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Morphological and Molecular Characterization of Three Edible Mushroom Varieties in Bangladesh

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National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

A comparative study of morphological and molecular traits was tested in the three cultivated variety of mushroom for identification of varieties. Morphological traits such as stalk length and diameter, pelius diameter and thickness, fruiting body/spawn, days to first harvest etc differed from each other. The biological efficiencies were approximately same. The genomic DNA of three species was analyzed using 4 decamer random primers. All the primers except OPA05 were polymorphic. The maximum polymorphism was produced by the primer OPA02_2 having polymorphism percentage 18%. The dendrogram based on similarity matrix differentiated the species into two distinct clusters A and B. Cluster A constituted species NBM-1 (*Agaricus bisporus*), cluster B contained species NRM-2 (*Ganoderma lucidum*) and NSM-1 (*Volvariella volvaceae*). The genetic similarities among the species ranged from 24 and 28%. The study showed that the molecular characters varied with the differences of morphological traits.

Key words: Genomic DNA, Morphological traits, RAPD.

INTRODUCTION

Mushrooms recognized as natural and healthy foods originating from an environmentally friendly organic farming system (Moore and Chiu, 2001). To make mushroom cultivation sustainable and highly productive, novel improved strains with improved characteristics are greatly needed. However, mushroom strains are very difficult to discriminate, due to lack of clearly distinguishable characters. Moharram *et al.* (2008) worked on characterization of Oyster mushroom on the basis of food supplements. Hyeon *et al.* (2007) studied the diversity of *Pleurotus eryngii* using RAPD and its sequence analysis and observed that, grouping based on physiological parameters is closely related to RAPD based grouping. Molecular markers of rDNA sequencing, RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), microsatellite and mitochondrial genotypes have all been used to discriminate mushroom species and/or strains of *Agaricus* (Castle *et al.*, 1987; Sonnenberg *et al.*, 1991; Khush *et al.*, 1992; Barroso *et al.*, 2000;Calvo-Bado *et al.*, 2000; Ramirez *et al.*, 2001), *Auricularia* (Yan *et al.*, 1999), *Ganoderma* (Hseu *et al.*, 1996), *Lentinula* (Chiu *et al.*, 1996) and *volvariella* (Chiu *et al.*, 1995). These technologies provide ways to obtain reliable data for mushroom species identification and protection. RAPD analysis was first developed to detect

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polymorphism between organisms, despite the absence of sequence information, to produce genetic markers and to construct genetic maps (Williams *et al.*, 1990). In this work, morphological traits and RAPD were tested in the three cultivated variety of mushroom species for identification of individual species.

MATERIALS AND METHODS

Agaricus bisporus (NAMDEC Button Mushroom 1), *Ganoderma lucidum* (NAMDEC Reishi Mushroom 2) and *Volvariella volvaceae* (NAMDEC Straw Mushroom 1) were collected from National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar, Dhaka and molecular analysis was done in the laboratory of NAMDEC.

Mushroom Cultivation: Composting material (using rice straw and chemical fertilizer), sawdust and rice straw were used as substrate for growth of Button, Reishi and Straw mushrooms respectively. Data for different morphological traits and yields were recorded from mushrooms grown on substrates.

DNA Extraction: Filamentous fungi have strong cell walls which are often difficult to rupture in traditional method. In the present investigation, modified method of Aljanabi *et al.* (1999) has been used to isolate the total genomic DNA from mushroom. DNA of three different mushroom genotypes was extracted from 0.2-0.3 g fruiting body of each genotype. It was grinded in extraction buffer (200 mM Tris-HCl-pH 8.5, 250 mM NaCl, 25 mM EDTA, 20% CTAB, 0.5% SDS) with a mortar pestle. The lysates were incubated at 65°C for 40 min in water bath and centrifuge 30 min at 10,000 rpm. DNA was precipitated from the supernatant by adding equal volumes of isopropanol and resultant pellet was washed with 70% ethanol. The DNA palette was air dried and dissolved in 50 µl TE buffer. DNA quantification was performed and a dilution of 50ng/µl was used in downstream application.

RAPD Analysis: Genomic DNA was amplified by the RAPD technique (Williams *et al.*, 1990) in which four sorts of arbitrary 10-base oligonucleotide primers (Operon technologies Inc.) such as OPA02_2 (sequence 5' TGCCGAGCTG 3'); OPA-03_2 (sequence 5' AGTCAGCCAC 3'); OPA-04_2 (sequence 5' AAT CGG GCT G 3'); OPA-05 (sequence 5' AGG GGT CTT G 3'). RAPD-PCR reaction was performed using a thermal cycler with an initial denaturation stage of 5 min at 94°C, followed by 40 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 36°C, extension for 2 min at 72°C and a final extension for 10 min at 72°C.

Gel Electrophoresis and RAPD Data Scoring: RAPD products were electrophoresed on 1.4% agarose gel in 1X TBE buffer for 1.15 hr at 100 V with 1kb DNA ladder as a size marker and then stained while agitated in an EtBr solution (0.5% µg/ml). The stained gels were visualized under a UV transilluminator and photographed using Bio-Rad gel documentation system. The amplification product generated by each RAPD primer was scored as '1' or '0' for presence or absence of specific allele respectively. To estimate the

similarity and genetics distance among different species, cluster analysis based on Nei's unweighted pair-group with arithmetic average (UPGMA) was performed using the 'statistica' software and a dendrogram was constructed.

RESULTS AND DISCUSSION

Morphological traits (Table 1) such as stalk length and diameter, pelius diameter and thickness, fruiting body/spawn, days to first harvest etc were differing from each other. The diameter of stalk of NBM-1 is about 2.05 cm, whereas it was absent in NSM-1 and about 1.0 cm in NRM-2. Days to final harvest (about 2-3 flushes) was the lowest (36.0 days) in NSM 1 and the highest (116.0 days) in NRM-2, Whereas the NBM-1 required about 62.0 days for final harvest. The biological efficiencies were approximately same.

Table 1. Morphological character of three mushroom variety cultivated at NAMDEC

Variety name	NAMDEC Button mushroom 1	NAMDEC Straw mushroom 1	NAMDEC Reishi mushroom 2
Strain accession No.	Ab-1	Vv(w)	GL-4
Color	White	Dim grey	Brick red
Stalk length (cm)	1.02	Egg shape	2.0
Stalk diameter (cm)	2.05	Absent	1.0
Pelius diameter (cm)	4.8	2.25	5.5
Pelius thickness (cm)	2.5	4.50	1.2
Fruiting body / spawn	95.6	92.0	4.7
Days to first harvest	25.0	12.0	45.0
Days to final harvest	62.0	36.0	116.0
Biol. yield g / compost packet (2.5 kg)	510.0	x	x
Biol. yield g / bed (4.5 kg)	x	1000.1	x
Biol. yield g / spawn(175 g)	x	x	40.0
Dry yield g / spawn	53.3	150.0	10.0
Biological efficiency (%)	20.4	22.2	22.8

The genomic DNA of three species was analyzed using 4 decamer random primers. All the primers except OPA05 were polymorphic. The number of bands and banding pattern (Fig. 1-4) were variable depending upon the primer and type of species tested and it ranged from 3 to 13 in counting. The maximum polymorphism was produced by the primer OPA02_2 having polymorphism percentage 18%. The dendrogram based on similarity matrix differentiated the species into two distinct clusters A and B. Cluster A constituted species NBM-1 (*Agaricus bisporus*), cluster B contained species NRM-2 (*Ganoderma lucidum*) and NSM-1 (*Volvariella volvaceae*) (Fig. 5). The genetic similarities (Table 2) among the species ranged from 24 and 28%. The highest linkage

distance (28.5) was recorded for NBM-1. The lowest linkage distance (24.0) was recorded between variety NRM-2 and NSM-1.

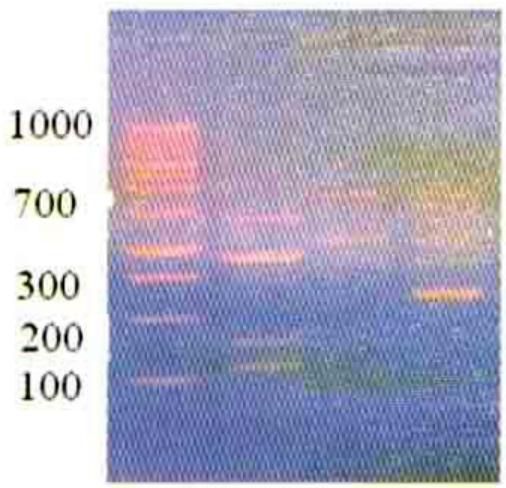

Fig. 1. RAPD profile of five different mushroom genotypes with primer OPA-02_2. Lane M, 1000 bp DNA ladder;Lane 1, NBM-1 (NAMDEC Button Mushroom-1); Lane 2, NRM-2 (NAMDEC Reishi Mushroom-2); Lane 3, NSM-1 (NAMDEC Straw Mushroom-1).

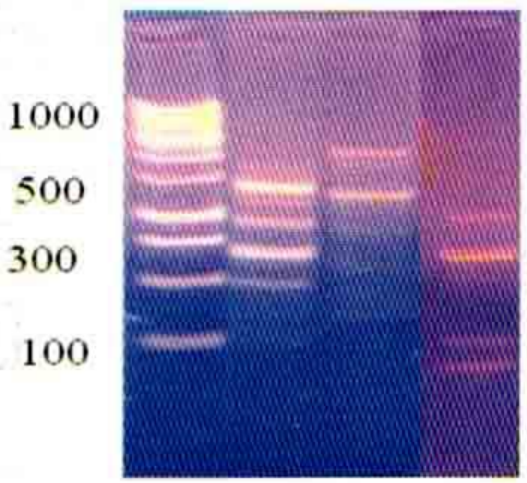

Fig. 2. RAPD profile of five different mushroom genotypes with primer OPA03_2. Lane M, 1000 bp DNA ladder;Lane 1, NBM-1 (NAMDEC Button Mushroom-1); Lane 2, NRM-2 (NAMDEC Reishi Mushroom-2); Lane 3, NSM-1 (NAMDEC Straw Mushroom-1).

Fig. 3. RAPD profile of three different mushroom genotypes with primer OPA-04_2. Lane M, 1000 bp DNA ladder;Lane 1, NBM-1 (NAMDEC Button Mushroom-1); Lane 2, NRM-2 (NAMDEC Reishi Mushroom-2); Lane 3, NSM-1 (NAMDEC Straw Mushroom-1).

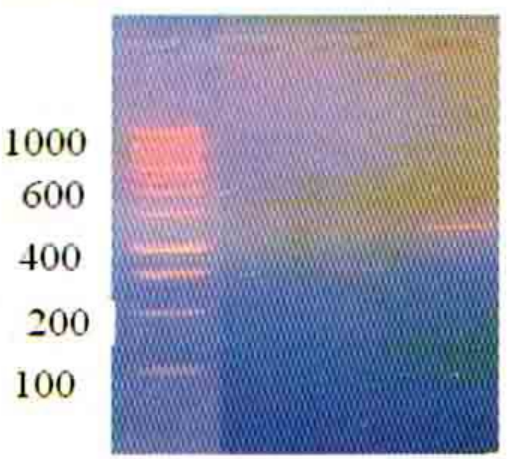

Fig. 4. RAPD profile of five different mushroom genotypes with primer OPA-05. Lane M, 1000 bp DNA ladder;Lane 1, NBM-1 (NAMDEC Button Mushroom-1); Lane 2, NRM-2 (NAMDEC Reishi Mushroom-2); Lane 3, NSM-1 (NAMDEC Straw Mushroom-1).

Table 2. Summary of linkage distances for different pairs of selected mushrooms by RAPD

Varieties	NBM-1	NRM-2	NSM-1
NBM-1	0	28.0	28.0
NRM-2	28.0	0	24.0
NSM-1	28.0	24.0	0

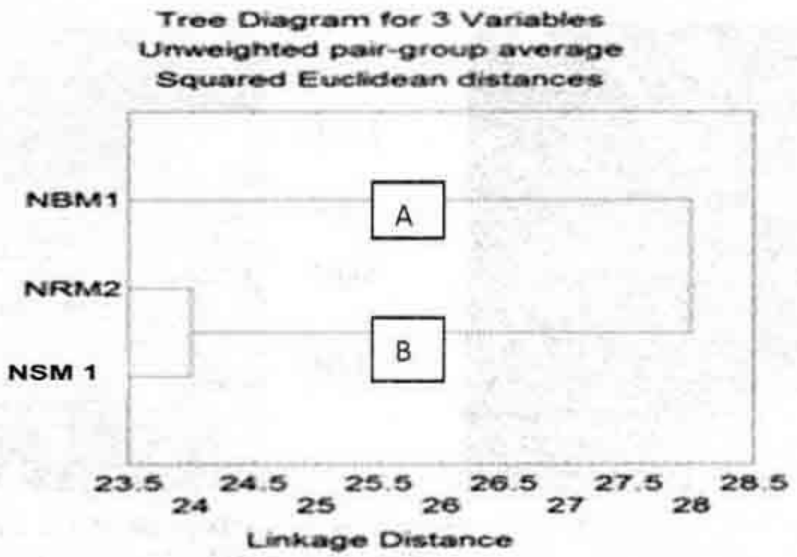

Fig. 5. Cluster analysis by unweighted pair group method of arithmetic means (UPGMA) of three mushroom genotypes based on RAPD. NBM- 1 (NAMDEC Button Mushroom 1), NRM- 2 (NAMDEC Reishi mushroom 2) and NSM-1 (NAMDEC Straw Mushroom 1).

The basic DNA sequence of an organism can be presumed to be insensitive to short term environmental change and thus should provide a more stable alternative for strain/ species identification. Therefore, the random and genomic wide nature of the RAPD technique is best to indicate over all genetic relatedness/dissimilarity than the morphological analysis (Alam, 2011 and Ravash *et al*., 2009). The different primers produced different number of bands in PCR. This variation in the number of bands may be due to the sequence of primer, availability of annealing sites in the genome and template quality (Alam *et al*., 2009 and Kernodle *et al*., 1993). The polymorphism produced by four RAPD primers except OPA-05 may be due to the base substitution, insertion and deletion or collection of genetic material from different sources (Chopra, 2005 and Jusuf, 2010). The maximum (28%) similarity was observed between NRM-2 (*Ganoderma lucidum*) and NSM-1 (*Volvariella volvaceae*) belonging to same major cluster B. This is may be due to their same environmental condition. They are both grown well at relatively higher (30-35°c) temperature than *A. bisporus (*15-20°c*)*. These findings revealed that the genetic make up is correlated with environmental heterogeneity (Alam *et al*., 2010 and Nevo, 1998). The results depicted that, there is strong correlation between molecular and morphological criteria (Zervakis *et al*., 2004). Jusuf (2010) suggested that, mushrooms belonging to *Pleurotus* specie have common growing habit that's why they may have common genetic background.

The mushroom genotypes belonging to ecological proximity or different geographical origins can be classified through morphological and molecular markers. The current study

demonstrated that, the RAPD analysis is useful for characterization, genetic diversity and identifying relationships among the mushrooms. Study also revealed that, RAPD analysis can be very useful tool for classification and maintenance of good quality spawns of mushrooms. The study also showed that the molecular characters are varying with the differences of morphological traits.

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Effect of Oyster mushroom (*Pleurotus ostreatus*) on Renal Function of Hypertensive Male Volunteers

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Abstract

As the kidneys are vital organs that filter impurities out of the bloodstream and the major attribute of mushrooms is their medicinal properties which have been the main focus of researchers around the world, the present study was carried out to evaluate the effect of *Pleurotus ostreatus* on renal function of hypertensive male volunteers. The study was conducted in the National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar Dhaka. Three grams of dried *Pleurotus ostreatus* powder as capsule form was taken by the subjects in three divided doses for three months. The finding of the study showed non-significant small reduction of plasma urea (21.95 ± 0.77 and 21.38 ± 1.03, p = 0.586), creatinine (0.92 ± 0.03 and 0.85 ± 0.04, p = 0.76) and uric acid (5.08 ± 0.22 and 5.00 ± 0.17, p = 0.707). These findings are suggestive of the Ameliorative Effect of *Pleurotus ostreatus* on kidney function of hypertensive male subjects.

Key words: Oyster mushroom, Creatinine, Urea, Uric acid.

INTRODUCTION

Mushrooms were used as food even before man understood the use of other organisms. Undoubtedly, mushrooms were one of man's earliest foods, and they were often considered an exotic and luxurious food reserved for the rich. Today mushrooms are food for both the rich and the poor. Now it is believable that mushroom eaters have a better nutrient profile than do those who do not eat mushrooms (Feeney, 2003).

For centuries, people have enjoyed mushrooms for their flavor, texture and mystique. Eastern cultures have revered mushrooms as both food and medicine for thousands of years. In recent years, some edible mushrooms are used as health foods, as well as a source for pharmaceutical compounds. In fact, these functional mushrooms are a source of biologically active substances with therapeutic effects due to their immunomodulating, anticancer and antivirus properties (Wasser and Weis, 1999), among others. Many commercially available mushrooms exhibit free radical scavenging, reducing power,

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chelating effects on metal ions, and antioxidant properties (Mau *et al.*, 2002; Yang *et al.*, 2002). It is now established that mushrooms are good source of high quality proteins and minerals (Pathak *et al.*, 1998).

Among the mushroom kingdom, oysters are one of the most versatile mushrooms. They are easy to cultivate and common all over the world. The Oyster mushroom (*Pleurotus ostreatus)*, first cultivated in Germany as a subsistence measure during First World War is now grown commercially around the world for food (Eger *et al.*, 1976). In Japanese, Korean and Chinese cookery, oyster mushroom is frequently used as a delicacy. It is a new addition of food crop in our country, needs no or minimum cultivable agricultural land for growth. It grows well in useless land with shadow and inside the home. Oyster mushroom has proven positive effects on the human body and spirit. Its main effects are based on active substances - β glucans, which have the ability to activate cells for natural immunity to the organism. In addition, oyster mushroom contains a variety of vitamins B, D, C, K, proteins, sterols, fatty acids and some trace elements of chromium, copper, iron, iodine, sodium, selenium and zinc.

Oyster mushrooms contain many things that fit the definition of food supplements. One kind of oyster mushroom may be richer in one of these materials while another kind will be richer in another. However, they are generally similar to each other in special food values. Oyster mushrooms are very good nutritionally and to explain what makes them so good. Any food with high nutritional value must be considered a health food. Mushroom of *Pleurotus* species are also rich in medicinal values and useful in preventing disease such as hypertension, hypercholesterolemia (Khatun *et al.*, 2007; Choudhury *et al.*, 2008) hyperglycemia and different types of cancer (Nayana, and Janardhanan, 2000). In China oyster mushroom is indicated for joint and muscle relaxation (Yang and Jong, 1989). Anecdotal reports suggest, oyster mushrooms improve liver and kidney function and help gastrointestinal disorders (Ying and Weil, 1987).

Oyster is also an important source of natural products from the statins (lovastatin, mevinolin), which protects against hardening of the blood vessel wall and has a positive effect on hypercholesterolaemia. Oyster mushroom is an important source of fiber, which consists of basic polysaccharide chitin and chitosan fibers, unlike higher plants, which is made up of cellulose and pectin. Chitin and chitosan inhibits the absorption of cholesterol and at the same time speeds up the metabolism. Human beings become more vulnerable to various diseases as age advances. Ageing-related diseases and disorders include hypercholesterolemia, obesity, hypertension and hyperglycemia. Now a days oyster mushroom are recognized as important food for their significant role in human health, nutrition and diseases. The use of mushroom nutrition as part of nutritional management to enhance the body's immune function is considered as standard practice in Japan, China and in other Asian cultures (Konno, 2003).

The vital organ kidneys filter impurities from the blood, which body then flushes out though the excretory system. Although Protein is an essential nutrient but its metabolic waste products are harmful for the body. Thus excessive amounts of protein can strain kidneys which are able to deteriorate chronic kidney disease (CKD) patients. Although protein is a necessary nutrient, CKD patients are often faced with the dilemma of having to limit protein intake. Again, high blood pressure makes the heart work harder and, over time, can damage blood vessels throughout the body. If the blood vessels in the kidneys are damaged, they may stop removing wastes and extra fluid from the body. The extra fluid in the blood vessels raises blood pressure even more. Current study was to investigate whether or not the oyster mushroom causes deleterious effect on renal function of hypertensive male volunteers.

MATERIALS AND METHODS

Subjects and Their Selection Criteria: The study was conducted in the laboratory of National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka. A total of 21 hypertensive male volunteers (systolic BP $\geq$ 140 mmHg and/or diastolic BP $\geq$ 90 mmHg) free from diabetes (fasting plasma glucose < 7 mmol/l) were included in the study. The age ranges of the subject were 27 to 62 years. After getting the written consent of the subjects with above mentioned criteria were included. Persons with renal impairment and other known acute or chronic diseases as well as history of addiction other than smoking were excluded in the study. They were allowed to continue the medication they were taking. Age, sex, occupation, educational status, marital status, family history and drug history were recorded in a preformed data collection sheet.

Performed Different Investigations: Subjects were evaluated for health status at the beginning of the study. Both systolic and diastolic blood pressure was measured following standard procedure using sphygmomanometer by a trained physician. Mean of duplicate measurements was taken. Ten milliliter of fasting blood sample was collected from median cubital vein with all aseptic precautions. Collected blood was poured into fluoride and EDTA containing test tube. It was then gently shaken for proper mixing with the anticoagulants. Within short time the anticoagulant-mixed blood was centrifuged at 3000 rpm for 5 minutes. Separated plasma was transferred into two eppendorf containing 1 ml in each. Fasting plasma glucose (FPG) was estimated by glucose oxidase method, plasma urea was estimated by enzymatic, colourimetric, endpoint – Berthelot method. Plasma creatinine was estimated by alkaline picrate method and plasma uric acid was estimated by uricase colorimetric method. Analysis was done by semi auto biochemical analyzer 3000 evaluation using commercially available reagent kit.

Preperation of Mushroom Capsule: Fresh fruiting bodies of *Pleurotus ostreatus* were harvested from culture house of National Mushroom Development and Extension Centre (NAMDEC). Collected mushrooms were dried at moisture level 4-5% using an electric drier machine. Dried mushrooms were grinded and poured into capsule shells, so that

each capsule contains 500 mg powder. Prepared capsules were ready to dispense and preserved into moisture free glass containers.

Quantity and Duration of Mushroom Capsule Supplementation: Subjects had directed to take two capsules three times daily for three months. So, each subject took three grams *Pleurotus ostreatus* powder daily. After the end of three months the subjects were re-evaluated and all the laboratory investigations were repeated.

Statistical Analysis: Results were expressed as mean ± SE. Paired Student's 't' test had done. 95% confidence limit was taken as the level of significance.

RESULTS AND DISCUSSION

The kidneys are vital organs that filter impurities out of the bloodstream. Lot of chemical substances available in different food may cause harm to the kidneys and for long ran responsible for chronic kidney diseases. Mushrooms are healthy and medicinal food; they contain various chemical compounds. Mushrooms of *Pleurotus* species is one of the most famous edible mushroom which have lot of ameliorative effect on health. But it is questionable, whether it has any detrimental effect on kidney. The present study was conducted to observe the effect of *Pleurotus ostreatus* on the kidney function of hypertensive male volunteers.

Hypertensive adult male subjects, free from diabetes were included in the study. The mean age (years) of the subjects were 42 ± 2.23, ranges from 27 to 62. The mean of fasting plasma glucose (mmol/l) was 6.21 ± 0.15 ranges from 4.5 to 6.9. The mean of systolic and diastolic blood pressure (mmHg) were 145 ± 3.43 and 90.95 ± 1.64 ranges from 120 to 180 and 75 to 100 respectively (Table 1).

Table 1. Evaluation of age, fasting plasma glucose, systolic and diastolic blood pressure, of the subjects

Parameter	N	Mean (± SE)	Range
Age (years)	21	42 ± 2.23	27 to 62
FPG (mmol/l)	21	6.21 ± 0.15	4.5 to 6.9
Systolic BP (mmHg)	21	145 ± 3.43	120 to 180
Diastolic BP (mmHg)	21	90.95 ± 1.64	75 to 100

In the study it was observed that the mean ± SE plasma urea before and 3 months after mushroom supplement was 21.95 ± 0.77 and 21.38 ± 1.03 respectively (Fig. 1). There was non-significant but small reduction of plasma urea ($p = 0.586$) between the two periods.

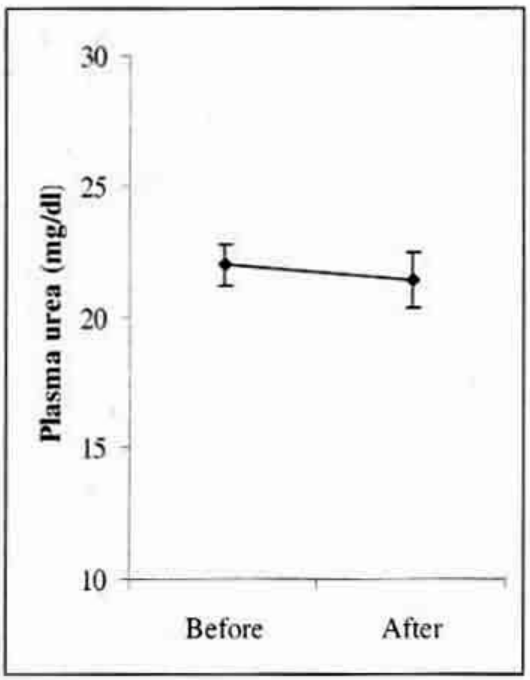

Fig. 1. Mean (± SE) plasma urea before and 3 months after mushroom supplementation.

