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Original Research Paper

Cultivation of *Pleurotus sajor-caju* using different agricultural residues

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Chandra P. Pokhrel*, Neelam Kalyan, Usha Budathoki and Ram Kailash P. Yadav

Central Department of Botany, Tribhuvan University, Kirtipur, Kathnadu, Nepal.

*Corresponding author
Email: chandraprkh@yahoo.com

Tel: +977-1-4332293

Mycelial growth, colonization period, primordial initiation, harvesting time, yield, mushroom size and biological efficiency (BE) of *Pleurotus sajor-caju* were assessed on three different substrates namely maize stalk, pea residue (tendrils) and banana leaves with and without supplementation of rice bran and chicken manure. The faster mycelial growth and highest yield (348.13 g per 25 cm × 15 cm bag) with 87.03% BE was obtained from maize stalk with rice bran and second best yield (299.53 g) with 74.88% BE was recorded from pea residue with rice bran. Among the substrates used, maize stalk appeared best followed by pea residue and banana leaves. Rice bran showed best supplementation for mycelial growth and yield with all substrates. The study revealed that faster mycelial growth is consistent with better yield and highest biological efficiency.

Key words: *Pleurotus sajor-caju*, agricultural residue, mycelial growth, yield, biological efficiency

INTRODUCTION

Pleurotus spp. are commercially important edible mushrooms commonly known as the oyster mushroom. Cultivation of oyster mushroom has recently increased tremendously throughout the world because of their abilities to grow at a wide range of agro-based residues. These white-rot fungi are useful decomposers of various agricultural wastes (Kurt and Buyukalaca, 2010). *Pleurotus* spp. are the most talented group among the cultivated mushrooms, which have ability to degrade many lignocellulosic substrates and are capable to colonize successfully on these substrates (Patrabansh and Madan, 1997). *P. sajor-caju* is one of the most successfully cultivated species of these mushrooms and it is considered to be delicious (Zhang *et al.,* 2002). Mushrooms have long been used for medicinal and food purposes. *Pleurotus* species contain high potassium to sodium ratio, which makes mushrooms an ideal food for patients suffering from hypertension and heart diseases. They are also rich source of proteins, minerals and vitamins (Caglarirmak, 2007). The carbohydrate content of mushrooms represents the bulk of fruiting bodies accounting for 50 to 65% on dry weight basis (Wani *et al*., 2010). Purkayastha and Chandra (1976) found 14 to 27% crude protein on dry weight basis in *Agaricus bisporus*, *Lentinus subnudus*, *Calocybe indica* and *Volvariella volvacea*. On a dry weight basis, mushrooms normally contain 19 to 35% proteins where as fat content is very low as compared to carbohydrates and proteins (Wani *et al.,* 2010).

Agriculture residues are the major source of lignocellulosic materials, which is best substrate for solid state fermentation of edible fungi such as *P. sajor-caju*. Zadrazil (1980) showed that *P. sajor-caju* has very high saprophytic colonizing ability and can degrade wheat straw efficiently. Bisaria *et al.* (1987) studied the growth of this mushroom on several different agricultural wastes, including paddy and wheat straws. Growing oyster mushrooms convert a high percentage of the lignocellulosic materials to fruiting bodies. Therefore, cultivation of *P. sajor-caju* on various agricultural residues offers high value products with nutritional and medicinal properties. Also, mushroom production gives additional or 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. In addition, mushrooms are excellent source of food to address the problem of malnutrition in developing countries like Nepal. Also, the demand of mushroom has been escalating due to changing consumer behavior, development and market expansions in recent times.

Rice straw is the chief substrate for oyster mushroom cultivation in Nepal.However, development of cost-efficient and alternate substrate to cultivate oyster mushroom without sacrificing mushroom quality is a major focus of many reaearchers and growers.

Table 1. Comparison of weekly mycelial growth of *P. sajor-caju* on different substrates **(**Mean ± SD, n**=**5).

