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Books:

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Volume 4 Number 1 June 2010

Contents

Bangladesh J. Mushroom. 4(1): 1-6, 2010 (June)

Differential Effect of *Pleurotus ostreatus* on Hepatocellular Markers Alanine Aminotransferase and Aspartate Aminotransferase in Adult Male *vs* Female during Ramadan Fast

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Abstract

The effect of oyster mushroom *(Pleurotus ostreatus)* on the serum level of alanine amino transferase (ALT) and aspartate aminotransferase (AST), the hepatic dysfunction marker enzymes, was determined. The experiment was conducted on male and female Muslim subjects before and after the Arabic month, Ramadan, during when there occurs a change both in the pattern and timing of dietary intake. Fresh *Pleurotus ostreatus*, taken along with the usual ifter items, showed noticeable reducing impact on both the levels of serum ALT and AST. However, the extent was much in case of males ($p < 0.001$ and < 0.05) compared to their female counterparts ($p > 0.05$ and > 0.05). In case of intra-male consideration, oyster mushroom lowered the level of ALT much than that of AST.

Key words: *Pleurotus ostreatus,* ALT, AST, Ramadan, Ifter, Male, Female.

INTRODUCTION

Recently, edible basidiomycetes have been considered by scientists as potential natural resources to develop antioxidant compounds. This is a result of the fact that they are good sources of secondary metabolites, vitamins & minerals (De Roman, *et al.,* 2006 and Pathak, *et al.,* 1998), proteins and carbohydrates, as well as being high in fiber and low in fat (Manzi, *et al.*, 1999). Since primordial days, mushrooms have been always consumed in human's daily diet as an additional supplementary item. Mushrooms are fleshy, spore-bearing fruiting bodies of a fungus, typically produced above ground on soil or on its food source. Mushroom consumption is growing worldwide due to the influence of Oriental culture, studies on their nutritional values and pharmacological properties (Yang, *et al.*, 2002). Most mushrooms contain vitamins; particularly niacin, thiamine, riboflavin, biotin and vitamin C. Mushrooms also contain a wide variety of bioactive molecules including terpenoids, steroids, phenols, nucleotides and the glycoprotein derivatives and polysaccharides (Borchers, *et al.*, 1999). In addition to their nutritional value mushrooms are claimed to exhibit antitumour, antimicrobial activities (Wasser and Weis, 1999,

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Gunde-Cinoerman, 1999 and Ooi, 2000), hypoglycemic and hypotensive properties (Choudhury, *et al.*, 2008).

Mushroom of *Pleurotus* species are rich in medicinal value and very much effective in reducing harmful plasma lipids and liver (Opletal *et al.*, 1997 and Jayakumar *et al.*, 2006) and improving the levels of different cellular enzymes (Alam *et al.*, 2007). It is generally known that lowering of plasma cholesterol levels reduces the risk of atherosclerosis (Bobek *et al.*, 1997 and Hossain *et al.*, 2003) and improves liver condition.

The Oyster mushroom contains statins such as lovastatin which works to reduce cholesterol (Gunde- Cinoerman and Cimerman 1995). It increases the levels of reduced glutathione in the liver and stimulates the activities of catalase and glutathione peroxidase in the liver (Pathak *et al.*, 1998). In a study it was observed that supplementation of 50 grams of *Pleurotus ostreatus* as Ifter item significantly reduced plasma ALT and AST at Ramadan (Choudhury *et al.*, 2009). Although mushroom is one of the top priority foods in different countries, still it is not established as food in Bangladesh. But its popularity is increasing to its nutritious and medicinal value.

Ramadan occurs in the ninth month of the lunar calendar, lasting for 29 or 30 days. Fasting during Ramadan represent one of the five pillars of the Islamic religion. So it is important to know the effect of this kind of fasting on people's life especially on their health. During these days people pay a lot to protect themselves against diseases and to reach this goal they should have an intact immune system. Islamic fasting during Ramadan is different from normal fasting plans because in Ramadan fasting, there is no malnutrition or inadequate calorie intake. The calorie intake of Muslims during Ramadan is at or slightly below the nutritional requirement guidelines. A balanced diet at Ramadan, even less in quantity than normal will be sufficient to keep a person healthy and active during the month. So the addition of edible mushroom as an ifter item is a fruitful purpose to improve the health and disease status of body such as the status of liver by which is reflected by lowering the hepatocellular enzymes as ALT, AST.

ALT is a transaminase enzyme. It is found in various bodily tissues, but is most commonly associated with the liver. It catalyzes the two parts of the alanine cycle. Estimation of ALT in plasma or serum is one of a group of tests known as liver function tests (LFTs) and is used to monitor damage to the liver parenchymal cells (Annon., 2010a). AST is an enzyme that is raised in the plasma in acute liver damage, as with liver cancer or hepatitis. It is also found in red blood cells, cardiac muscle, skeletal muscle, the pancreas, and the kidney. In LFTs, an elevated level of AST is a sign of serious liver damage, even before any other symptoms are seen in the patient (Annon., 2010b). So the aim of this investigation is to evaluate the effect of mushroom on hepatic markers as ALT & AST of fasting both male and female during Ramadan.

MATERIALS AND METHODS

The study was conducted during the period of 21st August 2009 to 17th September 2009 with the collaboration of Strengthening Mushroom Development Project, National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka.

Subjects: Total 53 subjects aged (years) from 25 to 80 who resides at the grip of the monitoring team wanting to be fast in the whole Ramadan were included in the study. They were divided into two groups. 24 male subjects in group-1 (G-1) and 29 female subjects in Group-2 (G-2) were included.

Selection criteria: The subjects were clarified about the study and after getting their written consent they were included. The details history was taken from the subjects who included age, sex, occupation, educational status, marital status, family history and drug history. Patients suffering from acute illness were excluded. In the study previously divided 2 groups, G-1 and G-2 were included. Both groups were studied with mushroom supplementation. If any drugs previously getting by the subjects, it was continued. Fifty grams of fresh *P. ostreatus* mushroom was ensured for each individual by the responsible workers daily. The mushrooms were collected from NAMDEC. At the beginning of Ramadan, subjects were evaluated for health status. Fasting blood sample was collected for analysis of ALT and AST. Just after ending of Ramadan the subjects were evaluated and all the investigation procedures were repeated. ALT and AST were estimated by semi-auto analyzer (3000 evaluation) using the reagent kit (Atlas, England).

Statistical analysis: The recorded characteristics of the subjects during Ramadan fasting analyzed by standard statistical methods using computer software, SPSS package programme.

RESULTS AND DISCUSSION

In G-1 the mean ± SE serum levels of ALT (U/L) before and after Ramadan were 20.33 ± 1.04 and 15.79 ± 0.73 respectively whereas in G-2 these levels were 15.41 ± 1.61 and 13.93 ± 1.22 respectively. In this result, it is observed that in G-1 there is a statistically significant mean difference of ALT ($p = 0.000$) in pre and post Ramadan state. On the other hand, in case of G-2 though there is no statistically significant mean difference of ALT between the two periods ($p = 0.102$), there is some reduction of ALT level during fasting of Ramadan (Table 1).

In G-1 the mean ± SE serum levels of AST of pre and post Ramadan samples were 28.83 ± 0.99 and 25.87 ± 1.06 respectively whereas in G-2 these levels were 26.52 ± 2.53 and 23.52 ± 1.67, respectively. In this result, it is observed that in case of G-1 there is a statistically significant mean difference of AST ($p = 0.019$) in pre and post Ramadan state. And in case of G-2 though there is no statistically significant mean difference of AST between the two periods ($p = 0.058$), there is some reduction of AST level during fasting of Ramadan (Table 1).

Table 1. Comparison of ALT and AST before and after Ramadan

Name of hepatocellular markers	Group	Number of subjects (n)	Period of observation (Ramadan)	Mean ± SE (U/L)	P
ALT	G-1	24	Pre	20.33 ± 1.04	0.000
			Post	15.79 ± 0.73	
ALT	G-2	29	Pre	15.41 ± 1.61	0.102
			Post	13.93 ± 1.22	
AST	G-1	24	Pre	28.83 ± 0.99	0.019
			Post	25.87 ± 1.06	
AST	G-2	29	Pre	26.52 ± 2.53	0.058
			Post	23.52 ± 1.67	

Results show mean ± SE. Data were analyzed by Student's Paired 't' test. Means were significantly different at $p<0.05$ at 95% confidence limit.

In this study, the mean serum levels of ALT of G-1 and G-2 before Ramadan were 20.33 ± 1.04 and 15.41 ± 1.61 respectively whereas after Ramadan those levels were 15.79 ± 0.73 and 13.93 ± 1.22 respectively. Simultaneously, the serum levels of AST of G-1 and G-2 before Ramadan were 28.83 ± 0.99 and 26.52 ± 2.53 respectively whereas after Ramadan those levels were 25.87 ± 1.06 and 23.52 ± 1.67. Here a statistically significant mean difference ($p = 0.018$) was observed in case of ALT levels of G-1 and G-2 (Table 2) before Ramadan indicating in normal condition ALT level persists significantly higher in G-1 then those of G-2. Though statistically not significant some mean difference ($p = 0.221$) was observed in case of ALT levels of G-1 and G-2 (Table 2) after Ramadan period. In case of both periods of observations the reduction of ALT level in G-1 and G-2 was observed. In case of AST levels of G-1 and G-2 before ($p = 0.433$) and after ($p = 0.263$) Ramadan periods though no statistically significant mean differences were observed, the reduction of AST level in G-I and G-2 during both period of observations was seen (Table 2).

Table 2. Comparison of ALT and AST in male and female subjects

Name of hepatocellular markers	Group	Number of subjects (n)	Period of observation (Ramadan)	Mean ± SE (U/L)	P
ALT	G-1	24	Pre	20.33 ± 1.04	0.018
	G-2	29		15.41 ± 1.61	
ALT	G-1	24	Post	15.79 ± 0.73	0.221
	G-2	29		13.93 ± 1.22	
AST	G-1	24	Pre	28.83 ± 0.99	0.433
	G-2	29		26.52 ± 2.53	
AST	G-1	24	Post	25.87 ± 1.06	0.263
	G-2	29		23.52 ± 1.67	

Results show mean ± SE. Data were analyzed by Student's unpaired 't' test. Means were significantly different at $p<0.05$ at 95% confidence limit.

In a study, Choudhury *et al.*, (2009) observed a significant reduction of both ALT and AST in serum of adult human subjects supplemented by oyster mushroom at Ramadan in comparison to control subjects who were not supplemented by mushroom. Considering this reference it is believable that supplementation of mushroom in Ifter table may cause sufficient reduction of ALT and AST in case of male and some reduction of ALT and AST in case of female at one month of Ramadan fasting. In the present study, it was found that the levels of both ALT and AST in male serum sample were comparatively more reduced than those in female serum sample. It is also noticeable that the mean (± SE) of both ALT (20.33 ± 1.04) and AST (28.83 ± 0.99) in male serum sample before Ramadan were higher than the ALT (15.41 ± 1.61) and AST (26.52 ± 2.53) levels in female sample.

So from these findings of this study it is assumed that the mean values of both ALT and AST in normal condition is little bit higher in male (20.33 ± 1.04 and 28.83 ± 0.99) in comparison to female (15.41 ± 1.61 and 26.52 ± 2.53) volunteers. And the supplementation of mushroom causes reduction of ALT and AST in both male (15.79 ± 0.73 and 25.87 ± 1.06) and female (13.93 ± 1.22 and 23.52 ± 1.67) sexes but the reduction rate of ALT (in case of male, $p = 0.000$ and in case of female, $p = 0.102$) is more marked in comparison to AST (in case of male, $p = 0.019$ and in case of female, $p = 0.058$). On the other hand the reduction rate of both ALT and AST is more prominent in case of male (p value of ALT = 0.000 and AST = 0.019) than that of female (p of ALT = 0.102 and AST = 0.058).

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Effect of Aging, Opening and Soaking of Spawn Packet on the Yield of Shiitake Mushroom (*Lentinus edodes*)

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Abstract

Different age, opening pattern and water soaking method of spawn packet were evaluated to determine the right spawn age, opening patterns and water soaking method for shiitake mushroom cultivation. A wide variation was observed in yield and biological efficiency in different ages, opening patterns and water soaking methods. The highest yield and biological efficiency were recorded in 110 days old spawn packet followed by 120 and 130 days old spawn packets and the lowest yield and biological efficiency were found in 80 days old spawn packet. The full opened spawn packet gave the highest yield followed by top opened spawn packet and no yield was obtained from the packets which were not opened. Water soaking after opening of spawn packet has a negative effect on yield and biological efficiency.

Key words: Shiitake mushroom, spawn aging, opening pattern, water soaking, biological efficiency and yield.

INTRODUCTION

Shiitake mushroom (*Lentinus edodes*) is commonly used in China and Japan as high valued food and medicine. It is produced on different kinds of lignocellulosic substrates. The production system of shiitake mushroom is quite different from other edible mushrooms. There are two period of mushroom cultivation, the incubation period and the harvesting period. During the incubation period, the mycelium colonizes on the substrates with some distinct stages, such as, mycelium running, thickening, bumping, pigmentation and hardening for growth improvement (Stamets, 1993). The incubation period of shiitake mushroom is very crucial which can affect the yield. If the bag opening is too early or too late, the crop may be failed. It is reported that a time period of 60 to 90 days is necessary for incubation of spawn packet (Kawai *et al.*, 1997 and Iizuka, 1997).

The production of shiitake mushroom varies depending on the opening pattern of spawn packet. Many growers produce shiitake mushroom with the opening of the bag partially or fully. Fan *et al.* (2005) suggested to open the bag at the places where primordia have formed. It will give higher yield of quality mushroom; but it time consuming and laborious task. However, Ramkumar *et al.* (2010) suggested to cut the top portion of polypropylene bag after browning of shiitake packet.

Soaking of shiitake bag in water is commonly used for primordia initiation (Oei, 1996). Przybylowicz and Donoghue (1988) soaked shiitake blocks in cold water at 16 to 26°C

temperature in a tank for 24 hours and then placed in a growing room. But Shiomi *et al.* (2007) and Stamets and Chilton (1983) reported that most of shiitake mushroom strains inducted nicely when submerged in water at 5 to 20°C temperature for 8 to 72 hours.

Considering the facts the present work was undertaken to determine the right age, appropriate opening pattern and right water soaking method of spawn packet of shiitake mushroom for higher yield and better quality.

MATERIALS AND METHODS

The experiment was conducted at National Mushroom Development and Extension Centre, Savar, Dhaka during October 2009 to March 2010.

Spawn packet preparation: The substrate of spawn packets were prepared by using sawdust and wheat bran at the ratio of 3:1 (dry basis). Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Polypropylene bags of 25 × 18 cm size were filled with 500g of substrate mixture and packet tied, plugged and covered with brown paper. Then the packets were sterilized in an autoclave at 121°C under 1.5 kg/cm^2 pressure for 2.0 hour. After sterilization the packets were cooled and transferred to an inoculation chamber. The packets were inoculated with the mother culture of *Lentinus edodes*-11 strain at the rate of two teaspoonfuls per packet. The inoculated packets were placed on a still rack at 22 ± 2°C temperature for incubation. Whitish mycelia began to grow on the substrate and after full colonization a thick mycelial coat forms on the outer surface of colonized substrate. Clumps of mycelia appeared as blister like bumps of various sizes on the surface of the mycelial coat.

Opening and soaking of spawn packet: To determine the right age of spawn, the packets were fully opened at 80, 90, 100, 110, 120 and 130 days after inoculation. The spawn packets were opened as- no opening, partial top opening, top opening, opening where bump is formed and full opening to determine the right opening pattern. Different soaking methods like- no water soaking, normal water soaking, ice cold water soaking, soil water soaking and wet cloth water soaking were practiced after fully open the spawn packet as treatments. The open spawn packets were kept in normal and ice cold water for 12 hours and in wet soil and cloth for 72 hours.

Cultivation conditions for fruiting: Treatment wise, the packets were moved to culture house and placed on racks. The temperature, relative humidity and light were maintained at 18-22°C, 70-80% and 10-20 lux respectively. Watering was done 3 to 4 times per day to maintain temperature and relative humidity. Excess CO_2 was removed by exhaust fan.

Data collection and analysis: The experiment was laid out in a completely randomized design (CRD) with 4 replications. Data on days required to harvest, number of fruiting body and biological and economic yield were recorded. Biological yield in g/packet was recorded by weighing the whole fruiting bodies without removing the lower hard and

dirty portion whereas economic yield was recorded by the removing of dirty portion of fruiting bodies. Biological efficiency was determined by following formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Total biological yield (g)}}{\text{Total dry substrate used (g)}} \times 100$$

Data were analyzed following MSTAT-c computer program. Means were computed following Duncan s Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Effect of aging of spawn packet: Significant variation was observed in days required from opening to first harvest (DROFH), number of fruiting body/packet, biological and economic yield (g/packet) and biological efficiency (%) at different age of shiitake spawn packet. The days required from opening to first harvest (DROFH) ranged from 8.50 to 15.00. The lowest DROFH was observed in spawn packets opened after 130 days of spawning which was statistically similar to all the spawn age except 80 and 90 days. The highest DROFH was recorded in 80 days spawn age.

The highest number of fruiting body (41.75) was recorded in 110 days old spawn packet which was statistically similar to 120 days old spawn packet. The lowest number of fruiting body 12.50 was recorded in 80 days old spawn which was significantly lower as compare to all the treatments (Table 1). The result is supported by Leatham (1985) who reported that shorter period of spawn run gave limited fruiting bodies and longer period 105 to 150 days gave more fruiting bodies.

