010). Both carbon and nitrogen play pivotal roles in fungal (mushroom) growth, with the optimal substrate's carbon-to-nitrogen (C: N) ratio varying among different fungal species. Bellettini *et al.* (2019) identified the C:N ratio range of 28:1 to 30:1 as optimal for achieving peak yields. While elevated nitrogen levels can expedite hyphal growth, they might also hinder the development of fruiting bodies (Tsegaye, 2015). According to Sharma *et al.* (2013), substrates that are rich in carbon and low in nitrogen content are particularly suitable for oyster mushroom growth. This is why a wide range of organic materials containing cellulose, hemicellulose, and lignin can be used as mushroom substrates. Examples of such substrates include rice and wheat straw, maize cobs, banana leaves, and pseudo stems, as well as sawdust from pine and other sources (Shah *et al.*, 2004; Rani *et al.*, 2008; Ahmed *et al.*, 2009; Sánchez, 2010; Buah *et al.*, 2010; Samuel and Eugene, 2012). *Pleurotus ostreatus* employs its lignocellulolytic enzymes to effectively break down lignocellulosic materials into potential substrates, converting them into usable carbohydrates that serve as a vital energy source for the fungus (Gateri *et al.*, 2009; Ali *et al.*, 2018).

Mushroom cultivation offers a practical approach for effectively managing agricultural and agro-industrial byproducts through bioconversion processes. This practice holds potential as a labor-intensive agro-industrial endeavor, creating avenues for income and employment, especially among women and youth in developing countries (Girmay *et al.*, 2016). The rapid growth rate of these mushrooms positions them as viable options for short-term agricultural enterprises, delivering immediate advantages to local communities. However, constraints on mushroom production in a naturalistic setting primarily revolve around the time of year and the availability of substrates or raw materials. Raut's (2019) assessment of Nepal's mushroom industry reveals several critical research gaps and pressing needs that warrant immediate attention. Despite possessing essential elements for mushroom production, such as low-cost labor, favorable climatic conditions, and abundant supplies of raw substrates, the industry remains in its infancy. The challenges identified include a lack of robust scientific research on mushrooms and a failure to adopt improved technology. Additionally, the unsuitability of rice production in higher regions (Castagnetti *et al.*, 2021), leading to the escalating prices of raw materials, especially rice straw, poses a significant obstacle to growth. To address these challenges and bridge the research gap, there is a compelling need for comprehensive research initiatives. One promising avenue is the exploration of alternative organic waste materials that align with local needs and agro-climatic conditions.

Therefore, addressing the identified challenges in Nepal's mushroom industry necessitates a concerted effort to diversify raw substrates and incorporate suitable technology. The proposed research seeks to contribute to this endeavor by exploring local, sustainable alternatives that can propel the industry forward while considering the unique agro-climatic conditions and resource availability in Nepal. This research endeavor could involve the utilization of readily available resources such as banana pseudo stems and leaves, sawdust, maize cobs, rice straw, and *Eulaliopsis species*. By assessing the suitability of these materials as substrates for oyster mushroom cultivation, this research aims to not only reduce dependence on expensive rice straw but also optimize mushroom growth and yield, ultimately establishing viable mushroom cultivation in the region to benefit the local community's well-being.

MATERIALS AND METHODS

Research site

The research was conducted in the Darchula district of Sudurpaschim province, located at coordinates 29°50'44" north and 80°32'43" east, from the 4th week of February to the 3rd week of May 2022. The experimental study was carried out under the supervision of the Agriculture Knowledge Center (AKC) in Darchula, within a temporary mushroom tunnel house. Figure 1 depicts the weekly temperature and relative humidity (RH) levels observed within the experimental tunnel during the experimental period.