Substrates	Supplements	First week (cm)	Second week (cm)	Third week (cm)
Pea residue	Control	4.70±0.40[b]	10.50±0.79[b]	16.50±0.26[b]
	Rice bran	6.04±0.28[a]	12.22±0.61[a]	17.44±0.43[a]
	Chicken manure	5.54±0.32[a]	11.14±0.62[b]	16.74±0.46[b]
Maize stalks	Control	5.73±0.40[b]	11.60±0.89[b]	17.27±0.21[b]
	Rice bran	6.46±0.32[a]	12.68±0.50[a]	18.24±0.64[a]
	Chicken manure	5.66±0.39[b]	10.92±0.69[b]	17.32±0.37[b]
Banana leaves	Control	3.84±0.35[b]	7.72±0.69[b]	11.28±1.07[b]
	Rice bran	4.86±0.30[a]	9.42±0.49[a]	13.86±0.90[a]
	Chicken manure	4.22±0.38[b]	8.42±0.70[b]	12.38±1.20[b]

Different letters along each column within each substrate indicate significant differences (each substrate) of the mean (p=0.05) according to the Duncan's multiple range test

The aim of the study is to determine the growth and yield of *P. sajor-caju* on different agricultural residues with supplement during the solid state fermentation**.**

MATERIALS AND METHODS

Wheat grain spawn of *P. sajor-caju* was obtained from National Agriculture Research Council (NARC), Khumaltar, Lalitpur, Nepal. Three substrates namely maize stalk, pea residue (tendrils) and banana leaves including two supplements viz. rice bran and chicken manure were used as ingredients for experiment. The agricultural residues such as pea residue (tendrils) and maize stalk were collected form Kirtipur, Kathmandu and banana leaves were obtained from Siraha district (tropical region) of eastern Tarai of Nepal. Rice bran was collected from local rice mill in Kirtipur and chicken manure was obtained from private livestock farm from the same area. A total of nine treatments, three from each substrate were prepared for experiment i.e. 100% pea residue (control); 90% pea residue + 10% rice bran; 90% pea residue waste + 10% chicken manure; 100% maize stalk (control); 90% maize stalk+ 10% rice bran; 90% maize stalk + 10% chicken manure; 100% banana leaves (control); 90% banana leaves+ 10% rice bran, 90% banana leaves+ 10% chicken manure.

All collected substrates were chopped into small pieces (2-4 cm long) and soaked in tap water for overnight. The excess water in the substrates was allowed to run off until set to required moisture. Each substrate was separately supplemented with 10 % chicken manure or rice bran and mixed thoroughly; substrate without supplement was considered as a control. Finally, moisture of each treatment was set to 60% (±2). Following this treatment, the wet substrates weighing 1 kg were placed in the polypropylene bags of 25 cm × 15 cm size and sterilized at 121^{o}C for 1 hr. After cooling, each bag was inoculated with 2.5% (w/w) spawn under aseptic conditions by placing the spawn on the substrate surface through the opening at the top of the bag. The inoculated bags were incubated at an ambient temperature of 25^{o}C (±2) and spawn run (mycelia extension) was observed regularly until appears white (colonization). Mouth of polypropylene bags were opened and the bags cut with a knife vertically from an upper point downward and removed carefully, when whitish mycelial growth (colonization) had spread to both lower and upper sides from the inoculated zone. Three times watering a day was done on the substrate before first harvest, one time during second flush. The day to colonization, primordial initiation and harvest were recorded. Harvesting was done by hand holding the stripes at the base and twisting lightly. Yield was determined by weighing and counting of fruit bodies. BE was calculated as fresh weight of harvested mushrooms (g)/ dry weight of substrate x 100. Size of mushrooms was calculated by total weight of fresh mushroom harvested / total number of mushroom harvested.

Mycelial growth was measured in centimeters as the length of the mycelium spreading form the mouth of the bag toward the bottom side at a weekly interval for three weeks. The data were analyzed using SPSS statistical program (Version 12.0). Analysis of variance (ANOVA) and the Duncan's multiples range tests were used to determine significant of differences between the means of yields of mushroom.

RESULTS

Three different substrates (maize stalk, pea residue, banana leaves) with two different supplements (rice bran and chicken manure) as well as substrate without supplement (control) were investigated to determine the growth and yield of oyster mushroom (*P. sajor-caju*). Weekly mycelial extension on different substrates is shown in Table 1. Maize stalks with rice bran showed significantly faster mycelial extension which were 6.46 cm, 12.68 cm and 18.24 cm in first, second and third week respectively. Pea wastes residue with rice bran also showed significantly faster mycelial extension in second and third weeks, respectively followed by chicken manure and control. Similarly slowest mycelial growth was observed in banana

Table 2. Comparison of colonization period, primordial formation and first harvest days of *P. sajar-caju* on different substrates (Mean ± SD, n= 5).