The mean ± SE plasma creatinine (mg/dl) before and three months after mushroom treatment was 0.92 ± 0.03 and 0.85 ± 0.04 respectively. Here also a non-significant small declining mean difference (p = 0.76) was observed (Fig. 2).

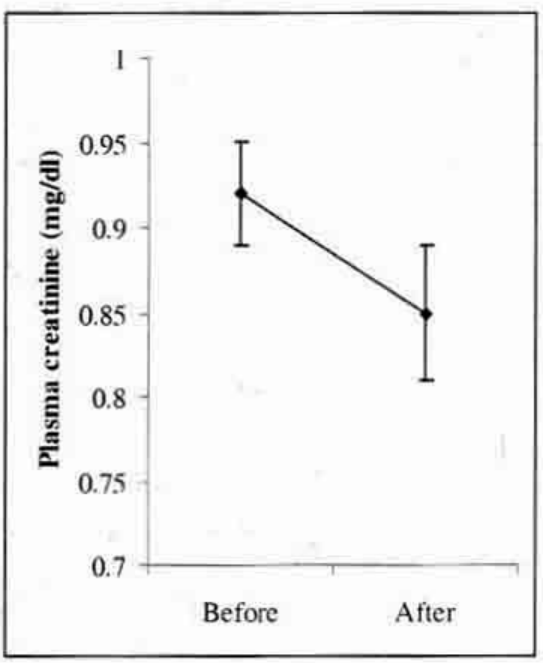

Fig. 2. Mean (± SE) plasma creatinine before and 3 months after mushroom supplementation.

The mean ± SE plasma uric acid (mg/dl) before and 3 months after mushroom treatment were 5.08 ± 0.22 and 5.00 ± 0.17 respectively. No statistically significant mean different was observed (p = 0.707), between the two periods (Fig. 3).

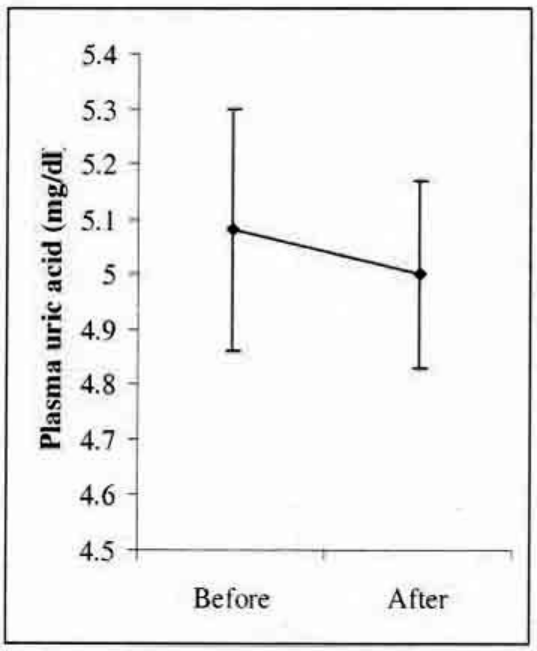

Fig. 3. Mean (± SE) plasma uric acid before and 3 months after mushroom supplementation.

Edible mushrooms have been widely utilized as human foods for centuries and they have been known for their nutritional and culinary values and used as medicines and tonics by humans for ages. In this study, it was observed that there is no significant change of plasma urea, creatinine and uric acid but it was noticeable that there is a small declining tendency of these three parameters. Previous studies shown that oyster mushroom is rich in high quality protein and this value is 19 to 39 gm in 100 gm dried matter (Breene, 1990). On the other hand urea and creatinine are protein metabolites. So, these two parameters normally can rise in increasing protein intake. But this study showed instead of rising, there is small reduction of urea and creatinine level in the blood, suggesting the ameliorative effect of oyster mushroom on kidney functions. On the other hand, non significant stable condition of plasma uric acid is suggestive of normal renal performance as uric acid is the final oxidation (breakdown) product of purine metabolism and it accumulates in the blood due to renal impairment.

One of the previous study shows *Pleurotus ostreatus* causes non significant small rising of palsma urea and creatinine and minute reduction of uric acid level of hypertensive diabetic male volunteers (Choudhury *et al.*, 2011). Current study is partially supportive of the previous one but instead of small rising here observed small reduction of these two parameters. This difference may be due to the subject's variation, where in previous study diabetic subjects were included. It is well known that persistent diabetes causes renal impairement. Another study of animal model, Alam *et al.* (2009) observed that there was

no significant difference in plasma bilirubin, creatinin and BUN levels in 5% mushroom-fed hypercholesterolemic rats after 40 days. Although there is no sufficient human data, in a study, Khatun *et al.* (2007) observed no detrimental effect of oyster mushroom on renal function. This trend was also supported by other previous study of this author (Choudhury *et al.*, 2008).

Anecdotal reports suggest, oyster mushrooms improve liver and kidney function and help gastrointestinal disorders (Ying and Weil, 1987). Jayakumar *et al.* (2008) studied the protective effect of the *Pleurotus ostreatus* on carbon tetrachloride (CCl_4)-induced toxicity in male Wistar rats. Histopathological studies confirmed the toxic effects of CCl4 on other organs such as kidneys, heart and brain and also tissue protective effect of the extract of *Pleurotus ostreatus*. These results suggest that an extract of *Pleurotus ostreatus* is able to alleviate the oxidative damage caused by CCl_4 in the kidneys, heart and brain of Wistar rats. Sirag (2009) was studied to investigate the putative protective effect with antioxidant potential of the *Pleurotus ostreatus* in glycerol-induced acute renal failure (ARF) in rats. He suggested that *Pleurotus ostreatus* may have ability to protect the renal damage involved in acute renal failure in rats. Our findings are supported by all of these findings.

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Market Chain Analysis of Button Mushroom in Dhaka area of Bangladesh

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Abstract

Present study was conducted in Dhaka city of Bangladesh to investigate the marketing channel, cost and margins and consumers preference of white button mushroom. The study also attempted to make recommendations for the improvement of marketing system of button mushroom in Bangladesh. The study was based on primary data of 48 samples purposively selected from the study area. The samples included 8 importers cum wholesalers and 40 retailers of Dhaka. Forty five tons of white button mushroom was consumed in 2009 while it was 19 MT in 2008 and 61 MT in 2007. The buying and selling prices of imported fresh white button mushroom at the wholesale level was Tk. 950.00 and 1450.00 / kg, respectively, whereas the prices for canned button mushrooms were Tk. 70.00 and 82.50 / can, respectively. The marketing cost of the wholesalers were Tk. 120.00 / kg for fresh and Tk. 8.15 / can for processed mushroom. At the retail level, the marketing costs were Tk.160.15 /kg for fresh and Tk. 8.71 / can for processed button mushrooms. The gross margins of the wholesalers were Tk. 500.00 / kg for fresh and Tk. 12.50 / can for processed mushroom. However the gross margins of the retailers were much higher than those of wholesalers, and were Tk. 800.00 / kg for fresh and Tk. 17.50 /can for processed button mushrooms. The corresponding net margins of the wholesalers were Tk. 340.00 / kg for fresh and Tk. 4.35/can for processed, and the retailers' net margins were estimated to be Tk. 639.85/kg for fresh and Tk. 8.79/can for processed button mushrooms. According to the consumers' opinion, they like mushroom for "Taste as Meat" followed by "Nutritious Food." Major problems were related to complexity of taking loan, lack of consumers, short shelf life, varietal colour, lack of processing facilities, quick spoilage of raw mushroom, religious taboo, ignorance, lack of knowledge on edible mushroom and lack of knowledge on cooking. The wholesalers and retailers also gave some suggestions for improving marketing system of button mushroom. Their views in this regard were to provide institutional credit, in country production and establishment of processing industry, build up awareness among the consumers for its nutrition, delicacy and therapeutic properties by government campaign.

Key words: Costs, Export, Import, Marketing, Margins, White button mushroom.

INTRODUCTION

The economy of Bangladesh is mainly agro-based. The country enjoys a salubrious climate without the extremes of either summer or winter. Nearly all the arable areas of the country have been brought under the plough and further agricultural expansion is almost impossible. On the other hand the unemployed population in our country is 2.2 million,

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out of which 0.8 million is female (Anon., 2002). Mushroom cultivation is labor intensive but land saving short duration crop, can be welcomed by the poor farmers of Bangladesh, as it is a profitable agribusiness.

In Bangladesh, people have been suffering from malnutrition. Mushrooms can solve the problem of malnutrition to some extent as it is a rich source of protein, vitamin and minerals. Besides these mushrooms possess medicinal properties which can prevent cancer and tumor formation, reduced blood cholesterol, control diabetes, ulcer and lungs diseases (Quimio, 1976). *Agaricus bisporus* (Large) is popularly known as white button mushroom has the widest acceptability as a food and extensively cultivated throughout the world and contributes about 40% of the total production of mushrooms (Flegg, 1992).

At present there is a large demand of white button mushrooms in Thai and Chinese restaurants, International hotels and other restaurants of our country. Moreover, about 1.1 million people of hilly areas consume mushroom from ancient time (Annual report, 2011). The rest of demand is fulfilled by imported fresh and canned button mushroom. For that yearly large amount of white button mushroom have been imported from China, Singapore, Oman, as fresh and canned. Bangladesh imports about 50 MT (EPB, 2010) of white button mushroom every year and this will be increased day by day as because the demand for button mushroom will continue to increase. For saving foreign currency as well as export potential it is very essential to cultivation button mushroom in commercial scale. But large-scale production is also dependant on its market demand and marketing facilities.

Several works have been done in different parts of the world on mushroom marketing. Alice Beetz & Lane Greer (1999) observed that the market for mushrooms continues to grow due to interest in their nutritional and health benefits. Their potential to serve in waste management has yet to be fully explored. The evaluation of a commercial mushroom enterprise should include a careful analysis of potential markets. Olumide and Oseni (2007) stated that the economics of mushroom marketing as a copping strategy for reducing poverty in Ondo state of Nigeria.

But in Bangladesh there is little or no organized research on mushroom marketing has been conducted. The present study is a modest attempt to find out problems of button mushroom marketing and which will fulfill the objectives of finding out the existing marketing channels of imported white button mushroom, the import cost, buying and selling prices, marketing cost and margins of importers and intermediaries, and identify the problems of white button mushroom marketing in Bangladesh and suggest some measures for its improvement.

MATERIALS AND METHODS

The methodology is related to selection of the study area, sample size and sampling techniques, preparation of survey schedule, collection of data, period of data collection, analysis of data and organization of the study. On the basis of

available information, Dhaka city corporation area was chosen for white button mushroom marketing of importers (fresh and canning) as wholesalers and retailers. A total of 48 intermediaries (8 importers cum wholesalers, 25 super shops, departmental store and other shops as retailer and 15 Chinese restaurants) involved in button mushroom marketing were selected from Dhaka. Apart from that 48 consumers were purposively selected to know their views on button mushrooms and their marketing.

Primary survey was conducted using interview schedule. The interview schedules for the respondents (importer cum wholesalers, retailers and consumers) were prepared keeping the objectives of the study in view. The period of data collection was during the period from January-July 2010. The researcher collected primary data through face to face interview.Apart from primary data, some secondary data were also collected from plant protection wing, Department of Agriculture Extension, Khamarbari, Dhaka.

The filled interview schedules were scrutinized and the collected data were edited to remove errors, inconsistencies and ambiguities. After necessary editing the collected data were then transferred to master sheets and summarized to facilitate tabulation. List of tables were prepared in accordance with the objectives of the study. Tabular technique was mainly followed for analysis of data. Average and percentages were used to interpret the results of the study.

RESULTS AND DISCUSSION

Marketing System of White Button Mushroom: Agriculture marketing is defined as the performance of all business activities involved in the flow of goods and services from the points of initial agriculture production until they are in the hands of the ultimate consumer (Khals and Uhl, 1972). Three functionaries found to be involved in marketing of white button mushroom in the study area, and they were importers as wholesalers, retailers and consumers. Marketing channels may be direct that is from grower to consumer or direct passing through several intermediaries. In this study button mushroom was imported and from importer to consumer passes through different traders.

Table 1. Imported white button mushroom in Bangladesh

Imported year	Amount imported (MT)
2007-08	00.61
2008-09	19.00
2009-2010	45.60

Source: DAE, 2010.

Amount of Button Mushroom Imported and It's Buying and Selling Price: According to the data collected from Plant Protection Wing (Quarantine unit),

Department of Agriculture Extension, Khamarbari, Dhaka, imported 0.61, 19.0 and 45.60 MT of white button mushrooms (Fresh and canned) from China, Singapore and Oman in the years 2007-08, 2008-09, and 2009-10 respectively (Table 1). In the present research, among the respondents, five canned mushroom importers imported 5500-7500 cans per month. Each can have the weight of 425 g, containing 200 g of button mushrooms. Three fresh mushroom importers imported 750-900 kg of fresh mushroom per month. Each small package (plastic foil) contains 250 g of fresh white button mushroom that was imported as ready packaging form. Buying price (including cost, insurance and freight) of imported (wholesalers) white button mushroom was Tk. 900-1000 and Tk. 65-75 per kg of fresh and canned white button mushroom, respectively. The selling price of the importers cum wholesalers to the retailers' (Chinese restaurants, Super shops, Departmental stores) was Tk. 1400-1500 and Tk. 75-90 per kg of fresh and per can, respectively (Table 2). Buying price of fresh white button mushroom of retailers was Tk. 1400-1500/kg and it was Tk. 75-90/can of canned white button mushroom. Their selling prices were Tk. 1800-2250/kg fresh (prepared Chinese dish) and Tk. 90-110/can, respectively. Retailers (Super shops, Departmental stores and others shops) sell canned mushroom at the Chinese restaurants, hotels and individuals. Furthermore, Chinese restaurants and hotels bought their fresh mushroom generally from importers (wholesalers) but Chinese restaurant and hotel serve themselves as retailers.

Table 2. Market scenarios of button mushroom in Bangladesh

Number of importer		Amount imported		Buying price of importers		Buying price of retailer		Selling price of retailers	
Fresh mushroom	Canned mushroom	Fresh mushroom (kg/month)	Canned mushroom (N / month)	Fresh mushroom (Tk./kg)	Canned mushroom (Tk./Can)	Fresh mushroom (Tk./kg)	Canned mushroom (Tk./can)	Fresh mushroom (Tk./kg)	Canned mushroom (Tk./Can)
3	5	750-900	5500-7500	900-1000	65-75	1400-1500	75-90	1800-2250	90-110

Marketing Cost and Margins:

Marketing cost: The cost which incurred to move the product from the producer to the consumer is known as marketing cost (Kohls, 1972). Marketing margin is the difference between the amount of consumers pay for the final product and the amount producers received. This margin includes all the costs incurred for moving the product to the consumption centers.

Marketing cost of wholesalers and retailers: Wholesalers purchased white button mushroom from China, Singapore and Oman through import processing and sold to the retailers and consumers. So, wholesalers were also involved the various marketing functions. Item wise breakup of total marketing cost of wholesalers have been shown in Table 3.The marketing cost incurred by wholesalers were Tk. 120/kg and Tk. 8.15/can for fresh and canned mushrooms, respectively. The cost for taxes was the highest (37.5%) for the wholesalers for

fresh mushroom. The others cost incurred by wholesalers were transportation (8.33%), personal expenses (25%) and others (12.5%). The marketing cost incurred by wholesalers for canned mushroom were 38.66, 24.53 12.26, 6.13 12.26 and 6.13% for taxes, transportation, labour charges, commission for broker, personal expenses and others, respectively. The total marketing cost incurred by retailers was Tk. 160.15/kg and Tk. 8.71/can for fresh and canned mushrooms, respectively. Tax was found to be the highest cost item comprising 40.75 and 42.59% of total the cost of marketing for fresh and canned button mushrooms, respectively. The other costs incurred by retailers were transportation (3.12 and 5.74%), labour charges (12.48 and 11.48%), commission of broker (0 and 5.74) personal expenses (31.22 and 28.70%) and others cost (12.48 and 5.74%) for fresh and canned button mushroom respectively (Table 3).

Table 3. Marketing cost of intermediaries

Cost items	Intermediaries			
	Fresh (Tk/kg)		Can (Tk/kg)	
	Wholesaler	Retailer	Wholesaler	Retailer
Taxes	37.5	40.75	38.65	42.59
Transportation	8.33	3.12	24.53	5.74
Labour charges	8.33	12.48	12.26	11.48
Commission of broker	8.33	-	6.13	5.74
Personal expenses	25	31.22	12.26	28.70
Others	12.5	12.48	6.13	5.74
Total (%)	**100**	**100**	**100**	**100**

Figure in the parenthesis indicate percentage.

Marketing margins of wholesalers retailer for canned mushroom: The wholesalers purchased white button mushroom at the Tk.70 per can mushroom and sale same at Tk. 82.50. So the gross margin for wholesalers Tk.12.50 per can. Total marketing cost incurred by wholesalers was Tk. 8.15 per can and therefore the net margin for wholesalers was Tk. 4.35 while the percentage of margin of wholesalers was 5.15%. The retailers purchased white button mushroom at Tk. 82.5 per can and sold the same at Tk.100. So per can gross margin for retailers were Tk.17.5. As they incurred a marketing cost of Tk. 8.71 per can. They earned net margin of Tk. 8.79. The percentage of margin of the retailers was the highest (Tk. 8.79%) among the intermediaries because their sale price was the highest (Table 4).

Table 4. Marketing margin of intermediateries of canned mushroom (Price in Taka)

Intermediaries	Average sale price	Average purchase price	Gross margin	Marketing cost	Net margin	Percentage of margin
Wholesaler	82.50	70.00	12.50	8.15	4.35	5.27
Retailer	100	82.50	17.50	8.71	8.79	8.79

Marketing margin of wholesaler and retailer for fresh mushroom: The wholesalers purchased fresh mushroom at Tk. 950 per kg and sold the same at the Tk. 1450. So the gross margin for wholesalers was the Tk. 500 per kg. Total marketing cost incurred by wholesalers was Tk. 120 per kg of fresh mushroom and therefore the net margin for wholesalers was Tk. 380. The percentage of margin was 26.20% which was much higher than the percentage margin of canned mushroom. The retailers purchased white button mushroom at Tk.1450/kg and sold the same at Tk. 2250 / kg (as Chinese dishes). So per kg gross margin for retailers was the Tk. 800. As they incurred marketing cost was Tk. 160.15/kg. They earn net margin Tk. 639.85 which was higher than wholesaler's net margin. The percentage of margin of retailer was Tk. 28.43% (Table 5) which is also higher than wholesaler percentage, because the fresh mushroom sold as Chinese soup, curry dish, salad and others (Table 5).

Table 5. Marketing margin of intermediateries of fresh mushroom (Price in Taka)

Intemediateries	Average sale price	Average purchase price	Gross margin	Marketing cost	Net margin	Percentage of margin
Whole saler	1450.00	950.00	500.00	120.00	380.00	26.20
Retailer	2250.00	1450.00	800.00	160.15	639.85	28.43

Gross margin=sale price–purchase price, Net Margin=Gross Margin-Marketing cost, Percentage Margin=Net Margin/Average sale price x 100.

Relationship between number of can purchased and net margin of retailer per month: The relationship between numbers of can purchase and net margin of retailer per month of white button mushroom was positive and linear and could be expressed by the equation, $y = 5.714x + 916.30$ ($R^2 = 0.637^{**}$) (Fig. 1). The regression equation stated that the net margin increased gradually at the rate of Tk. 5.714 per unit change of number of can purchased per month. The R^2 value indicated that 63.70% of net margin was attributed to number of can purchase per month.

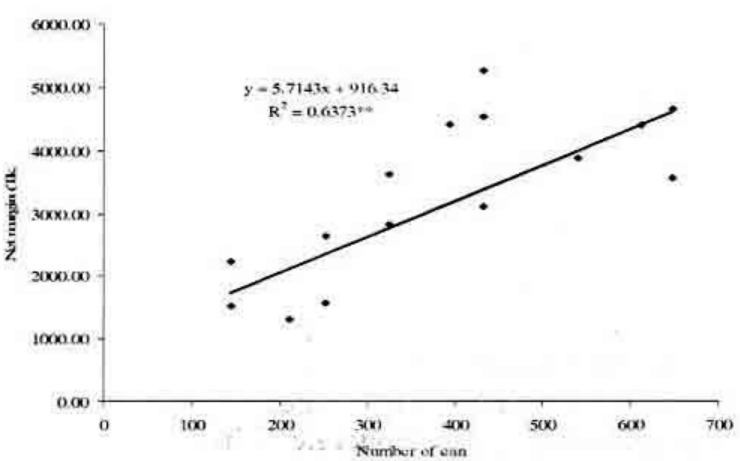

Fig. 1. Relationship between numbers of can purchased per month and net margin of white button mushroom of retailers.

Relationship between amounts of sold fresh mushroom and net margin of retailer per month: The relationship between amounts of sold fresh mushroom and net margin of retailer per month of white button mushroom was insignificant and could be expressed by the equation, $y = 148.30x + 71.76$ ($R^2 = 0.501^{NS}$) (Fig. 2).

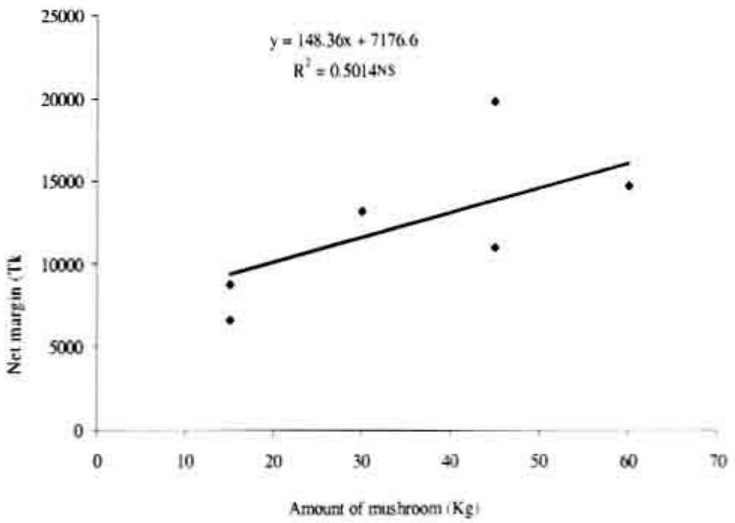

Fig. 2. Relationship between amount of sold fresh mushroom and net margin per month of white button mushroom of retailer.

Choice for white button mushroom: Mushroom consumption as food had been started very recent as when Chinese restaurant established in Dhaka city and as well as mushroom project started at Saver. It is found that the consumers preference in button mushroom were, feeling as meat, nutritional value, medicinal value, tastiness and others were 37.50, 29.20, 22.90, 6.24, and 4.16% respectively (Table 6).

Table 6. Choice of button mushroom by the consumers

Reasons for choice	Number of consumer	Percentage
Nutritive values	14	29.20
Medicinal values	11	22.90
Tastiness	3	6.24
Feeling as meat	18	37.50
Others	2	4.16

Problems Faced by Intermediaries: Problems faced by intermediaries during white button mushroom marketing are grouped in financial, marketing, technical and social problem with rank great, moderate, minimum and "not at all" problem (Table 7). The problems faced by intermediaries described as below.

Financial problem: Inadequate capital for intermediaries was reported to be moderate problems in this study of mushroom marketing. Among the 48 respondents 14.58, 35.36 20.80 and 29.14% stated inadequate capital as high, moderate, minimum and 'not at all', respectively. In the case of complexity of loan, higher percentage was in great rank (50%), and 31.35 and 16.65% for moderate and minimum rank, respectively (Table 7). In great rank, percentage was 52 and 31.35, 16.65 for the moderate and minimum rank, respectively.

Marketing problem: In button mushroom marketing in Bangladesh 52% intermediaries face great consumer problem where 25% and 16.65% face moderate and minimum consumer problem for marketing and 6.25% intermediaries face no problem at all. Fluctuation of price was also a problem of marketing. Highest opinion was on moderate ranking (31.25) followed by great (25%), moderate (23%) ad not at all (20.83%) (Table 7).

