PERFORMANCE AND NUTRITIONAL ATTRIBUTES OF DIFFERENT SPECIES OF OYSTER MUSHROOM *(Pleurotus spp.)* CULTIVATED UNDER DIFFERENT GROWTH SUBSTRATES

Ashish Ghimire[1*], Sundar Man Shrestha[2] and Gopal Thapa[3]

[1]School of Agriculture, Far Western University

[2]Agriculture and Forestry University

[3]Palika Agro Concern Pvt. Ltd.

[*]Corresponding author email: ashish.ghimire@fwu.edu.np

[*]ORCID: https://orcid.org/0000-0002-4125-8265)

ABSTRACT

Oyster mushroom (Pleurotus spp.) is cultivated on a wide range of substrates. However, the selection of appropriate species and substrate for its cultivation is crucial. This study, conducted in the two-factorial complete randomized design, was aimed at enhancing the performance of oyster mushrooms cultivated between November and March under sub-tropical conditions of Chitwan, Nepal through the selection of species and substrates. Three easily available substrates (paddy straw, water hyacinth, and corn cob) were used to cultivate three different species of oyster mushrooms (P. ostreatus, P. florida, and P. sajor-caju), and several parameters related to mycelial growth and yield were recorded. Water hyacinth was used along with paddy straw in the ratio 3:1 whereas paddy straw and corn cobs were used alone as separate treatments. The least number of days to spawn run completion was obtained from P. ostreatus grown on paddy straw (18 days) which was however statistically similar to several other combinations. The interaction between substrates and species of oyster mushroom showed no significant effect on the days to first harvest. In addition to early spawn run, the maximum Biological Efficiency (118.15%) and Benefit-Cost ratio (2.51) were also obtained from P. ostreatus on paddy straw. Besides, the combination of other species and substrates were also found superior based on nutritional attributes such as fiber, protein, and carbohydrate content. Cultivation of P. ostreatus on paddy straw can be considered optimal for the given conditions considering several growth, yield, and cost efficiency parameters however, some other locally available substrates such as water hyacinth and corn cobs can also be explored considering nutritional value and seasonal unavailability of paddy straw.

Keywords: *spawn, protein, fiber, Pleurotus ostreatus*

INTRODUCTION

Oyster mushrooms (*Pleurotus* spp.) are a group of edible basidiomycete fungi, cultivated and well known for their exceptional lignocellulolytic properties and biological effects attributed by the presence of several important bioactive molecules like phenolic compounds, terpenes, steroids, and polysaccharides (Bellettini et al., 2019; Chakravarty, 2011; Hoa & Wang, 2015). The huge popularity of oyster mushrooms is mainly contributed by their shorter growth period, cheap production, lower disease and pest incidence, wide temperature and chemical tolerance, possession of environmental bioremediation properties, medicinal benefits, and their ability to utilize a wide range of substrates, usually byproducts (Bellettini *et al.*, 2019; Shrestha et al., 2021; Singh et al., 2014; Chang & Wasser, 2017).

Oyster mushroom is the second most widely cultivated mushroom in the world after *Lentinula* sp. contributing around 19% of the world's production (Royse et al., 2017) and an annual production of 0.4 million tons (Raut, 2019). The production and consumption of oyster mushroom has increased steeply in the last decade (Chang & Wasser, 2017).

There are around 40 species of oyster mushrooms under the genus *Pleurotus* (Sidik et al., 2015) and several of these species such as *P. ostreatus*, *P. florida*, *P. eryngii*, *P. pulmonarius*, *P. djamor*, *P. citrinopileatus*, and *P. sajor-caju* are cultivated commercially worldwide. These species differ based on their yield potential, growth performance, temperature tolerance, and morphological characteristics (Patar et al., 2018; Hoa et al., 2015; Uddin et al., 2010).

Similarly, different growth substrates generate variable response in mushroom such as growth, yield, and nutritional composition, mainly due to the difference in nutritional value and quality of substrates (Miah et al., 2017). A wide range of agricultural wastes including cereal straw, cotton wastes, tea wastes, plant fibers, sawdust, banana leaves, sugarcane bagasse, and corn cobs can be used to cultivate oyster mushrooms (Hoa et al., 2015) since they are capable of secreting both cellulase and lignin-degrading enzymes (Chang & Wasser, 2017).

The cultivation of oyster mushroom in Nepal is mainly restrained by the unreliable availability of paddy straw and the lack of appropriate knowledge on the availability of efficient alternative to paddy straw (D. Maharjan, personal communication, February 2022). The use of locally available substrates is an important strategy to reduce the dependence of mushroom growers upon paddy straw in addition to the efficient conversion of non-edible wastes to the edible food. This study was conducted to optimize the production of oyster mushroom in the winter season of tropical Nepal through the selection of appropriate substrate and species.

MATERIALS AND METHODS

Research site

The experiment was carried out at Krishnapur, Chitwan (27.6732° N, 85.4223° E and about 245 meters above sea level) from November 2019 to March 2020. The temperature and RH within the experimental tunnel has been presented in figure 1.