Performance of different age of shiitake spawn in respect of biological and economic yield is presented in Table 1. The highest biological yield (148.80g/packet) was recorded from 110 days old spawn which was statistically similar to 120 (138.80g/packet) and 130 (127.30g/packet) days old spawn. The lowest biological yield (77.75g/packet) was recorded in 80 days old spawn which was significantly lower to all the treatments. Almost similar trend in relation to age was observed in economic yield of shiitake mushroom. The study revealed that yields were increased with the increases of age of spawn packet. The result was supported by Royse (1985).

The biological efficiency of shiitake mushroom in different ages of spawn packet ranged from 38.88 to 74.40% (Table 1). The highest biological efficiency (74.40%) was recorded in 110 days old of spawn which was statistically similar to that of 120 days and 130 days. The lowest biological efficiency (38.88%) was found in 80 days old spawn. Almost similar result was reported by Royse and Bahler (1986) stating that biological efficiency increased with the increases of incubation time.

Table 1. Effect of different age of spawn packet on days required from opening to first harvest, number of fruiting body, yields and biological efficiency of shiitake mushroom

Age of spawn (Days)	Days required from opening to first harvest	Number of fruiting body/packet	Biological yield (g/packet)	Economic yield (g/packet)	Biological efficiency (%)
80	15.00 a	12.50 d	77.75 d	71.75 c	38.88 c
90	13.50 ab	21.00 c	90.25 cd	82.75 c	45.13 c
100	11.50 bc	24.00 c	113.30 bc	107.50 b	56.67 bc
110	9.25 c	41.75 a	148.80 a	139.00 a	74.40 a
120	9.00 c	39.00 ab	138.80 ab	130.30 ab	69.40 ab
130	8.50 c	32.25 b	127.30 ab	116.80 ab	63.65 ab
CV (%)	19.62	20.30	16.41	16.46	16.55

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Effect of opening pattern of spawn packet: Days required from opening to first harvest (DROFH) in different opening pattern of shiitake spawn packet ranged from 9.75 to 20.75 (Table 2). The lowest DROFH (9.75) was found in spawn packet opened at bumping area which was statistically similar to top opening (12.25 days) and full opening (10.00 days). The partial top opening packet required more time for first harvest which was significantly higher as compare to all the treatments.

The highest number of fruiting body (36.75) was found on fully opened spawn packet which was significantly higher than all the treatments. The second highest number of fruiting body was observed on top opened spawn packet (27.25) followed by bumping area opened spawn packet (20.75). The lowest number of fruiting body was observed on partial top opened spawn packet (Table 2).

Biological and economic yield of shiitake mushroom influenced by opening pattern are presented in Table 2. The highest biological yield (152.00g/packet) and economic yield (142.00g/packet) were found in full opening packet followed by top opening and bumping site opening. The lowest biological yield (27.25g/packet) and economic yield (20.75g/packet) were recorded from partial top opening. No yield was obtained from spawn packet when it was not opened. Fan *et al.* (2005) stated that the spawn packets opened at the places of primordia initiation had produced higher quality and quantity of shiitake mushroom though it was time consuming and laborious.

The biological efficiency influenced by the opening pattern was ranged from 13.63 to 76.00% (Table 2). The full opening packet gave the maximum biological efficiency (76.00%) which was significantly higher to all treatments. The lowest biological efficiency (13.63%) was found in the spawn packet opened partially on the top.

Table 2. Effect of opening pattern of spawn packet on days required from opening to first harvest, number of fruiting body, yields and biological efficiency of shiitake mushroom

Treatment	Days required from opening to first harvest	Number of fruiting body/packet	Biological Yield (g/packet)	Economic Yield (g/packet)	Biological efficiency (%)
No opening	-	-	-	-	-
Partial top opening	20.75 a	3.50 d	27.25 d	20.75 c	13.63 d
Top opening	12.25 b	27.25 b	117.50 b	108.50 b	58.75 b
Opening at bumping area	9.75 b	20.75 c	99.50 c	92.00 b	49.75 c
Full opening	10.00 b	36.75 a	152.00 a	142.00 a	76.00 a
CV (%)	18.86	16.82	5.05	16.52	5.05

In a column, means followed by a common letter are not significantly different at 5% level by DMRT. (-) indicates no response/zero performance.

Effect of soaking methods of spawn packet: Days required from opening to first harvest (DROFH) in different soaking methods of shiitake spawn packet varied significantly (Table 3). The lowest DROFH (9.75) was recorded in non soaked spawn packet which was significantly lower to all the treatment. The highest DROFH (22.25) was recorded in ice cool water soaked spawn packet.

The highest number of fruiting body (37.25) was found in spawn packet which had not soaked at all and was significantly higher as compare to all the treatments. The lowest number of fruiting body (12.00) was found in spawn packet soaked in ice-cold water (Table 3).

Significant variations were observed in different soaking methods of shiitake spawn in respect of biological and economic yield (Table 3). The highest biological yield (145.30g/ packet) and economic yield (134.50g/ packet) were obtained from the packets which were not soaked at all. Soil water soaked and normal water soaked spawn packets performed moderately, while ice-cold water treatment gave the lowest yields. The study revealed that soaking method had not proved to be an effective technique to increase the shiitake mushroom yield. Similar findings were obtained by Royse and Shen (2005), who described that soaking for first flush was not required due to presence of sufficient water in the spawn packets of shiitake mushrooms.

The maximum biological efficiency (72.63%) was recorded from the spawn packets which were not soaked at all. The ice-cold water treated spawn packet showed minimum biological efficiency (33.63%) followed by wet cloth soaked spawn packet.

Table 3. Effect of different water soaking methods of spawn packet on days required from opening to first harvest, number of fruiting body, yields and biological efficiency of shiitake mushroom

Treatment	Days required from opening to first harvest	Number of fruiting body/packet	Biological Yield (g/packet)	Economic Yield (g/packet)	Biological efficiency (%)
No water soaking	9.75 c	37.25 a	145.30 a	134.50 a	72.63 a
Normal water soaking	15.75 b	23.50 b	100.50 b	92.25 b	50.25 b
Ice cold water soaking	22.25 a	12.00 c	67.25 c	58.25 c	33.63 c
Soil water soaking	13.25 b	22.50 b	102.80 b	94.00 b	51.38 b
Wet cloth water soaking	14.75 b	21.00 b	82.75 bc	77.75 bc	41.38 bc
CV (%)	14.64	20.32	15.19	15.25	15.19

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

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Cultivation of White Button Mushroom (*Agaricus bisporus*) under Evaporatively-cooled Mud House Condition

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Abstract

An evaporatively-cooled mud house structure of size 32' x 12' x 8'6" was developed and investigated for its suitability for growing white button mushroom (*Agaricus bisporus*). Evaporative cooling pads of size 10 mm thickness were made up of *partal* (*Abies pindrow*) wood shavings and installed in the side walls of mud house structure. Compost was prepared by standard long method of composting and cultivation trials of button mushroom strain S-11 were carried out in the newly developed evaporatively cooled mud house. The relative humidity and temperature inside the cropping rooms were significantly differed from the outside atmosphere. The outside atmospheric temperature and relative humidity ranged from 7 to 32.5°C and 9 to 92 %, respectively during the period of study where as the temperature and humidity inside the evaporatively-cooled mud house ranged between 17 to 24°C and 79 to 90 %, respectively. The button mushroom yield obtained from the evaporatively-cooled mud house was 13.4 %. The mud house was found quite suitable for seasonal cultivation of white button mushroom in the off-season for small and marginal growers.

Key words: Evaporatively-cooled mud house, button mushroom, *Agaricus bisporus*, temperature, relative humidity

INTRODUCTION

Button mushroom (*Agaricus bisporus*) in India is grown by small, marginal and large growers. The resource poor small and marginal growers largely depends upon season and they produce mushroom during winter season with old low cost production system but contribute significantly to the total output of mushroom (Arumuganathan *et al.*, 2005). Such type of seasonal mushroom growing in India is mainly confined to the hill state of Himachal Pradesh, Jammu & Kashmir, Uttarakhand and some part of Tamilnadu, Haryana, Punjab, Orissa, Karnataka and Delhi (Singh *et al.*, 1995). The seasonal growers, making use of natural low temperature in the winter season and cultivate a minimum of one button

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mushroom crop in their locality. But they have no control over the prevailing relative humidity and temperature of the atmosphere to maintain them inside the growing rooms, which is the basic and essential requirement to raise the button mushroom crop. In semi-urban areas, normally the seasonal growing rooms are built with simple brick walls with roof made of asbestos sheets and a false ceiling. These rooms are wholly depending upon the prevailing climatic condition (Dhar and Arumuganathan, 2005). In rural areas, the mushroom growing rooms are made by mud and button mushroom crop is raised in the months of November to January. The objective of the study was to introduce the evaporative cooling pads in the existing mud house structure so as to enable the seasonal growers to raise one more crop during the months of April to May. This paper deals in finding the suitability of cost effective mud-house installed with evaporative cooling pads for raising button mushroom crop.

MATERIALS AND METHODS

Mushroom growing room was built at National Research Centre for Mushroom, Solan, Himachal Pradesh, India using mud, which is available plenty in the rural areas. The property of the mud is to keep the room warmer in the winter and cooler during the summer. The overall dimensions of the mud house used for the study was 32′ x 12′ x 8′6″. The walls were made up of mud and cement plastering was done to protect the wall from environmental factors and also to provide stability to the walls. The thickness of the wall was 1′8″ in all the four sides. The roof was made with wooden frame work over which the mud was plastered to arrest the free fall of sunrays inside the growing rooms, to protect the room from temperature variation and to arrest the free entry of air into growing room. Besides the front door (7′ x 3′) and back door (7′ x 3′), two ventilators of size 1′6″ x 3′ and two windows of size 4′ x 3′ were provided on the side walls for proper exchange of air inside the room through the evaporative cooling pads. However, an exhaust fan was also fixed in the lower side of the room to ease the removal of carbon di-oxide gas from the growing room and another exhaust fan was fixed on the top left corner of the room for making the positive pressure inside the room. So that, during the working of evaporative cooling system, fresh air from outside will be sucked through the evaporative cooling pads and it provides the entry of huge quantity of chilled air inside the room. Four Iron racks of size 5′ x 3′x 5′6″ having 4 tires were kept in the room leaving a free space of 2 feet between the racks and sidewalls for accessibility and easy workability. These racks can hold a compost capacity of 2-3 tons of button mushroom compost in pp bags. Fig. 1a shows the picture of the mud house before installing the evaporative cooling system.

The evaporative cooling pads were provided in the windows (2 Nos.) and ventilators (2 Nos.). The cooling pads of size 10 mm thickness were made using the *partal* (*Abies pindrow*) wood shavings and bamboo. These cooling pads were installed in the windows and ventilators and over which a G.I pipe of ¾″ diameter was provided. Two mm holes at a distance gap of 10

cm were made on the pipe provided over the cooling pads and the pipe was finally connected with the overhead tank for continuous water supply by gravity flow. Fig. 1b shows the mud house with evaporative cooling pads and Fig. 1c shows the evaporative cooling pads with water supply pipe.

Fig. 1a. Mud house

Fig. 1b. Mud house with evaporative cooling pad system

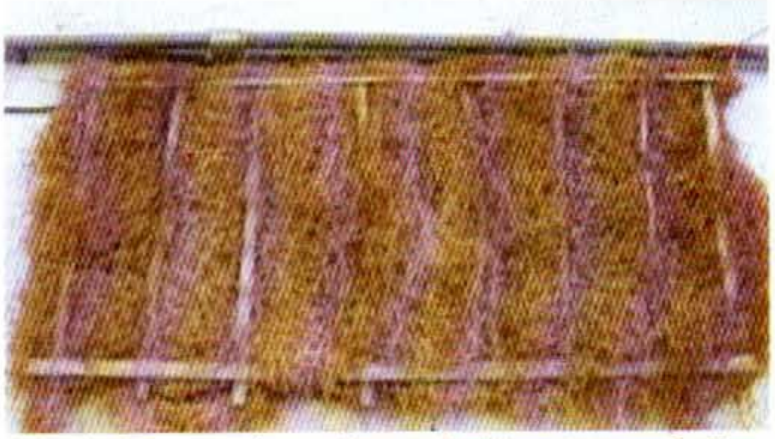
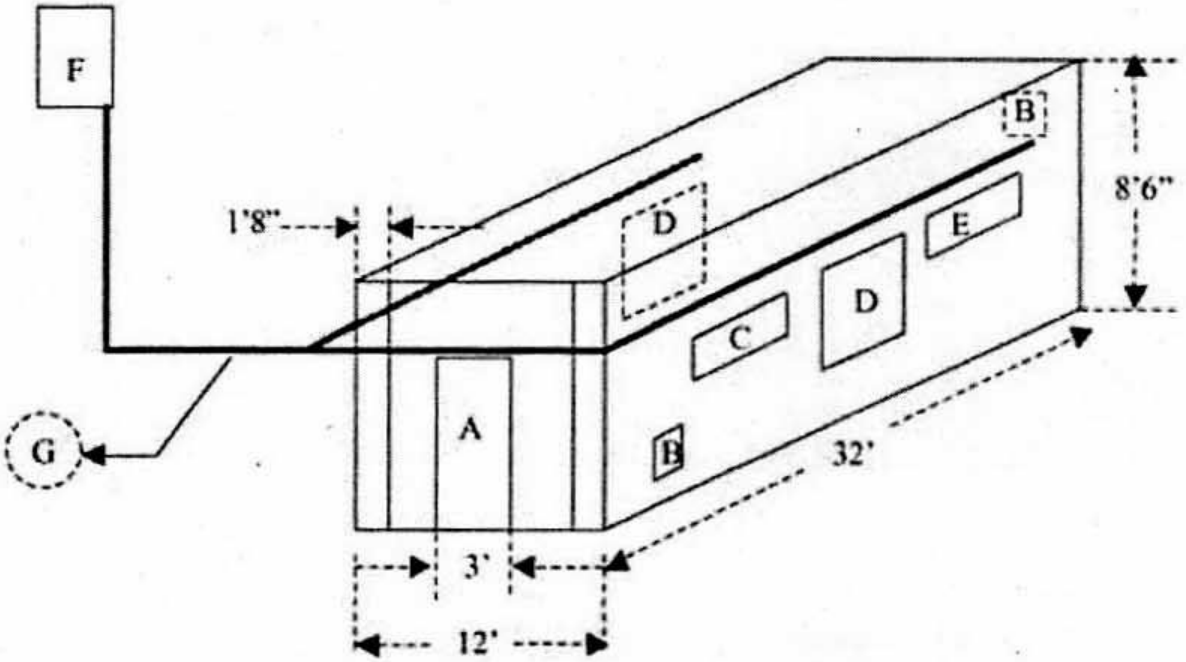


Fig. 1c. Evaporative cooling pad with water supply line.

In order to raise the button mushroom crop in the mud house, button mushroom compost was prepared by long method using raw material as per the standard method recommended by Vijay (2005). S-11 strain of *Agaricus bisporus* was evaluated during the study. The temperature and relative humidity were recorded daily using maximum-minimum thermometer and Dial type hygrometer, respectively. The mushroom yield in percentage was determined by the following formula:

$$\text{Mushroom Yield (\%)} = \frac{\text{Total mushroom harvested (kg)}}{\text{Total substrate used (kg)}} \times 100$$



Legends

A) Door	(7′ x 3′)
B) Exhaust Fan (Ventilator)	(1′ 6″ x 1′6″)
C) Ventilator (Evaporative cooling pad)	(1′6″ x 3′)
D) Window (Evaporative cooling pad)	(4′ x 3′)
E) Ventilator (Evaporative cooling pad)	(1′6″ x 3′)
F) Water tank	
G) GI Pipe	(3/4″)

Fig. 2. The schematic diagram showing the various components of the mud house with evaporative cooling system.

RESULTS AND DISCUSSION

The button mushroom crop was raised in the evaporatively cooled mud house during the month of April-May. During the period of study, the average minimum and maximum outside atmosphere temperature and relative humidity at NRC for Mushroom, Solan, Himachal Pradesh, India, ranged from 7 to 31.5°C and 9 to 87 %, respectively during the month of April and 12.5 to 32.5°C and 29 to 92 %, respectively during the month of May. The temperature and humidity inside the mud house ranged between 17 to 24°C and 79 to 90 %,

respectively. The temperature and humidity variation inside the mud house and atmosphere are shown in Fig. 3 and 4, respectively.

It is observed from the Fig. 3 that the maximum and minimum outside daytime temperature during the study period was 27 and 19°C. The similar temperature values observed inside the mud house were 24 and 17°C when the evaporative cooling pads system was not used in the mud house. The reduction in the temperature inside the mud house is due to the property of the mud, which keeps the room cooler in summer and warmer in winter. When the evaporative cooling pads were provided with the water supply, the maximum and minimum temperature values were drastically reduced. This is mainly due to the evaporative cooling effect of water which flows through the cooling pad come in contact with the incoming air, it evaporates into the air. The heat energy present in the air changes from water to water vapour, thus cooling of air takes place. During the working of evaporative cooling pad in the mud house, the temperature values were obtained between 21 and 15°C, which is the ideal temperature for the growth of button mushroom.

