ISSN 1995-0683

Bangladesh Journal of Mushroom

Volume 8 Number 2 December 2014

Mushroom Development Institute
Department of Agricultural Extension
Ministry of Agriculture
Sobhanbag, Savar, Dhaka-1340
Bangladesh

Published by : **Dr. Nirod Chandra Sarker**
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Mushroom Development & Extension Programme
Mushroom Development Institute
Department of Agricultural Extension, Ministry of Agriculture
Sobhanbag, Savar, Dhaka.

Printed by : **Panguchi Colour Graphics**
177, Fakirapool, Akbor Mansion (2nd Floor), Dhaka-1000
Phone: 01716-839396, 01711-991211

ISSN : 1995-0683
Key title : Bangladesh Journal of Mushroom
Abbreviated key title : *Bangladesh J. Mushroom*
Subscription rates : Individual : Tk. 100.00
(each issue) Institution : Tk. 200.00

Bangladesh Journal of Mushroom

Volume 8 **Number 2** **December 2014**

Board of Editors

Bangladesh Journal of Mushroom

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Books:

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

Roberts, D. W. 1980. Toxins of entomopathogenic fungi. **In : Microbial control of Pests and Plant Diseases** (Ed) H. D. Burgess, New York Academic Press. pp. 441-463.

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Bangladesh Journal of Mushroom

Volume 8 **Number 2** **December 2014**

Contents

Bangladesh J. Mushroom. 8(2): 1- 10. 2014 (December)

Effect of Harvesting Age on Texture and Nutritional Composition of the Fruiting Body of Milky White Mushroom (*Calocybe indica*)

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Abstract

The present study was conducted at Mushroom Development Institute (MDI) Savar, Dhaka during March to October 2014 to determine color, texture and some nutrients from different fruiting body parts of milky white mushroom at different age. Specific three parts of fruiting body of nine different ages were analyzed. Among the parameters, moisture, total ash, fat, protein, fiber, calcium, iron, zinc, cobalt, copper, selenium and molybdenum were estimated. Highest moisture content (90.34%) was observed at the 4 days whole fruiting body. Highest content of total ash (19.10%), lipid (8.23%), protein (5.06%) and fiber (2.31%) was observed in 7 days pileus, 6 days pileus, 8 days pileus and 10 days whole fruiting body respectively. The highest amount of calcium (1.55 mg/100g), cobalt (0.12 mg/100g%), copper (8.7 %), Iron (8.7%), molybdenum (459.0%), selenium (335.0%) and zinc (7.5%) was found in 12 days pileus, 10 days stalk, 10 days pileus, 5 days stalk, 4 days whole fruiting body, 7 days whole fruiting body and 12 days whole fruiting body respectively. Ideal white color of mushroom was shown in 5 days pileus after that period the color gradually deteriorated and turned towards brown. Findings of this study give idea to take decision about the proper harvesting period of milky white mushroom which might be helpful for mushroom growers.

Key words: Nutrient, Minerals, Texture, Color, Different Stages and Fruiting Body.

INTRODUCTION

Comparing other vegetables mushrooms have a short shelf life. Immediately after harvest, fresh mushrooms start to soften and its color turned into brown due to enzymatic breakdown of cells and losing moisture through respiration (Jolivet *et al.*, 1998; Brennan *et al.*, 2000; Zivanovic *et al.*, 2003; Zivanovic *et al.*, 2004; Lespindard *et al.*, 2009). The edible tropical mushroom *Calocybe indica* is grown in Bangladesh and used as a nutritious food supplement. It is commonly known as milky white mushroom. Its robust size, sustainable yield, attractive color, delicacy, relatively long shelf life and lucrative market value have attracted the attention of both mushroom growers and consumers. Milky white mushroom which contains low fat product is rich in protein, lipids, fiber, carbohydrates and vitamins. It also contains abundant amount of essential amino acids (Ruhul *et al.*, 2010). The size, shape, color and taste of the mushroom depends on the maturity of fruiting body. Nutritional composition is affected by many factors including strains, composition of growth substrate, method of cultivation, stages of harvesting, specific parts of fruiting bodies, duration between harvest and time of analysis (Benjamin, 1995). Premature harvesting of mushroom may cause reduction of yield. On the other

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hand, if it is harvested at over age, the mushroom shades spores, loses its attractive color, robust in size and sporophore become fibrous, leathery and ultimately tasteless (Sarker *et al.*, 2011).
The aim of the present study was to determine effect of mushroom age at harvest on texture, color and nutritional quality of fruiting body and to find out suitable age for harvesting the crop.

MATERIALS AND METHODS

Milky white mushrooms were grown in the culture house of Mushroom Development Institute (MDI), Savar, Dhaka, Bangladesh during March to October 2014. Fruiting bodies were harvested at the age of 4 to 12 days with 24 hours intervals. Whole fruiting body, pileus and stalk were separated after harvest. Color, texture and moisture content were determined immediately after harvest. After that, mushrooms were divided into small pieces and were oven dry up to moisture level 4%, and then they were pulverized. Lipid, crude fiber, total ash and minerals of whole fruiting body, pileus and stalk were analyzed from pulverized powder in the 'Quality Control and Quality Assurance' laboratory of MDI.

Moisture content: Moisture content of fresh mushroom was determined following oven dry method. Twenty grams of fresh mushroom was taken in a pre weighed moisture box (A&D company ltd. N 92; P1011656, Japan) and dried in an oven at 100^0C and cooled in a desiccators. The process of heating and cooling was repeated till a constant weight was achieved. The moisture content was calculated using following formula:
Moisture (%) = (Initial weight- final weight) × 100 /Initial weight (Raghuramulu *et al.*, 2003).

Determination of total protein: Five grams of dried of each sample was taken and mixed with 50 ml of 1N NaOH and boiled for 30 minutes. The solution was cooled at room temperature and centrifuged at 1000 rpm by a table centrifuge machine (DIGISYSTEM: DSC-200T; Taiwan). The supernatant was collected and total protein content was measured according to the Biuret method (Burtis and Ashwood, 2006) with a diagnostic kit (Total Protein: Colourimetric test- Biuret method/ Crescent Diagnostics, Saudi Arabia).

Determination of total lipid: Total lipid content in different parts of mushroom was determined following the methods of Folch *et al.* (1957) with slight modification. Five grams of grinding mushroom was suspended in 50 ml of chloroform: methanol (2:1 v/v) mixture. The content was mixed thoroughly and let stand for 3 days. The solution was filtrated through Whitman filter paper No. 2 and centrifuged at 1000 rpm by a table centrifuge. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid.

Determination of crude fibre: Five grams of moisture and fat-free sample was taken in a beaker and 200 ml of boiling 0.255N H_2SO_4 was added. The mixture was boiled for 30

minutes keeping the volume constant by frequent adding of water at regular intervals. The mixture was then filtered through a muslin cloth and the residue was washed with hot water till free from acid. The material was transferred to the same beaker, and 200 ml of boiling 0.313N NaOH was added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue was washed with hot water till free from alkali, followed by washing with some alcohol. It was transferred to a crucible, dried overnight at 80-100^{0}C and weighed in an electric balance (*KEYI: JY-2003; China*). The crucible was heated in a muffle furnace (*Nebertherm: Mod-L9/11/c6; Germany*) at 600^{0}C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber.
Crude fibre (g/100g sample) = [100-(moisture + fat)] × (We-Wa) / Wt. of sample (Raghuramulu *et al.*, 2003).

Determination of total ash: One gram of each sample was weighed accurately in a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5-6 hours at 600^{0}C. It was then cooled in a desiccators and weighed. To ensure completion of ashing, the crucible was then heated in the muffle furnace for 1h, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or grayish white in color. Total ash was calculated following the equation shown below:
Ash content (g/100g sample) = Wt. of ash × 100 / Wt. of sample taken (*Raghuramulu et al.*, 2003).

Mineral content: Content of Calcium (Ca), Copper (Cu), Molybdenum (Mo), Selenium (Se), Iron (Fe), Zinc (Zn) and Cobalt (Co) was determined following flame method of atomic absorption spectrophotometer (AAS 240, Varian) and that Molybdenum (Mo) was determined following graphite furnace method (GTA 120, Varian).

Total ash as determined earlier was taken in beaker. Two milliliter of concentrated HNO_3 was added to the ash and heated for 2 minutes. One drop of hydrogen peroxide was added into the solution to remove turbidity. The solution was transferred into a volumetric flask and total volume was made 100 ml by adding de-ionized water.

For each mineral, one milliliter 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. The solution was considered as the secondary stock solution of the respective mineral. Standard solution of the mineral was prepared as per the instruction of the AAS for the particular mineral (Fluka Analytical SIGMA-ALDRICH product of Switzerland).

Texture measurement: To determine texture, fresh samples were collected from culture house and stored at room temperature for 30 minutes. The texture was measured using a texture profile analyzers (Texvol, TVT-300XP, Sweden) in the laboratory of MDI.

Color measurement: The color of the mushroom was measured using a Chroma Meter (Model FLDO712, China) in the MDI laboratory. Mushroom color was commonly measured using the L value of the Hunter scale (Brennan, *et al.*, 2000; Jolivet, 1998; Cliffe-Byrnes and O'Beirne, 2007). However some studies indicated that changes in other parameters of the hunter scale (A and B) related to browning (Aguirre *et al.*, 2008; Vizhanyo and Felföldi, 2000: Burton, 1989).

RESULTS AND DISCUSSION

Moisture content: The water contents of milky mushroom gradually reduced in proceeding the harvested age. It was varied from 53.32 - 90.34% in whole fruiting body, 71.12 - 92.70 in pileus and 50.91 - 90.42 in stalk. The highest water content of all samples was found in the 4 days and lowest in 12 days. In general, the maximum water content was found in pileus and the minimum in stalk at every harvesting period (Fig. 1).

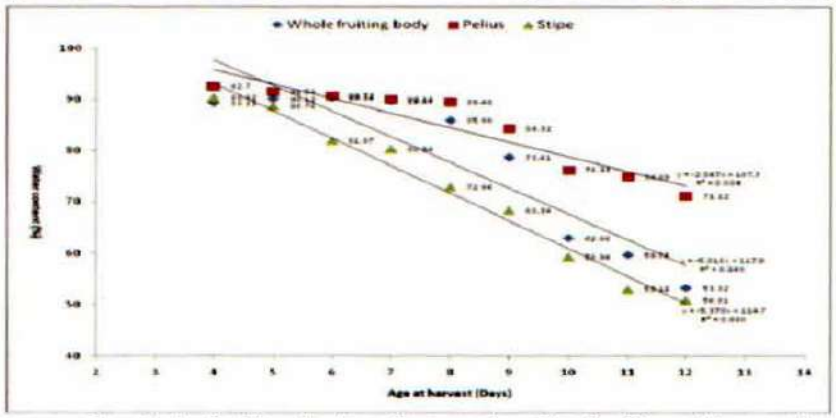

Fig. 1. Water content in whole fruiting body, pileus and stalk of milky white mushrooms harvested at the age of 4-12 days.

Protein content: The protein contents in whole fruiting body, pileus and stalk of milky white mushroom harvested at age of 4-12 days varied from 1.40 - 3.84, 0.78 - 5.06 and 1.20 - 1.95 g, per 100 g of dried sample respectively. The highest protein content was found in 8 days pileus and the lowest in 12 days pileus. In average, maximum protein content was found in pileus and the minimum in the stalk. Comparatively higher protein content was found in fruiting bodies harvested at the age of 6 - 8 days (Fig. 2).

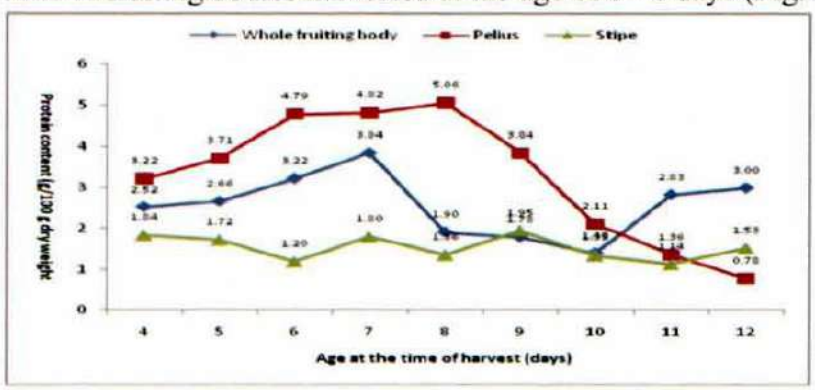

Fig. 2. Protien content in whole fruiting body, pileus and stalk of milky white mushroom harvested at the age 4-12 days

Total ash content: Total ash content was maximum in pileus followed by whole fruiting body and stalk. The ranges of ash content at 4 - 12 days of age were 8.60 - 19.1 mg in pileus, 8.00 - 11.67 mg in whole fruiting body and 4.82 - 9.32 mg in stalk per 100 g of dry sample. The highest total ash content was observed in 7 days pileus and lowest (4.82) in 10 days stalk (Fig. 3).

