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Books:

Gomez, K. A. & Gomez, A. A. 1984. **Statistical Procedures of Agricultural Research**, 2nd ed., John Wiley and Sons, Singapore. p. 21.

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Oyster Mushroom Ameliorates Lipid Profile of Bangladeshi Women during Ramadan Fast

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Abstract

To evaluate the effect of oyster mushroom on serum lipid profile status of women during Ramadan fast, the study was carried out in the National Mushroom Development and Extension Centre, Sobhanbag, Savar Dhaka in association with the Department of Pharmacy and Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka. The experiment was conducted before and after the Arabic month, Ramadan, during when there occurs a change both in the pattern and timing of dietary intake. Fifty grams of fresh oyster mushroom (*Pleurotus ostreatus*), taken along with the usual Ifter items at the whole Ramadan period significantly reduced serum total Cholesterol (TC) Triglyceride (TG) and Low Density Lipoprotein (LDL-C) ($p = 0.000$, $p = 0.041$ and $p = 0.000$) and raised serum High Density Lipoprotein (HDL-C) ($p = 0.006$). But considering the effect of oyster mushroom alone it was noticeable that *Pleurotus ostreatus* significantly reduced serum TC and LDL-C ($p = 0.035$ and $p = 0.049$) but there was no improvement of serum TG ($p = - 0.006$) and HDL-C ($p = 0.255$) rather there is significant raise of TG level observed which might be explained as additional intake of oils along with mushroom fry. These findings suggest that *Pleurotus ostreatus* may able to improve lipid profile status of women at Ramadan fast and hence can improve it all time in whole human population.

Key wards: Cholesterol, TG, HDL-C, LDL-C, *Pleurotus ostreatus*, Ramadan.

INTRODUCTION

Lipid profile is a group of blood tests that are used to measure the total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C)-good cholesterol and low density lipoprotein (LDL-C)-bad cholesterol level of an individual. Many test results will also included a VLDL cholesterol ratio as part of the final data.

Mushroom is now believed as the same origin of nutrients and medicinal properties. Relevant nutritional aspects of mushrooms include a high fiber supply, a low carbohydrate and fat content with low trans isomers of unsaturated fatty acids and a low concentration of sodium as well as the occurrence of components such as eritadenine, phenolic compounds, sterols (such as ergosterol), chitosan, triterpenes, etc., which are

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considered as important responsible agents for some hitherto healthy properties (Anon., 2010a). Among the various ameliorative effects of oyster mushroom, *Pleurotus ostreatus* (*P. ostreatus*) possesses antitumour activity (Yoshioka, *et al.*, 1985), hepatoprotective activity (Choudhury, *et al.*, 2009 and Choudhury, *et al.*, 2010), antihypertensive activity (Choudhury, *et al.*, 2008) and hypoglycaemic effects in experimentally induced diabetes (Chorvathova, *et al.*, 1993).

Oyster mushroom contains statins (or HMG-CoA reductase inhibitors), a class of drug used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. Increased cholesterol levels have been associated with cardiovascular diseases (CVD), and statins are therefore used in the prevention of these diseases. Randomized controlled trials have shown that they are most effective in those already suffering from cardiovascular disease (secondary prevention), but they are also advocated and used extensively in those without previous CVD but with elevated cholesterol levels and other risk factors (such as diabetes and high blood pressure) that increase a person's risk (Anon., 2010b). Statin drugs lowers cholesterol by slowing the production of LDL-C and by helping the liver to remove the bad cholesterol already in the blood. They also raise the HDL-C, which helps fight the bad cholesterol (Anon., 2011).

Ramadan fasting, in the ninth month of Islamic Hijri Calendar is a religious obligation of Islam, annually followed by millions of Muslims to fulfill their worship and to abstain from food and water from dawn to sunset. Ramadan fast is commonly seen as beneficial for health. Traditionally the practice is to eat 2 meals, 1 before dawn, Seheree, and 1 just after sunset, Ifter. Often Muslims eat a greater variety of foods in their meals during Ramadan than in other months. As a result, the Ramadan fast provides an excellent opportunity to study the effects of various diets on the human body and can serve as an excellent research model for metabolic and behavioral studies (Aldouni., 1997). Ramadan fasting and starvation are not synonymous. Many physiological and psychological changes take place during Ramadan, most probably due to the changes in eating patterns, eating frequency and sleep patterns (Akanji, *et al.*, 2000). Some studies in the eastern Mediterranean area have indicated improved HDL-C during Ramadan fast (Akhtar, *et al.*, 1991 and AL Hader, *et al.*, 1994). A balanced diet at Ramadan that is even less in quantity than normal, will be sufficient to keep a person healthy and active. 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 lipid profile.

MATERIALS AND METHODS

The study was conducted during the period of Ramadan with the help of Strengthening Mushroom Development Project, National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka.

Subjects: Total 56 female subjects were included in the study. They were divided into two groups. In G-1, 29 female aged (years) from 25 to 80 who were at the available location of the monitoring team wanting to be fast in the whole Ramadan were considered. And in G-2, 27 female volunteers aged from 27 to 75 also wanting to be fast in whole Ramadan were considered.

Study design: In the study previously divided 2 groups were included. G-2 was studied without mushroom supplementation. 50 grams of fresh *P. ostreatus* was ensured for each individual of G-1 by the responsible workers daily by home visits or from the research center. The mushrooms were collected from NAMDEC. If any drug previously getting by the subjects, it was continued. At the beginning of Ramadan, subjects were evaluated for health status. Fasting blood sample was collected for analysis of TC, TG, HDL-C and LDL-C. Just after ending of Ramadan the subjects were evaluated and all the investigation procedures were repeated. All the biochemical parameters for the measurement of lipid profile were estimated by semi-auto analyzer (3000 evaluation) using the available reagent kit.

Inclusion criteria: The subjects were clarified about the study and after getting their written consent showing willingness to participate in the study they were included. The details history was taken from the subjects which included age, sex, occupation, educational status, marital status, family history and drug history.

Exclusion criteria: Patients suffering from acute illness, malabsorption, alcoholism and non fast persons were excluded.

Anthropometry: Anthropometric measurements were taken by height in cm and weight in kg with the use of a manual machine. Participants were shoeless and wore light clothing. Body Mass Index (BMI) was calculated by taking subject's weight and height (BMI = weight in kg / Height in m^2). Blood pressure (systolic and diastolic) of subjects was measured by sphygmomanometer.

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-I who was supplemented with mushroom as ifter item, the mean ± SE serum Cholesterol (mg/dl) before and after Ramadan was 178.28 ± 7.08 and 144.46 ± 5.11 respectively. A highly significant mean difference of cholesterol ($p = 0.000$) observed in pre and post Ramadan state indicating supplementation of mushroom as Ifter item associated with Ramadan fast significantly reduced serum cholesterol level (Table-1). In G-II who was not supplemented with mushroom as Ifter item, the mean ± SE serum cholesterol (mg/dl) before and after Ramadan was 176.55 ± 9.69 and 162.44 ± 6.07 respectively. A statistically significant mean difference of cholesterol ($p = 0.045$) observed before and after Ramadan (Table-2). This finding indicates one month fasting state of Ramadan significantly reduced serum cholesterol level. The comparative mean of G-1 and G-2 (done by independents sample t test) shows – in pre Ramadan state the mean

± SE serum cholesterol of G-1 and G-2 was 178.58 ± 6.84 and 176.55 ± 9.69 respectively. No statistically significant mean difference (p = 0.863) between the two groups in pre Ramadan state observed, indicating there was no significant difference of mean of cholesterol of mushroom supplemented and non mushroom supplemented group (Table-3). In post Ramadan state the mean ± SE serum cholesterol of G-1 and G-2 was 145.44 ± 5.03 and 162.44 ± 6.07 respectively. A statistically significant mean difference (p = 0.035) between the two groups in post Ramadan state observed, indicating there was a significantly difference of mean cholesterol of mushroom supplemented and non mushroom supplemented group (Table-4). This finding suggests that supplementation of mushroom as Ifter item in Ramadan significantly reduces serum cholesterol level.

Table 1. Evaluation of serum lipid profile of G-1 subjects who were supplemented mushroom in Ifter

Parameter	Number of subjects (n)	Values		p
		Pre Ramadan (mean ± SE)	Post Ramadan (mean ± SE)	
Cholesterol (mg/dl)	29	178.28 ± 7.08	144.46 ± 5.11	0.000
Triglyceride (mg/dl)	29	160.68 ± 12.05	142.79 ± 5.99	0.041
HDL-C (mg/dl)	29	40.68 ± 1.62	43.55 ± 1.80	0.006
LDL-C (mg/dl)	29	105.72 ± 5.73	74.00 ±4.79	0.000

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 (HDL-C = High density lipoprotein and LDL-C = Low density lipoprotein).

In mushroom supplemented group (G-1), the mean ± SE serum TG before and after Ramadan was 160.68 ± 12.05 and 142.79 ± 5.99 (mg/dl) respectively. A significant mean difference of TG (p = 0.041) observed in pre and post Ramadan state indicating supplementation of mushroom associated with Ramadan fast significantly reduced serum TG level (Table-1). In non mushroom supplemented group (G-2), the mean ± SE serum TG (mg/dl) before and after Ramadan was 136.77 ± 4.27 and 121.70 ± 4.07 respectively. A highly significant mean difference of TG (p = 0.001) observed before and after Ramadan (Table-2). This finding indicates one month fasting state of Ramadan significantly reduced serum TG level. The comparative mean of G-1 and G-2 (done by independents sample t test) shows – in pre Ramadan state the mean ± SE serum TG of G-1 and G-2 was 160.68 ± 12.05 and 136.77 ± 4.27 respectively. No statistically significant mean difference (p = 0.075) between the two groups in pre Ramadan state observed, indicating there was no significant difference of mean TG of mushroom supplemented and non mushroom supplemented group (Table-3). In post Ramadan state the mean ± SE serum TG of G-1 and G-2 was 142.79 ± 5.99 and 121.70 ± 4.07 respectively. A statistically significant mean difference (p = 0.006) between the two groups in post Ramadan state observed, indicating the mean serum TG level of non mushroom supplemented group (G-2) is significantly lower then mushroom supplemented group (G-1). This opposite diversity of result may be due to additional intake of edible oil with mushroom fry during Ifter time in whole one month of Ramadan period (Table-4).

Table 2. Evaluation of serum lipid profile of G-2 subjects who were not supplemented mushroom in Ifter

Parameter	Number of subjects (n)	Values		p
		Pre Ramadan (mean ± SE)	Post Ramadan (mean ± SE)	
Cholesterol (mg/dl)	27	176.55 ± 9.69	162.44 ± 6.07	0.045
Triglyceride (mg/dl)	27	136.77 ± 4.27	121.70 ± 4.07	0.001
HDL-C (mg/dl)	27	38.62 ± 1.04	41.00 ± 1.24	0.046
LDL-C (mg/dl)	27	110.49 ± 9.31	90.03 ± 6.50	0.011

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 (HDL-C = High density lipoprotein and LDL-C = Low density lipoprotein).

In G-1 who was supplemented mushroom as Ifter item, the mean ± SE serum HDL-C (mg/dl) before and after Ramadan was 40.68 ± 1.62 and 43.55 ± 1.80 respectively. A significant mean difference of HDL-C (p = 0.006) observed in pre and post Ramadan state indicating supplementation of mushroom as Ifter item associated with Ramadan fast significantly raised serum HDL-C level, which is termed as good cholesterol (Table-1). In G-2 who was not supplemented with mushroom as ifter item, the mean ± SE serum HDL-C (mg/dl) before and after Ramadan was 38.62 ± 1.04 and 41.00 ± 1.24 respectively. A statistically significant mean difference of HDL-C (p = 0.046) observed before and after Ramadan (Table-2). This finding indicates one month fasting state of Ramadan significantly raised serum HDL-C level. The comparative mean of G-1 and G-2 (done by independents sample t test) shows – in pre Ramadan state the mean ± SE serum HDL-C of G-1 and G-2 was 40.68 ± 1.62 and 38.62 ± 1.04 respectively. No statistically significant mean difference (p = 0.298) between the two groups in pre Ramadan state observed, indicating there was no difference of mean of mushroom supplemented and non mushroom supplemented group (Table-3). In post Ramadan state the mean ± SE serum HDL-C of G-1 and G-2 was 43.55 ± 1.80 and 41.00 ± 1.24 respectively. Here also no statistically significant mean difference (p = 0.255) between the two groups in post Ramadan state observed, indicating there was no significantly difference of mean of mushroom supplemented (G-1) and non mushroom supplemented (G-2) group (Table-4). This finding suggests that supplementation of mushroom alone as Ifter item in Ramadan has no significant effect on HDL-C.

In mushroom supplemented group (G-1), the mean ± SE serum LDL-C before and after Ramadan was 105.72 ± 5.73 and 74.00 ± 4.79 (mg/dl) respectively. A highly significant mean difference of LDL-C (p = 0.000) observed in pre and post Ramadan state indicating supplementation of mushroom associated with Ramadan fast significantly reduced serum LDL-C level (Table-1). In non mushroom supplemented group (G-2), the mean ± SE serum LDL-C (mg/dl) before and after Ramadan was 110.49 ± 9.31 and 90.03 ± 6.50 respectively. A significant mean difference of LDL-C (p = 0.011) observed before and after Ramadan (Table-2). This finding indicates one month fasting state of Ramadan also significantly reduced serum LDL-C level. The comparative mean of G-1 and G-2 (done by independents sample t test) shows – in pre Ramadan state the mean ± SE serum LDL-

C of G-1 and G-2 was 105.72 ± 5.73 and 110.49 ± 9.31 respectively. No statistically significant mean difference (p = 0.660) between the two groups in pre Ramadan state observed, indicating there was no difference of mean of LDL-C of mushroom supplemented and non mushroom supplemented group (Table-3). In post Ramadan state the mean ± SE serum LDL-C of G-1 and G-2 was 74.00 ± 4.79 and 90.03 ± 6.50 respectively. A statistically significant mean difference (p = 0.049) between the two groups in post Ramadan state observed, indicating there was a significantly difference of mean of LDL-C between mushroom supplemented and non mushroom supplemented group (Table-4). This finding suggests that supplementation of mushroom as Ifter item in Ramadan significantly reduces serum LDL-C level, which is considered as bed cholesterol.

Table 3. Evaluation of serum lipid profile of G-1 (with mushroom) and G-2 (without mushroom) subjects before Ramadan state

Parameters	G-1 (with mushroom) n = 29 (mean ± SE)	G-2 (without mushroom) n = 27 (mean ± SE)	p
Cholesterol (mg/dl)	178.58 ± 6.84	176.55 ± 9.69	0.863
Triglyceride (mg/dl)	160.68 ± 12.05	136.77 ± 4.27	0.075
HDL-C (mg/dl)	40.68 ± 1.62	38.62 ± 1.04	0.298
LDL-C (mg/dl)	105.72 ± 5.73	110.49 ± 9.31	0.660

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 (HDL-C = High density lipoprotein and LDL-C = Low density lipoprotein).

Table 4. Evaluation of serum lipids profile of G-1 (with mushroom) and G-2 (without mushroom) subjects after Ramadan state

Parameters	G-1 (with mushroom) n = 29 (mean ± SE)	G-2 (without mushroom) n = 27 (mean ± SE)	p
Cholesterol (mg/dl)	145.44 ± 5.03	162.44 ± 6.07	0.035
Triglyceride (mg/dl)	142.79 ± 5.99	121.70 ± 4.07	0.006
HDL-C (mg/dl)	43.55 ± 1.80	41.00 ± 1.24	0.255
LDL-C (mg/dl)	74.00 ± 4.79	90.03 ± 6.50	0.049

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 (HDL-C = High density lipoprotein and LDL-C = Low density lipoprotein).

Considering the obtained findings of the study it was observed that supplementation of a considerable amount (50 grams per day) of *P. ostreatus* regularly (1 month) as ifter item significantly reduces the serum total cholesterol and bed cholesterol LDL-C in comparison to non mushroom supplemented control subjects. But from the above findings it was noticeable that there is no effect on serum TG and HDL-C at the fasting state of Ramadan. In a study Bobek *et al.* (1997) observed a significant reduction of cholesterol in serum (31-46%) and liver (25-30%) in Wister rats fed a diet containing 5% *P. ostreatus* for 52 weeks. These observations were supported by the findings of Hossain *et al.* (2003).

They suggested that 5% *P. ostreatus* supplementation provides health benefits, at least partially, by acting on the atherogenic lipid profile in the hypercholesterolaemic condition. It is now established that excess lipid accumulation in the liver causes fatty change and ultimately responsible for hepatocellular injury.

Considerable experimental evidence suggests that one of the most important food components that help in reducing serum cholesterol is its polyunsaturated fatty acid (PUFA) content (Hashimoto, *et al.*, 2001; Gamoh, *et al.*, 2001 and Hossain, *et al.*, 1999). In a study Hossain (2002) shown that *P. ostreatus* contains the sufficient amount of n-3 linolenic acid (LNA) which acts as a precursor of the physiologically important PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Schmidt, *et al.*, 2001). There is considerable data supporting the belief that the health benefit obtained through the lowering of blood cholesterol may derive from the effects of EPA and DHA (Hashimoto, *et al.,* 1998 and Hashimoto, *et al.,* 1999).

Although lots of study conducted in different corner of the World with *P. ostreatus* but most of them were limited in animal subjects. In this respect this study might be pioneer as the study was conducted among the targeted human population. This study is consistent with Bobek *et al.* (1997) and Hossain *et al.* (2003) which gives the guidelines of hypolipidemic effects of oyster mushroom.

Considering the findings of the study it is believable that regular consumption of edible mushroom *P. ostreatus* can improve lipid profile status of blood and hence able to improve atherosclerotic disease which include hypertension, ischemic heart disease, stroke etc.

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Effects of Amount of Rice Straw on the Growth and Yield of *Pleurotus cystidiosus*

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Abstract

Different amounts of rice straw per packet were used to evaluate their effects on the growth and yield of *Pleurotus cystidiosus*-1 (Pcys-1) and *Pleurotus cystidiosus*-2 (Pcys-2). The minimum days required from opening to primordia initiation (DROPI) (4.75) and first harvest (DROFH) (8.00) were recorded on 500g rice straw in both the strains. The maximum DROPI (11.00) and DROFH (14.00) were recorded in (Pcys-1) on 1500g rice straw. The highest number of effective fruiting bodies (103.50), length of stipe (9.19 cm), diameter of stipe (1.70cm) and diameter of pileus (11.63 cm) found in Pcys-1 on 1500g rice straw. The biological yield was increased with increasing amount of rice straw. The highest biological yield 870.30g and lowest biological yield 164.00g was recorded in Pcys -1 on 1500g and 250g rice straw respectively. The highest biological efficiency (BE) (207.40 %) and lowest BE (135.50 %) was observed in Pcys-2 on 250g and 1000g rice straw respectively.

Key words: *Pleurotus cystidiosus,* rice straw, growth and yield.

INTRODUCTION

Mushrooms of *Pleurotus* spp. are commonly called 'oyster mushrooms'. They are the second most popular mushrooms after button mushroom all over the world (Adejoye *et al.,* 2006) and are most popular in Bangladesh. Now, in Bangladesh different species of *Pleurotus* mushrooms are being cultivated. Among those, *Pleurotus cystidiosus* have a great prospect because of its better yield performance along with medicinal and nutritional properties. Protein and ash content of this mushroom are preferable in comparison to other *Pleurotus ostreatus* (Shelly *et al.*, 2008). Moreover, *Pleurotus cystidiosus* contain non volatile component (Mau *et al.*, 1997, Tseng and Mau, 1999 and Yang *et al.*, 2001) and are active against anthracnose caused by *Colletotrichum gloeosporioides* (Inoka *et al.*, 2009). *Pleurotus cystidiosus* has the longest storage life among the species of *Pleurotus* (Djajanegara and Masduki, 2010) and the cultivation of this mushroom is also very easy.

Many substrates, depending upon their availability and low cost, are being used for its cultivation. Among the substrates used for oyster mushroom cultivation, rice straw is one of the best performing substrates (Sarker *et al.,* 2007). The amount of substrates is a matter of discussion as it affects the yield largely (Zhang, *et al.* 2002, Amin *et al.,* 2008). Jebunnahar *et al.* (2007) reported that the yield of button mushrooms have increased with increasing the amount of substrates. But at higher amount of substrates, the biological efficiency decreased in case of *Pleurotus ostreatus* (Amin, *et al.* 2008). So, present piece of work has undertaken to determine the right amount of substrate per packet for better yield or biological efficiency and higher benefits from *Pleurotus cystidiosus* cultivation on rice straw in Bangladesh.

MATERIALS AND METHODS

The experiment was conducted in the culture house of National Mushroom Development and Extention Centre (NAMDEC), Sobhanbag, Savar, Dhaka from December to March 2010. Six different strains of *Pleurotus cystidiosus*, Pcys-1, Pcys-2, Pcys-3 Pcys-4, Pcys-5 and Pcys-6 are available in NAMDEC, Savar, Dhaka. Among them two best performing strains, Pcys-1 (T_1) and Pcys-2 (T_2) were selected and grown on different amounts of rice straw such as 250g (A_1), 500g (A_2), 750g (A_3), 1000g (A_4), 1250g (A_5) and 1500g (A_6). Rice straw was chopped in to 3-4 inch length and poured into a net bag and treated at 60°C temperature hot water for one hour and allowed to drain out the excess water by hanging the bag for 16 hours. Then the straw spread over a polythene sheet to attain the moisture level approximately 65 %. The moisture content of the straw was measured by an electric moisture analyzer and it was 65.12 %.

Then the above mentioned amount of rice straw mixing with mother culture of selected strains of *Pleurotus cystidiosus* was poured into the polypropylene (PP) bags. The mouths of the PP bags were plugged by inserting water absorbing cotton with the help of plastic necks. The packets were kept in a room at about 25°C temperature. After inoculation when colonization was completed, the spawn packets were taken into the culture house and were opened by 'D' shaped cut on different part of the bags. The relative humidity and the temperature of the culture house were maintained at 80-90% and 18-20°C respectively by spraying water 3-4 times daily. Diffused day light and proper ventilation in culture house were maintained.

The experiment was laid out following Completely Randomized Design (CRD) with 4 replications. Data on yield parameters and yield were collected from three flushes. Data were recorded on days required from opening to primordia initiation and first harvest, number of fruiting body, length and diameter of stipe, diameter and thickness of pileus and biological yield and efficiency. Biological efficiency was estimated following a standard formula (Sarker *et al.*, 2007).