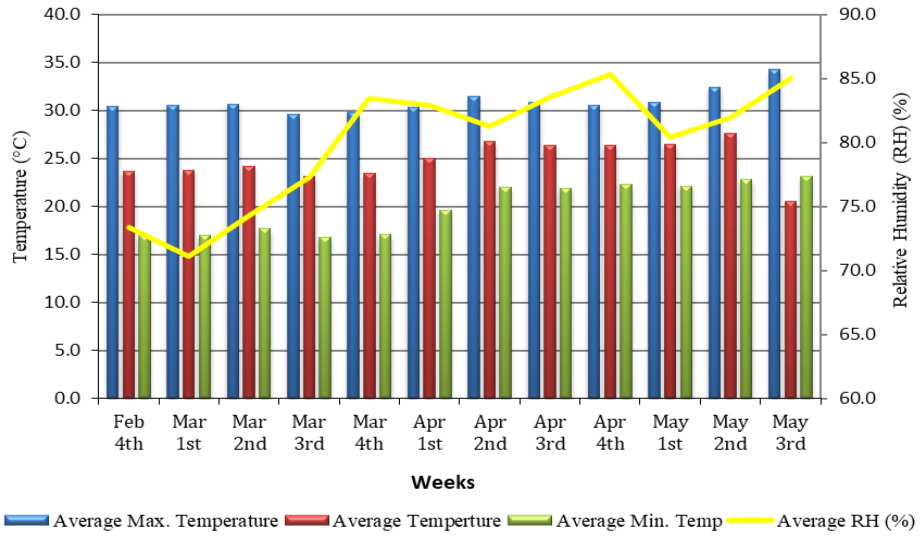

Figure 1. *Weekly temperature (minimum, maximum, and average) and relative humidity recorded inside the experimental tunnel from the 4th week of February to the 3rd week of May, 2022.*

Table 1. List of substrate materials used as treatments, their composition, dry weight, and soaking duration.

Treatments	Substrate materials	Dry weight (kg)	Soaking duration
T1	Rice straw	9	Overnight
T2	Banana leaves and pseudostem	9 (3:1) (6.75kg:2.25kg)	Overnight
T3	Maize cob	9	2 days
T4	Pine sawdust	9	2 days
T5	Grass: "Gajjo" (*Eulaliopsis* sp.)	9	Overnight
T6	Spent Mushroom Substrate supplemented with 1.27 kg of wheat flour and 0.9 kg of sugar	9	Overnight

Selection of species and substrates for mushroom production

Oyster mushroom (*Pleurotus ostreatus*) spawns grown on wheat grains were obtained from the Regional Agriculture Training Center in Sundarpur, Kanchanpur. Six treatments were designated as follows: T1 (rice straw), T2 (banana leaves), T3 (maize cobs), T4 (pine sawdust), T5 (abundant grass, *Eulaliopsis* sp.), and T6 (spent mushroom substrate), as presented in Table 1. The substrates necessary for the experimental study were sourced from various localities. Rice straw was gathered from the rice cultivation areas of the district; dried banana leaves were collected from nearby farmers' banana orchards; maize cobs were acquired from a village called Tigram; pine sawdust was obtained from nearby furniture sawmills; and *Eulaliopsis* sp., a locally abundant grass commonly called "gajjo," was sourced from nearby farmers' fields. The spent substrate, consisting of rice straw that had undergone four previous harvests and was on the verge of disposal, was collected from previously cultivated substrates within the same mushroom house at AKC. The widespread availability of *Eulaliopsis* grass within the region primarily drove its selection as a substrate for oyster mushroom production, effectively addressing the issue of limited access to conventional substrates like rice straw. Its local abundance not only provides a consistent and cost-effective source for mushroom cultivation, benefiting local farmers, but also reduces dependence on more expensive alternatives. Moreover, *Eulaliopsis* grass is not restricted to the study area; it is found across several Asian countries, including China, Nepal, India, Pakistan, Bhutan, Myanmar, Thailand, Malaysia, and the Philippines (Qingfeng, 1993; Sahu *et al*., 2010). This extensive geographical distribution implies that the potential benefits of using *Eulaliopsis* grass as a substrate for mushroom production extend beyond the study area region, offering practical advantages to mushroom cultivators in multiple countries. Similarly, incorporating spent mushroom substrate as a treatment aims to maximize resource utilization to its full capacity. Typically, after completing the initial four harvests, farmers were reported to either burn the leftover spent mushroom substrate or use it as a smoke generator in their farm sheds to ward off animal flies. However, supplementing such spent substrates with additional nutrient resources for subsequent cycles of mushroom production could potentially facilitate the exploration of their resource efficiency to its fullest extent.

Experimental design, substrate preparation, spawning, and cultivation

Six treatments were organized in a one-factor Completely Randomized Design (CRD), with a total of four replications per treatment. The substrates were chopped into pieces roughly 3-5 cm in size (corn cobs were granulated to about 0.5 cm) and then dried for two days under direct sunlight to eliminate moisture. The dry substrates were weighed, with a consistent quantity of 9 kg (dry weight) selected for each substrate under investigation. Within each substrate treatment, there were four replications, each weighing 2.25 kg (dry weight). These substrates were then soaked in water for the durations specified in Table 1.