It can also be seen from the Fig. 4 that the maximum and minimum outside daytime relative humidity during the study period was 92 and 80 %. The similar humidity values observed inside the mud house were 90 and 79 % when the evaporative cooling pad system was not used in the mud house. On providing water supply over the evaporative cooling pads, the maximum and minimum relative humidity values were raised. This is mainly because of the evaporative cooling effect of water which flows through the cooling pad comes in contact with the incoming air, it evaporates into the air. The heat energy present in the air changes from water to water vapour and thus adiabatic cooling of air takes place, which eventually increased the relative humidity inside the mud house. During the working of evaporative cooling pad in the mud house, the humidity values were obtained between 90 and 83 %, which is the conducive relative humidity for the growth of button mushroom.

Excellent spawn run was observed in all the bags during the study period in the mud house and it took 17 days for the completion of spawn run. The button mushroom yield obtained in the study was 13.4 %. This result is on par with Singh (1997), who has also reported that button mushroom crop yield of 10 to 15 % obtained in the seasonal mushroom growing condition by small and marginal growers of Uttar Pradesh using the long method compost. Such type of mushroom growing house can be constructed for Rs. 6000 to 8000, as per the prevailing rates in the locality. It can be concluded that evaporatively-cooled mud house is highly suitable for cultivating button mushrooms in the temperate regions and North Western Plains in India during the month of April-May which enables the seasonal growers to take up one more crop in the off season apart from the regular crop at winters.

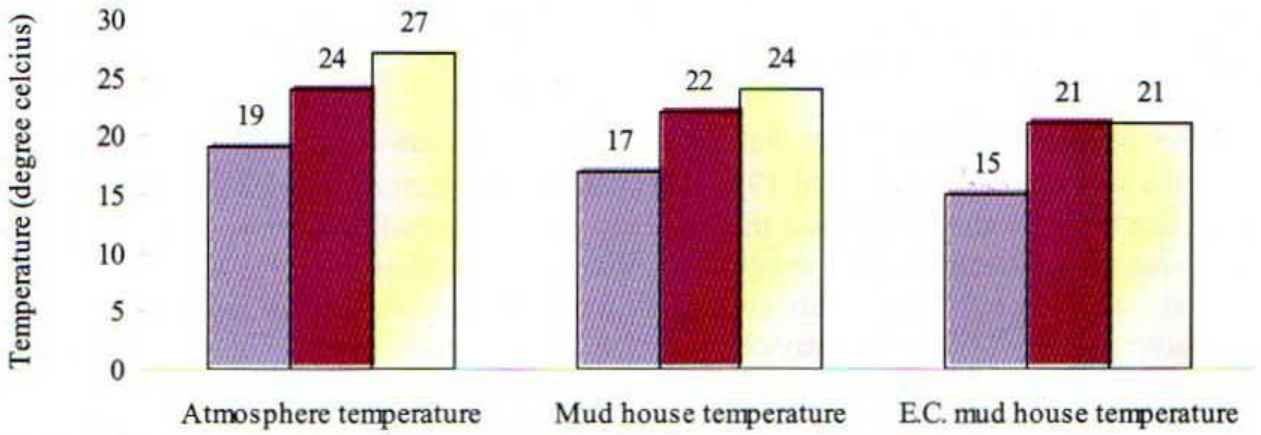


Fig. 3. Temperature variation at Evaporatively cooled mud house and atmosphere.

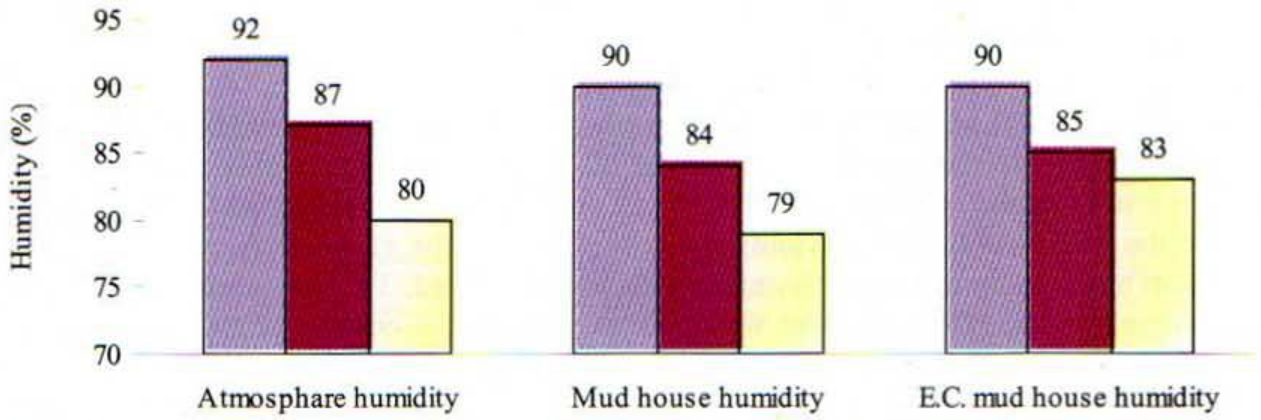


Fig. 4. Relative humidity variation in the Evaporatively cooled mud house and atmosphere.

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Effect of Scrapping, Thinning and Harvesting Time on Yield and Yield attributes of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract

Effect of three management practices viz., thinning, harvesting and scrapping time on yield and yield attributes of *Pleurotus ostreatus* was investigated during the month of June to December 2009. Remarkable variation was found in yield as well as yield attributes of the mushroom. Cluster thinning of primordia from the open surface of spawn packet after 2 days of initiation gave the highest biological yield (78.88 g/packet) as well as economic yield (72.20 g/packet). The lowest yield was obtained from control (no thinning). Potential biological yield (137.80 g/packet) as well as economic yield (126.80 g/packet) were obtained from the packets harvested just after starting of splitting of one pileus in a packet and the yield was affected when the mushroom was collected after splitting the edges of all pileus. The highest biological yield (76.20 g/packet) as well as economic yield (70.20 g/packet)was obtained from the spawn packets scrapped after 12 hours of first harvest and the lowest yield was recorded under control where no scrapping was done. Most of the yield attributes showed similar results.

Key words: Oyster mushroom, scrapping, thinning, harvesting time and yield.

INTRODUCTION

Oyster mushroom (*Pleurotus ostreatus*) is one of the widely accepted edible mushrooms that can be cultivated in the tropical region (Quimio *et al.* 1990). The cultivation technique of oyster mushroom is easy and cheap due to its wide adaptability and availability of its growing substrates (Hal, 1994). In Bangladesh, this mushroom is widely cultivated on sawdust based substrates in polypropylene bags. In the life cycle of *Pleurotus ostreatus* there are two stages- the vegetative stage and the reproductive stage. To shift the mycelium growth stage to reproductive stage for the formation of fruiting body generally some kinds of stimuli are needed. These stimuli can be initiated by some management practices like scrapping, thinning, etc. Scrapping is a process to remove some whitish portion from open surface of the sawdust bags with a sharp blade. This process stimulates the mycelium for the formation of fruit body and removes the dry or waste primordia, small and dead fruit bodies without causing harm to mycelium. It is necessary to induce fructification of mushroom in spawn bags containing sawdust supplemented with wheat bran. Some growers of Bangladesh scrap off some portion of the substrates to free the spawn packets from small and undeveloped desiccated primordia.

Thinning refers to the removal of primordial cluster partially or totally or individual primordia from spawn packet before its maturity in order to improve the size, shape and quality of the remaining fruiting body. Mushroom thinning is necessary when uniform fruiting body is required for processing and canning or for table purpose or fancy trade.

Harvesting stage of *Pleurotus* spp. can enhance the economic returns (Croan, 2000) and good quality of mushrooms can be obtained when harvesting is done at the proper stage of maturity. Immature fruit body when harvested will give poor yield. On the other hand, delayed harvesting of fruit body may increase their susceptibility to decay, resulting in poor quality and hence low market value.

The present experiment was conducted to determine the scrapping time, thinning style and harvesting stage of *Pleurotus ostreatus.*

MATERIALS AND METHODS

The experiment was conducted at National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh during June to December 2009.

Spawn packets preparation: The substrate of spawn packets were prepared using sawdust and wheat bran mixture at the ratio of 2:1 (dry weight/weight basis). Water was added to make the moisture content at 60% and $CaCO_3$ was added at the rate of 0.2% (w/w) of the total mixture to maintain the pH level at 6.5 to 7.0. Polypropylene bags of 25 × 18 cm were filled with 500 g of prepared substrate. The packets were tied, plugged with absorbent cotton and covered with brown paper. Then the packets were sterilized in an autoclave for 1.0 hour at 121°C under 1.5 kg/cm^2 pressure. After sterilization the packets were cooled and transferred to an inoculation chamber and inoculated with the mother culture of *Pleurotus ostreatus* at the rate of one teaspoonful per packet. The inoculated packets were placed on a still rack at 23 ± 2°C temperature for incubation.

Cultivation method for fruiting: After completion of mycelium running the packets were opened by D shape cut on the shoulder side and removed the sheet with a blade. Then the packets were scrapped and placed separately on the rack in the culture house. The temperature and relative humidity were maintained at 22-28°C and 80-90% respectively. Proper management practices such as watering, aeration, light arrangement and other operations were done carefully.

Thinning, harvesting and scrapping: Two to three days after opening huge number of primordia appeared on the open surface of spawn packet. Thinning of primordia was done as: T_1 = No thinning, T_2 = Total cluster thinning just after primordia initiation, T_3 = Partial thinning just after primordia initiation, T_4 = Total cluster thinning after 1 day of primordia initiation, T_5 = Partial thinning after 1 day of primordia initiation, T_6 = Total cluster thinning after 2 days of primordia initiation and T_7 = Partial thinning after 2 days of primordia initiation

Three to four days after primordia initiation, the fruiting bodies were harvested as: T_1 = Harvesting before maturation or premature stage, T_2 = Before splitting the edges of pileus, T_3 = Just splitting on one pileus, T_4 = After splitting the edges of pileus and T_5 = Farmer's practice (Control).

After mushroom harvest scrapping of spawn packet was done as: T_1 = No scrapping (control), T_2 = Just after harvesting, T_3 = 12 hours after harvesting, T_4 = 24 hours after harvesting, T_5 = 36 hours after harvesting, T_6 = 48 hours after harvesting, T_7 = 60 hours after harvesting and T_8 = 72 hours after harvesting. Treatment wise scrapping started after first harvest.

Data collection and analysis: The experiment was laid out in a completely randomized design with 4 replications. Data were collected from first two flushes. But, in case of scrapping effect study, data were collected from second and third flushes. Data on number of primordia initiation, number of effective fruiting body, biological yield, economic yield, length of stalk, diameter of stalk, diameter of pileus and thickness of pileus were recorded. Biological yield in g/500 g packet was recorded by weighing the whole fruiting bodies without removing the lower hard and dirty portion whereas economic yield was recorded by the removing of lower hard and dirty portion of fruiting bodies. Data were analyzed following standard method (Gomez and Gomez, 1984) using the MSTAT-C computer program. Means were compared following Duncan's Multiple Range Test (Gomez and Gomez, 1984) using the same computer program.

RESULTS AND DISCUSSION

Effect of thinning: The highest number of 13.64 effective fruiting bodies per packet was harvested under control where no thinning was done. It was statistically similar to all the treatments except T_3 and T_5 where partial thinning was done just after primordia initiation and 1 day after primordia initiation (Table 1).

All treatments gave significant increase in biological and economic yield of the mushroom. The two parameters under different thinning practices ranged from 61.81 to 78.88g and 56.25 to 72.20g/ packet, respectively. The highest biological yield (78.88g/ packet) and economic yield (72.20g/ packet) were recorded in T_6 where the primordia were thinned cluster wise after 2 days of initiation. The second highest economic yield was observed under T_4 which was statistically similar to all other treatments except T_1. The lowest economic yield was recorded under control where no thinning was done. The higher yield observed under cluster thinning might be due to the minimal disturbance of the remaining primordia which got the sufficient space for their growth and development.

Length and diameter of stalk of fruiting bodies under different treatments ranged from 2.71 to 3.16 cm and 1.15 to 1.46 cm, respectively. Differences in length of stalk under various treatments including control were not significant. The diameter of stalk under control was statistically similar to T_3-T_7, but significantly lower compared to T_2 only (Table 1). The lowest pileus diameter of 5.69 cm was found under control (T_1) which was

statistically similar to T_2, T_4 and T_7. The highest diameter of pileus was found under T_5 followed by T_3 and T_6. The pelius diameter under T_3, T_5 and T_6 was statistically similar and significantly higher compared to control. Significantly the highest thickness of pelius was recorded from T_2. The parameter under T_1 and T_3-T_7 was statistically similar (Table 1). The positive effect of thinning on size of fruit body might be due to availability of space for the remaining priomrdia.

Effect of harvesting time: The number of effective fruiting body of oyster mushroom was recorded in different harvesting stages. The highest number of effective fruiting bodies/ packet was observed under T_1 treatment when they were collected at pre-mature stage, which was statistically similar to T_3 and T_2. The lowest number of effective fruiting body/ packet was recorded from T_4 where the fruiting body was harvested after splitting the edges of pileus i. e. at over mature stage, which was statistically similar to T_5 (Table 2).

The highest biological yield of 137.80g/packet and economic yield of 126.80g/packet were obtained when the fruiting bodies were harvested just after splitting of one pileus (T_3), which were significantly higher compared to all other treatments. The lowest biological yield of 78.25g/packet and economic yield of 72.00g/packet were recorded from T_4 where the fruiting bodies were harvested after splitting the edges of pileus. Both the parameters under T_1, T_2 and T_5 were statistically similar (Table 2). The lower yield in both immature and over mature stages might be due to shortage of accumulation of nutrient materials in immature stage and loss of nutrient materials especially the carbohydrates for respiration in over mature stage.

Length and diameter of stalks of fruiting bodies under different treatments were not significantly different. The highest stalk length of 4.75 cm was found under T_2, which was followed by T_3 and T_4. The lowest stalk length of 4.10 cm was obtained from T_5. The diameter of stalk was maximal under T_2, while it was minimal under T_3. The lowest pelius diameter of 3.65 cm was found under T_1, which was statistically similar to T_2, T_3, and T_5. The highest diameter was recorded from T_4. The highest pileus thickness of 1.25 cm was recorded under T_1, which was statistically similar to T_5 and T_2. The minimum thickness of pileus was found under T_4 followed by T_3 (Table 2).

Table 1. Effect of thinning style of primordia on yield and yield contributing characters of oyster mushroom (*Pleurotus ostreatus*) (Two flushes)

Treatments	Number of effective fruiting body/packet	Biological yield (g/packet)	Economic yield (g/packet)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1=No thinning (Control)	13.64 a	61.81 c	56.25 c	3.16 a	1.17 b	5.69 c	0.98 b
T_2= Total cluster thinning just after primordia initiation	12.94 ab	72.69 ab	66.38 ab	3.02 a	1.46 a	5.97 bc	1.21 a
T_3= Partial thinning just after primordia initiation	10.94 b	71.44 ab	64.63 ab	2.71 a	1.35 ab	6.75 ab	0.97 b
T_4= Total cluster thinning after 1 day of primordia initiation	12.19 ab	74.13 ab	66.57 ab	2.91 a	1.25 ab	6.26 abc	1.04 b
T_5= Partial thinning after 1 day of primordia initiation	10.63 b	68.94 bc	62.05 bc	2.98 a	1.29 ab	6.82 a	1.03 b
T_6= Cluster thinning after 2 days of primordia initiation	13.00 ab	78.88 a	72.20 a	2.86 a	1.36 ab	6.58 ab	1.02 b
T_7=Partial thinning after 2 days of primordia initiation	12.25 ab	70.38 b	64.88 ab	3.11 a	1.15 b	5.00 bc	0.98 b
CV (%)	12.82	7.22	7.36	9.80	11.28	7.96	8.31

Means within the same column followed by a common letter are not significantly different (P=0.05) by DMRT.

Table 2. Effect of harvesting time on yield and yield contributing characters of oyster mushroom (*Pleurotus ostreatus*) (Two flushes)

Treatments	Number of effective fruiting body/packet	Biological yield (g/packet)	Economica l yield (g/packet)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1= Harvesting before maturation or premature stage	31.25 a	104.80 b	95.75 b	4.18 a	1.23 a	3.65 b	1.25 a
T_2= Before splitting the edges of pileus	26.50 ab	106.80 b	98.25 b	4.75 a	1.28 a	4.55 ab	1.15 ab
T_3= Just splitting the one pileus	30.50 a	137.80 a	126.80 a	4.38 a	1.07 a	4.33 ab	1.10 b
T_4= After splitting the edges of pileus	17.75 c	78.25 c	72.00 c	4.20 a	1.13 a	5.30 a	1.08 b
T_5= Farmer's practice(Control)	23.50 bc	100.80 b	92.00 b	4.10 a	1.25 a	4.05 b	1.18 ab
CV (%)	16.99	13.02	12.40	10.50	14.39	14.53	7.61

Means within the same column followed by a common letter are not significantly (P=0.05) different by DMRT.