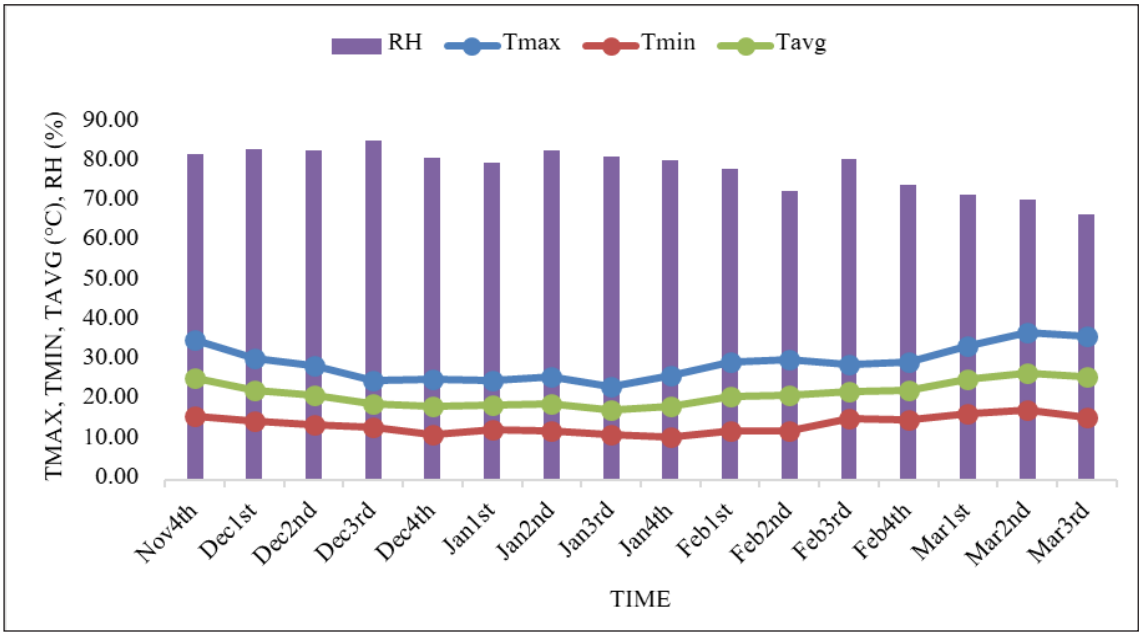


Figure 1. Weekly minimum, maximum, average temperatures, and RH inside the cultivation house during 4th week of November 2019 to 3rd week of March 2020

Source of pure cultures and substrates

Three different species of *Pleurotus* viz. *P. ostreatus* (strain: 8801), *P. florida* (strain: BMRP 136), and *P. sajor-caju* (strain: AX) were obtained from two different spawn producers at Kathmandu valley namely Quality Seed Spawn, Balambu and Mushroom Seed Nepal and Research Center, Lalitpur. Similarly, three different locally available substrates viz. paddy straw, corn cobs, and a combination of paddy straw and water hyacinth (3:1) were collected and used for the experiment.

Spawn production

Spawn was produced at the Central Plant Pathology laboratory at the Agriculture and Forestry University on wheat grains. The grains were cleaned and soaked overnight

followed by boiling for 20 minutes. The boiled grains were supplemented with 2% $CaCO_3$ and excess moisture was reduced to around 60-70% by air drying before packing 200 grams of grains in autoclavable plastics. The mouths were covered with cotton plugs and autoclaved at 121°C and 15 psi for 20 minutes, for 2 consecutive days. The grains were cooled off before inoculating the mycelial discs containing mycelia of individual *Pleurotus* species cultured in PDA media and were finally incubated in a BOD incubator until the mycelia completely colonized the grains.

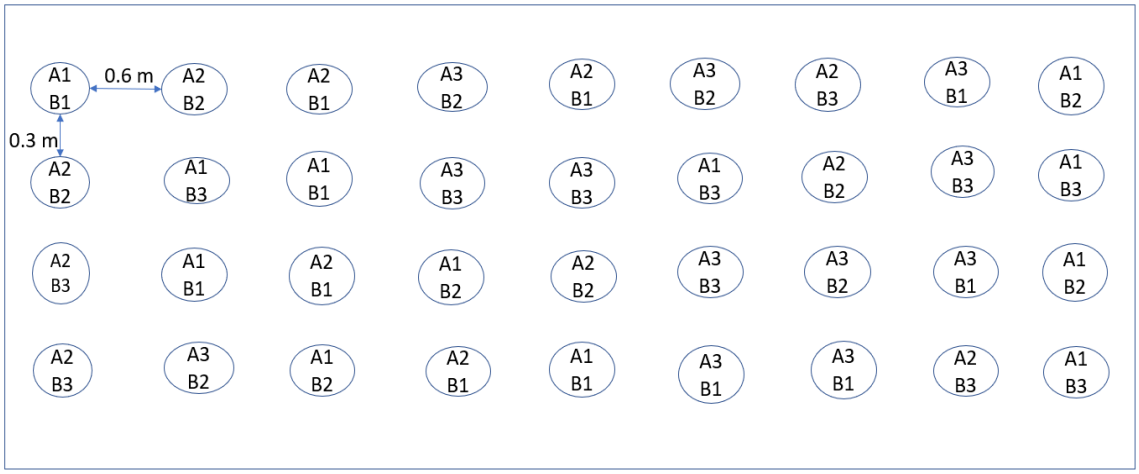
Preparation of substrate and spawning

Substrates were chopped to about 3-5 cm and soaked overnight followed by steam sterilization at temperatures above 100 °C for 2 hours. Then the substrates were left to cool down and packed into plastic bags (30*50cm) at a moisture of 60-70%. 1 kg substrate (dry weight basis) was filled per bag and spawning was done in three layers with a single generation wheat grain spawn at a rate of 7% on a dry weight basis. The bag was finally tied with ropes and 10 pin holes were made per bag before transferring the bags to a dark incubation room.

Experimental design and cultivation

Two factorial Complete Randomized Design (CRD) was adopted as the experimental design. First factor was the species of *Pleurotus* mushroom while the second factor was the substrates used for cultivation of these different species. Both species and substrates were employed at three levels. A total of 4 replications per treatment was maintained.