Table7. Problems of button mushroom marketing in Bangladesh and their status

	Problems	No. of interviewee	Status of problem							
			Great		Moderate		Minimum		Not at all	
			No.	%	No.	%	No.	%	No.	%
Financial	Inadequate capital	48	7	14.58	17	35.36	10	20.8	14	29.14
	Complexity of loan	48	25	52	15	31.35	8	16.65	-	
Marketing	Consumer	48	25	52	12	25	8	16.65	3	6.25
	Fluctuation of selling price	48	12	25	15	31.25	11	22.92	10	20.83
Technical	Shelf life	48	26	54.16	19	39.58	3	6.26	-	
	Varietals colour	48	28	58.33	15	31.35	5	10.42	-	
	Processing system	48	20	41.66	15	31.25	10	20.84	3	6.25
	Packaging	48	10	20.83	12	25	23	47.92	3	6.25
	Spoilage of raw mushroom	48	30	62.50	15	31.25	3	6.25	-	
Social	Religious taboo	48	18	40	10	20.84	10	20.84	7	15.55
	Ignorance	48	28	58.33	15	31.25	5	10.42	-	
	Lack of knowledge of edible mushroom	48	25	52	10	20.84	8	16.65	5	10.41
	Lack of knowledge of cooking	48	28	58.33	12	25	5	10.42	5	6.25

Technical problem: Shelf life of button mushroom was the major problem in mushroom marketing. Highest was in great rank (54.16%) which followed by moderate (39.85%) and minimum rank (6.26%).Varietals was colour also the major problem in mushroom marketing. Intermediaries expressed their opinion, 58.33% under great rank and other opinion was moderate (31.25%), minimum (10.42%). In case of processing system problem, 41.66%, 31.257%, 20.84% and 6.25% were under great, moderate, minimum and not at all rank respectively. Packaging was not a problem in button mushroom marketing. The highest was under rank minimum (47.92%). Spoilage of raw mushroom during marketing was

major problem. The highest was 62.50% under great rank followed by 31.25% and 62.25% for moderate and minimum rank respectively (Table 7).

Social problem: Religious taboo was a problem for mushroom marketing. In case of religious taboo the highest opinion was 40% under great rank which was followed by 20.84%, 20.84% 15.55% under rank moderate, minimum and not at all respectively. In case of ignorance problem highest opinion was 58.33% under great rank which was followed by 31.25% and 10.42% under moderate and minimum rank respectively. Lack of knowledge about edible mushroom was also a marketing problem which was observed during this study (Table 7).

Suggestion to Minimize Problems Faced by Intermediaries: To overcome the problems and to make button mushroom demandable, profitable, and also to encourage the farmers to grow button mushrooms, number of suggestion were made by the respondents and are summarized in the following (Table 8).

Table 8. Measures Suggested by intermediaries

Suggestions	No. of respondent	%	Rank
Easy terms of institutional credit	48	100	1
In country production of button mushroom	48	100	1
Transportation and communication system	37	77	3
Establishment of processing industry	42	88	2
Publicity through Government institutions	48	100	1

Social problem like religious taboo, ignorance, lack of knowledge on edible mushroom and its cooking methods were the major problems for button mushroom marketing. For minimizing these problems, publicity through government institutions about mushroom food value, tastes and its various other medicinal values, is essential, and this was supported by all the respondents. Government and also non-government organizations, mass media like newspapers, television, radio, etc. can play vital role to popularize mushroom as a vital food for better and sound health of the country.

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Yield Performance of Oyster Mushroom Variety at Different Locations in Bangladesh

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Abstract

Investigations on the performance of seven oyster mushroom variety such as *Pleurotus ostreatus* (PO-2), *Pleurotus ostreatus* (WS) *P. djmour* (POP-1 & POP-2), *P. salmoniostraminus* (PSS) and *P. florida* (FLO-1 & FLO-2) with five locations like Dhaka, Dinajpur, Rangamati, Faridpur and Jessore were carried to find out the growth and yield performance. Considerable variations on different parameters related to yield and yield attributes were recorded. The minimum days required from opening to harvesting (2.67 days), was observed from the treatment combination Savar and PO-2. The maximum days (53.00) required from opening to first harvest were observed from Faridpur with WS variety. The highest number of fruiting body (51.75) was observed from the treatment combination Savar and PSS variety. The lowest number of fruit body was observed in Faridpur and FLO-1. The highest yield (151.30g) and biological efficiency (75.63%) were found in Dinajpur with the strain of PSS followed by Dinajpur and POP-2 and the lowest yield (50.00) and biological efficiency were found at Rangamati with the strain of FLO-1. Results suggested that all varieties are not suitable for all locations or each variety is suitable for specific location.

Key Words: Growth, Oyster mushroom, Strain, Yield.

INTRODUCTION

Oyster mushrooms are ideally suitable for cultivation under both temperate and tropical climatic conditions, oyster mushrooms are cultivated and harvested all over the year (Amin *et al*, 2007).The oyster mushroom is one of the useful mushrooms and can easily be grown, if proper requirements of food and humidity are maintained for its growth (Amin *et al*., 2007). In the life cycle of *pleurotus* mushrooms there are two stages; the vegetative stage and reproductive growth stage. Generally some kinds of stimuli are needed for the shift from mycelial (Vegetative) growth to the fruiting body formation (reproduction) phase. These stimuli include abrupt changes in temperature, humidity, gas concentration, light and nutrient reserves etc. Mushroom species requiring light for primordia formation are said to be photosensitive. Although light in not necessary to induce fructification in all mushrooms, certain spectra have proven to be stimulatory to pinhead initiation and are critical for the normal development of the fruiting body. *Pleurotus ostreatus* is a photosensitive species. Pinning induction includes cold shock, watering and lighting. Once the pins come out, growers stop pinning induction and maintain environmental conditions that are favorable to fruiting. *Pleurotus ostreatus*

needed 85 to 90% relative humidity and 1000-1500 lux light for fruiting (Stamets, 1993). As the growing room temperature is raised, relative humidity decreases.

The mushroom cultivation is a profitable agribusiness and oyster (*Pleurotus ostreatus*) mushroom is an edible mushroom having excellent flavour and taste (Shah *et al.*, 2004). Growing oyster (*Pleurotus ostreatus*) mushrooms is becoming more popular throughout the world, because of their abilities to grow at a wide range of temperatures utilizing various ligno–cellulose substrates (Khan and Garcha 1984). Environmental factors such as temperature, O_2, CO_2, humidity, light and pH have been reported also to affect mycelia growth in the spawn preparation (Nwanze *et al.*, 2005). Optimization of industrial mushroom production depends on improving the culture process (Larraya *et al.*, 2003). A range of abiotic parameters including temperature, light, carbon dioxide concentration, humidity and pH have been shown to influence carpophore production (Wessels *et al.*, 1987). Nutrient and other factors that affect mushroom growth include moisture content, temperature, pH and light intensity (Stamets, 1993; Kadiri and Kehinde, 1999). The environmental factors vary from location to location. The present study was designed to evaluate the growth and yield performance of different oyster mushroom (*Pleurotus* spp.) varieties at different locations of Bangladesh.

MATERIALS AND METHODS

A 7x5 factorial experiment in completely randomized design with 4 replications was conducted at different locations during the period from March to June 2012. The first factor was different locations, the second factor was different oyster mushroom strains / variety. Five locations viz. National Mushroom Development and Extension Centre, Savar, Dhaka, Mushroom sub-centre Dinajpur, Mushroom sub-centre Rangamati, Mushroom sub-centre Faridpur, Mushroom sub-centre Jessore and seven oyster mushroom variety namely PO-2, POP-1,POP-2,PSS, WS, FLO-1, FLO-2. The factors were tested in different combinations.

Preparation of Pure Culture: Pure culture of above mentioned variety (tested variety) were prepared on Potato Dextrose Agar (PDA) medium containing infusion of 200g of peeled and sliced potato, 20g of dextrose and 20g of agar. The mixture was boiled on gas burner until the agar dissolved. The medium was poured into test tube (30ml) at 10 ml/tube. The medium in test tube was sterilized in an autoclave for 20 minutes at 120°C under 1.1 kg/cm^2 pressure. After sterilization and solidification, the tubes were inoculated with the inocula of the test materials. Pieces of inner tissues of joint of stalk and pileus were used as inocula. A fresh and full grown sporophore of oyster mushroom was surface-sterilized with 70% ethanol by rubbing cotton soaked in alcohol. The stalk was peeled from outside. Tissues were collected from inner region of the sporophore. The tissues were cut into small pieces and placed on the solidified test tube containing PDA. After inoculation, the tubes were covered with cork. All operations were done under sterile condition in a clean bench. The inoculated tubes were transferred to a growth chamber maintaining temperature at 20-25°C and incubated for 8-15 days. Then pure culture was used for inoculation of mother culture.

Preparation of Mother Culture: Mother culture was prepared by mixing sawdust and wheat bran at the ratio of 2:1. Calcium carbonate was used at the rate of 0.2% of the mixture. The moisture level of the mixture was maintained at 60% by adding tap water. Polypropylene bags of 18 cm × 25 cm size were filled with 300 g of the above prepared mixture and packed tightly. The neck of the bag was prepared by using heat resistant plastic neck. A hole of about 2/3 deep of the volume of the bag was made at the center with a sharp end stick for space to put inoculums. The neck was plugged with cotton and covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for one hour at 120°C under 1.1 kg/cm^2 pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. A piece of pure culture medium containing mycelium of different oyster mushroom variety according to treatments were placed aseptically in the hole of mother culture packet and the packet was again plugged as mentioned before. Then the inoculated packets were placed on a wooden rack in the laboratory at 25 ± 2°C temperature for incubation. The medium of the mother culture was colonized by the strains as manifested by white colony growth of mycelium within 10-20 days of inoculation. The fully colonized packets were used for spawning.

Spawn Packets Preparation: The substrate of spawn packets were prepared using sawdust and wheat bran mixture at the ratio of 2:1 (dry weight/weight basis). Water was added to make the moisture content at 60% and $CaCO_3$ was added at the rate of 0.2% (w/w) of the total mixture to maintain the pH level at 6.5 to 7.0. Polypropylene bags of 18cm x 25 cm were filled with 500 g of prepared substrate. The packets were tied, plugged with absorbent cotton and covered with brown paper. Then the packets were sterilized in an autoclave for 2.0 hour at 121°C under 1.1 kg/cm^2 pressure. After sterilization the packets were cooled and transferred to an inoculation chamber and inoculated with the mother culture of test materials at the rate of one teaspoonful per packet. The inoculated packets were placed on a still rack at 25 ± 2°C temperature for incubation.

Experimental Condition: The packets were kept in a dark room at 25°C for incubation. When colonization of mycelium was completed, the spawn packets were taken to a culture house and were opened by 'D' shaped cut on the shoulder and removed the sheet. The relative humidity and temperature of the culture house were maintained at 80-90% and 20-25°C respectively by spraying water. Diffused light, about 200 lux and proper ventilation in culture house were maintained. After harvesting of mushroom, the residues were removed from the packet and temperature and relative humidity were maintained as before. The yield was obtained from single, flush in the harvest period. Yield in g/packet was recorded by weighing all the fruiting bodies in a packet after removing the lower dirty portion. The biological efficiency was determined using the following formula:

$$\text{Biological efficiency}(\%) = \frac{\text{Total biological yield (g / packet)}}{\text{Total dry weight of the substrate used (g / packet)}} x100$$

Data Collection and Statistical Analysis: The experiment was laid out following completely randomized design (CRD) with 4 replications. Data on days required from opening to first harvest, number of fruiting bodies, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, days required to harvesting, yield, and biological efficiency were recorded and analyzed following Gomez and Gomez (1984) using MSTAT-C computer program. Means separation were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Main Effect of Locations: Main effect of locations on different parameters is shown in Table 1. Ranges of days required from opening to first harvest, stalk length and diameter, pileus diameter and thickness, fruit number and yield of mushroom per packet under different treatments with locations were 4.96-17.16, 2.30-4.17.cm and 0.63- 3.11 cm , 4.73 -20.61 cm and 0.37-1.47cm, 6.04-30.86 / packet and 45.79-104.50 g / packet, respectively. The differences in every parameter under all treatments were significant. The lowest stalk length of 2.30 cm was recorded in NAMDEC, Savar, which was statistically similar to Dinajpur and Faridpur sub-centre. The maximum stalk length of 4.17 cm was recorded from Jessore sub-centre followed by Rangamati. The lowest pelius diameter of 4.73 cm was found in Faridpur sub-centre. Maximum pileus diameter of 20.61 cm was found in Rangamati. Significant variation in days required from opening to first harvest was achieved with all locations. The maximum days were observed in Faridpur followed by Savar and the lowest days were obtained in Jessore sub-centre. Biological efficiency (BE) of mushroom growing substrate varied from 22.89 to 52.23% under different treatments. The lowest BE was recorded from Faridpur. Significantly the highest BE was achieved from Dinajpur followed by Savar (Table 1).

Table 1. Effect of location on growth and yield of oyster mushroom variety

Locations	Required days from opening to first harvest	Number of fruit body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield per packet (gm)	Biological Efficiency (%)
Savar	8.95b	24.70b	2.30b	0.72bc	5.91bc	0.37d	75.38b	37.69b
Dinajpur	4.64c	30.86a	2.70b	1.18b	7.20b	0.67b	104.5a	52.23a
Rangamati	7.39	17.18c	3.69a	3.11a	20.61a	1.47a	54.25c	27.13cd
Faridpur	17.16a	14.66c	2.49b	0.63c	4.73 c	0.57bc	45.79c	22.89d
Jessore	4.96 c	6.04 d	4.17a	0.74bc	5.16 c	0.46cd	69.22b	34.61bc
CV (%)	**13.35**	**21.81**	**20.24**	**24.44**	**13.91**	**14.68**	**14.95**	**15.76**

In a column do not differ significantly at 5 % level according to DMRT.

Main Effect of Different Oyster Mushroom Variety: Main effect of the factor, different variety on various growth, yield and yield attributes of oyster mushroom is shown in Table 2. It was found that ranges of days to first harvest, stalk length, stalk diameter, pileus diameter, pileus thickness, number of fruiting body per packet, yield and biological efficiency of substrate were respectively 4.36-16.80, 1.79-3.69 cm, 1.04-1.61

cm, 7.62-9.35 cm, 0.62-0.84 cm, 11.90-25.00, 52.43-95.41 g / packet and 26.22%-47.41%. Difference in every parameter under the factor significant. The lowest stalk length of 1.79 cm was recorded in PSS variety, which was statistically different to other variety. The maximum stalk length of 3.69 cm was recorded in FLO-1 which was statistically similar to other variety except PSS. The lowest pelius diameter of 7.62 cm was found in PO-2 while maximum pileus diameter of 9.38 cm was found in FLO-2 and this variation was not significant. Significant variation in days required from opening to first harvest was achieved with all varieties. The maximum days were observed in WS followed by FLO-2 and the lowest days were obtained in POP-1. The lowest BE was recorded from FLO-1. Significantly the highest BE was achieved from PSS variety followed by POP-2 (Table 2).

Table 2. Yield contributing characters and yield of different oyster mushroom variety

Oyster mushroom variety	Required days from opening to first harvest	Number of fruit body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield per packet (gm)	Biological Efficiency (%)
PO-2	8.61c	14.76cd	3.42a	1.29ab	7.62a	0.63b	60.33bc	30.16bc
POP-1	3.81d	20.07bc	3.33a	1.19ab	9.35a	0.84a	73.75b	36.88b
POP-2	4.36d	25.00ab	2.93a	1.04b	8.71a	0.72ab	74.30b	37.15b
PSS	4.75d	29.48a	1.79b	1.24ab	7.98a	0.62b	95.41a	47.71a
WS	16.80a	14.72cd	2.90a	1.23ab	8.68a	0.75ab	64.25bc	32.13bc
FLO-1	8.15c	11.90d	3.69a	1.61a	9.34a	0.74ab	52.43c	26.22c
FLO-2	13.88b	14.89cd	3.44a	1.35ab	9.38a	0.66b	68.26c	34.13bc
CV (%)	**13.35**	**21.81**	**20.24**	**24.44**	**13.91**	**14.68**	**14.95**	**15.76**

In a column do not differ significantly at 5 % level according to DMRT.

Interaction Effect of the Factors Locations, and Oyster Mushroom Variety: The analysis of variance for days to first harvest, number of fruit body, stalk length, stalk diameter, pileus diameter,and thickness of pileus of oyster mushroom indicated significant differences between the locations and variety (Table 3).

Days Required to First Harvest: The minimum days (2.67) required from opening to first harvest was observed from the treatment combination of NAMDEC, Savar and PO-2.The maximum days (53.00) required from opening to first harvest were observed from Faridpur and WS variety which was statistically dissimilar to other treatment combinations.

Number of Fruiting Body: The number of fruiting body under different treatment combinations varied significantly (Table 3). The highest number of fruiting body was observed from the treatment combination Savar and PSS variety (51.75).The lowest number of fruit body was observed in Faridpur and FLO-1.

Size of Fruiting Body: The length of stalk ranged from 1.50 to 6.50 cm with significant difference (Table 3). The highest length of stalk was found in FLO-1 when cultivated at Rangamati (6.50 cm) which was statistically identical to the treatment combination

Jessore and PO-2. The lowest length of stalk was found from the treatment combination Dinajpur and PSS variety (1.50).The diameter of stalk differed significantly and ranged from 0.55 to 4.63 cm (Table 3). The highest diameter was found in Rangamati and FLO-1 variety (4.63 cm) followed by Rangamati and FLO-2 (.3.53 cm) while it was lowest (0.55 cm) in .Savar and WS variety.

Table 3. Interaction effect of location and different oyster mushroom variety

Treatment combination		Required days from opening to first harvest	Number of fruit body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield per packet (gm)	Biological efficiency (%)
PO-2	Savar	6.63hij	19.75e	2.69ghij	0.73ghij	5.06ghij	0.30mn	50.38ijk	25.19kl
	Dinajpur	6.00ijkl	23.00e	3.25efg	1.29f	8.13de	0.71efg	92.50bcd	46.25bcd
	Rangamati	7.00hi	11.25ghi	2.88fghi	3.13bc	14.75c	1.23d	42.50jkl	21.25lm
	Faridpur	17.17d	11.52ghi	2.29ghijk	0.71ghij	4.64hij	0.63efghi	41.25kl	20.6lm
	Jessore	6.25ijk	8.25ij	6.00a	0.58j	5.50ghij	0.28n	75.00efg	37.50efg
POP-1	Savar	2.67n	32.58d	2.19ghijk	0.90fghij	5.99fghij	0.55ghij	47.08bcd	32.58d
	Dinajpur	4.00mn	30.00d	2.75ghi	1.21fg	7.88def	0.73efg	96.25bcd	48.13bcd
	Rangamati	5.00jklm	16.50efgh	4.63bcd	2.43d	23.25a	1.85a	50.00ijk	25.00kl
	Faridpur	4.13lmn	17.77efg	2.23ghijk	0.77fghij	4.62hij	0.56ghij	54.17hijk	27.09jkl
	Jessore	2.67n	3.50j	4.85bc	0.63ij	5.00ghij	0.50hijk	74.18efg	37.09efg
POP-2	Savar	4.25lmn	39.88bc	1.88ijk	0.61j	5.63ghij	0.33lmn	88.88cde	44.44cde
	Dinajpur	4.00mn	41.50bc	2.88ghi	1.18fgh	6.63defgh	0.68efgh	107.5b	53.75b
	Rangamati	5.00jklm	19.00ef	4.10cde	1.98e	22.00ab	1.58b	50.00ijk	25.00kl
	Faridpur	5.05jklm	20.88e	1.81ijk	0.56j	4.79ghij	0.52hijk	52.13ijk	26.06kl
	Jessore	3.50mn	3.75j	4.00cde	0.85fghij	4.50ij	0.48ijkl	73.00efg	36.50efgh
PSS	Savar	4.38klmn	51.75a	1.81ijk	0.66hij	5.31ghij	0.35klmn	100.9bc	50.44bc
	Dinajpur	4.00mn	43.00b	1.50k	1.18fgh	6.25efghij	0.43jklmn	151.3a	75.63a
	Rangamati	5.00jklm	31.50d	1.98hijk	2.88c	21.25b	1.38c	100.0bc	50.00bcd
	Faridpur	6.60hij	17.65efg	1.64jk	0.57j	4.71hij	0.52hijk	42.00kl	21.00lm
	Jessore	3.75mn	3.50j	2.00hijk	0.93fghij	2.38k	0.45jklm	82.93def	41.46def
WS	Savar	14.19e	11.35ghi	2.54ghijk	0.55j	5.73ghij	0.33lmn	66.97fghi	33.49fghijk
	Dinajpur	4.00mn	36.00cd	2.63ghij	1.00fghij	5.25ghij	0.74ef	103.8bc	51.88bc
	Rangamati	8.25gh	6.25ij	2.75ghi	3.25bc	21.50ab	1.58b	22.25m	11.13 n
	Faridpur	53.30a	12.50fghi	4.08cde	0.58j	4.78ghij	0.59fghij	46.05jkl	23.02lm
	Jessore	4.25lmn	7.50ij	2.50ghijk	0.78fghij	6.13fghij	0.53hijk	41.13def	7.50ij
FLO-1	Savar	11.63f	6.63ij	2.20ghijk	0.95fghij	6.81defg	0.41jklmn	55.63hijk	27.81hijkl
	Dinajpur	6.00ijkl	21.00e	2.05hijk	1.15fghi	8.25d	0.64efghi	72.50efg	36.25efghi
	Rangamati	7.50ghi	17.75efg	6.50a	4.63a	20.75b	1.58b	55.00hijk	27.50jkl
	Faridpur	9.10g	5.86ij	2.58ghij	0.65hij	4.36j	0.52hijk	30.17lm	15.09mn
	Jessore	6.50hij	8.25ij	5.10b	0.65hij	6.50defghi	0.58fghij	48.85jk	24.42kl
FLO-2	Savar	18.88c	11.00hi	2.81ghi	0.64ij	6.81defg	0.31lmn	70.75fgh	35.38fghij
	Dinajpur	4.50klmn	21.50e	3.83def	1.23fg	8.00def	0.78e	107.5b	53.75b
	Rangamati	14.00e	18.00 efg	3.00fgh	3.53 b	20.75b	1.13d	60.00ghij	30.00ghijkl
	Faridpur	24.77b	16.45efgh	2.83ghi	0.58j	5.21ghij	0.67efgh	54.75hijk	27.38ijkl
	Jessore	7.25hi	7.50ij	4.75bcd	0.78fghij	6.13efghij	0.43jklmn	48.33jk	24.16l
CV (%)		**13.35**	**21.81**	**20.24**	**24.44**	**13.91**	**14.68**	**14.95**	**15.76**

In a column do not differ significantly at 5 % level according to DMRT.

The diameter of pileus ranged from 2.38 cm to 23.25 cm with significant difference among the treatments (Table 3). The highest diameter of pileus was found in Rangamati and POP-1 variety (23.25 cm) followed by Rangamati and POP-2 (22.00cm) and the lowest diameter of pileus was found in treatment combination of Jessore and PSS variety

(2.38 cm).The thickness of pileus in different species differed significantly and ranged from 0.28 cm to 1.85 cm (Table 3). The highest thickness was found in Rangamati and POP-1 variety (1.85 cm) which was statistically different to other treatment combinations. The lowest thickness was found in Jessore and PO-2 variety.

Yield / Packet (g): Significant variation was observed in yield under different treatment combinations (Table 3). The highest yield (151.30g) was found in Dinajpur and PSS followed by Dinajpur and POP-2 and the lowest yield was found in (50.00) Rangamati and FLO-1.

Biological efficiency (%): Significant variation was observed on biological efficiency (BE) (Table 3). The highest biological efficiency (75.63%) was found in Dinajpur and PSS followed by Dinajpur and POP-2 and the lowest biological efficiency was found in (15.09%) Rangamati and FLO-1.

Many other investigators found variations on growth yield and yield contributing characters of oyster mushrooms. Hawlader *et al.* 2010 reported that oyster mushroom needs 3 to 17.75 days for pin head initiation. Amin *et al.* 2007 also reported that DRPI for oyster mushroom ranged from 3-4 days. This might be attributed to different environmental factors and management practices. Patra and Pani (1995) reported that oyster mushroom took 4-8 days for initiation of fruiting bodies. Shelly *et al.* 2010 observed that days required from stimulation to pinhead initiation in different species of oyster mushroom ranged from 3.25 to 10.25.

Sarker *et al.* (2008) observed that the number of fruiting body of oyster mushroom ranged from 20 to 98.25/packet on wheat straw supplemented with different levels of wheat and rice bran. Ahmed 1998 observed that incase of *pleurotus citrinopileatus* the number to primordia per packet from 6 flushes ranged form 150 to 350 while Shelly et al. described 110.50 fruit body per packet was obtained. Alam *et al.* (2007) observed that thickness of pileus ranged from 0.502 to 0.80 cm in case of oyster mushroom. Number of effective fruiting bodies and number of primordia initiation had a linear relationship. In the present study some abnormal fruiting bodies were found. Those abnormal fruiting bodies may be caused due to the presence of glucose, fructose and trehalose in the substrate, reported by Kitamoto *et al.* (1995).