Substrates	Supplements	Colonization (days)	Primordial formation (days)	First harvest (days)
Pea residue	Control	29.33±1.15[a]	36.00±1.00[a]	38.67±0.58[a]
	Rice bran	23.20±1.30[c]	30.60±1.14[b]	34.20±0.84[b]
	Chicken manure	27.40±1.14[b]	35.20±1.30[a]	38.80±1.48[a]
Maize stalks	Control	24.33±0.58[a]	31.33±0.58[a]	35.00±1.00[a]
	Rice bran	22.80±1.10[b]	29.00±1.58[b]	32.80±1.79[b]
	Chicken manure	25.20±0.84[a]	31.80±0.84[a]	35.20±0.84[b]
Banana leaves	Control	35.00±1.00[a]	41.80±3.49[b]	44.40±3.29[a]
	Rice bran	28.20±0.84[c]	36.00±1.22[a]	39.60±1.14[b]
	Chicken manure	33.20±1.30[b]	40.40±2.07[b]	43.20±2.17[a]

Different letters along each column within each substrate indicate significant differences of the mean (p=0.05) according to the Duncan's multiple range test.

Table 3. First and second flush of *P. sajar-caju* on different substrates (Mean ± SD, n= 5)

Substrates and supplement		Total yields (g)	First flush (g)	Second flush (g)	Size of mushroom(g)	Biological efficiency (%)
Pea residue	Control	224.23±23.36[b]	137.51±12.61[b]	86.72±13.07[b]	5.51±0.28	56.06±5.84
	Rice bran	299.53±13.12[a]	185.23±11.49[a]	114.30±10.28[a]	6.78±0.24	74.88±3.27
	Chicken manure	234.30±16.49[b]	128.51±10.59[b]	105.79±7.22[a]	4.52±0.71	58.57±4.12
Maize stalk	Control	280.66±33.06[b]	158.96±18.56[b]	86.72±13.07[b]	6.29±0.76	70.16±8.26
	Rice bran	348.13±15.73[a]	213.17±12.68[a]	114.30±10.28[a]	7.28±0.37	87.03±3.93
	Chicken manure	271.57±6.58[b]	156.50±6.97[b]	105.79±7.22[a]	4.40±0.29	67.89±1.64
Banana leaves	Control	94.90±13.81[c]	70.92±13.19[c]	23.98±5.19[b]	5.01±1.07	23.72±3.45
	Rice bran	153.46±4.35[a]	102.36±4.74[a]	51.10±6.06[a]	6.32±0.56	38.37±1.08
	Chicken manure	131.28±16.00[b]	84.50±7.93[b]	46.78±8.64[a]	4.93±0.35	32.82±4.00

Different letters along each column within each substrate indicate significant differences of the mean (p=0.05) according to the Duncan's multiple range test.

leaves. Our study observed that pea residue with addition of rice bran was the best for mycelium extension after maize stalk with rice bran as supplement. The study also revealed that rice bran significantly support the mycelial growth in all substrates used in the experiment. However, mycelial growth did not differ significantly between control and with chicken manure in all substrates used.

Days to colonize the substrates, primordial formation and first harvest are presented in Table 2. The fastest colonization (22.80 days), primordial initiation (29.00 days) and first harvest (32.80 days) were observed in maize stalk with rice bran followed by pea waste with rice bran. Thus rice bran as supplement supported early colonization, primordial formation and early harvest of the mushrooms. Overall, maize stalk showed better results for colonization period, primordial formation days and first harvest days followed by pea waste, whereas banana leaves demonstrated delayed results.

For each of the treatments, two flushes were harvested during the study. The study revealed that different substrates showed varying yields and BE with or without supplements. Yield, mushroom size and BE are presented in Table 3. In the first flush, maize stalk yielded 213.17 g, 158.96 g, and 156.50 g with rice bran, control and chicken manure respectively. The total yield in maize stalk with rice bran was 348.13 g followed by 280.66 g and 271.57 g in control and chicken manure respectively.