During the colonization period, the spawned bags were maintained in a dark room ranging between 17°C and 26.5°C whereas the average RH was maintained between 80.67 to 88.5%. After colonization, the bags were transferred to a ventilated room with the exposure to diffused light. An average temperature of at least 21°C and 77% RH was maintained during the cropping period. Regular sprinkling of water during the cropping period for 2-3 times a day was done to maintain higher RH whereas covering of tunnel with jute bags from outside was done to reduce temperature inside the tunnel during hot periods.



A1= P. ostreatus, A2= P. florida, A3= P. sajor-caju, B1= Paddy straw, B2= Paddy straw+ Water hyacinth (3:1), B3= corn cob

Figure 2. Layout of the experiment to test the effectiveness of substrates and species on the growth and yield of oyster mushroom

Harvesting and data recording

Harvesting was done after fruiting body maturity before the fruiting bodies showed any splitting on the edges and after the gills were open. The following parameters were recorded.

A. Growth behavior in days

I. Spawn run period

II. Days to first harvest

B. Yield attribute

I. Biological Efficiency

BE was calculated using the formula:

BE= (wt. of fresh mushroom fruiting bodies) × 100/ wt. of dry substrate

Nutrient analysis

Estimation of moisture content

About 10 g of each sample was weighed separately and then dried in a hot air oven at 105°C with pre-weighed petri-plates till the weight were constant. The moisture content in percentage was expressed using the following formula.

$$\text{Moisture content (\%)} = \frac{(\text{weight of fresh sample} - \text{weight of dry sample}) * 100}{\text{weight of fresh sample}}$$

Estimation of Protein content

Kjeldahl method was used to first calculate the N content which was then multiplied by 6.25 to obtain crude protein content.

Estimation of fiber content

For the estimation of fiber content in the mushroom sample, moisture free and fat free sample (coming out of the process involving ether extraction) was taken. 10 g of the sample was reacted with three different chemicals for different durations. Firstly, the sample was taken in a beaker and 200 ml of boiling 0.255 N H_2SO_4 was added and boiled for 30 minutes. The mixture was then filtered through a muslin cloth and the residue was washed with hot water until it became free from acid. The material was transferred in the beaker and 200 ml of boiling 0.313 N NaOH was added, boiled for 30 minutes and again filtered using a muslin cloth. The residue was again washed with hot water to make the sample free from alkali. The working material was then washed with 15-20 ml ethanol and ether. It was then transferred to a crucible, dried at 80-100°C overnight and weighed. The remnants can only contain fiber and minerals. The sample was then heated in a muffle furnace at 500°C for half an hour to remove fiber and weighed. The difference in the weights before and after heating in a muffle furnace gives the weight of the fiber which is then converted to percentage.

$$\text{Crude fiber (\%)} = \frac{(\text{Dry weight after digestion} - \text{Weight of ash}) * 100}{\text{weight of moisture and fat-free sample}}$$

Estimation of total ash content

A 50 g of fresh mushroom sample was taken in a clean and properly weighed porcelain crucible and then the sample was turned to ash by placing the crucible containing sample in a muffle furnace for 0.5 hours at 600°C. It was then cooled in a desiccator and weighed. To ensure the completion of ash formation, the crucible was again placed in a muffle furnace, heated for half an hour, cooled, and then weighed. This was repeated until two consecutive readings were equal.

Estimation of lipid content

The dried sample (1g) was weighed into a conical flask and extracted with anhydrous ether in the ether extraction set. The ether extract was filtered into another weighed conical flask. The flask containing the original ether extract was washed for around 5 times with small quantities of ether and the washings were also transferred to the filter paper. Ether present in the conical flask was removed by evaporation and the flask with residual was dried in an oven at 80-100°C, cooled in desiccator and weighed the percentage of lipid was calculated using the formula:

Lipid content (g per 100 g of dried sample) =

$$\frac{(\text{weight of ether extract} * \text{percentage of dried sample})}{\text{weight of dried sample taken}}$$

Estimation of carbohydrate content

Carbohydrate content was calculated and expressed in % using following formula: Carbohydrate (g/l00g sample or %) = 100 - [(Moisture + Fat + Protein + Ash + Crude Fiber) g/100g]

Statistical analysis

Data entry was done using MS-Excel. Statistical analysis was conducted using Rstudio. Data were analyzed using Analysis of Variance (ANOVA). Mean comparison was done with Duncan's Multiple Range Test (DMRT). Descriptive analysis was also performed.

RESULTS AND DISCUSSION

Effect of species of oyster mushroom and substrates on mean days to spawn run completion

Spawn run duration was significantly affected by the species, substrates, and their interaction. Among different substrates, corn cob was found to exhibit the least time (19.67 days) to reach mycelial colonization which was statistically at par with paddy straw (19.92 days). Among the species however, *P. sajor-caju* took the longest time (21.58 days) whereas *P. ostreatus* took least time (19.92 days) for mycelial colonization (Table 1).

Table 1. Effect of species of oyster mushroom and substrates on mean days to spawn run completion

Species	**Mean days to spawn run completion**
P. ostreatus	19.92[b]
P. florida	20.50[b]
P. sajor-caju	21.58[a]
Probability	0.004288 **
SEM (±)	0.49
Substrate	**Mean days to spawn run completion**
Paddy straw	19.92[b]
Paddy straw + water hyacinth	22.42[a]
Corn cob	19.67[b]
Probability	2.389e-06 ***
LSD (<0.05)	0.95
CV (%)	5.47
SEM (±)	0.88

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

P. ostreatus grown on paddy straw took the least number of days (18.25) whereas *P. sajor-caju* grown on the combination of paddy straw and water hyacinth took the maximum number of days (23.25). *P. florida* on paddy straw (19.50 days), *P. sajor-caju* on corn cob (19.50 days), and *P. florida* on corn cob (19.25 days) also showed lower spawn run duration, statistically at par with that of *P. ostreatus* on paddy straw (Table 2).