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

RESULT AND DISCUSSION

The growth parameters, yield attributes and yield of *Pleurotus cystidiosus*-1 (Pcys-1) and *Pleurotus cystidiosus*-2 (Pcys-2) varied significantly by different amount of rice straw.

Days required from opening to primordia initiation: The days required from opening to primordia initiation (DROPI) ranged from 4.75 to 11.00 (Table1). The maximum

DROPI (11.00) was found in T_1A_6, where Pcys-1 grown on 1500 g of rice straw which was statistically similar to T_1A_5 (10.25) and were significantly higher than other treatments. The minimum DROPI (4.75) was recorded in T_1A_2 and T_2A_2 *i.e.* 500 g of rice straw irrespective of strains. The DROPI increased with increases of amount of straw. The result agreed with Amin *et al.* (2008) who reported that the days required from opening to primordia initiation was increased with increasing the amount of rice straw for the cultivation of *Pleurotus ostreatus* and was ranged from 3.25 to 13.50days. The result was also supported by Patra and Pani (1995) and Shah *et al.* (2004) who observed that day for primordia initiation was 4 to 10 days.

Days Required from opening to First Harvesting: Significant variation on days required from opening to first harvesting (DROFH) was observed on different amount of rice straw and was ranged from 8 to 14 days. The maximum DROFH (14.00) was found in T_1A_6 followed by T_1A_5 (10.25) when 1500g and 1250g rice straw were used for the cultivation of Pcys-1. The minimum DROFH (8.00) was found in T_1A_2, T_2A_2, and T_2A_3 preceded by 8.25 in T_2A_4 and 8.50 in T_1A_1. The result for DROFH agreed with Amin *et al.* (2008).

Number of effective fruiting bodies: The number of effective fruiting bodies (NEFB) in different treatments differed significantly (Table 1). The highest NEFB (103.50) was found in T_1A_6 followed by 51.75 in T_2A_6 where 1500g rice straw was used for the cultivation of Pcys-1 and Pcys-2 respectively. The lowest NEFB (16.75) was found in T_1A_1 and T_2A_1 which was statistically similar with T_2A_2. The result was supported by Amin *et al.* (2008) in case of oyster mushroom (*Pleurotus ostreatus*).

Biological yield (g/packet): Significant variation was observed in biological yield (BY) (Table 1). The BY increased with the increases of the amount of rice straw. The highest BY, 870.30 g was found in T_1A_6 where Pcys-1 was grown on 1500 g of rice straw which was significantly higher than all the treatments. The second highest BY, 709.8 g was observed in T_2A_6 where Pcys-2 was grown on 1500 g of rice straw. The lowest biological yield 164.0 g was found in T_1A_1 preceded by 181.8g in T_2A_1 when 250g rice straw was used for the cultivation of Pcys-1 and Pcys-2 respectively. The results revealed that the BY increased with the increases of the amount of substrate irrespective of mushroom strains. Amin *et al.* (2008) supported the result who observed that the BY increased with the increases of the amount of rice straw for the cultivation of *Pleurotus ostreatus.*

Biological efficiency (%): The biological efficiency was estimated from 3 flashes of mushroom. The highest biological efficiency 207.40 % was found in T_2A_1 where Pcys-2 was cultivated on 250 g of rice straw followed by 188.10 % in T_1A_1 (Fig. 1). The lowest biological efficiency 135.50 % was recorded in T_2A_4 preceded by 135.70 % in T_2A_6 where 1000g and 1500g rice straw was used for the cultivation of Pcys-2. The results revealed that the BE is almost inversely proportional to the amount of substrates per packet. The result differed with the findings of Amin *et al.* (2008) might be due to environmental factors and cultural management of the crop.

Table 1. Days required from opening to primordia initiation and to first harvest, the number of effective body and biological yield of *Pleurotus cystidiosus* -1 and *Pleurotus cystidiosus*-2 grown on different amounts of rice straw

Treatments	Days required from opening to primordia initiation	Days required from opening to first harvest	Number of effective fruiting body	Biological yield (g)
T_1A_1	5.25 d	8.50 e	16.75 h	164.00 j
T_1A_2	4.75 d	8.00 e	21.25 g	263.80 i
T_1A_3	6.25 cd	10.25 bc	36.75 f	357.30 h
T_1A_4	7.75 b	11.25 b	46.25 cd	534.50 e
T_1A_5	10.25 a	13.00 a	48.25 c	677.80 c
T_1A_6	11.00 a	14.00 a	103.50 a	870.30 a
T_2A_1	5.50 d	8.75 de	16.75 h	181.80 j
T_2A_2	4.75 d	8.00 e	18.25 h	272.80 i
T_2A_3	5.25 d	8.00 e	39.75 e	442.50 g
T_2A_4	5.00 d	8.25 e	44.75 d	472.50 f
T_2A_5	6.25 cd	9.00 cde	48.25 c	634.30 d
T_2A_6	7.25 bc	10.00 bcd	51.75 b	709.80 b
CV (%)	13.76	8.72	4.62	2.92

In a column, means followed by a common letter are not significantly different at 5% level by DMRT (T_1=Pcys-1, T_2=Pcys-2, A_1=250g, A_2=500g, A_3=750g, A_4=1000g, A_5=1250g and A_6=1500g).

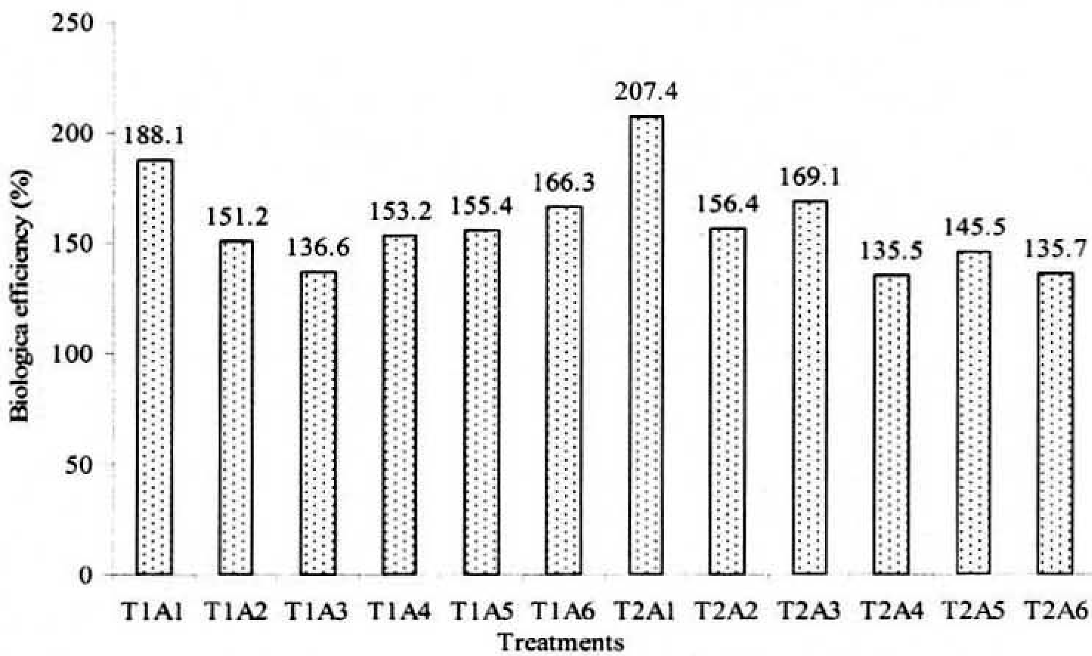

Fig. 1. Biological efficiency of of *Pleurotus cystidiosus* -1 and *Pleurotus cystidiosus*-2 on different amounts of rice straw (T_1=Pcys-1, T_2=Pcys-2, A_1=250g, A_2=500g, A_3=750g, A_4=1000g, A_5=1250g and A_6=1500g).

Length of stipe: The length of stipe (LS) ranged from 4.81 to 9.19 cm with significant difference (Table 2). The highest LS, 9.19 cm was found in T_1A_6 followed by 7.63 cm in T_2A_5 and the lowest LS, 4.81 cm was found in T_2A_2 preceded by 5.75 cm in T_1A_1.

Diameter of stipe: The diameter of stipe (DS) differed significantly and ranged from 1.13 to 1.70 cm (Table 2). The highest DS, 1.70 cm was found in T_1A_6 followed by 1.54 cm in T_2A_1. The lowest DS, 1.13 cm was found in T_2A_4 when 1000g rice straw was used for the cultivation of Pcys-2.

Diameter of pileus: The diameter of pileus (DP) ranged from 7.30 to 11.63 cm with significant difference among the treatments (Table 2). The highest DP, 11.63 cm was found in T_1A_6 followed by 10.25 cm in T_1A_2 and the lowest DP, 7.30 cm was found in T_2A_4 preceded by 7.50 cm in T_1A_4.

Thickness of pileus (cm): The thickness of pileus (TP) differed significantly and ranged from 1.00 to 1.50 cm (Table 2). The highest TP, 1.50 cm was found in T_1A_3, T_1A_6 and T_2A_1 which was statistically similar to the other treatments except T_1A_4, T_1A_5 and T_2A_4. The lowest TP, 1.00 cm was found in T_1A_5.

The results for LS, DS, DP and TP were almost similar with the findings of Amin *et al.*, (2007) and Sarker *et al.*, (2007).

Table 2. Combind effects of different amounts of rice straw on the growth and yield of *Pleurotus cystidiosus* -1 and *Pleurotus cystidiosus* -2

Treatments	Length of stipes (cm)	Diameter of Stipes (cm)	Diameter of Pileus (cm)	Thickness of Pileus (cm)
T_1A_1	5.75 fg	1.33 bcd	8.00 cde	1.40 a
T_1A_2	6.06 ef	1.43 bc	10.25 b	1.40 a
T_1A_3	5.31 g	1.30 cd	8.25 cde	1.50 a
T_1A_4	7.38 b	1.31 bcd	7.50 e	1.03 b
T_1A_5	7.56 b	1.40 bc	8.50 cde	1.00 b
T_1A_6	9.19 a	1.70 a	11.63 a	1.50 a
T_2A_1	6.75 cd	1.54 ab	9.00 bcd	1.50 a
T_2A_2	4.81 h	1.49 abc	8.63 cde	1.45 a
T_2A_3	6.63 cd	1.30 cd	8.50 cde	1.35 a
T_2A_4	6.38 de	1.13 d	7.30 e	1.03 b
T_2A_5	7.63 b	1.40 bc	9.16 bc	1.35 a
T_2A_6	6.88 c	1.48 abc	7.75 de	1.36 a
CV (%)	4.61	10.16	9.61	11.82

In a column, means followed by a common letter are not significantly different at 5% level by DMRT (T_1=Pcys-1, T_2=Pcys-2, A_1=250g, A_2=500g, A_3=750g, A_4=1000g, A_5=1250g and A_6=1500g).

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Performance of Different Casing Materials on the Yield Attributes and Yield of White Button Mushroom

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Abstract

Performance of four different casing materials such as Farm Yard manure: Burnt Rice Husk (FYM: BRH) (2:1), Peat soil, Poultry compost and Soil: Sand (3:1) were evaluated for white button mushroom cultivation. The highest number of fruiting body, biological yield and biological efficiency were recorded in farm yard manure and burnt rice husk (2:1) casing materials. The highest economic yield (332.07g/ 3 kg bag) was estimated from farm yard manure and burnt rice husk (2:1) followed by peat soil (313.62 g/ 3kg bag) and soil and sand (3:1) (301.25g/ 3 kg bag). The lowest economic yield was observed in poultry manure (292.01kg/ 3 kg bag).

Key words: Casing materials, yield and white button mushroom

INTRODUCTION

Agaricus bisporus (Lange) Singer is the most popular cultivar among the artificially grown fungi of the world. It contributes about 31.8% to the global mushroom production (Angrish *et al.*, 2003). It requires two different substrates to form its fruiting bodies, *i.e.*, the compost in which it grows vegetatively and the nutritionally poor casing materials which provide suitable physical, chemical and biological conditions that stimulate the initiation of fruiting body formation (Coskuner and Ozdemir, 1997 and Segula *et al.*, 1987). Casing is a mixture designed to cover the nutritional composted substrate colonized with mycelium and has an essential function in stimulating and promoting the developments of sporophores (Pardo *et al.,* 2003 and Noble *et al.,* 2005). It is normally believed that fruiting bodies of mushrooms are produced when some stress is provided. Application of casing layer, which is not nutritionally as rich as compost, creates condition of stress, necessary for induction of fruiting bodies. Besides, the casing layer fulfils several functions (Stames and Chilton, 1983 and Wuest and Beyer, 1996): it constitutes the physical support of the emerging carpophores and contributes to the maintenance of a moist microclimate to help feed the mycelium and support the formation of primordia; it acts as a suitable medium for the development of bacteria which stimulate fructification; it provides water for the growth and development of mushrooms, supplementing the water provided by the compost; it provides the mycelium with a

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suitably aerated environment, permitting gas interchange; and finally, it provides an environment of low osmotic value unlike compost, whose osmotic value is too high for mushrooms (Wuest and Beyer, 1996, Hayes, 1981 and Flegg and Wood, 1985). However, a proper casing material has to have some special criteria such as it must be sufficiently resistant and deep enough to provide adequate support for mushroom growth, have a high capacity to absorb and release water, be able to withstand frequent irrigation without losing its structure and possess a structure which permits good permeability for water and gases. As peat moss, which is universally accepted as best casing material in mushroom cultivation due to high water holding capacity and other favorable traits (Vijay and Gupta, 1995), is not available in Bangladesh, different casing mixtures based on locally available materials have to be tested. The aim of the present study was to evaluate the performance of different locally available casing materials on the cultivation of *Agaricus bisporus* and to find out the best casing materials for yield attribute and yield of white button mushroom.

MATERIALS AND METHODS

Preparation of casing materials: Six different casing mixtures were selected and collected from local villages of Savar, Dhaka area and National Mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka. The casing materials were prepared in the following manner: T_1 = Farm Yard manure: Burnt Rice Husk (FYM: BRH) (2:1), T_2 = Peat soil, T_3 = Poultry manure and T_4 = Soil: Sand (3:1).

Preparation of compost: Paddy straw was used as the main substrate (compost) and the compost was prepared by long method of composting (LMC) using rice straw (300 kg), wheat bran (30 kg), gypsum (15 kg), Calcium carbonate (10 kg), Urea (9 kg), triple super phosphate (6 kg), muriate of potash (3 kg), Furadan (250 g) and Bavistin (150 g). The ready compost was deep brown colour, free from bad smell and had 65-67% moisture.

Spawning and incubation: Three kilograms of compost were mixed with 75 g of mother culture of *Agaricus bisporus* and poured in a polypropylene bag. The open top of the bags were covered with wetted news papers and incubated in the incubation room at $24^0 \pm 2^0$C temperature for 20 days.

Casing: After completion of mycelium run, the news paper sheet was removed and the surface of the compost was uniformly layered with 3.5- 4.0 cm casing formulations. Before use, the casing materials were sterilized by autoclaving at 121^0C temperature and 1.1 kg/cm^2 pressure for 1 hour. The packets were incubated in the same incubation room at $24^0 \pm 2^0$C temperature for 10 days.

Cropping and harvesting: Case run was considered complete when mycelia appeared in the valleys of casing layer. After case run, the spawn packets were transferred to the culture house where the temperature, relative humidity and low CO_2 concentration were maintained by bringing down the temperature to 15-17°C (air), RH to 85% by opening of the fresh air ventilation and exhaust CO_2. This change in environmental parameters induced pinhead formation in 3-4 days time. The pinheads developed into solid button sized mushrooms in another 3-4 days.

Mushroom was harvested before the fruiting body showed any detachment of the cap from the stripe. The yield of mushrooms and their parameters were recorded regularly. The number of fruiting body, biological and economic yield was estimated. Biological efficiency (BE) was estimated by the formula:

BE (%) = Total biological yield (g) X 100/Total compost used (g).

Data analysis: The experiment was laid out following completely randomized design with 5 replications. Data were analyzed following MSTAT-C computer program. Means was computed following Duncan's Multiple Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Days to primordia initiation (DPI): All the four casing mixtures were evaluated for their yield potential. Days to primordia initiation in compost bag ranged from 14.80-15.60 days on different casing materials and no significant difference was observed among the treatments. The highest DPI was observed on soil and sand (3:1) (Table 1). This finding correlates that of Amin *et al.* (2007).

Number of primodia in first flush (NPFF): Significant variation was observed in the number of primordia in first flush (NPFE) on different casing materials tested in the present experiment (Table 1). The highest NPFF was found on peat soil which is statistically similar to FYM+BRH (2 :1) and soil and sand (3:1). The lowest NPFF was recorded on poultry. Gupta and Dhar (1993) also found the mixture to be good but equally good yields obtained with farm yard manure alone as well as with mixture of FYM + spent compost + loam Soil (1:1:1), which support the present experiment.

Number of total fruiting body (NTFB): Significant variation was found in number of total fruiting body (NTFB) on different treatment tested in this experiment. The treatment FYM and BRH (2:1) showed the highest NTFB which is statistically similar to the peat soil casing. The lowest NTFB was recorded in soil and sand (3:1) which did not differ with poultry manure. This supports the results of Amin *et al.* (2007) that soil: sand: CD: FYM casing treatment produced the highest number of fruiting bodies. The results also satisfied the experimental results of Angrish *et al.* (2003). They found that FYM: SC (spent compost) casing mixture gave the highest number of fruiting bodies followed casing mixture, FYM+BRH (2:1).

Biological yield (BY) and economic yield (EY): From the Table 1, it is obvious that there was significant variation among biological yield (BY) and economical yield (EY) ranging from350.80 to 399.60 g and 292.01 to 332.07 g/bag respectively. The highest biological yield was recoded in FYM+BRH (2:1) which was significantly higher as compared to all the treatments except peat soil. The lowest BY was observed in poultry manure. Almost similar trend was observed in EY.

Angrish *et al.* (2003) evaluated five casing materials of *Agaricus bisporus*: biogas plant slurry, burnt rice husk (BRH), farm yard manure (FYM), Sandy Soil (SS), spent compost(SC) and observed that FYM+SS (1:1) was the best which was followed by FYM+SC (1:1) and FYM + SS (2:1)

Table 1. Effect of different casing materials on the yield attributes and yield of white button mushroom

Treatment	Days to primordia initiation	Number of primordia in first flush	Number of total fruiting body	Biological yield (g)	Economic yield (g)
Farm Yard Manure and burnt rice husk (2:1)	14.80 a	24.20 a	95.6 a	399.60 a	332.07 a
Peat soil	14.80 a	24.60 a	85.20 a	382.00 ab	313.62 ab
Poultry manure	14.80 a	16.20 b	59.60 b	350.80 c	292.01 b
Soil and Sand (3:1)	15.60 a	22.40 a	50.0 b	371.80 bc	301.25 b
CV (%)	5.16 a	9.97	9.82	3.54	4.05

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

Biological efficiency in different casing materials: The biological efficiency (BE) of *Agaricusl bisporus* influenced by different casing materials were ranged from 11.69 to 13.32% (Fig. 1). The highest BE (13.32%) was observed in FYM+BRH (2:1) which was followed by peat soil (12.73%) and sand and soil (3:1) (12.39%). The lowest BE was recorded in poultry manure (11.69%).

Relation between number of fruiting body (NFB) and economic yield (EY): A positive linear relationship was observed between number of fruiting body and economic yield per packet (3 kg of compost) (Fig. 2). The equation $y = 0.7202x + 257.45$ gave a good fit to the data and the value of co-efficient of determination ($R^2 = 0.788^*$) showed that the fitted regression line had a significant regression co-efficient. So, it indicated that economic yield per packet increased as the number of fruiting body increased.

Relationship between biological yield (BY) and economic yield (EY): A positive linear relationship was observed between biological and economic yield per bag (Fig. 3). It was observed that the equation $y=0.8291x-2.0367$ gave a good fit to the data and the co-efficient of determination ($R^2 = 0.952^{**}$) showed that the best fitted regression line had a significant regression co-efficient. It indicated that the economic yield per bag increased with the increase of biological yield. More over its value also indicate that 95.2% economic yield was attributed by the biological yield.

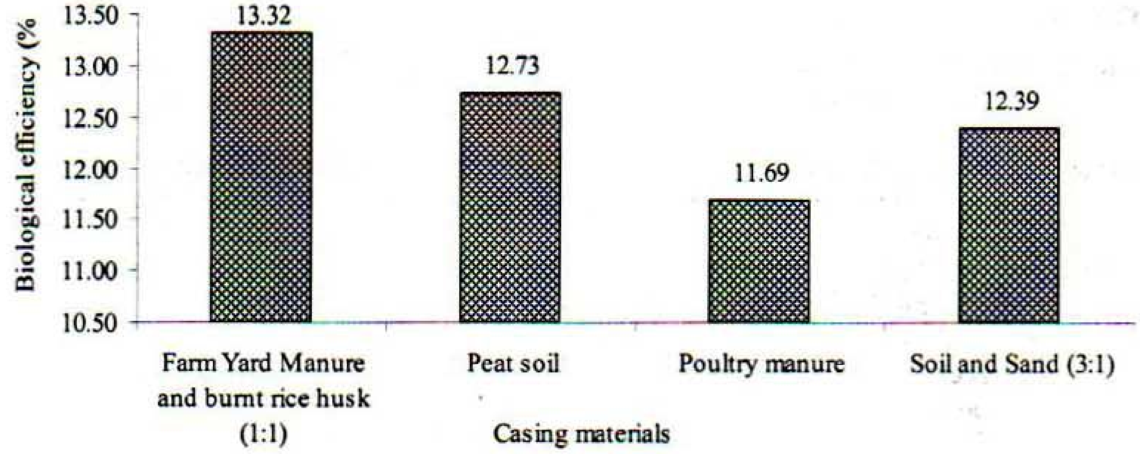

Fig. 1. Biological efficiency of white button mushroom in different casing materials.