Sarker *et al.* (2004) reported that the thickness of oyster mushroom was ranged from 0.53cm to 0.62 cm. Sarker *et al.* (2007) observed that diameter of pileus ranged from 4.00cm to 5.50 cm and diameter of stipe ranged form 0.70 to 0.88 cm incase of oyster mushroom. Amin *et al.* (2007) reported that in some species of oyster mushroom yield ranged from 43.00 g to 58.00 g/packet from one flush. Alam *et al.* (2007) found that the biological yield of oyster mushroom ranged from 120.60gm per packet to 221.80 gm per packet. The results clearly show that effect of location, had a positive effect on growth, yield and yield attributes of oyster mushrooms.

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Characteristics of Tray-Dried and Freeze-Dried Oyster Mushroom

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Abstract

Attempts were made to study the feasibility of tray drying and freeze drying to dehydrate the oyster mushroom (*Pleurotus* spp.) and to study the effect of various pretreatments on the drying characteristics of the dried oyster mushroom. Samples of the oyster mushroom were sun-dried after giving various washing pretreatments such as blanching and soaking in citric acid (40 g/l) with 0.5 % sodium benzoate. The time taken for drying of oyster mushroom was more in freeze drying when compared to tray drying. The time taken for drying of oyster mushroom in tray dryer was 4 hours at 60°C drying temperature where as in case of freeze drying time varied from 225 min to 300 min. The drying characteristics involving the drying rate, drying time with moisture content were also studied.

Key words: Freeze drying, Drying character, Drying rate, Oyster mushroom, *Pleurotus* Spp, Tray drying.

INTRODUCTION

Oyster mushrooms (*Pleurotus* Spp.) are rich in proteins and their mineral content is higher than meat/fish and twice that of vegetables. It contains less fat and carbohydrates. Oyster mushrooms contain approximately 90 per cent moisture and hence are highly perishable in nature. They keep respiring after harvest and many changes like browning, liquefaction, loss of moisture and texture occurs, resulting in reduced market value and acceptability. At ambient conditions mushrooms have a short shelf life of 1-2 days (Arumuganathan *et al.*, 2003).

The aim of commercial food preservation is to prevent undesirable changes in the wholesomeness, nutritive value, or sensory quality of food by economical methods which can control growth of microorganisms. Preservation of food can be accomplished by biological, chemical and physical means. Drying of mushrooms can benefit both consumers and mushroom producers. Consumers would benefit by having mushroom products that could be stored for a longer period of time making it a more convenient ingredient to be used in recipes. The mushroom producers would benefit by having a means to reduce the amount of waste from the slicing process. Drying is a relatively simple process that has been used for many years as a means to preserve the shelf life of products. Though there are other methods that can be used such as freeze-drying, the low cost of drying highly outweighs the slightly higher quality that is achieved by freeze-drying.

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Quality has acquired paramount importance internationally. Colour of dried mushroom is an important criteria for evaluating the quality. Mushroom browning during drying may be brought about by the action of polyphenol oxidase enzyme and/or by non-enzymatic maillard reactions between carbohydrates and amino acids at elevated temperatures. Polyphenol oxidase is very active with an activation energy of 48 kJ / mole between the temperatures of 30 to 50°C (Svensson, 1971). As mushrooms contain more proteins and carbohydrates, they are susceptible to browning by maillard reactions occurring between reducing sugars and amino acids. At elevated temperatures and reduced moisture, caramelisation or partial thermal breakdown of the sugar molecules, also cause browning.

Extending the shelf life of mushrooms is important to mushroom producers and consumers. Currently mushrooms, regardless of the species, can only be stored for a few days before they diminish greatly in quality. The information on the related aspects of pretreatments and method of drying to improve the shelf life of oyster mushroom is scanty. Hence, the present study is undertaken with the specific objective to determine the drying characteristics of mushroom under tray drying and vacuum freeze-drying.

MATERIALS AND METHODS

Freshly harvested oyster mushroom (*Pleurotus* Spp.) was procured from local market of Coimbatore, Tamil Nadu. Washed and cleaned mushrooms were selected for experimental studies. Pretreatment is necessary to check the discoloration during processing of mushrooms (Pruthi *et al.*, 1974 and Mudahar and Bains, 1982). The following pretreatments (Blanching, Soaking in citric acid 40 g/l with 0.5 % sodium benzoate, Fresh Control) were given for 100 g fresh mushroom samples.

Blanching was done by wrapping the fresh mushrooms in a clean white cloth, immersing in boiling water for 2 minutes then immediately the samples were immersed in cold water and draining the water by spreading on perforated cloths. For treatment 2, the fresh mushroom was soaked in 0.5 % sodium benzoate with citric acid (40 g/l) solution for 10 min. The unbound surface moisture available in the treated samples was removed by placing the samples in perforated nets with ambient air drying.

The lab model tray dryer was used in this study. The drier consists of blower, heater, plenum chamber and exhaust port. Hundred gram of fresh mushroom sample was taken and its initial moisture content was determined. The sample was kept in the trays and the trays were kept inside the drying chamber. The drying was carried out with an air velocity of 1.4 m/s. Drying air temperature was adjusted to 40°C using a thermostat. During drying, weight of the different treated samples was recorded for every 30 min until a constant weight is reached. Similar drying experiments were conducted at 50°C as well as 60°C by adjusting the thermostat. The drier was operated by a 0.5 hp electric motor and the capacity of the heating coil was 0.5 kw.

The lab model shell freezer (FD-5505) was also used in this study to conduct freeze-drying experiment. It consists of control unit, cold chamber, shell product, heater and drying chamber. The drying temperature was - 40°C and the pressure was 10 mTorr. Mushroom samples taken for freeze drying were subjected to pretreatment following air drying for removing the excess moisture available on its surface.

RESULTS AND DISCUSSION

Drying Characteristics of Oyster Mushroom: The drying behavior of oyster mushroom under tray drying is shown in figures 1, 2 and 3. From the Figures it is clear that the time taken to bring down the moisture content of mushroom from an initial value or 90.58 per cent (w. b.) to nearly 10 per cent (w. b.) was 4 hour for a higher temperature of 60°C. The time taken to reach nearly 10 per cent (w. b.) moisture under freeze-drying was considerably high, i.e. 225 min to 300 min. The following figures give the details of drying characteristics of oyster mushroom at different temperature.

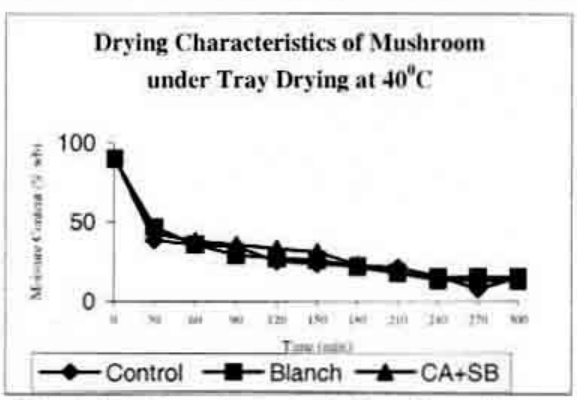

Fig. 1. Drying characteristics of mushroom with different pretreatment at 40°C.

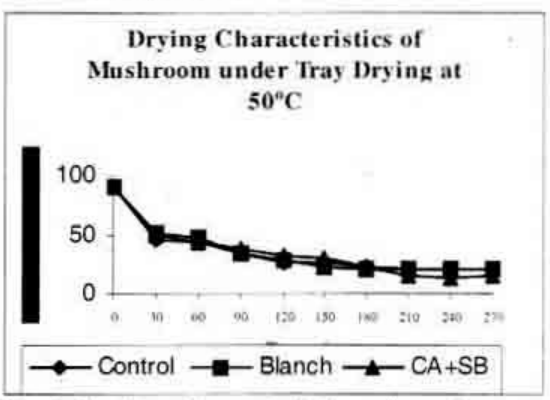

Fig. 2. Drying characteristics of mushroom with different pretreatment at 50°C.

Effect of Temperature on Drying Rate: Figures 4, 5 and 6 show the drying rate of oyster mushroom in tray drying at different temperatures. It is observed from the figures that the temperature did not have significant effect on moisture evaporation during initial stages of drying in both tray dying and freeze drying. From these results, it can be concluded that in low moisture range, drying rate was significantly higher at higher temperature. This may be due to the redistribution of internal moisture which is the drying rate determining factor in this phase as the material attains the dry bulb temperature of air during this region of drying and high temperature of material could be an accelerating factor for internal movement of moisture.

It was also observed that drying rate was maximum for higher temperature. It can also be observed that mushrooms were dried under constant rate period at the initial period of drying and falling rate period followed subsequently. In constant rate period, the rate of evaporation under any given set of drying conditions is independent of the solid and is essentially the same as rate of evaporation from a free liquid surface under the same conditions.

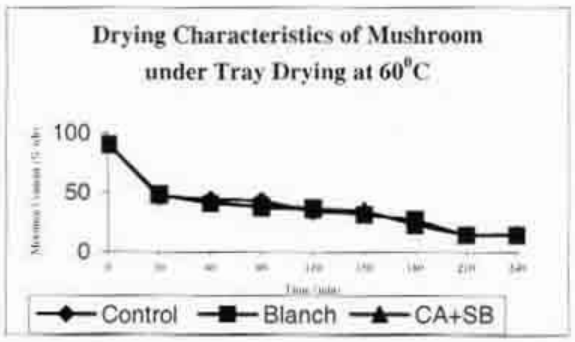

Fig. 3. Drying characteristics of mushroom with different pretreatment at 60°C.

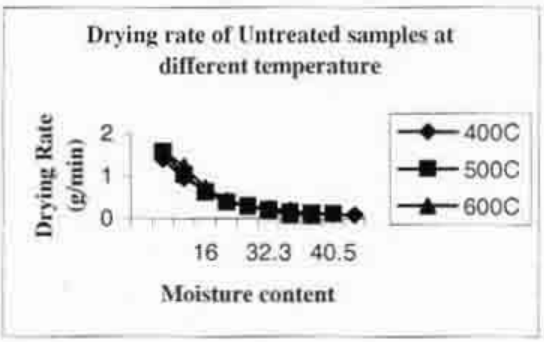

Fig. 4. Drying rate of untreated oyster mushroom at different temperatures.

Effect of Pretreatments on Drying Rate: The effect of pretreatment on drying behavior of oyster mushroom (Fig. 4, Fig. 5, Fig. 6). In all the cases, untreated samples exhibited higher drying rate than treated samples. Samples treated with sodium benzoate with citric acid showed higher drying rate than blanched samples. The slower drying rate of blanched mushrooms may be because of the moisture that would be more strongly bound to the samples. Even though blanching increases permeability of cell wall, and strong shrinkage of volume during subsequent drying, which steadily decreases the distance through which water must travel to reach the surface, the number of cell walls through which it must pass is not changing. Hence, samples received blanching treatment showed slower drying rate.

Drying time of untreated oyster mushroom was relatively less than the treated one in all cases might be due to the increased bound moisture available in the treated samples.

From this present study following conclusions are drawn: The drying rate curves showed a characteristics constant rate period and falling rate period in tray drying and the drying rate was less in freeze drying. The time taken for drying of oyster mushroom was more in freeze drying when compared to tray drying. The time limit for drying of oyster mushroom in tray dryer was 4 hours for 60°C similarly for freeze drying the drying time varied from 225 min. to 300 min.

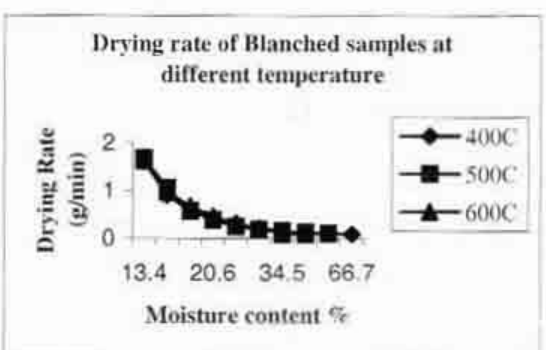

Fig. 5. Drying rate of blanched oyster mushroom at different temperatures.

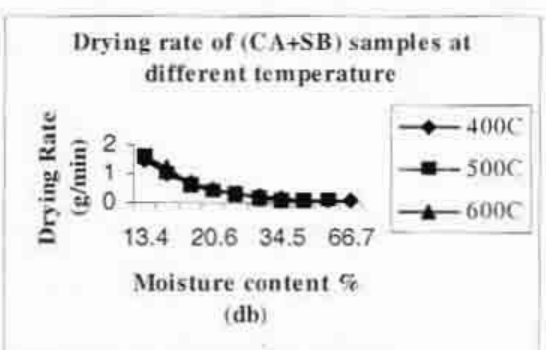

Fig. 6. Drying rate of Citric acid + Sodium benzoate treated oyster mushroom at different temperatures.

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Study on Mycelial Growth, Yield and Yield Attributes in Different Strains of *Volvariella volvaceae*

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Abstract

This study was initiated to assess the performance of eight different strains i.e. VV-1, VV-2, VV-3, VV-4, VV-5, VV-6, VV-7, and VV-8 of *Volvariella volvaceae* for their appropriate vegetative growth, yield and yield contributing characters. Among the tested strains mycelial growth and run rate were observed on PDA medium and mother culture. Highest days required for completing the mycelial growth and run rate were recorded in VV-4 (8.55 days) and VV-1 (22.75 days), while lowest days required for completing the mycelial growth and run rate were observed in VV-7 (7.00 days) and VV-2 (17.72 days), respectively. Minimum days required to primordia initiation (DRPI) was found in both VV-3 and VV-5 (6.33 days), whereas maximum DRPI was recorded in VV-6 (9.33 days). Optimum days required for first harvest was recorded in VV-7 (10 days). The lowest and highest numbers of effective fruiting bodies were observed in VV-2 (85.00) and VV-8 (147.30) respectively. Maximum length (3.83 cm) and diameter (2.20 cm) of fruiting bodies were observed in VV-6 strain. Highest biological yield (1045.10 g) and biological efficiency (26.13%) were observed in VV-5, whereas lowest biological yield and biological efficiency were recorded in VV-1 strain of *V. volvaceae*. These results indicate that VV-5 strain of *V. volvaceae* is suitable for the commercial cultivation in Bangladesh.

Key words: Biological efficiency, Fruiting bodies, Strains, Vegetative growth, *Volvariella volvaceae*.

INTRODUCTION

Volvariella volvaceae is one of the eminent edible mushrooms in Bangladesh and it is very much preferable for its attractive fruiting bodies as well as unique taste (Sarker *et al.*, 2012). It is the third most important mushroom cultivated in the world with an annual production of 287 tones (Thakur *et al.*, 2002). Although more than 100 subspecies and varieties of *V. volvaceae* have been described throughout the world (Shaffer, 1957), four species including *V. esculenta*, *V. bakeri*, *V. diplasia* and *V. volvaceae* are reported to be cultivated in Asia and Africa. The monokaryotic and dikaryotic mycelia are capable of indefinite growth, allowing for the maintenance and duplication of the genotype of each ploidy state. It is generally recognized that growing mycelia has a defined medium to obtained fungal biomass for further use (Alam *et al.*, 2010a).

Mushroom cultivation on simple substrate alone sometimes cannot provide enough nitrogen required for optimal growth. Supplements may be added to obtain higher yields.

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Many kinds of waste materials such as wheat bran, rice bran and maize powder are used for supplementation with the substrate at different level. These supplements also raise the nutritional status of the mushroom (Ahmed *et al.*, 2011). An and Awan (1966) obtained the higher yield with the use of rice straw as the main substrate and rice bran as additives. Supplementation of substrates with nutrient was reported to increase the yields of *P. sajor-caju* (Jadhav *et al.*, 1991). Rice straw is widely used as the substrate for the commercial cultivation of *V. volvaceae*, in Bangladesh this substrate is available, cheap and has no proper utilization (Amin *et al.*, 2010). The biological efficiency of *V. volvaceae* is very poor, may be due to lack of suitable strain, mother culture, and substrate. In order to increase the production of mushroom, there is need to screening the suitable strains for the commercial cultivation. Therefore, the present study was aimed to screening the suitable strains *V. volvaceae* for the commercial cultivation in agro climatic conditions of Bangladesh.

MATERIALS AND METHODS

Mushroom Strains: Eight different strains such as VV-1, VV-2, VV-3, VV-4, VV-5, VV-6, VV-7 and VV-8 of *Volvariella volvaceae* was obtained from the National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar, Dhaka. This experiment was carried out from June 2012 to November 2012 at NAMDEC.

Mycelial Growth on PDA Medium: Pure culture of each strain was prepared on PDA medium (20 g glucose, 200 g potato, 20 g agar and 1 litter of distilled water) and sterilized in an autoclave for 20 minutes at 120°C temperature under 1 kg / cm^2 pressure. A 5 mm diameter agar plug in each inculum was removed from 10 days old culture grown on PDA Petri dish and incubated at 30°C temperature. Radial growth of mycelia on each Petri dish was measured at three directions. Average value of mycelial growth of each Petri dish was calculated.

Preparation of Mother Culture: Mother culture was prepared by chopping the rice straw into 3-4 inch size and mixed with wheat bran at the ratio of 3:1 with equal amount of water. Polypropylene bags of 7"×10" size were filled with 300 g of the above mentioned mixture and packed tightly. The necks of the bags were heat resistant plastic. A hole of about 2/3 deep of the volume of the bags was made at the centre with a sharp end stick for space to put inoculums. The neck was plugged with cotton and covered with brown paper and tied with rubber. The packets were sterilized in an autoclave for one hour at 120°C under 1 kg/cm^2 pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. Pieces of PDA culture medium containing mycelium of above mentioned strains were placed aseptically in the hole of mother culture packet and again plugged as mentioned before. Then the inoculated packets were placed in the culture house at room temperature (30 ± 4°C).

Substrate and Preparation of Bed: Rice straw was used as substrate and chopped into 4-5 cm lengths and poured into a net bag then treated with hot water at 60°C for one hour and allowed to drain out the excess water by hanging the bag for 20 hours.

Transparent polythene sheet was placed in the incubation room and then both end open box was placed on the sheet. The size of the box was 100 cm (length) × 30 cm (width) × 30 cm (height). The first layer was filled with 4 cm sterilized rice straw then inoculated with mother culture and covered with 4 cm pasteurized rice straw and again inoculated with mother culture covered with 3 cm pasteurized rice straw and after inoculating with mother culture the 3rd layer was covered with 1cm pasteurized rice straw. The mother culture was taken out of the packet followed by placing on a tray breaks into small pieces and placed in the each layers at 2-3 cm intervals at peripheral region. The whole bed was covered with a transparent polythene sheet. After 8 days, the sheet was removed for sufficient aeration, light, temperature and humidity, so that fructifications could be ensure. Temperature and relative humidity during the experiment were 27-38°C and 85-95%, respectively and mushrooms were harvested at egg stage.

Experimental Design and Data Collection: The experiment had a completely randomized design with three replications. The following data were collected: mycelium growth and run rate, the number of days required for the initiation of primordia, the days required for first harvest, length and diameter of the fruiting bodies, the number of effective fruiting bodies, biological yield and biological efficiency. The data were analyzed according to standard methods using the MSTAT-C program. Means were compared using Duncan's multiple range test. Biological efficiency was measured using the following formula-

Biological efficiency (%) = total biological yield/total substrate used × 100.

RESULTS AND DISCUSSION

Mycelial growth and run rate were observed on PDA medium and mother culture. Highest days required for completing the mycelial growth and run rate were recorded in VV-4 (8.55 days) and VV-1 (22.75 days), while lowest days required for completing the mycelial growth and run rate were observed in VV-7 (7.00 days) and VV-2 (17.72 days), respectively (Fig. 1 and 2), which resembles with Amin *et al.* (2010) findings for *Calocybe indica*. Adejoye *et al.* (2006) reported that PDA medium enhance the mycelial growth of *Pleurotus florida*. In case of mycelial growth of Hennerberg, it shows slightly slower growth than that of in PDA and Hamada. It may be due to the ineffective utilization of microelements by the mushrooms. Qumio (1981) reported that the following substrates supported very well mycelial growth of *V. volvacea*e, rice straw, ipil-ipil leaves, sigadillas leaves, newspaper prints, coconut coir dust and banana bracts. It took 8 days for mycelium to fill up the entire diameter of the Petri dish containing the substrate. This only confirmed the fact *Volvariella*, unlike *Agaricus* species, could grow directly on uncomposted substrates and therefore could be considered less specific in growth requirements than the later mushroom. Generally it is recognized that growing mycelia in a defined medium is a rapid and alternative method to obtain fungal biomass for further use. Moreover, it is also well known that the mycelium cultivation of mushrooms is enhanced by different environmental and nutritional factors as well as

propagation of mycelia is an earlier and essential step to cultivate fruiting bodies of mushrooms.

Result on the performance of eight different strains i.e. VV-1, VV-2, VV-3, VV-4, VV-5, VV-6, VV-7 and VV-8 of *V. volvaceae* for their yield and yield contributing characters have been presented in Table 1. The days required for primordia initiation (DRFPI) was found minimum in VV-3 and VV-5 (6.33 days) and maximum in VV-6 (9.33 days), which was statistically higher than all other strains. The days required for first harvest (DRFFH) observed between 10.00 to 13.33 days in VV-7 and VV-6 respectively. The fruiting bodies of eight different strains of *V. volvaceae* presented in Fig. 4. The maximum number of effective fruiting bodies (NEFB) observed in VV-8 (147.30) and minimum NEFB observed in VV-2 (85.00). The highest lengths (3.83 cm) of fruiting bodies were found in VV-7 and VV-8 respectively and highest diameter of fruiting bodies were recorded in VV-6 (2.20 cm). The lowest length (3.03 cm) and diameter (1.40 cm) of effective fruiting bodies were found in VV-1. The length and diameter of effective fruiting bodies were significantly higher as compared to other strains. The maximum and minimum biological yield was recorded in VV-5 (1045.00 g) and VV-1 (551.00 g) respectively. Biological efficiency ranged from 13.74 to 26.13% (Fig. 3). Highest biological efficiency was found in VV-5 followed by VV-8 and lowest biological efficiency was found in VV-1. The screening results of *V. volvacae* showed significant variations among the different yield parameters which were discussed before. The growth and yield performance of *V. volvacae* depend on the genotypic structure of the strains. The selected best performing strain might significantly increase the biological yield of rice straw mushroom under suitable growth condition of mycelium. Among the tested strains of *V. volvacae*, highest mycelium run rate and lowest days required for primordia initiation were observed in VV-5 with highest biological yield and biological efficiency. The days required for primordia initiation recorded 6.33 to 7.33 days which was supported by Kumari *et al.* (2008) and Moonmoon *et al.* (2008). The biological yield of this study is comparatively higher than the previous study of Moonmoon *et al.* (2008) and Haq (2009). According to Kumari *et al.* (2008) the evaluated ten strains of *V. diplasia* along with *V. volvacae*, reported a significant variation on the biological efficiency ranged from 0.70 to 16%, where VV-w was the best performing strain on rice straw substrate. Haq (2009) recorded highest biological yield in VvPk (Pakistan) on cotton waste substrate. The variation among the different *Genoderma* isolates showed that the biological efficiency was ranged from 15.35-20% reported by Mishra *et al.* (2012). These differences in strains yield on different substrates may depend on the physical conditions and chemical composition of the substrates along with the adaptive capacity of the strain in different agro-climatic condition.

In comparison to the performance of mycelial run rate, biological yield and biological efficiency the results suggests that among the studied strains VV-5 of *V. volvacae* was the best strain for the commercial cultivation.