Table 2. Effect of interaction of oyster mushroom species and substrates on mean days to spawn run completion

Species	Substrate		
	Paddy straw	Paddy straw + water hyacinth	Corn cob
P. ostreatus	18.25^{e}	21.25^{bc}	20.25^{cd}
P. florida	19.50^{de}	22.75^{ab}	19.25^{de}
P. sajor-caju	22.00^{ab}	23.25^{a}	19.50^{de}
Probability	0.006709 **		
LSD(<0.05)	1.64		
CV (%)	5.47		
SEM (±)	0.58		

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

Figure 3. Status of spawn running of different species of *Pleurotus* under different substrates on the 15th day of spawning

Under the study, it took 18.25 to 23.25 days for different species under different substrates to colonize mycelium. Kalita (2015) also suggested that the spawn colonization can require up to 3 weeks. A negative linear relationship was observed between the spawn run period and temperature [r^2=0.96] (Kibar & Peksen, 2008). The mean temperature recorded during the spawn colonization duration (21.58 days) of *P. sajor-caju* was 21.95°C. Bano and Rjarathnam (1982) as cited in Kibar and Peksen (2008), also suggested 22-24 days for spawn colonization by *P. sajor-caju* at 15-25°C. However, different strains of *P. sajor-caju* could contribute significantly to the difference in the number of days to complete the spawn run (Kibar & Peksen, 2008). Kibar and Peksen (2008) reported that prolonged spawn run duration under an unheated high plastic tunnel since the experiment was carried out during late autumn and winter seasons and temperature did not exceed 20°C. Similar were the conditions during the progression of our research.

Similar to our findings, Dhakal et al. (2020) also found the least days for spawn run completion (17.5) by *P. ostreatus* on corn cobs, which the researcher suggested could be due to the substrate's higher moisture retention capacity. Corn cobs also have a low percentage of carbon ranging from 47-49 % (Shariff et al., 2016). In contrast, Liu et al. (2014) suggested 1.89% Nitrogen making C:N ratio 24.86 to 25.93 : 1 very suitable for spawn. Hoa et al. (2015) observed the least amount of carbon (39.98%) and C:N ratio (34.57:1) from 100% corn cobs and suggested that a negative correlation existed between C:N ratio and total colonization period.

In contrast to the findings from our study, Shubhra and Jaitly (2011) pointed out lower days to spawn run completion for *P. sajor-caju* in comparison with other oyster mushrooms. The variation in the duration to mycelial colonization could be due to the variation in the strain of oyster mushrooms used. The optimum temperature for *P. sajor-caju* spawn colonization is considered to be around 24-29°C (Stamets, 1993). The variation in recorded and optimal temperature probably describes the effect of temperature on prolongation of spawn colonization. Also, several researches have reported paddy straw to be the best substrate for cultivation of oyster mushroom due to easier nutrient solubilization in cellulosic substances once they start to degrade (Ponmurugan et al., 2007). The earliest spawn run completion of *P. ostreatus* when grown on paddy straw seems to be affected by some intrinsic interaction effect. However, the mean spawn run days achieved from paddy straw and corn cobs are statistically at par. The colonization period is directly related to the rate of uptake of nutrients by the fungus which in turn is greatly affected by the quality of substrate which in turn is determined by the chemical

form, concentration, and availability of nutritional compounds (Kashangura, 2008). In addition, the difference in colonization period of mushroom may also be contributed by the level of production of enzymes such as cellulases, hemicellulases, and ligninases (Oseni et al., 2012). The poor performance of *Pleurotus* spp. on substrate containing PS and WH could be due to the lower amount of carbon sources compared to other substrates (Ruan *et al.*, 2016; Nageswaran *et al.*, 2003).

Effect of species and substrates of oyster mushroom on the days to first harvest

The species and substrates showed significant effect on the duration of first harvest from inoculation whereas the interaction effect of substrate and species on duration to first harvest was found statistically non-significant. *P. sajor caju* showed the least days (46.58) for first harvest which was statistically at par with *P. florida* (51.33) whereas *P. ostreatus* showed the maximum days (54) for acquiring first harvest which was also statistically at par with *P. florida*. Among the substrates, paddy straw showed the least days (45.92) which was statistically at par with corn cob as substrate (46.58). The combination of PS and WH however took the highest number of days (62.17) to reach first economical maturity stage (Table 3).

Table 3. Effect of species of oyster mushroom and substrates on mean days to first harvest

Species	**Mean days to fist harvest**
P. ostreatus	54.00[a]
P. florida	51.33[ab]
P. sajor-caju	46.58[b]
Probability	0.01628 *
SEM (±)	2.17
Substrate	**Mean days to first harvest**
Paddy straw	45.92[b]
Paddy straw + water hyacinth	62.17[a]
Corn cob	46.58[b]
Probability	3.789e-08 ***
LSD (<0.05)	4.96
CV (%)	11.71
SEM (±)	5.80

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

Effect of species and substrates of oyster mushroom on the biological efficiency BE)

Species, substrates and their interaction were all found to significantly affect the yield and consequently BE of oyster mushroom. Among the species, P. ostreatus showed the highest average BE (96.03%) which was followed by P. florida (92.02%). Among substrates, paddy straw exhibited the highest BE (101.72%) followed by the combination of substrates paddy straw and water hyacinth (85.68%) whereas the least BE was obtained with corn cob (80.04%). All of the substrates showed BE that were significantly different from each other (Table 4).