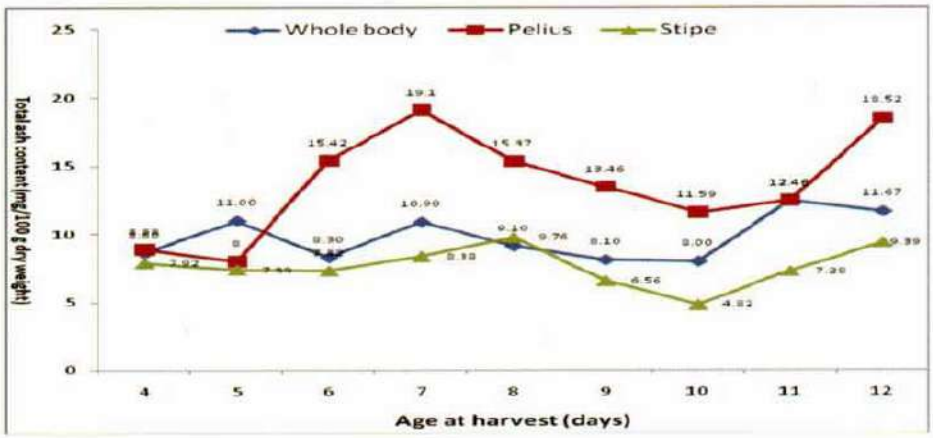

Fig. 3. Total Ash contents in whole fruiting body, pileus and stalk of milky white mushroom at 4-12 days of harvesting period.

Lipid content: Lipid content was varied at different stages and ranged from 3.16 to 8.23 g per 100g of sample. The highest lipid (8.23) content was observed in 6 days pileus followed by 10 days pileus and the lowest (3.16) in 9 days pileus (Fig. 4).

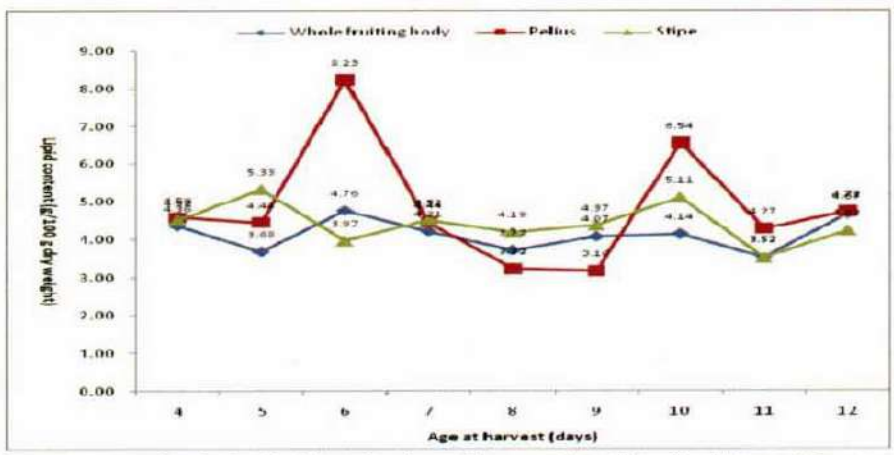

Fig. 4. Lipid contents of whole fruiting body, pillus and stalk of milky white mushrooms at different harvesting age.

Fiber content: Fiber contents differ from 0.53 to 2.31 mg per 100g of sample. The highest fiber content (2.31mg) was observed in 10 days whole fruiting body and the lowest (0.53) in 5 days whole fruiting body (Fig. 5).

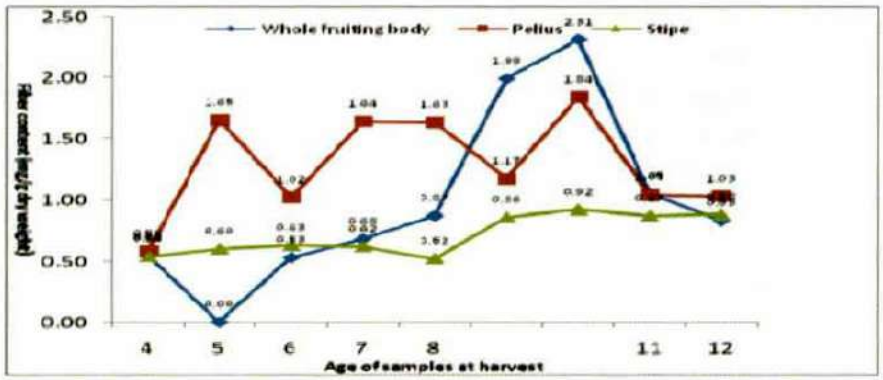

Fig. 5. Fiber content in whole fruiting body, pileus and stalk of of milky white mushrooms at 4-12 days of harvest.

Mineral content: The mineral contents in different parts of fruiting body harvested at the age of 4-12 days are summarized in Table 1.

Table1. Mineral contents in different parts of fruiting body of milky white mushroom harvested at the age of 4-12 days

Age of samples at harvest	Mineral content						
	Ca mg/100g	Co mg/100g	Cu mg/100g	Fe mg/100g	Mo Mcg/100g	Se Mcg/100g	Zn mg/100g
whole fruiting body							
4	5.8	0.8	6.6	2.7	459.0	263.0	3.9
5	6.7	0.6	5.3	2.2	181	261.0	3.6
6	6.0	0.8	5.6	2.2	238.9	212.0	4.1
7	3.4	0.7	6.1	3.2	72.5	335.0	4.0
8	3.2	0.7	5.1	3.1	277.0	159.0	4.1
9	4.7	0.6	5.7	2.6	173.3	116.0	6.1
10	3.9	0.8	6.5	2.5	232.2	179.0	6.1
11	4.7	1.0	7.0	2.8	272.3	153.3	4.3
12	3.8	0.1	4.9	8.7	37.5	16.0	4.3
Pileus							
4	5.5	0.8	6.0	3.2	433.3	262.3	4.9
5	1.4	0.2	3.4	2.4	189	259.8	3.1
6	5.2	0.2	5.9	5.7	140.7	210.1	7.0
7	4.3	0.5	5.0	2.3	173.4	133.4	3.4
8	1.5	0.7	7.8	2.0	113.7	34.0	3.8
9	15.3	0.7	7.0	4.7	130.2	36.0	6.9
10	4.7	0.8	8.7	2.8	360.1	224.0	6.3
11	3.9	0.7	7.4	2.6	372.2	124.5	6.2
12	15.5	0.5	3.6	3.3	56.0	33.0	7.5
Stalk							
4	4.9	0.8	6.1	3.0	383.5	226.7	4.2
5	3.3	0.7	5.2	8.9	112.1	144.0	3.3
6	5.2	0.6	2.4	2.7	160.9	33.0	3.4
7	5.7	0.3	5.0	3.0	238.8	43.3	3.3
8	6.6	0.3	3.6	4.9	45.	14.0	6.7
9	3.7	0.7	7.2	2.8	296.2	104.0	6.8
10	4.1	1.2	5.1	2.7	241.9	11.0	7.1
11	2.7	1.0	7.1	3.0	232.2	157.0	5.3
12	3.5	0.4	4.3	3.9	77.8	24.0	5.2

Calcium: Among all the samples the highest amount of calcium found (15.5 mg/100g) in 12 days pileus followed by 9 days pileus and the lowest (1.4mg/100g) in 5 days pielus.

Cobalt: The cobalt contents in different parts of milky mushroom at different stages were estimated and ranged from 0.1 to 1.2 mg/100g of 100g of dried mushroom sample. The highest (1.2mg/100g) amount of cobalt content was observed in 10 days stalk followed by 11 days stalk, 11 days whole fruiting body and the lowest (0.1) in 12 days whole fruit body. Very small amount of Co is required for human subjects which might be fulfilling by mushroom consumption. The average daily intake of cobalt is about 5-8 mcg. A deficiency of cobalt is equivalent to a deficiency of vitamin B12, and can cause anemia, nerve disorders, and abnormalities in cell formation. Also "scaly" skin and atrophy.

Copper: Copper content in different part of the milky fruiting body were 2.4 to 8.7 mg/100g of dried mushroom harvested at different stages. The highest amount (8.7 mg/100g) of Copper was observed in 10 days old pileus and the lowest (2.4mg/100g) in 6 days stalk.

Iron: Among all the samples the highest (8.9 mg/100g) amount of Iron was found in 5 days stalk and the lowest (2.0 mg/100g) iron in 8 days pileus. Daily requirement of iron for man is 8 mg and 18 mg for woman, so one hundred gram of *C. indica* is enough to fulfill the daily need.

Molybdenum: The highest amount (456 Mcg/100g) of Mo was found in 4 days whole fruit body and the lowest (37.5 Mcg/100g) in 12 days whole fruit body.

Selenium: The highest amount (335 Mcg/100g) of Se was found in 7 days whole fruiting body and the lowest (11 Mcg/100g) amount in 10 days stalk.

Zinc: The highest amount of Zn (7.5 mg/100g) was found in 12 days whole fruiting body and the lowest (3.1 mg/100g) in 5 days pileus. Thus Mushrooms are said to be good biological accumulators of zinc which is biologically very vital to the human body (Bano, 1981).

Instrumental texture measurement: The textural hardness of different parts of milky white mushroom shown in Table 2. Textural hardness decreased when milky mushroom harvested after 7 days in respect of pileus and stalk. Textural hardness comparatively higher in stalk than pileus. After 9 days of fruiting body formation acceptability of mushroom were reduced due to deterioration of their color and texture.

Table 2. Texture and color of pileus and stalk of milky white mushroom harvested at the age of 4-8 days

Age of harvest (Days)	Color			Texture			
	L	A	B	Height	weight	Hardness	Resilience
				Pileus			
4	97.24	1.20	4.10	18	17	0.74	0.13
5	98.78	1.05	4.21	24	22	0.76	0.13
6	97.11	1.37	4.45	19	17	0.83	0.18
7	96.56	1.65	9.48	16	18	1.22	0.31
8	84.63	7.04	13.24	20	22	0.86	0.45
				Stalk			
4	82.54	3.90	8.88	11	11	2.98	0.35
5	87.59	1.82	7.59	23	20	1.70	0.46
6	86.35	4.12	10.87	18	10	2.27	0.47
7	84.71	2.39	6.48	10	9	3.36	0.45
8	85.64	3.69	11.14	18	13	1.85	0.42

Color measurement: The color measurement of different parts of the milky mushroom was shown in Table 2. The figure shows that the best white color shown in 5 days pileus. After 5 days the white color gradually decreases. After 9 days the mushroom fruiting bodies were less acceptable. The loss of hardness and browning of mushroom were governed by enzymatic activities. Color and appearance attract the consumer to a product and can help in impulse purchases. Consumers have a preferred color for a specific item (Crisosto *et al.*, 2003). Color may be determined using nondestructive methods founded on visual or physical measurements. These methods are based on evaluation of either the light reflected from the surface of a product or transmitted through it. There are three components necessary to the perception of color. 1. a source of light, 2. an object that modifies light by reflection or transmission and 3. the eye/brain combination of an observer (Leggett,2004). Color space may be divided into a three-dimensional (L, a and b) rectangular area (Fig. 1) such that L (lightness) axis goes vertically from 0 (perfect black) to 100 (perfect white) in reflectance or perfect clear in transmission (HunterLab, 1996; Leggett, 2004). The a axis (red to green) considers the positive values as red and negative values as green; 0 is neutral. The b axis (blue to yellow) expresses positive values as yellow and negative values as blue; 0 is neutral. Fruits and vegetables are often described in terms of their L, a, and b values.