The following day, the soaked substrates underwent thorough rinsing with clean water, repeating the process 3–4 times until the water ran clear. Subsequently, the excess water was either pressed out of the substrates or allowed to drain until reaching the desired moisture level, thus preparing them for the sterilization phase. Steam sterilization was performed using a specialized drum setup. Inside the drum, a water level of 4–6 inches was maintained, and a perforated round tin plate was placed just above the water surface, supported by a stand. Moist substrates were then carefully layered into the drum, with the layers separated using jute sacks. The drum's upper opening was sealed using a plastic sheet fastened to the rim, and a fire was ignited underneath. This process involved steaming for 2 hours at temperatures exceeding 100°C, effectively eradicating all insects and wild fungi. After the sterilization process, the substrates were allowed to cool by being spread on a sanitized plastic mat. Once the temperature had dropped to approximately 25°C, the substrates were deemed ready for the filling stage. The substrates were carefully packed into plastic bags measuring 20 inches in length and 8 inches in width, with a moisture content ranging from 60% to 70%. Spawning was carried out using a single generation of wheat grain spawn at a rate of 9% based on dry weight, distributed across four layers. For each replication of the mushroom ball, 200 g of spawn was evenly distributed in portions of 50 g across each layer. Each substrate-spawned bag was tightly closed, ensuring that its shape became circular, punctured with 12 holes for aeration, and then transferred to a completely sterilized, dark tunnel. The average temperature inside the experimental tunnel ranged between 20 °C and 27 °C, with the relative humidity maintained between 71 and 85%.

Sample and sampling technique

A total of 24 mushroom bundles (balls), each consisting of 2.25 kg of substrate by dry weight, were prepared. These mushroom balls were considered samples for the study. Data for each parameter under consideration were recorded from every replication and later averaged for the analysis.

Incubation and mycelium run

Each bundle was individually suspended on bamboo frames using plastic ropes. The room was kept in darkness and sealed until the mycelium had fully colonized the substrate. This process took around 20–29 days, during which nearly all substrate bundles became covered with mushroom mycelium. Once the white mycelium had developed, the room was ventilated to facilitate air circulation. Upon completion of the mycelium colonization, the outer plastic covering of each bundle was removed using a sterilized blade, making a D-shaped cut. Throughout the mycelium colonization phase, the average relative humidity and temperature were maintained at around 75% and 24–25°C, respectively.

Harvesting

Harvesting was carried out when the fruiting bodies reached full size, which was determined by observing the downward curling of the margin. The harvesting process was executed with the utmost care, limited only to fully mature fruiting bodies. The process involved delicately twisting and gently pulling the stalk upwards, ensuring neighboring fruiting bodies remained unaffected.

Data collection and analysis

Prior to harvest, various growth behavior parameters, such as the spawn run period, days to pinhead formation, and days to the first harvest, were collected from each replication. After harvest, yield-related characteristics, including total yield from the first, second, and third flushes, along with the average pileus diameter of the fruiting body, average stalk diameter, and average stalk length, were recorded. The raw data recorded were input into MS Excel, processed, and subsequently analyzed using R-Studio. A comprehensive analysis was performed, including an analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) to facilitate mean separation. The differences among the means were tested using the least significant difference (LSD) at a 5% level of significance (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The production of oyster mushrooms was assessed across six distinct substrate types. These substrates exhibited significant variations in parameters such as spawn run period, days to pinhead formation, days to first flush harvesting, stipe length, stipe diameter, pileus diameter, and total yield. The results obtained from the research work are presented in Tables 2-5.

Spawn run period

The spawn run period represents the interval between spawning and the initial emergence of pinheads, denoting complete mycelium colonization of the substrate. The duration of this period varies with substrate type, environmental factors, and spawn quantity, with a shorter period indicating an earlier production start. This parameter was assessed by tracking the time from packaging to complete substrate bundle colonization, which was significantly affected by the substrate type used in the experiment. Among the substrates examined, maize cob exhibited the earliest spawn spread (20.50 days), followed by spent mushroom substrate (21.75 days), pine sawdust (22.50 days), rice straw (23.00 days), *Eulaliopsis* sp. (25.00 days), and banana leaves (28.00 days). This variation in spawn run period aligns with the findings by Yang *et al.* (2013), who attributed it to the influence of substrate-specific chemicals. A pronounced mycelium spread, especially in pine sawdust, might be due to increased moisture retention from a 2-day soak, leading to a shorter spawn run period. Pathmashini *et al.* (2008) also demonstrated shorter mycelium run periods in sawdust, consistent with our study. The higher mycelium running rate in rice straw could be attributed to its balanced presence of alpha-cellulose, hemicellulose, and lignin (Mondal *et al.*, 2010). However, unlike our findings, they reported faster colonization and harvesting with banana leaves, potentially due to differences in substrate composition and soaking duration before sterilization. Rice straw's greater water-holding potential (Rangel *et al.*, 2006) might have facilitated an early spawn run in T1 compared to T2.