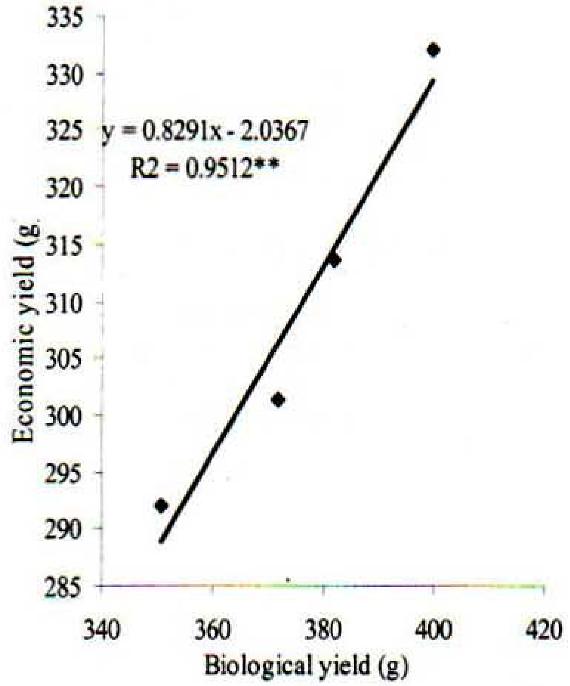

Fig. 2. Functional relationship between number of fruiting body and economic yield of white button mushroom.

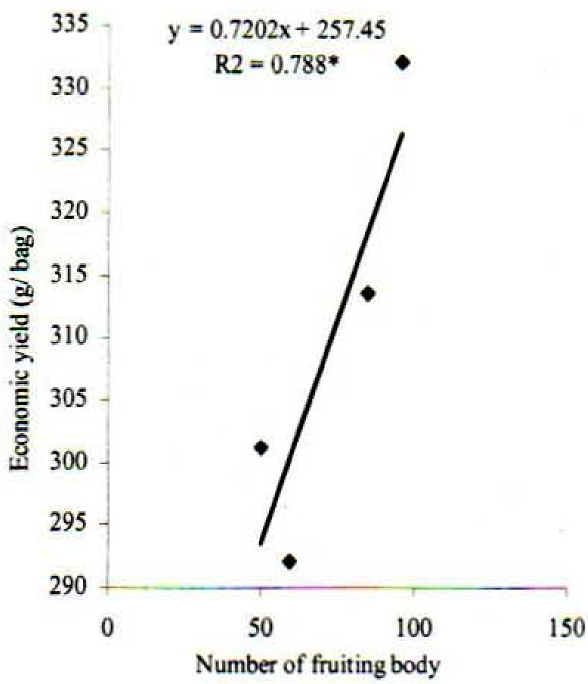

Fig. 3. Functional relationship between biological yield and economic yield of white button mushroom.

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Effect of Wheat Bran Supplements with Sugarcane Bagasse on the Yield and Proximate Composition of *Pleurotus ostreatus*

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Abstract

The performance of different levels of wheat bran (0, 10, 20, 30 and 40 %) as supplement with sugarcane bagasse on the yield and proximate composition of oyster mushroom was studied. The highest mycelium growth rate (0.96 cm/day), number of primordia/ packet (70.67) and number of fruiting body/ packet (61.00) were observed in sugarcane bagasse supplemented with 40% wheat bran. The lowest time from primordia initiation to harvest (3.23 days) and the highest weight of individual fruiting body (3.69 g) were observed in 30% level of wheat bran. The highest biological yield (254.7 g/ 500g wet substrate), marketable yield (243.3 g), dry matter (23.40 g), biological efficiency (87.82%) and benefit cost ratio (8.29) were also observed in 30% wheat bran. The highest content of protein (30.31 %), ash (9.15 %) and crude fiber (24.07 %) and the lowest content of lipid (3.90 %) and carbohydrate (32.57 %) were recorded in 30% wheat bran.

Key wards: Oyster mushroom, wheat bran, sugarcane bagasse, yield and proximate composition.

INTRODUCTION

Oyster mushrooms are large reproductive structures of edible fungi belong to genus *Pleurotus* under the order Agaricales, the family Tricholomataceae and the class Basidiomycetes. In the developed countries, mushrooms have become one of the most important horticultural crops (Alam and Saboohi, 2001). Mushroom reduces serum cholesterol and high blood pressure (Mori, 1986). Edible mushrooms have been treated as important tool in modern medicine for their medicinal values (Kovfeen, 2004).

Mushroom production converts agricultural wastes to a protein rich food of human being (Labuschagne *et al.*, 2000). It grows fast and does not require any fertile land. It grows on composted or non-composted agro-wastes like wheat or paddy straw, banana leaves, sugarcane bagasse and leaves, wheat bran, rice husk, sawdust, etc. Sarker *et al.* (2007a) reported that the yield potential of oyster mushroom on waste paper, wheat straw, rice straw, ulu (*Imperata cylindrica*), kansh (*Saccharum spontaneum*) and sugarcane bagasse were satisfactorily. Sarker *et al.* (2008) achieved higher yield on waste paper and wheat straw using wheat bran and rice bran as supplement. They did not study the effect of wheat bran as supplement to sugarcane bagasse on the yield and proximate composition of oyster mushroom (*Pleurotus ostreatus*). So, the present experiment was undertaken to find out the effect of different levels of wheat bran supplements with sugarcane bagasse on the yield and proximate composition of oyster mushroom (*Pleurotus ostreatus*).

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MATERIALS AND METHODS

The experiment was carried out at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka. Five different levels of wheat bran, T_1= 0% (Controlled), T_2= 10%, T_3= 20%, T_4= 30%, T_5= 40% were evaluated as the supplement to sugarcane bagasse substrate of oyster mushroom. The experiment was laid out in Completely Randomized Design with three replications.

Wheat bran was mixed to sugarcane bagasse according to the treatments. The spawn packets preparation, sterilization, inoculation, incubation and culture house activities were done using the method described by Sarker *et al.* (2007a). The wet weight of each spawn packet was 500 g. The light intensity and temperature of the culture house was around 300-500 lux and 22 to 25°C respectively.

The first primordia appeared 2-4 days after scrapping depending upon the levels of supplement. The harvesting time also varied depending upon the levels of supplement. Data were collected on mycelial growth rate, time from stimulation to primordia initiation and harvest, number of primordia and fruiting body/ packet, weight of individual fruiting body, biological, marketable and dry yield, biological efficiency and cost benefit ratio. Biological efficiency and dry yield were estimated following standard formulas (Sarker *et al.,* 2007b).

Proximate analysis of the mushrooms

Moisture and dry mater were determined by the following formulas.

Moisture (%) = (Initial weight-Final weight) X 100/ weight of mushroom sample

Dry mater (%) = 100- % Moisture content.

Crude fiber, total lipid, carbohydrate and ash were determined by Raghuramulu *et al.* (2003). Total nitrogen was determined by using the standard Micro kjeldhal procedure of AOAC (1975) and total crude protein was estimated by multiplying the nitrogen content by a factor of 6.25.

Determination of Ca, Mg, K, Fe, and P: The sample was digested with nitric acid to release of Ca, Mg, K, Fe, and P. Calcium, Mg, and Fe were determined by atomic absorption spectrophotometry, K was determined by flamephotometry and P was determined by spectrophotometry.

Statistical analysis of data: The recorded data were analyzed statistically with the help of computer MSTAT-c programme and means following least significant difference (LSD) test at 1% and 5% level of probability for interpretation of results as and when required (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Mycelium growth: The highest growth rate of mycelium (0.96 cm/day) was observed in T_5 which was significantly higher than all other treatments. The lowest growth rate was observed in control treatment (0.72 cm/day). The lowest time from stimulation to primordia initiation was observed in the treatment T_3 (7.17 days) which was statistically similar to all other treatments except the control. The time from primordia initiation to harvest was lowest (3.23 days) in the treatment T_4 which was significantly lower than other treatments and it was the highest in the treatment T_1 (5.17days). The present findings corroborated with the findings of previous workers (Sarker *et al.,* 2008). They found that the mycelium growth rate of oyster mushroom greatly influenced by the supplement, wheat bran in different levels.

Table 1. Effect of different levels of wheat bran with sugarcane bagasse on mycelial growth of *Pleurotus ostreatus*

Treatments	Mycelium growth rate in spawn packet (cm)	Time from stimulation to primordial initiation (days)	Time from primordia Initiation to harvest (days)
T_1	0.72e	11.5a	5.17a
T_2	0.78d	7.83b	4.2b
T_3	0.87c	7.17b	4.1b
T_4	0.91b	7.57b	3.23c
T_5	0.96a	7.3b	5.16a
CV (%)	0.71	5.16	3.01
Level of significance	**	**	**
LSD (0.05)	0.028	1.169	0.358

Means followed by same letter are not significantly different at 1% or 5% level of significance. ** Significant at 1% level; T_1= 0% (Controlled), T_2= 10%, T_3= 20%, T_4= 30%, T_5=40% level of wheat bran.

Yield attributes: The highest number of primordia/ packet (70.67) was observed in treatment T_5 which was significantly higher than all the treatments except T_4 (69.00). The lowest number of primordia/packet was recorded in the treatment T_1 (48.00) (Table 2). Almost similar trend was observed in number of fruit body/ packet. The weight of individual fruit body in different treatment ranged from 3.06 g to 3.69 g and the highest weight of individual fruit body was observed in the treatment T_4 (3.69 g) which was significantly higher to other treatments. The lowest weight of individual fruit body was recorded in the treatment T_1 (3.06 g).

Yields, biological efficiency and benefit cost ratio: The highest biological yield (BY) was observed under treatment T_4 (254.7 g) which was significantly higher than other treatments. The lowest BY was recorded in T_1 (147.0 g). The result revealed that the BY increased with the increases of supplement level up to 30% and then decreased (Table 3). Similar trend was observed in marketable yield, dry yield and biological efficiency. The highest benefit cost ratio was observed in T_4 treatment. Similar results were observed by Sarker *et al.* (2008).

Table 2. Effect of different levels of wheat bran with sugarcane bagasse on the yield contributing characters of *Pleurotus ostreatus*

Treatments	Number of primordia/ packet	Number of fruit body/packet	Weight of individual fruit body (g)
T_1	48.00d	41.00d	3.06d
T_2	59.00c	51.00c	3.33b
T_3	66.00b	53.00c	3.33b
T_4	69.00a	58.00b	3.69a
T_5	70.67a	61.00a	3.15c
CV (%)	1.49	1.80	0.91
Level of significance	**	**	**
LSD (0.05)	2.55	2.60	0.087

Means followed by same letter are not significantly different at 1% or 5% level of significance. ** Significant at 1% level; T_1= 0% (Controlled), T_2= 10%, T_3= 20%, T_4= 30%, T_5=40% level of wheat bran.

Table 3. Effect of different levels of wheat bran with sugarcane bagassee on the yield, biological efficiency and cost benefit ratio of *Pleurotus ostreatus*

Treatments	Biological yield (g)	Marketable yield (g)	Dry yield (g)	Biological efficiency (%)	Benefit cost ratio
T_1	147.0d	142.0d	14.13d	50.69d	6.09e
T_2	196.3c	192.3c	19.37c	67.70c	7.21c
T_3	219.7b	214.7b	21.13b	75.75b	7.67b
T_4	254.7a	243.3a	23.40a	87.82a	8.29a
T_5	222.7b	215.0b	20.53b	76.78b	6.72d
CV (%)	0.93	0.44	2.10	0.93	0.43
Level of significance	**	**	**	**	**
LSD (0.05)	5.32	2.42	1.13	1.83	0.087

Means followed by same letter are not significantly different at 1% or 5% level of significance. ** Significant at 1% level; T_1= 0% (Controlled), T_2= 10%, T_3= 20%, T_4= 30%, T_5=40% level of wheat bran.

Proximate composition of mushroom: The highest moisture percent was observed in treatment T_5 (90.45 %) which was statistically similar to T_4 (90.38 %). The lowest moisture percent was observed in T_2 (89.93 %) followed by T_1 (90.05 %) (Table 4). The dry matter percentage was inversely proportional to the moisture percentage. The highest content of protein was found in treatment T_4 (30.31 %) which was significantly higher to other treatments and the lowest content of protein was found in T_1 (17.40 %). The higher amount of ash and crude fiber was observed in T_4 and they decreased with the increases or decreases of the level of supplement. Almost opposite feature was observed in case of lipid and carbohydrate content. The results agreed with the findings of Moni, *et al.* (2004) and Alam *et al.* (2007). Moni, *et al.* (2004) found 88.15-91.64% moisture, 18.46-27.78% crude protein, 1.49-1.90% crude fats, 40.54-47.68% carbohydrates in oyster mushroom. Alam *et al.* (2007) reported 87-87.5% moisture, 4.30-4.41% lipids, 22.87-23.29 g/100g of fiber, 39.82-42.83% of carbohydrates and 8.28-9.02% of ash in *Pleurotus spp.*

Table 4. Effect of different levels of wheat bran with sugarcane bagassee on chemical composition of *Pleurotus ostreatus*

Treatment	Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	CHO (%)	Crude fiber (%)
T_1	90.05bc	9.95ab	17.40e	6.16a	7.05e	55.22a	20.17d
T_2	89.93c	10.07a	22.50d	5.75b	8.20d	42.49b	21.06c
T_3	90.16b	9.84b	24.30c	4.15d	8.75b	39.65c	23.15b
T_4	90.38a	9.62c	30.31a	3.90e	9.15a	32.57e	24.07a
T_5	90.45a	9.55c	27.13b	4.43c	8.55c	36.85d	23.03b
CV (%)	0.23	2.14	0.35	1.39	0.44	0.15	0.18
Level of significance	**	**	**	**	**	**	**
LSD (0.05)	0.17	0.173	0.23	0.19	0.087	0.171	0.123

Means followed by same letter are not significantly different at 1% or 5% level of significance. ** Significant at 1% level; T_1= 0% (Controlled), T_2= 10%, T_3= 20%, T_4= 30%, T_5=40% level of wheat bran.

The highest percentage of phosphorus content (0.92) was observed under treatment T_1 which was followed by T_2 (0.88) but the lowest phosphorus percentage was found at T_3 and T_5 (0.82). Sarker *et al.* (2007c) found 0.97% phosphorus, in oyster mushroom grown on sugarcane bagasse based substrates. The higher amount of K, Ca, Mg, S and Fe was observed in T_4 treatment which decreased with the increases or decreases of supplement level to the substrate. The results matched with the findings of Alam *et al.* (2007).

Table 5. Effect of different levels of wheat bran with sugarcane bagassee on elemental contents of *Pleurotus ostreatus*

Treatment	P (%)	K (%)	Ca (mg/100g)	Mg (mg/100g)	S (mg/100g)	Fe (mg/100g)
T_1	0.92a	1.12d	20.20d	18.13d	0.013	40.53c
T_2	0.88b	1.18c	20.82c	18.87c	0.019	41.84b
T_3	0.82c	1.26b	21.15b	19.40b	0.037	42.40ab
T_4	0.83c	1.39a	22.08a	20.21a	0.042	43.11a
T_5	0.82c	1.28b	21.06b	19.23b	0.035	42.27b
CV (%)	2.17	0.83	0.2	0.41	3.63	0.66
Level of significance	**	**	**	**	NS	**
LSD (0.05)	0.027	0.026	0.151	0.212	0.028	0.76

Means followed by same letter are not significantly different at 1% or 5% level of significance. ^{NS} Not significant ** Significant at 1% level; T_1= 0% (Controlled), T_2= 10%, T_3= 20%, T_4= 30%, T_5=40% level of wheat bran.

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Effect of Hot Water Extract of *Calocybe indica* on Acute Metabolic Study

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Abstract

Calocybe indica, an edible mushroom, is being used as a nutritious food supplement in Bangladesh. In this study effect of hot water extract of *Calocybe indica* (HWEC) on normal metabolic processes, acute metabolic study was carried out utilizing female Swiss-Webster mice. In the 24 hrs acute metabolic study, six parameters of normal metabolic process, i.e. water intake, urination, food intake, defecation, water content of the stool and weight of the dry stool were taken into account. Water intake was significantly ($p<0.012$) decreased at the 3[rd] hour after administration of the extract at the dose of 20 mg/kg body weight. And, the weight of the dry stool though not significantly but yet was noticeably increased during the period of 9[th] to 12[th] hours with an overall decrease in the total period of the observation. Overall it can be concluded that the HWEC showed a mild decreasing effect on water intake, food intake and urination, with an insignificant increase in defecation and water content in stool during the period of 3[rd] to 12[th] hours after the administration.

Key words: *Calocybe indica,* hot water extract, acute metabolic study.

INTRODUCTION

Mushroom, an edible fungi, is a newly introduced culinary and medicinal as well as food supplement in Bangladesh. Milky white (*Calocybe indica*), the white summer mushroom is a nutritious and delicious edible fungi (Quimo *et al.*, 1995). The mushroom almost resembles button mushroom in shape and appearance at early stages of growth. It is easy to grow and involves less cost as compare to button mushroom. The mushroom grows well at temperature range of 30-35^{0}C (Krishnamoorthy and Amutha, 2007) which prevails at least 8 months of a year in Bangladesh. The medicinal values of various types of mushrooms have been elucidated; *Calocybe indica* is yet to get such study. Due to their immense nutritional values, mushrooms stand a good stead to meet the nutritional demand of the people of Bangladesh. Our present study, aimed at the elucidation of the metabolic study of *Calocybe indica,* is an innovative approach and is destined to pave a pathway whether the edible mushroom of Bangladeshi climate-prone, poses any threat to the physique.

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MATERIALS AND METHODS

Experimental animal: Female mice (Swiss-Webster strain, 20-25gm body weight) bred in the animal house of the Department of Pharmacy, Jahangirnagar University, were used for the acute metabolic study. The mice were housed in plastic cages (having dimensions of 30×20×13 cm and bedding was soft wood chips) under controlled conditions of 12 hours dark-light cycles. They all received a basal diet of food preparation formulated by the Bangladesh Council of Scientific and Industrial Research (BCSIR) and tap water *ad libitum*. The mice were divided into two groups- Control and hot water extract of *Calocybe indica* (HWEC) treated group.

Collection of *Calocybe indica*: Dried Milky mushrooms were collected from the National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh. The mushrooms were powdered using a grinder and preserved in air tight polythene packet.

Preparation of the water Extract of *Calocybe indica*: Ten g powder of *Calocybe indica* was mixed in 200 ml distilled water. The mixture was heated to reduce the volume. The condensed mixture was filtered off through a cotton cloth to get approximately 40 ml filtrated solution. Again 200 ml water was added with the residue and the total procedure was repeated to get 80 ml (40ml+40ml) filtrate, which was then heated and ultimately condensed to 40 ml. This liquid is the water extract of the *C. indica*. The extract was administered orally at the dose of 20 ml/ kg body weight by using gastric gavage needles.

Acute Metabolic Study: Utilizing a 'Nalgene Metabolic Case' the effect of the extract (HWEC) on acute metabolism was performed. After a period of one day (*i.e.* 24 hours) of adjustment, they were administrated with graded dosage of the extract. The rate of food and water intake as well as defecation and urination were measured for as period of 3 days (72 hours) maintaining a 3 days of rest before each days (24 hours) of test (Khan and Choudhuri, 1998). Eighteen mice were randomly selected and equally divided into 3 groups. These mice were placed in 3 different cases. The mice of one case were treated as control (administering distillated water) and the mice of the remaining 2 cases were treated with the same extract maintaining the same dose as duplicate. Within the next 24 hours, food and water intake as well as urination and defecation were measured with an interval of one hour for the first 4 hours, interval of two hours for the next 4 hours; next measurement was after another 4 hours and the final measurement after another 12 hours (*i.e.* a total of 24 hours). The test was repeated in the next two days with alternating control group (according to the Latin Square Design). Then the percent deviation of the drug treated group was compared with the corresponding control group.

Statistical analysis: The results are expressed as mean ± SEM (Standard error of mean). Means were compared by independent sample t-test. The statistical program "SPSS 12.0 for Windows" was used to test the level of significance. Probability (p) value of 0.05 or less ($p<0.05$) was considered as significant. Here * indicates $p<0.05$.

RESULTS AND DISCUSSION

Effect of *Calocybe indica* on water intake: Water intake was decreased significantly at the 3rd hour after administration of hot water extract of *Calocybe indica* (HWEC). And, overall a slight decrease in the water intake was observed in the total period of observation with an exception (i.e. increase) during the period of 1st hour and 7th to 8th hour after administration of it (Table 1.1). The cumulative water intake calculation reveals that it has overall slight decreasing effect on water intake (Table 1.2).

Effect of *Calocybe indica* on urination: A slight decreasing effect on urination was observed in the total period of the observation after administration of the HWEC (Table 2.1). The cumulative urination calculation also expresses the same information (Table 2.2).

Effect of *Calocybe indica* on food Intake: Food Intake was slightly decreased all throughout total period of the observation after administration of the HWEC (Table 3.1 and Table 3.2).

Effect of *Calocybe indica* on defecation: A slight increase in defecation was observed in 3rd to 12th hours after the administration of HWEC. But it decreased slightly in first 2 hours and the period of 13-24 hours time interval (Table 4.1 and Table 4.2).

Effect of *Calocybe indica* on water content in the stool: Water content in the stool increased slightly during the period of 3rd to 12th hours. But, insignificant decrease in it was observed in first 2 hours and the period of 13-24 hours (Table 5.1). The cumulative water content in the stool calculation expresses its slightly increasing effect on the water content in stool (Table 5.2).

Effect of *Calocybe indica* on weight of the dry stool: The weight of the solid mass present in the stool though not significantly but yet was noticeably increased during the period of 9th to 12th hours with an overall decrease in the total period of the observation (Table 6.1).The cumulative weight of the dry stool calculation reveals that HWEC causes an overall decrease in the weight of dry stool (Table 6 .2).

Table 1.1. Effect of *Calocybe indica* on water intake (hourwise)

Type	Rate of water intake at different hours (g/100 g body weight of the mice)							
	1st	2nd	3rd	4th	5-6th	7-8th	9-12th	13-24th
Control	0.971± 0.189	0.883± 0.383	1.830± 0.373	1.888± 0.477	2.344± 0.524	2.186± 0.463	2.729± 0.424	2.598± 0.223
HWEC	1.220± 0.366	0.815± 0.188	0.659± 0.167*	1.301± 0.360	2.253± 0.294	2.465± 0.236	2.147± 0.155	2.487± 0.202
t/p	-0.449/ 0.667	0.183/ 0.860	3.390/ 0.012	0.958/ 0.370	0.166/ 0.872	-0.605/ 0.564	1.620/ 0.149	0.339/ 0.745

HWEC= Hot water extract of *Calocybe indica* treated.

