Growth and Yield of Oyster mushroom (*Pleurotus ostreatus*) on different substrates

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ABSTRACT

Cultivation of *Pleurotus ostreatus* on different substrates such as rice straw, rice straw + wheat straw, rice straw+ paper, sugarcane bagasse and sawdust of alder was investigated. All the substrates except rice straw were supplemented with 10% rice bran. The substrate without supplement was considered as control. The effects of various substrates on mycelial growth, colonization time, primordial appearance time, mushroom yield, biological efficiency (BE), size of the mushroom and chemical composition were analyzed. Among all aspects, rice straw (control) was found as a best substrate with yield (381.85 gm) and BE (95.46%) %) followed by rice plus wheat straw, rice straw plus paper waste for the production of mushroom. The nutritional composition was also better from mushroom fruit grown on rice straw.

Key Words: Lignicelloulosic residue, Mushroom Cultivation, *Pleurotus ostreatus*, Yield, Biological efficiency.

INTRODUCTION

Cultivation of oyster mushroom (*Pleurotus ostreatus*) has increased tremendously throughout the world because of their abilities to grow at a wide range of temperature and utilizing various agro-based residues. *Pleurotus* species are efficient lignin degraders, which can grow on different agricultural wastes with broad adaptability to varied agro-climatic conditions (Jandiak & Goyal 1995). Growing oyster mushrooms convert a high percentage of the lignocellulosic substrate to fruiting bodies increasing profitability. Of them, *Pleurotus ostreatus* demands few environmental controls, and their fruiting bodies are not often attacked by diseases and pests, and they can be cultivated in a simple and economic way (Kues & Liu 2000). It requires a short growth time in comparison to other edible mushrooms. All this makes *P. ostreatus* cultivation an excellent alternative for production of mushrooms when compared to other mushrooms (Kausar 1998).

Mushrooms with their flavor, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries (Eswaran & Ramabadran 2000). *P. osteratus* are rich source of proteins, minerals & vitamins (Caglarirmak 2007).

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Apart from food value, its medicinal value for diabetics and in cancer therapy has been emphasized (Sivrikaya *et al*. 2002). *Pleurotus* species contain high potassium to sodium ratio, which makes mushrooms an ideal food for patients suffering from hypertension & heart diseases. The practice of mushroom cultivation not only produces medicinal and nutritious food but also improves the straw quality. This takes place by reducing lignin, cellulose, hemicelluloses, tannin and crude fiber content of straw making it ideal for animal feed (Ortega *et al*. 1992).

Most of the world's poor are in, or employed mainly on family farms. Strengthening mushroom production sector could be essential in order to enable the rural economy to keep its vibrancy and development, increasing and diversifying business and employment opportunities in the rural areas, and providing income opportunities for disadvantageous groups, small family farms. Also, mushroom production gives additional / alternative income to farmers looking for a value-added product and a way to supplement farm income while making use of by products or co-products from other crops. Since mushrooms can be grown on nearly any type of agricultural and forest residues, they are an ideal crop for rural areas with large amounts of cultivated hectare and residue from field crops.

Mushrooms require carbon, nitrogen and inorganic compounds as their nutritional sources and the main nutrients are carbon sources such as cellulose, hemicellulose and lignin. Oyster mushrooms require

less nitrogen and more carbon source. Thus, most organic matters containing cellulose, hemicellulose and lignin can be used as mushroom substrate i. e. rice and wheat straw, cottonseed hulls, corncob, sugarcane baggase, sawdust, waste paper, leaves, and so on. However, demanded amount of each nutritional source differs according to mushroom species and substrate used. The demand of mushroom has been mounting day by day due to population growth, market expansions, changing of consumer behavior, and developments. Rice straw is the principal substrate for oyster mushroom cultivation in Nepal. Hence, in this study, we attempt to identify the alternative substrates from various agricultural and forest residue and to assess the growth performance and yield as well as the nutrient content of *P. ostreatus*.