Table 4. Effect of species of oyster mushroom and substrates on the BE (%) of mushroom

Species	**Mean BE**
P. ostreatus	96.03[a]
P. florida	92.02[a]
P. sajor-caju	79.38[c]
Probability	2.785e-09 ***
SEM (±)	5.02
Substrate	
Paddy straw	101.72[a]
Paddy straw + water hyacinth	85.68[b]
Corn cob	80.04[c]
Probability	9.910e-12 ***
LSD (<0.05)	3.78
CV (%)	5.06
SEM (±)	6.49

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

Based on the interaction between substrate and species, the highest BE was obtained from *P. ostreatus* cultivated on paddy straw (118.15%), followed by *P. florida* on paddy straw (99.73%) and *P. florida* on corn cob (89.90%) whereas it was least for *P. sajor-caju* on corn cob (69.28%) (Table 5).

Table 5. Effect of interaction of oyster mushroom species and substrates on the BE (%)

Species	Substrate		
	Paddy straw	Paddy straw + water hyacinth	Corn cob
P. ostreatus	118.15[a]	89.00[c]	80.95[d]
P. florida	99.73[b]	86.43[cd]	89.90[c]
P. sajor-caju	87.28[cd]	81.60[d]	69.28[e]
Probability	3.925e-06 ***		
LSD (<0.05)	6.54		
CV (%)	5.06		
SEM (±)	4.54		

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

Results suggested that the yield of individual species of oyster muhsroom varied from substrate to substrate. Higher biological efficiency of *P. florida* over *P. sajor-caju* was also reported by Patar et al. (2018) when cultivated on wheat straw. However, a study by Shubhra and Jaitly (2011) showed higher BE of *P. sajor-caju* compared to *P. florida* on wheat straw (65.61% and 65.05% respectively). This could be due to the difference in strains of mushroom species as well as substrates used between the studies. Dhakal et al. (2020) reported significantly higher yield of *P. ostreatus* on paddy straw compared to corn cobs when cultivated at an average daily temperature and RH between 12-21°C and 80-90% respectively. The C:N ratio of corn cob is suggested to be 97:1 (Paudel & Dhakal, 2020). Hoa et al. (2015) however suggested the optimum C:N ratio to be 35.7 for *P. ostreatus* and 40.6 for *P. florida* for optimum production whereas Cueva et al. (2017) suggested a C:N ratio of 47.99:1 to be optimum for the growth of *Pleurotus* spp. Higher C:N ratio of substrate compared to recommended value could be the reason for poor performance of *Pleurotus* spp. under corn cob as substrates.

The temperature of the mushroom house throughout the cropping period was low, which could be another reason why *P. ostreatus* exceled other species in terms of yield since *P. ostreatus* produces basidiomes at low temperature (Kashangura, 2008). Patar et al. (2018) also reported higher yield of *P. florida* compared to *P. sajor-caju* on wheat straw. The quality and nutritional value of substrate can significantly lead to variation

in yield of mushroom (Kuhad et al., 1997; Miah et al., 2017). *P. sajor-caju* does best at higher temperatures at around 25°C whereas *P. ostreatus* does best at 12-20°C (Kibar & Peksen, 2008). Throughout the cropping period of *P. sajor-caju*, an average of lower than 20°C temperature was maintained which could be an imporant reason why *P. sajor-caju* failed to perform well in our experiment. The mean average temperature for *P. ostreatus* and *P. florida* were 20.77 and 20.45°C respectively.

On cultivating different species of *Pleurotus* on different substrates, the BE ranged between 69.28% to 118.15%. Atila (2016) observed the BE of oyster mushroom on different substrates to be in the range between 46.9% and 92% whereas Paudel and Dhakal (2020) suggested a range of 50.8%- 106.2% for *P. ostreatus* under different substrates. The BE of *P. sajor-caju* grown on paddy straw obtained from our research was 90.30% which was close to the findings by (Rajak, 2011). High yield of *P. ostreatus* on paddy straw has also been reported by Dhakal et al. (2020). %. A good performance by *P. florida* grown on paddy straw is supported by Chandravanshi et al. (2012). Studies have revealed higher efficiency of the combination of PS and WH compared to PS alone (Nageswaran et al., 2003). In our study however, the combination of PS and WH (3:1) has been found less effective for the growth of each species. *Pleurotus* spp. are mostly cellulolytic meaning they grow well on substrates with higher cellulose content (Bellettini et al., 2019). Since the cellulose content of paddy straw is suggested to be higher compared to water hyacinth, substrates containing only paddy straw might have exhibited better yield. Rajak (2011) find out that maximum yield of *P. sajor-caju* from paddy straw however statistically at par with a treatment combination of paddy straw with other grasses at the ratio of 3:1. Our study also suggested higher yield of *P. sajor-caju* on paddy straw over the aquatic weed, *E. crassipes,* but statistically at par with each other. Water hyacinth originally contains about 95.8% water which can only be reduced to up to 72% after even 15 days with temperatures and humidity 25°C and 68% (Akendo et al., 2008). The higher moisture content of the substrate could be another reason for poor performance under substrates involving the combination of paddy straw and water hyacinth since mushroom mycelium doesn't not grow well in too wet substrates. Since, in addition to environmental effect, BE is strain and substrate-specific, it is possible that certain species can show synergistic effect with specific substrates (Kashangura, 2008; Miah et al., 2017; Shrestha et al., 2021). The BEs from our study can't be compared with those from another study involving different strains of mushroom or substrate composition. However, the present study has identified strains that have shown high yield and BE under specific substrates

that are readily available in Nepal. Paddy straws are widely used as animal fodder in India as well as Nepal and are costly (Chandravanshi et al., 2012). Thus, the substrates involving relatively cheap resources such as water hyacinth can be used in case of poor availability of paddy straw or to reduce the cost of production.