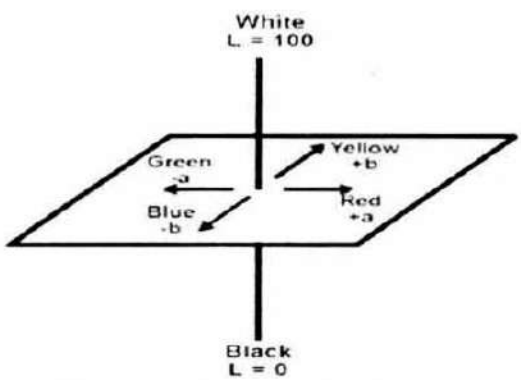

Fig. 1. Diagram depicting three dimensional L, a and b color space.

Observation of different study show that, most of the mushrooms contain around 90% moisture, 10-40% protein, 2-8% fat, 3-28% carbohydrate, 3-32% fiber, 8-10% ash and some vitamins and minerals. The results show that the pileus and gills are richer in protein than stalk. Alam *et al.* (2008) also found variations in nutritional parameters among the different parts of mushrooms. They recorded that pileus and gills are richer in protein (about 40~60%), lipid (30~60%) and ash content (5~10%) than stalk. On the other hand the stalk is richer in fiber (40~50%) and carbohydrate content (10~15%). Findings of the present study support the findings of other investigators (Justo *et al.*, 1999; Shin *et al.*, 2007; Dundar *et al.*, 2008).

Among all, potassium, calcium, phosphorus, magnesium, selenium, iron, zinc and copper are accounting for most of the mineral content (Borchers *et al.*, 1999). According to Alam et al. 2008. *C. indica* contained 2.6 to 2.9 g of proteins, 0.6 to 0.7 g of lipids, 1.5 to 1.8 g of fiber and 6.3 to 7.3 g of carbohydrates. Other investigators found that the fat, protein, carbohydrate, dietary fiber, moisture and ash (g/100 g dried matter) content of *C. indica* were 0.66 ± 0.02g, 31.29 ± 1.56g, 58.40 ± 1.75g, 38.21 ± 1.91g, 84.91% and 8.47 ± 0.25g, respectively, when cultivated in paddy straw (Lakshmipathy *et al,*. 2011). According to Alam *et al.* (2008) 100 g of dried *C. Indica* contained 20~23 g of proteins, 4.6~5.3 g of lipids, 11~15 g of fiber and 46~51 g of car-bohydrates and hudred gram *C. Indica* also contained Ca (20.7 mg), Fe (56.2 mg), Mg (12.8 gm), Mn (1.65 mg), Se (13.2μg) and As (54 μg). Results of the present study reveal that the pileus and gills are richer in protein (about 40~60%), lipid (30~60%) and ash content (5~10%) than stalk. On the other hand the stalk is richer in fiber (40~50%) and carbohydrate content (10~15%). The findings are agreeable with the findings of other investigators (Watanabe *et al.*, 1994; Justo *et al.*, 1999; Shin *et al.*, 2007; Dundar *et al.*, 2008). The variations in mineral content in the present study may be due to variations of environmental condition, soil, water, etc, which influence the quality of mushrooms. The findings also reveal that best age of harvesting mushroom is 5-7 days after initiation of fruiting bodies.

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Bangladesh J. Mushroom. 8(2): 11-18. 2014 (December)

Comparative Study on Nutrients and Minerals Content of Mushroom Ketchup and Other Ketchup Available in Market

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Abstract

The present study was conducted to compare nutrient and mineral content of mushroom ketchup and other ketchups persisting at local market. Eleven different ketchup samples were included in this study, which were named as T-1 (50% oyster mushroom enriched ketchup), T-2 (50% oyster mushroom enriched ketchup), T-3 (50% shiitake mushroom enriched ketchup), T-4 (50% shiitake mushroom enriched ketchup), T-5 (30% oyster mushroom enriched ketchup), T-6 (30% oyster mushroom enriched ketchup), T-7 (30% shiitake mushroom enriched ketchup), T-8 (30% shiitake mushroom enriched ketchup), T-9 (Ahmed ketchup), T-10 (Shezan ketchup) and T-11 (Ruchi katchup). To make them free from moisture, all the samples were dried in hot air oven, which were analyzed. The highest and lowest content of moisture, ash, crude fiber, fat and protein was observed in T1 (5.28%) and T10 (2.09%), T1 (18.13) and T9 (3.2), T7 (2.08) and T11 (0.01), T9 (22.48) and T4 (12.86), T-9 (13.20) and T-5 (4.80) respectively. Some important minerals contents such as calcium, cobalt, copper, iron, molybdenum, selenium and zinc were also observed. The highest amount of Ca (51.80 mg/100g) observed in T-9 and lowest in T-11 (9.60 mg/100g), highest amount of Co (1.25 mg/100g) was observed in T-7, and lowest in (0.10 mg/100g) in T-9, highest Cu (3.39 mg/100g) was observed in T-1 and lowest (1.30 mg/100g) in T-11, highest Fe was found in T-7 (7.08 mg/100g) and lowest (1.35 mg/100g) in T-11, highest Mo was observed in T-7 (542.11 μg/100g) and lowest (57.22 μg/100g) in T-9, highest Se was observed in T-1 (389.35 μg/100g) and lowest (4.89 μg/100g) in T-9, highest Zn was observed in T-9 (5.78 mg/100g) and lowest (127.20 mg/100g) in T-6. Considering the obtained findings it is mentionable that mushroom enriched ketchup enhances the nutritional status in comparison with the other ketchups available at the market.

Key Words: Mushroom, Tomato, Local market, Ketchup, Nutrient and Minerals.

INTRODUCTION

Ketchup or catsup is a table sauce. Traditionally, different recipes featured ketchup made of mushrooms, oysters, mussels, walnuts, or other foods, but in modern times the term without modification usually refers to tomato ketchup, often called tomato sauce, or, occasionally, red sauce. It is a sweet and tangy sauce, typically made from tomatoes, a sweetener, vinegar, and assorted seasonings and spices. Seasonings vary by recipe, but commonly include onions, allspice, cloves, cinnamon, garlic, and sometimes celery. Mushroom ketchup is a style of ketchup that is prepared with edible mushrooms as its primary ingredient. Originally, ketchup in the United Kingdom was prepared with mushrooms as a primary ingredient, instead of tomato, the main ingredient in

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contemporary preparations. Geo Watkins Mushroom Ketchup is a contemporary, commercially mass-produced product that is marketed to consumers. The company was founded in 1830. Contemporary preparation of Geo Watkins Mushroom Ketchup use mushroom powder as a primary ingredient (Bell & Annie, 1999). Joshi *et al.* (1991) developed sweet chutney from button mushroom and the storage life of the product was more than a year. Mushroom ketchup is by concentrating mushroom mass along with necessary ingredients. Ketchup generally does not flow freely and highly viscous in nature. It contains more sugar and less acid. It is a common and popular product relished for its typical taste and texture as accompaniment with snacks (Mujib *et al.*, 2013). It is made by concentrating the juice or pulp of the fruits or vegetables without seeds and pieces of skin.

Mushrooms are valuable health food, which are low in calories, high in good quality vegetable proteins, chitin, fiber, vitamins (folic acid, biotin, niacin, B complex, A, D and C) and minerals such as phosphorus, potassium, calcium, zinc, sodium, iron and magnesium (Royse and Schisler, 1980; MoA and JICA, 2000; Oei, 2003). The protein value of dried mushroom has been found to be 30-40% containing all the essential amino acids (Chang, 1991). Edible mushrooms are recommended by the FAO as food, to meet protein requirement of developing countries, the large portion of which depends mainly on cereals (World Bank, 2004). Fresh mushrooms cannot be stored for more than 24 h at ambient temperature (Abhijit *et al.*, 2004) and this make difficulty in distribution and marketing of fresh product thus extending post harvest storage is constant quest. Desirability of a food product is not necessary correlated with its nutritional value. Appearance, taste and aroma are often important in stimulating the appetite and determining preference. Thus, in addition to nutritional value, edible mushrooms possess unique characteristics in terms of color, taste, aroma and texture, which make them attractive for human consumption (Chang and Miles, 2004). Presently, long-term preservation of mushroom by drying, canning and pickling are in vogue (Chandrasekar *et al.*, 2002).

For long term preservation, processing is considered to be the best method. Therefore ketchup may be one of the best methods for processing mushroom. The present study was conducted to find out the nutrient and minerals content of mushroom ketchup in comparison with local market's ketchup.

MATERIALS AND METHODS

The experiment was conducted in the Quality Control and Quality Assurance (QCQA) laboratory of Mushroom Development Institute, Savar, Dhaka during November to December 2014.

Sample collection: Eleven different samples were collected among them eight were prepared by the method of Mujib *et al.* (2013) and three were collect from local market such as T-9 (Ahmed ketchup), T-10 (Shezan ketchup) and T-11 (Ruchi katchup) which is

given below in Table 1. Moisture, lipid, crude fiber, total ash and some minerals were estimated from the collected samples.

Table 1. Composition of different mushroom ketchups

Amount of Different Ingredients (approx) gram	Treatments							
	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8
Oyster mushroom (g)	500	500	---	---	300	300	---	---
Shiitake mushroom (g)	---	---	500	500	---	---	300	300
Tomato (g)	--	--	--	--	200	200	200	200
Common salt (g)	80	40	15	15	40	20	20	20
Sugar (g)	250	250	250	250	250	300	250	200
Vinegar (ml)	15	15	15	15	15	15	15	15
Sodium benzoate (g)	0.50	0.70	0.50	0.50	0.50	0.70	0.50	0.70
Onion paste (g)	100	100	100	100	100	100	100	100
Garlic paste (g)	5	10	5	5	5	10	5	10
Ginger paste (g)	30	30	30	30	30	30	30	30
Cumin seed paste (g)	10	10.	10.	10	10	10	10	10
Black pepper (powder) (g)	1	1	1	1	1	1	1	1
Red chili powder (g)	10	8	5	5	8	5	5	5
Arrarote (g)	2	2	2	2	2	2	2	2
Total (g)	1000	1000	1000	1000	1000	1000	1000	1000

Content of local market ketchups:
Ahmed (T-9): Tomato, Sugar, Salt, Hot pepper, Different spices, Modified starch, Acidity regulator.

Shezan (T-10): Tomato paste, water, Sugar, Hot pepper, Acetic acid, Starch powder, Different spices, Potassium, Natural food color.

Ruchi (T-11): Tomato, Sugar, Corn strach, Salt, Acetic acid, Citric acid, Hot pepper, Na benzoate, Different spices.

Moisture analysis: Twenty gram of fresh mushroom was weighed taken into a weighed moisture box (*A&D company ltd. N 92; P1011656, Japan*) and dried in an oven at 100^{0}C and cooled in a dessicator. The process of heating and cooling was repeated till a constant weight was achieved. The moisture content was calculated as following equation:
Moisture (%) = (Initial weight- final weight) × 100 /Weight of sample (Raghuramulu *et al.*, 2003).

Determination of total protein: Five gram of dried ketchup was taken with 50ml of 1N NaOH and boiled for 30 minutes. The solution was cooled in room temperature and centrifuged at 1000 rpm by a table centrifuge machine (DIGISYSTEM: DSC-200T;

Taiwan). The supernatant was collected and total protein content was measured according to the Biuret method (Burtis and Ashwood, 2006) with a diagnostic kit (Total Protein: Colourimetric test- Biuret method, Crescent Diagnostics, Saudi Arabia).

Determination of total lipid: Total lipid was determined by slight modified method of Folch *et al.* (1957). Five gram of grinded mushroom was suspended in 50ml of chloroform: methanol (2:1 v/v) mixture then mixed thoroughly and let stand for 3 days. The solution was filtrated and centrifuged at 1000rpm by a table centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid. For the determination of total lipid from fresh mushroom, 5g was taken with 50ml phosphate buffer and homogenized with a tissue homogenizer. Five ml of homogenized was taken with 50 ml of chloroform: methanol (2:1 v/v) mixture and lipid content was determined as mentioned above.