Table 3. Effect of scrapping of oyster spawn packet on yield and yield contributing characters (Two flushes)

Treatments	Number of primordia/ packet	Number of effective fruiting body/packet	Biological yield (g/packet)	Economic yield (g/packet)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1= No scrapping	15.20 b	11.20 b	47.40 b	44.40 b	3.90 a	1.28 ab	6.40 ab	1.22 a
T_2= Scrapping just after harvesting	17.80 ab	11.80 b	68.20 a	62.60 a	3.96 a	1.22 bc	6.72 ab	1.20 a
T_3= 12 hours after harvesting	21.20 ab	14.60 b	76.20 a	70.20 a	4.22 a	1.02 c	5.72 b	1.16 a
T_4= 24 hours after harvesting	20.40 ab	14.40 b	75.20 a	69.20 a	3.74 a	1.08 bc	6.94 ab	1.12 a
T_5= 36 hours after harvesting	23.60 a	18.40 a	70.60 a	64.60 a	3.78 a	1.48 a	6.84 ab	1.28 a
T_6= 48 hours after harvesting	18.20 ab	11.80 b	67.80 a	61.80 a	3.48 a	1.10 bc	6.96 ab	1.12 a
T_7= 60 hours after harvesting	17.20 ab	11.60 b	65.00 a	61.00 ab	3.70 a	1.16 bc	6.92 ab	1.18 a
T_8= 72 hours after harvesting	20.00 ab	13.60 b	63.20 ab	68.80 ab	4.36 a	1.28 ab	7.86 a	1.20 a
CV (%)	25.75	19.73	19.26	19.97	17.90	13.47	17.46	15.15

Means within the same column followed by a common letter are not significantly (P=0.05) different by DMRT.

Effect of scrapping: The highest number of 23.60 primordia per packet was found where the packets were scrapped after 36 hours of harvest, which was significantly higher compared to only T_1 (control). The lowest number of 15.20 primordia per packet was found under T_1 where no scrapping was done, which was statistically similar to other treatments. Significantly the highest number of 18.40 effective fruiting bodies per packet was obtained from the spawn packets scrapped 36 hours after first harvest. The lowest number of 11.20 effective fruiting bodies per packet was recorded from control (Table 3). The superiority of scrapping over control might be due to stimulation effect of scrapping on primoridia initiation.

The biological and economic yields of oyster mushroom under different treatments of scrapping ranged from 47.40 to 76.20g and 44.40 to 70.20g/packet, respectively. The highest biological yield of 76.20g/packet and economic yield of 70.20g/packet were obtained when spawn packet was scrapped 12 hours after harvest, which was statistically similar to all other treatments except the control (T_1), which gave the lowest biological yield of 47.40g/packet and economic yield of 44.40g/packet (Table 3).

Stalk length of fruiting bodies under all treatments including control ranged 3.70-4.36 cm. Their differences were not significant. The highest stalk diameter of 1.48 cm was found under T_5, which was statistically similar to T_8 and T_1 (Control). The lowest stalk diameter of 1.02 cm was recorded from T_3. Pelius diameter ranged 5.32-7.86 cm and their difference was significant. The highest pileus diameter of 7.86 cm was observed under T_8, which was statistically similar to all other treatments except T_3. The lowest pileus diameter of 5.32 cm was recorded from T_3. The thickness of pileus ranged 1.12-1.20 cm under various treatments and were statistically similar (Table 3).

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Effect of Different Size and Shape of Spawn Packet on the Yield of Shiitake Mushroom (*Lentinus edodes*)

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Abstract

Five different sizes (500g, 750g, 1000g, 1250g and 1500g) and two shapes (cylindrical and square block) of spawn packet were used for the cultivation of shiitake mushroom. The growth, yield and yield contributing characters of shiitake mushroom were significantly influenced by the size and shape of spawn packets. The time required for mycelium running and total harvest were increased with the increases of packet size in both shapes of the packets. The number of fruiting body was highest in 1500g size of spawn packet and it was lowest in 500g size of spawn packet when packets were cylindrical. The maximum yields of both biological (245.50g) and economic (229.50g) were recorded from cylindrical packet when cultured on 1500g size of spawn packet and the lowest yields were obtained in 500g size of spawn for similar shape of the packet. The biological efficiency of square block shape packet showed 60.38% in 500g size of spawn which was better than others. The biological efficiency decreased with the increase of size of spawn packet. The block shape of spawn packet performed better in small size of spawn packet, whereas, cylindrical shape is better in larger size of spawn packet.

Key words: Shiitake mushroom, size and shape of spawn packet and yield.

INTRODUCTION

Shiitake is the most popular and important edible medicinal mushroom in many countries (Chen, 2001 and Royse, 2001). It is the second most cultivated edible mushroom, comprising 25.4% of the world production (Chang, 1999). The commercial cultivation of shiitake mushroom on artificial substrates, based on enriched sawdust, has increased in the last few years (Donoghue and Denison, 1995). The productivity of this mushroom depends on growing techniques, amount of supplementation, types of spawn and temperature ranges. One common technique involves heat-sealed larger bags which filled with 2-3 kg or more substrate for shiitake cultivation and produce more flushes of mushroom. Larger bag size may be suitable for reducing the labour cost and increasing the flushes and yield. The mushroom cultivation literature relealed that its production depends not only on its bag size but also on its shape. The latest development in the U. S. is the use of much larger sawdust-substrate blocks in sealed polypropylene bags. This methodology leads itself to faster and greater productivity by mixing the spawn thoroughly with the substrate, which produces more flushes of mushroom in much shorter growing cycles. European growers use larger bags and more substrate. Each bag contains 15kg of substrate in a flat slate shaped. The growing cycle in Europe is usually longer

than in the United States (Oei, 2003). Today, a common cylindrical bag method is used in Southeast Asia. Longer cylindrical bags seem to produce better than the same weight of substrate closely packed together. Hence, it is essential for growers to identify the suitable size and shape of spawn packet. Thus, the present study is aimed at determining the appropriate size and shape of spawn packet in which shiitake mushroom could be grown better.

MATERIALS AND METHODS

The experiment was conducted at National Mushroom Development and Extension Centre, Savar, Dhaka during October to March 2010 to determine the appropriate size and shape of spawn packets of shiitake mushroom. Two shapes of spawn packet *viz.* T_1 = cylindrical and T_2 = square block shape combination with five amount of substrate *viz.* R_1 = 500g, R_2 = 750g, R_3 = 1000g, R_4 = 1250g and R_5 = 1500g were used as treatments.

Spawn packet preparation: The substrate of spawn packets were prepared by using sawdust and wheat bran at the ratio of 3:1 (dry basis). Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Different sizes of polypropylene bags were filled with substrate mixture as above treatments. After filling the bags, the mouths of the packet were plugged by inserting absorbent cotton with the help of plastic neck and autoclaved at 121^0C and 1.5 kg/cm^2 pressure for 2 hours. After autoclaving and cooling, the bags were inoculated separately with the mother culture of *Lentinus edodes*-12 strain. Then, the packets were incubated in the laboratory at about 22 ± 2^0C temperatures.

Mycelial colonization and bump formation: In incubation period, whitish mycelia begin to grow on the substrate. After full colonization of spawn packet, a thick mycelial coat forms on the outer surface of colonized substrate. Blister like bump of various sizes appeared on the surface of the mycelial coat. Bumping began after browning of colonized white mycelia developed on the packets.

Culture condition and management: After mycelium maturation and bump formation, all the packets were fully opened by removing the polypropylene bags. Then the packets were placed on the rack of a growing house. The temperature, relative humidity and light intensity of the growing house were maintained at 18 - 22°C, 70 - 80% and 10 - 20 lux respectively. Watering was done 3 to 4 times/ day to maintain the temperature and relative humidity.

Experimental design, data collection and analysis: The experiment was laid out in a completely randomized design (CRD) with 4 replications. Data were collected on the time required for mycelium running and harvest (days), number of fruiting body/ packet and biological and economic yield (g/ packet). The biological yield in g/ packet was recorded by weighing the whole fruiting bodies collected from only first flush and the economic yield was recorded by removing the lower hard and dirty portion of the fruiting bodies. The biological efficiency (%) was determined by the following formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Total biological yield (g)}}{\text{Total dry substrate used (g)}} \times 100$$

The data were analyzed following MSTAT-C computer programme and means were computed and separated following Duncan s multiple range test (DMRT) using the same computer programme.

RESULTS AND DISCUSSION

Time required for mycelium running and total harvest: Among the treatments, significant variations were observed in time required for mycelium running (TRMR) and in time required for harvest (TRH) (Table 1). The minimum TRMR (24.75 days) was recorded in T_1R_1, where the packets were cylindrical shape 500g, which was statistically similar to all the treatment except T_1R_4 and T_1R_5. The highest TRMR was observed in T_1R_5 where packets were cylindrical 1500g. The lowest TRH was recorded in T_2R_1, where the packets were square block 500g which was statistically similar to all the treatment except T_2R_5. The larger packets took more time in both the shapes.

Effect on number of fruiting body: The number of fruiting body ranged from 13.25 to 32.00 among the treatments (Table 1). The highest number of fruiting body (32.00) was recorded under T_1R_5, 1500g cylindrical spawn packet, which was statistically similar to the rest of the treatments, except T_1R_1. The lowest number of fruiting body (13.25) was recorded from T_1R_1, 500g cylindrical spawn packet.

Biological yield and economic yield: The highest biological yield (245.50 g/ packet) was recorded in T_1R_5, 1500g cylindrical spawn packet, followed by T_1R_4, T_2R_5, T_1R_3 and T_2R_4 (Table 1). The lowest biological yield (100.80g/ packet) was obtained from 500g cylindrical spawn packet. Similar trend was observed in economic yield. It was evident from the present study that the mushroom yield increased with the increasing amount of substrate in the spawn packet, i.e., yield was positively correlated to the substrate amount. The results of the present study corroborated the findings reported by Chen (2001).

Biological efficiency: Biological efficiency was significantly affected by the size and shape of spawn packet (Fig. 1). Square block shape packet gave the highest biological efficiency (60.38%) when cultivated with 500g size of spawn packet. The similar size of cylindrical packet showed 50.38% biological efficiency. The lowest biological efficiency (32.71%) was recorded in 1500g of spawn packet when cultured in block shape of packet. The study revealed that the biological efficiency decreased with the increase of size of spawn packet. The block shape of spawn packet performed better in small size of spawn packet, whereas, cylindrical shape is better in larger size of spawn packet.

Table 1. Interaction effect of different sizes and shapes of spawn packet on growth, yield contributing character and yield of shiitake mushroom

Shape × Size	Time required for mycelium running (days)	Time required for harvest (days)	Number of fruiting body/packet	Biological yield (g/packet)	Economic yield (g/packet)
T_1R_1	24.75 c	107.00 b	13.25 b	100.80 d	91.00 d
T_1R_2	35.00abc	114.00 b	23.25 ab	154.00bcd	144.50bcd
T_1R_3	44.00abc	115.50 b	26.00 ab	193.50abc	182.00abc
T_1R_4	50.75 ab	121.00 b	29.75 ab	233.50 ab	223.00 ab
T_1R_5	59.25 a	125.50 ab	32.00 a	245.50 a	229.50 a
T_2R_1	34.25 bc	105.30 b	19.50 ab	120.80 cd	110.80 cd
T_2R_2	38.00abc	121.30 b	23.00 ab	143.00 cd	134.50 cd
T_2R_3	42.50abc	120.00 b	25.00 ab	153.80bcd	143.50bcd
T_2R_4	44.50abc	124.30 ab	26.25 ab	190.00abc	179.80abc
T_2R_5	45.75abc	146.00 a	27.50 ab	196.30abc	184.30abc
CV (%)	6.37	3.92	11.76	14.64	16.65

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.
T_1 = cylindrical, T_2 = square block, R_1 = 500g, R_2 = 750g, R_3 = 1000g, R_4 = 1250g and R_5 = 1500g.

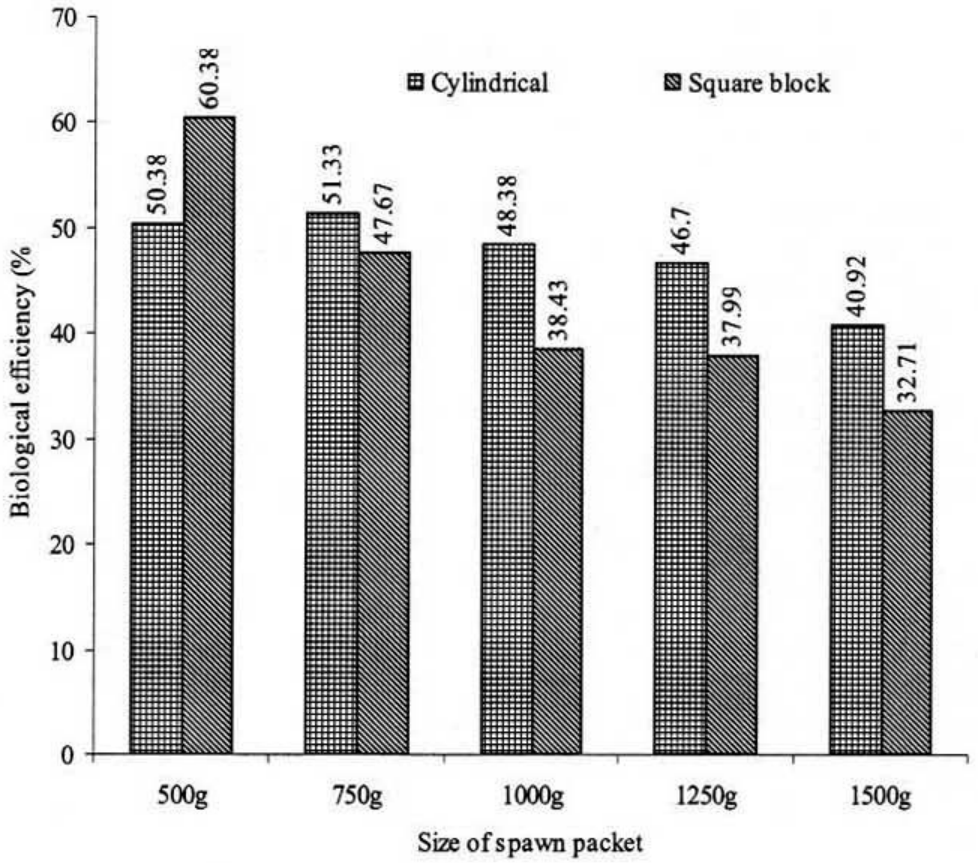

Fig. 1. Biological efficiency of shiitake mushroom on five different sizes and two shapes of spawn packet.

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Development of a Soya-Mushroom Curry Cake (Bori) and Its Comparison with Traditional Bori

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Abstract

The nutritional value of a newly developed curry cake (soya-mushroom bori), prepared from the composite mixture of oyster mushroom (*Pleurotus ostreatus*), soybean, mushkolai and papya, was compared with that of the traditional one. The proximate composition of this novel soya-mushroom bori (Sample A) and those of others (Two home-made bori- samples Band C; two commercially available bori-samples D, E) were determined according to the standard AOAC (1995) methods. The mean ± SD for moisture, ash, protein, fat, fiber and carbohydrate per 100g of the newly developed bori were 4.57±0.17, 3.76±0.03, 36.13±0.61, 4.14±0.19, 3.8±0.57 and 47.34±0.72, respectively. Energy content of the soya-mushroom product was 352 Kilocalorie per 100g. The mean ± SD for moisture, ash, protein, fat, crude fiber, and carbohydrate per 100g of traditional bori were (10.55±0.19, 2.92±0.2, 26.8±0.94, 0.88 ±0.05, 2.1±0.53 and 56.72±0.95,respectively) for sample B, (9.25±0.05, 2.99±0.39, 28.40±0.5, 0.61±0.05, 1.3±0.46 and 57.46±0.53,respectively) for sample C, (13.59±0.5, 2.84±0.54, 22.52±0.56, 0.67±0.06, 1.01±0.34 and 59.38±1.59, respectively) for sample D and (13.08±0.34, 2.85±0.32, 22.60±0.77, 0.60±0.07, 1.0±0.15 and 59.87±1.53, respectively) for sample E. Energy content of sample B, C, D and E were 337, 345, 330 and 332 Kilocalorie per 100g, respectively.

Key words: Curry cake, bori, oyster mushroom, and soybean.

INTRODUCTION

In Bangladesh, nearly forty percent of the population lives below the food consumption-based poverty line, lacking sufficient nutritional supplements to afford diet of 2,122 Kilocalories (Kcal) per person per day, along with other basic necessities (Hossain *et al.* 2005). Apart from the prevailing deficit in total caloric intake, the normal diet of Bangladeshi people is seriously imbalanced, with inadequate consumption of protein, fat, fruits and vegetables. Women and children are especially vulnerable to their greater nutritional requirements. Animal foods, which are the richest sources of protein and many micronutrients, are beyond most people's means in Bangladesh. Hence, promoting the production and consumption of comparatively cheap food is an important strategy for combating nutritional deficiency. Considering these facts, an attempt was made to prepare a protein-, fiber- and mineral-enriched curry cake from mushroom, soyabean, mushkolai, and papaya.

Mushroom is considered to be as a complete and safest food, suitable for all age groups. It is one of the richest sources of protein, vitamins and minerals (Kurtzman, 1997).The climatic condition of Bangladesh especially facilitates the cultivation of oyster mushroom

(*Pleurotus ostreatus*) well. Soybean, a staple food in many Asian countries, contains valuable constituents, including protein, isoflavones, saponins, and phytosterols (Allison *et al.*2003). Soybeans are considered to be a source of complete protein (Henkel, 2000). Papaya, a culinary-cum-fruit item in Bangladesh, is grown all the year round and all over the country. Bori, a taste enhancer, is a popular food in some part of Bangladesh. Thus, the present study is aimed at developing a protein, fiber and minerals enriched novel curry cake (soya-mushroom bori), measuring the nutritional value of the resultant product and comparing this value with those of traditional ones.