Effect of species and substrates of oyster mushroom on nutritional attributes of fruiting bodies

Protein content

Both the species of oyster mushroom and substrates showed significant effect on the protein content of mushroom fruiting bodies. Among the species, highest mean protein content was recorded from P. sajor-caju (23.42%) followed by P. florida (22.00%) and P. ostreatus (21.86%). The latter two were statistically at par with each other. Among the substrates tested, highest mean protein content was recorded from fruiting bodies grown on corn cobs (27.60%) followed by the combination of PS and WH (22.40%) and paddy straw used alone (17.29%) (Table 6).

Table 6. Effect of species of oyster mushroom and substrates on mean protein content of fruiting bodies

Species	**Mean protein content**
P. ostreatus	21.86[b]
P. florida	22.00[b]
P. sajor-caju	23.42[a]
Probability	0.01049 *
SEM (±)	0.50
Substrate	
Paddy straw	17.29[c]
Paddy straw + water hyacinth	22.40[b]
Corn cob	27.60[a]
Probability	3.219e-13 ***
LSD (<0.05)	1.05
CV (%)	4.74
SEM (±)	2.97

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

Interaction effect between substrates and species also brought significant difference between protein content among treatments. The highest protein content was recorded from *P. florida* grown on corn cobs (28.77%) followed by *P. ostreatus* grown on corn cob (27.54%). The least protein content was recorded from *P. florida* grown on paddy straw (16.62%), statistically at par with *P. ostreatus* grown on paddy straw (17.57%) and *P. sajor-caju* grown on paddy straw (17.69%) (Table 7).

Table 7. Effect of interaction between oyster mushroom species and substrates on the mean protein content of the fruiting body

Species	**Substrate**		
	Paddy straw	Paddy straw + water hyacinth	Corn cob
P. ostreatus	17.57^{d}	20.47^{c}	27.54^{ab}
P. florida	16.62^{d}	20.63^{c}	28.77^{a}
P. sajor-caju	17.69^{d}	26.09^{b}	26.49^{b}
Probability	4.212e-05 ***		
LSD (<0.05)	1.83		
CV (%)	4.74		
SEM (±)	1.59		

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

Fat content

Neither substrate nor species was found to significantly affect the fat content of the mushroom. Similarly, the interaction effect of species and substrate was also not found to affect the fat content of the mushroom fruiting body.

Fiber content

Among species, *P. sajor-caju* was found to have highest fiber content (25.29%) followed by P. *ostreatus* (22.72%). Similarly, among substrates, highest fiber content was obtained from paddy straw as the substrate (25.28%) followed by corn cob (22.39%), and combination of PS and WH (22.07%) (Table 8).

Table 8. Effect of species of oyster mushroom and substrates on mean fiber content of fruiting bodies

Species	Mean fiber content
P. ostreatus	22.72[b]
P. florida	21.72[b]
P. sajor-caju	25.29[a]
Probability	0.007380 **
SEM (±)	1.062
Substrate	
Paddy straw	25.28[a]
Paddy straw + water hyacinth	22.07[b]
Corn cob	22.39[b]
Probability	0.01 **
LSD (<0.05)	2.14
CV (%)	9.29
SEM (±)	1.021

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

The interaction between species and substrates was also found to significantly influence the fiber content of oyster mushroom. Highest fiber content was observed from *P. sajor-caju* on paddy straw (27.10%), statistically at par with *P. ostreatus* on the combination of substrate (25.13%), and *P. florida* on paddy straw (25.07%). Least fiber content was observed from *P. florida* on combination of substrate (16.27%), statistically at par with *P. ostreatus* on corn cob (19.37%) (Table 9).

Table 9. Effect of interaction between oyster mushroom species and substrates on the mean fiber content of fruiting body

Species	Substrate		
	Paddy straw	Paddy straw + water hyacinth	Corn cob
P. ostreatus	23.67[ab]	25.13[ab]	19.37[cd]
P. florida	25.07[ab]	17.20[d]	22.90[bc]
P. sajor-caju	27.10[a]	23.87[ab]	24.90[ab]

Probability	0.002865 **
LSD (<0.05)	3.71
CV (%)	9.29
SEM (±)	1.032

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

Ash content

Species of oyster mushroom showed significant effect on the mean ash content of the fruiting bodies whereas substrates failed to generate significant effect on mean ash content of fruiting bodies. Among the species, *P. sajor-caju* showed the highest ash content (11.32%) whereas *P. florida* showed the least ash content (8.93%) (Table 10).

Table 10. Effect of species of oyster mushroom and substrates on mean ash content of fruiting bodies

Species	Mean ash content
P. ostreatus	9.39^{b}
P. florida	8.93^{c}
P. sajor-caju	11.32^{a}
Probability	6.548e-13 ***
LSD (<0.05)	0.73
CV (%)	2.76
SEM (±)	0.09

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

The interaction between substrates and species also significantly contributed on the difference in mean ash content between treatments. Maximum mean ash content was recorded from P. sajor-caju grown on paddy straw (12.27%) followed by P. sajor-caju cultivated on cob cobs (11.30%) whereas the least from P. florida cultivated on the combination substrate involving paddy straw and water hyacinth (8.50%) (Table 11).