Table 1. Performance of yield and yield contributing characters of eight different strains of *Volvariella volvaceae*

Strains	Required days for primordia initiation	Required days for first harvest	Number of effective fruiting bodies / bed	Length of fruiting bodies (cm)	Diameter of fruiting bodies (cm)	Biological yield g / bed
VV-1	7.00 bc	11.67 abc	124.33 abc	3.03 c	1.40 d	551.00 e
VV-2	7.33 bc	11.33 bcd	85.00 d	3.39 abc	1.58 c	561.70 e
VV-3	6.33 c	10.67 cd	119.70 bc	3.52 abc	1.77 b	710.70 d
VV-4	8.00 ab	12.00 abc	136.30 ab	3.67 ab	1.64 bc	874.30 bc
VV-5	6.33 c	10.33 cd	136.30 ab	3.19 bc	1.76 b	1045.10 a
VV-6	9.33 a	13.33 a	90.33 d	3.79 a	2.20 a	711.30 d
VV-7	7.66 bc	10.00 d	100.00 cd	3.83 a	1.50 cd	777.70 cd
VV-8	7.66 bc	12.67 ab	147.30 a	3.83 a	1.51 cd	976.70 ab
CV%	**11.00**	**8.93**	**11.95**	**8.27**	**5.67**	**8.61**

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

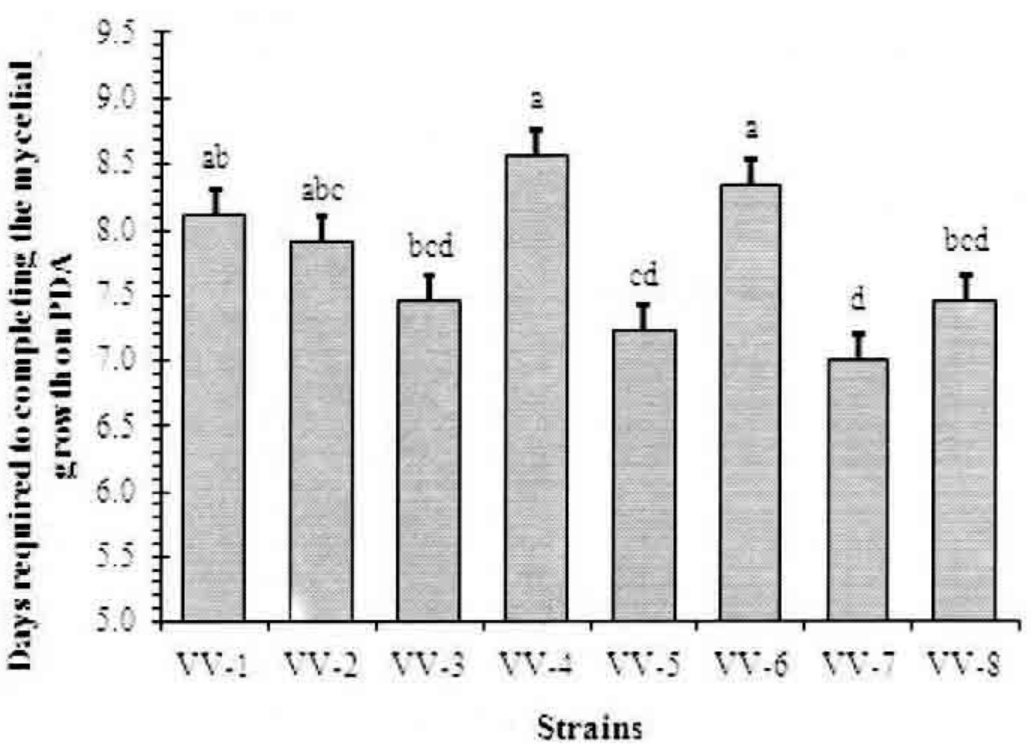

Fig. 1. Mycelial growth of eight different strains of *Volvariella volvaceae* on PDA medium. Values on the bar that do not share a common superscript are significantly different at $p < 0.05$.

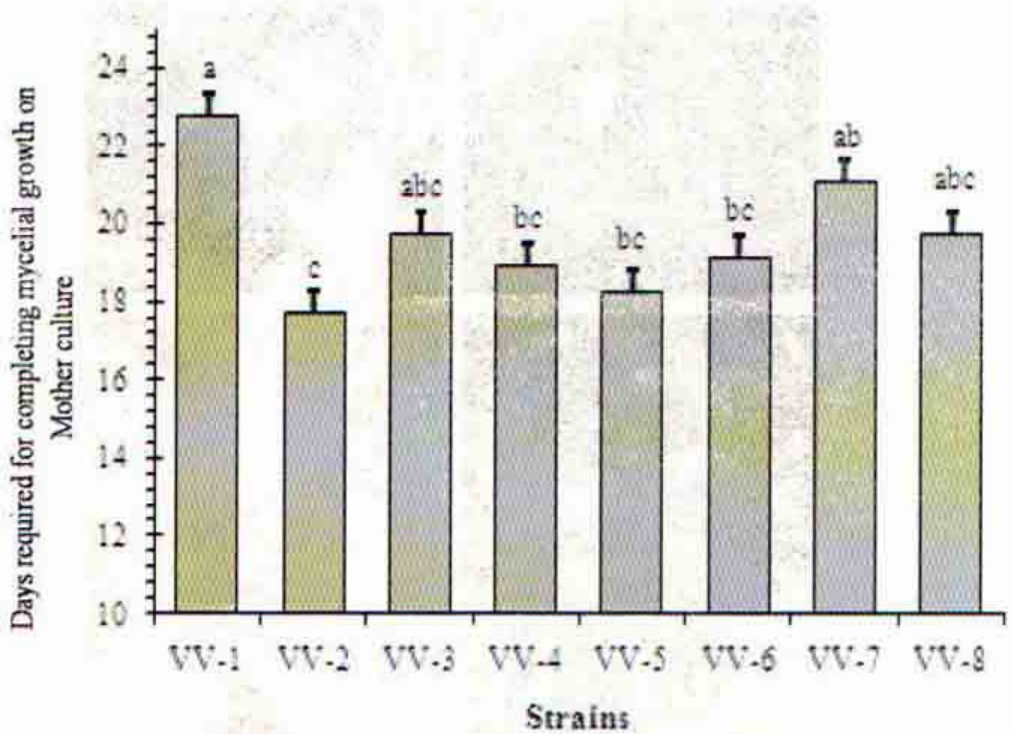

Fig. 2. Mycelial growth of eight different strains of *Volvariella volvaceae* on mother culture. Values on the bar that do not share a common superscript are significantly different at $p < 0.05$.

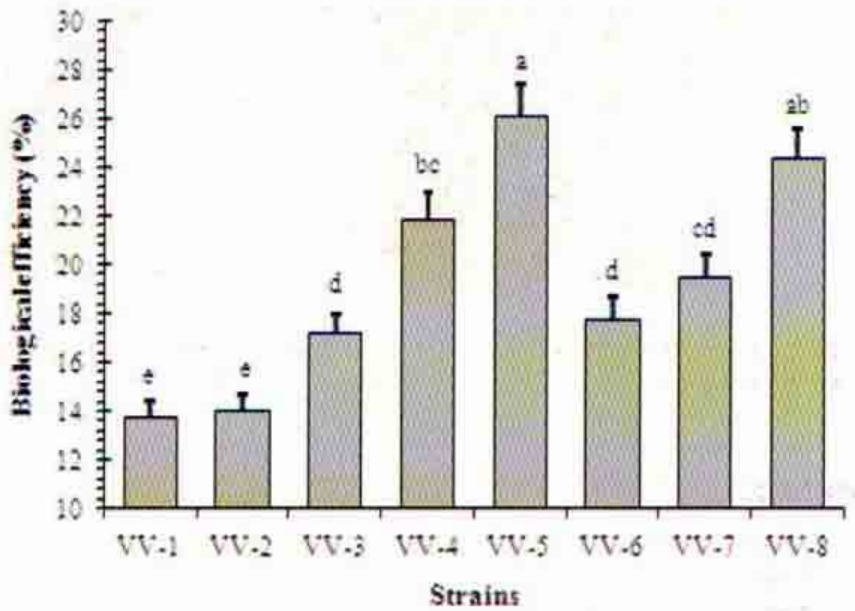

Fig. 3. Biological efficiency of eight different strains of *Volvariella volvaceae*. Values on the bar that do not share a common superscript are significantly different at $p < 0.05$.

Fig. 4. Fruiting bodies of eight different strains of *Volvariella volvaceae*. A: VV-1; B: VV-2; C: VV-3; D: VV-4; E: VV-5; F: VV-6; G: VV-7; H: VV-8.

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Study on Knowledge and Attitude of Mushroom Growers at Selected Upazilas of Dhaka

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Abstract

A study was conducted to determine the extent of knowledge and attitude of the mushroom growers towards mushroom production, the study was also aimed to determine the socio demographic characteristics of the respondents and to find out the relationship between selected characteristics of mushroom growers and their knowledge and attitude towards mushroom production. Data were collected from 70 mushroom growers sampled randomly from 280 growers from Savar and Dhamrai Upazila under Dhaka district. Interview schedule was used for collecting data from 24th February to 31st March, 2012. Most of the respondents were middle aged (72.9%) with small to medium family (77.2%) and had secondary to above secondary level (73%) of education. Majority (68.6%) of the respondents had medium knowledge and moderate favorable attitude (71.4 %) towards mushroom cultivation. Coefficient of correlation showed that education, annual income, extension participation and innovativeness had positive significant relationship with knowledge and attitude towards mushroom cultivation.

Key words: Attitude, Knowledge, Mushroom.

INTRODUCTION

In Bangladesh context ever increasing population, declining agricultural land, changes in environment, water shortage and need for quality food product at competitive rates etc. are going to be important issues. Mushrooms are such component that helps in addressing the problems of quality food, health and environment related issues. According to Chang and Miles (1991), the amount of nutrient and protein in mushroom is double than any other vegetables. Mushroom cultivation is a labor intensive work. It might serve as means of generating employment, particularly for rural women and youths in order to raise their social status. By practicing mushroom cultivation farmers can contribute successfully and significantly to the economic development.

Although mushroom is a popular and nutritious food in many countries of the world, but it had long been ignored in Bangladesh .However, peoples impression on mushroom has been gradually changed through mushroom projects' activity publication and publicity.

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The technology of mushroom production is not too hard. It can be cultivated by using spawn. Spawn is derived from different substrate and supplements by using tissue culture technology. It can be grown within short time and needs less capital. People having poor financial ability can easily be involved in mushroom cultivation. Besides poverty alleviation, we can earn foreign currency through exporting it. Once people treated it only as fungus and waste. Their view was fully negative about mushroom. But now it is cultivated in large scale in many countries and they take it one of the major commercial crops. Even the production is not satisfactory in our country but people have started to uptake it for its food and medicinal value. And many places the demand for mushroom is increasing. For this, the knowledge of the mushroom growers should be high. Otherwise production will not be increased. So they should be trained appropriately. As mushroom is a good source of protein, low fat content, improves immune system, people should keep it in their daily diet chart. The attitude of growers should be known to disseminate it to the people. Mushroom has tremendous prospects. A strong effort should be made both by Government organization and NGO to mitigate the severity of unemployment problem by intensive program on mushroom cultivation and getting more and more people involved in those programs. But it is unfortunate that adequate research has not yet focused on this field. So it is timely attempt to analyze the knowledge and attitude of the growers to the mushroom cultivation. By conducting the study it will be possible to find out the factors which keep the people away from adopting mushroom cultivation. Considering the above mentioned facts the study was undertaken to determine selected socio-demographic characteristics of the mushroom growers of the concerned area; to determine their extent of knowledge and attitude towards mushroom cultivation and to explore the relationship of selected characteristics of the respondents with their extent of knowledge and attitude towards mushroom cultivation.

MATERIALS AND METHODS

The study was conducted in two villages (Joypara and kagujipara) of Savar and Dhamrai Upazila of Dhaka District. Mushroom beneficiaries of Savar and Dhamrai Upazila of Dhaka district were the population of the study whose number was 280, among them 25% was selected as sample using simple random sampling technique. Thus the total numbers of respondents became 70. In order to collect relevant information, an interview schedule was carefully designed keeping the objectives of the study in view and the data were collected from the selected respondents though personal face to face interview. Data were collected by using a personal interview schedule from 24th February to 31st March, 2012.

Variables of the Study and Their Measurement: Ezekiel and Fox (1959) stated variable as "any characteristics which can assume varying or different values in successive individual cases". In any descriptive research, the selection and measurement of variables constitute an important task. A hypothesis if properly formulated will contain at least two elements namely an independent variable and a dependent variable. An independent variable is that factor which is manipulated by the researcher in his attempt to ascertain its relationship to an observed phenomenon (Townsend, 1953). A dependent variable is that factor which appears, disappears or varies as the researcher introduces, removes, or varies the independent variables (Dalen, 1977).

Table 1: Measurement of dependent and independent variables

Variables	Terminology	Measurement
Age	Age of a respondent referred to the period from his birth to the time of data collection.	Age of a farmer was measured in terms of actual years on the basis of his/her statement. A score of one (1) was assigned for each years of age.
Education	Education was defined as the number of years successfully spent by the respondent in receiving formal education.	A score of one (1) was given to each respondent who could sign his name reasonably assumed as an equivalent to class 1. Similarly a farmer who could not read and write fall under illiteracy criteria having a score of zero.
Annual income	Annual income referred to the total earning of a respondent and other members of his family from agriculture and other occupation. Thus, income was calculated by adding all income received by other socially acceptable sources such as business, service etc. during the previous years.	Annual income was expressed in Taka.
Family size	Family size of a respondent was measured in terms of actual number (all dependents) of members of his/her family (including him / herself) during the interview period.	Family size was operationally measured by assigning a score of one (1) for each member of the family who jointly lived and ate together.
Extension participation	Extension contact scores of the respondents were computed on the basis of their extension participation with different sources of information.	A respondent's extension participation was obtained by adding the weights for his responses to all the six sources listed in the instrument like demonstration, extension tour etc.
Risk taking willingness	The term risk commonly referred to all outcome which lead losses or derivation of realization from expectation (Heady *et al.*, 1937).	Risk taking willingness is divided into three categories where six statements were asked to the respondents and their opinion is described in the form of agree, undecided and disagree which score 3 to 1 respectively. The total score is divided into low, moderate and more risk taking willingness according to mean ± SD.
Innovativeness	It refers to the degree to which an individual is relatively earlier in adopting new ideas than the other members of a social system (Rogers, 1995).	To measure the innovativeness, each respondent was asked if they agree or disagree with each of those innovations mentioned in the instrument. A score of 2 is for agree and a score of 1 is for disagree statements. Each respondent was given a total score consisting of the sum of the scores for the individual innovations.
Cosmopolite-ness	The term cosmopoliteness was used to refer to orientation of an individual external of his/her own social system for the purposes of agriculture, personal, entertainment and general information.	Cosmopoliteness scores of the respondents were computed on the basis of respondents' visit to different places inside or outside of his own area. The respondents indicated whether they visited those places often, sometimes or seldom.
Mushroom farming experience.	Mushroom farming experience was measured in terms of actual numbers in years. Production quantity of Mushroom was measured in terms of actual production in kilogram.	A score of 1 was assigned to a respondent for less than 12 months, a score of 2 was assigned for 12-24 months, a score of 3 was assigned for over 24 more months.

Knowledge	Knowledge about mushroom cultivation of a respondent was measured by 20 questions related to the different aspects of mushroom like nutrient requirement, light requirement, spawn packet, time for cultivation and collection, preservation, disease.	Each of the questions was assigned to two score. The total assigned score of all questions was 40. Full score (2) was assigned for each correct answer, 1 for partially correct answer and '0' (zero) for each wrong answer. Therefore, the level of knowledge was measured by summing up his or her obtained scores against each of the questions.
Attitude	Attitude is the degree of positive and negative effect associated with some psychological object.	Twenty statements in relation to cultivation, social and economic return, marketing were taken to measure the level of attitude. Five point liker type scale was used for measuring the attitude. The selected statements were presented either in positive or in negative form. The respondents used to express their opinion against each statement as 'strongly agree undecided, disagree and strongly disagree. Score was assigned to each of the five responses for positive statement in the following manner while for negative statements scoring was put in reverse.

Statistical Technique: The analysis was performed using SPSS (Statistical Package for Social Science) computer package. Descriptive analysis such as range, frequency count, number and percentage, mean, standard deviation and rank order used whenever possible. Pearson's Product Moment Coefficient of Correlation (r) was used in order to explore the relationship between the concerned variables.

RESULTS AND DISCUSSION

Characteristics Profile of the Respondents: Characteristics Profile of the Respondents were determined and presented (Table 2).

Knowledge and Attitude towards Mushroom Cultivation Training increases knowledge, skill and change the attitude of the farmers in a specific area. Ttraining provided by NAMDEC, respondents were practically informed about the mushroom production and supposed to get enough information which increased their knowledge (Table 3-6). Table 3 revealed that most of the respondents (more than three-fifths) in the study area were medium to high knowledge group. It is observed that (Table 4), the mushroom growers have enough knowledge on the required temperature for mushroom production, appropriate time to collect mushroom, spacing between racks and spawns, time of collection of mushroom, infected spawn packet. The data also revealed that (Table 5) majority (84.3 percent) of the farmers had medium to high level of favorable attitude toward Mushroom cultivation. It is found in Table 6 that mushroom growers showed more favorable attitude on the cooking procedure, production, career opportunity of mushroom. But the respondents showed less favorable attitude to the market value, availability of technologies, input cost, space that is needed to produce mushroom and so on.

Table 2. Socio demographic characteristic profile of the respondents

Variables	Measurement	Categories	Respondents number	Respondents (%)	Mean	SD
Age	Years	Young(up to21)	2	2.9		
		Middle (21– 41)	51	72.9	35.64	13.21
		Old (> 41)	17	24.2		
Education	Rated score	No education	4	5.7		
		Primary	15	21.4		
		Secondary	29	41.4	8.11	3.73
		Higher secondary	22	31.4		
Family size	Rated score	Small (< 5)	24	34.3		
		Medium (5– 7)	30	42.9	5.37	1.44
		Large (> 7)	16	22.8		
Extension participation	Rated score	Low (< 6)	5	7.1		
		Medium (6-10)	57	81.4	8.3	1.58
		High (> 10)	8	11.4		
Annual income	Rated score	Low (< 175$)	37	52.9		
		Medium (175-225$)	21	30.0	177.1	53.79
		High (> 225$)	12	17.1		
Innovativeness	Selected scale	Poor (< 12)	17	24.3		
		Moderate (12-16)	34	48.6	14.66	1.52
		High (> 16)	19	27.1		
Cosmopolitenes s	Selected scale	Low (< 4)	24	34.3		
		Medium (4-8)	34	48.6	6.56	2.29
		High (> 8)	12	17.1		
Mushroom farming experience	Rated score	Low (< 2 yr)	9	12.9		
		Medium (2-4yr)	42	60.0	25.7	7.44
		High (> 4 yr)	19	27.1		
Risk taking willinness	Selected scale	Poor (< 13)	11	15.7		
		Moderate (13-17)	28	40.0	15.54	2.29
		High (> 17)	31	44.3		

Table3. Distribution of the mushroom growers according to their knowledge

Category	Mushroom Growers		Mean	SD
	Number	Percent		
Poor knowledge (< 26)	17	24.3		
Medium knowledge (27-33)	48	68.6	31.24	6.68
High knowledge (> 33)	5	7.1		

Table 4. Rank order of Knowledge of the growers about mushroom cultivation

SL.	Questions	Total marks	Rank order
1.	Which nutrient that mushroom contains more?	84	17th
2.	Which temperature is favorable for oyster mushroom production?	128	1st
3.	Name two mushrooms that can grow in winter.	89	16th
4.	What amount of light oyster mushroom needs?	114	10th
5.	What are the things that need to be written on a mushroom packet?	96	15th
6.	What condition is required to justify a standard spawn packet?	118	7th
7.	What is the size of opening area of a spawn packet?	120	6th
8.	When it is appropriate time to collect mushroom?	127	2nd
9.	How many times can you collect mushroom from a single spawn?	124	3rd
10.	What is the time duration you can cultivate or produce milky white mushroom?	104	14th
11.	What amount of mushroom can you get from 1 kg of dried straw?	106	12th
12.	Say two ways through which you can protect mushroom spawn.	122	5th
13.	What is your opinion about shelf life of mushroom?	115	9th
14.	Name two varieties of mushroom cultivated in our country.	116	8th
15.	What are the spacing between racks and spawn packet?	127	2nd
16.	How many times do you irrigate spawn packet per day?	123	4th
17.	How do you collect and preserve mushroom?	105	13th
18.	When do you stop water spaying at harvesting to ensure good quality mushroom?	110	11th
19.	Mention two diseases of mushroom.	110	11th
20.	Mention two colors of infected spawn packet.	122	5th

Table 5. Distribution of the mushroom growers according to their attitude

Category	Mushroom growers		Mean	SD
	Number	Percent		
Less favorable attitude (< 83)	11	15.7		
Moderate favorable attitude (83-92)	50	71.4	89.13	5.5
Highly favorable attitude (> 92)	9	12.9		

Table 6. Rank order of attitude of the growers about mushroom cultivation

SL	Statements	Extent of attitude					Total	Rank
		SA	A	UD	DA	SDA		
1. (+)	I think it is needed to eliminate the undesired and predatory spawn packet from my culture house to prevent contamination.	170	144				314	7th
2. (+)	I believe good varieties of oyster mushroom are needed to maximize production.	190	128				318	5th
3. (-)	I believe mushroom does not work as a nutritional food or does not ensure food security.			12	120	180	312	8th
4. (+)	I think mushroom cultivation ensures extra income.	275	60				335	3rd
5. (+)	I observe after mushroom cultivation my social status increases.	135	152	52			302	13th
6. (+)	I believe mushroom cultivation can remove unemployment problem.	225	40	15			320	4th
7. (-)	I think for mushroom cultivation it is needed a very large space.			24	104	175	303	12th
8. (+)	I observe our female family members play role in earning after mushroom cultivation.	180	104	24			308	11th
9. (+)	I think mushroom needs minimum infrastructure.	185	100	24			309	9th
10. (-)	I observe mushroom needs high input cost.		8	12	136	140	296	15th
11. (+)	I think preserved mushroom has a good market value.	65	192	12	14		283	17th
12. (+)	I think return from mushroom cultivation my home becomes well furnished and I have some electronic device now.	120	152	18	10		300	14th
13. (+)	I believe mushroom cultivation is feasible because of the availability and affordability of the technologies that encourage the growers to cultivate more and more.	120	152	18	10		300	14th
14. (-)	I think scale is friendly for mushroom cultivation.	5			52	135	292	16th
15. (+)	I think mushroom has medicinal value and helps to prevent diabetes.	175	104				315	6th
16. (-)	I believe maintaining spacing between spawn packets (5.08cm) is not a requirement to grow mushroom smoothly.				128	190	318	5th
17. (+)	I observe that by maintaining temperature (20-30c) humidity (80%) production can be increased a lot that helps growers keep continuing mushroom cultivation.	285	52				337	2nd
18. (+)	I believe with my neighbors that stop water spraying before 12 hours of harvesting is conductive to ensure quality spawn packet.	125	160	18	4		307	10th
19. (+)	I observe if care is taken we can get 1-1.5kg of straw mushroom per bed.	278	60				335	3rd
20. (+)	I think mushroom is very tasty and easy to cook.	300	40				340	1st

Relationship between Selected Characteristics of Mushroom Growers and Their Knowledge and Attitude towards Mushroom Cultivation: In order to explore between the selected characteristics of the respondents and their knowledge and attitude towards mushroom cultivation, the correlation of co-efficient (r) was computed. The relationships of the selected characteristics of the respondents with their knowledge and attitude towards mushroom cultivation is shown in Table 7.

Table 7. Relationships between the selected characteristics of the respondents and their knowledge and attitude towards mushroom cultivation

Characteristics of mushroom growers	Knowledge (r value)	Attitude (r value)
Age	0.151	0.262*
Education	0.579**	0.362**
Family size	-0.043	0.043
Experience	0.267*	0.112
Income	0.275*	0.237*
Extension contact	0.346**	0.282*
Innovativeness	0.276*	0.238*
Cosmopoliteness	0.225	0.219
Risk orientation	0.189	0.060

NS=Not significant, *=Correlation is significant at 0.05 level of probability, **= Correlation is significant at 0.01 level of probability.

The result indicated that education, mushroom cultivation experience, annual income, extension participation and innovativeness showed positive significant relationship while the rest of characteristics like age, family size, cosmopoliteness, and risk orientation had no significant relationship with knowledge about mushroom cultivation. Relationship of nine select characteristics of mushroom growers with their attitude towards mushroom cultivation was investigated in the study. The result indicated that age, education, annual income, extension participation and innovativeness showed positive significant relationship with attitude towards mushroom cultivation, while the rest of characteristics like family size, mushroom cultivation experience, cosmopoliteness, and risk orientation had no significant relationship with attitude towards mushroom cultivation.

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Influence of *Pleurotus ostreatus* on Glycemic Status of Normotensive Diabetic Female Volunteers

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Abstract

To fiend out the effect of *Pleurotus ostreatus* on glycemic status of normotensive diabetic subjects, the study was undertaken on 32 diabetic female volunteers in National Mushroom Development and Extension Center (NAMDEC) Sobhanbag, Savar, Dhaka. Fasting plasma glucose and glycated hemoglobin (HbA1c) were estimated before and three months after administration of mushroom capsule. The feeding of three grams mushroom capsule daily showed a significant reduction of fasting plasma glucose ($p = 0.000$) and glycated hemoglobin ($p = 0.000$). These findings suggest that *Pleurotus ostreatus* may able to improve type 2 diabetes mellitus of non-hypertensive diabetic females.

Key words: Diabetes, Fasting plasma glucose, Females, HbA1c, *Pleurotus ostreatus*.

INTRODUCTION

The serious global problem diabetes is increasing due to population growth, aging, urbanization, increasing prevalence of obesity and physical inactivity (Wild *et al.*, 2004). The estimated diabetes prevalence for 2010 was 285 million and is expected to affect 438 million people by 2030. It showed an increase of diabetic population by 35%, which has been attributed to the rise of worldwide prevalence of diabetes from 4.0% to 4.5%. The overall rise was predicted to be much higher in the developing countries than those of the developed counterparts. In developing countries, the majority of people with diabetes are in the 45 to 64 years age range, similar to the finding reported previously (King *et al.*, 1998).

Diabetes is caused by a defect in insulin secretion or insulin action. Insulin is the main hormone regulating glucose uptake from blood into muscle and fat cells (Schinner *et al.*, 2005; Lanner *et al.*, 2008). When diabetes is not well managed, it is associated with serious complications including heart disease, stroke, blindness, kidney disease, nerve damage and amputations leading to disability and premature mortality. Complications may begin five to six years before diagnosis and the actual onset of diabetes may be ten years or more before clinical diagnosis (Harris *et al.*, 1992). Good diabetes management

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has been shown to reduce the risk of complications (Stratton *et al.*, 2000). Diabetes has a 2-fold higher death rate than in the nondiabetes population. Most risks of diabetes are from its complications that often result in death (Fox *et al.*, 2004).