Table 1.2. Effect of *Calocybe indica* on water intake (cumulative study)

Type	Water intake in g/100 g body weight of the mice at different hours						
	00-02	00-03	00-04	00-06	00-08	00-12	00-24
Control	1.854± 0.569	3.684± 0.918	5.572± 1.311	7.916± 1.783	10.102± 2.169	12.831± 2.589	15.429± 2.385
HWEC	2.033± 0.397	2.692± 0.538	3.993± 0.697	6.246± 0.748	8.711± 0.719	10.858± 0.730	13.345± 0.699
t/p	-0.259/ 0.803	0.647/ 0.350	1.184/ 0.275	1.043/ 0.332	0.787/ 0.457	0.984/ 0.358	1.116/ 0.301

HWEC= Hot water extract of *Calocybe indica* treated.

Table 2.1. Effect of *Calocybe indica* on urination (hourwise)

Type	Rate of urination at different hours (g/100 g body weight of the mice)							
	1st	2nd	3rd	4th	5-6th	7-8th	9-12th	13-24th
Control	0.875± 0.195	0.186± 0.036	0.311± 0.181	0.275± 0.068	0.453± 0.207	0.277± 0.130	0.882± 0.289	1.272± 0.294
HWEC	0.824± 0.098	0.187± 0.061	0.150± 0.059	0.206± 0.082	0.331± 0.037	0.169± 0.066	0.740± 0.171	1.328± 0.268
t/p	0.265/ 0.799	-0.010/ 0.992	1.102/ 0.307	0.536/ 0.608	0.576/ 0.620	0.843/ 0.427	0.451/ 0.666	-0.129/ 0.901

HWEC= Hot water extract of *Calocybe indica* treated.

Table 2.2. Effect of *Calocybe indica* on Urination (cumulative study)

Type	Urination in g/100 g body weight of the mice at different hours						
	00-02	00-03	00-04	00-06	00-08	00-12	00-24
Control	1.061± 0.168	1.372± 0.336	1.647± 0.301	2.000± 0.468	2.377± 0.447	3.258± 0.405	4.530± 0.682
HWEC	1.011± 0.134	1.161± 0.152	1.367± 0.136	1.699± 0.142	1.867± 0.173	2.607± 0.166	3.935± 0.256
t/p	0.222/ 0.830	0.676/ 0.521	0.998/ 0.351	1.083/ 0.315	1.316/ 0.230	1.811/ 0.113	1.021/ 0.341

HWEC= Hot water extract of *Calocybe indica* treated.

Table 3.1. Effect of *Calocybe indica* on food intake (hourwise)

Type	Rate of food intake at different hours (g/100 g body weight of the mice)							
	1st	2nd	3rd	4th	5-6th	7-8th	9-12th	13-24th
Control	1.32± 0.269	1.56± 0.527	2.24± 0.614	2.161± 0.423	2.74± 0.561	4.14± 1.02	4.636± 0.557	4.79± 0.647
HWEC	1.31± 0.21	1.32± 0.237	1.50± 0.307	1.99± 0.289	2.922± 0.542	3.39± 0.259	4.271± 0.234	4.102± 0.318
t/p	0.019/ 0.985	0.485/ 0.642	1.232/ 0.258	0.337/ 0.746	-0.209/ 0.841	0.718/ 0.540	0.729/ 0.489	1.095/ 0.310

HWEC= Hot water extract of *Calocybe indica* treated.

Table 3.2. Effect of *Calocybe indica* on food intake (cumulative study)

Type	Food intake in g/100 g body weight of the mice at different hours						
	00-02	00-03	00-04	00-06	00-08	00-12	00-24
Control	2.879± 0.795	5.119± 1.308	7.280± 1.726	10.019± 2.042	14.164± 3.051	18.800± 3.586	23.591± 3.911
HWEC	2.635± 0.364	4.133± .536	6.123± 0.716	9.045± 0.770	12.434± 0.937	16.705± 1.141	20.807± 1.230
t/p	0.328/ 0.753	0.849/ 0.424	0.750/ 0.477	0.557/ 0.595	0.714/ 0.498	0.727/ 0.491	0.889/ 0.403

HWEC= Hot water extract of *Calocybe indica* treated.

Table 4.1. Effect of *Calocybe indica* on defecation (hourwise)

Type	Rate of defecation at different hours (g/100 g body weight of the mice)							
	1^{st}	2^{nd}	3^{rd}	4^{th}	$5\text{-}6^{th}$	$7\text{-}8^{th}$	$9\text{-}12^{th}$	$13\text{-}24^{th}$
Control	0.290± 0.038	0.329± 0.124	0.662± 0.165	0.596± 0.094	1.038± 0.262	1.412± 0.108	2.461± 0.075	4.685± 1.514
HWEC	0.244± 0.047	0.267± 0.079	1.093± 0.719	0.622± 0.115	1.181± 0.238	1.390± 0.070	2.898± 0.190	3.697± 0.364
t/p	0.639/ 0.543	0.436/ 0.676	-0.406/ 0.697	-0.144/ 0.890	-0.370/ 0.722	0.177/ 0.864	-2.143/ 0.074	0.634/ 0.585

HWEC= Hot water extract of *Calocybe indica* treated.

Table 4.2. Effect of *Calocybe indica* on defecation (cumulative study)

Type	Defecation in g/100 g body weight of the mice at different hours						
	00-02	00-03	00-04	00-06	00-08	00-12	00-24
Control	0.619± 0.138	1.282± 0.288	1.878± 0.359	2.915± 0.581	4.327± 0.631	6.788± 0.602	11.473± 2.102
HWEC	0.511± 0.122	1.604± 0.732	2.226± 0.812	3.407± 0.841	4.797± 0.900	7.695± 0.880	11.392± 0.983
t/p	0.541/ 0.605	-0.296/ 0.776	-0.287/ 0.782	-0.382/ 0.714	-0.340/ 0.744	-0.674/ 0.522	0.040/ 0.969

HWEC= Hot water extract of *Calocybe indica* treated.

Table 5.1. Effect of *Calocybe indica* on water content in stool (hourwise)

Type	Water content in stool at different hours (g/100 g body weight of the mice)							
	1^{st}	2^{nd}	3^{rd}	4^{th}	$5\text{-}6^{th}$	$7\text{-}8^{th}$	$9\text{-}12^{th}$	$13\text{-}24^{th}$
Control	0.127± 0.020	0.144± 0.070	0.335± 0.083	0.289± 0.072	0.422± 0.226	0.661± 0.071	1.296± 0.092	2.278± 0.836
HWEC	0.123± 0.027	0.126± 0.044	0.844± 0.717	0.350± 0.081	0.618± 0.125	0.637± 0.073	1.563± 0.181	1.852± 0.170
t/p	0.101/ 0.923	0.217/ 0.834	-0.485/ 0.643	-0.471/ 0.652	-0.833/ 0.433	0.208/ 0.841	-1.312/ 0.232	0.499/ 0.664

HWEC= Hot water extract of *Calocybe indica* treated.

Table 5.2. Effect of *Calocybe indica* on water content in stool (cumulative study)

Type	Water content in stool of the mice at different hours						
	00-02	00-03	00-04	00-06	00-08	00-12	00-24
Control	0.272± 0.078	0.606± 0.150	0.896± .211	1.318± 0.438	1.979± 0.497	3.275± 0.550	5.553± 1.364
HWEC	0.250± 0.069	1.094± .711	1.443± 0.780	2.061± 0.781	2.698± 0.827	4.261± 0.810	6.113± 0.821
t/p	0.190/ 0.854	-0.466/ 0.655	-0.476/ 0.648	-0.631/ 0.548	-0.574/ 0.584	-0.796/ 0.452	-0.374/ 0.719

HWEC= Hot water extract of *Calocybe indica* treated.

Table 6.1. Effect of *Calocybe indica* on weight of dry stool (hourwise)

Type	Weight of dry stool at different hours (g/100 g body weight of the mice)							
	1st	2nd	3rd	4th	5-6th	7-8th	9-12th	13-24th
Control	0.163± 0.018	0.185± 0.056	0.328± 0.088	0.307± 0.036	0.616± 0.157	0.751± 0.075	1.165± 0.063	2.407± 0.704
HWEC	0.121± 0.024	0.140± 0.035	0.175± 0.063	0.272± 0.045	0.563± 0.113	0.753± 0.050	1.336± 0.042	1.845± 0.226
t/p	1.147/ 0.289	0.708/ 0.502	1.399/ 0.205	0.496/ 0.635	0.268/ 0.797	-0.024/ 0.981	-2.317/ 0.054	0.992/ 0.354

HWEC= Hot water extract of *Calocybe indica* treated.

Table 6.2. Weight of Dry Stool (cumulative study)

Type	Weight of dry stool in g/100 g body weight of the mice at different hours						
	00-02	00-03	00-04	00-06	00-08	00-12	00-24
Control	0.348± 0.064	0.675± 0.144	0.982± 0.157	1.598± 0.168	2.349± 0.193	3.513± 0.170	5.920± 0.843
HWEC	0.261± 0.055	0.436± 0.102	0.709± 0.129	1.272± 0.214	2.025± 0.218	3.361± 0.235	5.206± 0.423
t/p	0.961/ 0.368	0.756/ 0.217	1.275/ 0.243	0.979/ 0.360	0.944/ 0.324	0.422/ 0.686	0.861/ .418

HWEC= Hot water extract of *Calocybe indica* treated.

The hot water extract of *Calocybe indica* showed a mild decreasing effect on water intake, food intake and urination, with an insignificant increase in defecation and water content in stool during the period of 3rd to 12th hours after the administration. So, the mushroom might had no significant change in normal metabolic processes. So, *Calocybe indica* may be an ideal vegetable since it showed no constipating or laxative effect.

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An Acute Metabolic Study and Neuropharmacologic Findings of *Pleurotus ostreatus* on Rat

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Abstract

A neuropharmacologic and acute metabolic study was carried out with the water extract of *Pleurotus ostreatus* (Jacquin ex Fr. Kummer) (oyster mushroom) on laboratory mice. Open field, hole board, hole cross and hypoxia tests were carried out. Treatment with water extract of *Pleurotus ostreatus* (Jacquin ex Fr. Kummer) showed somewhat depressing feature but this depressing effect was not statistically significant. In acute metabolic study, there was no significant change in the parameters observed in the tested animals as compared with the control group.

Key words: *Pleurotus ostreatus,* water extract, neuropharmacologic, metabolic study.

INTRODUCTION

The use of mushrooms as medicine or nutrient-rich diet is increasing day by day. So the global cultivation of mushroom is greater than ever in recent years (Chang, 1999). Oyster mushroom (*Pleurotus ostreatus*) is the widely used edible mushroom all over the world. In Bangladesh, at present *Pleurotus spp.* are widely cultivated for the suitability of climate (Amin *et al.,* 2007), cultivation facilities and its nutritional and medicinal value (Hossain *et al*., 2003). It contains high quality protein (15-25%) which is nearly equal to animal derived protein. In addition, it is enriched with carbohydrates, fiber, vitamins e.g. thiamin, pyridoxine, riboflavin, niacin, pantothenic acid, folates, minerals especially iron, phosphorus, magnesium, zinc and manganese and an antioxidant. It contains a low amount of fat (2.6%) and most of them are unsaturated fatty acid (Dundar *et al*., 2008). Although many scientific researches are conducted on the nutritional composition and medicinal values of different mushrooms in different climatic conditions, it is also necessary to investigate the medicinal value and safety of *P. ostreatus* cultivated in Bangladesh. *Pleurotus ostreatus* has many beneficial effects e.g. anticancer (Jedinak and Sliva, 2008), hypocholesterolemic, anticataractogenic (Isai *et al*., 2009), etc. on experimental animals as well as on human being (Choudhury *et al.,* 2009). Edible mushrooms are not only good for the stomach alone but also nourishing a person. Though some cultivated mushrooms have some undesirable effects (Nieminen *et al.,* 2009). However, little research has been done into the long-term effects of mushrooms. On this point of view an acute metabolic study and a neuro-pharmacologic study was carried out on this mushroom to investigate its possible side-effect on laboratory mice.

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MATERIALS AND METHODS

Experimental animal: Female mice (Swiss-Webster strain, 20-25gm body weight) bred of the animal house of the Department of Pharmacy, Jahangirnagar University, were used for the neuropharmacologic study. The mice were housed in plastic cages (dimension 30×20×13cm, soft wood chips bedding) under controlled conditions of 12 hours dark-light cycles. They all received a basal diet of food preparation formulated by the Bangladesh Council of Scientific and Industrial Research (BCSIR) and tap water *ad libitum*. The mice were divided into two groups- the Control and *P. ostreatus*- treated group.

Collection of *P. ostreatus*: *Pleurotus ostreatus* mushrooms were collected from the National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh. The mushrooms were powdered using a grinder and preserved in air tight polythene packet. This powder was used in the experiment.

Preparation of the hot water extract of *P. ostreatus*: *P. ostreatus* powder (10gm) was mixed in 200 ml distilled water. The mixture was heated to reduce the volume. The condensed mixture had been filtered off through a cotton cloth to get approximately 40 ml filtrated solution. Then, 200 ml water was added with the residue and the total procedure was repeated to get 80 ml (40ml+40ml) filtrate, which was then heated and ultimately condensed to 40 ml. This liquid was the water extract of *P. ostreatus*. The extract was administered orally at the dose of 20 ml/kg body weight by using gastric gavage needles.

Hole cross test: In this experiment, the method of Takagi *et al.* (1971) was employed. In a box (dimension 30 X 20 X 14 cm), a hole of 3 cm in diameter at a height of 4.5 cm from the floor was constructed on the dividing wall. Spontaneous movement of the animals through the hole from one chamber to the other was counted for a period of 2 minutes. The observation was conducted at 30, 60, 120 and 240 minutes after oral administration of test drugs and was compared with control animal administered with the normal saline water.

Open field test: The method of Gupta *et al.* (1971) was employed in this experiment. The floor of an open field of half square meter was divided into a series of squares, each alternatively colored black and white. The apparatus had a wall of 40 cm. The number of squares, travelled by the animal, was recorded for a period of two minutes.

Hole board test: The Hole board test has been conceived to study the behavior of the mouse confronted with a new environment (head plunging stereotype) according to the methods described by Boissier and Simon (1964), Boissier *et al.* (1964) and Boissier *et al.* (1967). The test enables the initial exploratory activity of the animal and its variations brought about by psychotropic elements of a drug to be unmistakably assessed. The hole board test reveals the effect of the drug on the exploratory behavior of the animals. Exploration can be defined as a broad category of behavior, the consequences of which are to provide the organism with information about the exteroceptive environment.

The principle of the test is that a novel situation of open field evokes in the animals a pattern of behavior characterized by exploration (head dipping through the holes), locomotion (ambulation past the holes) and emotional defecation. It has been considered that the exploration evoked under an unfamiliar environment is modified with physiological factors such as curiosity, fear and anxiety and the modulation of these factors after the administration of a drug (Nakama *et al.*, 1972).

In this procedure, a total of 16 holes (diameter 3 cm each) were presented to the mouse in a flat space of 25 sq cm. Each of the animal was transferred carefully to one corner of the field and the number of ambulation (expressed as the number of holes passed), head dipping and number of fecal boluses excretion was recorded for a period of 2 minutes at pre 30 minutes and post 30, 60, 120 and 240 minutes intervals and the oyster mushroom-treated animals were compared with the controls administered distilled water.

Hypoxia time: The method of Caillard *et al.* (1975) was employed to measure the hypoxia time. Three sets of animals (ten mice per group) had been used. The hypoxia time was recorded individually for all the animals, 2 hr after the treatment of the oyster mushroom extract. The animals had been placed in an empty glass jar of 300 mL capacity attached with an electronic watch. The jars were made air tight with greased glass stoppers and the time until the onset of convulsion was recorded.

Acute Metabolic Study: Utilizing a 'Nalgene Metabolic Case' the effect of the hot water extract of *Pleurotus ostreatus* (HWEP) on acute metabolism was performed. After a period of one day (*i.e.* 24 hours) of adjustment, they were administrated with graded dosage of the extract. The rate of food and water intake as well as defecation and urination were measured for as period of 3 days (72 hours) maintaining a 3 days of rest before each days (24 hours) of test (Khan and Choudhuri, 1998). Eighteen mice were randomly selected and equally divided into 3 groups. These mice were placed in 3 different cases. The mice of one case were treated as control (administering distillated water) and the mice of the remaining 2 cases were treated with the same extract maintaining the same dose as duplicate. Within the next 24 hours, food and water intake as well as urination and defecation were measured with an interval of one hour for the first 4 hours, interval of two hours for the next 4 hours; next measurement was after another 4 hours and the final measurement after another 12 hours (*i.e.* a total of 24 hours). The test was repeated in the next two days with alternating control group (according to the Latin Square Design). Then the percent deviation of the drug treated group was compared with the corresponding control group.

Statistical analysis: The results are expressed as mean ± SEM (Standard error of mean). Means were compared by independent sample t-test. The statistical program "SPSS 12.0 for Windows" was used to test the level of significance. Probability (p) value of 0.05 or less ($p<0.05$) was considered as significant.

RESULTS AND DISCUSSION

Hole cross test: The hole cross test was designed to evaluate effects on the exploratory behavior. In order to investigate the effects of the drug on the exploratory behavior of the treated animals, this test was performed. Results of this test presented in Fig. 1, indicate that administration of test extract reduced the exploratory activity of the treated animals at the first time. But, at last period of observation (2-4 hr), the test animals showed more interest in crossing the hole, in comparison to that of the control animals.

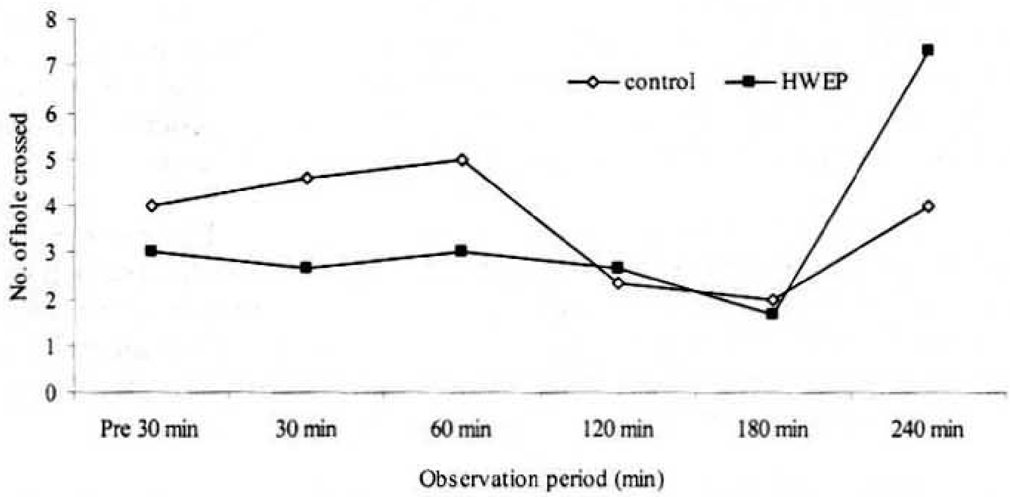

Fig. 1. Graphical Presentation on Hole cross test after administration of water extract of *Pleurotus ostreatus* (20 ml/ kg body wt). HWEP= Hot water extract of *Pleurotus ostreatus* treated.

Open field test: The overall results of the open field test are summarized in Fig. 2, Fig. 3 and Fig. 4. In the open field test, administration of water extract of *Pleurotus ostreatus*, decreased the number of squares crossed. However this decrease is not statistically significant. Also, mice treated with the water extract did not show any statistically significant alteration in their standing up tendency and fecal dropping behaviors.

Hole board test: The Hole Board Test is somewhat related to the open field situation, but here animals are provided with a stronger stimulus for exploratory behavior, represented by the holes, which the animals explore by inserting their head into them. A pattern of behavior characterized by exploration, (head dipping through the holes), locomotion (ambulation past the holes) and emotional defaecation are evoked in the hole-board test. The hole-board test is a measure of exploratory behaviour (File and Wardill, 1975). An agent that decreases this parameter is considered a sedative (File and Pellow, 1985). Anxiolytics have been shown to increase the number of head dips in the holeboard test (Takeda *et al.*, 1998). The results of the hole board test presented in Fig. 5, Fig. 6 and Fig. 7, showed that administration of extract caused a decrease in the exploratory behaviour and also head dipping in mice but this decrease is not statistically significant. No significant effect was observed on defecation.

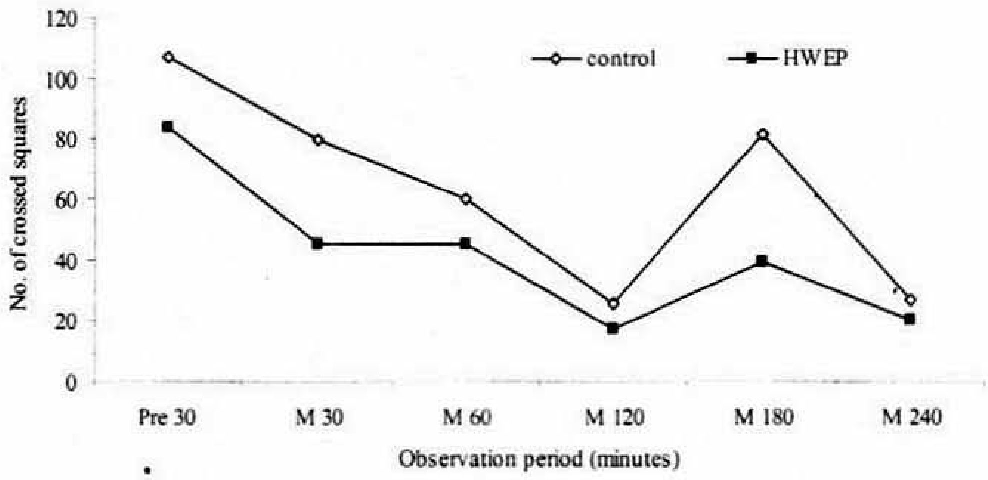

Fig. 2. Graphical presentation on open field test (movement) after administration of water extract of *Pleurotus ostreatus* (20 ml/ kg body wt). HWEP= Hot water extract of *Pleurotus ostreatus* treated.