Table 2. Effect of different substrates on spawn run period, days to pinhead formation, and days to first harvesting of *P. ostreatus* in Darchula, 2022.

Treatments	Spawn run period (days)	Days to pinhead formation	Days to first harvesting
Rice straw	23.00^{c}	25.75^{c}	35.25^{c}
Banana leaves and pseudostem	28.00^{a}	33.00^{a}	40.00^{a}
Maize cob	20.50^{d}	25.00^{c}	35.50^{bc}
Pine sawdust	22.50^{c}	25.75^{c}	35.00^{c}
Local grass (*Eulaliopsis* sp.)	25.00^{b}	28.25^{b}	37.50^{b}
Spent mushroom substrate	21.75^{cd}	27.25^{b}	34.00^{c}
LSD (0.05)	1.333	1.262	2.12
SEm (±)	0.183	0.173	0.292
F-probability	<0.001	<0.001	<0.001
CV (%)	3.833	3.090	3.946
Grand mean	23.42	27.50	36.21

Treatment means separated by DMRT and columns represented with different letter (s) are significant based on DMRT P = 0.05. *** Significant at 0.001 P value, LSD: Least Significant Difference, SEm: Standard error of the mean deviation, CV: Coefficient of Variance.

Table 3. Effect of different substrates on stipe diameter, stipe length, and diameter of pileus *P. ostreatus* in Darchula, 2022.

Treatments	Stipe Diameter (cm)	Stipe Length (cm)	Pileus Diameter (cm)
Rice straw	0.97^{b}	5.63^{a}	6.69^{abc}
Banana leaves and pseudostem	0.99^{b}	4.52^{b}	7.43^{ab}
Maize cob	0.97^{b}	4.85^{b}	6.51^{bc}
Pine sawdust	1.23^{a}	3.71^{c}	7.72^{a}
Local grass (*Eulaliopsis* sp.)	0.67^{c}	4.52^{b}	6.50^{bc}
Spent mushroom substrate	0.93^{b}	4.43^{b}	5.79^{c}
LSD (0.05)	0.233	2.10	1.135
SEm (±)	0.032	0.090	0.156
F-probability	<0.01	<0.001	<0.05
CV (%)	16.404	9.57	11.29
Grand mean	0.96	4.61	6.77

Treatment means separated by DMRT and columns represented with different letter (s) are significant based on DMRT P = 0.05. *** Significant at 0.01, 0.001 or 0.05 P value, LSD: Least Significant Difference, SEm: Standard error of the mean deviation, CV: Coefficient of Variance.

Pinhead formation

The findings revealed a significant difference (p<0.001) among the substrates in the time it took from spawning to the first pinhead formation. Maize cob exhibited the earliest pinhead formation (25.00 days), followed by pine sawdust and rice straw (25.75 days), spent mushroom substrate (27.75 days), local grass (28.25 days), and banana leaves and pseudostem treatment (33.00 days). The duration of pinhead formation varies depending on the substrate and is influenced by factors such as moisture content and nutrient availability (Muswati *et al*., 2021; Iqbal *et al*., 2016), a trend also evident in our findings. Variations in the duration of pinhead formation observed across different substrates can be attributed to nutrient and moisture availability. Substrates like maize cob, with faster pinhead formation, might provide favorable conditions for mycelial growth and the initiation of fruiting body formation. Additionally, Shah *et al.* (2004) also reported early mycelium colonization and pinhead formation in sawdust. In contrast to the sawdust and maize cob, both soaked for two days, the delayed pinhead formation in banana leaves and pseudostem might result from their shorter soaking periods, possibly causing slower substrate decomposition and reduced nutrient availability. This could have probably delayed the transition of mycelium to the fruiting stage in T2.

Days to the first flush harvesting

Pleurotus ostreatus displayed a distinct behavior in response to various substrate treatments, significantly affecting the time required for the first flush to reach maturity. Muswati *et al.* (2021) demonstrated that varying substrates result in varying growth rates and maturity periods. Treatment T2 resulted in the longest duration, taking 40 days from spawning, including an additional 7 days after pinhead formation, to reach maturity. This significant difference was observed when compared to all other treatments. In contrast, T6, which utilized spent mushroom substrate, had the shortest time to reach the harvesting stage, requiring 34.00 days from spawning and 6.75 days following pinhead formation. This result significantly differed from T3 and T5, where maturity was achieved in 35.50 days and 37.50 days from spawning, respectively. T6 was statistically comparable to T1 (35.25 days from spawning and 9.5 days after pinhead formation) and T4 (35.00 days from spawning and 9.25 days after pinhead formation). The longer time required for T2 can be attributed to the specific characteristics of the substrate used, which may impact mycelial colonization and fruiting body development. Conversely, T6's faster maturity may be attributed to the presence of residual nutrients in their available form in the spent mushroom substrate. Variances in cropping duration and harvesting time across different substrates may stem from differences in the periods required for pinhead formation and fruiting body maturation, which are, in turn, influenced by substrate composition, as indicated by Tan (1981).