The total yield in pea residue with rice bran was 299.53 g, followed by 234.30 g and 224.23 g in chicken manure and control, respectively. The total yield in banana leaves with rice bran was 153.53 g, 131.28 g with chicken manure and 94.90 g in control. Among all, maize stalk with rice bran showed the highest yield with 87.03 % BE and 74.88 % BE on pea residue with rice bran as a second best. The yield was significantly low in second flush in all substrates.The biggest size of *P. sajor-caju* was found in maize stalk with rice bran (7.28 g) whereas smaller size of *P. sajor-caju* was found in maize stalk with chicken manure. Among the substrates, the biggest size of the mushroom was recorded with rice bran.

DISCUSSION

In the present study, different substrates were examined with or without addition of supplement for the growth and yield of *P. sajor-caju*. Mycelial growth is a preliminary step that creates suitable internal conditions for fruiting. Thus,

outstanding growth of mycelia is a vital factor in mushroom cultivation (Pokhrel et al., 2009). Mushroom mycelia (vegetative phase) are important in the ecosystem because they are able to biodegrade the substratum and therefore, use the wastes of agricultural products (Manzi et al., 2001). On comparing three substrates used in this study, maize stalk was the best substrate for mycelial extension followed by pea residue and banana leaves. Pea residue with addition of rice bran appeared better substrate after maize stalk supplemented with rice bran. Furthermore, rice bran presented better results as supplement for mycelial growth.

Oei (2003) reported that substrate having high quality lignin and cellulose contents takes a longer time to start pinning and fruit body formation. Similarly in mycelia extension, maize stalk showed rapid colonization, fast primordial initiation and earliest harvest day with addition of rice bran. In the present study, the fruiting bodies appeared 32-44 days after inoculation of spawn. These findings are in conformity with the results of Quimio et al., (1999) who reported that formation of fruiting bodies was 3-4 weeks after inoculation of spawn. Similarly, Tan (1981) reported that *P. ostreatus* and other species on cotton waste took 2-3 weeks for fruit body formation after spawn running. According to Baysal et al., (2003), the fastest mycelial growth, pin head formation and fruit body formation were recorded with the supplementation of 20% rice husk.

The study revealed that the addition of rice bran to substrates could be beneficial as a nutrient supplement and promoter to growth and yield. The higher mushroom yield and biological efficiency correspond to the mycelia growth, colonization period and harvest period. Baysal et al., (2003) obtained an increase in the biological efficiency with the increasing concentration (10 and 20%) of rice bran during the production of *P. ostreatus*. Mane et al., (2007) reported that organic supplements such as groundnut oilseed cake, gram powder and rice bran not only affected growth parameters but also increased yields of *P. sajor caju*. They also found that the best yield in addition of rice bran supplement with various lignocellulosic substrates yielded better than without supplement. Similar observations have also been made by several other researchers (Bano et al., 1993). This fact can be related to our research where the addition of rice bran with different substrates resulted in an increase in productivity and biological efficiency. Among nine treatments tested for mushroom growth of *P. sajor-caju*, banana leaves with or without supplementation found to be the least responsive in terms of yield and biological efficiency. Low performance by banana leaves may be due to lower break down of cellulosic and lignin substrates. In a similar study, Bonatti et al., (2004) reported 7.51% biological efficiency of *P. sajor-caju* on banana tree straw with 5% rice bran supplement. However, our study showed better results on banana leaves than reported by Bonatti et al., (2004). Not only yield but also size of mushroom was better with rice bran supplements in all substrates.

In conclusion, cultivation of oyster mushroom on various agricultural residues offers economic initiatives for agribusiness to examine these residues as valuable resources and use them to produce protein rich mushroom products. In this study, highly prized edible mushroom, namely *Pleurotus sajor-caju* was tested to grow on different agricultural residues such as banana leavepea residue and maize stalk as base materials with rice bran and chicken manure as supplements. Nepal as an agricultural country, large quantity of these residues has been produced. Maize stalk was the best and cheap alternative substrate for the cultivation of oyster mushroom (*P. sajor-caju*) with rice bran supplement followed by pea residue and banana leaves. The addition of rice bran with different substrates resulted in an increase in yield and biological efficiency. The study revealed that pea residue can also be used as alternative substrate in the cultivation of *P. sajor-caju*.

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