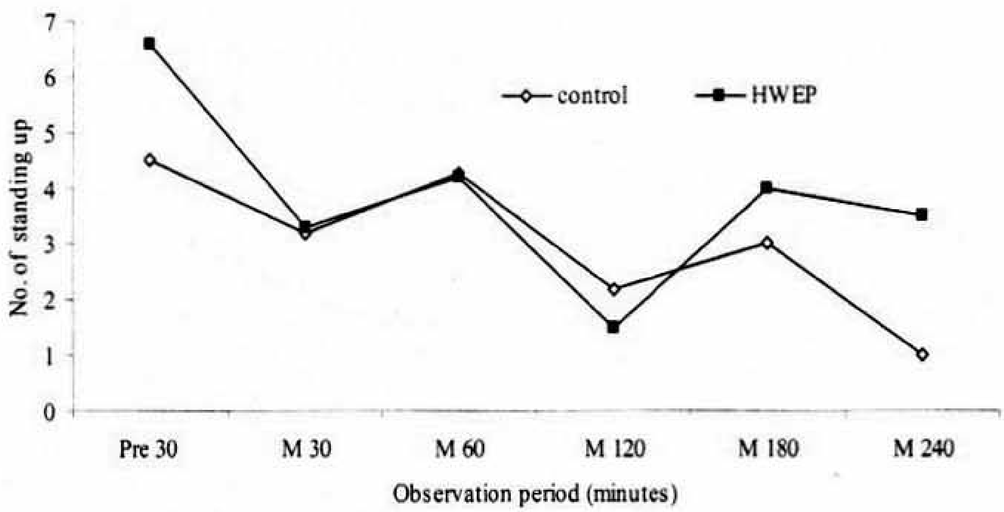

Fig. 3. Graphical presentation on open field test (standing up tendency) after administration of water extracts of *Pleurotus ostreatus* (20 ml/ kg body wt). HWEP= Hot water extract of *Pleurotus ostreatus* treated.

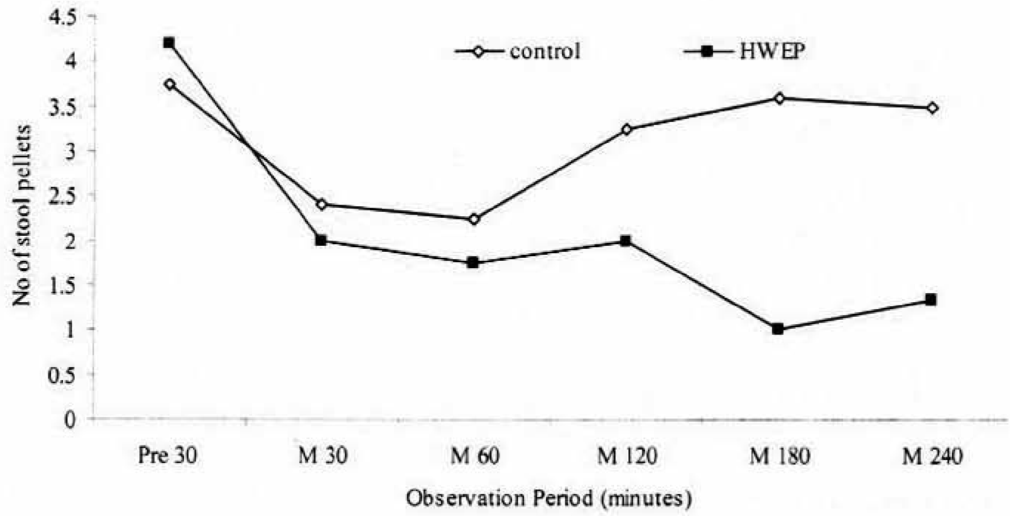

Fig. 4. Graphical presentation on open field test (emotional defaecation) after administration of water extracts of *Pleurotus ostreatus* (20 ml/ kg body wt). HWEP= Hot water extract of *Pleurotus ostreatus* treated.

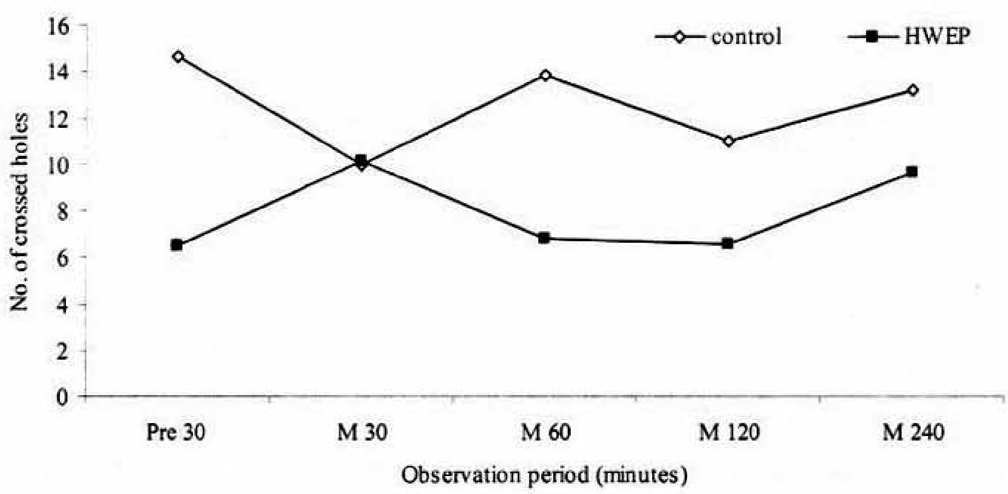

Fig. 5. Graphical presentation on hole board test (movement) after administration of water extract of *Pleurotus ostreatus* (20 ml/ kg body wt). HWEP= Hot water extract of *Pleurotus ostreatus* treated.

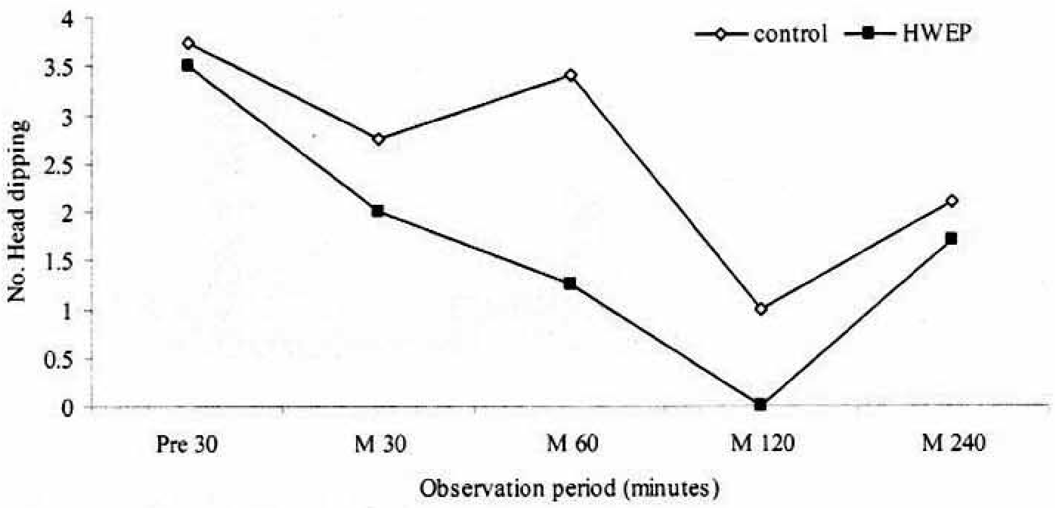

Fig. 6. Graphical presentation on hole board test (Head dipping) after administration of water extract of *Pleurotus ostreatus* (20 ml/ kg body wt). HWEP= Hot water extract of *Pleurotus ostreatus* treated.

Hypoxia time test: There was insignificant decrease in the survival time of *Pleurotus ostreatus* extract fed mice than the control group. It may suggest that the drug has no adverse impact on the survival time of hypoxia test.

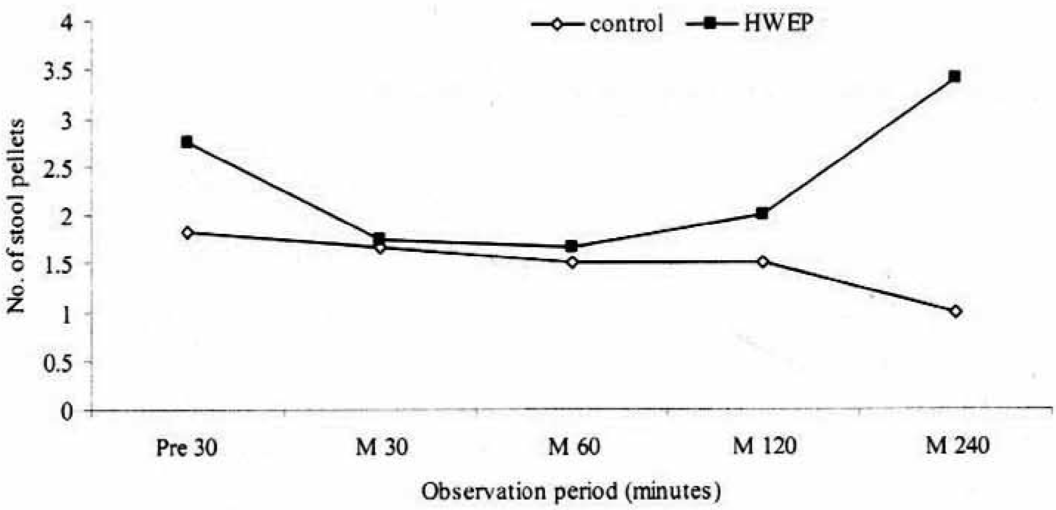

Fig. 7. Graphical presentation on hole board test (emotional defecation) after administration of water extract of *Pleurotus ostreatus* (20 ml/ kg body wt). HWEP= Hot water extract of *Pleurotus ostreatus* treated.

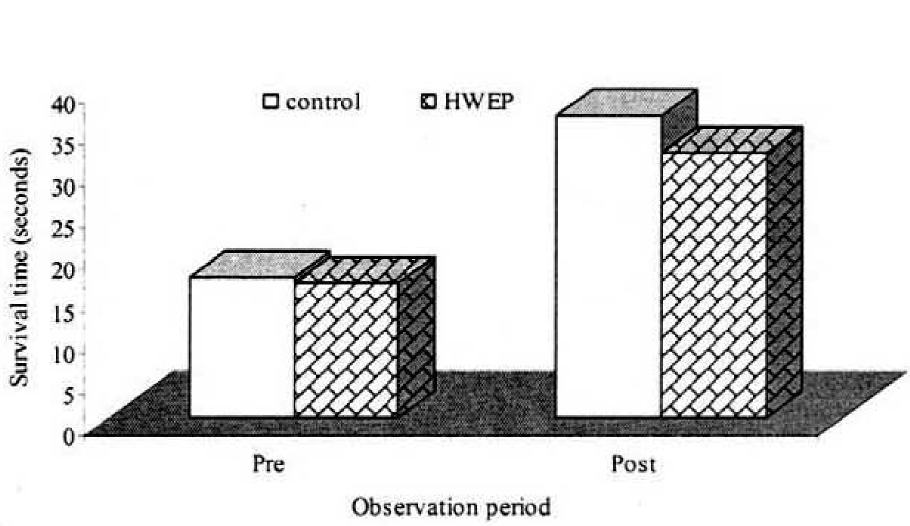

Fig. 8. Graphical presentation on hypoxia test after administration of water extract of *Pleurotus ostreatus* (20 ml/ kg body wt). HWEP= Hot water extract of *Pleurotus ostreatus* treated.

Acute metabolic study: The main purpose of testing the effect of the water extract of *Pleurotus ostreatus* on acute metabolic rate was to investigate whether *Pleurotus ostreatus* had any effect, when administered acutely, on the normal rate of metabolism. The experimental data (Table 1 to Table 4) indicates that the administration of water extract exhibited a decreased, though not statistically significant, rate of food and water intake. The data also revealed that the administration of *P. ostreatus* results in an insignificant lowering of the defecation and urination.

Table 1. Food intake (g/100 g body weight of the mice) in hour

Type	Hours							
	1st	2nd	3rd	4th	5-6th	7-8th	9-12th	13-24th
Control	1.72± 0.53	0.98± 0.39	1.23± 0.50	0.43± 0.19	1.70± 0.65	2.06± 0.76	3.92± 0.75	6.29± 1.33
HWEP	1.45± 0.46	1.36± 0.22	1.00± 0.22	1.35± 0.43	1.98± 0.24	3.96± 0.66	4.29± 0.12	5.83± 0.41
t/p	0.350/ 0.735	-0.923/ 0.387	0.490/ 0.639	-1.969/ 0.092	-0.505/ 0.629	-1.742/ 0.125	0.702/ 0.506	0.441/ 0.673

HWEP= Hot water extract of *Pleurotus ostreatus* treated.

Table 2. Water intake (g/100 g body weight of the mice) in hour

Type	Hours							
	1st	2nd	3rd	4th	5-6th	7-8th	9-12th	13-24th
Control	0.92± 0.23	1.04± 0.56	1.40± 0.41	0.53± 0.21	1.79± 0.76	2.23± 0.74	4.70± 0.74	7.95± 1.14
HWEP	0.87 ± 0.18	1.22± 0.24	0.97± 0.23	1.28± 0.29	2.20± 0.26	3.10± 0.70	3.56± 0.44	7.10± 0.55
t/p	0.147/ 0.887	-0.363/ 0.727	0.999/ 0.351	-1.746/ 0.088	-0.663/ 0.529	-0.769/ 0.467	1.411/ 0.201	0.771/ 0.466

HWEP= Hot water extract of *Pleurotus ostreatus* treated.

Table 3. Urination (g/ 100 g body weight of the mice) in hour

Type	Hours							
	1st	2nd	3rd	4th	5-6th	7-8th	9-12th	13-24th
Control	1.22± 0.39	0.31± 0.30	0.41± 0.12	0.17± 0.01	0.82± 0.23	0.32± 0.14	1.03± 0.27	1.57± 1.39
HWEP urine	0.853± 0.371	0.41± 0.10	0.29± 0.06	0.49± 0.01	0.49± 0.16	0.42± 0.04	1.19± 0.33	1.76± 0.51
t/p	0.637/ 0.548	-0.424/ 0.689	1.064/ 0.323	-1.772/ 0.093	1.216/ 0.270	-0.900/ 0.419	-0.319/ 0.761	-0.163/ 0.877

HWEP= Hot water extract of *Pleurotus ostreatus* treated.

Table 4. Defecation (g/100 g body weight of the mice) in hour

Type	Hours							
	1st	2nd	3rd	4th	5-6th	7-8th	9-12th	13-24th
Control	0.267± 0.101	0.136± 0.036	0.243± 0.101	0.239± 0.042	0.661± 0.283	0.63± 0.17	1.60± 0.35	8.70± 3.80
HWEP defecation	0.231± 0.103	0.283± 0.077	0.254± 0.057	0.422± 0.139	0.674± 0.130	1.85± 0.63	2.68± 0.50	5.60± 1.19
t/p	-0.296/ 0.777	-1.278/ 0.242	-0.105/ 0.919	-0.978/ 0.366	-0.048/ 0.963	-1.309/ 0.232	-1.415/ 0.200	1.023/ 0.340

HWEP= Hot water extract of *Pleurotus ostreatus* treated.

In conclusion, the result of the investigation of the aqueous extract of *Pleurotus ostreatus* shows that it is relatively safe and well tolerated in mice when given orally.

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Effect of Cow Dung Supplements with Rice Straw on the Yield and Proximate Composition of *Pleurotus ostreatus*

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Abstract

The effect of different levels of cow dung (0, 5, 10, 15 and 20%) on yield and proximate composition of *Pleurotus ostreatus* were studied. The highest number of primordia (70.63) and fruiting body (51.92) per packet were observed in rice straw supplemented with 5% level of cow dung. The highest weight of individual fruiting body (4.71g), biological yield (234.24g), economic yield (227.72g), dry yield (22.83g) per 500 g packet, biological efficiency (140.26%) and benefit cost ratio (5.69) were observed in 10% cow dung. The highest protein content (30.90%), crude fiber (24.03%) and the lowest lipid (3.34%) was found in 10% cow dung.

Key words: *Pleurotus ostreatus,* cow dung, rice straw, yield and proximate composition.

INTRODUCTION

Mushroom is a highly nutritious, delicious, medicinal and economically potential vegetable. As a vegetable, mushroom can play an important role to meet up the nutritional requirements of the country. Mushroom reduces the diabetic on regular feeding (Anderson and Ward, 1979). It also reduces the serum cholesterol in human bodies which reduces hypertension (Suzuki and Oshima, 1979). Mushroom inhibits the growth of tumor and cancer (Mori, 1986). Edible mushrooms have been treated as important tool in modern medicine for their medicinal values (Kovfeen, 2004). Oyster mushroom (*Pleurotus ostreatus*) contains 19-35% protein on dry weight basis as compared to 7.3% in rice 13.2% in wheat and 25.2% in milk (Chang & Miles, 1988). It contains 4.0% fat having good quantity of unsaturated fatty acids which are essential in our diet (Holman, 1976). It is rich in essential minerals and trace elements (Chandha and Sharma 1995). Oyster mushroom is widely cultivated in Bangladesh because of the suitable weather and climatic condition.

Substrate plays an important role in the yield and nutrient content of oyster mushroom. The substrates on which mushroom spawn is grown, affects the mushroom production (Klingman, 1950). Oyster mushroom can grow on sawdust, rice and wheat straw and other agro-waste. Sarker *et al.* (2007) observed a remarkable variation in nutrient content of oyster mushroom in different substrates. The National Mushroom Development and Extension Centre (NAMDEC), Savar grows oyster mushroom using sawdust. But, sawdust in our country has been becoming scarce due to its use in huge amount in developing poultry industries and its price is also increasing day by day. Therefore, it is

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necessary to identify the alternative suitable substrate for mushroom production that will be easily available, low cost and more productive. Considering the facts the present experiment was undertaken to find out the effect of different levels of cow dung supplements with rice straw on the yield and proximate composition of oyster mushroom (*Pleurotus ostreatus*).

MATERIALS AND METHODS

The experiment was carried out at the, Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka. Five different levels, T_1= 0% (Control), T_2= 5%, T_3= 10%, T_4=15%, T_5= 20% of cow dung were evaluated as the supplement to rice straw substrate of oyster mushroom. The experiment was laid out in Completely Randomized Design with three replications.

Preparation of Spawn packets: Cow dung was mixed to rice straw according to the treatments. The spawn packets preparation, sterilization, inoculation and incubation were done using the method described by Sarker *et al.* (2007). The weight of each spawn packet was 500 g.

Cultivation of spawn packet: Two ends, opposite to each other of the upper position of polypropylene (PP) bag were cut in "D" shape with a blade and opened by removing the PP sheet. The opened surface of the substrate was scraped slightly with a tea spoon for removing the thin whitish mycelial layer. Then the spawn packets were soaked in water for 15 minutes and inverted to remove excess water for another 15 minutes. The packets of each type were placed separately on the floor of culture room and covered with newspaper. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3 times a day. The light of culture house was maintained around 300-500 lux and ventilation was kept properly. The temperature of culture house was maintained within 22°C to 25°C. The first primordia appeared 2-4 days after scribing depending upon the levels of supplement. The harvesting time also varied depending upon the levels of supplement. Data were collected on mycelial growth, time from stimulation to primordial initiation, time from primordial initiation to harvest, average number of primordia/packet, average number of fruiting body/packet, average weight of individual fruiting body, biological yield, economic yield, dry yield, biological efficiency and benefit cost ratio. Dry yield and Biological efficiency were determined by the following formulas.

$$\text{Dry yield (g/500g packet)} = \text{Economic yield} \times \frac{\text{Oven dry weight of sample (g)}}{\text{Fresh weight of sample (g)}}$$

$$\text{Biological efficiency} = \frac{\text{Total biological weight (g)}}{\text{Total dry weight of substrate (g)}} \times 100$$

Proximate analysis of the mushrooms

Moisture and dry matter: Moisture and dry mater were determined by the following formulas.

Moisture (%) = (Initial weight - final weight) ×100/ Weight of sample
Dry matter (%) = 100 - % Moisture content

Determination of crude fiber: Crude fiber (g/l00g sample) = [100-(moisture + fat)] x (We-Wa)/ Weight. of sample (Raghuramulu *et al.*, 2003).

Total lipid: Total lipid was estimated by using the method described by Raghuramulu *et al.* (2003)

$$\textbf{Lipid} = \frac{\text{Weight of ether extract} \times \text{Percentage of dried sample}}{\text{Weight of the dried sample taken}}$$

Total carbohydrate estimation: The content of the available carbohydrate was determined by the following equation: Carbohydrate (g/l00g sample) = 100 - [(Moisture + Fat + Protein + Ash + Crude Fiber) g/100g] (Raghuramulu *et al.*, 2003)

Determination of ash: Ash (%) content = Weight of ash × 100 / Weight of sample taken (Raghuramulu *et al.*, 2003)

Determination of total nitrogen: Total nitrogen was determined by using the standard Micro kjeldhal procedure of AOAC (1975) and total crude protein was estimated by multiplying the nitrogen content by a factor of 6.25.

Determination of Ca, Mg, K, Fe, Zn and P: The content of Ca, Mg, K, Fe, and P was estimated by Perchloric acid digestion method as proposed by Yamakawa (1992).

Statistical analysis of data: The recorded data were analyzed statistically with the help of computer MSTAT-c programme and means following least significant difference (LSD) test at 1% and 5% level of probability for interpretation of results as and when required (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Mycelium growth and yield attributes: The highest mycelium running rate was observed in T_3 (0.70 cm/day) and the lowest running rate of mycelium was observed in T_1 (0.52 cm/day). The other treatments varied significantly over control (Table 1). The highest time from stimulation to primordia initiation was observed in T_1 (7.23 days) and the lowest time from stimulation to primordia initiation was in the treatment T_4 & T_5 (6.03 days). The time from primordia initiation to harvest was lowest in the treatment T_3 (3.63 days) and it was the highest in the treatment T_1 (5.06 days) followed by T_5 (5.16 days). The highest average number of primordia/packet was observed in the treatment T_2

(70.33) and the lowest average number of primordia/packet was in the treatment T_1 (57.35). The highest average number of fruiting body/packet was observed in the treatment T_2 (51.92) and the lowest average number of fruiting body /packet was in the treatment T_1 (39.67). The average weight of individual fruiting body in different treatment ranged from 3.73 g to 4.71 g. The highest average weight of individual fruiting body was observed in the treatment T_3 (4.71 g) and the lowest average weight of individual fruiting body was in the treatment T_1 (3.73 g). The other treatments varied significantly over control in terms of average weight of individual fruiting body.