MATERIAL AND METHODS

Spawn of *P. ostreatus* was obtained from National Agriculture Research Council (NARC), Khumaltar, Lalitpur, Nepal for experiment. Five treatments were prepared for experiment i.e. rice straw, rice plus wheat straw, rice straw plus paper, sugarcane bagasse and sawdust, all agricultural or plant-based residues. Rice straw was taken as control. Except control, other four treatments were supplemented with rice bran. The straws of rice and wheat were obtained from agricultural field of local farmers in Kirtipur and chopped into small pieces (3-2 inches long). Sugarcane bagasse was collected from local juice shop, Kirtipur that was sun dried and chopped into small pieces. The newspaper was collected from house and shredded into small pieces manually. Sawdust of Alder (*Alnus nepalensis*) was obtained from Patan Industrial Area, Lalitpur. After that, wheat straw and newspaper were separately combined with rice straw in the ratio of 1:1 (w:w). Each treatment except rice straw was supplemented with 10% rice bran obtained from local rice mill. The mixture of substrates and supplements were mixed thoroughly. The substrates were soaked in tap water and about 60% moisture was set to each substrate.

One kg wet substrate was filled in the polypropylene bag of 25cm×15cm in size and autoclaved at 121°C at 15 lbs pressure for an hour and allowed to cool overnight. After cooling, about 2.5 % grain spawn were inoculated on the surface of substrate and incubated in a dark at controlled temperature of 20-25°C. After colonization, the plastic bag was removed from the substrate and was placed in the growing room of temperature between 15-18°C, relative humidity 70-80 % and light intensity of 200-500 lux. Mycelium growth, the total time taken for the colonization, primordial formation and first harvest were recorded. Mycelial extension was vertically measured (from four faces) at weekly interval for three weeks. The number and weight of fruit bodies were recorded. Total weight of all the fruiting bodies harvested from two flushes was measured separately and calculated as total yield. The Biological Efficiency (BE) was defined as the percentage ratio of the fresh weight of harvested mushroom over dry weight of substrate (Pokhrel & Ohga 2007). The harvested mushrooms were oven dried and used for nutritional analysis. The ash, protein, fat and fiber contents of the mushroom samples were determined using standard methods (Chang *et al*. 1981, Singh & Pradhan 1981). The total moisture was determined by drying the mushroom to a constant weight at 105°C (Madan *et al*. 1987). Total carbohydrate and energy was calculated by using following equations (Nilsen 2010):

Carbohydrate (%) = [100 – (moisture – total ash – fiber – protein – fat)].

Energy (Kcal/100gm) = [(protein×4) + (Carbohydrate×4) + (fat×9)]

Data were analyzed by using one-way ANOVA. Five replicas were used for each treatment (n=5). Significant differences between treatments were determined by Tukey's B test at ($P< 0.05$).

RESULTS AND DISCUSSION

Five different types of substrates were investigated to determine the growth and yield of *P. ostreatus*. Weekly mycelia extension on different substrates is shown in Table. 1. The fastest mycelia extension was observed in rice straw substrate followed by mixture of rice plus wheat straw, sugarcane bagasse, mixture of rice straw plus paper and sawdust, respectively. Mycelial growth is a preliminary step that creates suitable internal conditions for fruiting. Thus, outstanding growth of mycelium is a vital factor in mushroom cultivation (Pokhrel *et al.* 2009).

Colonization of the substrate was completed in between 22.40-26.00 days of incubation. Similarly primordial initiation on various substrates was also observed in between 26.40-31.60 days of incubation. The total day for the first harvest of mushroom took between 32-37 days, depending on substrate used. The fastest colonization period (22.40 days), primordial formation time (26.40 days) and first harvest period (32.40 days) were also recorded from rice straw. The colonization, primordial initiation and harvest time are presented in Table 2.

The main function of rice straw is to provide a reservoir of cellulose, hemicelluloses and lignin which is utilized during the growth and fructification (Yildiz *et al.* 2002). Therefore, our study also showed the similar results, might be because rice straw contained sufficient amount of necessary nutrients for the growth of *P. ostreatus*. Kumari & Achal (2008) stated that colonization of the substrate was completed within 20 days of inoculation.

Table 1. Comparison of weekly mycelial growth of *P. ostreatus* on different substrates.