Determination of crude fiber: Five gram of moisture and fat-free sample was taken in a beaker and 200ml of boiling 0.255N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker, and 200ml of boiling 0.313N NaOH added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100^{0}C and weighed (We) in an electric balance (*KEY1: JY-2003; China*). The crucible was heated in a muffle furnace (*Nebertherm: Mod-L9/11/c6; Germany*) at 600^{0}C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber.
Crude fibre (g/100g sample) = [100-(moisture + fat)] × (We-Wa) / Wt. of sample (*Raghuramulu et al.*, 2003).

Determination of total ash: One gram of the sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5-6 hours at 600^{0}C. It was then cooled in desiccators and weighed. To ensure completion of ashing, the crucible was then heated in the muffle furnace for 1h, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or grayish white in color. Then total ash was calculated as following equation:
Ash content (g/100g sample) = Wt. of ash × 100 / Wt. of sample taken (*Raghuramulu et al.*, 2003).

Mineral analysis: Total ash was taken for the analysis of mineral contents. Two ml of concentrated HNO_3 was added to the ash and heated for 2 minutes. One drop of hydrogen peroxide was added into the solution to remove turbidity. The solution was then transferred into a volumetric flask and total volume was made 100 ml by adding 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 Calcium (Ca), Copper (Cu), Selenium (Se), Iron (Fe), Zinc (Zn) and Cobalt (Co) was done in flame method of AAS and the analysis of Molybdenum (Mo) was done in graphite furnace method.

RESULTS AND DISCUSSION

Moisture: The moisture contents from different ketchups were varied from 2.09% to 5.28%. The highest moisture content was observed in T-1 (5.28%) and lowest in T-10 (2.09 %) (Table 2).

Total protein: Protein contents varied ketchups 4.80% to 13.20% per 100gm of dried sample. The highest protein content (13.20%) was observed in T-9 and the lowest (4.80%) was observed in T-5 (Table 2).

Lipid: The lipid contents varied in different ketchups from 12.86% to 22.48% per 100g of dried sample. The highest lipid content (22.48%) was observed in T-9 and the lowest (12.86%) was observed in T-4 (Table 2).

Table 2. Nutrient contents of different mushroom ketchups and local market ketchups

Treatment	Moisture (%)	Proteinn (%)	Ash (%)	Fat (%)	Fiber (%)
T-1	5.28	7.04	18.13	15.27	0.89
T-2	4.28	8.40	12.13	20.60	1.03
T-3	2.43	6.12	5.28	16.44	1.46
T-4	3.04	7.56	5.23	12.86	1.86
T-5	3.30	4.80	11.27	18.21	1.02
T-6	2.99	5.20	6.37	15.51	0.88
T-7	3.19	3.80	4.83	20.15	2.08
T-8	3.15	6.84	6.00	16.72	1.13
T-9	3.03	13.20	3.52	22.48	0.15
T-10	2.09	5.40	6.27	19.04	0.23
T-11	2.73	10.08	7.30	15.70	0.10

Fiber: The fiber contents differ from 0.10% to 2.08% per 100g dried samples of different ketchups. The highest fiber content (2.08%) was observed in T-7 and the lowest (0.10%) was observed in T-11 (Table 2).

Total Ash: The total ash contents differ from 3.52% to 18.13% per 100g dried samples of different ketchups. The highest total ash content (18.13%) was observed in T-1 and the lowest (3.2%) was observed in T-9 (Table 2).

Afsary *et al.* 2013 reported that the maximum protein (25.1%), lipid (6.5%), fiber (22.5%), total ash (13.2%) and carbohydrate (43.5%) were observed in *P. ostreatus, P. eryngii, P. cystidiosus,* and *P. djamor* respectively. Alam *et al.* (2008) reported the similar results on *P. sajor-caju, P. florida* and *Calocybe indica.* Fiber is important in preventing colon cancer, as it helps in movement of food particles in the gut and ultimately protect the gut wall. Fiber may even helps the immune system for function properly. Fiber-rich plant foods are also important for prevention of cancer.

Table 3. Mineral contents of different mushroom ketchups and local market katchups

Treatments	Ca mg/100g	Co mg/100g	Cu mg/100g	Fe mg/100g	Zn mg/100g	Se µg/100g	Mo µg/100g
T-1	49.80	0.67	3.39	3.12	3.90	389.35	220.61
T-2	46.63	0.60	2.06	2.44	7.61	298.23	130.20
T-3	43.22	0.75	2.57	3.23	6.80	239.65	164.60
T-4	29.70	0.54	2.52	2.27	5.43	96.87	128.00
T-5	31.10	1.01	2.62	2.68	4.23	303.23	183.20
T-6	32.10	0.67	2.25	3.12	3.84	212.67	127.20
T-7	43.30	1.25	2.08	7.06	4.11	244.19	542.11
T-8	37.90	1.13	2.98	2.83	5.24	197.11	329.53
T-9	51.80	0.10	2.75	7.01	5.78	4.89	57.22
T-10	23.10	0.08	3.80	3.32	4.43	30.38	83.30
T-11	9.60	0.46	1.30	1.35	4.52	20.47	136.04

Calcium: Among all the samples the highest amount of calcium was found in 51.80 mg/100g in T-9 and the lowest calcium was observed in (9.60 mg/100g) in T-11. Calcium is one of the most important nutrients for adolescents. If teens get enough calcium while they are young, they can strengthen their bones and reduce the risk of osteoporosis later in life (Table 3).

Cobalt: The cobalt contents of different ketchups were 0.10 to 1.25 mg/100g of dried sample. The highest amount of cobalt was observed in T-7and the lowest in treatment T-9 (Table 3).

Copper: The copper contents of different ketchups and local market ketchups were 1.30 to 3.39 mg/100g of dried sample. The highest amount of Copper was observed in T-1 and the lowest amount in T-11(Table 3).

Iron: Among all the samples the highest amount of Iron found in T-7 and it was 7.08 mg/100g, and the lowest was found in T-1 which was 1.35 mg/100g. It is well known that the daily requirement of iron for man is 8 mg / day and for woman is 18 mg / day (Table 3).

Molybdenum: The highest amount of Mo was found in T-7 and it was 542.11 μg /100g and the lowest amount in T-9 which was 57.22 μg /100g (Table 3).

Selenium: The highest amount of Se was observed in T-1 (389.35 μg/100g) and the lowest amount was found in T-9 (4.89 μg/100g) (Table 3).

Zinc: Mushrooms are said to be good biological accumulators of zinc which is biologically vital for human body (Bano, 1981). The highest amount of Zn was observed in T-9 (5.78 mg/100g) and the lowest amount in T-6 (3.84 mg/100g).

It is observed that mushrooms are also a good source of some important minerals like selenium, potassium and copper. Selenium, an antioxidant, can keep the body's cells healthy and prevent heart disease and cancer. Selenium also promotes a strong immune system and fertility in men. Mushrooms are the richest vegetable source of selenium. It is especially good news for vegetarians since selenium is often found in animal foods. Copper keeps our bones and nerves healthy. Moreover, copper helps in formation of red blood cells which carry oxygen throughout the body. Potassium helps in controlling blood pressure since it helps to maintain normal fluid and mineral balance. It also helps the nerves, the heart, and other muscles function normally (Gerger, 2013). A recent study in 2013 examined the effect of substituting red meat with mushrooms. The results of this interesting study showed that the participants who consumed mushrooms instead of red meat showed more weight loss, lower waist circumference, lower blood pressure, and they were also able to maintain their weight when compared to the participants who ate red meat! (Ren, Perera, and Hemar). Afsary *et al.* (2013) reported that the maximum Ca (37.45 mg/100g), Fe (45.25 mg/100g), Zn (22.82 mg/100g) and Se (0.45 mg/100g) were recorded in *P. eryngii*, *P. ostreatus* and *P. geesteranus* respectively, similar result also shown Alam *et al.* (2008) on mineral content.

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Bangladesh J. Mushroom. 8(2): 19-22. 2014 (December)

Performance of Hybrid Strains of Oyster Mushroom Developed at Mushroom Development Institute

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Abstract

A breeding approach to hybrid production from cultivated *Pleurotus spp.* were used. Eight different types of oyster mushroom were used for hybridization. Basidiospores were collected and suspended in sterile distilled water. After germination, colony of each isolate transferred into the PDA medium. Growth rate and colony type of each strain was determined and then 8 strains were selected. Consequently screening monokaryons were fused to each other. Some characteristics such as morphological interaction in the contact zone of mycelium, increasing in growth rate of hybrid, change of colony morphology and the presence of clamp connections between dikaryotic cells used to distinction of monokaryons from dikaryons. Ten hybrids were recognized by these characteristics. Five hybrids were preliminary selected from 10 hybrids on the basis of their morphology and yield. These selected hybrids were tested along with five parents at different locations. The hybrid $C_3F_1P_2$ (Pop-1xPo_2) produced the highest yield than all the hybrids.

Key words: Monokaryote, Dikaryote, Single spore, Crossing , Yield.

INTRODUCTION

In Bangladesh, mushroom is being cultivated for about 30 years. Especially oyster mushroom is cultivated in the country and the main strain is PO2. Recently some strains of the mushroom have been collected from different countries and being tested to adopt in Bangladesh condition. Some strains also giving good yield. On the other hand Due to repeated subculture the PO2 strain of oyster mushroom getting lost its productivity. In this situation it is necessary to develop new high yield variety of oyster mushroom. Optimization of industrial mushroom production depends on improving the culture process and breeding new strains with higher yields and productivities (Gharehaghaji *et al.*, 2007). The first commercial hybrid variety of mushroom was released in 1980. Subsequent commercial hybrids were identical or very similar to the first hybrid. It is clear that some "new" varieties were generated by making copies of the first hybrid via tissue cultures of mushroom (Sonnenberg. *et al.*, 2011).

However, the yield of different mushroom species is not yet satisfactorily. Poor yielding cannot be profitable for the growers. As there is no such breeding programme in Bangladesh to develop high yield of mushroom, it is a prime need to develop new varieties specially for Bangladeshi environment. National Mushroom Development and Extension Centre (NAMDEC) have been engaged about more than 30 years for

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developing technology on mushroom. A lot of works have been done in cultural management practices to improve the yield of mushroom and some good technologies are being used by the growers. Mushroom breeding is also important discipline to develop new varieties through hybridization technique. In this study hybridization technique is being used. It is well known that hybridization is the method of producing new crop varieties with desired traits in which two or more plants of unlike genetically constitution are crossed together. The main objectives of the study were to develop new genotypes or varieties better than exiting cultivars in respect of yield and adaptability under the climatic conditions of Bangladesh.

MATERIALS AND METHOD

The experiment was carried out at National Mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka during 2013-2014. Before going to initiate any hybridization programme, parent should be selected at least one character which is better than the existing cultivars. The following genotypes were taken as parents for crossing.

i. Pop-1 (*Pleurotus djmour*): Quick growing (3-4 days) at summer season, attractive pink colour, rapid completion of mycelium (16-18 days). ii. Py-1 (*Pleurotus citrinopileatus*): Pileus golden in colour, small, thin, cluster form. iii. PO-2 (*Pleurotus ostreatus*): Popular cultivated variety, cream white in color, grown round the year with acceptable yield. iv. Flo (*Pleurotus florida*): Absolute white, large pelius. v. Pcys-2 (*Pleurotus cystidiosus*): Thick pileus, grey colour (called maple oyster). vi. Po-10 (*Pleurotus ostreatus*): Good performance at high temp. (35-38^{0}C), pileus is thin in comparison to PO2. vii. PG-4 (*Pleurotus geesternus*): Grey colour, grown round the year except very high temperate period (May-July in Bangladesh). viii. HK-51 (*Pleurotus highking*): Large pileus (weight of a single pileus-120-150 g), violet colour, performance is good in winter.

In this study single spore isolation technique was used for hybridization.

Single spore isolation technique: Selected lines/strains/genotypes were grown in culture house for collecting spores. Collection of spore was done in aseptic condition using clean bench. Serial dilution method was followed to reduce heavy spore loads. The distance between two spores of two different strains were kept about 1-2 cm distances in PDA media and placed in growth chamber for growing mycelia. After 3-4 days mycelia grow and touch/cross over each other (compatible lines only). At the junction point of the two different strains mycelia, it seems to be deep colour. From the thick portion of junction point a small sample was collected and transferred to test tube for pure culture. Mother culture and spawns were prepared from the pure culture. After few days whole spawn packet was covered by mycelium which was cut on both sides like inverted "D". It was placed in a isolated mushroom culture house and required amount of water was sprayed for getting fruit body. After 4-6 days several F_1 fruit bodies were found. F_1 fruit body was selected directly for Hybrid varieties. In this case selections were made in F_1 population as hybrid strains. In F_2 segregating population: F_2 spores were collected from F_1 fruiting body. F_2 segregating generations were grown to obtained more variability and selection

was made according to the objectives. Selected fruit body were grown in progeny rows and selection were done in the following generation i.e. F_3, F_4, F_5, F_6 etc until homogeneity obtained.