Cardiovascular disease is a major cause of death and disability in people with diabetes, accounting for 44% of fatalities in people with Type 1 diabetes and 52% in people with Type 2 (Morrish *et al.*, 2001). The risk of death from coronary heart disease associated with Type 2 diabetes is about 50% greater in women than it is in men (Huxley *et al.*, 2006). People with Type 2 diabetes have a two-fold increased risk of stroke within the first five years of diagnosis compared with the general population (Jeerakathil *et al.*, 2007).

A recent epidemiological study reported the prevalence of Type 2 diabetes (T2DM) and impaired fasting glucose (IFG) and their risk factors in the urban population of Bangladesh exceeding 11.2% and 5.9%, respectively. The prevalence of diabetes in the urban population has increased alarmingly in recent years. Older age, obesity, higher income, family history of diabetes, and reduced physical activity proved to be the significant risk factors for diabetes and IFG (Sayeed *et al.*, 2007).

Traditional medicines such as mushrooms are very useful for treatment of certain health problems. Mushrooms are edible fungi which traditionally have been used in the treatment and prevention of diabetes, obesity, heart disease, hyperacidity, constipation, cancer, blood pressure and hypertension (Suguna and Usha, 1995).

In recent years, some edible mushrooms are used as health foods, as well as a source for pharmaceutical compounds. In fact, these functional mushrooms are a source of biologically active substances with therapeutic effects due to their immunomodulating, anticancer and antivirus properties (Wasser and Weis, 1999), among others. Many commercially available mushrooms exhibit free radical scavenging, reducing power, chelating effects on metal ions, and antioxidant properties (Mau *et al.*, 2002; Yang *et al.*, 2002).

Mushrooms are not only sources of nutrients but also have been reported as therapeutic foods. Mushroom of *Pleurotus* species are also rich in medicinal values and useful in preventing disease such as hypertension, hypercholesterolemia (Khatun *et al.*, 2007; Choudhury *et al.*, 2008) hyperglycemia (Choudhury *et al.*, 2011; Choudhury *et al.*, 2012) and different types of cancer (Nayana and Janardhanan, 2000).

MATERIALS AND METHODS

The study was conducted in the laboratory of National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka. After getting written consent, a total 32 normotensive female subjects (systolic BP < 130 mmHg and/or diastolic BP < 85 mmHg) aged > 26 years, suffering from diabetes mellitus were included in the study. The details history was taken from the subjects including age, sex, occupation, educational

status, marital status, family history and drug history. Subjects suffering from renal impairment were not included in the study. During the study period any acute or chronic disease, medication or malabsorption were excluded.

At the beginning of study, health status was evaluated. Fasting blood sample was collected from the subjects for analysis. Mushroom powder was supplied as capsule form. Subjects took two capsules three times daily. Each capsule contained 500 mg *Pleurotus ostreatus* powder, so that each subject took 3 gm mushroom powder daily. After three months the subjects were re-evaluated and all the investigation procedures were repeated. If any drug previously getting by the subjects, it was continued.

Ten ml fasting blood sample was collected with all aseptic precaution. Collected blood was poured immediately into test tube containing fluoride and EDTA. The test tube then gently shaken so that anti coagulant and fluoride mix with the blood properly. Then it was centrifuged by 3000 rpm for 5 minutes. Plasma was separated which were transferred into two eppendorfs containing 1 ml in each. All the tests were carried out within short period.

Fruiting body of fresh *Pleurotus ostreatus* was collected from NAMDEC culture house. Collected mushrooms were dried at moisture level 4-5% in electric dryer, then grinded and pour into capsule shell which contains 500 mg powder in each. Prepared capsules were preserved for distribution into moisture free glass containers.

Blood pressure was measured using sphygmomanometer by trend physician. Plasma creatinine was estimated by 'alkaline picrate' and plasma level of glucose was estimated by 'Glucose oxidase' method using commercially available reagent kits. Glycated hemoglobin was estimated by a photometric method using 'Stanbio reagent kit'. Analysis was done by semi auto biochemical analyzer 3000 evaluation.

Results were expressed as mean ± SE. Paired Student's 't' test was used to see the level of significance. 95% confidence limit was taken as level of significance.

RESULTS AND DISCUSSION

Most widely eaten *Pleurotus ostreatus* mushrooms have been revered for thousands of years as both a food and a medicine in the world. For people with hypertension, obesity and diabetes, oyster mushroom can form part of a diet that is low in sodium, starch, fat and calories. Oyster mushrooms help in reducing the cholesterol level. In addition to their nutritional value oyster mushrooms are claimed to exhibit hypoglycemic and hypotensive properties. The present study was performed to observe the effect of oyster mushroom (*Pleurotus ostreatus*) on glycemic control of normotensive female subjects suffering from type 2 diabetes.

The mean (± SE) age (years) of subjects in the study was 40.37 ± 1.84, ranges from 26 - 64. The mean (mmHg) of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were 113.59 ± 1.62 and 74.53 ± 0.96, ranges from 100 - 130 and 65 - 85

respectively. The mean of plasma creatinine (mg) was 0.62 ± 0.03, ranges from 0.4 – 1.1 (Table 1).

Table 1. Evaluation of age, fasting plasma glucose, systolic and diastolic blood pressure of the subjects

Parameter	N	Mean (± SE)	Range
Age (years)	32	40.37 ± 1.84	26 to 64
Systolic BP (mmHg)	32	113.59 ± 1.62	100 to 130
Diastolic BP (mmHg)	32	74.53 ± 0.96	65 to 85
Creatinine (mg/dl)	32	0.62 ± 0.03	0.4 to 1.1

In this study the mean ± SE fasting plasma glucose (mmol/L) before and three months after mushroom treatment was 10.85 ± 0.56 and 8.66 ± 0.45 respectively. A highly significant mean difference of glucose ($p = 0.000$) was observed between the two periods (Fig. 1).

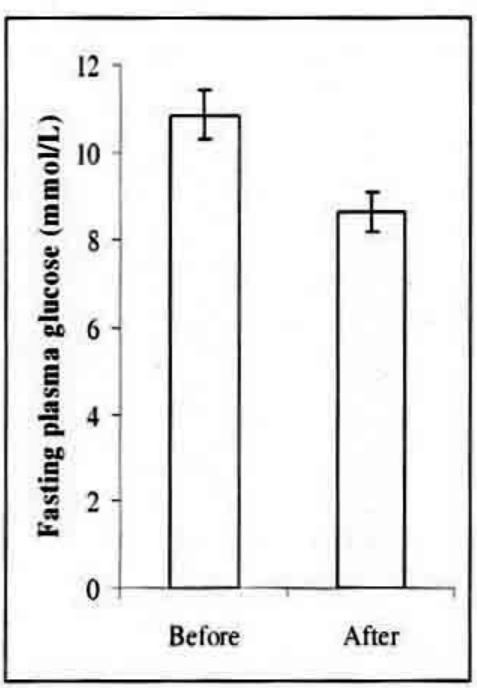

Fig. 1. Mean (± SE) fasting plasma glucose before and 3 months after mushroom supplementation.

The mean ± SE HbA1c (%) before and 3 months after mushroom treatment were 8.17 ± 0.22 and 7.47 ± 0.19 respectively. Here also, a significant mean difference of HbA1c ($p = 0.000$) was observed (Fig. 2).

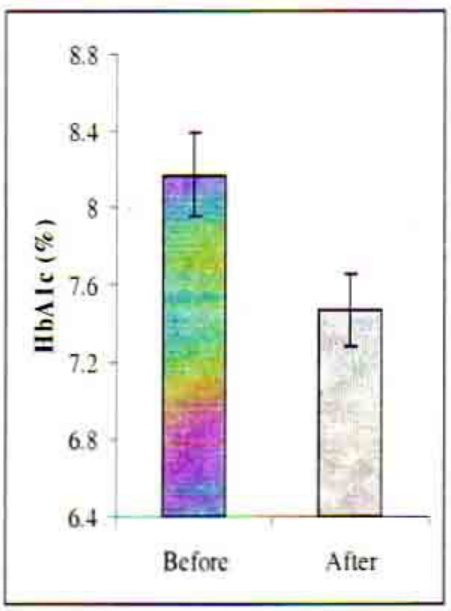

Fig. 2. Mean (± SE) HbA1c before and 3 months after mushroom treatment.

Findings in the study showed that after 3 month period, the fasting plasma glucose level was significantly reduced in non-hypertensive diabetic female patients ($p = 0.000$). There was also a significant reduction of HbA1c ($p = 0.000$). The effect of *Pleurotus ostreatus* on reduction of blood glucose in diabetic patients has been evaluated in this clinical investigation of 32 subjects. In a similar study, the same author observed that *Pleurotus ostreatus* significantly improved glycemic status of normotensive diabetic male volunteers (Choudhury *et al.*, 2011). Our current trial is fully agreement with the previous one and it is observable that irrespective of sex, oyster mushroom reduces blood glucose as well as HbA1c and hence it causes improvement of diabetes. In a study Oyster mushroom consumption significantly reduced systolic and diastolic blood pressure, lowered plasma glucose, total cholesterol and triglycerides significantly, whereas there was no significant change in body weight, there were no deleterious effects on liver or kidney function (Khatun *et al.*, 2007).

The blood glucose and triglyceride (TG) lowering effects of water soluble extracts from *Lentinus edodes, Pleurotus ostreatus* and *Phellinus linteus* in the streptozotocin-induced diabetic model have been clearly demonstrated (Kim *et al.*, 1997 and Kim *et al.*, 2001). Such results strongly suggest that these mushrooms have potential preventive and therapeutic action in diabetes mellitus (type 1 and type 2). Our findings are supportive with them. Reduction in glycated hemoglobin in streptozotocin-induced diabetic mice after mushroom supplement was observed by others (Swanston *et al.*, 1989). Current study showed reduction of fasting plasma glucose and there was significant effect on glycemic control (HbA1c) by supplementation of *Pleurotus ostreatus*. The significant fall in fasting blood glucose and HbA1c may be attributed to the hypoglycemic potential of the oyster mushroom supplement. Further trials with large sample size are needed to verify these observations.

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Comparative Study on Minerals Content of Some Mushroom Species and Common Vegetables

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Abstract

Mushrooms are nutritious foods but people of many districts of Bangladesh put a little care on this delicious vegetable. The present study was conducted on five different mushroom varieties as well as five different widely used vegetables. The objective of this study was to determine the amount of the mineral contents of mushrooms with different vegetables. Total ash, Iron, Zinc, Cobalt, and Molybdenum, were checked and reported. The mineral contents were determined by atomic absorption spectrometer from the ash prepared by a muffle furnace. Results of analysis of five edible species of mushroom and vegetables in Bangladesh indicated that each mushroom was a very good source of Iron, Zinc, Cobalt, and Molybdenum. Findings of this study suggests that mushrooms may be used as a part of daily dishes to fulfill the requirements of analyzed minerals.

Key words: Cobalt, Iron, Molybdenum, Zinc, Mushrooms, Vegetables.

INTRODUCTION

In recent times, mushrooms have assumed greater importance in the diets of both rural and urban dwellers, unlike previously when consumption was confined to hilly areas of Bangladesh. Mushrooms are now marketed along major shopping mall in city area and all the district of Bangladesh. They are also relatively much cheaper than beef, mutton and chicken that contain similar nutrients.

Fasidi and Kadiri (1991) reported that potassium and phosphorus are the major mineral elements in *Pleurotus* species. However, the lipid content of mushrooms has been found to be generally low. Mushrooms have high mineral content reported to be almost twice that of vegetables, while its protein value doubles that of cabbages, potato and six times more than that orange. Iron, copper, potassium and calcium have also been reported to be available in moderate amount in mushrooms (Zakhary *et al.*, 1983). The quality of mushroom protein is valued better than that of cereal grains, legumes, meat, eggs and milk (Jonathan *et al.*, 2009).

Nutritional values of locally cultivated mushrooms remain speculative. Moreover, nutritional composition is affected by many factors; these include differences among

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strains, the composition of growth substrate, the method of cultivation, stage of harvesting, specific portion of fruiting bodies used for analysis (Benjamin, 1995).

Generally, people in Bangladesh are still not very aware of nutritional and medicinal importance of mushrooms. But now it is increasing its demand in people of Bangladesh due to its nutritional and medicinal importance. The aim of this investigation was to analyze and compare the nutritional values of these mushrooms and vegetables cultivated in Bangladesh, with a goal of increasing awareness of the beneficial effects of edible mushrooms among the consumers.

MATERIALS AND METHODS

Sample Collection and Preparation: Different species of mushrooms are cultivated in the culture house of National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar, Dhaka, Bangladesh and harvested. The mushrooms were sun dried carefully. The vegetables were collected from a raw vegetable market of Savar and sun dried in the similar ways.

Determination of Total Ash Content: One gm of each sun dried sample of mushrooms and vegetables were taken in a crucible and burnt on a burner. After open burning for about 1 hour, the samples were placed in a muffle furnace about 5-6 hours at 600°C. After that the samples were cooled in desiccators and weighted. The total ash content was calculated as per the equation described by Raghuramulu *et al.*, 2003:

Ash Content (g/100g sample) = Wt. of Ash ×100 / Wt. of sample taken.

Determination of Mineral Content: Total ash was used for the analysis of mineral contents. Two ml of concentrated nitric acid was added to the ash and placed for 2 hours. One drop of hydrogen peroxide was added to the solution to observe whether any turbidity occurred. The solution was then transferred to a volumetric flask and the volume was adjusted to 100 ml with de-ionized water.

For each mineral, one ml of the primary standard was taken in a 100 ml volumetric flask and the volume was adjusted up to 100 ml with de-ionized water and mixed properly. This solution was the secondary stock solution of the particular mineral. Standard solution of the mineral was prepared as per the instruction of the AAS for the particular mineral. The analysis of Iron (Fe), Zinc (Zn) and Cobalt (Co) was done in flame method of AAS and the analysis of and Molybdenum (Mo) was done in graphite furnace method.

Instruments and Working Conditions: In the present study the Atomic Absorption Spectrophotometer (AAS) was Varian AAS 240 and Graphite Furnace was Varian GTA 120. Flame temperature was 2200°C. Acetylene and air flow rates were respectively 1.5 L/min and 3.5 L/min and recording time was 10 sec/ drop.

RESULTS AND DISCUSSION

Minerals represent the ash left behind after complete incineration of the dry mushroom. The mineral composition reflects on the growth conditions of the mushroom. To overcome the inaccuracies borne in differences in moisture contents from different parts of the world, on fresh weight basis, and to ensure universal comparison of data, all the values obtained were expressed here on moisture free basis. The findings on the total ash and mineral contents of 5 vegetables and 5 mushrooms were represented in the Table 1.

Table 1. Minerals content of different vegetables and mushrooms (mg/100 g dry weight basis)

Vegetables/ Mushrooms	Total ash content	Fe	Zn	Co	Mo
Vegetables					
Pumpkin (*Cucurbita moschata*)	5	5.55	1.09	6.79	4.8
Carrot (*Daucus carota*)	9	10.76	2.25	5.01	89.7
Radish (*Raphanus sativus*)	19	15.70	5.82	4.56	36.7
Radish leaf	15	35.55	4.64	4.97	11.5
Amaranth (*Amaranthus caudatus*)	18	72.6	5.41	5.06	17.2
Spinach (*Spinacia oleracea*)	25	45.82	24.55	4.15	27.1
Mushrooms					
Calocybe indica (Cid)	7	9.69	6.82	0.35	88.5
Volveriella volvaceae (Vv)	10	13.81	9.43	1.74	45.6
Pleurotus ostreatus (po9)	27	12.05	7.28	0.33	58.0
Pleurotus florida (flo2)	5	5.52	4.55	2.10	108.7
Pleurotus citrinopilatus (Py2)	3	20.65	6.00	1.57	157.4

Total Ash Content: The highest ash content found in *Pleurotus ostreatus* (Po 9) and it was 27 mg/100g, which was very close to the ash content of *Spinacia oleracea* (25 mg/100g). However, the lowest ash content found in pumpkin among the vegetables and it was 5 mg/ 100g which was identical to *Pleurotus florida* (Flo 2), while the lowest amount of ash found in *Pleurotus citrinopilatus* (Py 2) and it was 3 mg / 100g. The ash content of *Daucus carota* of our study was 9 mg / 100 gm dry weight which is higher than that of Shyamala and Jamuna, 2010 (5.78 ± 0.06 mg / 100 gm dry weight).

Iron Content: Among all the samples the highest amount of Iron found in *Amaranthus caudatus* and it was 72.5 mg/100g, whereas the highest iron content in 5 mushroom varieties was in *Pleurotus citrinopilatus* (Py 2) and it was 20 mg/100g. The iron content of *Pleurotus citrinopilatus* was higher than that of *Cucurbita moschata, Daucus carota* and *Raphanus sativus*. Shyamala and Jamuna (2010) found that iron content of pulp of carrot was 11.66 ± 0.00 mg/ 100 gm dry weight. Whereas, our finding was 10.76 mg / 100 gm dry weight. The observations were similar in that result. On the other hand, Mallikarjuna *et al.* (2012) found 6.27 ± 0.41 mg / 100 g iron in *Pleurotus florida*, but we found 5.52 mg / 100 g, which is similar to that one. As, we know that the daily

requirement of iron for man is 8 mg / day and for woman is 18 mg / day, so in these cases, hundred gram *Pleurotus* sp is enough to fulfill the daily need.

Zinc Content: Mushrooms are said to be good biological accumulators of zinc which is biologically very vital to the human body (Bano, 1981). Among the mushroom varieties, the highest Zn content was found in *Volveriella volvaceae* (Vv) and the lowest in *Pleurotus florida* (Flo 2). Among all the mushrooms and vegetables, the highest content of zinc was found in *Spinacia oleracea* and it was 24.55 mg / 100g. Moreover, the zinc content found in all mushroom species was higher than that of *Cucurbita moschata* and *Daucus carota* and almost similar to that of *Raphanus sativus* and *Amaranthus caudatus*. Mnkeni *et al.* (2006) found that the zinc content of Amaranths varies between 3.99 mg/100 gm to 5.3 mg/ 100 gm of dry weight. In the present study the mineral content found 5.41 mg / 100 gm dry weight, which is very close to the limit. Murphy *et al.* (1975) mentioned more than 5 mg / 3-oz zinc present in oyster mushrooms whereas, we found 7.28 mg / 100 gm. On the other hand, Mallikarjuna *et al.* (2012) found 5.06 ± 0.04 mg / 100 g zinc in *Pleurotus florida*, but we found 4.55 mg / 100 g, which is nearly similar to the results.

Cobalt Content: Almost all vegetables contained cobalt nearly 5 mg / 100g which were much higher than that of mushroom contained. Mushrooms contained less than or around 2 mg / 100 g of cobalt. Very small amount of Co is required for humen subjects which might be fulfill by mushroom consumption. The cobalt content in mushrooms depend on the availability of the metal in the substrate on which these mushrooms grow.

Molybdenum Content: The highest aomunt of Mo was found in *Daucus carota* and the figure were 89.7 mg / 100g, whereas, the lowest amount of Molybdenum was found in the *Cucurbita moschata* and it was 4.8 mg / 100g. On the other hand, among the mushroom varieties the highest Mo content was found in *Pleurotus citrinopilatus* (Py 2) and the lowest in *Volvariella volvacea* (Vv). In general, among the five varieties four mushrooms contained relatively higher amount of Mo than those of vegetables.

Finally, it may be said that all the mushroom and vegetables contain minerals in respective amount so the dietary requirements of these minerals can be fulfilled through extensive use of these mushrooms in our daily dishes. In addition, from the discussion, it can be say that there are some minerals present in mushroom is lower than vegetables but the amount present is enough to fulfill the daily requirement. Accordingly, these mushroom species are good supplementary health foods from the angle of human nutrition. More importantly, the present mineral values add to the safe consumption of mushrooms as supplementary foods to the populations predominantly dependent on cereal diets.

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Cultivation of Different Oyster Mushroom Variety on Agricultural Byproducts

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Abstract

Five lignocellulosic substrates (saw dust, cotton seed hull, rice straw, water hyacinth, sugarcane, bagasse, waste paper, press mud and spent mushroom substrate) were used for oyster mushroom cultivation. The utility value of the substrates was assessed in terms of growth rate, yield, cultivation period, and benefit cost ratio of the cultivated mushrooms. The highest days (31.75) required for complete mycelium running was recorded in sugarcane bagasse substrate and the lowest (11.00) was found in rice straw. The highest biological yield (216 g/packet) and biological efficiency (108%) was recorded in waste paper and the lowest biological yield (48.25 g/packet) and biological efficiency (14.43%) was observed in water hyacinth. The highest benefit cost ratio was found in rice straw substrate and the lowest in water hyacinth. The results indicated through oyster mushroom cultivation byproducts/wastes can be managed efficiently.

Key words: Bioconversion, *Pleurotus ostreatus*, Substrate.

INTRODUCTION

Billions of tones of agricultural wastes are yearly produced around the world as residues of high yield crops. They are mainly destroyed by burning, burying or uncontrolled disposed in the environment (Kumar, 2005). Organic residues currently are conceived as a negative factor in the world, since they generate adverse environmental and economic effects related to their disposal (Mshandete *et al.*, 2008). Hence, the utilization of waste is necessary for ecological sustainability, environmental safety, economic stability and well being of human society. It has been reported that, cultivation of mushroom can help to solve agro-industrial and agricultural residue disposal problems and increase domestic proteinaceous human food production (Salmones *et al.*, 1996). There are several mushroom varieties is cultivating in Bangladesh like oyster, milky, reishi, straw, shiitake and button. The oyster mushroom is common and favourite in Bangladesh. The main raw material for growing mushroom is rice straw, sugarcane bagasse and a mixture of rice straw and wheat bran, saw dust, cotton waste, and some other agro-residues.

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Most environmental wastes are cellulose or lignocelluloses materials. Vast quantities of lignocellulosic and other organic waste residues are generated annually through the activities of agricultural, forest and food processing industries. In 1999, more than 3,000 million tons of cereal straws were available in the world, and about half of these residues remain unused. In addition, the world produced 952 million tons of bagasse; 6,476 thousand tons of coffee pulps; 6,152 thousand tons of coffee wastes; 9,386 thousand tons of cottonseed hulls; 14,073 thousand tons of sunflower seed hulls; and 325 thousand tons of sisal wastes. Million tons of sawdust, wood chips, and water hyacinth are also available worldwide. All these lignocelluloses waste residues can be used as substrate for growing mushrooms. Most cellulose residues have been found to stimulate the growth of wood rotting fungi. These fungi are capable of degrading cellulose materials and ultimately oxidize lignin content of the materials (Kadiri, 2005). The cultivation of edible mushroom might be a way of reducing agro-waste in the environment first as reported by Kuyper *et al.*, 2002. In such a direction, mushroom production technology may be an eco-friendly, effective tool in reduction of waste quantities, reuse of waste materials or recovery of this materials. So, environmental waste management is to be a productive option by the cultivation of mushroom.

However, Bangladesh is an agro-based country. Solid wastes are produced in enormous quantities from industries like sugar industries, food industries, agro industries and dairy industries etc which have no proper utilization. In this case, these wastes if not managed scientifically pose a sequel of environmental and health hazards. Considering these wastes as the misplaced resource they can be scientifically managed by mushroom cultivating and transforming them into a marketable value added product. It provides a viable option for pre treatment of different type environmentally hazardous waste materials. So, there is a scope to manage the environmental waste through oyster mushroom cultivation, which can help people to keep their surroundings clean besides developing a reasonable income. Considering this fact the study was undertaken with the following objectives to utilize different wastes by oyster mushroom (*Pleurotus ostreatus*) cultivation as good waste management practices and to find out the suitability or performance of different wastes as substrate of oyster mushroom (*Pleurotus ostreatus*) cultivation.

MATERIALS AND METHODS

The experiment was conducted in the National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar, Dhaka, Bangladesh during January 2011 to January 2012.

Experimental Waste Materials: The following byproducts were used as basal materials in the present study: ie. saw dust, cotton seed hull, rice straw, water hyacinth, sugarcane, bagasse, waste paper, press mud and spent mushroom substrate.

Preparation of Pure Culture: Pure culture of the selected mushroom fungus was prepared on Potato Dextrose Agar (PDA) media containing infusion of 200 g of peeled

and.sliced potato, 20 g of dextrose and 20 g of agar. The mixture was boiled on gas burner until the agar dissolved. The medium was poured into petri dishes (90 mm diameter) at 15 ml/dish.