Table 11. Effect of interaction of oyster mushroom species and substrates on the mean ash content of the fruiting body (%)

Species	Substrate		
	Paddy straw	Paddy straw + water hyacinth	Corn cob
P. ostreatus	9.016^{de}	10.33^{c}	8.82^{ef}
P. florida	8.90^{ef}	8.50^{f}	9.40^{d}

P. sajor-caju	12.27[a]	10.40[c]	11.30[b]
Probability	3.907e-08 ***		
LSD (<0.05)	0.47		
CV (%)	2.76		
SEM (±)	0.43		

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

Carbohydrate content

Among the species, *P. florida* showed the maximum carbohydrate content (45.26%), also statistically at par with P. ostreatus (43.95%), followed by *P. sajor-caju* (37.82%) whereas among the substrates, paddy straw showed the highest carbohydrate content (45.21%), statistically at par with the combination of PS and WH (45.18%) (Table 12).

Table 12. Effect of species of oyster mushroom and substrates on mean carbohydrate content of fruiting bodies

Species	**Mean carbohydrate content**
P. ostreatus	43.95[a]
P. florida	45.26[a]
P. sajor-caju	37.82[b]
Probability	0.0001268 ***
SEM (±)	2.29
Substrate	
Paddy straw	45.21[a]
Paddy straw + water hyacinth	43.64[a]
Corn cob	38.17[b]
Probability	0.0002830 ***
LSD (<0.05)	3.07
CV (%)	7.18
SEM (±)	2.13

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

The interaction between species and substrates also showed significant effect on the carbohydrate content of mushroom fruiting body. The highest carbohydrate content was recorded from *P. florida* on the substrate combining paddy straw and water hyacinth

(51.45%) which was statistically at par with *P. ostreatus* on paddy straw (47.81%) and *P. florida* on paddy straw (47.30%). Least carbohydrate content was observed for the treatment involving *P. sajor-caju* and corn cob (35.22%) followed by *P. florida* on corn cob (37.03%) (Table 13).

Table 13. Effect of interaction between oyster mushroom species and substrates on the mean carbohydrate content

Species	Substrate		
	Paddy straw	Paddy straw + water hyacinth	Corn cob
P. ostreatus	47.81^{a}	41.76^{c}	42.26^{bc}
P. florida	47.30^{ab}	51.45^{a}	37.03c^{d}
P. sajor-caju	40.53^{cd}	37.71^{cd}	35.22^{d}
Probability	0.0054709 **		
LSD (<0.05)	5.32		
CV (%)	7.18		
SEM (±)	1.83		

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

The nutritional attributes of mushroom varied with species and substrates used. The mean protein content on the fruiting bodies of different species as suggested by different studies ranged between 21.86% and 23.42% (Kurtzman, 2005; Salami et al., 2017; Hoa et al., 2015). Shubhra and Jaitly (2011) also reported higher average protein content of *P. sajor-caju* compared to *P. florida* grown on wheat straw, similar to our findings. The consistently lower protein content in mushroom fruting bodies grown on paddy straw could possibly suggest that the substrate contained poor level of nutrients contributing to protein content. Among the substrates, corn cob-grown mushrooms showed the highest average protein content whereas paddy straw showed the least. Furthermore, *P. florida* grown on corn cobs showed the highest proetin content that was statistically at par with *P. ostreatus* on corn cobs. Hoa et al. (2015) also reported highest protein of *P. ostreatus* (29.70%) and *P. cystidiosus* (24.54%) grown on corn cobs, along with an increasing trend of protein content with increase in the level of corn cobs in the substrate. Negative correlation exists between protein content of fruiting bodies and C:N ratio of the substrate, which suggests higher protein content from corn cobs is due to higher nitrogen content of the substrate (Hoa et al., 2015). Nageswaran et al. (2003) reported higher N content of 100% paddy straw (4.2%) compared to paddy straw in combination with water hyacinth

at a 3:1 ratio (3.6%) however the protein content in our study was recorded higher for the substrate containing 25% water hyacinth. This could be due to higher N content in the used species of water hyacinth or due to several environmental factors during growth, increasing its N content including higher amount of N in the pond water resulting in higher phytoextraction of N in the plant tissues (Ting et al., 2018). Mshandete and Cuff (2007) found that the protein content of mushroom can be influenced by the substrate used as well as the species and strains employed. Moreover, the nutrient composition of substrate also varies based on season of cultivation and environmental factors during cultivation (Tucker & DeBusk, 1981).

Hoa et al. (2015) presented a significant effect of substrates fat content of mushroom. Our study however found no significant effect of substrates on fat content which could be due to the use of limited substrates leading to no huge differences in fat content among them.

Among the species, *P. sajor-caju* was found to possess highest fiber (25.29%) followed by *P. ostreatus* (22.72%). Hoa et al. (2015) also gave an account a similar range (22-29.75%) of fiber content of *P. ostreatus* when grown on different substrates. *P. ostreatus* however showed lowest fiber content from corn cob contrasting the findings from Hoa et al. (2015) which could be due to the difference in strain used. Miah et al. (2017) however reported only 13.33-14.03% crude fiber content of *P. ostreatus* from different substrates whereas Wang et al. (2001) showed even lower range of carbohydrate content (5.97%-6.42%), which contradicted with our findings. However, these variable results on fiber content from different researches suggest that fiber content is influenced by several factors including substrate composition and nutritional level, strains used, time of cultivation, and nutrient supplementation (Jeznabadi et al., 2016). The substrate combination constituting paddy straw and water hyacinth also showed lower fiber content. Tucker and Debusk (1981) observed that the acid detergent fiber (ADF) content of water hyacinth was lower in winter compared to summer, which could be the reason for the lower contribution of WH as the substrate to fiber content in mushroom fruiting bodies.