Table1. Effect of different levels of cow dung with rice straw on mycelium growth of *Pleurotus ostreatus*

Treatments	Mycelium running rate in spawn packets(cm)	Time from stimulation to primordial initiation (days)	Time from primordial initiation to harvest (days)	Average number of primordia /packet	Average number of fruiting body /packet	Average weight of individual fruiting body (g)
T_1	0.52 d	7.23 a	5.06 a	57.35 d	39.67 d	3.73 b
T_2	0.64 b	6.50 b	4.40 b	70.63 a	51.92 a	3.80 b
T_3	0.70 a	6.13 bc	3.63 c	63.33 b	48.33 b	4.71 a
T_4	0.65 b	6.03 c	4.30 b	66.67 b	45.67 c	4.61 a
T_5	0.61 c	6.03 c	4.36 b	61.00 c	49.50 b	3.78 b
CV (%)	2.92	3.08	5.48	3.73	4.50	4.90
Level of Significance	**	**	**	**	**	**
LSD(0.05)	0.01883	0.3718	0.4495	2.630	2.170	0.5156

T_1=0% (Controlled), T_2=5%, T_3=10%, T_4=15%, T_5=20%; Means followed by same letter are not significantly different at 1% or 5% level of significance. ** Significant at 1% level.

Yields, biological efficiency and benefit cost ratio: The supplementation of rice straw with cow dung had great effect on the yield. The highest biological yield was recorded under treatment T_3 (234.24 g) and the lowest biological yield was recorded under T_1 (157.36 g). Baysal *et al.* (2003) found the highest yield of oyster mushroom (*Pleurotus ostreatus*) with the substrate composed of 20 % rice husk in weight. The highest economic yield was recorded under treatment T_3 (227.72 g) and the lowest economic yield was recorded under T_1 (148.21 g). The dry yield of mushroom was maximum under the treatment T_3 (22.83 g) and the lowest dry yield was recorded under T_1 (14.19 g). The highest biological efficiency (140.26 %) was calculated in the treatment T_3 and the lowest biological efficiency (100.54 %) was calculated from T_1 (Table 2). The highest benefit cost ratio was calculated in treatment T_3 (5.69) and the lowest benefit cost ratio (3.70) was calculated from T_1. Sarker *et al.* (2007) mentioned the performances of substrates were significantly differed based on benefit cost ratio.

Proximate composition of mushroom: The highest moisture percent was observed in treatment T_5 (90.64) followed by T_5 (90.51) and the lowest moisture percent was observed in treatment T_1 (90.15). The highest dry matter percentage was observed in treatment T_1 (9.85) and the lowest dry matter percentage was observed in treatment T_5 (9.36). The other treatments were statistically similar (Table 3). The highest content of protein was found in the treatment T_3 (30.90%) which was followed by T_4 (27.53%) and the lowest protein was found in T_1 (18.43%). The lowest lipid percentage was observed under treatment T_3 (3.34) followed by T_2 (3.70) and the highest lipid percentage was observed under T_1 (5.13). The highest percentage of ash was observed in the treatment T_3 (8.23) and the lowest percentage of ash was in the treatment T_1 (6.33). The lowest percentage of carbohydrate was observed under treatment T_3 (33.50) and the highest carbohydrate percentage was observed under T_1 (49.58). The highest percentage of crude fiber was observed under treatment T_3 (24.03) followed by T_2 (23.27) and the lowest crude fiber percentage was observed under T_1 (20.53). The results of the present study keep in with the findings of previous studies (Chang *et al.*, 1981; Moni, *et al.*, 2004; Alam *et al.*, 2007). Chang *et al.* (1981) reported that the fruit bodies of mushrooms contained 26.6-34.1% crude protein, 1.1-8.0% fat, 4.30-50.7% carbohydrate. Moni, *et al.* (2004) found 88.15 to 91.64% moisture, 18.46 to 27.78% crude protein; 1.49 to 1.90% crude fats, 40.54 to 47.68% carbohydrates in oyster mushroom. Alam *et al.* (2007) reported 87 to 87.5% moisture; 4.30 to 4.41% lipids; 22.87g/100g to 23.29g/100g fiber; 39.82 to 42.83% carbohydrates and 8.28 to 9.02% ash in *Pleurotus spp.*

Table 2. Effect of different levels of cow dung with rice straw on the yield, biological efficiency and cost benefit ratio of *Pleurotus ostreatus*

Treatments	Biological yield (gm)	Economic yield (gm)	Dry yield (gm)	Biological efficiency (%)	Benefit cost ratio
T_1	157.36 e	148.21 e	14.19 e	100.54 d	3.70 d
T_2	204.52 c	196.43 c	19.80 c	126.64 b	4.91 c
T_3	234.24 a	227.72 a	22.83 a	140.26 a	5.69 a
T_4	218.35 b	210.55 b	20.80 b	126.94 b	5.26 b
T_5	196.63 d	187.02 d	18.03 d	111.41 c	4.67 c
CV (%)	0.79	0.65	1.29	0.79	0.78
Level of Significance	**	**	**	**	**
LSD(0.05)	2.893	2.328	0.4495	1.090	0.2793

T_1=0% (Controlled), T_2=5%,T_3=10%,T_4=15%,T_5=20%; Means followed by same letter are not significantly different at 1% or 5% level of significance. ** Significant at 1% level.

The highest percentage of nitrogen was observed under treatment T_3 (4.944) followed by T_4 (4.404) and the lowest nitrogen percentage was observed under T_1 (2.948). The highest percentage of phosphorus was observed under treatment T_1 (0.926) and the lowest phosphorus percentage was observed under T_3 (0.82). The highest percentage of potassium was observed under treatment T_3 (1.353) and the lowest potassium percentage was observed under T_1 (1.137). Sarker *et al.* (2007) also found 1.3% potassium, in oyster mushroom grown on sawdust based substrates. The highest amount of calcium was observed under treatment T_3 (23.50 mg/100g) and the lowest amount was observed under T_1 (21.47 mg/100g). The highest amount of magnesium was observed under treatment T_3 (18.70 mg/100g) and the lowest amount was observed under T_1 (13.60 mg/100g). The highest amount of sulfur was observed under treatment T_3 (0.045 mg/100g) and the lowest amount was observed under T_1 (0.014 mg/100g) (Table 4). The highest amount of iron was observed under treatment T_3 (44.20 mg/100g) and the lowest amount was observed under T_1 (40.33 mg/100g). The highest amount of zinc was observed under treatment T_3 (16.53 mg/100g) and the lowest amount was observed under T_1 (13.57 mg/100g).

Table 3. Effect of different levels of cow dung with rice straw on chemical composition of *Pleurotus ostreatus*

Treatments	Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	CHO (%)	Crude fiber (%)
T_1	90.15 c	9.85 a	18.43 d	5.13 a	6.33 d	49.58 a	20.53 e
T_2	90.37 bc	9.63 ab	24.61 c	3.70 d	8.40 a	40.02 b	23.27 b
T_3	90.24 c	9.76 a	30.90 a	3.34 e	8.23 ab	33.50 c	24.03 a
T_4	90.51 ab	9.49 bc	27.53 b	4.23 b	8.07 bc	37.80 b	22.37 c
T_5	90.64 ab	9.36 c	25.65 c	4.01 c	7.95 c	40.56 b	21.83 d
CV (%)	0.15	1.41	2.51	2.65	1.69	4.81	0.42
Level of Significance	*	*	**	**	**	**	**
LSD(0.05)	0.2526	0.2526	1.145	0.2063	0.2455	3.654	0.1786

T_1=0% (Controlled), T_2=5%,T_3=10%,T_4=15%,T_5=20%; Means followed by same letter are not significantly different at 1% or 5% level of significance. * Significant at 5% level; ** Significant at 1% level.

Table 4. Effect of different levels of cow dung with rice straw on elemental contents of *Pleurotus ostreatus*

Treatment	N (%)	P (%)	K (%)	Ca (mg/100g)	Mg (mg/100g)	S (mg/100g)	Fe (mg/100g)	Zn (mg/100g)
T_1	2.948 e	0.926 a	1.137e	21.47 e	13.60 e	0.014 b	40.33 c	13.57 e
T_2	3.937 d	0.883 b	1.353a	22.73 b	15.67 d	0.038 a	43.50 ab	14.87 d
T_3	4.944 a	0.82 d	1.310b	23.50 a	18.70 a	0.045 a	44.20 a	16.53 a
T_4	4.404 b	0.853 c	1.240c	22.30 d	17.50 b	0.034 a	43.50 ab	15.77 b
T_5	4.104 c	0.866bc	1.160d	22.50 c	16.70 c	0.028 ab	42.87 b	15.23 c
CV (%)	1.25	1.03	1.01	0.27	0.67	1.95	0.23	0.51
Level of Significance	**	**	**	**	**	*	*	**
LSD(0.05)	0.0188	0.0266	0.0188	0.1975	0.2147	0.01883	0.9693	0.2663

T_1=0% (Controlled), T_2=5%,T_3=10%,T_4=15%,T_5=20%; Means followed by same letter are not significantly different at 1% or 5% level of significance. * Significant at 5% level; ** Significant at 1% level.

Correlation study: A highly significant correlation between average number of fruiting body and biological yield was observed when rice straw was supplemented with different levels of cow dung (Fig. 1). The relationship showed a quadratic equation as $y = -1.0565x^2 + 100.13x - 2151.5$ ($R^2 = 0.8155^{**}$), Where y = biological yield and x = average number of fruiting body. The majority of total variation in biological yield of the oyster mushroom can be explained by this equation. The R^2 value indicated that 81.55% of biological yield of *Pleurotus ostreatus* was attributed to the average number of fruiting body.

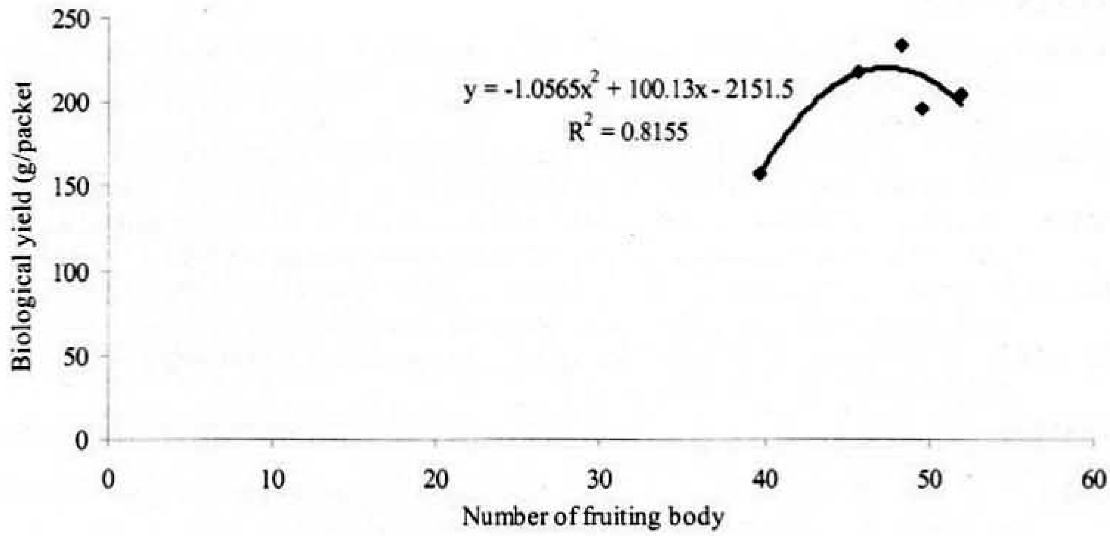

Fig. 1: Relationship between average number of fruiting body and biological yield.

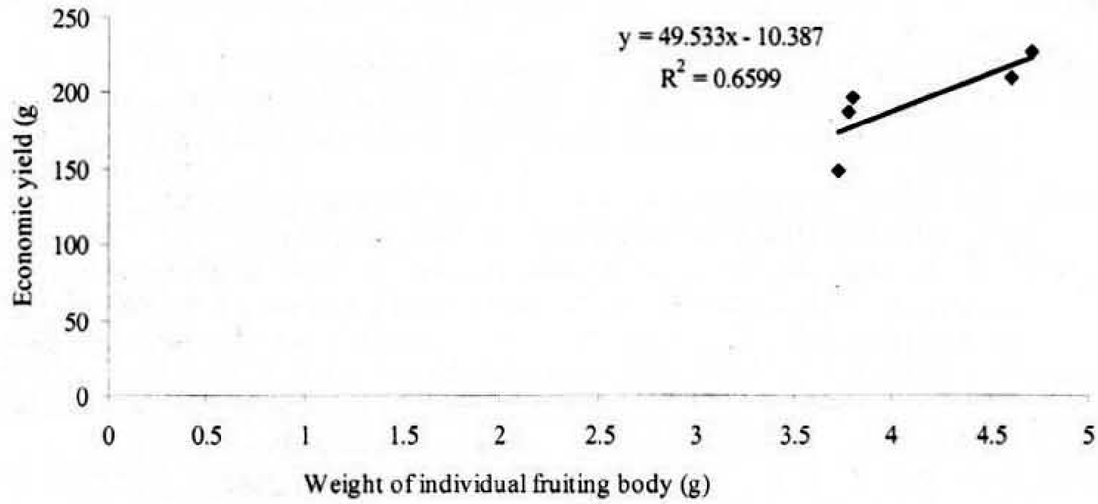

Fig. 2. Relationship between average weight of individual fruiting body and economic yield.

Among the yield contributing characters maximum biological yield (234.24g), economic yield (227.72g), dry yield (22.83g) and highest biological efficiency (140.26 %) and benefit cost ratio (5.69%) was recorded in rice straw supplemented with 10% cow dung. The highest percentage of nitrogen and protein was observed in rice straw supplemented with 10% cow dung. Therefore observing all the yield contributing characters, yield, biological efficiency and nutritional composition it can be concluded that rice straw supplemented with 10% cow dung is the best among the applied treatments for locally grown popular oyster mushroom (*Pleurotus ostreatus*) in Bangladesh.

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Comparative Study on the Growth and Yield of *Pleurotus cystidiosus* on Different Substrates

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Abstract

An experiment was carried out to investigate the effect of different composts on the growth and yield performance of oyster mushroom (*Pleurotus cystidiosus*). The composts were composed of sawdust of mango (*Mangifera indica*) tree sawdust, gamar (*Gmelina arborea*) tree sawdust, acccia (*Accacia dicurrins*) tree sawdust, mixed sawdust (mango + gamar + acacia), rice straw, sugarcane bagasse, waste paper, mixed sawdust + rice straw, mixed sawdust + sugarcane bagasse, mixed sawdust + waste paper, rice straw + sugarcane bagasse, rice straw + waste paper, and sugarcane bagasse + waste paper. The materials were supplemented with wheat bran at $1/3^{rd}$ of the total dry matter. The minimum days required from stimulation to primordial initiation (DRSPI) (3.75) was found in sugarcane bagasse, waste paper and rice straw + waste paper and the maximum DRSPI (17.75) was found in acacia sawdust. The minimum days required from stimulation to first harvest (DRSFH) (6.75) was found in mixed sawdust + waste paper and the maximum DRSFH (23.00) was found in acacia sawdust. The number of primordia was highest (76.50) in sugarcane bagasse and the lowest (47.50) in mixed sawdust. The number of fruiting bodies was highest (62.75) in sugarcane bagasse + waste paper and the lowest (29.00) in mixed sawdust + rice straw. The numbers of effective fruiting bodies was highest (55.50) in sugarcane bagasse + waste paper and the lowest (24.75) in mango sawdust. The weight of individual fruiting body was highest (7.67) in mango sawdust and the lowest (4.44) in sugarcane bagasse + waste paper. The highest biological yield (331.00g/500g packet), economic yield (326.00g/500g packet) and biological efficiency (132.40%) were observed in mixed sawdust + waste paper.

Key Words: *Pleurotus cystidiosus*, substrates, growth, yield and biological efficiency.

INTRODUCTION

Oyster mushroom (*Pleurotus cystidiosus*) cultivation is gaining popularity in Bangladesh as a commercial variety for its attractive size, shape and color. In Bangladesh, mostly sawdust is used as basic materials of substrates to grow the mushroom. Alternate basic material to prepare mushroom substrates is yet to identify in the country.

Various agricultural wastes are also used as substrates for the cultivation of oyster mushroom. Some of these wastes include banana leaves, peanut hull, corn leaves, mango seeds, sugarcane leaves and wheat and rice straw (Cangy & Peerally, 1995). Sarker *et al.* (2007) tested waste paper, wheat straw, rice straw, sugarcane bagasse and *Saccharam spontaneum* as the substrates of *Pleurotus ostreatus* and indicated possibility of their commercial use. Report from other country reveal that the most extensively used agro-wastes for the production of edible mushrooms are wheat or rice straw, sawdust, wood chip, sugarcane bagasse, cotton waste, cotton seed hull, corn cob, rice and wheat bran, chicken and horse manure and other green materials like cotton stalk and soybean straw (Panjabrao *et al.*, 2007). The widely used substrate for cultivation of the oyster mushroom in Asia is rice straw (Thomas *et al.*, 1998). However, attempt to select suitable substrates for cultivation of *Pleurotus cystidiosus* has not yet made in Bangladesh. Considering the above facts, is the present investigation was undertaken to find out suitable substrates available in Bangladesh for cultivation of the species of oyster mushroom, *Pleurotus cystidiosus*.

METERIALS AND METHODS

The experiment was conducted at the National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka during the months of August - November 2010.

Preparation of substrates and spawn packets: Thirteen different substrates were tested in the present investigation. They were (T_1) mango (*Mangifera indica*) tree sawdust, (T_2) gamar (*Gmelina arborea*) tree sawdust, (T_3) acacia (*Accacia dicurrins*) tree sawdust, (T_4) mixed sawdust (mango + gamar + acacia), (T_5) rice straw, (T_6) sugarcane bagasse and (T_7) waste paper. Other substrates tested in the present investigation were mixture of sawdust and other materials (1:1) viz. (T_8) mixed sandiest + rice straw, (T_9) mixed sawdust + sugarcane bagasse, (T_{10}) mixed sawdust + waste paper, (T_{11}) rice straw + sugarcane bagasse, (T_{12}) rice straw + waste paper, and (T_{13}) sugarcane bagasse + waste paper. Wheat bran at $1/3^{rd}$ of total dry matter and $CaCO_3$ at 0.2% of the total mixture were commonly thoroughly mixed with each of the substrate materials. Required quantity of water was added to materials to make the moisture content 65%. Polypropylene bags of 7" x 10" size were filled with 500 g of substrate mixture. Their mouths were plugged by plastic necks and water absorbing cotton, covered with brown paper and tied with a rubber band. The bags were autoclaved at 121^0 C and 1.1 kg/cm^2 for 2 hours. After cooling, each spawn packet was inoculated with the mother culture of *P. cystidiosus* at the rate of two tea spoonful per packet. Bags were incubated for mycelium running at 25 ± 2^0C temperature. After completion of mycelium running the spawn packets were transferred to culture house. The culture house activities performed following the method described by Sarker *et al.* (2007).

Data collection and statistical analysis: The experiment was laid out following completely randomized design with four replications. Data on days required from stimulation to primordia initiation and stimulation to first harvest, approximate number of primordia, number of total and effective fruiting body, average weight of individual fruiting body, length and diameter of stipe, diameter and thickness of pileus, biological yield in 1^{st} harvest (g/packet), total biological yield (g/packet), economic yield (g/packet) and biological efficiency (%) were recorded. The biological efficiency was measured by the following formula.

$$\text{Biological Efficiency (\%)} = \frac{\text{weight of fresh mushroom fruiting bodies}}{\text{weight of dry substrate}} x100$$

Data were analyzed following standard methods (Gomez and Gomez, 1984) using MSTAT-C computer program. Means was compared following Duncan's multiple ranges test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Days required from stimulation to primordia initiation: Days required from stimulation to primordia initiation (DRSPI) ranged from 3.75 to 17.75 in different substrates. The DRSPI was minimum in sugarcane bagasse, waste paper and rice straw + waste paper, which was statistically similar to mango tree saw dust, mixed sawdust + waste paper, sugarcane bagasse + waste paper and rice straw + sugarcane bagasse. The maximum DRSPI (17.75) was found in acacia sawdust, which was statistically similar to mango tree sawdust + rice straw and mixed sawdust. The results are in agreement with the findings of Sarker *et al.* (2007), who reported that oyster mushroom took minimum days from stimulation to primordia initiation in case of waste paper, sawdust, sugarcane bagasse and wheat straw substrates (Table 1).

Days to require stimulation to first harvest: Days required from stimulation to first harvest (DRSFH) on different substrates ranged from 6.75 to 23.00. The DRSFH was minimum on mixed sawdust + waste paper, which was statistically similar to sugarcane bagasse + waste paper, waste paper, rice straw + waste paper and mango sawdust. The maximum DRSFH was found in acacia sawdust, which was significantly higher than other treatments except mixed sawdust and mixed sawdust + rice straw. The results of present investigation are in agreement with the findings of Bugarski *et al.* (1994) who found that the first fruit occurred on different days depending on substrates. Sarker *et al.* (2007) reported that oyster mushroom (*P. ostreatus*) took minimum days from stimulation to first harvest in case of waste paper (7.00), sawdust (7.00), sugarcane bagasse (6.75) and wheat straw (7.00) substrates. Baysal *et al.* (2003) reported almost similar results (Table 1).

Number of primordia: The number of primordia (NP) ranged from 47.50 to 76.50 on different substrates and varied considerably. The NP was the highest in bags with sugarcane bagasse, which was statistically similar to rice straw and it was lowest in mango sawdust , which was significantly lower compared to all other treatments. The result was approximately similar to Amin *et al.* (2007) who observed that the number of primordia/packet in four selected substrate such as saw dust, sugarcane bagasse, wheat straw and rice straw ranged from 41.7 to 57.3 in case of oyster mushroom cultivation (Table 2).

Number of fruiting body: The number of fruiting bodies (NFB) obtained from different substrates ranged from 29.00 to 62.75. The highest NFB was found in sugarcane bagasse + waste paper, which was significantly higher as compared to all other treatments. The lowest NFB was found in mixed sawdust + rice straw, which was statistically similar to mango sawdust, gamar sawdust and mixed sawdust. Sarker *et al.* (2007) observed the highest number of fruiting body of oyster mushroom (*P. ostreatus*) on waste paper (Table 2).