MATERIALS AND METHODS

Raw materials: Soyabean was collected from Bangladesh Agricultural Research Institute. Oyster mushroom (*Pleurotus ostreatus*) was collected from National Mushroom Development and Extension Center, Savar. Mushkolai and papaya were collected from local market.

For comparative study, two samples of home made bori (sample B and sample C) were collected from a village of Jessore and two samples of commercial bori (sample D and sample E) were collected from the local market.

Preparation of raw materials: Soyabean and mushkolai, after being soaked for 12 hours,were dehulled and finally blended through blender. Mushroom was dried properly and made into fine powder through blender. Papaya, collected from local market, were washed properly, cut into small pieces and blended.

Preparation of soya-mushroom bori: Blended soyabean (30%) and mushkolai (56%) were mixed together and whipped for afew minutes to incorporate air in the mixture. When the mixture became lighter than water,mushroom powder (6%) and blended papaya (8%) were added and again mixed for several minutes. Then small cake like bori was made from the paste and dried in a hot air oven at 100°C for 2 hours and then at 80°C for another 3 hours (Fig. 1). After proper drying, the bori was ready to use with curry or individually as curry.

Proximate analysis: The proximate composition (i.e. moisture, protein, fat, fiber, carbohydrate) and total energy of soya-mushroom bori and different traditional bori were determined according to the standard analytical methods (AOAC, 1995). At least four samples of each category were analyzed.

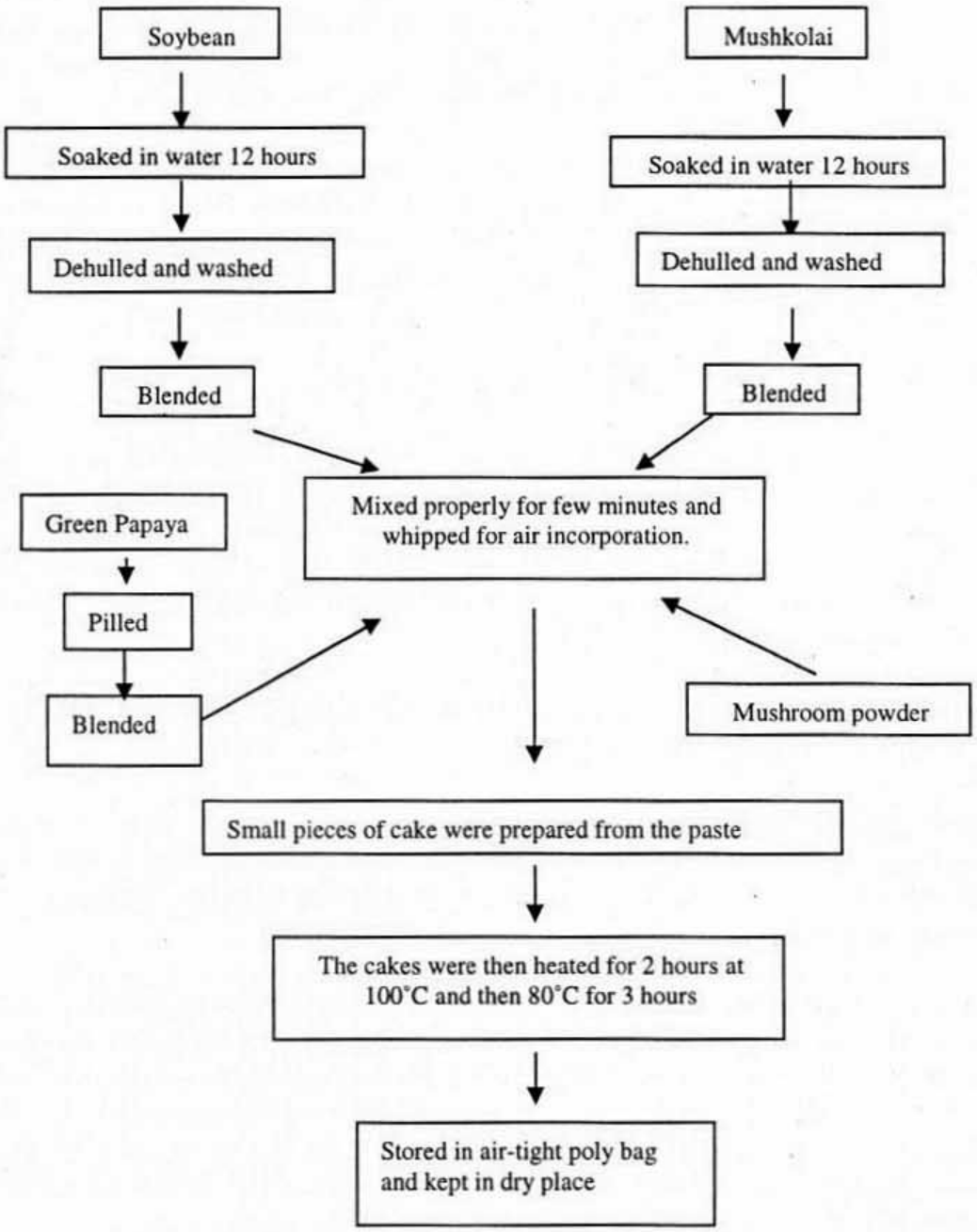

Fig 1. Flow chart for the preparation of soya-mushroom bori.

Determination of moisture: Moisture content was determined by drying a sample in an oven at 100°C for 12 hours, the weight loss incurred was calculated as:

% moisture = (Weight loss on drying/ Weight of the sample) X 100

Determination of crude protein: Crude Protein content of the samples was determined using the Kjeldahl method. The method consists of three basic steps: 1. digestion of the sample in sulfuric acid with a catalyst, which results in conversion of nitrogen to ammonia; 2. distillation of the ammonia into a trapping solution; and 3. quantification of the ammonia by titration with a standard solution. According to this method,

% crude protein content of the samples = % nitrogen x 6.25.

Determination of total ash: To determine Ash content, a dried and ground sample was ignite in a furnace at 600°C for 4 hours to oxidize all organic matter. Crucibles were first dried for about 2 hours at 100°C in an oven and placed in a desiccator. They were cooled and about 2.0g of sample was weighed into the crucible. The samples were then placed in a furnace at 600°C for four hours. Percentage ash content was determined by weighing the resulting inorganic residue.

Weight of ash g = [{(Weight of the crucible +ash) - (Weight of the crucible)}/ Weight of the sample] X 100

Determination of fat: Fat content was determined using Soxhlet extraction method. In this method, fat was determined by extracting the dried materials (food samples) with a light petroleum fraction in a continuous extraction apparatus. The solvent was distilled off and extract was dried and weighed.

Determination of crude fiber: Moisture and fat free sample was boiled with 0.255N H_2SO_4 and 0.313N NaOH, consecutively, for 30 minutes under a reflux condenser and each time the sample was washed with boiling water properly to remove acid and alkali residue. The sample was then transferred in a crucible, dried overnight at 100°C and weighed (W_1) in an analytical balance. The crucible was heated in a muffle furnace at 600°C for 20 minutes, cooled and weighed again (W_2). The difference in the weights (W_1-W_2) represents the weight of crude fiber.

Crude fiber (g/100g) = {(W_1-W_2)/ Weight of the dried sample} X 100

Determination of total carbohydrate: The content of the available carbohydrate was determined by the following equation:

Total carbohydrate (g/100g of sample) = {100-(moisture + ash + protein + fat + crude fiber)}

Determination of energy content: Metabolizable energy was calculated following the formula below:

Energy (kcal/100g) = (4 X Carbohydrate) + (4 X Protein) + (9 X Fat) (Joshi, 2002)

RESULTS AND DISCUSSION

The nutritional analysis of the developed soya-mushroom bori (sample A) compared with those of the traditional ones are presented in the table 1. The content of the moisture, ash, protein, fat, fiber and carbohydrate per 100g of the newly developed bori were 4.57±0.17, 3.76±0.03, 36.13±0.61, 4.14±0.19, 3.8±0.57 and 47.34±0.72, respectively. Energy content of the developed product was 352 Kcal per 100g.

Table 1. Nutritional value of traditional bori as compared with newly developed bori

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E
Moisture (%)	4.57 (±0.17)	10.55 (±0.19)	9.25 (±0.05)	13.59 (±0.5)	13.08 (±0.34)
Fat (%)	4.14 (±0.19)	0.88 (±0.05)	0.61 (±0.05)	0.67 (±0.06)	0.60 (±0.07)
Protein (%)	36.13 (±0.61)	26.80 (±0.94)	28.40 (±0.5)	22.52 (±0.56)	22.60 (±0.77)
Fiber (%)	3.80 (±0.57)	2.10 (±0.53)	1.30 (±0.46)	1.01 (±0.34)	1.00 (±0.15)
Ash (%)	3.76 (±0.03)	2.92 (±0.2)	2.99 (±0.39)	2.84 (±0.54)	2.85 (±0.32)
Carbohydrate (%)	47.34 (±0.72)	56.72 (±0.95)	57.46 (±0.53)	59.38 (±1.59)	59.87 (±1.53)
Energy Kcal	352	337	345	330	332

Sample A= Developed bori, Sample B= Home made bori 1, Sample C= Home made bori 2, Sample D= Local market bori 1, Sample E= Local market bori 2.

The moisture content of all the traditional bori (sample B, C, D and E) was higher than that of the newly developed product. However, protein, fat, fiber and ash content of the developed bori (36.13±0.61, 4.14±0.19, 3.8±0.57 and 3.76±0.03) were higher than those of sample B (26.8±0.94, 0.88 ±0.05, 2.1±0.53 and 2.92±0.2), sample C (28.40±0.5, 0.61±0.05, 1.3±0.46 and 2.99±0.39), sample D (22.52±0.56, 0.67±0.06, 1.01±0.34, and 2.84±0.54) and sample E (22.60±0.77, 0.60±0.07, 1.0±0.15, and 2.85±0.32). Although, carbohydrate content of the soya-mushroom bori was lower, the energy content was higher than those of the samples B, C, D and E.

The higher content of protein, fat, fiber and total minerals in the soya-mushroom based curry cake might be attributed to the addition of soybean, mushroom and papaya. As the prepared cake contain significant amount of protein, it may be an alternative protein source for the vegetarians and for the people suffering from protein energy malnutrition. The increased amount of dietary fiber present in the soya-mushroom bori may aid in ameliorating diabetes, constipation and peptic ulcers. Thus, this complex nutritional supplement might stand a good stead in providing nutritional support towards malnourished population of different age groups. Besides, this novel endeavor, will open a new vista for the local entrepreneurs and aid in developing new food industries leading to the economic gain of our national economy.

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Effect of Different Supplements to Different Substrates on Growth and Yield of *Pleurotus citrinopileatus*

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Abstract

To find out an effective supplement, *Pleurotus citrinopileatus* was grown on sawdust (SD), cotton waste (CW) and paddy straw (PS) and their combinations supplemented with rice bran (RB), wheat bran (WB), maize powder (MP) and sesame oil seed cake (SOSC) at 1% level. The days required from opening to first harvest (DROFH) varied from 3.00 to 15.50 days and it was maximum (15.50) in SD + CW (1:1) supplemented with SOSC. The minimum DROFH (3.00) was required in SD + PS (1:1) supplemented with SOSC. The maximum yield (166.00g/ 200g dry substrate) was obtained from SD supplemented with SOSC and the lowest yield (24.00g/ 200g dry substrate) was obtained from SD supplemented with MP. The highest biological efficiency (BE) (83.0%) was recorded in SD supplemented with SOSC that was significantly higher than all other treatments. The lowest BE (12.0%) was recorded in SD supplemented with MP.

Key words: Supplements, *Pleurotus citrinopileatus,* yield and biological efficiency.

INTRODUCTION

Pleurotus citrinopileatus is an edible mushroom having beautiful colour, excellent flavour and taste. It can be grown on agricultural and industrial wastes though the yield in Bangladesh is not satisfactorily. To increase the productiveness of mushroom supplementation of substrates is commonly practiced (Moda *et al.*, 2005). Supplementations of main substrates with nutrient or combination of two or more substrates increase the yields of *P. sajor-caju* (Jadhav *et al.*, 1998). Supplements or additives usually change the decomposition rate and also the sequence of decomposition of substrates components (Zadrazil, 1993). Organic supplements viz. soybean meal, alfalfa meal, and cotton seed powder increase not only yields but also proteins of mushrooms (Zadrazil, 1980). *Pleurotus citrinopileatus* and *P. sajor caju* when cultivated on paddy straw supplemented with different supplement, Krishnamoorthy (1997) observed that neem cake increased yield 48.7 and 75% respectively. Various basic raw materials or nutritive supplements have been tried to increase the productivity of *P. eryngii* (Olivier *et al.*, 1999). In case of *Pleurotus spp.* it was found that supplementing the growth substrate with oil seed cakes could greatly influence the production of mushroom yields (Bano *et al.*, 1993). Several nitrogen sources tried as supplements to the rice straw, yeast mud, cotton seed powder and rice bran proved ideal in increasing the mushroom yields (Jandaik, 1989). Rana and Subag (1990) recorded that gram pod waste in combination with wheat straw (1:1) improved mushroom yields by 5.26% over the

wheat straw alone. Yoshida *et al.* (1993) reported the highest yield with substrates (chopped straw or sawdust) mixed with wheat bran, rice bran and bean curd at 45% level. The substrate of sawdust of *Mangifera indica* supplemented with 50% sawdust waste of *L. edodes* appeared to be the best substrate for growing *P. citrinopileatus* (Liang *et al.*, 2005). In *P. citrinopileatus*, there is generally low yield achieved when grown or cultivated on substrates. To improve the yield, supplementation is necessary. This study was under taken to investigate the best supplement on the growth and yield of *P. citrinopileatus.*

MATERIALS AND METHODS

The experiment was conducted at the National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka during the months of June to November 2009.

Saw dust (SD), cotton waste (CW) and paddy straw (PS) were used as substrates. The straw was cut into small pieces (3-4 cm) and all the substrates were supplemented with 1% rice bran (RB), wheat bran (WB), maize powder (MP) and sesame oil seed cake (SOSC). Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Polypropylene bags of 7″×10″ size were filled with 200g of dry substrate mixture and their mouth were plugged by absorbent cotton and covered with brown paper and tied with a rubber band. Then bags were autoclaved at 121^0C and 15 PSI for 2 hours and then allowed to cool. Each spawn packet was inoculated with the mother culture at the rate of two teaspoonfuls per packet. Bags were then incubated for mycelium running at 25 ± 2^0c temperature. After completion of mycelium running, the polypropylene bags were cut open on both the side by D-shaped (1.5″× 0.5″) and were sprayed with water using a sprayer to maintain high relative humidity in the culture house. The spray of water was discontinued a day before the harvest of the fruiting bodies.

Statistical Analysis: The experiment was laid out in completely randomized designs (CRD) with 4 replications. Data on days required from opening to first harvest, biological yield and biological efficiency were collected and analyzed following Gomez and Gomez (1984) using MSTAT-C computer Programme. Means were separated by Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Days required from opening to first harvest (DROFH): The DROFH varied from 3.00 to 15. 50 days. The highest DROFH (15.50) was observed in combination of SD + CW (1:1) substrate supplemented with SOSC which was statistically similar to all the treatments except WB supplemented to SD and PS + CW (1:1), MP supplemented to CW and SOSC supplemented to CW and PS + CW (1:1). The lowest DROFH (3.00) was recorded in combination of PS+ CW (1:1) supplemented with SOSC.

Table 1. Effect of different supplements to different substrates on days required from opening to first harvest of *Pleurotus citrinopileatus*

Substrates	Supplements				
	No supplement (Control)	Rice bran (RB)	Wheat bran (WB)	Maize powder (MP)	Sesame oil seed cake (SOSC)
Saw dust (SD)	7.00 abc	11.00 abc	5.25 bc	7.25 abc	5.75 abc
Cotton waste (CW)	6.50 abc	5.75 abc	6.75 abc	5.25 bc	3.50 c
Paddy straw (PS)	14.25 ab	9.50 abc	10.25 abc	13.00 abc	7.25 abc
SD+ CW (1:1)	6.50 abc	6.25 abc	14.75 ab	7.25 abc	15.50 a
SD+PS (1:1)	11.25 abc	11.0 abc	8.00 abc	10.50 abc	15.00 ab
PS+ CW (1:1)	8.25 abc	10.0 abc	5.25 bc	8.50 abc	3.00 c
SD+CW+PS (1:1:1)	9.50 abc	8.75 abc	6.75 abc	9.50 abc	7.00 abc
CV (%)			7.85		

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Yield: The yield of mushrooms was positively affected by different supplements. The highest yield (166.0g/200g dry substrate) was obtained on SD supplemented with SOSC which was statistically similar to all the treatments except RB, WB and MP supplemented to SD; MP and SOSC supplemented to CW; WB and SOSC supplemented to SD + PS (1:1) and on SD, CW and SD + CW (1:1) without any supplement. The lowest yield was recorded on the SD supplemented with MP. Krishnamoorthy (1997) reported that among 15 different organic supplements neem cake increased the yield of *P. citrinopileatus and P. sajor caju* by 48.7 and 75% respectively.