Total eight cross combinations were made. These are as follows- C_1(Pop-1x Pg-4), C_2(Pop-1xPy-1), C_3(Pop-1xPo_2), C_4(Po_2xPo-96), C_5(Po_2x Hk-51), C_6(Po_2 x Pcys-2), C_7(Po_2 x Po-10), and C_8 (Pop-1x Flo). From this experiment total 10 F_1 plants were isolated (table 1). All the F_1 plants were grown in culture house at NAMDEC. Out of 10 F_1 fruit body, only six were grown with their parents at different locations i.e. NAMDEC culture house, Comilla sub centre and farmers culture houses.

Table 1. Showing the cross combination and characteristics

Hybrid number	Cross number & Cross combination	Characters
Hybrid 1	$C_1F_1P_1$(Pop-1x Pg-4)	Excellent colour
Hybrid 2	$C_1F_1P_2$ (Pop-1x Pg 4)	Big pileus with thickness
Hybrid 3	$C_2F_1P_2$(Pop-1xPy-1)	Coloured pileus
Hybrid 4	$C_3F_1P_2$(Pop-1xPo_2)	Excellent colour and Yield
Hybrid 5	$C_4F_1P_1$(Po_2xPo- 96)	Large pileus and thickness
Hybrid 6	$C_4F_1P_1$(Po_2xPo- 96)	High yield
Hybrid 7	$C_5F_1P_1$(Po_2x Hk-51)	Large pileus
Hybrid 8	$C_6F_1P_1$(Po_2 x Pcys-2)	Good looking
Hybrid 9	$C_7F_1P_3$ (Po_2 x Po-10)	High fruit bodies
Hybrid 10	$C_8F_1P_5$(Pop-1x Flo)	Good looking

RESULTS AND DISCUSSION

Analysis of variances of fruit yield, number of fruit bodies, stalk diameter, pileus diameter and pileus thickness showed significant differences among the strains in Table 2.

Table 2. Mean squares (ANOVA) of six traits of mushroom grown at different locations

Source of Variation	Df	Yield	No. of fruit bodies	Stalk diameter	Stalk length	Pileus diameter	Pileus thickness
Replication	4	28.12	3.52	0.007	0.026	0.048	0.004
Strain	9	3865.80**	464.86**	0.142**	0.073**	3.712**	0.037**
Error	36	80.55	16.44	0.012	0.074	0.309	0.004

** Significant at 1% level.

It is evident in Table 3 that the number of fruit bodies is significantly different among the genotypes. The hybrid strain $C_3F_1P_2$ (Pop-1xPo_2) showed the highest number of fruit bodies (28.6) among all the genotypes and followed by Po-96 (Parent-3), Po-2 (Parent-1) and Pop1 (Parent-2). On the other hand, the hybrid strain $C_7F_1P_1$ (Po2 xP0-10) showed the lowest number of fruit bodies. In case of stalk diameter the hybrid strain $C_4F_1P_1$ (Po_2xPo- 96) and Py-1 (Parent-5) had the highest and lowest stalk diameter respectively. The strain Po-96 (Parent-3) and the hybrid strain $C_3F_1P_2$ (Pop-1xPo_2) showed the longest

and shortest stalk length, respectively. The hybrid strains $C_4F_1P_1$ (Po_2xPo- 96) and Po-96 (Parent-3) showed the highest and the lowest pileus diameter, respectively. The strain $C_3F_1P_2$ (Pop-1xPo_2) had the maximum thickness of the pileus. The main objective of the breeder is the fruit yield/ spawn packet. In this study, the hybrid strain $C_3F_1P_2$ (Pop-1xPo_2) produced the highest fruit yield (127.8 g/spawn packet) among all the tested genotypes. Performance of the hybrid strain was better than all other parents and genotypes. It is needed to mention here that the total yield is not good enough compare to the yield during summer season.

Table 3. Summarized values of different mushroom traits cultured at different locations

Mushroom Strains	No. of fruit bodies	Stalk diameter (cm)	Stalk length (cm)	Pileus diameter (cm)	Pileus thicknes (cm)	Yield (g)
Po-2 (Parent-1)	19.2b	0.66c-e	1.36b	5.70b	0.52ab	87.2c
Pop1(Parent-2)	19.2b	0.72b-d	1.12de	4.74c	0.54ab	107.4b
Po-96(Parent-3)	19.8b	0.50ef	2.94a	4.28c	0.38d	84.8c
Po-10 (Parent-4)	13.2b	0.68b-e	2.06bc	4.60c	0.56ab	86.4c
Py-1 (Parent-5)	12.2b	0.44f	1.60cd	4.44c	0.36d	111.6b
$C_2F_1P_2$(Pop1xPy1)	15.0b	0.52d-f	1.08e	4.68c	0.40cd	73.4c
$C_3F_1P_1$(Pop1xPo_2)	15.8b	0.66c-e	1.48de	4.68c	0.46b-d	88.4c
$C_3F_1P_2$(Pop1xPo_2)	28.6a	0.88ab	1.06e	6.00ab	0.60a	127.8a
$C_4F_1P_1$(Po_2xPo96)	4.2c	0.96a	2.28b	6.74a	0.58ab	51.8d
$C_7F_1P_1$(Po2xP01)	2.6c	0.82a-c	2.36b	6.14ab	0.50a-c	33.4e

Letters are common in a column do not differ significantly at 5% level according to DMRT.

Mushroom hybridization was done for the first time at NAMDEC and hybrid varieties are going to be released very soon. More number of crosses will be needed for better selection. Farmers' field trial will be conducted with the developed hybrids strains. Better hybrid strains will be selected and to be registered as varieties. Moreover, crop diversification and changes of food habit is an urgent need to improve our national health. Mushroom cultivation does not compete with other crops, can be grown with the active participation of family members. The agricultural and other wastes can be used as substrates. Thus it can be produced in large quantity within a short time, provides large quantity of proteins per unit area and can provide some income generating source in unemployed sector. New varieties can give more effort to minimize the above issues.

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Bangladesh J. Mushroom. 8(2): 23-28. 2014 (December)

The Use of Genetic Markers for Detecting DNA Polymorphism, Genotype Identification and Phylogenetic Relationship among Shiitake Mushroom (*Lentinus edodes*) Strains

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Abstract

The aim of this study was to analyze the genetic divergent of fourteen strain of *Lentinus edodes* mushroom based on random amplified polymorphic DNA (RAPD) pattern. Observation showed that all the species tested could be differentiated by RAPD data. The maximum polymorphism was produced by the primer OPA07 and OPA08 having polymorphism percentage 100%. The highest linkage distance (17.0) was found in Le3 vs. Le15 and Le15 vs Le18 strain pairs. The lowest linkage distance (1.0) was found in Le1 vs. Le2 strain pair. The dendrogram based on similarity matrix differentiated the strains into two distinct clusters A and B. Cluster A constituted strain Le15, cluster B contained rest of the strains. Cluster B subdivided into sub cluster B1 and B2. Sub cluster B1 comprises strain Le10 and Le11. Sub cluster B2 belongs to cluster B2a and B2b; cluster B2a contained only strain Le4 whereas cluster B2b sub divided into B2b1 and B2b2. Cluster B2b1 contain only strain Le21 and cluster B2b2 also sub divided into cluster B2b2a and B2b2b. Cluster B2b2a belongs to the strains such as Le3, Le16 and Le5H; whereas cluster B2b2b comprises the strains Le1, Le2, Le8, Le17 and Le6. The results of the present investigation revealed that the RAPD primers were able to identify and classify the fourteen shitake strains based on their genetic relationship. Therefore, DNA fingerprint and molecular relationship of all the varieties as well as entire mushroom germplasm core collection should be done using RAPD markers in order to determine their genetic relationships for variety development.

Key words: Genetic markers, Polymorphism, Linkage distance, Germplasm.

INTRODUCTION

Lentinus edodes popularly known as shiitake, the black oak mushroom or xiang-gu, is extensively cultivated in Japan, China, Europe and USA. In the wild forest, *L. edodes* distributes widely in Asia and Australia (Kobayashi and Shimizu, 1951; Kobayashi, 1966; Aoshima and Furukawa, 1980). Shiitake mushroom (*Lentinus edodes*) is very much popular in all over the world for their nutritional value, taste and medicinal importance (Wasser, 2005). This mushroom has anti-tumor, antihypertensive, hypocholesterolemic and antibacterial activities (Chihara, 1970; Kabir *et al.*, 1987; Hirasawa, 1999; Kim *et al.*, 1999).

The taxonomy of the genus is traditionally based on the morphological characteristics. Though they are really useful for differing species, there are still difficulties for

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distinguishing the close groups such as the populations or strains of the same species. According to Zheng *et al.* (2009), environmental factors, variability, inter hybridization and morphological propensity makes the accurate identification hard for *Ganoderma* species. However, the new techniques using molecular markers have turned easy the identification not only for *Ganoderma* species, but also for other organisms which has the same taxonomic difficulties.

Several techniques for molecular studies have been used for analyzing the genetic diversity in basidiomycetes, such as isoenzymes (Lan *et al.*, 1998), AFLP (*Amplified Fragment Length Polymorfism*) (Qi *et al.*, 2003), RFLP (*Restriction Fragment Length Polymorfism*) (Park *et al.*, 1996), ITS (*Internal Transcribed Spacers*) (Kindermann *et al.*, 1998) and RAPD (*Random Amplified Polymorphic DNA*) (Wang *et al.*, 2003). Among these techniques, RAPD is still one of the cheapest and quickest methods for accessing the variability at DNA level, being especially useful on intraspecific analysis. These markers have the advantage of amplifying both regions of the genome which may be transcript/translated, and non-coding regions. This is important when the objective is to evaluate the variation along with the biggest part of the species genome (Williams *et al.*, 1990; Ferreira and Grattapaglia, 1996; Ro *et al.*, 2007). This study aimed to select the RAPD molecular markers for *Lentinus edodes*, evaluating the genetic similarity pattern among the strains available at NAMDEC.

MATERIALS AND METHODS

Fourteen *Lentinus edodes* strains were collected from Mushroom Development Institute (MDI), Sobhanbag, Savar, Dhaka and molecular analysis was done in the laboratory of MDI.

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 65^0C for 40 min in water bath and centrifuged 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 five sorts of arbitrary 10-base oligonucleotide primers (Operon technologies Inc.) such as OPA06 (5' GGT CCC TGA C 3'); OPA-07 (5' GAA ACG GGT G 3'); OPA08 (5' GTG ACG TAG G 3'); OPA09 (5' GGG TAA CGC C 3'); OPA10 (5' GTG ATC GCA G 3'). RAPD-PCR reaction was performed using a thermal cycler with an initial denaturation stage of 5 min at 94^0C, followed by 40 cycles of denaturation for 1

min at 94^{0}C, annealing for 1 min at 31.5^{0}C, extension for 2 min at 72^{0}C and a final extension for 10 min at 72^{0}C.

Gel Electrophoresis and RAPD Data Scoring: Electrophoresis was done from RAPD products on 1.4% agarose gel in 1X TBE buffer for 1 hr and 15 minutes at 100 V with 1kb DNA ladder as a size marker, and then stained while agitated in an EtBr solution (0.5%). The stained gels were visualized under a UV transilluminator and photographed using 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

The genomic DNA of fourteen strains was analyzed using 5 decamer random amplified polymorphic DNA markers. All the markers except OPA06 produced significant bands. The number of bands and banding pattern were variable depending upon the marker and type of species tested and it ranged from 15 to 50 in counting (Table 1). The maximum polymorphism was produced by the primer OPA07 and OPA08 having polymorphism percentage 100%.