Table 4. Effect of different substrates on yield from different flushes and biological efficiency of P. *ostreatus*.

Treatments	Yield from the 1st flush (kg/bag)	Yield from the 2nd flush (kg/bag)	Yield from the 3rd flush (kg/bag)	Total yield (kg/bag)	Biological Efficiency (BE)
Rice straw	1.65[a]	1.17[a]	0.32[a]	3.14[a]	139.63[a]
Banana leaves and pseudostem	1.04[b]	0.67[b]	0.34[a]	2.05[b]	91.17[b]
Maize cob	0.75[c]	0.23[c]	0.17[b]	1.14[d]	50.84[d]
Pine sawdust	0.34[d]	0.17[c]	0.12[c]	0.63[e]	28.06[e]
Local grass (*Eulaliopsis* sp.)	0.68[c]	0.57[b]	0.18[b]	1.43[c]	63.58[c]
Spent mushroom substrate	0.63[c]	0.24[c]	0.07[c]	0.95[d]	42.11[d]
LSD (0.05)	0.25	0.21	0.05	0.26	11.41
SEm (±)	0.034	0.028	0.007	0.035	1.57
F-probability	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	19.67	27.19	16.83	11.10	11.09
Grand mean	0.8476	0.51	0.20	1.56	69.23

Treatment means separated by DMRT and columns represented with different letter (s) are significant based on DMRT P = 0.05. *** Significant at 0.001 P value, LSD: Least Significant Difference, SEm: Standard error of the mean deviation, CV: Coefficient of Variance.

Table 5. Economics (cost, income, gross margin, and benefit-cost ratio) of different substrates used in the experiment.

Substrate (Treatments)	Cost per 10 kg of substrate (NRs.)	Yield per 10 kg of substrate	Income per 10 kg of substrate (NRs.)	Gross margin (NRs.)	Benefit-cost ratio
Rice straw	1225	13.96	3070.22	1845.22	2.51
Banana leaves and pseudostem	1020	9.11	2004.44	984.44	1.97
Maize cob	640	5.07	1114.67	474.67	1.74
Pine sawdust	523	2.80	616.00	93.00	1.18
Local grass (*Eulaliopsis* sp.)	660	6.36	1398.22	738.22	2.12
Spent mushroom substrate	655	4.22	928.89	273.89	1.42

(1 USD = NRs. 131.40).

Stipe length, stipe diameter, and pileus (cap) diameter

The longest average stipe length was observed in the fruiting bodies cultivated on rice straw substrate (5.63 cm), and this length significantly differed from all other substrates, including maize cob, pine sawdust, banana leaves, local grass, and spent substrate. This finding aligns with the results of an experiment conducted by Neupane *et al.* (2018), who obtained similar results concerning stipe length using various substrates. Another study conducted by Muswati *et al.* (2021) investigated mushroom performance on different substrates and noted that the fruiting bodies of mushrooms cultivated on rice straw exhibited the highest stipe length among all substrates. The largest average stipe diameter was observed in pine sawdust (1.23 cm), which showed statistical significance when compared to all other substrates. Stipe diameters for banana leaves and pseudostem (0.99 cm), rice straw (0.97 cm), maize cobs (0.97 cm), and spent mushroom substrate were statistically similar, with the smallest average diameter of 0.67 cm observed in *Eulaliopsis* grass substrate. Muswati *et al.* (2021) also reported significant variations in stipe diameter among different substrates used in their experimental study, which can be attributed to several factors, including variations in nutrient content, substrate structure, and the interaction between the substrate and mushroom species. The findings also align with Girmay *et al.* (2016), who similarly observed a significantly larger stipe diameter in sawdust compared to other substrates. The average pileus diameter was observed to be greatest in the pine sawdust substrate (7.72 cm), a value that was statistically comparable to T1 (rice straw) as well as the combination of banana leaves and pseudostem (T2). Interestingly, despite producing the largest fruiting bodies, pine sawdust yielded the lowest total yield among all substrates, potentially indicating an early nutrient depletion effect. Conversely, the local grass substrate resulted in the smallest cap diameter (5.79 cm), which might be attributed to the gradual release of nutrients over time. The findings from a study by Dubey *et al.* (2019), exploring the comparative advantages of various substrates for oyster mushroom production, aligned with our results. The authors also concluded that rice straw and banana leaves led to better cap diameter outcomes compared to other substrates. Additionally, the experiment conducted by Hoa *et al.* (2015) reported a significant variance in cap diameter resulting from diverse nutrient formulations combined with different substrate combinations. The marked distinctions in stipe length, stipe diameter, pileus diameter, and overall quality attributes across various substrate treatments strongly indicate that the type of substrate plays a pivotal role in influencing the growth, development, fruiting, and quality of oyster mushrooms. These findings are consistent with those observed by several other researchers, including Chukwurah *et al.* (2013), Tsegaye and Tefera (2017), Besufekad *et al.* (2020), and Onyeka *et al.* (2018), who also documented significant variations among different substrates. Furthermore, it's noteworthy that mushroom size appears to be contingent on substrates with lower levels of cellulose, hemicellulose, and lignin, as these components constitute physical barriers that are challenging to break down in the absence of lignin-degrading enzymes, as elucidated by Sanjel *et al.* (2021).