Substrates	Supplements	First Week (cm)	Second Week (cm)	Third Week (cm)
Rice straw	Control	6.80±0.07[a]	7.30±0.41[a]	9.20±0.46[a]
Rice straw + Wheat straw	Rice bran	5.80±0.78[ab]	7.00±0.50[a]	7.22±0.38[b]
Rice straw + Paper	Rice bran	5.62±0.68[ab]	5.92±0.25[b]	7.00±0.5[b]
Sugarcane bagasse	Rice bran	5.68±0.62[ab]	6.06±0.18[b]	7.18±0.3[b]
Saw dust	Rice bran	5.34±0.95[b]	5.80±0.40[b]	6.80±0.07[b]

Different letters along the column indicate significant differences of the mean (P = 0.05) according to Tukey's test (mean ± sd, n = 5).

Table.2. Comparison of colonization period, primordial initiation time and mushroom harvest time of *P. ostreatus* on different substrates.

Substrates	Supplements	Colonization period days	Primordial formation days	First harvest days
Rice straw	Control	22.40±1.10[a]	26.40±1.67[a]	32.40±1.67[a]
Rice straw+ Wheat straw	Rice bran	23.20±0.83[b]	28.40±1.51[b]	34.40±2.07[ab]
Rice straw + paper	Rice bran	24.00±0.70[bc]	29.60±0.54[c]	35.40±1.14[bc]
Sugarcane bagasse	Rice bran	24.80±0.83[cd]	30.80±0.83[bc]	36.60±1.14[bc]
Sawdust	Rice bran	26.00±0.70[d]	31.60±1.14[c]	37.80±1.48[c]

Different letters along the column indicate significant differences of the mean (P = 0.05) according to Tukey's Btest (mean ± sd, n = 5).

Table 3. The mushroom yield, size and biological efficiency of *P. osteratus* on different substrates.

Substrates	Total yields (gm)	First flush (gm)	Second flush (gm)	T-test (first and second flash) (gm)	Size of mushroom (gm)	Biological efficiency (%)
Rice straw	381.85±8.36[a]	253.83±6.16[a]	128.02±2.33[a]	t = 35.482 (P < 0.001)	7.15±0.54	95.46±2.09[a]
Rice straw+ Wheat straw	309.29±9.70[b]	191.96±2.27[b]	117.33±8.44[b]	t = 17.070 (P < 0.001)	6.63±0.46	77.32±2.42[b]
Rice straw+ Paper	298.59±6.88[b]	188.60±7.36[b]	110.99±5.38[b]	t = 17.843 (P < 0.001)	6.70±0.28	74.89±1.71[b]
Sugarcane bagasse	268.17±17.45[c]	168.73±13.99[c]	99.44±4.76[c]	t = 10.378 (P < 0.001)	6.47±0.40	67.04±4.36[c]
Saw dust	247.87±26.14[c]	158.91±21.53[c]	88.96±7.03[d]	t = 8.273 (P < 0.001)	6.51±0.45	61.96±6.55[c]

Different letters along the column indicate significant differences of the mean ($P = 0.05$) according to Tukey's B test (mean ± sd, n = 5).

Table 4. Determination of Total Ash, Fibre, Protein, Fat, Carbohydrate and Energy of *P. ostreatus* grown on different substrates.

Sample	Total Ash %	Fibre %	Protein %	Fat %	Carbohydrate %	Energy (kcal/100gm)
Rice straw (Control)	9.73	14	25.97	1.09	42.26	282.73
Rice straw + Wheat straw	7.67	13	25.38	1.03	30.248	231.780
Rice straw + Paper	10.34	12	24.72	1.03	32.650	263.010
Sugarcane bagasse	7.75	12	22.89	1.08	41.577	267.588
Saw dust	8.50	14	23.87	1.50	38.740	263.970

Quimio *et al.* (1999) reported that good harvest of *P. ostreatus* was 3-4 weeks after incubation. In our results, colonization and harvest time are not consistent with their results. Moreover, Shah *et al.* (2004) reported that primordial formation of *P. ostreatus* appears 27-34 days of inoculation which is consistent with the results of this study. The size of fruiting bodies was higher in case of rice straw (control) than in all other substrates. This is probably due to higher degradation of various constituents of the substrate rice straw and consumption of high nutrients by *P. ostreatus.* Average mushroom yield of the two flushes, mushroom size and biological efficiency are given in Table 3.