Table 1. RAPD primers with corresponding bands scored, number of monomorphic and polymorphic bands and polymorphism

Primer	Total number of bands scored	Number of polymorphic bands	Polymorphism (%)
OPA-07	25	25	100
OPA-08	15	15	100
OPA-09	43	41	95.35
OPA-10	50	47	94

The genetic similarities (Table 2) among the species ranged from 1 and 17%. The highest linkage distance (17.0) was found in Le3 vs. Le15 and Le15 vs. Le18 strain pairs. The lowest linkage distance (1.0) was found in Le1 vs. Le2 strain pair. The dendrogram based on similarity matrix differentiated the strains into two distinct clusters A and B. Cluster A constituted strain Le15, cluster B contained rest of the strains (Fig.1). Cluster B subdivided into sub cluster B1 and B2. Sub cluster B1 comprises strain Le10 and Le11. Sub cluster B2 belongs to cluster B2a and B2b; cluster B2a contained only strain Le4 whereas cluster B2b sub divided into B2b1 and B2b2. Cluster B2b1 contain only strain Le21 and cluster B2b2 also sub divided into cluster B2b2a and B2b2b. Cluster B2b2a belongs to the strains such as Le3, Le16 and Le5H; whereas cluster B2b2b comprises the strains Le1, Le2, Le8, Le17 and Le6.

Information on the genetic relationships among strains based on the molecular markers can help to decide which strain is highly variable. Plant breeders get the idea of breeding program and goal directed towards the selection of diverse parents to produce heterotic

hybrid strain. The reduced values of distance estimates between the strains seemed to suggest a downward trend on the level of genetic diversity present in the strain evaluated. This can be further enhanced by the use of related or limited number of parents in the hybridization program. The low level of genetic diversity in the germplasm collection is not beneficial to breeding because the use of genetically related parents can boost the effects of inbreeding depression like susceptibility to biotic and abiotic stresses, narrow adaptation and decreased productivity. When the level of genetic diversity available in the germplasm materials is low, progress in selection will be slow. The presented tree diagram demonstrates clearly the ability of the RAPD markers to detect the genetic variation in fourteen *Lentinus edodes* strains.

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

	Le 1	Le2	Le3	Le 4	Le5H	Le6	Le8	Le10	Le11	Le15	Le16	Le17	Le18	Le21
Le1	0.0	1.0	7.0	6.0	3.0	3.0	2.0	10.0	10.0	14.0	5.0	2.0	9.0	10.0
Le2	1.0	0.0	6.0	7.0	4.0	4.0	3.0	11.0	11.0	15.0	6.0	3.0	8.0	11.0
Le3	7.0	6.0	0.0	13.0	4.0	8.0	7.0	9.0	9.0	17.0	2.0	9.0	2.0	7.0
Le4	6.0	7.0	13.0	0.0	9.0	7.0	8.0	10.0	14.0	10.0	11.0	6.0	13.0	12.0
Le5H	3.0	4.0	4.0	9.0	0.0	4.0	3.0	7.0	7.0	13.0	2.0	5.0	6.0	7.0
Le6	3.0	4.0	8.0	7.0	4.0	0.0	3.0	9.0	11.0	13.0	6.0	3.0	8.0	9.0
Le8	2.0	3.0	7.0	8.0	3.0	3.0	0.0	10.0	10.0	14.0	5.0	4.0	9.0	10.0
Le10	10.0	11.0	9.0	10.0	7.0	9.0	10.0	0.0	6.0	12.0	9.0	8.0	9.0	8.0
Le11	10.0	11.0	9.0	14.0	7.0	11.0	10.0	6.0	0.0	10.0	9.0	10.0	11.0	10.0
Le15	14.0	15.0	17.0	10.0	13.0	13.0	14.0	12.0	10.0	0.0	15.0	12.0	17.0	14.0
Le16	5.0	6.0	2.0	11.0	2.0	6.0	5.0	9.0	9.0	15.0	0.0	7.0	4.0	5.0
Le17	2.0	3.0	9.0	6.0	5.0	3.0	4.0	8.0	10.0	12.0	7.0	0.0	9.0	8.0
Le18	9.0	8.0	2.0	13.0	6.0	8.0	9.0	9.0	11.0	17.0	4.0	9.0	0.0	7.0
Le21	10.0	11.0	7.0	12.0	7.0	9.0	10.0	8.0	10.0	14.0	5.0	8.0	7.0	0.0

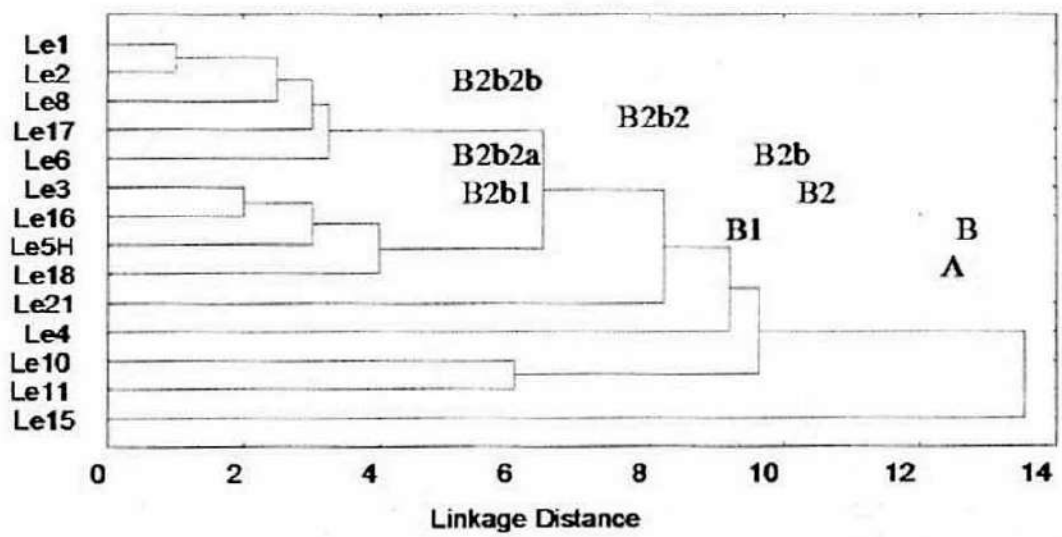

Fig. 1. Cluster analysis by unweighted pair group method of arithmetic means (UPGMA) of fourteen mushroom genotypes based on RAPD

The RAPD technique has also been successfully used to distinguish *Lentinus* and other genera of cultivated mushrooms, such as for the discrimination of different strains of *Agaricus bisporus* (Khush *et al.*, 1992). *Ganoderma lucidum* complex (Hseu *et al.*, 1996), *Lentinula edodes* (Chiu *et al.*, 1996), and for the identification and genetic evaluation of single-spore progenies of *Agaricus bisporus* (Calvo-Bado *et al.*, 2000) and *Stropharia rugoso-annulata* (Yan *et al.*, 2003). Therefore, the RAPD genetic variability in Shitake mushroom will also provide useful information for breeding of commercial strains.

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Bangladesh J. Mushroom. 8(2): 29-37. 2014 (December)

Effect of Amount of Substrate on the Growth and Yield of Shiitake (*Lentinus edodes*) Mushroom

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Abstract

The experiment conducted to study the effect of amount of substrate on the growth and yield of shiitake (*Lentinus edodes*) mushroom. Four strains (Le 8, Le 11, Le 12 and Le 16) of shiitake mushroom with various amounts (300g, 500g, 750g and 1000g) of substrate were used as treatments. The growth, yield and yield contributing characters of mushroom were significantly influenced by changing the amount of substrate and strains, Mycelial running time and duration of harvesting period become longer with the increasing substrate volume. The highest count of fruit body (81.50) was obtained in 500g size of spawn packet with Le 12 whereas it was lowest (1.25) in 1000g size of spawn packet with Le 8 and Le 16. The maximum yield (135.80 g) was recorded from 500g sized Le 12 spawn packet and the lowest yield (24.50 g) was obtained from 750g size of spawn packet with similar strain. The highest biological efficiency (85.47%) was shown in 300g spawn packet with Le 11. The biological efficiency decreased with the increasing size of spawn packet. Considering all the strains and amount of substrate, 500g sized spawn packet of Le 12 shows better performance than others.

Key words: Substrate, Amount, Growth, Yield, *Lentinus edodes*.

INTRODUCTION

Shiitake is the most popular and important edible medicinal mushroom in many countries (Chen, 2001 and Royse, 2001). It is the second most cultivated edible mushroom, comprising 25.4% of the world production (Chang, 1999). The commercial cultivation of shiitake mushroom on artificial substrates, based on enriched sawdust, has increased in the last few years (Donoghue and Denison,1995). The productivity of this mushroom depends on growing techniques, amount of supplementation, types of spawn and temperature ranges. One common technique involves heat-sealed larger bags which filled with 2-3 kg or more substrate for shiitake cultivation and produce more flushes of mushroom. Larger bag size may be suitable for reducing the labour cost and increasing the flushes and yield. The mushroom cultivation literature revealed that its production depends on many factors. Amount of substrate i.e. bag size is an important factor. The latest development in the U. S. i.e. the use of much larger sawdust-substrate blocks in sealed polypropylene bags. This methodology leads itself to faster and greater productivity by mixing the spawn thoroughly with the substrate, which produces more flushes of mushroom in much shorter growing cycles. European growers use larger bags and more substrate. Each bag contains 15kg of substrate in a flat slate shaped. The

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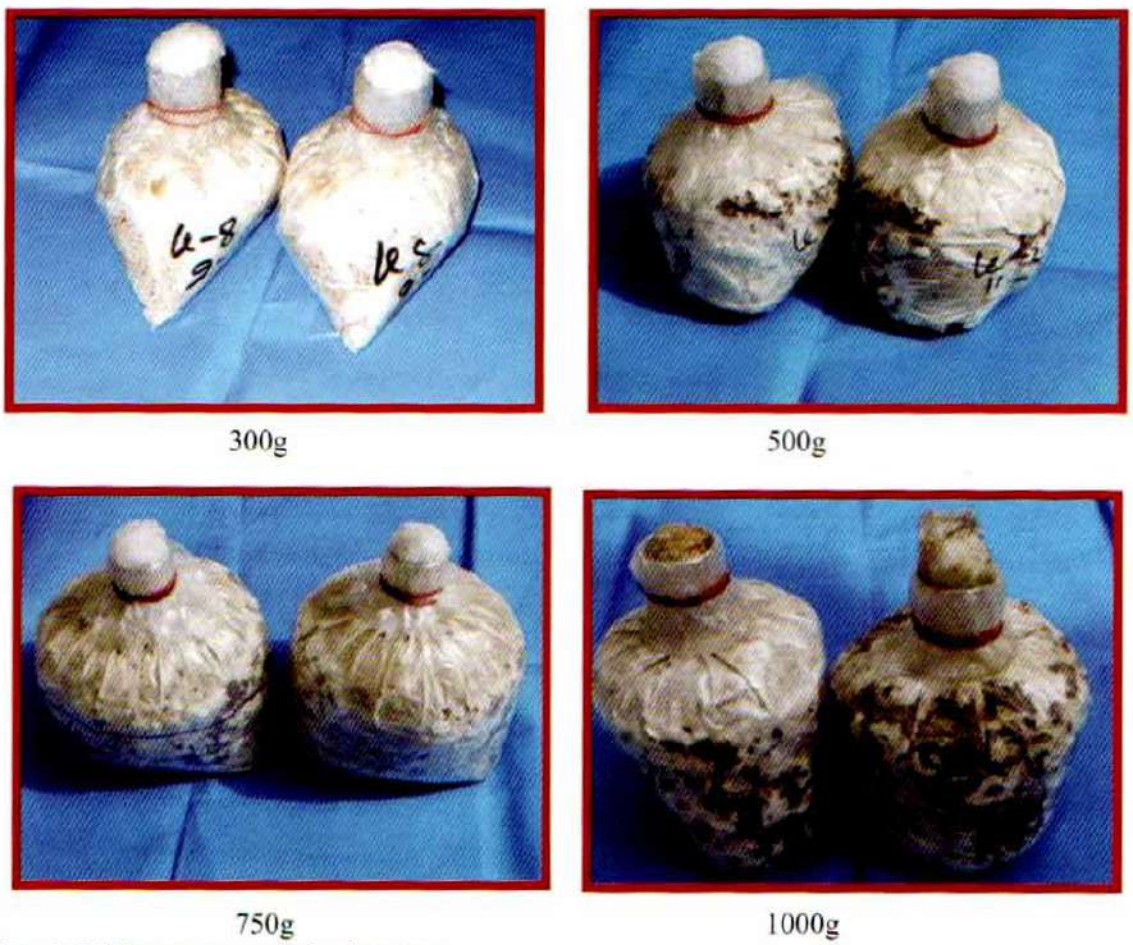
growing cycle in Europe is usually longer than in the United States (Oei, 2003). Today, a common cylindrical bag method is used in Southeast Asia. Longer cylindrical bags seem to produce better than the same weight of substrate closely packed together. Hence, it is essential for growers to identify the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

MATERIALS AND METHODS

The experiment was conducted at National Mushroom Development and Extension Centre, Savar, Dhaka during September 2011 to January 2012 to determine the appropriate size of spawn packets and better strain of shiitake mushroom. Four strains of shiitake (Le 8, Le 11, Le 12 and Le 16) combination with four amount of substrate viz. 300g, 500g, 750g and 1000g were used as treatments (Plate 1).