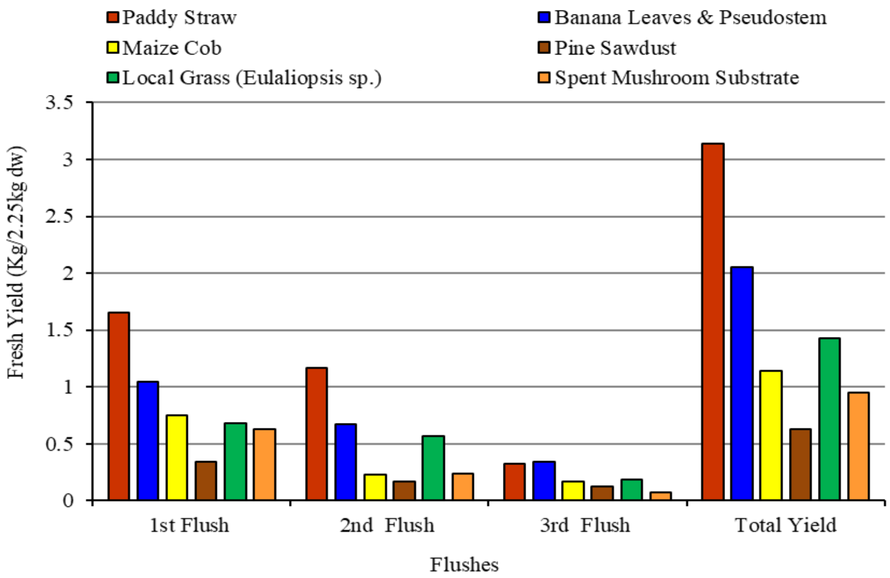

Figure 2. *Total mushroom yield (kg per 2.25 kg of dry weight of substrate) of P. ostreatus across three flushes for each of the six substrates used in the research.*

Yield from the first flush

Significant differences in mushroom yield were observed among the various substrate treatments during the first flush. The treatment with the highest yield, T1 (rice straw), produced 1.65 kg in the initial flush, displaying highly significant variation from the yields of all other treatments. T2 followed with a yield of 1.04 kg in the same period. However, T3 (maize cob) yielded 0.75 kg, which showed no statistical significance compared to the yields of T5 (0.68 kg) and T6 (0.63 kg) during this phase. Conversely, the lowest yield (0.34 kg) during the first flush was obtained in T4 (pine sawdust), possibly due to the limited degradation of lignocellulosic substances present in the sawdust. This finding aligns with Sharma *et al.* (2013) conclusions, where they also observed a low yield in sawdust. They attributed this outcome to the inefficiency of *P. ostreatus* in degrading the lignocellulosic constituents of sawdust. Similarly, Ashraf *et al.* (2013) also reported the highest yield in rice straw during the first flush, attributing it to the rapid degradation and efficient release of nutrients from rice straw in comparison to other substrates.

Yield from the second flush

The yield during the second flush was also found to be significantly influenced by the substrate type used. The highest yield, obtained from T1 (1.17 kg), outperformed all other treatments in terms of yield. This superior performance might be attributed to the continuous and efficient release of nutrients from this substrate compared to others. Conversely, during the second flush, the lowest yield (0.23 kg) was obtained from T4 (pine sawdust), which was statistically comparable to the yields from T3 (0.23 kg) and T6 (0.24 kg).