None of the researches supported the results of average ash content from our experiment. Our findings suggest higher ash compared to other researches. This could be suggesting that the strains of *Pleurotus* spp. used in our experiment might contain higher minerals such as K, P, Na, Ca, Mg, Cu, Zn, Fe, Mo, and Cd or even higher efficiency to uptake these nutrients. The proportion of minerals present in the fruiting bodies vary according to the species, age, diameter of mushroom and substrates used (Agarwal et al., 2017). Our findings suggested significant effect of species on the ash content of the mushroom. The substrates were unable to generate significant effect on the difference in ash content in spite of the interaction factor showing significant effect. Gogavekar et al. (2014) as cited in Agarwal et al. (2017) reported ash content of *P. ostreatus* (9.36%) close

to our findings. The study also obtained a range of values of ash content from several species of oyster mushroom. Variation could also be observed within the same species. This suggests that various conditions such as substrate quality and environmental factors can also determine ash content (Agarwal et al., 2017).

Various studies have reported varying range of carbohydrate content from oyster mushrooms cultivated under different sources. Different studies have suggested various range within which the carbohydrate level of *P. ostreatus* falls when grown on different substrates (Hoa et al., 2015; Sharma et al., 2013; Patil et al., 2010). From our study, the highest mean carbohydrate content was obtained from paddy straw and the least from corn cobs. Paddy straw contains high amount of carbohydrates and lower amount of lignin (Lee et al., 2017).

Effect of species and substrates on the benefit cost ratio of production

The benefit cost ratio was significantly affected by the species of mushroom and substrates used. The average BC ratio was highest for *P. ostreatus* (2.20) among species, statistically at par with *P. florida* (2.12) whereas it was lowest for *P. sajor-caju* (1.82). Also, among the substrates, highest BC ratio was obtained from paddy straw (2.16) as substrate whereas least for the combination of paddy straw and water hyacinth (1.94 (Table 14).

Table 14. Effect of species of oyster mushroom and substrates on benefit cost (BC) ratio of production

Species	**Benefit Cost ratio**
P. ostreatus	2.20[a]
P. florida	2.12[a]
P. sajor-caju	1.82[b]
Probability	1.984e-09 ***
SEM (±)	0.11
Substrate	
Paddy straw	2.16[a]
Paddy straw + water hyacinth	1.94[c]
Corn cob	2.05[b]
Probability	5.445e-05 ***
LSD (<0.05)	0.08
CV (%)	4.94
SEM (±)	0.06

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

The interaction effect between species and substrates also significantly contributed on the difference between the benefit-cost ratio of production of oyster mushroom. *P. ostreatus* grown on paddy straw showed the highest BC ratio (2.51) followed by *P. florida* on corn cob (2.31). The least BC ratio was obtained from the treatment involving *P. sajor-caju* grown on corn cob (1.78) (Table 15).

Table 15. Effect of interaction between oyster mushroom species and substrates on the benefit-cost ratio of production

Species	Substrate		
	Paddy straw	Paddy straw + water hyacinth	Corn cob
P. ostreatus	2.51[a]	2.01[cd]	2.08[cd]
P. florida	2.11[c]	1.95[de]	2.31[b]
P. sajor-caju	1.85e[f]	1.84[ef]	1.78[f]
Probability	3.939e-06 ***		
LSD (<0.05)	0.08		
CV (%)	4.94		
SEM (±)	0.08		

SEM= Standard error of mean, LSD= Least significant difference, and CV= Coefficient of variation

Nelima et al. (2021) also suggested that oyster mushroom's cultivation suits developing and least-developed countries, accessible with agricultural by-products as substrates. The species of oyster mushroom, substrates and the interaction between substrate and species significantly affected the benefit-cost ratio of production of oyster mushroom. Nelima et al. (2021) found a similar BC ratio (1.97) when water hyacinth was combined with sawdust and used as substrate. Although, water hyacinth was procured free of cost but the extraction of water hyacinth from the pond increased the cost of cultivation. In addition, since only 25% water hyacinth was used whereas rest 75% was paddy straw, the cost of paddy straw was not significantly reduced. Based on the interaction effect, *P. ostreatus* grown on paddy straw showed the highest BC ratio which could be due to the highest yield leading to the highest gross return. Second highest BC ratio was obtained from *P. florida* grown on corn cob (could be due to higher yield and lower cost for the purchase of corn cobs). The least BC ratio was obtained from *P. sajor-caju* on corn cob which could be mainly because of the lowest yield. Farm size also contributes significantly to BC ratio (Acharya & Dhungel, 2021). Smaller scale of

production associated with our study could be the reason for lower BC ratio. Acharya and Dhungel (2021) found a BC ratio of 2.58 from the cultivation of oyster mushroom under paddy straw, close to our findings.

CONCLUSION

This study has tried to identify the optimal conditions in terms of the selection of species and substrates for oyster mushroom cultivation under sub-tropical condition in order to contribute to the enhancement of oyster mushroom production. The study has identified *P. ostreatus* (strain: 8801) grown on paddy straw to be best suited for oyster mushroom cultivation under sub-tropical condition during winter in Nepal in terms of yield, earliness in spawn colonization, and benefit-cost ratio. Furthermore, *P. ostreatus* cultivated on paddy straw has also shown good nutritive benefits suggested by high fiber and carbohydrate content. Substrate combining paddy straw and water hyacinth (3:1) also showed good yield and yield attributing characters for *P. sajor-caju* (strain: AX) under aforementioned conditions. Corn cobs and substrates constituting water hyacinth could also be explored in further research to look for the profitability analysis since these substrates are cheaper than paddy straw.

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