300g 500g

750g 1000g

Plate 1. Different amount of substrates.

Spawn packet preparation: The substrate of spawn packets were prepared by using sawdust and wheat bran at the ratio of 3:1 (dry basis). Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture.

Different sizes of polypropylene bags were filled with substrate mixture as above treatments. After filling the bags, the mouth of the packet were plugged by inserting absorbent cotton with the help of plastic neck and autoclaved at 121^{0}C and 1.5 kg/cm^2 pressure for 2 hours. After autoclaving and cooling, the bags were inoculated separately with the mother culture of Le 8, Le 11, Le 12 and Le 16 strain. Then, the packets were incubated in the laboratory at about 22 ± 2^{0}C temperatures.

Mycelial colonization and bump formation: During incubation period, whitish mycelia started to grow in the substrate.

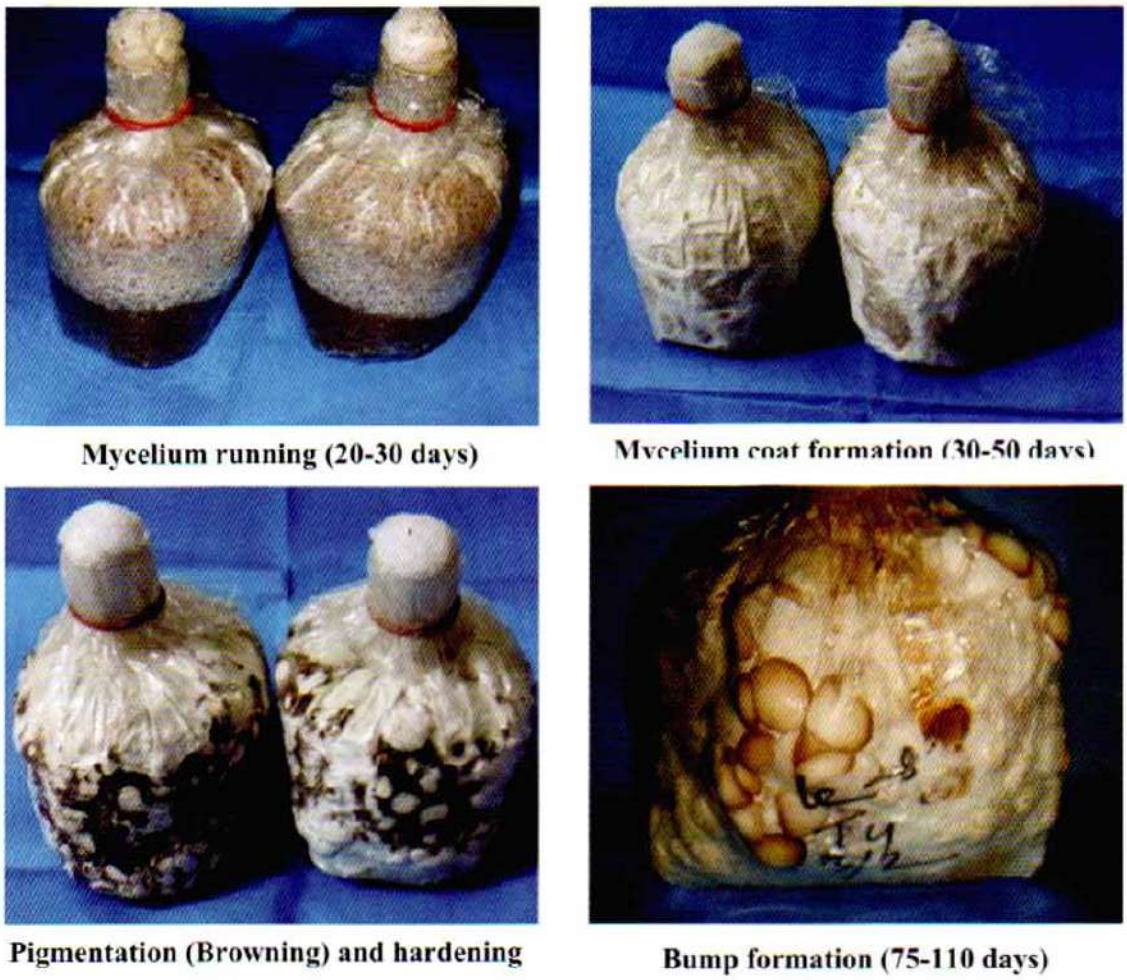


Plate 2. Mycelial growth phase of shiitake mushroom.

All the strains showed optimal mycelial growth at 22 ± 2^{0}C temperatures and 60-70% relative humidity. After full colonization of 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 colonization of white mycelia changed to brown. There are four phase steps involved in mycelial growth phase such as mycelium running, mycelium coat formation, pigmentation and bump formation (Plate 2).

Cultivation conditions for fruiting: After mycelium maturation and bump formation, all the packets were fully opened by removing the polypropylene bag. Then the packets were placed separately on the rack of culture house. Temperature, relative humidity was maintained as per required and light was maintained at 10-20 lux. Sufficient water was applied per day and proper aeration was maintained in culture house for the release of excess CO_2 and supply of sufficient O_2 as required for the development of primordia and fruiting body.

Experimental design, data collection and analysis: The experiment was laid out in a completely randomized design (CRD) with 4 replications. Data were collected on mycelium growth rate, time required for mycelium running, time required for bump formation, time required from opening to first harvest, time required for harvest, number of fruiting body & number of effective fruiting body/ packet, length & diameter of stalk, diameter of pileus, thickness of pileus, yield (g/packet) and biological efficiency. The yield was recorded by removing the lower hard and dirty portion of the fruiting bodies. The biological efficiency (%) was determined by the following formula:

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

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

RESULTS AND DISCUSSION

Mycelium growth rate (MGR): Significant variation in mycelium growth rate was observed by the different strains of shiitake mushroom against different amount of substrates. The highest mycelium growth rate (4.23 mm/day) was recorded in Le 11 with 1000g of spawn packet which was statistically similar to the combined effect of Le 12 with 1000g of spawn packet. The lowest mycelium growth rate (2.60 mm/day) was recorded in Le 8 with 500g weight of spawn packet (Table 1).

Time required to completion of mycelium running: Significant variations were found on time required to completion of mycelium running by the combined effect of strain and different amount of substrates. The highest time (114.80 days) required to completion of mycelium running was obtained from the strain Le 12 with 1000g spawn packet. The lowest time (33.25 days) required to completion of mycelium running was obtained from the strain Le 11 with 300g spawn packet which was statistically similar to the treatment combination of Le 8 with 300g spawn packet and Le 16 with 300g spawn packet (Table 1).

Time required for bump formation: Significant variations were found on time required for bump formation by the combined effect of strain and different amount of substrates. The highest time (149.30 days) required for bump formation was obtained from the

treatment of strain Le 11 with 1000g spawn packet. The lowest time (79.50 days) required for bump formation was obtained from the strain Le 8 with 300g spawn packet which was statistically similar to the treatment combination of Le 16 with 300 g spawn packet (Table 1).

Time required from opening to first harvest: Significant variations were found on time required from opening to first harvest by the combined effect of strain and different amount of substrates. The highest time (16.50 days) required from opening to first harvest was obtained from the Le 12 with 1000g spawn packet. The lowest time (4.00 days) required from opening to first harvest was obtained from the strain Le 16 with 300g spawn packet (Table 1).

Time required for harvest: Time required for harvest was significantly influenced by different amount of substrates and different strains of shiitake mushroom (Table 1). The highest time (160.00 days) required for harvest was recorded from the Le 11 with 1000g of spawn packet which was statistically similar to the treatment combination of Le 12 with 1000g of spawn packet. The lowest time (84.00 days) required for harvest was found from the treatment combination of Le 8 with 300g weight of spawn packet which was statistically similar to the Le 16 with 300g spawn packet.

Table1. Effect of strains and different amount of substrates on growth and development of shiitake mushroom

Amount of substrate	Mycelium growth rate (mm/day)	Time required to completion of mycelium running (days)	Time required for bump formation (days)	Time required from opening to first harvest (days)	Time required for harvest (days)
Strain of shiitake (Le 8) mushroom					
300g	2.90fh	35.25i	79.50i	4.50fg	84.00h
500g	2.60i	41.50g	90.50h	4.50fg	95.00g
750g	2.73hi	75.50c	104.30g	5.75df	110.00f
1000g	3.60b	95.25b	123.00d	6.50de	129.00cd
Strain of shiitake (Le 11) mushroom					
300g	3.40bc	33.25i	92.00h	5.00efg	97.00g
500g	3.13def	38.75h	123.30d	6.75d	130.00c
750g	2.90e-h	71.50d	137.00b	7.00d	144.00b
1000g	4.23a	93.50b	149.30a	10.75bc	160.00a
Strain of shiitake (Le 12) mushroom					
300g	3.53b	54.75f	104.50g	5.25efg	109.80f
500g	3.23cd	66.25e	112.00f	6.00df	118.00e
750g	3.03d-g	93.75b	128.50c	11.50b	140.00b
1000g	4.15a	114.80a	138.00b	16.50a	157.00a
Strain of shiitake (Le 16) mushroom					
300g	3.18cde	34.00i	84.00i	4.00g	88.00h
500g	2.85f-i	40.75gh	105.00g	5.00fg	110.00f
750g	2.78ghi	73.50cd	117.80e	7.25d	125.00d
1000g	3.43bc	94.00b	132.00c	10.00c	142.00b
CV (%)	**5.42**	**2.33**	**2.81**	**12.99**	**2.55**

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

Number of fruiting body: The combined effect of different strain with different amount of substrates on number of fruiting body was varied significantly. The highest number (81.50) of fruiting body was recorded from the Le 12 with 500g of spawn packet and the lowest number (1.25) of fruiting body was recorded from the treatment combination of Le 8 with 1000g weight of spawn packet which was statistically similar to the treatment combination of Le 16 with 1000g weight of spawn packet and Le 8 with 1000g weight of spawn packet (Table 2).

Table 2. Effect of strains and different amount of substrates on yield attributes and yield of shiitake mushroom

Amount of substrates	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)
Strain of shiitake (Le 8)							
300g	19.00d	14.50c	3.95fg	1.78cd	6.15c	1.05efg	85.75e
500g	21.00cd	17.50bc	4.90bcd	1.25efg	5.93c	1.10ef	112.30b
750g	4.00fg	3.75de	7.18a	1.40de	7.55b	1.78b	56.50h
1000g	1.25g	1.25e	3.95fg	2.45b	10.38a	2.33a	70.00f
Strain of shiitake (Le 11)							
300g	24.00c	17.75bc	5.25bc	0.83fg	5.88c	0.90gh	89.75de
500g	29.00b	18.75b	5.33bc	1.20efg	6.00c	1.15ef	94.00d
750g	11.00e	6.25d	3.38g	0.98efg	4.43def	0.88h	29.50k
1000g	6.00f	3.75de	3.88fg	0.80g	5.70cd	0.83h	38.00j
Strain of shiitake (Le 12)							
300g	23.75c	16.00bc	3.85fg	0.98efg	5.05c-f	1.05fg	63.50g
500g	81.50a	53.00a	4.28def	0.88fg	3.75f	0.80h	135.80a
750g	2.75fg	1.75e	5.05bc	2.33b	4.13ef	1.38cd	24.50l
1000g	1.75g	1.00e	4.13efg	10.25a	1.28g	1.43c	36.00j
Strain of shiitake (Le 16)							
300g	23.50c	17.50bc	5.50b	1.05efg	4.33def	1.20ef	72.75f
500g	23.75c	15.75bc	4.85b-e	1.43de	5.50cde	1.23de	104.50c
750g	4.25fg	3.50de	5.55b	2.00bc	5.20cde	1.38cd	43.00i
1000g	1.25g	1.25e	4.73cde	1.33def	9.60a	1.50c	57.00h
CV (%)	13.39	17.92	10.01	16.31	15.30	8.89	4.45

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

Number of effective fruiting body: The number of effective fruiting body was also highly significant by the combined effect of different strain and different amount of substrates. The highest number (53.00) of effective fruiting body was recorded from the treatment combination of Le 12 with 500g weight of spawn packet. The lowest number (1.00) of effective fruiting body was found from the treatment combination of Le 12 with 1000g weight of spawn packet which was statistically similar to the treatment combination of Le 8 with 1000g spawn packet, Le 12 with 750g spawn packet and Le 16 with 1000 g spawn packet (Table 2).