Yield from the third flush

In the third flush, the yields were considerably diminished across all treatments, potentially due to the progressive nutrient depletion over successive flushes, leading to reduced nutrient availability for subsequent flushes. The highest yield, 0.34 kg, was attained from T2 (banana leaves and pseudostem substrate), displaying no significant difference from the yield of T1 (0.32 kg). Third flush yields from T3 (0.18 kg) and T5 (0.17 kg) showed no significant difference between them, but both of these treatments significantly differed from the yield of T4 (0.12 kg). The lowest yield (0.07 kg) was observed during the third flush of spent mushroom substrate (T6), which was statistically comparable to the yield from T4. The lowest yield in the third flush from the spent mushroom substrate (T6) reflects the diminishing nutrient reservoir in this substrate after previous flushes. This trend corresponds with the study by Cunha Zied *et al.* (2020), which discussed the gradual exhaustion of nutrients in spent substrates.

Total yield

A higher biological yield is the primary goal of mushroom production. The results of the experimental study demonstrated that different substrate treatments had varying effects on yield. The total yield was calculated from the harvests of the first three flushes. The highest total yield, 3.14 kg, was obtained from T1 (rice straw), significantly outperforming all other treatments in terms of yield. Following closely was T2 (banana leaves and pseudo stems), with an average yield of 2.05 kg per bag. In comparison, the total yield from locally available grass (*Eulaliopsis sp.*) substrate significantly surpassed that of maize cob (1.14 kg), spent mushroom substrate (0.95 kg), and pine sawdust (0.63 kg). Importantly, only T1 (rice straw) and T2 (banana leaves and pseudostem) achieved yields greater than the average yield of 1.56 kg across all substrate treatments, with T5 (local grass) yielding close to this mean. These results indicate that rice straw, followed by banana leaves, pseudostem, and local grass, are excellent local substrate sources for oyster mushroom production. The cost-effective *Eulaliopsis* grass demonstrated a significantly higher advantage in total yield, further benefiting from its low production cost. To optimize mushroom production, a substrate with a strong water-holding capacity is essential (Rangel *et al*., 2006). Significantly, rice straw outperforms other substrates in this aspect due to its superior water-retaining ability, which aligns with our findings. Furthermore, the delayed emergence of pinheads had a noteworthy impact on the final mushroom yield on both banana and *Eulaliopsis* substrates, resulting in a significantly lower yield compared to the rice straw substrate. These findings align with the studies conducted by Sharma *et al*. (2013), Neupane *et al*. (2018), and Bhandari *et al*. (2021), all of which reported the highest yields in rice straw among the various treatments. The superior yield of rice straw may be attributed to its ease of sugar extraction from cellulosic materials, as reported by Ponmurugan *et al*. (2007). The faster mycelium growth and higher yield in rice straw could be due to the balanced proportions of alpha-cellulose, hemicellulose, and lignin in rice straw, resulting in an optimal C:N ratio (Mondal *et al*., 2010). Overall, a declining yield trend was observed from the first to the third flush of harvest, as illustrated in Figure 2. These findings are consistent with the research conducted by Philippoussis and Diamantopoulou (2011) as well as Tsegaye (2015). According to Mensah (2015), this trend can be attributed to the decreasing nutrient content within the substrate, which mushrooms consume and utilize during their growth across flush stages. This is a consequence of the mushrooms' lignocellulolytic nature, involving the breakdown and utilization of cell wall constituents. Previous research by Daba *et al*. (2008) suggested that substrates rich in nitrogen, such as cereal husks and sawdust, tend to yield fewer mushrooms compared to carbon-rich substrates. Considering the preference of Pleurotus species for high-carbon and low-nitrogen substrates, rice straw substrate contains abundant carbon-based components such as lignin, cellulose, and hemicellulose while maintaining low nitrogen levels, rendering it an excellent choice (Hasan *et al*., 2010). The lower mycelium running rate and yield in sawdust could be attributed to the presence of various polyphenol compounds (Chang and Quimio, 1982). Furthermore, the results revealed that the yield from spent mushroom substrate was lower compared to all other substrates except sawdust. This difference in yield could potentially be attributed to a decrease in nutrient availability stemming from the prior utilization of maximum nutrients during the previous mushroom production cycle. Conversely, the higher yield observed in the spent mushroom substrate compared to sawdust may be due to the supplementation of additional nutrients such as wheat flour and sugar. These nutrients are relatively more accessible for mycelium growth, as nutrient extraction from such sources is typically easier. This perspective aligns with Oei and Mass's (1996) suggestion that mushroom mycelia require specific nutrients for optimal growth, and the addition of supplements can consequently enhance mushroom yields by providing these essential nutrients.