For each of the treatments, two flushes of mushroom were harvested. The yield was significantly highest obtained in the first flush of all treatments. The study revealed that the yield for rice straw without supplementation was significantly highest yield among all treatments. The mushroom yield was not significantly different from mixtures of rice straw plus wheat straw and the mixture of rice straw and paper. The highest yield (381.85gm) and biological efficiency (95.46%) of this mushroom was obtained from rice straw followed by rice straw plus wheat straw, rice straw plus paper and sugarcane bagasse. The lowest yield 247.87g and biological efficiency 61.96 % was obtained from sawdust. The increase in the yield of mushroom in paddy straw is due to easier way of getting sugars from the cellulosic substances (Ponmurugan *et. al.* 2007). Kumari & Achal (2008) cultivated *P. ostreatus* on different substrates and reported the highest yield on wheat straw, followed by the combination of paddy and wheat straw. But our study results are not similar with their findings. Das & Mukherjee (2007) also found that when weed plants were mixed with rice straw in the ratio 1:1, there is increase in the yield than when used individually. Further, Yildiz *et al.* (2002) mentioned that the mixtures which included the paper generally produced higher yields. But our result is not consistent with the findings of Das & Mukherjee (2007) as well as that of Yildiz *et al.* (2002). Taurachand (2004) reported that sugarcane bagasse contains cellulose and sucrose which is easily degraded by oyster mushroom. It is also rich in nitrogen content. Even though it is rich in cellulose, sucrose and nitrogen, its yield was found low in our experiment in comparison to other substrates. Further, Yildiz *et al.* (2002) reported that the natural substrates (woods on which *Pleurotus* species grow) are very poor in nitrogen content, nevertheless the fruit bodies are produced.

Thus, the lack of nitrogen may be one of the factors affecting the overall yield values in sawdust. Sawdust and sugarcane bagasse also contain high amount of lignin. Low degradation of lignocellulosic substances of sawdust by *P. ostreatus* might be another factor affecting the overall low yield values from sugarcane bagasse and sawdust.

As fundamental food characteristics: ash, fiber, protein, fat, carbohydrate content and energy value of analyzed of mushroom grown on different substrates are presented in Table 4.

The study indicates that the fruit bodies are quite rich in protein, ranged between 22.89 % - 25.97 % on dry weight basis. Highest protein, fiber, carbohydrate and energy were recorded from fruit body grown on rice straw. It has been reported that not only the protein content in fruit body but also the nature of protein depends on used substrate (Wang *et al.* 2001). Similarly fat and fiber content ranged between 1.03 % - 1.50 % and 12 – 14 % respectively. The highest fat was observed from the fruit body grown on sawdust, whereas total ash was highest obtained from the mushroom grown on mixture of rice straw and paper. The fat and fiber content obtained in the study are lower than that reported by Wang *et al.* (2001) as 2.50% - 2.82% and 5.97- 6.42% respectively. The total carbohydrate content found in this study (30.24% - 42.26%) is lower than the finding of Patil *et al.* (2010) as 50.50% - 55.33%. The total energy contribution of the sample ranged between 231.78kcal/100gm - 282.73kcal/100gm. Manzi *et al.* (2001) reported that energy contribution of *P. ostreatus* was 30 kcal/100gm but our values of energy in kcal/gm were higher in comparison to his findings. In this experiment, different substrates have affected nutrient composition of *P. ostreatus*. This result may be probably due to biological, chemical differences and the C/N ratio of the substrates which is also indicated by several authors (Sangwan & Saini 1995). Despite differences in the chemical composition of *P. ostreatus* grown on different substrates, the overall nutritional potential of the mushrooms were relatively superior to several other research finding.

CONCLUSION

Mushroom cultivation is one of the efficient ways by which residues can be recycled. *P. ostreatus* grown on different substrates are nutritious with high protein, fiber and low fat. It may also offer economic incentives for agribusiness to examine these residues as valuable resources and develop new enterprises to use them to produce nutritious mushroom products. Therefore, the mushroom cultivation may become one of the most profitable agribusiness that could produce food products from different substrates and help to dispose them in an environment friendly manner.

Almost all producers use rice straw for the production of *P. osteratus,* which is also one of the best substrate in this study. Therefore, use of a variety of the substrates is essential. Although the amount of yield is lower than in rice straw, other substrates such as rice straw plus wheat straw, rice straw plus paper and sugarcane bagasse can also be used as alternative substrates with supplement in the cultivation of *P. osteratus.*

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