Table 2. Effect of different supplements to different substrates on yield (g/packet) of *Pleurotus citrinopileatus*

Substrates	Yield (g/ substrate)				
	No supplement (Control)	Rcie bran (RB)	Wheat bran (WB)	Maize powder (MP)	Sesame oil seed cake (SOSC)
Saw dust (SD)	48.75 bc	59.0 bc	48.0 bc	24.00 c	166.0a
Cotton waste (CW)	56.25 bc	63.75 abc	62.75 abc	56.75 bc	51.50 bc
Paddy straw (PS)	89.0 abc	107.3 abc	76.75 abc	103.5 abc	79.50 abc
SD+ CW (1:1)	46.75 bc	123.5 ab	68.50 abc	105.5 abc	88.0 abc
SD+PS (1:1)	83.0 abc	102.3 abc	41.50 bc	98.75 abc	42.0 bc
PS+ CW (1:1)	76.5 abc	93.0 abc	95.75 abc	95.0 abc	95.75 abc
SD+CW+PS (1:1:1)	94.75 abc	107.0 abc	86.25 abc	86.0 abc	88.25 abc
CV (%)			9.36		

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Biological efficiency (BE): The BE was worked out against the dry weight of substrates. Different supplements increased the BE and it was highest in SD supplemented with SOSC followed by SD + CW (1:1) supplemented with RB; PS supplemented with RB. The lowest BE (12%) was recorded in SD supplemented with MP.

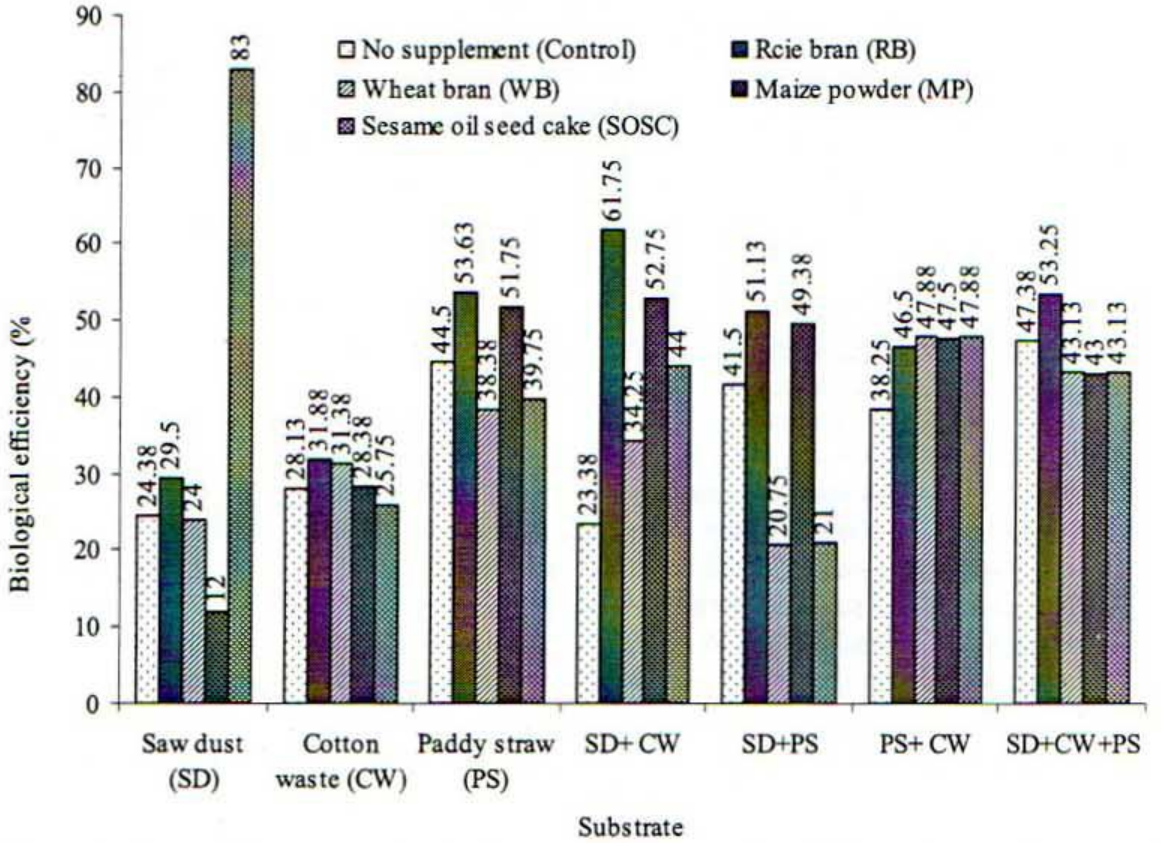

Fig. 1. Effect of different supplements to different substrates on biological efficiency of *Pleurotus citrinopileatus*.

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Prevalence of Mycoflora Associated with Oyster Mushroom (*Pleurotus ostreatus*) Substrates and Evaluation of Formalin and Bavistin against Them

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Abatract

The present study was carried out to identify weed mycoflora associated with *Pleurotus ostreatus* (*Oyster mushroom*) substrate during culture in the spawn packet and to evaluate Formalin and Bavistin % (Cabendazim) 50WP against the weed mycoflora. A total of 50 spawn packets colonizing substrate of *Pleurotus ostreatus* were collected randomly at different growth stages Ten weed mycoflora namely *Aspergillus flavus, A. fumigatus, A. niger, A. nidulans, A. terreus, Penicillium citrinum, P. thiersii, Penicillium.* sp., *Rhizopus stolonifer* and *Trichoderma harzianum* were found to be associated with the substrate. Formalin, Bavistin and their combination were tested against the idenfied weed mycoflora at 100, 250 and 500 ppm in vitro following poison food technique. All treatments caused radial mycelium growth inhibition of all isolated weed mycoflora over control. The rate of inhibition corresponded to higher doses of the chemicals. The combine application of Formalin and Bavistin at their highest concentration (500+75 ppm) gave the maximum inhibition of gowth of all the identified fungi.

Key words: Mushroom substrate, weed mycoflora, Bavistin and Formalin.

INTRODUCTION

Mushrooms are large reproductive structure of edible fungi belonging to Basidiomycotina. They comprise a large heterogeneous group having various shapes, sizes, colours; appearance and edibility (Chang and Miles, 1991). Of the various kinds, oyster mushroom (*Pleurotus ostreatus*) is cultivated in Bangladesh. Mushroom is used as food for human consumption in the country. It is a good source of protein, vitamins and minerals (Khan *et al.,* 1981) and contain about 85-95% water, 3% protein, 4% carbohydrates, 0.1% fats and 1% minerals and vitamins (Tewari, 1986). Mushrooms have medicinal properties like anti-cancerous, anti-cholesteral and anti-tumorous activities and are useful against diabetes, ulcer and lungs diseases (Quimio, 1976).

Mushroom crops are attacked by weed moulds or associated mycoflora. Most of them act as competitor moulds thereby spawn run is adversely affected either by competition for food material or through production of toxic substances (Vijay and Sohi, 1986). Yield losses due to the associated weed molds on the basidiocarps of oyster mushrooms (*Pleurotus sajor-caju* and *P. ostreatus*) may vary between 10 and 20% (Alameda and Mignucci, 1998). The most common weed molds associated with green mould disorder of edible mushrooms is *Trichoderma,* which is resistant to pasteurization. Emerged fruiting

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bodies in the affected portion of the substrate were badly spotted, brownish in colour and reduced in both growth rate and yield performance. In general, when weed moulds were at its worst, crop yields were reduced an average of 35%, with a range of 20% to 80% (Singh *et al.* 1999).

The management of weed mycoflora associated with substrate of mushroom is very difficult because both the host and the parasites are fungi. Improved sanitation, hygiene and the application of chemicals to mushroom spawn before the spawn is mixed with the substrate may reduce the continuing threat. Chemical treatments with 1.0% formaldehyde and that using 2.0% copper sulphate gave satisfactory mushroom yields. The sterilization method had little effect on the rate of mycelial development. Chemical disinfection is recommended as it is cheaper than steam sterilization (Afyon, 1988). Highest yield (95% biological efficiency) was recorded with substrates treated with Bavistin (carbendazim) at 75 ppm + formalin (formaldehyde) at 500 ppm, which was equivalent to the yields obtained from substrates pasteurized with hot water (70-80^{0}C) or Bavistin at 75 ppm (Pani and Das, 1998). In Bangladesh, reports on weed mycoflora of mushroom substrate and their control are not available Keeping these view in mind, the present investigation was undertaken to identify weed mycoflora associated with the substrates of oyster mushroom and to find out the appropriate chemicals and their doses to control them.

MARERIAL AND METHODS

Identification of weed mycoflora: An attempt was made to isolate and identify different mycoflora associated with the substrate colonized with oyster mushroom. The experiment was conducted at National Mushroom Development and Extension Centre, Department of Agriculture Extension, Sobahanbag, Savar, Dhaka, Bangladesh during 2006-2007. Oyster mushroom growing substrates were sampled at five different growth stages viz. mother spawn, commercial spawn, 1st flush, 2nd flush and 3rd flush. To prepare a stalk solution, 10 g samples from each of the substrates were mixed with 100 ml sterile distilled water. A series of dilutions were made by taking 1 ml from the stalk solution to add with 9 ml sterile distilled water consecutively in 10 successions (Dhingra and Sinclair, 1985). From each of the different substrate dilutions 0.5 ml volumes were pipetted on melted poatato dextrose agar medium (PDA). The substrate solution was thoroughly mixed with the medium and allowed to solidify. After solidification the plates were incubated at 30^{0}C for 72 hours. The mycoflora grown as the mixed colony in the culture plate were isolated. To prepare pure culture sufficient number of sub culturing were made following hyphal tip technique (Hyakumachi, 1994) and maintained in PDA slants at 10^{0}C. Individual mycoflora were identified using CMI description (Barnett, 1980).

Invitro evaluation of Bavistin and Formalin: Bavistin (Carbendazim) 50WP and Formalin were tested *in vitro* to evaluate their effect on the radial growth of isolated weed fungi were evaluated against the weed mycoflora isolated from mushroom substrate). Three concentrations of the materials viz. 100, 250 and 500 ppm of Formalin, 25, 50 and 75 ppm of Bavistin, and 100 +25, 250 + 50 and 500 + 75 ppm of Formalin + Bavistin were used and poison food technique (Begum, 2000) was followed. Freshly prepared

PDA was poured into Petri dishes at 20 ml/dish. Before solidification the PDA was amended with required amounts of fungicides to have the selected concentrations and mixed thoroughly. PDA in plates under control did not recieve any fungicides.

Efficiency of chemicals at different concentrations were evaluated on the basis of percent inhibition of the radial colony growth of the isolated fungi, and computed as a proportional measurement of colony diameter on control plate expressing in percentage, following the formula given by Sundar *et al.*, 1995. Wheat grains were used as substrates for the preparation of inocula of isolated weed mycoflora. Wheat grains were soaked in water over night and taken in the 500 ml Erlenmeyer (Pyrex) flasks up to 1/3 from the bottom and autoclaved under 1.1 Kg/cm^2 pressure at 121^0C for an hour. Separate flasks were used for each isolated fungus. Ten mycelial discs each of 5 mm diameter were cut from the edge of three days old PDA culture of individual fungus and added to autoclaved wheat grain in the flasks. They were incubated at 25^0C for 20 days. The incubated flasks were shaken by hand at 2-3 days interval for uniform colonization. The colonized wheat grains were air dried for 2 weeks and stored at 10^0C for use in the test.

After solidification, amended PDA in plates was inculated with the inocula of individual mycoflora. For inoculation one wheat grains colonized with individual weed mycoflora was placed at the centre of a plate. Five plates were used for each concentration. The plates were placed in an incubator following completely randomized design and incubated at 30^0C. When a fungus covered the plates under control radial colony diameter was measured. Efficiency of two chemicals at different concentrations were evaluated on the basis of percent inhibition of the radial colony growth, and computed as a proportional measurement of colony diameter on control plate expressing in percentage, following the formula given by Sundar *et al.*, 1995.

$$\% \text{ Inhibition} = \frac{X - Y}{X} \times 100$$

Where, X= radial colonydiameter (mm) on unamended PDA in control plates,
Y = radial colony diameter (mm) on fungicide treated PDA.

RESULTS AND DISCUSSION

Isolation and identification of associated mycoflora: A total number of 10 weed mycoflora were isolated from the substrates of oyster mushroom. They were identified as *Aspergillus flavus, A. fumigatus, A. niger, A. nidulans, A. terreus, Penicillium citrinum, P. thiersii, Penicillium.* sp., *Rhizopus stolonifer* and *Trichoderma harzianum.* Thei prevalence with growth stages of musroom. (Table 1). They are also identified as common weed fungi of mushroom.

Table 1. Prevalence of associated mycoflora at different stages of Oyster mushroom

Associated mycoflora	% Contaminated packets with mycoflora				
	Mother spawn	Commercial spawn	1st harvest	2nd harvest	3rd harvest
Aspergillus flavus	2.67	5.33	14.67	18.0	26.67
A. fumigatus	2.00	4.00	7.33	8.00	12.00
A. niger	0.00	1.00	12.00	7.33	13.33
A. nidulans	1.00	6.00	8.00	4.67	12.00
A. terreus	0.67	4.00	5.33	6.00	8.67
Penicillium citrinum	0.67	2.00	4.00	7.33	9.33
P. thiersii	2.67	5.33	5.33	8.67	10.00
Penicillium sp.	2.00	6.00	14.67	17.33	24.67
Rhizopus stolonifer	0.67	7.33	2.00	.33	6.00
Trichoderma harzianum	2.67	6.00	13.33	16.67	20.67

Invitro evaluation of fungicides against associated weed mycoflora: The results of the laboratory evaluation of Formalin, Bavistin and Formalin + Bavistin at three different concentrations are presented in the Table 2. The highest concentrations of Formalin, Bavistin and Formalin + Bavistin are appeared to be superior in inhibiting the radial growth of all the tested 10 mycoflora ranging from 85.80 to 100% inhibition. Among the tested fungicides, the combination of Formalin and Bavistin was found to be superior at all the selected concentrations in comparison to the individual fungicide, Formalin and Bavistin. At the lowest dose of combined Formalin and Bavistin the highest 80.00% inhibition was found against *R. stolonifer* followed by *T. harzianum* (73.86%) while the minimum 44.11% inhibition was observed against *Penicillium* sp. In case of all the isolated fungi above 92.00% inhibition was obtained at the maximum concentration of Formalin and Bavistin combination. The complete inhibition of *Aspergillus niger* was observed at the highest concentration when Formalin and Bavistin was used together.

At the lowest dose of Formalin, the maximum 78.00% inhibition was achieved against *Rhizopus stolonifer* followed by *Trichoderma harzianum* (69.28%), while the minimum 39.20% inhibition was recorded against *Penicillium* sp. The highest inhibition (96.80 %) of *T. harzianum* followed by *A. niger* (94.70%), *R. stolonifer* (94.00%) was achieved at the maximum concentration of Formalin while the minimum 85.80% inhibition was obtained against *Penicillium* sp.

In general, Bavistin was found to be superior in comparison to Formalin but inferior to the combination of Formalin and Bavistin at all the selected concentrations. At the lowest dose of Bavistin, the highest 84.00% inhibition of the radial growth of *R. stolonifer* was observed followed by *T. harzianum* (70.66%) while the lowest 44.65% reduction was obtained against *P. thiersii*. The maximum 97.52% inhibition of *A. nidulans* followed by *R. stolonifer* (97.00%) was obtained at the highest concentration of Bavistin while the minimum 91.25% inhibition was observed against *P. thiersii*.

Table 2. Laboratory evaluation of chemicals against mycoflora associated with *Pleurotus ostreatus* grown substrate

Fungicides with conc. (ppm)	% Inhibition									
	A. flavus	*A. fumigatus*	*A. niger*	*A. nidulans*	*A. terreus*	*P. citrinum*	*P. thiersii*	*P.* sp.	*R. tolonifer*	*T.harzianum*
Formalin										
100	46.15	44.45	61.19	59.23	57.33	40.21	45.52	39.20	78.00	69.28
250	64.94	66.75	83.54	82.52	80.25	72.24	70.96	73.52	86.00	84.87
500	89.74	87.35	94.70	93.16	92.21	91.24	86.33	85.80	94.00	96.80
Bavistin										
25	51.89	49.54	64.72	63.14	62.46	45.64	44.65	40.08	80.00	70.66
50	64.10	63.20	82.66	81.26	79.56	69.45	69.58	71.08	85.00	78.00
75	92.74	91.50	97.00	97.52	96.89	91.52	91.25	92.14	97.00	97.25
Formalin+Bavistin										
100+25	56.40	55.62	67.89	66.23	67.33	46.24	47.15	44.11	84.00	73.86
250+50	77.35	76.23	80.29	78.21	76.58	76.33	75.32	77.94	95.00	90.13
500+75	94.02	93.25	100.00	98.42	98.26	92.45	93.4	93.82	98.00	98.17
Untreated										
Control	3.90 (cm)	3.7 (cm)	5.67 (cm)	4.10 (cm)	3.80 (cm)	3.3 (cm)	3.4 (cm)	3.4 (cm)	8.50 (cm)	7.27 (cm)

However, the results of the present study prevailed that both the fungicides Formalin and Bavistin are effective against the associated molds of oyster mushroom. But, when they are used in combination were more effective. The results are in agreement with several investigators (Rai and Vijoy 1992, Bermudez *et al.* 1994, Anandh *et al.* 1999 and Pani and Das 1998).