Biological efficiency

The biological efficiency (BE) of the substrate treatments was significantly influenced by the differences in yield. Substrates yielding higher quantities also demonstrated higher BE values. The highest total yield from the first three flushes and the corresponding highest BE value (139.63%) were achieved with substrate treatment T1, followed by T2 (91.17%), T5 (63.58%), and T3 (50.84%). Conversely, the lowest yield and BE value (28.06%) were recorded for T4 (pine sawdust). Muswati *et al*. (2021) similarly found a direct relationship between substrate yield variations and BE values, with higher-yielding substrates resulting in greater BE values. Sitaula *et al*. (2018) observed a comparable trend, reporting the highest BE value of 96.296% in rice straw and the lowest in combined substrates, which included sawdust. The high BE values in rice straw (T1) and banana leaves and pseudo stems (T2) could be attributed to their successful combination of the substrate's nutrient content, carbon-to-nitrogen ratio (C:N ratio), and structural components like cellulose and lignin, which facilitated mycelial colonization and mushroom development. On the other hand, substrates like pine sawdust (T4) could have had lower nutrient availability due to their low cellulose content and possibly contained inhibitory compounds, resulting in lower yields and BE values (Chang and Quimio, 1982; Mondal *et al*., 2010; Neupane *et al*., 2018). These findings additionally propose that, except for sawdust, all the substrate treatments utilized in this study have the potential to serve as viable alternatives to rice straw for cultivating oyster mushrooms. This assertion gains support from their demonstrated attainment of a biological efficiency (BE) surpassing 40.0%, as stated by Gume *et al*. (2013).

Economics of mushroom production

The mushrooms produced were marketed at an average price of NRs. 220 per kilogram, regardless of the type of substrate used for their production. Gross margin was calculated by deducting the average cost from the average gross return in order to evaluate the profitability of oyster mushrooms grown on various substrates. Oyster mushroom production in rice straw yielded the highest gross margin at NRs. 1,845.22, while the lowest gross margin was observed in spent mushroom substrate, amounting to NRs. 273.89. Similarly, the benefit-cost ratio (B:C ratio) was determined by dividing the total revenue from ten kg of substrate by the cost per ten kg of substrate. Benefit-cost analysis of these substrates revealed that growing mushrooms in rice straw was the most profitable, with a B:C ratio of 2.51. Furthermore, the analysis indicated that cultivating mushrooms in substrates such as local grass, banana leaves, maize cob, spent mushroom substrate, and pine sawdust also proved profitable, with B:C ratios of 2.12, 1.97, 1.74, 1.42, and 1.18, respectively. Bhandari *et al*. (2021) also reported similar findings, highlighting the highest B:C ratio in rice straw among the substrates used in the experiment. Given the limited availability and rising cost of rice straw, locally abundant and cost-effective substrates like banana leaves and pseudo stems, as well as local grass or maize cob, could provide more economically viable and climate-suited alternatives for sustainable oyster mushroom cultivation in the area.

Conclusion

This study represented substantial progress stride in addressing critical gaps within Nepal's mushroom industry by delving into economically feasible alternative substrates for oyster mushroom cultivation through a comparative analysis. The outcomes emphasized the considerable potential of alternative local substrates, such as banana leaves, pseudostem, *Eulaliopsis sp*., and maize cobs, in mitigating the limitations posed by expensive and scarce rice straw, particularly in regions where unfavorable climatic conditions hinder rice cultivation. While yield attributes did not show significant differences among treatments, the economic performance of the substrates varied considerably. Rice straw demonstrated the highest total yield and gross margin, reaffirming its profitability. However, promising alternatives, including banana leaves, pseudo stems, and local grass, exhibited competitive yields and attractive benefit-cost ratios, suggesting their viability as sustainable and cost-effective substitutes. The findings also suggest maximizing resource utilization by supplementing spent rice straw-mushroom substrates with additional nutrient sources. This approach also yielded a positive benefit-cost ratio, supporting a sustainable resource utilization strategy. These findings have important implications for the mushroom industry's growth, as they reduce the reliance on costly rice straw and offer profitable cultivation options, particularly beneficial in resource-constrained regions. Moreover, investigating the potential of substrate combinations and amendments to enhance yield and biological efficiency would be beneficial. Further investigations into the nutritional and sensory qualities of mushrooms grown on different substrates could expand the value proposition of these alternatives.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contribution statement

Krishna Raj Pandey; Conceptualized, designed, and conducted experiments, analyzed data, and led manuscript preparation, review, and submission, Yagya Raj Joshi; Conducted experiments, analyzed data, contributed to writing and reviewing, Prakash Kumar Pant; Sobita Subedi; Sharwari Bhattarai; Dharmendra Joshi; Sushil Khatri; Contributed to experiments and manuscript writing.

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