Number of effective fruiting body: The number of effective fruiting bodies (NEFB) obtained from different substrates varied appreciably and ranged from 24.75 to 55.50. The highest NEFB was found in sugarcane bagasse + waste paper, which was significantly higher as compared to all other treatments. The lowest NEFB was found in mango sawdust, which was statistically similar to mixed sawdust + rice straw (Table 2).

Weight of individual fruiting body: Weight of individual fruiting body (WIFB) grown on different substrates ranged from 4.44 to 7.67 g. The highest WIFB was found in mango sawdust, which was statistically similar to gamar sawdust, mixed sawdust + waste paper, mixed sawdust and mixed sawdust + rice straw. The lowest WIFB was found in sugarcane bagasse + waste paper, which was statistically similar to rice straw + sugarcane bagasse and sugarcane bagasse alone (Table 2). The WIFB was inversely proportional to the number of fruiting body. Almost similar result was reported by Sarker *et al.* (2007).

Length and diameter of stipe: The length of stipe (LS) ranged from 4.50 to 6.93 cm. The highest LS were found in rice straw + sugarcane bagasse, which was significantly higher as compared to all the treatments except paper. The lowest LS was found in mixed sawdust + rice straw. The diameter of stipe (DS) differed ranged from 0.82 to 1.60 cm. The highest DS was found in mango sawdust, which was significantly higher compared to all the treatments and the lowest diameter was found in sugarcane bagasse + waste paper, which was statistically similar to rice straw (Table 3).

Table 1. Time required from stimulation to primordia initiation and stimulation to first harvest as influenced by different substrates

Substrates	Days required from scrapping to primordia initiation (DRSPI)	Days required from scrapping to first harvest (DRSFH)
Mango sawdust	4.00 d	8.75 de
Gamar sawdust	10.25 c	15.75 c
Acacia sawdust	17.75 a	23.00 a
Mixed sawdust	16.50 ab	21.50 ab
Rice straw	15.50 b	20.75 b
Sugarcane bagasse	3.75 d	9.75 d
waste paper	3.75 d	7.25 e
Mixed sawdust + Rice straw	17.00 ab	21.25 ab
Mixed sawdust + Sugarcane bagasse	15.00 b	20.00 b
Mixed sawdust + waste paper	4.00 d	6.75 e
Rice straw + Sugarcane bagasse	5.00 d	9.75 d
Rice straw Rice straw + waste paper	3.75 d	7.25 e
Sugarcane bagasse + waste paper	4.00 d	7.00 e
CV(%)	15.26	10.00

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

Table 2. Comparative Study on different substrates on some yield attributes of *Pleurotus cystidiosus* grown on different substrates

Substrates	Number of primodia	Number of fruiting body	Number of effective fruiting body	Weight of individual fruiting body (g)
Mango sawdust	47.50 f	30.50 fg	24.75 h	7.67 a
Gamar sawdust	60.25 de	34.00 efg	30.00 f	7.52 a
Acacia sawdust	60.00 de	34.75 ef	31.00 f	5.28 c
Mixed sawdust	56.00 e	34.00 efg	29.00 fg	7.04 ab
Rice straw	72.75 ab	37.00 de	36.50 e	5.52 c
Sugarcane bagasse	76.50 a	52.25 b	47.25 b	4.99 cd
waste paper	65.50 c	41.75 cd	41.50 cd	6.36 b
Mixed sawdust + Rice straw	64.25 cd	29.00 g	25.50 gh	7.01 ab
Mixed sawdust + waste paper	64.25 cd	37.25 de	38.00 de	5.38 c
Mixed sawdust + waste paper	60.50 de	44.75 c	41.00 cd	7.41 a
Rice straw + Sugarcane bagasse	59.75 de	51.25 b	43.75 bc	5.18 cd
Rice straw + waste paper	68.50 bc	37.75 de	35.25 e	6.45 b
Sugarcane bagasse Sugarcane bagasse + waste paper	64.75 cd	62.75 a	55.50 a	4.44 d
CV(%)	4.93	7.95	7.61	8.11

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

Diameter and thickness of pileus: The diameter of pileus (DP) ranged from 5.45 to 8.53 cm. The highest DP was found in rice straw + waste paper, which was significantly higher compared to all the treatments and the lowest DP was found in rice straw alone, which was statistically similar to sugarcane bagasse. The thickness of pileus (TP) ranged from 0.69 to 0.91 cm. The highest TP was found in mango sawdust, which was statistically similar to waste paper, rice straw + waste paper, mixed sawdust + waste paper, sugarcane bagasse + waste paper and sugarcane bagasse alone. The lowest TP was found in rice straw (Table 3). The results are in agreement with Islam *et al.* (2009) who observed the highest TP in mango sawdust.

Biological yield in first harvest: The biological yield from the first harvest (BYFH) ranged from 91.00 g to 176.5 g/packet. The highest BYFH was observed in waste paper, which was statistically similar to sugarcane bagasse + waste paper. The lowest BYFH was found in acacia sawdust (Table 4).

Biological and economic yield in total harvest: Significant variation was observed in biological yield from total harvest (BYTH) and ranged from 182.3 g to 331.0 g/packet. The highest BYTH was found in mixed sawdust + waster paper, which was significantly higher as compared to all the treatments. The lowest BYTH was recorded in acacia sawdust (Table 4). Almost similar trend was observed in case of economic yield. Sarker *et al.* (2007) observed the highest biological and economic yield on waste paper. Almost similar results were obtained by Baysal *et al.* (2003).

Biological efficiency: The highest biological efficiency (BE) was found in mixed sawdust + waste paper, which was followed by sugarcane bagasse + waster paper. The lowest BE was observed in acacia sawdust (Table 4).

Table 3. Physical properties of *Pleurotus cystidiosus* in different substrates

Substrates	Length of stipe	Diameter of stipe	Diameter of pileus	Thickness of pileus
Mango sawdust	5.45 de	1.60 a	6.83 c	0.91 a
Gamar sawdust	5.15 e	1.21 bc	7.43 b	0.73b cd
Acacia sawdust	5.49 de	1.15 bc	7.68 b	0.70 cd
Mixed sawdust	6.28 b	1.20 bc	6.14 d	0.74 bcd
Rice straw	5.85 c	0.83 d	5.45 f	0.69 d
Sugarcane bagasse	5.70 cd	1.20 bc	5.73 ef	0.81 abc
Waste paper	6.68 a	1.26 bc	6.55 c	0.87 a
Mixed sawdust + Rice straw	4.50 f	1.10 c	6.78 c	0.70 cd
Mixed sawdust + Sugarcane bagasse	5.23 e	1.20 bc	6.57 c	0.75 bcd
Mixed sawdust + Waste paper	6.22 b	1.23 bc	6.60 c	0.82 ab
Rice straw + Sugarcane bagasse	6.93 a	1.26 bc	6.61 c	0.73 bcd
Rice straw + waste paper	5.20 e	1.33 b	8.53 a	0.83 ab
Sugarcane bagasse + waste paper	5.15 e	0.82 d	6.10 de	0.81 abc
CV(%)	4.03	11.33	3.98	9.29

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

Table 4. Study of different substrates on the biological and economic yield, biological efficiency of *Pleurotus cystidiosus* grown on different substrates

Substrates	Biological yield in first harvest (g)	Biological yield in total harvest(g)	Economic yield (g)
Mango sawdust	111.8 f	232.0 f	225.8 f
Gamar sawdust	132.8 bc	254.0 d	248.0 d
Acacia sawdust	91.00 h	182.3 h	175.5 h
Mixed sawdust	116.0 ef	238.3 ef	232.0 e
Rice straw	117.3 ef	203.0 g	197.0 g
Sugarcane bagasse	127.5 cd	259.5 cd	254.3 c
Waste paper	176.5 a	264.8 c	259.3 c
Mixed sawdust + Rice straw	104.0 g	201.5 g	196.5 g
Mixed sawdust + Sugarcane bagasse	121.0 de	199.5 g	194.3 g
Mixed sawdust + waste paper	134.3 b	331.0 a	326.0 a
Rice straw + Sugarcane bagasse	135.3 b	264.5 c	258.8 c
Rice straw + waste paper	121.3 e	242.0 e	235.8 e
Sugarcane bagasse + waste paper	175.3 a	277.8 b	273.5 b
CV (%)	3.41	2.12	6.26

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

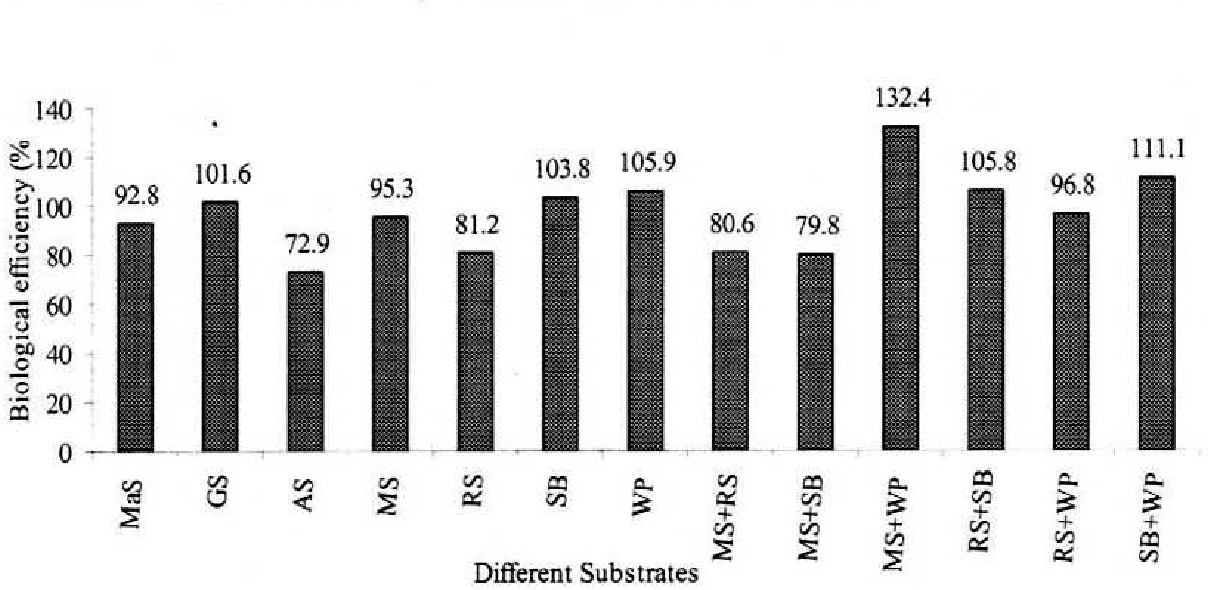

Fig. 1. Biological efficiency of *Pleurotus cystidiosus* on different substrates (MaS= Mango sawdust, GS= Gamar sawdust, AS= . Acacia sawdust, MS= Mixed sawdust, RS= Rice straw, SB= Sugarcane bagasse and WP= Waste paper).

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Effect of Manganese Chloride as Post Composting Supplement on the Yield of White Button Mushroom

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Abstract

An experiment was conducted to evaluate the effect of manganese chloride ($MnCl_2$) as post composting supplement on yield and nutrient content of white button mushroom (*Agaricus bisporus*). Solution of the chemical was prepared at the rate of 0, 20, 50, 100 and 200 ppm in water. The compost was supplemented with individual solution @ 50 ml per 3 kg compost. The spawn packets were placed in the mushroom house and allowed to grow maintaining proper conditions. The highest number of fruiting body, biological yield, economic yield and biological efficiency were achieved with 100 ppm of $MnCl_2$. The highest economic yield (457.60 g/ 3 kg bag) was also recorded from 100 ppm followed by 200 (424.60 g/ 3 kg bag), 50 (420.40 g/ 3 kg bag) and 20 (382.60 g/3 kg bag) ppm of $MnCl_2$. The level 100 ppm gave the highest benefit cost ratio of 8.07 compared to the lowest BCR of 5.99 recorded under control. The protein content of fruiting body was highest (15.07%) at 200 ppm followed by 100, 50, and 20 ppm. Contents of different minerals were not appreciably influenced by the supplement.

Key words: Manganese chloride, post composting supplement and button mushroom.

INTRODUCTION

Agaricus bisporus (Lange) Singer, popularly known as white button mushroom has the widest acceptability as a food item world wide. It is extensively cultivated in many countries, ranked the first in terms of production and popularity and contributes about 40% of the total world production of mushrooms (Flegg, 1992). The yield of the mushroom is comparatively lower as compare to other mushrooms like oyster. Supplementation of composts with different materials is one of the ways to increase yield of the mushroom (Randep, 1985). The supplements used in the mushroom cultivation is of both animal and plant origin which may be carbohydrate-rich, protein-rich or oil-rich substances. Of them, protein-rich materials give better results. However, supplementation at spawning time may create some hazards. Growers who are not having cooling facilities at their farm should not be dependent on this practice as temperature tends to increase 3-4^0C in the compost. Sometime the temperature may be too high, resulting in death of mycelium. The high temperature may generate secondary metabolites including ammonia, which lethal to mushroom mycelium. Supplementation may increase temperature, which increases the risk of weed moulds incidence.

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Supplementation with inorganic fertilizers before spawning has no hazard. It is easy to apply and cost effective and it increases the yield of *A. bisporus*. Racz and Tasnadi (1998) observed that addition of manganese increases the activity of manganese dependent peroxides enzyme, enhances lignin degradation and increases the availability of carbohydrates for mushroom production. Weil *et al.* (2006) reported that the addition of manganese to the compost has a stimulatory effect on the growth of mushroom and the yield increases by 9.6% - 11.8% over the control.

Under the above circumstances, the present investigation was undertaken to study the effect of supplementation of compost before spawning with manganese chloride on yield attributes and yield of *A. bisporus* and to find out the best level of the material as post composting supplement.

MATERIALS AND METHODS

The experiment was conducted in the laboratory and culture house of National Mushroom Development and Extension Centre, Savar, Dhaka during December 2006 to March 2008.

Manganese chloride ($MnCl_2$) solutions were prepared in water at the concentrations of 20, 50, 100, 200 ppm. Before spawning, $MnCl_2$ solutions were mixed with the compost @ 50 ml/3kg compost. Pure culture of *A. bisporus*, mother spawn and compost were prepared and inoculation, casing and watering were done following standard procedures as suggested by Amin *et al.* (2007). The spawn packets were placed in the mushroom house and allowed to grow maintaining proper conditions. The experiment was laid out in a completely randomized design with 5 replications.

Collection and analysis of data: fruiting: Mature fruiting bodies were harvested and data on yield attributes, yield and quality of mushroom were recorded. Data on days to primordial initiation, number of total fruiting body, biological yield, economical yield and benefit cost ratio (BCR) were recorded. The BCR was computed based on present market price of imported white button mushroom and cost of different inputs including price of $MnCl_2$ used in the study. Chemical analysis of the mushroom was performed to determine contents of protein and minerals as the criteria of quality.

Protein content estimation: Protein content of harvested mushroom was estimated to observe the effect of $MnCl_2$ on quality of mushroom. To estimate the protein content, nitrogen content of mushroom was estimated by "Colorimetric method" as described by Linder (1944). Mushroom sample was digested in 'Kjeldahl' digestion flask with salicylic sulfuric acid and digestion catalyst. After digestion, color of the solution was developed with four different reagents. Then absorbance of the solution was measured at 625 nm wavelengths with a Double Beam Spectrophotometer (Model 200-20, HITACHI).

Mineral content: Content of different mineral elements in mushroom, viz. Fe, Zn, Ca, Cu, Mg, K and Na were estimated following "Perchloric acid digestion method" as proposed by Yamakawa (1992). Phosphorus was determined following "Venamolybdate colorimetric method" proposed by Yamakawa (1992).

Data were analyzed statistically following standard procedures (Gomez and Gomez, 1984) using MSTAT-C computer program. Means were compared following Duncan's multiple ranges test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Days to primordial initiation: Days to primordial initiation varied from 14.40 to15.40 at different concentration (0-200 ppm) of $MnCl_2$. However, effect of different levels of the post composting supplement on the variation of days to primordial initiation was not significant (Table 1). Similar findings also reported by Racz and Tasnadi (1998).

Number of total fruiting body: The number of total fruiting body (NTFB) ranged from 70.00 to 84.20 per spawn packet at different concentrations (0-200 ppm) of $MnCl_2$. The highest NTFB was observed at 100 followed by 50 and 100 ppm. Effect of three higher concentrations on this parameter was statistically similar but significantly higher compared to only control.. The lowest NTFB was observed under control, which was not significantly different from 20 ppm. The results reveal that the NTFB increase with the increase of $MnCl_2$ level up to 100 ppm and decreased thereafter (Table 1). The results of the present experiment supported the findings of Racz and Tasnadi (1998).

Biological and economical yield: Supplementation of composts with $MnCl_2$ at 20-200 ppm caused significant increase in both biological yield (BY) and economic yield (EY) of button mushroom over control (0 ppm). The BY and EY ranged from 385.80 to 510g and 339.60 to 457.60 g/3kg compost, respectively. The highest BY was observed at 100 ppm, which was statistically similar to 50 and 200 ppm. Significantly the highest EY was found at 100 ppm. The second highest EY was obtained with 200 ppm, which was statistically similar to 50 ppm. The lowest increase of BY as well as EY was observed at 20 ppm (Table 1). The results of the present experiment supported the findings of Racz and Tasnadi (1998). They reported that $MnCl_2$ at 100 mg/kg produced the highest yield when 1 litre of $MnCl_2$ or $MnS0_4$ solution containing 20, 50, 10, 200 or 400 mg/kg of Mn was added to 20 kg of compost of *Agaricus bisporus* before inoculation. The higher yield in higher concentration of $MnCl_2$ might be due to the ability of manganese to degrade the lignin of compost like cellulose and hemicelluloses. Degradation of lignin increases the availability of carbohydrate for growth of mushroom mycelium which ultimately increases the production. Adenipekun (2006) and Kerem and Hadar (1995) also supported the result.

Benefit cost ratio: The benefit cost ratio (BCR) was 5.99, 6.75, 7.41, 8.07 and 7.48 at 0, 20, 50, 100 and 200 ppm $MnCl_2$, respectively. The results reveal that supplementation increases BCR up to 100 ppm level and decreased thereafter. The highest BCR was at 100 ppm $MnCl_2$ and the lowest at 0 ppm (Table1).

Table 1. Effect of manganese chloride as post composting supplement on the yield attributes and yield of white button mushroom (*Agaricus bisporus*)

Level of $MnCl_2$ (ppm)	Days to primordia initiation	Number of total fruiting body	Biological yield (g)	Economical yield (g)	Benefit cost ratio
0 (Control)	15.20 a	70.00 c	385.80 c	339.60 d	5.99
20	15.20 a	73.40 bc	433.80 b	382.60 c	6.75
50	15.40 a	81.00 a	496.60 a	420.40 b	7.41
100	14.80 a	84.20 a	510.00 a	457.60 a	8.07
200	14.40 a	80.20 ab	498.80 a	424.60 b	7.48
CV (%)	5.33	5.37	5.43	3.29	-

Means within the same column with a common letter(s) are not significantly different (P=0.05).

Protein Content: The content of protein in white button mushroom varied from 10.39 to 15.07% (w/w) at 0-200 ppm concentration of $MnCl_2$. The highest content of protein was estimated at 200 ppm followed by 100, 20 and 50 ppm. The lowest content of protein was found under 0 ppm (control). The results reveal that supplementation of compost with $MnCl_2$ caused protein content in white button mushroom over control (Table 2).

Minerals: The iron content in mushroom increased with the increase of $MnCl_2$ level in compost. The highest Fe content of 260 ppm was recorded at 200 ppm of $MnCl_2$. Similar trend was observed in case of Zn. Effect of $MnCl_2$ supplementation in compost on P, Ca, Cu, Mg, K and Na content of mushroom fruit bodies was considerable (Table 2).

The results of the present study reveal that $MnCl_2$ is a suitable post composting supplement to grow white button mushroom and to increase content of protein, iron and zinc, which are important nutrient of human diet.

Biological efficiency: The maximum biological efficiency (BE) of 17.00% was observed at 100 ppm of $MnCl_2$. It was decreased with the increase or decrease of $MnCl_2$ level. The lowest BE of 12.87% was recorded under control (Fig. 1).

Relationship between level of manganese chloride and number of total fruiting body: Highly significant relationship between the level of manganese chloride and number of fruiting body was observed. The relationship showed a quadratic equation as $y = -0.001x^2 + 0.2486x + 69.78$ ($R^2 = 0.9789^{**}$). The majority of total variation in the number of total fruiting body of the mushroom can be explained by this equation. The R^2 value indicated that 97.89% of the number of fruiting body was attributed to the level of manganese chloride (Fig. 2). The equation also stated that the number of fruiting body was the highest at 100 ppm level of $MnCl_2$.

Table 2. Effect of different concentrations of manganese chloride on the nutrient content of white button mushroom (*Agaricus bisporus*)

Concentration (ppm)	Protein (%)	Fe (ppm)	Zn (ppm)	P (%)	Ca (%)	Cu (ppm)	Mg (%)	K (%)	Na (%)
0	10.39	170	60	0.35	0.14	60	0.05	1.22	0.06
20	12.06	200	70	0.40	0.12	40	0.04	1.16	0.04
50	11.05	250	80	0.36	0.14	60	0.04	1.18	0.05
100	13.74	250	80	0.39	0.13	40	0.04	1.20	0.06
200	15.07	260	80	0.40	0.14	60	0.04	1.18	0.04

Relationship between level of manganese chloride and economic yield: The functional relationship between level of manganese chloride and economic yield of white button mushroom was shown in Fig. 3. It was clearly observed that economic yield of white button mushroom increased gradually with the increased level of manganese chloride used as post supplement to the compost up to 100 ppm level and decreased thereafter.