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Performance of Different Species of Oyster Mushroom on Rice Straw

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Abstract

Ten different species of oyster mushroom have been cultivated on rice straw and the yield and yield related attributes were compared. The minimum days required from stimulation to primordia initiation (DRSPI) (3.50) was recorded in *Pleurotus ostreatus* (whitesnow), *Pleurotus geesteranus* and *Pleurotus citrinopileatus* and the maximum DRSPI (10.25) was recorded in *Pleurotus erryngi* and *Pleurotus sajor-caju*. The minimum days required from stimulation to first harvesting (DRSFH) was found in *Pleurotus citrinopileatus* (5.50) and the maximum DRSFH (15.25) was found in *Pleurotus erryngi* closely followed by *Pleurotus sajor-caju* (14.25). The number of effective fruiting bodies was highest (47.00) in *Pleurotus citrinopileatus* and it was lowest (1.00) in *Pleurotus erryngi*. The length of stipe ranged from 3.45 to 6.80 cm. The highest length of stipe (6.80cm) was found in *Pleurotus erryngi* followed by *Pleurotus ostreatus* (5.50cm) and the lowest length of stipe was found in *Pleurotus sajor-caju* (3.45cm). The diameter of stipe, pileus and thickness of pileus ranged from 0.35 to 4.60 cm; 4.00 to 11.00 cm and 0.35 to 1.40 cm respectively. The highest diameter of stipe (4.60 cm) and pileus (11.00 cm) were found in *Pleurotus erryngi*. The biological and economic yields and biological efficiency were the highest, 191.00g and 183.5g and 127.30% respectively, in *Pleurotus ostreatus* (whitesnow).

Key Words: Rice straw, growth, yield and oyster mushrooms.

INTRODUCTION

Long ago mushrooms had drawn attention of human beings as a food, nutrition and medicine. Now-a-days they are the leading food component. There are so many mushroom species being cultivated in the world. Among them, oyster mushrooms (*Pleurotus* spp.) occupied a leading position in Bangladesh as these are easy to grow and the environmental factors of the country are very much favourable for their cultivation. The small and marginal farmers of the country can easily grow the mushroom in small scale in their house with minimum initial investment. *Pleurotus* spp. are efficient lignin-degrading mushroom and can grow well on different types of lignocellulolosic materials *viz*. paddy straw, maize stalks/cobs, vegetable plant residues, sawdust etc. with slightly variation in the range and combination of the substrates in different part of world based on their availability in abundant and being cheaper in the respective region (Royse, 1998 and Schmidt, 1986). Different species of oyster mushrooms available in Bangladesh are usually cultivated on sawdust and only *Pleurotus ostreatus* is cultivated on rice straw on a small scale. Zhang *et al.* (2002) and Mathew *et al.* (1996) reported that rice straw was a very good substrate for oyster mushroom cultivation. Park *et al.* (1995) used rice straw

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and wheat straw as substrates of oyster mushroom and found better result on rice straw. Singh (1998) reported that *P. sajar-caju* could be grown successfully on paddy straw. Moreover, rice straw is very much available and cheap in comparison to other substrates in Bangladesh. With this view, the present experiment was undertaken to evaluate the performance of different species of oyster mushroom on rice straw and to find out the best species of oyster mushroom for cultivation on rice straw.

MATERIALS AND METHODS

The experiment was conducted in the culture house of National Mushroom Development and Extention Centre, Sobhanbag, Savar, Dhaka, Bangladesh from January to April 2010. In this experiment rice straw was used as substrate for the cultivation of 10 different species of oyster mushrooms. The straw was chopped to 3-4 inch length and then poured in water at 60°C for one hour and then drained out the water and kept it to get cool slowly. After about 20 hours the straw was spread over floor to reduce the moisture level at 65%. The polypropylene bags of 9" x 12" size was filled with pasteurized straw inoculating the mother culture of 10 different species of oyster mushroom, *Pleurotus ostreatus, Pleurotus geesteranus, Pleurotus high king-51, Pleurotus florida, Pleurotus ostreatus* (whitesnow) *Pleurotus citrinopileatus, Pleurotus erryngi, Pleurotus cystidiosus, Pleurotus sajor-caju, Pleurotus sapidus* and their mouths were plugged by inserting absorbent cotton with the help of plastic necks. The packets were kept in a dark room at 25°C for incubation. When colonization of mycelium was completed, the spawn packets were taken to a culture house and were opened by 'D' shaped cut on the shoulder and removed the sheet. The relative humidity and temperature of the culture house were maintained at 80-90% and 20-25°C respectively by spraying water. Diffused light, about 200 lux and proper ventilation in culture house were maintained. After harvesting of mushroom, the residues were removed from the packet and temperature and relative humidity were maintained as before.

The experiment was laid out following completely randomized design (CRD) with 4 replications. Yield and yield parameters were taken on the basis of two flushes except in *Pleurotus erryngi* where only one flush was obtained. Data on days required from stimulation to primordia initiation and first harvest, number of primordia and effective fruiting body, length of stipe, diameter of stipe and pileus, thickness of pileus, biological and economic yield (g/packet) were recorded. Biological efficiency was calculated according to the formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Total biological yield (g)}}{\text{Total dry substrate used (g)}} \times 100$$

Data were analyzed following Gomez and Gomez (1984) using MSTAT-C computer program. Means separation were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Days required from stimulation to primordia initiation and first harvest: Appreciable variation was found in days required from stimulation to primordia initiation (DRSPI) in different species of oyster mushroom and ranged from 3.25 to 10.25 (Table 1). The DRSPI was maximum (10.25) in *Pleurotus erryngi* and *Pleurotus sajor-caju* followed by *Pleurotus sapidus* (8.25). The lowest DRSPI (3.50) was found in *Pleurotus geesteranus, Pleurotus ostreatus* (whitesnow) and *Pleurotus citrinopileatus* which were statistically similar to *Pleurotus ostreatus, Pleurotus high king*-51, *Pleurotus florida* and *Pleurotus cystidiosus.* Almost similar trend was observed in days required from stimulation to first harvesting (DRSFH) and was ranged from 5.50 to 15.25. The maximum DRSFH was found in *Pleurotus erryngi* (15.25) which was significantly higher from all other species except *Pleurotus sajor-caju* (14.25). The minimum DRSFH (5.50) was recorded in *Pleurotus citrinopileatus* preceded by *Pleurotus geesteranus* (6.50). The results were almost similar to the findings of Patra and Pani (1995) and Shah *et al.* (2004) who reported that the oyster mushroom took 4-8 days for primordia initiation. But the results varied with the findings of Amin *et al.* (2007) who reported that DRSPI for oyster mushroom ranged 3- 4 days. This might be attributed to different environmental factors and culture substrates. However, the present findings are in concert with those of Bugarski *et al.* (1995) and Baysal *et al.* (2003).

Number of primordia: The number of primordia of different oyster mushroom species varied significantly (Table 1). The highest number of primordia was observed in *Pleurotus citrinopileatus* (110.50) followed by *Pleurotus ostreatus (whitesnow)* (91.50) and *Pleurotus cystidiosus* (90.50). The lowest number of primordia was observed in *Pleurotus erryngi* (2.00) preceded by *Pleurotus sapidus* (7.50) and *Pleurotus sajor-caju* (8.50). The number of primordia in *Pleurotus citrinopileatus* was similar to Ahmed (1998) who observed that the number of primordia/ packet ranged from 150 to 350 in case of oyster mushroom.

Number of effective fruiting bodies: The number of effective fruiting bodies obtained from 2 flushes (except in *Pleurotus erryngi)* in different species of oyster mushroom differed significantly (Table 1). The highest number of effective fruiting bodies (47.00) was found in *Pleurotus citrinopileatus* followed by *Pleurotus ostreatus* (30.25) which was statistically similar to *Pleurotus cystidiosus* (28.75). The lowest numbers of effective fruiting bodies were found in *Pleurotus erryngi* (1.00). The finding of the present study was supported by Sarker *et al.* (2008) who observed that the number of fruiting body of oyster mushroom ranged from 20 to 98.25/packet on wheat straw supplemented with different levels of wheat and rice bran.

Table1: Days required from stimulation to primordia initiation and to first harvest and the number of primordia and effective fruiting body of different oyster mushroom species on rice straw

Mushroom Species	Days required from stimulation to primordia initiation	Days required from stimulation to first harvest	Number of primordia	Number of effective fruiting body
Pleurotus ostreatus	4.25 c	7.00 de	67.50 c	30.25 b
Pleurotus geesteranus	3.50 c	6.50 e	58.75 d	13.50 e
Pleurotus high king-51	4.25 c	8.25 cd	55.50 d	18.50 d
Pleurotus florida	4.25 c	8.25 cd	66.75 c	20.25 cd
Pleurotus ostreatus (whitesnow)	3.50 c	6.50 e	91.50 b	23.25 c
Pleurotus citrinopileatus	3.50 c	5.50 e	110.50 a	47.00 a
Pleurotus erryngi	10.25 a	15.25 a	2.00 f	1.00 g
Pleurotus cystidiosus	5.25 c	9.25 c	90.50 b	28.75 b
Pleurotus sajor-caju	10.25 a	14.25 a	8.50 e	6.25 f
Pleurotus sapidus	8.25 b	12.25 b	7.50 e	4.00 fg
CV (%)	20.73	11.70	5.15	11.59

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Length of stipe (cm): The length of stipe ranged from 3.45 to 6.80 cm with significant difference (Table 2). The highest length of stipe was found in *Pleurotus erryngi* (6.80cm) which was significantly different from others followed by *Pleurotus ostreatus* (5.50cm) which was statistically identical to *Pleurotus highking*-51 (4.75 cm) and *Pleurotus florida* (4.70). The lowest length of stipe was found in *Pleurotus sajor-caju* (3.45cm).

Diameter of stipe (cm): The diameter of stipe differed significantly and ranged from 0.35 to 4.60 cm (Table 2). The highest diameter was found in *Pleurotus erryngi* (4.60 cm) followed by *Pleurotus high king-51* (1.48 cm) while it was lowest (0.35 cm) in *Pleurotus citrinopileatus.*

Diameter of pileus (cm): The diameter of pileus ranged from 4.00 cm to 11.00 cm with significant difference among the species (Table 2). The highest diameter of pileus was found in *Pleurotus erryngi* (11.00 cm) followed by *Pleurotus sapidus* (9.00cm) and the lowest diameter of pileus was found in *Pleurotus citrinopileatus* (4.00 cm).

Thickness of pileus (cm): The thickness of pileus in different species differed significantly and ranged from 0.35 cm to 1.40 cm (Table 2). The highest thickness was found in *Pleurotus erryngi* (1.40 cm) which was statistically similar to *Pleurotus highking*-51 (1.30 cm) and it was lowest in *Pleurotus citrinopileatus* (0.35 cm).

The results for length of stipe, diameter of stipe, diameter of pileus and thickness of pileus were more or less similar to Amin *et al.* (2007) and Sarker *et al.* (2007) in most of the species of oyster mushrooms except in *Pleurotus erryngi.*

Table 2: Yield and yield attributes of different species of oyster mushroom on rice straw

Mushroom Species	Length of stipe (cm)	Diameter of stipe (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Biological Yield (g/packet)	Economic Yield (g/packet)	Biological efficiency (%)
Pleurotus ostreatus	5.50 b	1.02 bcd	7.30 c	0.90 b	182.00 b	176.30 b	121.30 b
Pleurotus geesteranus	3.90 cd	1.25 bc	7.20 c	0.83 b	167.80 d	163.30 c	111.80 d
Pleurotus high king-51	4.75 bc	1.48 b	7.60 bc	1.30 a	176.00 c	166.00 c	117.30 c
Pleurotus florida	4.70 bc	1.15 bcd	6.30 c	0.93 b	162.50 e	151.00 d	108.30 e
Pleurotus ostreatus (whitesnow)	4.00 cd	1.02 bcd	6.80 c	0.85 b	191.00 a	183.50 a	127.30 a
Pleurotus citrinopileatus	4.00 cd	0.35 e	4.00 d	0.35 d	155.50 f	149.00 d	103.70 f
Pleurotus erryngi	6.80 a	4.60 a	11.00 a	1.40 a	86.50 g	80.00 e	57.67 g
Pleurotus cystidiosus	4.30 cd	0.85 cd	6.50 c	0.83 b	176.30 c	167.30 c	117.50 c
Pleurotus sajor-caju	3.45 d	0.70 de	7.50 bc	0.50 c	60.50 h	56.50 f	40.33 h
Pleurotus sapidus	4.00 cd	0.90 cd	9.00 b	0.48 cd	45.75 i	42.00 g	30.50 i
CV (%)	15.59	21.85	13.66	10.49	2.52	3.03	2.52

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Biological yield (g/packet): Significant variation was observed in biological yield (BY) of different oyster mushroom species (Table 2). The BY from 2 flushes was counted except *Pleurotus erryngi* where only one flush was counted. The highest BY (191.00g) was found in *Pleurotus ostreatus* (whitesnow) followed by *pleurotus ostreatus* (182.00g) and the lowest biological yield was found in *Pleurotus sapidus* (45.75g). *Pleurotus cystidiosus* recorded 3rd highest BY (176.30g) which performed statistically similar to *Pleurotus highking*-51 (176.00 g). The result differed with Amin *et al.* (2007) in some species who found that the BY of oyster mushroom ranged from 43.00 g to 58.00 g/packet from one flush but the result was approximately similar with Balasubramanya (2007) who got the biological yield of oyster mushroom within the range of 307 to 453 g/packet from three flushes.

Economic yield (g/ packet): The economic yield obtained from 2 flushes in different species varied significantly and ranged from 42.00 to 183.50g/ packet (Table 2). It was highest (183.50g) in *Pleurotus ostreatus* (whitesnow) followed by *Pleurotus ostreatus* (176.30g) and was lowest in *Pleurotus sapidus* (42.00 g) preceded by *Pleurotus sajor-caju* (56.50g) and *Pleurotus erryngi* (80.00g). The present findings are similar with Sarker *et al.* (2007).

Biological efficiency (%): Significant variation was observed on biological efficiency (BE) (Table 2). The highest biological efficiency (127.30%) was found in *Pleurotus ostreatus* (whitesnow) followed by *pleurotus ostreatus* (121.30%) and the lowest biological efficiency was found in *Pleurotus sapidus* (30.50%) preceded by *Pleurotus sajor-caju* (40.33%) and *Pleurotus erryngi* (57.67%). The result was approximately similar with Balasubramanya (2007).

Considering all the parameters, *Pleurotus ostreatus* (whitesnow) performed best among the species on rice straw.

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Performance of Vermicomposts Derived from Different Organic Wastes as Casing Material of Milky White Mushroom (*Calocybe indica*)

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Abstract

The experiment was conducted at National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh to evaluate the performance of vermicomposts derived from different sources like rice straw (RS), sawdust (SD), cow dung (CD), SD + CD (1:1) and SD + RS (1:1) as the casing material of milky white mushroom. The vermicomposts were added to sand + soil mixture (1:1) at the ratio of 2:1. All the vermicomposts have profound effects on yield and yield contributing characters of the mushroom. The minimum days required from casing to primordia initiation (14.75) and the maximum number of fruiting body (5.25) was found in the bags cased with vermicompost derived from sawdust + cow dung (1:1) source. The highest yield (247g/ 500g bag) and biological efficiency (98.8 %) was recorded in vermicompost derived from rice straw.

Key words: Vermicompost, Milky white mushroom, Biological yield, Biological efficiency.

INTRODUCTION

Milky white mushroom (*Calocybe indica*) is an edible white summer mushroom. It can be cultivated in the temperature range of 25-35°C with 60-70% biological efficiency. The protein content of this tropical mushroom is 32.3% and has about 41% crude fiber (Krishnamoorthy, 2003). In milky mushroom cultivation, casing is an important event for enhancing the transformation of vegetative phase to reproductive phase. It is also very important for obtaining proper development of the fruiting bodies (Nagaratna & Mallesha, 2007). Casing provides support to the fruiting bodies and protects the substrates from desiccation (Flegg and Wood, 1985). It can also provide some nutrient and micro environment suitable for milky mushroom cultivation. In western countries, sphagnum moss and peat are being used as casing media. But, its availability is not abundant in all the places. Many researchers used various things as casing materials such as soil: sand (3:1), soil: cow dung (1:1), soil: farm yard manure (1:1) (Amin *et al.*, 2007), well decompost spent compost (Mantel, 1973), 2-3 years old farm yard manure (Hayes and Shandilya, 1977). But the most suitable casing material for cultivation of milky white mushroom in Bangladesh is not yet identified. Therefore, it is necessary to find out the suitable and alternative casing materials available for its production in Bangladesh.

Vermicompost is an organic fertilizer that helps in the maintenance of environment and results in sustainable agriculture (Senapati, 1996). This may be an alternative material for

casing of milky white mushroom. Buchanan *et al.* (1988) observed that most vermicompost had higher values of available nutrients than the wastes from which they were formed. Vermicompost contains mineral nutrients like N, P, K, Fe, Zn, Ca, Mg and Cu (Kale, 1998). This compost can be produced very easily in abundance within a few days. Therefore, the present study was undertaken to know the suitability of vermicomposts as casing materials in milky mushroom cultivation.

MATERIALS AND METHODS

The experiment was conducted at National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh during the month of March to May, 2010.

Rice straw was chopped to 3-4 inch length and then poured in water at 60°C for one hour and then the water was drained and the straw was placed where it would cool slowly. After about 20 hours the straw was spread over a floor to reduce the moisture level at 65%. The polypropylene bags of 9" x 12" size were filled with 500 g of pasteurized straw inoculating the mother culture of *Calocybe indica* at the rate of 5 percent. The inoculated bags were incubated for 20 days at 27 to 32°C temperature. After completion of mycelium running the polypropylene cover of the bags were opened and covered with casing materials according to treatments.