The medium in petri dish was sterilized in an autoclave for 20 minutes at 120^0C under 1.1 kg / cm^2 pressure. After sterilization and solidification, the plates were inoculated with the inocula of the strain. Pieces of inner tissues of stalks were used as inocula. A fresh and full grown sporophore of oyster mushroom was surface sterilized with 70% ethanol by rubbing cotton soaked in the alcohol. The stalk was peeled from out site. Tissues were collected from inner region of stalk of the sporophore. The tissues were cut into small pieces and placed on the solidified petri dish containing PDA. After inoculation, the PDA plates were covered with cellophane paper. All operations were done under sterile condition in a clean bench. The inoculated petri dishes were transferred to a growth chamber maintaining temperature 27^0C and incubated for 10 days. This ten days old PDA culture was used for inoculation of mother culture.

Preparation of Mother Culture (Sawdust): Mother culture was prepared by mixing sawdust and wheat bran at the ratio of 2:1. Calcium carbonate was used at the rate of 0.2% of the mixture. The moisture level of the mixture was maintained at 65% by adding tap water. Polypropylene bags of 18 X 25 cm size were filled with 300 g of the above prepared mixture and packed tightly. The neck of the bag was prepared by using heat resistant plastic neck. A hole of about 2/3 deep of the volume of the bag was made at the center with a sharp end stick for space to put inoculum. The neck was plugged with cotton and covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for one hour at 120° C under 1.1 kg/cm2 pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. A piece of pure PDA culture medium containing mycelium of oyster mushroom was placed aseptically in the hole of mother culture packet and the packet was again plugged as mentioned before. Then the inoculated packets were placed on a wooden rack in the laboratory at 25 ± 20°C temperature for incubation. The medium of the mother culture was colonized by the fungus as manifested by white colony growth of mycelium within 10-15 days of inoculation. The fully colonized packets were used for spawning.

Preparation of Mother Culture (Wheat): Mother culture was prepared by mixing boiled wheat and calcium carbonate. For 300gm mother, calcium carbonate was used at the rate of 0.5gm per packet. Polypropylene bags of 18 X 25 cm size were filled with 200 g of the above prepared mixture and packed tightly. Then packets preparation, inoculataion and incubation were done as same as mother culture (sawdust).

Preparation of Mother Culture (Rice): Mother culture was prepared by mixing boiled rice, calcium carbonate and bavestin. Per 300 gm mother calcium carbonate was used at 1gm /p and bavistin is used 0.3gm / p. Polypropylene bags of 18 X 25 cm size were filled with 300 g of the above prepared mixture and packed tightly. Then packets preparation, inoculataion and incubation were done as same as mother culture (wheat).

Preparation of Substrates and Spawn Packets: Different substrates were prepared separately using selected waste materials.

In case of saw dust waste: Saw dust was mixed with nutrient material of wheat bran at the ratio of 2:1. Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Then 500 g of substrate mixtures were filled in polypropylene bags. The procedure of packet preparation, plugging, sterilization and incubation were the same as mentioned under the section preparation of mother culture. Each spawn packet was inoculated with the mother culture at the rate of two tea spoonfuls per packet. After inoculation, the packets were incubated in the laboratory.

In case of rice straw: Dried straw residue was chopped into 2-4 cm long. In the hot water method, water is heated in a large container to 60°C (140°F). Then dry substrate materials were added to the water. Ideally the water should just cover the substrate. Let that stand for 60 minutes. At that time drain the substrate and place it where it will cool slowly. After pasteurizing the straw materials were transferred to cool for 16-20 hrs and if necessary placed on cemented floor for removal of excess water to make the moisture content 60%. Then 500 g of substrates were filled individually in polypropylene bags. During bagging the packets were inoculated individually with the mother culture at the rate of 20% of substrate. After completion of spawning, all packets were plugged by inserting water-absorbing cotton with the help of plastic ring, rubber band and placed in the mushroom culture house for incubation.

In case of sugarcane bagasse: Fresh sugarcane bagasse residue was sun dried and chopped into 2-4 cm lengths using a chopper machine and mixed with wheat bran at the ratio of 2:1. Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Then 500 g of substrate mixture was filled in polypropylene bags. The procedure of packet preparation, plugging, sterilization, inoculation and incubation were the same as mentioned under the preparation of saw dust spawn.

In case of press mud: Press mud residue was sun dried and mixed with saw dust at the ratio of 2:1. Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Then 500 g of substrate mixture was filled in polypropylene bags. The procedure of packet preparation, plugging, sterilization, inoculation and incubation were the same as mentioned under the preparation of saw dust spawn.

In case of cotton seed hull: Cotton seed waste was mixed with saw dust at the ratio of 2:1. Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Then 500 g of substrate mixture was filled in polypropylene bags. The procedure of cotton seed hull packet preparation was same as mentioned under the preparation of press mud spawn.

In case of water hyacinth: Water hyacinth was collected from locally aquatic region. Then the residue was sun dried for 3 days and chopped into section 2 to 5 cm long using a locally made manual chopper. Chopped hyacinth was mixed with wheat bran at the ratio of 2:1. Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Then 500 g of substrate mixture was filled in polypropylene bags. The procedure of packet preparation, plugging, sterilization, inoculation and incubation were the same as mentioned under the preparation of saw dust spawn.

In case of waste paper: Waste paper was cut into 2 to 5 cm pieces and mixed with wheat bran at the ratio of 2:1. Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Then 500 g of substrate mixture was filled in polypropylene bags. The procedure of packet preparation, plugging, sterilization, inoculation and incubation were the same as mentioned under the preparation of saw dust spawn.

In case of spent mushroom substrate: Spent cotton seed hull was reused as spent mushroom substrate which collected from straw bed after harvesting of straw mushroom. This substrate mixed with saw dust at the ratio of 2:1. Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Then 500 g of substrate mixture was filled in polypropylene bags. The procedure of cotton seed hull spawn preparation was same as mentioned under the preparation of press mud spawn. After completion of mycelium running, spawn packets were opened by 'D' shape cut on both the shoulder of the packets and transferred to culture house at 14-20^{0}C and 60-70% relative humidity.

Data Collection and Analysis: The experiment was laid out in a completely randomized design (CRD) with four replications. Yield and yield parameters were taken on the basis of three flashes except in pcys-6 where only one flash was obtained. Data on mycelial growth rate, days required from stimulation to primordia initiation, number of primordia, number of fruiting body, number of effective fruiting body, length of stipe, diameter of stipe, diameter of pileus, thickness of pileus, biological yield (g/packet), economic yield (g/packet) and benefit cost ratio were recorded. Data were analyzed followed by Gomez and Gomez (1984) using MSTAT-C computer program. Means were computed following Duncan's Multiple Ranges Test (DMRT).

RESULTS AND DISCUSSION

Eight different byproducts like- saw dust, rice straw, sugarcane bagasse, press mud, cotton seed hull, water hyacinth, waste paper and spent mushroom substrate were evaluated by observing the effect of yield and yield related characters.

Duration to Complete Mycelium Running: The effect of different byproducts on mycelium running time was significantly different. Duration to complete mycelium running in spawn packet ranged from 11.00 to 46.00 days on different wastes (Table 1). Significantly the lowest days to complete mycelium running was recorded on rice straw which was followed by waste paper, spent mushroom substrate and cotton seed hull. Maximum days to complete mycelium running was found in water hyacinth which was statistically highest to all other wastes. Days to complete mycelium running on sawdust and press mud were statistically similar but insignificantly lower to sugarcane bagasse. These variations have been reported by different Scientists. Patra and Pani (1995) recorded the duration of complete mycelium running in 13-16 days on paddy straw. Tan (1981) reported the completion of spawn running in 21 days on the cotton waste. The duration of different growth stages of cultivated mushrooms depends on the type of substrate and substrate formulation like composted or non-composted or spent mushroom substrate and supplements (Mamiro and Royse, 2008; Olfati and Peyvast, 2008). The appreciable days to complete mycelium running of oyster mushroom on different substrates might be due to variations in their chemical composition and C: N ratio as reported by Bhatti *et al.* (1987).

Table 1: Effect of different biproducts on the duration to complete mycelium running of oyster mushroom (*Pleurotus ostreatus*)

Wastes	Duration to complete mycelium running (days)
Saw dust	23.25 c
Rice straw	11.00 e
Sugarcane bagasse	31.75 b
Press mud	24.00 c
Cotton seed hull	16.75 d
Water hyacinth	46.00 a
Waste paper	16.25 d
Spent mushroom substrate	16.75 d
CV (%)	**10.39**

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

Biological Yield: The effect of different wastes on yield of oyster mushroom (*Pleurotus ostreatus*) varied significantly (Table 2). Biological yield on different wastes ranged from 48.25-216.80 g/packet. The highest biological yield was found on waste paper, which was statistically similar to rice straw. The effects of sugarcane bagasse, cotton seed hull and press mud on this parameter were statistically similar but significantly lower as compared to saw dust. The lowest biological yield and economical yield were recorded from the packet containing water hyacinth, which was statistically lower to spent mushroom substrate. In the present study maximum yield was obtained from waste paper supplemented with wheat bran. Almost similar results were obtained by Baysal *et al.* (2003).

In the study, the yield of mushroom was affected by different substrates or wastes and it's indicate the mycelia of mushroom have different colonizing potentials for the substrates in which they are grown and directly related to the correspond yield. These results similar within the mushroom yield range of 61-796 g/kg wet substrate reported by Mane *et al.*, 2007; Vetayasuporn, 2006) on *Pleurotus* species cultivated on various non-composted substrates, with and without supplements. Besides, Gerrits and Muller (1965) and Quimio (1987) found that cellulose rich organic substrates are good for the cultivation of mushroom. High cellulose waste enhanced cellulose enzyme production and increased yield of mushroom (Ramasamy and Kandaswary, 1976). However, in the study all lignocelluloses wastes were performed as mushroom substrates and showed positively response on the yield. Similar results were found by Obodai, *et al.* (2003).

Table2. Effect of different biproducts on the economic yield, dry yield, biological yield and biological efficiency of oyster mushroom (*Pleurotus ostreatus*)

Biproducts	Economic yield	Dry yield	Biological yield	Biological efficiency
Saw dust	188.00 b	19.25 b	192.80 b	96.38 b
Rice straw	192.80 ab	17.25 b	201.30 ab	100.60 ab
Sugarcane bagasse	155.00 c	13.88 c	162.00 c	81.00 c
Press mud	138.00 c	12.75 c	143.00 c	71.50 c
Cotton seed hull	146.50 c	13.00 c	151.50 c	75.75 c
Water hyacinth	44.75 e	4.75 d	48.25 d	24.43 d
Waste paper	208.30 a	23.00 a	216.80 a	108.40 a
Spent mushroom substrate	62.75 d	6.37 d	65.75 d	32.88 d
CV (%)	**8.59**	**11.27**	**8.83**	**8.83**

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

Biological Efficiency: The biological efficiency was analyzed in order to find out the suitability of the tested wastes. It was positively correlated with the biological yield and it's depending on amount of dry substrate used. Significant variation was found in biological efficiency of oyster mushroom grown on different wastes. It was ranged from 24.43-108.40% (Table 2). The highest biological efficiency (108.40%) was recorded on waste paper followed by rice straw (100.60%), sawdust (96.38%), respectively. The lowest biological efficiency (24.43%) was recorded on water hyacinth followed by spent mushroom substrate (32.88%). Many works have been developed on different wastes against this character. Sarker *et al.* (2007) reported that highest biological efficiency obtained on waste paper (145.66%) for *Pleurotus ostreatus* against the different substrates. They also found the biological efficiency of rice straw and saw dust were 115.66% and 102.76% with the supplement of 50% wheat bran. Cotton and bagasse also gives a productive waste for oyster mushroom. Cotton waste has a biological efficiency of 56-86% for oyster mushroom. For *Pleurotus* species the biological efficiency of the pure bagasse is 15%. Comparable biological efficiency of 61% was obtained from sawdust using *P. ostreatus* strain EM-1 (Obodai *et al.*, 2003). Spent *Volvariella* compost dried and re-used for *Pleurotus* species with biological efficiency of 80% (Chang & Miles, 1989). The differences of biological efficiency is an indication that the nature of the wastes.

Patra and Pani (1995) reported that substrates for the cultivation of oyster mushrooms should have biological efficiency values at least 50%. In this context, maximum wastes in the study supported satisfactory biological efficiency.

Economic Yield and Dry Yield: Remarkable differences were observed in economical yield and dry yield on spawn packets of the different wastes. It was ranged from 44.75 to 208.30 g/packet and 4.75 to 23 g/packet respectively (Table 2). The highest economical yield and dry yield were found on waste paper, which was statistically similar to rice straw and saw dust. The effects of sugarcane bagasse, cotton seed hull and press mud on this both parameters were statistically similar. The lowest economical yield and dry yield were recorded from the packet containing water hyacinth, which was followed by spent mushroom substrate. In the present study, positively relation was observed between economical yield and dry yield on different substrates.

Benefit Cost Ratio: The benefit cost ratio was analyzed in order to find out the profitability of the different wastes. For this purpose the total cost per packet and total income per packet were considered in the study. The highest benefit cost ratio was obtained with rice straw (4.20), which was followed by saw dust (4.10), waste paper (3.33) and press mud (3.31) respectively. The lowest benefit-cost ratio was recorded with water hyacinth, which was followed by spent mushroom substrate. Similar results were also obtained by Sarker *et al.* (2007) observed the benefit cost ratio of 4.90, 5.58, 5.90 and 5.65 in case of *P. ostreatus* on saw dust, rice straw, sugarcane bagasse and waste paper. To maximize profit from mushroom production, the increases in the use of rice straw and saw dust and decreases in the use of labor must be adjusted to reach the optimum level. In the study, waste paper produced highest yield among the tested wastes but the benefit cost ratio was less than rice straw and saw dust based on present market price. The cause of these variations might be due to consideration of other costs involved in the production of oyster mushroom and price of substrate. On the other hand, all wastes showed the profitable for oyster mushroom cultivation in the respect of benefit cost ratio. So, the utilization of these wastes for the production of oyster mushroom could be more economically and ecologically practices.

As 85 percent of the direct beneficiaries of the project's mushroom development activities are women, this experiment has showed that women can be involved successfully in a small cash-generating enterprise. Before being involved with mushroom production, the majority of the female farmers spent most of their time on household activities. The proceeds from the sales of mushroom are now kept by these female farmers, which they use to meet their family needs. Furthermore, all the project's mushroom farmers are now maintaining accounts and keeping records of their small enterprise.

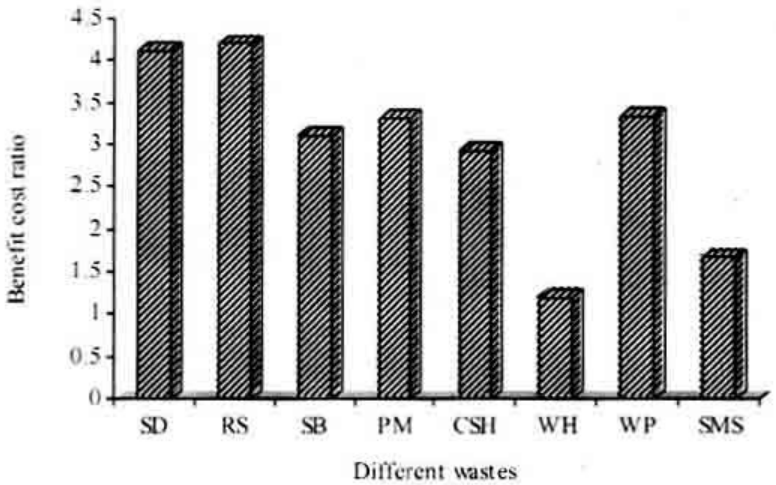

Fig. 3. Effect of different byproducts on benefit cost ratio of oyster mushroom (*Pleurotus ostreatus*) (SD = Saw dust, RS = Rice straw, SB = Sugarcane bagasse, PM = Press mud, CSH = Cotton seed hull, WH = Water hyacinth, WP = Waste paper and SMS = Spent mushroom substrate).

In the present experiment, oyster mushroom successfully produced on all agricultural biproducts or wastes and gave the positive results against the tested characters. The results were supported with the results of Chang, 1999, He reported that mushroom cultivation is a world wide practice which utilizes the almost all agricultural and agro-industrial residues or wastes as substrate. So, it might be a way of reducing the waste by the cultivation of mushroom in which creates a solution of reducing environmental pollution

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Effect of Media of Mother Culture on Growth and Yield of Shiitake Mushroom

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Abstract

An experiment was conducted to determine the effect of different media of mother culture containing sawdust, wheat grain, paddy, millet on growth, yield and yield attributes of shiitake mushroom (*Lentinus edodes*). Wheat grain required shortest duration of 20.00 days to complete mycelium running. The strains required up to 32 days to complete mycelium running on other substrates or media. The highest DMR (38.00days) was recorded when spawn packet inoculation with millet containing mother whereas the lowest (30.00days) DMR, days to bump formation (76.00), minimum days required from opening to harvest, minimum days to harvest (TDH), highest no of fruit body and effective fruiting body maximum yield and biological efficiency was observed when paddy grain containing mother culture throughly mixed with pasteurized sawdust. Sawdust containing mother culture ie.T_1 required the highest duration to form bump, pinhead initiation, highest DOH and maximum days to harvest (TDH) The minimum days required from opening to pin initiation (PI), lowest no of fruitbody and effective fruiting body, minimum yield and biological efficiency in T_4 treatment i e, millet containing mother culture.

Key words: Grain, Growth, Inoculation, *Lentinus edodes*, Mother culture, Sawdust, Spawning.

INTRODUCTION

Lentinula. edodes (Berk.) Pegler, commonly known as the shiitake mushroom, is one of the most widely grown species of mushroom and a very efficient biodegrader of wood. Although the mushroom is traditionally grown on wood logs (Harris, 1986), this procedure has been largely replaced by artificial log cultivation that utilizes heat-treated supplemented substrates enclosed in plastic bags. The shorter time required to complete a crop cycle and the higher yields, resulting in consistent market supply, are the main advantages of this procedure (Miller and Jong, 1986; Przybylowicz and Donoghue, 1990).

Substrate formulation, strain genotype and length of the incubation period have already been identified as important variables in the efficient production of shiitake on synthetic substrates used in bag-log cultivation (Royse and Bahler, 1986; Zadrazil, 1973; Kalberer, 1995). World wide, the most popular basal ingredient used in synthetic formulations of substrate for the commercial production of *L. edodes* is hardwood (preferably oak-wood)

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sawdust, supplemented at a rate of 20-30% (dry weight). by wheat or rice bran, millet etc. (Miller and Jong, 1986; Royse, 1996; Pire *et al.*, 2001). Wheat straw and other agricultural by-products resulting from processing maize, cotton, sunflower, flax, grape coffee, cacao etc. have been examined as alternative substrates for their cultivation (Przybylowicz and Donoghue, 1990; Levanon *et al.*, 1993; Bis'ko and Bilay, 1996; Philippoussis *et al.*, 2001).

The actual organism that produces the mushroom is called mycelium, a strand like mass of white cells found in the growing substrate. The mycelial growth of different mushroom species greatly influenced on media (Fasidi and Olorunmaiye, 1994; Eswaran and Ramabadran, 2000). Cereal grains such as wheat (Elhami *et al.*, 2008; Chang, 2009; Stanley, 2010), rye (Chang, 2009), sorghum (Chang, 2009; Stanley, 2010), rice (Oei, 1996), millet (Oei, 1996; Elhami *et al.*, 2008; Stanley, 2010) and white maize (Stanley, 2010) are used as mother spawn. Optimization of industrial mushroom production depends on improving the culture process (Larraya *et al.*, 2003). A range of abiotic parameters including temperature, light, carbon dioxide concentration, humidity and pH have been shown to influence carpophores production (Wessels *et al.*, 1987). Fruiting may also be stimulated by mechanical injury and chemical treatments (Hibbitt *et al.*, 1994). Highly proteinaceous materials such as ground pigeon pea and soybean have been reported to stimulate high fruit yield. Wheat, rye and millet that are used in making spawn also belong to this genre (Royse and May, 1982).

Grain spawn is in common use because of its ability to ramify the substrate faster and ease of planting (Bahl, 1988). Spawn grains such as wheat, millet and corn have been reported to affect carpophores production (Nwanze *et al.*, 2005b) and there are various additives that are known to stimulate fruiting. They include rice bran, cassava peels and soybean powder, Nwanze *et al.* (2005a) examined the effect of spawn grains such as wheat, millet and corn on the culture of *Lentinus squarrosulus*. The results showed that corn spawn induced highest yield and dry weight of fruiting as compared to wheat and millet spawn. The present work focuses on the effect of the above factor on the culture of *Lentinus edodes*.

MATERIALS AND METHODS

The experiment was conducted at National Mushroom Development and Extention Centre, Sobhanbag, Savar, Dhaka, Bangladesh from January to May 2013. In this experiment different grains and sawdust were used as media of mother for the cultivation of shiitake mushroom. The treatments were T_1 = Spawn packet inoculation with sawdust, T_2 = Spawn packet inoculation with Paddy T_3 = Spawn packet inoculation with wheat T_4 = Spawn packet inoculation with millet T_5 = Spawn packet prepared by through spawning method with paddy grain containing mother culture.

Pasteurization of Sawdust: Sawdust used as a main substrate for preparation of spawn packet. The sawdust was supplemented with wheat bran, rice husk and $CaCO_3$ @ 27.27, 4.56 and 0.2% (w/w) for spawn packet preparation in each treatment except T_5. Incase of

treatment T_5 without any supplement and pasteurized sawdust was used. The sawdust was poured in net's bag (8-10 kg) and soaked in warm water at 60^0C for one hour. After soaking excess water was drained off and the sawdust was air dried to fix the moisture content at 62%. To estimate the moisture content of air dried sawdust with moisture meter. If no water drop was released from the sawdust the moisture content was considered to be appropriate. Then drained out the water. was used without any supplementation. Strains of shiitake mushroom (*Lentinus edodes*), namely Le-8 (NAMDEC shiitake-1) was used as test materials.

Preparation of Mother Culture: To prepare mother culture of the test mushroom (Le-8) sawdust and wheat bran were mixed together at 2:1 (v/v) and supplemented with $CaCO_3$ at 0.2% (w/w) of the mixture. The moisture level of the mixed substrate was maintained at 65% with tap water. Incase of grains (paddy, wheat, millet) substrate grains collected which was free from diseases and cereal grains which were not broken, old, and insect damaged. The grains were thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses, weeds, etc. Washed grains were then soaked in sufficient water for 2-3hours and boiled in a container for 25-45 minutes. Excess water from the boiled grains was removed by spreading on sieve made of fine wire mesh. The grains were left as such for few hours on the sieve so that the water on surface was evaporated. Then spread on polythene sheet and grains were thoroughly mixed with calcium carbonate at 0.2% so that the pH of the grains was around 7.0 to 7.8. This mixing was done on a smooth surface after wearing gloves.

The substrate was poured into polypropylene bags (18 cm × 25 cm) according to treatments at 500 g / bag. The substrate in bags was sterilized in an autoclave for 2 h at 121^0C under 1.1 kg / cm^2 pressures and allowed to cool for 24 h. Pure cultures of strain Le-8 was grown on potato dextrose agar (PDA) following hyphal tip method. A piece of the PDA culture of Le-8 containing mycelium was placed aseptically in the opening of the mother culture packets. The inoculated packets were placed on a rack in the laboratory at 22 ± 2^0C for incubation. The substrate of the mother culture was covered by whitish mycelium of test strain within 20-32 days after inoculation according to treatments. The fully colonized packets were used for spawning or inoculation.

Preparation of Spawn Packets: The spawn packets were prepared separately according to individual treatment. The substrate mixture was poured into 18 cm × 25 cm polypropylene bags at 500 g / bag. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the bag was made for space to introduce the inocula. The neck of each poly bags was plugged with cotton, covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for 2 h at 121^0C under 1.1 kg / cm^2 pressures. After sterilization, the packets were cooled and transferred to an inoculation chamber. The packets were inoculated separately with different mother culture of the test strain at the rate of two tea spoonful per packet. Incase of T_5 treatment pasteurized sawdust and paddy grain containing mother mixed thoroughly without supplementation. Sawdust and paddy mother mixed at the ratio of 2:1. Then the substrate mixture was poured into 18 cm × 25 cm polypropylene bags at 500 g / bag. The

neck of the bag was prepared by using heat resistant plastic pipe. The neck of each poly bags was plugged with cotton and tied with a rubber band. The inoculated packets were incubated at 25 ± 2°C.

Mycelial Colonization and Bump Formation: During incubation period, whitish mycelium started to grow in the inoculated substrate. The strain showed optimal mycelial growth at 22 ± 2°C and 60-70% relative humidity in the culture house. After full colonization of the spawn packets, a thick mycelial coat formed on the outer surface of colonized substrate. Clumps of mycelia appeared as blister like bumps of various sizes on the surface of the mycelial coat in each packet. Bumping usually started when color of the colonized white mycelia became brown.

Cultivation for Fruiting Body: After mycelium maturation and bump formation, all the packets were fully opened, and placed separately on the rack in the culture house. Temperature, relative humidity and light intensity of the culture house were maintained at 18-22 °C, 60-70 % and 10-20 lux, respectively. Sufficient water was sprayed every day and proper aeration was maintained in culture house for the release excess CO_2 and supply of sufficient O_2 as required for the development of primordia and fruiting bodies.