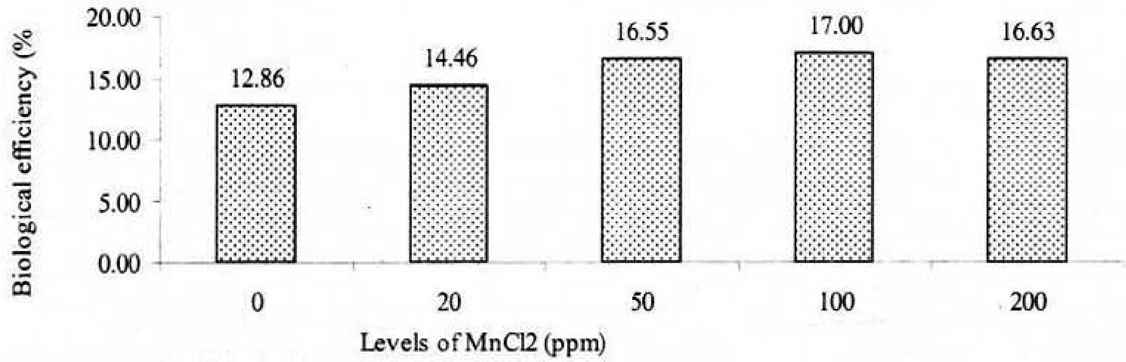

Fig. 1. Effect of different levels of $MnCl_2$ on the biological efficiency of white button mushroom.

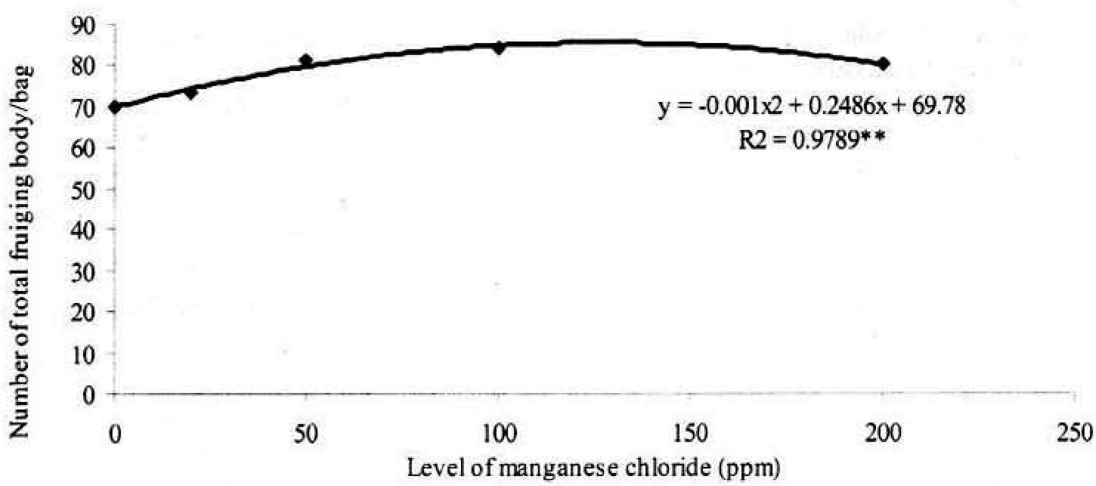

Fig. 2. Functional relationship between levels of manganese chloride and number of total fruiting body of white button mushroom.

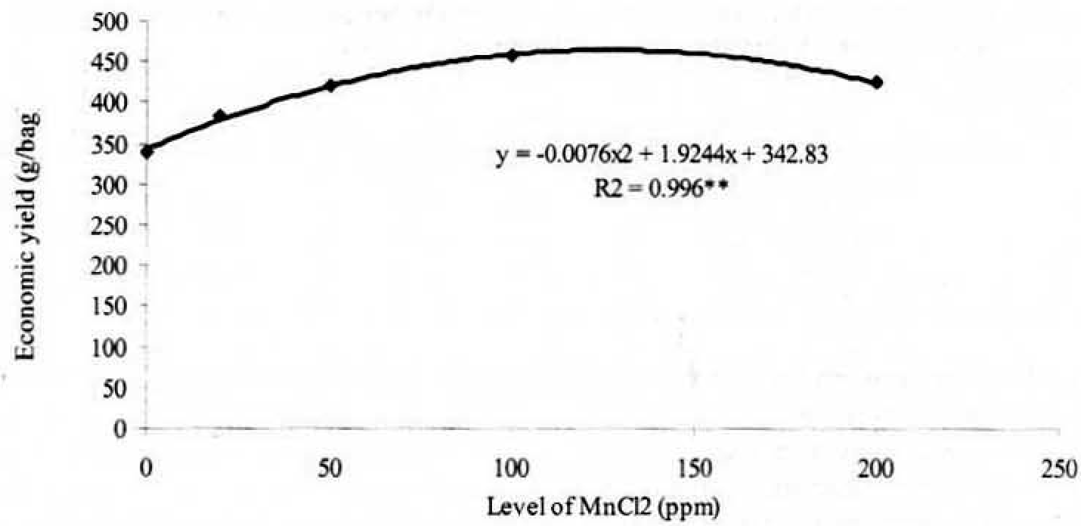

Fig. 3. Functional relationship between levels of $MnCl_2$ and economic yield of white button mushroom.

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Occurence of *Coprinus lagopus* (Fr.): A Potential Weed Fungus as a Contaminant of Mushroom Cultivation in Bangladesh

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Abstract

Coprinus lagopus, a common weed fungus for mushrooms was found to occur in a mushroom farm at Savar, on sawdust based substrate prepared for spawn production of *Pleurotus ostreatus*. Various taxonomic characters and fruiting period, mode of reproduction, various types of propagules of this weed fungus have been studied thoroughly. Regenerative propagules such as oidia, sclerotia, basodiospore are commonly found. In addition, the whole tissue system of sporophore showed its highly regenerative capability on substrate used for mushroom cultivation.

Key words: *Coprinus lagopus*, weed fungus, characterization, propagules and survivality.

INTRODUCTION

Coprinus lagopus belonging to the family Coprinaceae is a delicate and short-lived weed fungus of which the fruit bodies last only a few hours before dissolving into a black ink- a process called deliquescence (Jolles & Muzzarelli, 1999). It is also known as hare-foot mushroom due to the vague resemblance of the young fruiting body to the paw of a white rabbit (Crosier *et. al.*, 1949). Black spore of this potential weed characterize the well-known genus *Coprinus*, whose members are commonly known as the inky cap mushrooms. *Coprinus lagopus* can be grown in culture and has become an important experimental organism in biological science. Due to its very short life cycle and easy fructification on various agricultural wastes it has been established as important weed in mushroom cultivation. As a potential weed of various mushrooms it produces several propagules round the year which act as a survival units of its life cycle. Weeds have become adapted for their survival capacity at every step of their life cycle and interfere with man's utilization of land for specific purpose (Moore, 1954). The interference depends on some limiting factors of the environment, such as moisture, nutrient and sunlight (King, 1966). Due to weed competition in the crop, a significant yield loss occurs. Biological research of weed is essential for the purpose to minimize yield loss (Dennis, 1984). This sort of research can provide knowledge about their reproductive capacity, germination behaviour, adaptive power, dispersal mechanism, competitiveness, life cycle and also of fundamental importance to manage mushroom crop field for weed eradication. In Bangladesh, study on fungal weed biology is still completely virgin. Therefore, the present investigation was undertaken with purpose to fill up these lacunae of knowledge in the field of fungal weed science in Bangladesh,.

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MATERIALS AND METHODS

The experiment was conducted at National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh during February 2010 to December 2010. For study of various biological characters, *Coprinus lagopus* occuring as a weed species was selected and its fructification period, mode of reproduction, germination behaviours were investigated. The propagules, both asexual (oidia, sclerotia) and sexual (basidiospore) parts which were able to reproduce new individuals were recorded to determine the mode of reproduction. Beyond common propagules, the fruit body and vegetative tissues of its all tissue system demonstrates as uncommon survivable propagule particularly on substrate like sawdust, straw rather than culture media..

Taxonomic identification of the studied organism: The studied organism was identified according to the Kew Bulletin Additional series VI, A Preliminery Agaric Flora of East Africa by David Norman Pegler, 1977.

Organism and conditions of growth: The weed fungus *Coprinus lagopus* was collected from Ethnomushroom (pvt) Ltd, Savar, Dhaka. Fresh specimen was studied for taxonomic investigation and it was grown in pure culture on PDA medium in 125 ml Erlenmeyer flasks. The inoculum consisted of fragments of the vegetative mycelium. All cultures were maintained at room temperature (28-30°C). Mature fruiting bodies were obtained in 8 to 12 days.

Spore collection: Mature caps were placed in flasks of distilled water and refrigerated until the caps auto-digested. The slury was gently homogenized in a high speed (4000 rpm) homogenizer (Model-Glas-coal, Polytron: PT 1200) and filtered twice through cheesecloth. The spore in the filtrate was washed with distilled water and concentrated by centrifugation at 4000 rpm using the centrifuge of Digisystem: DSC-200T (Taiwan). Prior drying collected spore was preserved at 4°C in a refrigerator.

Reproductive units: The different propagules, both asexual and sexual parts which were able to produce new individuals were recorded to determine the mode of reproduction.

Count of germination: The criterion of germination was the appearance of germ tube from the spore were observed under an Olympus (Model CX 41) compound microscope. The number of spores germinated and counted and the percent of germination was calculated.

Observation of force of expansion in sawdust spawn: Vegetative tissue of *Coprinus lagopus* was inoculated on sawdust based substrate. After fully colonization of packet, the expansion of fruit body was observed.

Determination of competitive capacity as weed : Dual culture method was conducted against each of *Pleurotus ostreatus*, *Calocybe indica*, *Volvariella volvacea* and *Agaricus bisporus* for *Coprinus lagopus* to determine the competition or competition rate as weed. The PDA medium was used for this purpose.

Zoom Stereo microscopic observation: Morphological and cultural characteres of *Coprinus lagopus* were observed microscopically in a zoom sterio microscope (Model SZ 61).

Measurement of vegetative structure: Microscopic structures were measured by calibrating stage-micrometer with an oculo-micrometer.

Photomicrography:
Photograph was taken with an Olympus DP 20 camera attaching with both compound and sterioscopic microscope.

RESULT AND DISCUSSION

Identification: All the characteristics features of *Coprinus lagopus* described in the the Kew Bulletin Additional series VI, A Preliminery Agaric Flora of East Africa by David Norman Pegler, 1977; were recorded. All the important characters to match with the key for taxonomic identification were white veil, hyaline hyphae, white and fibrillose stipe, pileal margin soon revolute, spores with the dimension of 11–13 X 6–8 µm and showed pleurocystidia between the basidia.

Description of the sporophore

Cap: Size 1.5-5 cm, conical, finally with recurved margin, split and curled over on itself, completely white, flocose and mealy, often squamose. As the mushroom matures, the shape of the cap becomes more conical or convex, and finally flattens out, with edges curved upward. The veil is initially whitish, then turns to a silvery grey or grey-brown; it eventually splits up, becoming hairy (fibrillose) (Fig. 1).

Gills: White, then flesh-colored, finally blackish, adnexed, crowded, deliquecent. The gills are freely attached to the stem, very thin and crowded closely together. Initially the color of the gills is white then progresses to grayish brown then to black as the spores mature. In maturity the gill edges dissolve (*deliquesce*) into a black liquid. Lasting only a few hours before death, the autodigestive process is enhanced in humid environments (Jolles & Muzzarelli, 1999) .

Hymenium of *Coprinus lagopus* : Detail of section through two gills of unexpanded fruit-body showed short and long both type of basidia; paraphyses; and pleurocystidia in interlamellar space.The cystidia found on the sides of the gills (*Pleurocystidia*) are abundant in large fruiting bodies, fewer in number in the smaller specimens. These cells are oval, rounded at the apex with a bulge in the middle, and contracted into a stalk at the base (Fig. 2). The length of these cells is typically 100–130 µm, with a width of 35–45 µm. Before the cap expands, each cystidium completely branches an interlamellar space, with both ends attached to the gills, help together by clasping paraphyses. As the gill expands the cystidium breaks away from one gill and projects from the other gill. The basidia (spore-bearing cells) comes in two sizes; long basidia have dimensions of 40 × 8–10 µm, while the shorter basidia have dimensions of 23 × 8–10 μm. The basidia have four spores, which are attached by short sterigmata.

Fig. 1. Sporophore of *Coprinus lagopus*

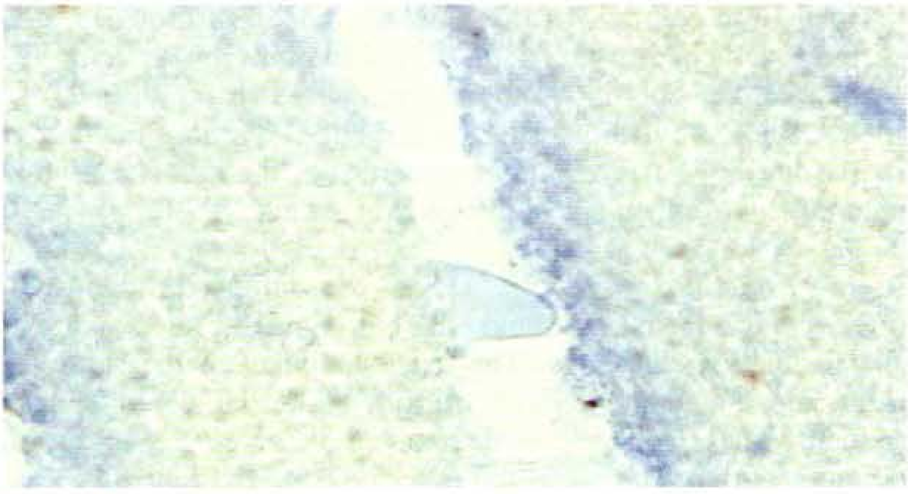

Fig. 2. Pleurocystidia of *Coprinus lagopus* (40x)

Stem: The stem is whitish in color, and is hollow, hairy (flocculose) over the whole surface but especially at lower part and becomes smooth (glabrous) with age. Size 2.5-7.5 x 0.3-0.6 cm, narrowing towards top (Pegler, 1977) .

Flesh: White, thin, undefined odor and flavour.

Spore: The spore print is violet-black. Spores have dimensions of 11–13 x 6–8 µm (Pegler, 1977). They are ellipsoid or ovoid in shape, with a rounded base and apex, dark red-brown in color, and nonamyloid. The process of autolysis of mature fruiting bodies of *Coprinus lagopus* is accomplished by the action of chitinases which are formed shortly before spore release begin (Niederpruem, & Cox, 1975).

Autolysis of the fruitbody: The process of autolysis of mature fruiting bodies of *Coprinus lagopus* is accomplished before release of spore begins (Buller, 1958).

Damaging symptoms: Ink cap *Coprinus lagopus* appear in the substrate during spawn run or newly placed cultured spawn and outside the substrate piles during storing. This fungus sometimes grows in clusters in beds and has a long sturdy stem which often reaches deep into the compost layer. Several days after their appearance ink caps decay and form a blackish slimy mass due to auto-digestion.

Propagules recorded in this study

A. Common propagules

Basidiospore: The spore of *Coprinus lagopus* easily can germinated in PDA medium within 2 days in room temperature. In microscopic field average germination of spore was 91% (Fig. 3) within 2 days. Unlike some other coprophilous fungi of the same genus, the spore of *Coprinus lagopus* has no dormancy in germination (Jolles & Muzzarelli, 1999).

Oidia: *Coprinus lagopus* produces distinct asexual phase that are thick walled more resistant spores are regarded as survival structure (memnospore) resembling mycelial fragments called oidia are produced by special, short hyphal branches, the oidiophores, which cut off oidia in succession, from the tip of the oidiophore. Such oidia serve a dual purpose; they may either germinate and produce uninucleate, primary mycelia, or they may act as spermatia, uniting with somatic hyphae, thus behaving like the microconidia of Neurospora (Alexopoulos & Mims, 1979). The spore and oidia are structurally unlike; however, the end product of this germination is the same is that monokaryotic hyphae as formed (Heintz & Niederpruem, 1971) (Fig. 4).

Sclerotia: Sclerotia were formed in both aerial and submerged parts of the mycelium. In addition a layer of cells with pigmented thick walls (called brown matting) which differentiated at the air/agar interface was interpreted as an aspect of sclerotial behaviour since it was regularly formed by strains which produced submerged sclerotia and was composed of cells of similar structure to those of the outermost layer of the submerged sclerotium (Alexopoulos, & Mims, 1979). Apart from producing sclerotia and oidiospores the cells of the aerial mycelium remained undifferentiated. In contrast, cells of the submerged mycelium, though initially indistinguishable from those of aerial hyphae, became individually differentiated within about 5 days of growth producing two further novel cell types. Submerged sclerotia were pale brown in colour, irregularly shaped and about 0.5-1.0 mm in diameter. In sharp contrast the aerial sclerotia were highly organized structures composed of distinct and compact tissues. Mature aerial sclerotia were dark brown to black spheroidal structures up to 0.5 mm in diameter. An outer layer of dead and moribund hyphae surrounded the main body of the sclerotium which was bilayered with an outer rind and inner medulla. The rind was multilayered and consisted of small cells with thick pigmented walls; intercellular spaces were cuticularized. The medulla was a closely packed tissue composed predominantly of hyaline thick-walled cells of the same type as were encountered in the submerged mycelium (Fig. 5 & 6).

Fig. 3. Germinated basidiospore (40X)

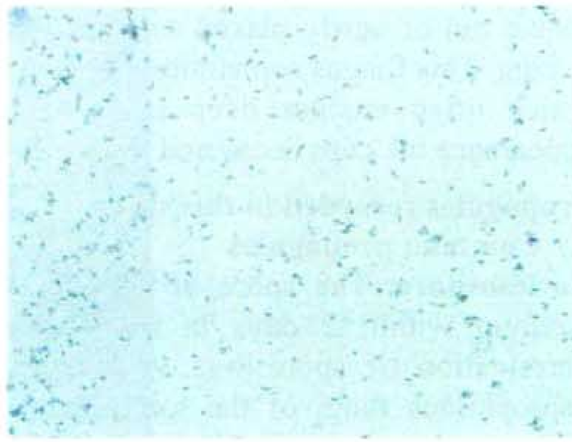

Fig. 4. Oidia (40X)

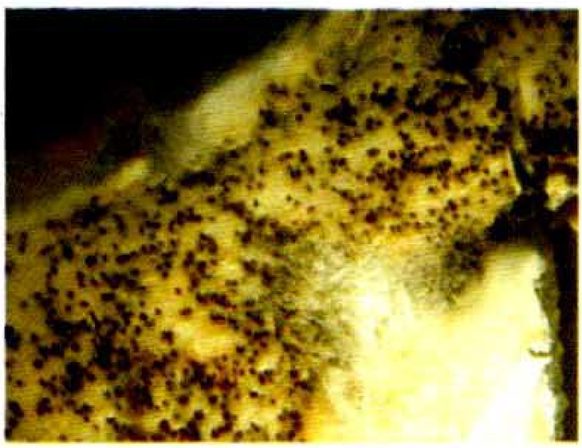

Fig. 5. Sclerotia: Stereioscopic view (0.67X)

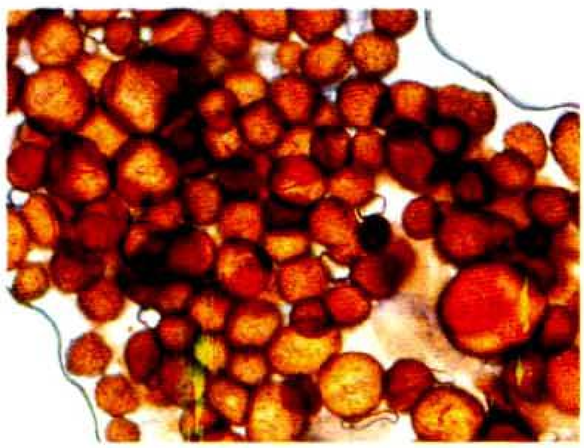

Fig. 6. Sclerotia (Microscopic view,10X)

B. Un-common propagules

Fruit body: The fruit body of *Coprinus lagopus* not only releases spore but also initiate mycelial body before ending its life cycle in Petri dishes (Fig. 7).

Vegetative tissue: The whole tissue system of *Coprinus lagopus* sporophore enable to initiate mycelial running direct on sawdust based traditional substrate of Oyster mushroom that proves its weed potentiality over cultivated mushrooms (Fig. 8).

Fig. 7. Mycelia generated from margin of pileus attached to the lid of petri dish, sterioscopic view (0.67 X).

Fig. 8. Propagation from tissue on sawdust

***In vitro* Competitiveness of *Coprinus lagopus*:** Dual culture of *Coprinus lagopus* and *Pleurotus ostreatus, Volvariella volvacea, Agaricus bisporus* and *Calocybe indica* at 22 ± 2 ^{0}C over a period of 9 days. *Coprinus lagopus* had been growing 7 days before *Calocybe indica* and *Agaricus bisporus* was started on the left side of the plate. Growth of *Pleurotus ostreatus* and *Volvariella volvacea* was satisfactorilly but finally fail to competitiveness of *Coprinus lagopus* as it overgrew and its force of mycelial colonization revert the growth direction of cultivated mushroom. Dual culture of every Petri dish shows the merrits of *Coprinus lagopus* as a potential weed to cultivated mushrooms and the type of competitivenes is a clear evident (Fig. 9).

Fig. 9. Competitive mycelial growth of *Coprinus lagopus* against cultivated mushrooms.

Fig. 10. *Coprinus lagopus* emerged by cracking the cotton plug.

The expansion force of fruit body: The force of expansion is quite considerable, *Coprinus lagopus* could not grow within cotton plug of experimental spawn packet, it initiates its fruiting by cracking the cotton plug (Fig. 10). *Coprinus atramentarius* has been demonstrated to be capable of cracking asphalt paving and *Coprinus sterquilinus* has been reported to lift a weight of over 200 g, many times of its own weight (Buller, 1958). Furthermore it was also recorded that the final rapid stage of expansion in many Agarics, eg *Agaricus bisporus* and *Coprinus cenerius* is almost entirely because of the extension of cells already laid down in the young primordium (Webster, 1980).

Current observations indicate that *C. lagopus* is a potential weed of various mushrooms world wide. Morris *et al.* (1995) reported that *C. logopus* reduces mushroom yield by 57.6 %. *Coprinus lagopus* completes its life cycle in a shorter time than does even the straw mushroom. *Coprinus lagopus* taking only 1 week, whereas *V. volvacia* takes 9 to 10 days (Chang & Quimio, 1982). Our study has been an evidence in Bangladesh that this weed fungus might be treat for our expanding mushroom cultivation and industry. Therefore proper attention and care to be paid for appropriate pasteurization and sterilization of the substrate used for developing spawn.

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