Six different vermicomposts derived from different sources like rice straw (RS), sawdust (SD), cow dung (CD), SD + CD (1:1) and SD + RS (1:1) were used as the casing materials of milky white mushroom. The vermicomposts were added to sand + soil mixture (1:1) at the ratio of 2:1 to prepare the casing materials, i. e. the final ratio of the casing materials was sand: soil: vermicompost = 1:1:1. Before use, the casing materials were pasteurized by steam in a closed drum for 6 hours. After cooling, the casing materials were spread over the mycelium coated open spawn bags making 2.50 cm thick layer and kept in the culture house at 30 to 35°C temperature, above 80% relative humidity (RH) and 500-800 lux light intensity. The RH was maintained 80% by watering 3 to 4 times a day. Pin head appeared after 14-17 days of casing and developed into mature fruiting bodies within 5 to 6 days.

Data were collected on days required from casing to primordia initiation, number of effective fruiting body, yield, biological efficiency (BE), length of stipe, diameter of stipe and pileus and thickness of pileus. The BE was estimated by the formula:

$$\text{BE (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

The experiment was laid out in completely randomized design with 4 replications. The data for the characters considered in the present experiments were statistically analyzed following Gomez and Gomez (1984) using MSTAT-C computer programme. Means were computed following Duncan's Multiple Range Test (DMRT) using the same computer programme.

RESULT AND DISCUSSIONS

Days required from casing to primordia initiation (DRCPI): Vermicompost has profound effects on the DRCPI and it was ranged from 14 to 17 days. The minimum days (14.75) required in SD + CD (1:1) vermicompost which was statistically similar to all the treatment except the control where no vermicompost was used in casing material. The maximum DRCPI (16.75) was recorded in the control treatment (Table 1). The result of the present study corroborates the result of Tandon *et al.* (2006). They found that casing with vermicompost requires 15.6 days for primordial initiation.

Number of effective fruiting body/bag: The number of effective fruiting bodies (NEFB) was significantly influenced by the vermicomposts of different sources. The highest NEFB (4.25) was found in RS and SD + CD (1:1) vermicompost followed by CD vermicompost. The lowest NEFB (3.50) was found in SD vermicompost which was statistically similar to SD + RS (1:1) (Table 1). Almost similar results were reported by Nagaratna and Mallesha (2007). They found the maximum number of fruiting body (7) with sand+soil+coir pith and sand+soil+crop residues vermicomposts and lowest number of fruiting body (4) with crop residues and sand+soil+compost vermicomposts.

Yield and biological efficiency: The highest yield of milky mushroom (247.0 g/bag) was recorded in RS vermicompost which was significantly higher compared to other treatments (Table 1). The second highest yield (242.8 g/bag) was observed in SD vermicompost and the lowest yield (178.0 g/bag) was recorded in the control treatment where no vermicompost was used in the casing materials. Similar trend was observed in biological efficiency (BE) of milky mushroom with casing materials supplemented with vermicomposts of different sources. The highest BE (98.80%) was recorded in RS vermicompost which was significantly higher compared to all the treatments. The second highest BE (97.12%) was observed in SD vermicompost. The lowest yield (71.2%) was recorded in the control treatment where no vermicompost was used in the casing materials. The higher yield and BE of milky white mushroom with vermicomposts derived from different organic wastes may be due to presence of high nutrient content and good physical condition. Almost similar results were reported by Tandon *et al.* (2006) and Nagaratna and Mallesha (2007).

Dimension of fruiting body: Length and diameter of stipe and diameter and thickness of pileus of milky white mushroom were significantly influenced by different vermicomposts derived from different organic wastes (Table 2). The highest length of stipe (10.73 cm) was observed in CD vermicompost followed by SD + CD (1:1) (10.45cm) and RS (9.88cm) vermicomposts. The lowest stipe length (7.50 cm) was found in the control treatment. The highest diameter of stipe was found in SD (3.70 cm) which was significantly higher as compared to all the treatments and the lowest diameter of stipe was observed in the control treatment.

The highest diameter of pileus (11.38 cm) was observed in RS vermicompost which did not differ significantly with other treatments except the control. The lowest diameter of pileus (7.50 cm) was observed in the control treatment. The highest thickness of pileus was recorded in SD + RS vermicompost (2.78 cm) which was statistically similar to all the treatment except the control (2.40 cm).

Table 1. Effect of different vermicompost derived from different organic wastes on days required from casing to primodia initiation, number of effective fruiting body, yield and biological efficiency of milky white mushroom (*Calocybe indica*)

Sources of organic wastes for vermicomposts	Days required from casing to primodia initiation	Number of effective fruiting body	Yield (g/bag)	Biological efficiency (%)
Rice straw (RS)	15.25ab	5.250a	247.0a	98.8a
Saw dust (SD)	16.25ab	3.500b	242.8b	97.12b
Cow dung (CD)	15.75ab	4.250ab	225.5c	90.2c
SD + CD (1:1)	14.75b	5.250a	209.3d	83.72d
SD + RS (1:1)	16.00ab	3.750b	208.0d	83.2d
No vermicompost (Control)	16.75a	3.750ab	178.0e	71.2e
Level of significance	0.10	0.01	0.01	0.01
CV (%)	7.43	15.49	0.82	0.82

In acolumn, the figures having common letters do not differ significantly where as figures having dissimilar letters at 5% and 1% level of significance according to DMRT. (Ratio of the casing materials was sand: soil: vermicompost = 1:1:1).

Table 2. Effect of different vermicompost derived from different organic wastes on the dimension of milky white mushroom (*Calocybe indica*) fruiting bodies

Sources of organic wastes for vermicomposts	Length of stipe (cm)	Diameter of stipe (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Rice straw (RS)	9.88ab	3.28b	11.38a	2.48ab
Saw dust (SD)	9.13bc	3.70a	11.00a	2.60ab
Cow dung (CD)	10.73a	3.03bc	10.68a	2.63ab
SD + CD (1:1)	10.45a	3.25b	10.38a	2.45ab
SD + RS (1:1)	8.38cd	3.23b	11.00a	2.78a
No vermicompost (Control)	7.5d	2.70c	7.500b	2.40b
Level of significance	0.01	0.01	0.01	0.05
CV (%)	4.88	5.89	6.69	7.9

In column the figures having common letters do not differ significantly where as figures having dissimilar letters at 5% and 1% level of significance according to DMRT.

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Cultivation of *Auricularia polytricha* in Bangladesh Condition

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Abstract

The effect of different media and pH levels on the mycelial growth and different substrates on growth and yield of *Auricularia polytricha* was investigated. The best mycelial growth rate was observed on malt extract agar medium while the least growth rate was recorded on potato dextrose yeast agar medium. The optimum pH level for mycelial growth of *Auricularia polytricha* was observed in pH 7.0 while it was the lowest in pH 4.0. Among the substrates, the best yield (137.30 g/500g packet) and biological efficiency (68.63%) were obtained from mixed sawdust followed by mango sawdust. No fruit body was produced on paddy straw substrate.

Keywords: *Auricularia polytricha,* environmental factor, substrate, yield and biological efficiency.

INTRODUCTION

Auricularia polytricha is an edible jelly fungus that invades and live on the woodcut or fallen logs of some specific trees. It is the most suitable mushroom species to cultivate in tropical and subtropical regions of the world (Zoberi, 1972 and Well, 1984). It is frequently consumed as a food and a traditional medicine in the Far East. Its fungal mycelia and fruitbody have high content of fiber, protein, vitamin and carbohydrate than that of many vegetables and fruits and low content of lipid, so it adds many nutritious ingredients to different dishes and often used in Asian cooking (Cheng and Tu, 1978 and Kim *et al.*, 2004). The fruitbodies of this fungus are highly valued as medicinal herb, owing to their biological and pharmacological activities, such as immuno-stimulating and anti-tumour activities (Borchers *et al.*, 1999 and Lee *et al.*, 1996). Mycelial culture gives rise to potential advantages of higher production in a small space and in short time with lesser contamination rate (Friel and McLoughlin, 2000 and Yang and Liau, 1998). So, it is very important to develop a commercial cultivation system of this mushroom. The most common practice of this mushroom cultivation in different countries is wood log cultivation. Freshly cut *Acacia koa* (koa), *Aleurites* (kukui) and *Hibiscus tiliaceus* (hau) logs are generally used for the production of this mushroom. But due to the scarcity of suitable logs, the cultivation practice of this mushroom shifted from log to bag culture and is gaining popularity. In case of bag cultivation, types of substrates are most important for fructification. *Auricularia polytricha* can be cultivated on the sawdust which may originate from different kinds of trees. A successful synthetic cultivation has been reported on solid substrates, utilizing sawdust and agricultural wastes as the main media components (Triratana *et al.*, 1991 and Malarvizhi *et al.*, 2003). But the suitable substrate

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and culture condition are not yet determined in Bangladesh. The present study was undertaken to investigate the effects of different media and pH on mycelial growth and to screen out the suitable substrates for *Auricularia polytricha* cultivation in Bangladesh.

MATERIALS AND METHOD

This experiment was conducted at the National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh during June to December 2009.

Media: Eight different culture media, *viz.* potato dextrose agar (PDA), potato dextrose yeast agar (PDYA), malt extract agar (MEA), malt extract yeast agar (MEYA), wheat extract agar (WEA), wheat extract yeast agar (WEYA), corn extract agar (CEA) and corn extract yeast agar (CEYA) were evaluated for the mycelial growth of *Auricularia polytricha* and were prepared according to Moonmoon *et al.* (2008).

pH: Eleven different pH levels, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 of the media were evaluated for getting the best pH level for *Auricularia polytricha* cultivation. The pH levels were adjusted by adding 1N HCl or NaOH before autoclaving. The MEA medium was used as the base medium.

Spawn packet preparation, innoculation and incubation: Five different substrates, mango (*Mangifera indica*) sawdust, garjan (*Dipterocarpus alatus*) sawdust, acacia (*Acacia auriculiformis*) sawdust, mixed sawdust and paddy straw were tested in this experiment. Spawn packets of 500 g size were prepared, innocuculated and incubated following the procedure that developed and explained by Sarker *et al.* (2007).

Culture condition: After completion of mycelium running, spawn packets were opened by rectangular cut (3.0 × 1.5 cm) on the shoulder at both the sides of the packet and transferred to the culture room at 25-30^{0}C temperature and 85-95% relative humidity. Water was sprayed 4-5 times per day to maintain the temperature and relative humidity.

Experimental design, data collection and analysis: The experiment was laid out in completely randomized design (CRD) with 4 replications. Data on mycelium growth rate (cm/ day) in different media and pH, days required to completion of mycelium running in different substrates, days required from opening to primordia initiation and primordia initiation to first harvest, number of effective fruiting body/packet, weight of individual fruit body (g), yield (g/ packet), dry yield (g/ packet), and biological efficiency (%) were collected and analyzed following Gomez and Gomez (1984) using MSTAT-C computer programme. Means were separated by Duncan's Multiple Range Test (DMRT) using the same computer programme.

RESULTS AND DISCUSSION

Mycelial growth rate on different media: Mycelium growth rate of *Auricularia polytricha* was significantly influenced by different growth media (Table 1). The highest

growth rate (0.39 cm/ day) was observed in malt extract agar (MEA) medium which was statistically similar to potato dextrose agar (PDA), wheat extract agar (WEA), wheat extract yeast agar (WEYA) and corn extract yeast agar (CEYA). The lowest mycelium growth rate (0.26 cm/ day) was observed in potato dextrose yeast agar (PDYA) medium. This result is similar to Khan *et al.* (1991) who reported that MEA, WEA and PDA were the best media for cultivation of *Auricularia polytricha*.

Table1: Effect of different media on mycelial growth rate (cm/day) in *Auricularia polytricha*

Media	Mycelium growth rate (cm/day)
Potato dextrose agar (PDA)	0.35 ab
Potato dextrose yeast agar (PDYA)	0.26 c
Malt extract agar (MEA)	0.39 a
Malt extract east agar (MEYA)	0.22 c
Wheat extract agar (WEA)	0.35 ab
Wheat extract yeast agar (WEYA)	0.38 a
Corn extract agar (CEA)	0.31 b
Corn extract yeast agar (CEYA)	0.37 a
CV(%)	9.88

In a column means followed by a common letter are not significantly different at 5% level by DMRT.

Table 2: Effect of pH on mycelial growth rate (cm/day) in *Auricularia polytricha*

pH	Mycelium growth rate (cm/day)
4.0	0.28 e
4.5	0.34 d
5.0	0.36 cd
5.5	0.39abc
6.0	0.40abc
6.5	0.41abc
7.0	0.43 a
7.5	0.42 ab
8.0	0.41 ab
8.5	0.41abc
9.0	0.38bcd
CV(%)	7.20

In a column means followed by a common letter are not significantly different at 5% level by DMRT.

Different pH on mycelial growth: Table 2 represents the mycelial growth rate of *Auricularia polytricha* on MEA media with different pH level (4-9). The highest mycelial growth (0.43 cm/ day) was observed in pH 7.0 followed by pH 7.5 (0.42 cm/ day) and pH 8.0 (0.41 cm/ day). The lowest mycelial growth rate (0.28 cm/ day) was recorded in pH 4.0. Similar observation has earlier been reported by Khan *et al.* (1991) who observed that *Auricularia polytricha* grows well at pH 7.0. Elvira and Marharyta (2005) also reported that the mycelium growth rate of *Hypsizygous marmoreus*, *Auricularia polytricha* and *Grifola frondosa* was higher at pH above 6.5. Xu & Yun (2003) observed the best growth of *Auricularia polytricha* at pH 8.0.

Days required to completion of mycelium running in spawn packet: Significant difference was observed in days to completion of mycelium running (DCMR) in spawn packet (Table 3). The lowest DCMR (16.75) was recorded on paddy straw substrate which was significantly lower to all the treatments. The highest DCMR (30.50) was observed on garjan sawdust followed by mango sawdust (30.00).

Days required from opening to primordia initiation (DROPI): The DROPI ranged from 9.00 to 10.50 (Table 3). The lowest DROPI (9.00) was found in mixed sawdust which was statistically similar to acacia sawdust. The highest DROPI (10.50) was found in garjan sawdust followed by mango sawdust. No primordia was initiated on paddy straw substrate.

Days required from primordia initiation to first harvest (DRPIFH): No significant difference was observed in DRPIFH among the substrates. The maximum DRPIFH (28.50) was recorded in acacia sawdust and it was minimum (25.75) in mixed sawdust.

Number of effective fruiting body/packet: The number of effective fruiting bodies (NEFB) obtained from different treatments are shown in Table 3. The highest NEFB (25.75) was observed in mango sawdust which was statistically similar to mixed sawdust (24.0) and the lowest number of fruiting bodies (14.25) was found in garjan sawdust. No fruiting body was produced on paddy straw substrate.

Weight of individual fruiting body (WIFB): The WIFB in different treatments ranged from 4.35 g to 5.78 g (Table 3) and the highest WIFB (5.78 g) was observed in mixed sawdust which was statistically similar to that in acacia sawdust. The lowest WIFB (4.35 g) was observed in garjan sawdust.

Yield, dry yield and biological efficiency: Significant difference was observed in yield and dry yield of *Auricularia polytricha* mushroom in sawdust of different wood species (Table 3). The highest yield (137.30 g/packet) was obtained from mixed sawdust followed by mango sawdust (120.30 g/packet) while lowest yield (61.25 g/packet) was observed in garjan sawdust. The dry yield obtained from different treatments ranged from 16.70 g to 37.43 g/packet (Table 3). The highest dry yield (37.43 g/packet) was observed in mixed sawdust and the lowest (16.70 g/packet) in garjan sawdust. No yield was observed in paddy straw substrate. The highest biological efficiency (68.63%) was obtained from mixed sawdust followed by mango sawdust (60.13%) while the lowest biological efficiency (30.63%) was observed in garjan sawdust.

The results are in agreement with Islam *et al.* (2009), who stated that mango sawdust gave maximum yield of *Pleurotus flabellatus.* Thakur *et al.* (2003) also reported that no fruitbody of *Auricularia polytricha* was formed on different types of straw.

Table 3. Effect of different substrate on growth, yield attributes, yields and biological efficiency of *Auricularia polytricha*

Substrates	Days required to completion mycelium running	Days required from opening to primordia initiation	Days required from primordia initiation to first harvest	Number of effective fruiting body/ packet	Weight of individual fruit body (g)	Yield (g/packet)	Dry yield (g/packet)	Biological efficiency (%)
Mango sawdust	30.00 a	10.00 ab	26.75 a	25.75 a	4.69 b	120.3 a	32.59 b	60.13 b
Garjan sawdust	30.50 a	10.50 a	27.75 a	14.25 b	4.35 b	61.25 d	16.70 d	30.63 d
Acacia sawdust	20.75 c	9.50 bc	28.50 a	17.00 b	5.11 ab	85.75 c	23.38 c	42.88 c
Mixed sawdust	28.50 b	9.00 c	25.75 a	24.00 a	5.78 a	137.3 a	37.43 a	68.63 a
Paddy straw	16.75 d	-	-	-	-	-	-	-
CV (%)	1.91	6.62	12.85	14.74	15.59	9.10	9.11	9.10

In a column means followed by a common letter are not significantly different at 5% level by DMRT.

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Volume 4 **Number 1** **June 2010**

Contents