Collection and Analysis of Data: The packets were arranged in culture house following completely randomized design with 4 replications. Data on days to complete mycelium running, bump formation, days to pin head initiation, days required from opening to harvest, days for total harvest, number of fruiting body and effective fruiting body, length and diameter of stalk, diameter and thickness of pileus, yield (g/packet) and biological efficiency were recorded. Weight of fruiting body was recorded after removing the lower hard and dirty portion of stipe. The biological efficiency was determined using the following formula:

$$\text{Biological efficiency}(\%) = \frac{\text{Total biological yield (g / packet)}}{\text{Total dry weight of the substrate used (g / packet)}} x100$$

Data were analyzed using MSTAT-C computer program. Means were compared following Duncan's multiple range test using the same computer program.

RESULTS AND DISCUSSION

Days to Complete Mycelium Running in Mother Culture: Days to complete mycelium running (DMR) on different media ranged 20.0-32.0 (Fig. 1). The highest DMR was recorded from sawdust containing mother culture followed by millet. The lowest DMR was observed in wheat grain followed by paddy. The number of days from inoculation to the total colonization of a substrate is related to the mycelial growth rate on the substrate. A faster growth rate results in a corresponding reduction in the days required for complete colonization of the substrate by the mycelia. The days until total colonization of the grains by the mycelia varied for the various treatments, these differences were significantly different.

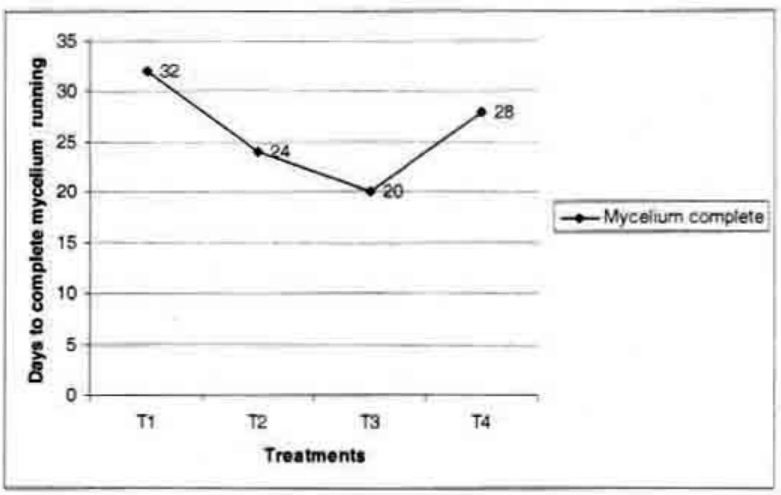

Fig. 1. Effect of media on days required to complete mycelium running in mother culture.

Days to Complete Mycelium Running in Spawn Packet: Days to complete mycelium running (DMR) on different media and spawning method ranged 30.00-38.00 and significantly different (Table 1). The highest DMR was recorded when spawn packet inoculation with millet containing mother culture followed by sawdust. The lowest DMR was observed when paddy grain thoroughly mixed with pasteurized sawdust that is thorough spawning method.

Days to Bump Formation: Days required bump formation (DBF) in different treatments ranged 76.0-115.0 and significantly different. In paddy grain containing mother culture and through spawning method developed bump earlier than inoculation with other grains. Paddy grain containing mother culture required lowest duration to form bump in through spawning method followed by inoculation with wheat and millet. Sawdust required the highest duration to form bump in inoculation method i.e. T_1 treatment (Table 1).

Days to Pin Head Initiation: The duration from opening of spawn packet to pin head initiation on different treatments ranged 1.50-3.50. The minimum days required from opening to pin initiation (PI) in T_4 treatment where inoculation with millet followed by paddy and wheat. Significantly the highest PI was recorded in sawdust i.e. T_1 treatment (Table 1).

Required Days from Opening to Harvest: The duration from opening of spawn packet to first harvest on different treatments ranged 4.68-9.00. The minimum days required from opening to harvest (DOH) in T_5 treatment when paddy grain used as mother culture and through spawning method which was statistically similar to T_2, T_3 and T_4. Significantly the highest DOH was recorded in sawdust i.e. T_1 treatment (Table 1).

Total Days for Harvest: In case of paddy grain, the minimum days to harvest (TDH) was recorded from through spawning method (T_5) followed by other grains. Effect of those treatments on DH was significantly different. Significantly the highest duration

observed in T_1 treatment where sawdust containing mother used as inoculation to obtain spawn packets (Table 1).

Table 1. Effect of different media on growth of shiitake mushroom

Treatments	Days to complete mycelium running	Days to bump formation	Days to pin head initiation	Days required from opening to harvest	Total days for harvest
T_1	37.00ab	115.00a	3.50a	9.00a	124.00a
T_2	35.00c	80.00c	2.50b	5.50b	85.50bc
T_3	36.00bc	83.00b	2.75b	5.25b	88.25b
T_4	38.00a	85.00b	1.50c	5.00b	90.00b
T_5	30.00d	76.00d	2.38b	4.68b	81.69c
Average	35.20	87.80	2.53	5.89	93.89
CV (%)	**3.11**	**1.59**	**10.42**	**8.54**	**3.92**

Figures within the same column with a common letter(s) do not differ significantly (P = 0.05). T_1 = Inoculation with sawdust, T_2 = Inoculation with Paddy T_3 = Inoculation with Wheat T_4 = Inoculation with Millet T_5 = Through spawning with Paddy.

Number of Total and Effective Fruiting Body: Number of total fruiting body ranged 5.25-20.31 per packet and significantly different. The lowest number was found on millet followed by wheat, paddy and sawdust. The highest number was found T_5 treatment where through spawning with paddy grain containing mother culture. Number of effective fruiting body varied from 3.75-13.81 with mean 7.56 per packet. Significantly the highest number of effective fruiting body was recorded fromT_5 treatment followed by T_1. Their differences were significant. The lowest number of effective fruiting body was found in T_3 containing wheat grain which was statistically similar to T_2 and T_4 treatments (Table 2).

Length and Diameter of Stalk: T_4 treatment (millet) produced the longest stalk (6.62 cm) which was statistically similar to wheat and paddy. The shortest stalk (3.58 cm) was found in T_5which was statistically different to other treatments. The maximum stalk diameter (1.32 cm) was found in sawdust which was statistically different to other treatments. The lowest diameter of 0.56 cm was recorded from paddy grain and through spawning method (Table 2).

Diameter and Thickness of Pileus: The diameter of pileus ranged 5.12-6.12 cm with mean 5.49 cm and its thickness varied from 0.42 to 0.92 cm with mean 0.68 cm. The highest pileus diameter was found in T_4 treatment. The minimum diameter was recorded from T_2 treatment where inoculation with paddy grain. Significantly the highest pileus thickness was found in T_1 treatment and the lowest in T_5 (Table 2).

Table 2. Effect of different media of mother culture and spawning on yield attributes and yield of shiitake mushroom

Treatments	Number of fruit body	Number of effective fruit body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield per packet (g)	Biological efficiency (%)
T_1	13.00b	11.50b	5.05b	1.32a	5.35ab	0.92a	62.50b	32.90b
T_2	7.00c	4.50c	6.12a	0.92b	5.12b	0.65b	50.00c	26.32c
T_3	6.00cd	3.75c	6.52a	0.87b	5.62ab	0.60b	43.00cd	22.63cd
T_4	5.25d	4.25c	6.62a	0.95b	6.12a	0.82a	39.00d	20.53d
T_5	20.31a	13.81a	3.58c	0.56c	5.25ab	0.42c	75.81a	39.90a
Average	10.31	7.56	5.58	0.92	5.49	0.68	54.06	28.46
CV (%)	**10.59**	**10.24**	**7.32**	**7.62**	**9.95**	**10.76**	**9.71**	**9.71**

Figures within the same column with a common letter(s) do not differ significantly (P = 0.05). T_1 = Inoculation with sawdust, T_2 = Inoculation with paddy, T_3 = Inoculation with wheat, T_4 = Inoculation with millet, T_5 = Through spawning with paddy.

Yield per Packet and Biological Efficiency: The yield of fruiting body per packet ranged 39.0-75.81 g with mean 54.06 g and biological efficiency ranged 20.53-39.90% with mean 28.46%. The maximum yield and biological efficiency were recorded from paddy grain and through spawning method (T_5). Differences in both the parameters under those five media were significant (Table 2).

Results of the present experiment reveal that there are appreciable variations in growth, yield and yield contributing attributes with the variation of media and spawning method of shiitake mushroom. In terms of yield and yield attributes, performance of spawning method, through spawning was better than inoculation. Among the media of mother culture paddy and sawdust give maximum yield and yield contributing characters. Millet is not suitable as media and other grains except paddy also not suitable for through spawning to cultivate shiitake mushroom. Many other investigators also found variations in effect of different media on growth, yield and yield contributing characters of mushroom.

Spawn grains were used to introduce pure fungal cultures into different growth substrates as well as to increase mushroom yield. Kadiri (1999) showed a preference for millet while producing spawn for *L. squarrosulus*. Nwanze *et al.* (2004) examined the effect of corn, millet and wheat spawn on some growth parameters of *L. squarrosulus*. Narh *et al.* (2011) observed that sorghum grains better than millet for mycelial growth due to large surface area. Larger surface area and pore of substrates support faster mycelium growth rate (Tinoco *et al.*, 2001). This could account for the significant difference between the mycelial growths rates recorded for the treatments. Wheat grains have a larger surface area compared to millet grains and sawdust. Since smaller particles are generally more compact than larger particles, wheat and paddy would have larger air spaces than millet and sawdust. This increased ventilation within the wheat grain treatment resulting in improved respiration by the mycelia. Respiration rate is directly related to O_2 concentration of substrate (Mehravaran, 1993). Hence, significantly higher growth rate of mycelia in the wheat grain treatment compared to sawdust. The present study, however,

examined the effect of media of mother like sawdust, paddy, millet and wheat grain on some growth parameters of *Lentinus edodes* (Le-8).

Nwanze *et al.* (2004) observed that in the case of *P. atroumbonata*, wheat and corn spawn were similar andinduced the longest stipe lengths and heaviest carpophore wet weights. However, with *L. squarrosulus*,only corn spawn had that effect. In addition, wheat spawn produced the widest pileus diameters with *P. atroumbonata*, but with *L. squarrosulus*, grains had no significant effect on pileus diameter. It can be inferred from the results that Fasidi and Kadiri (1993) and Kadiri (1999) could have gotten superior fruit body production and mycelia density of *L. squarrosulus* if they had used corn or millet as opposed to rice or sorghum.

Earlier works reported that mycelial growth, earliness, crop quality and productivity vary among strains on sawdust-based substrates (Diehle and Royse, 1986; Royse and Bahler, 1986). Furthermore, the close relationship between the rate of mycelial development during substrate colonization, CO_2 production and crop yield and the role of sufficient aeration have been demonstrated in sawdust based experiments (Leatham and Stahmann, 1987; Kalberer, 1995; Donogue and Deninson, 1995). Nevertheless, little is known concerning the performance of selected strains on different substrates composed of various lignocellulosic residues, especially regarding mycelial growth and the parameters affecting it.

Media composition has a significant effect on mushroom production whether it is at the mycelia orcarpophore level (Klemmer and Lenny, 1965; Schisler and Sinden, 1962). Animal bedding and rice was the best media for *L. squarrosulus*, producing superior weights and dimensions. This was in contrast to *P. atroumbonata* that favours sawdust media (Nwanze *et al.*, 2004). The one component that the two media have in common is brown rice. This observation supports the finding that rice protein, which is rich in phenylalanine, leucine, isoleucine and valine is important in increasing mushroom yield (Schisler and Sinden, 1962).

The results clearly show that effect of media, and spawning method have a positive effect on growth yield and yield attributes of *L. edodes* (Le-8). The possibilities of economic exploitation should be examined since this particular specie can easily be cultured.

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Microbial Status of Different Mushroom Products Available in Bangladesh

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Abstract

The experiment was conducted to study comparative microbial load of mushroom and mushroom enriched products available in market. The highest total viable microbial count of powder mushroom recorded was 3.0×10^5 cfu/g in powder-1 and the lowest was found 1.0×10^3 cfu/g in powder-7. The results indicated that approximately 80, 90 and 80% powder mushrooms were safe for consumption with Coliform, Fecal coliform and *E.coli* respectively as well as about 60% powder mushroom was free from salmonella sp. The findings show that the highest microbial load of pickle was found 8.0×10^2 cfu/g and the lowest 1.5×10^2 cfu/g in pickle 3 and pickle 1 respectively. Moreover, nearly 85% pickle samples were found free from coliform, fecal coliform and *E. coli* contamination. On the other hand, the microbial load of other products both halua and cosmetics were contain < 10 cfu/g and were free from *Salmonella* sp. So, it was revealed that in most of the cases powder and pickles were safe for consumption in view of total viable count, total coliform, fecal coliform, *E.coli* and even *Salmonella* sp. but it will be better to consume those after processing. In addition, halua was fully safe for consumption and cosmetics products were also safe for use.

Keywords: Assessment, Mushroom product, Quality, Coliform, *E. coli*, *Salmonella* sp.

INTRODUCTION

Mushrooms have gained popularity all over the world due to its pleasant aroma, taste and fleshy nature although the nutritional and medicinal values of mushrooms have long been recognized (Lucas *et al.*, 1957; Suzuki *et al.*, 1976). Now, mushrooms are being cultivated in more than 100 countries of the World. In Bangladesh, interest in mushroom began in the late 1960's (Majid *et al.*, 1968). Now more than ten thousand tons fresh mushroom is being produced in Bangladesh per year (Annual report-2012, NAMDEC). Mushrooms are now being available in fresh pack, processed pack, powdered form, capsule form, canned products as well as mushroom enriched cosmetics in most upazilla level, town's shopping malls and mega shops of the country.

The microbial load and the presence of the bacterial pathogens in foods are a good indicator of the food quality and the potential health risk they pose to consumers (Rosmini *et al.*, 2004). Food borne infections and illnesses is a major international health problem with consequent economic reduction. It is a major cause of illness and death worldwide (Adak *et al.*, 2005). According to Clarence *et al.* (2009), food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by micro

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organisms present in food. *Escherichia coli*, particularly serotype O157:H7 has become an important food borne pathogen responsible for gastroenteritis epidemics in North America, Europe, Asia and Africa (Altekruse *et al.*, 1997). Various serotypes of *Salmonella* sp. including *S. enteritidis*, have been reportedly responsible for food borne epidemics in various countries, emphasizing the importance of the pathogen as a food safety concern (Todd, 1997).

Improvements in food security will bring significant socioeconomic benefits by checking microbial status (kell *et al.*, 2011). So the present investigation was conducted to determine the microbial status of mushroom and mushroom based products in Bangladesh.

MATERIALS AND METHODS

The present study is to evaluate the microbiological quality of the mushroom powder and mushroom based products such as pickles, halua, cosmetics etc. The experiment was conducted at microbiology laboratory of National Mushroom Development and Extention Centre (NAMDEC), Sobhanbagh, Savar, Dhaka.

Collection of Samples: Samples of mushroom including powdered, capsules, pickles, halua and cosmetic products were collected from randomized sampling from the marketing section of NAMDEC and different local producer in Bangladesh.

Table 1: General description of collected samples

Sl. No.	Collected sample No.	Samples derived from	Samples condition when it was collected		
			Type	Package	Weight
1.	10	Oyster / reishi mushrooms	Powder	Plastic jar	100 g
2.	10	Oyster mushrooms	Pickle, Halua, and Cosmetics	Glass / plastic jar	400 / 20 g

Procedure:

Total viable count (TVC): Twenty five grams (25 g) of each sample was diluted in 225 ml of sterile distilled water (diluents) and mixed vigorously by shaking. One ml of the resultant mixture was aseptically transformed to 9 ml of sterile water in a test tube. The dilution was continued serially until the required dilution was attained. One milliliter (1 ml) of each dilution was inoculated into a sterile Plate Count Agar (PCA). This action was carried out under sterile aseptic conditions. Immediately, inoculated samples and agar medium was mixed thoroughly by alternate rotation and back-and-forth motion of plates on flat level surface. Let agar be solidified. Solidified petri dishes were kept in invert position and incubated promptly for 24 ± 2 h at 35 ± 2^{0}C. Then total viable organisms were counted.

Presumptive test of total coliform (TC), fecal coliform (FC) and *E. coli*: Twenty five gram sample was weighed and added into 225 ml of Butterfield's phosphate-buffered water and decimal dilutions were prepared. Numbers of dilutions were prepared depending on anticipated coliform density. All suspensions were shaked 25 times in vortex mix for 7 second. One ml of each dilution was transferred to 3 McCartney bottles containing LST broth and inverted Durham's tubes. Inoculated McCartney bottles were incubate at 35 ± 2^{0}C for 24-48 ± 2 h. Gas production were examined and recorded in tubes. Gas-negative tubes were re-incubated for an additional 24 h and examined (BAM, 1998).

Confirmed test for coliforms: A loopful of suspension was transferred into a tube of BGBB broth from each gas positive LST tube and pellicle was being avoided if present. BGBB tubes were incubated at 35 ± 2^{0}C and examined for gas production at 24-48 ± 2 hour. Then most probable number (MPN) of coliforms was calculated based on proportion of confirmed gas positive tubes for 3 consecutive dilutions using MPN charts (BAM, 1998).

Confirmed test for fecal coliforms and *E. coli*: From each gas positive LST tube of the presumptive test, a loopful of each suspension was transferred to tube containing BGBB and EC broth. Inoculated tubes were incubated at 44°C for 24 ± 2 h and examined for gas production. If negative, reincubated and examined again at 48 ± 2 h. Observing the gas production, the number of fecal coliform and *E. coli* were calculated using MPN charts (BAM, 1998).

Qualitative Detection of *Salmonella* Species and Isolation of Salmonella: Twenty five gram sample was weighed and homogenated in 225 ml Buffered Peptone water and incubated at 35°C for 24 h. Three (03) mm loopful (10 μl) of incubated broth medium was streaked on bismuth sulfite (BS) agar and xylose lysine desoxycholate (XLD) agar, and Incubated plates for 24 ± 2 h at 35°C. The presence of colonies were examined that may be Salmonella. Following test was done to identify Salmonella:

Triple Sugar Iron (TSI) agar test: Each presumed-Salmonella culture was inoculated into tubes of TSI with sterile needle by stabbing and streaking and again inoculated LIA slant in the same manner without flaming the loop. Salmonella gave positive reaction in TSI slants and produced H_2S as shown by the blackening of the media. The butt of the slant would turn to yellow.

Lysine Iron agar test: In LIA slant, the butt color was purple for positive test and was considered a distinct yellow LIA butt as negative result.

Simmons citrate agar: Containing growth from unclassified TSI agar slant was inoculated into the medium using needle by streaking slant and stabbing butt. It was Incubated 96 ± 2 h at 35°C. Presence of growth, usually accompanied by color change from green to blue was considered as positive. Most cultures of Salmonella were citrate-positive. No growth or very little growth and no color change were considered as negative.

RESULTS AND DISCUSSION

The results of total viable count (TVC), colifrom, fecal colifrom, *E.coli* and salmonella obtained from different mushroom products are summarized below:

Powder Mushroom: Among the 10 analyzed powder samples, powder-1 contains higher microbial load (log_{10} value 5.477 or 3.0×10^5 cfu/g) and the lowest microbial load (log_{10} value 3 or 1.0×10^3 cfu/g) was found in powder-7. According to the Bangladesh standard (BDS) 1829:2010, the acceptable microbial load is 1000 cfu/g and the fig.1 shows that the maximum analyzed mushroom powder sample were not acceptable in quality, if it does not undergoes any further thermal treatment. The analyzed sample in the industry may be contaminated due to improper handling and processing techniques as well as unhygienic production floor.

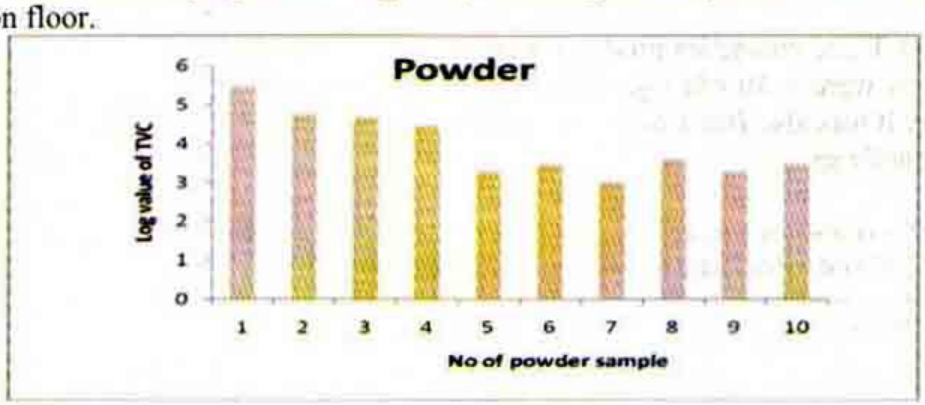

Fig. 1. Total Viable Count (Log value) of powdered mushroom samples.

In case of total coliform, fecal coliform and *E. coli*, 80% of analyzed powder samples (Table 2) were acceptable and can be consumed directly. But the remaining 20% samples were not acceptable due to high microbial load. About 60% of analyzed powder mushroom was free from *Salmonella* sp. and rest 40% was contaminated by *Salmonella* sp.

Table 2. The results of processed mushrooms (powder) of coliform, fecal coliform, *E. coli* and salmonella

Sl. No.	Product ID	Test Results			
		Total Coliform (MPN / g)	Fecal Coliform (MPN / g)	*E.coli* (MPN / g)	*Salmonella*
1	Powder 1	240	43	9.2	Present
2	Powder 2	< 3	< 3	< 3	Absent
3	Powder 3	75	9.2	9.2	Present
4	Powder 4	< 3	< 3	< 3	Absent
5	Powder 5	< 3	< 3	< 3	Absent
6	Powder 6	< 3	< 3	< 3	Absent
7	Powder 7	< 3	< 3	< 3	Absent
8	Powder 8	< 3	< 3	< 3	Present
9	Powder 9	< 3	< 3	< 3	Present
10	Powder 10	< 3	< 3	< 3	Absent

Pickles: In this study, the six pickles were analyzed. Among them, pickle-3 contains higher microbial load (8.0×10^2 cfu/g) and the lowest microbial load (1.5×10^2 cfu / g) was found in pickle 1. In bacteriological point of view, about 80% of the analyzed pickle samples (Table 3) were acceptable in case of total coliform and 20% of them were contaminated. But 100% products were acceptable for fecal coliform and *E. coli*.

Halua: In this study, halua was contained < 10 cfu/g, so in accordance to BDS 1829:2010, the halua was acceptable. It was free from any other contamination. The presence of *Salmonella* sp. was not found in this product. It might be followed proper handling and processing.

Cosmetics: Three cosmetics products were analyzed, among them, the microbial load of the products were < 10 cfu / g. According to BDS 1829:2010, the 100% samples were acceptable. It was also free from other contamination like coliform, fecal coliform, *E. coli* and *Salmonella* sp.

Table 3. The results of preserved mushrooms (pickle and other) of coliform, fecal coliform, *E.coli* and salmonella halua and cosmetic

Sl No.	Product ID	Test Results			
		Total Coliform (MPN / gm)	Fecal Coliform (MPN / gm)	*E.coli* (MPN / gm)	*Salmonella*
1	Pickle 1	< 3	< 3	< 3	Absent
2	Pickle 2	36	< 3	< 3	Absent
3	Pickle 3	36	< 3	< 3	Absent
4	Pickle 4	9.2	< 3	< 3	Absent
5	Pickle 5	< 3	< 3	< 3	Absent
6	Pickle 6	< 3	< 3	< 3	Absent
7	Mushroom halua	< 3	< 3	< 3	Absent
8	Cosmetic product 1	< 3	< 3	< 3	Absent
9	Cosmetic product 2	< 3	< 3	< 3	Absent
10	Cosmetic product 3	< 3	< 3	< 3	Absent

Results of TVC demonstrated that powder mushroom contain higher cfu/g and those of pickle and others contain less cfu/g, which is more or less similar with the findings of others (Kamal *et al.*, 2010; Van-Kampen *et al.*, 1998; Gilbert *et al.*, 1996; Patricia and Azanza, 2004). It may be due to the lack of suitable moisture level, proper drying method, hygienic condition, packaging and over all handling process. On the other hand, coliform, fecal coliform, *E. coli and salmonella* sp. also found comparatively higher in powder than pickle. Because pickle contain huge amount of oil and it is a very good preservative and also may be heat treatment is done perfectly. Burton (1989) found that bacterial counts were consistently lower depending on processing and storage conditions. In this experiment, it was clearly prove that the harmful or pathogenic organisms like coliform, fecal coliform, *E. coli* and *Salmonella* sp. were comparatively higher in powder mushrooms than those of processed or preserved mushrooms.

So, contaminated mushrooms or mushroom based products require minimal processing to consume. Conducting such type of experiments would enable us to decide as to what kind of processing methods should be adopted.

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