Length of stalk: Length of stalk was significantly influenced by different amount of substrate and different strain of shiitake mushroom. The highest length (7.18 cm) of stalk was found from the of Le 8 with 750g weight of spawn packet followed by Le 16 with 750g weight of spawn packet. The lowest length (3.38 cm) of stalk was recorded from the treatment combination of Le 11 with 750g weight of spawn packet (Table 2).

Diameter of stalk: Diameter of stalk was significantly influenced by different amount of substrates and different strains of shiitake mushroom. The highest diameter (10.25 cm) of stalk was observed from the treatment combination of Le 12 with 1000g weight of spawn packet. The lowest diameter (0.80 cm) of stalk was observed from the treatment combination of Le 11 with 1000g weight of spawn packet (Table 2).

Diameter of pileus: Diameter of pileus was also highly significant influenced by the combined effect of different strains and different amount of substrates. The highest diameter (10.38 cm) of pileus was recorded from the treatment combination of Le 8 with 1000g weight of spawn packet which was statistically similar to the treatment combination of Le 16 with 1000g spawn packet. The lowest diameter (1.28 cm) of pileus was recorded from the treatment combination of Le 12 with 1000 g weight of spawn packet (Table 2).

Thickness of pileus (TP): Thickness of pileus was also highly significant influenced by the combined effect of different strains and different amount of substrates. The highest thickness (2.33 cm) of pileus was recorded from the treatment combination of Le 8 with 1000g weight of spawn packet. The lowest thickness (0.80 cm) of pileus was recorded from the treatment combination of Le 12 with 500g weight of spawn packet which was statistically similar to the treatment combination of Le 11 with 750g and 1000g weight of spawn packet (Table 2).

Yield: The yield was significantly influenced by the combined effect of different strains and different amount of substrates. The highest yield (135.80g) was recorded from the Le 12 with 500g spawn packet followed by Le 8 with 500g weight of spawn packet. The lowest yield (24.50g) was recorded from the treatment combination of Le 12 with 750g weight of spawn packet (Table 2).

Biological efficiency (BE %): The biological efficiency was also significantly influenced by the combined effect of different strains and different amount of substrates. The highest biological efficiency (85.47%) was obtained from Le 11and 300g substrate. The second highest biological efficiency (81.66%) was obtained from the treatment combination Le 8 with 300g substrate. The lowest biological efficiency (9.31%) was recorded in Le 12 with 750g substrate which was statistically similar to Le 11 and Le 16 with 750g substrate as well as also similar to Le 11, Le 12 and Le 16 with 1000g substrate (Fig. 1).

Results of the present experiment reveal that there are appreciable variations in yield and yield contributing attributes with the variation of amount of substrates and strain of shiitake mushroom. In terms of yield and yield attributes performance of the strain Le 12

was the best among the strains. In case of different amount of substrates 500g weight spawn packet gave the highest yield while the highest biological efficiency obtained when spawns packets prepared with 300g substrates. The yield decreased with the increase of amount of substrates. On the other hands the larger spawn packets delay the harvesting period. Many other investigators also found variations in effect of amount of substrates on growth, yield and yield contributing characters of shiitake mushroom.

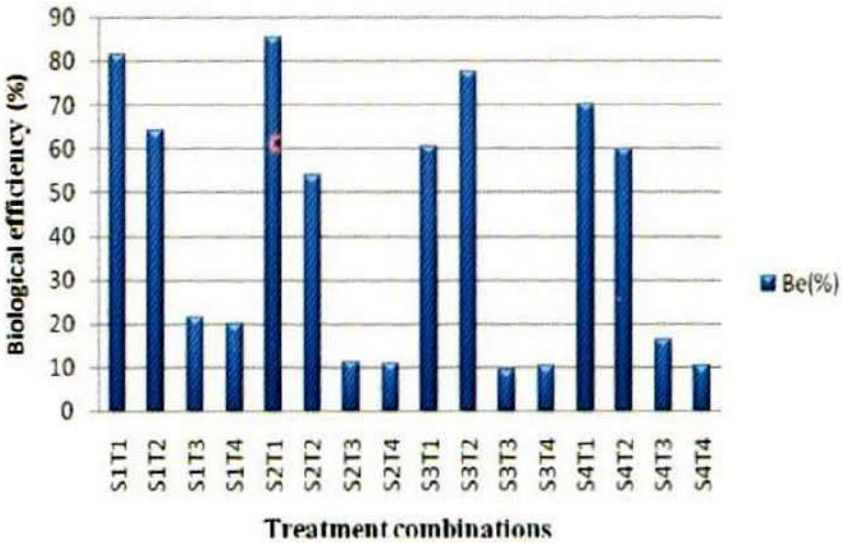


Fig.1. Combined effect of strain and amount of substrates on biological efficiency.
S_1T_1 = Le 8 × 300g, S_1T_2 = Le 8 × 500g, S_1T_3 = Le 8 × 750g, S_1T_4 = Le 8 × 1000g, S_2T_1 = Le 11×300g, S_2T_2 = Le 11 ×500g S_2T_3 = Le 11 × 750g S_2T_4= Le 11 × 1000g S_3T_1 = Le 12×300g S_3T_2= Le 12× 500g S_3T_3 = Le 12× 750g S_3T_4 = Le 12× 1000g S_4T_1 = Le 16 ×300g S_4T_2= Le 16 × 500g S_4T_3=Le16×750g, S_4T_4=Le16×1000g.

Hossain *et al.* (2010) reported that minimum days required when minimum amount of substrate were used for spawn packet preparation. He observed that minimum days required when the packets were cylindrical shape with 500g substrate and maximum days required where packets were cylindrical 1500g. He also (2010) reported that larger packets took more time than small packets for harvest, the lowest number of fruiting body was recorded from 500g cylindrical spawn packet and the lowest yield was obtained from 500g cylindrical spawn packet while highest yield was recorded 1500g cylindrical spawn packets. It was evident from Hossain *et al.* (2010) who studied that mushroom yield was positively correlated to the substrate amount and the results of Hossain study corroborated with the findings of Chen (2001).

Hossain *et al.* (2010) reported that biological efficiency decreased with the increase of size of spawn packet. He also reported that highest biological efficiency recorded in 500g size square block shape packet and lowest biological efficiency from 1500g block shape packet. The block shape of spawn packet performed better in small size of spawn packet whereas cylindrical shape is better in larger size of spawn packet. The present study more or less similar to the cited vales and little bit deviation may be due to different genotypes used.

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Bangladesh J. Mushroom. 8(2): 39-44. 2014 (December)

Effect of *Pleurotus ostreatus* on Reduction of Body Weight of Obeys Females Suffering from Diabetes

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Abstract

The present study was carried out in Nationl Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka, on diabetic obeys females, in association with the Department of Pharmacy and Department of Biochemistry & Molecular Biology, Jahangirnagar University, 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 a non significant small reduction of the body weight (63.38 ± 1.32 and 63.05 ± 1.28, p = 0.238) and body mass index (BMI) (28.18 ± 0.48 and 28.03 ± 0.47, p = 0.224). Findings of the study suggest that regular consumption of a considerable amount of *Pleurotus ostreatus* may able to reduce the body weight of diabetic female human subjects.

Key words: BMI, Body weight, Diabetes, Females, Obesity, *Pleurotus ostreatus*.

INTRODUCTION

Body mass index (BMI) of 25 or higher is defined as overweight and obesity is defined as a BMI of 30 or higher. Obesity, diabetes and cardiovascular diseases, clustered in a single word "the metabolic syndrome", have emerged as a major health problem. Obesity is one of the significant risk factors for metabolic syndrome, which include hypertension and hyperlipidaemia, potentially leading to type - 2 diabetes, cardiovascular disease, and nonalcoholic fatty liver disease (Kopelman, 2000; Funahashi and Matsuzawa 2007).

Overweight and obesity is being recognized as a global public health problem by the World Health Organization (WHO, 2000). Over the last few decades, there has been an alarming upward trend in the prevalence of obesity both in developed and developing countries (Popkin, 2001 and York, 2004). Research has shown that as weight increases to reach the levels referred to as "overweight" and "obesity," the risks for the following conditions also increases: Coronary heart disease, Type 2 diabetes, Cancers (endometrial, breast, and colon), Hypertension, Dyslipidemia, Stroke, Liver and Gallbladder disease, Sleep apnea and respiratory problems, Osteoarthritis, Gynecological problems (abnormal menses, infertility) (NHLBI, 1998).

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Hypertension, dyslipidemia and diabetes mellitus runs parallelly in relation to mortality and morbidity. But there is no easy procedure to overcome them. Now a day, the use of complementary and alternative medicine (CAM) is increasing rapidly. The World Health Organization classifies 65-80% of the world's health care services as 'traditional medicine' (Jonas, 1997). Recently, mushrooms have become the target of studies trying to determine whether fungi have nutritional and medicinal benefits which could improve the health status. Mushrooms are being used as an anti-diabetic drug since ancient time. They have traditionally been used in the treatment and prevention of diabetes, obesity, heart disease, hyperacidity, constipation, cancer, blood pressure and hypertension (Suguna and Usha, 1995).

By virtue of having high fibre, low fat and low starch, edible mushrooms have been considered to be ideal food for obese persons and for diabetics to prevent hyperglycaemia. They are also known to possess promising antioxidative, cardiovascular, hypercholesterolemia, antimicrobial, hepato-protective and anticancer effects. But little is known about the anti- obesity efficacy of oyster mushroom. Choudhury *et al.* (2008) conveyed the message that oyster mushroom may reduce body weight of an obese person, relieve constipation and hence can prevent related diseases.

Diabetes, cardiovascular disease, hypertension and obesity creating vigorous pressure on global health sector. Thus the present study was conducted to observe the effect of *Pleurotus ostreatus* on diabetic obeys females.

MATERIALS AND METHODS

The study was conducted in the laboratory of National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka in association with the department of Pharmacy Jahangirnagar University, Savar, Dhaka. After taking informed written consent a total 26 diabetic (fasting plasma glucose 7 to 19.8 mmol/l) obeys (and over weight) females age range (years) from 28 to 65 were included in the study. They were free from renal impairment (plasma creatinine 0.4 to 1.3 mg/dl) and other known acute or chronic diseases. The subjects were allowed to continue the medication (if any) they were taking. Age, sex, occupation, educational status, marital status, family history and drug history were recorded in a preformed data collection sheet.

At the beginning of the study, subjects were evaluated for health status. Mean of duplicate measurements was taken. Height and weight of the subjects were taken with light cloths without shoes. Trained workers took these measurements using the standard measuring tools. Body Mass Index (BMI) of the subjects was calculated.

BMI = Weight in kg / Height in meter^2.

With all aseptic precautions, 10 ml of fasting blood was collected from median cubital vein. Immediately after collecting, blood was poured into fluoride and EDTA containing test tube. The test tube was then gently shaken for proper mixing with the anticoagulants.

This anticoagulant-mixed blood was centrifuged at 3000 rpm for 5 minutes. Separated plasma was transferred into two eppendorf containing 1 ml in each. Plasma glucose level was estimated using enzymatic Glucose oxidase method and plasma creatinine was estimated by alkaline picrate method. Analysis was performed by semi auto biochemical analyzer 3000 evaluation using commercially available reagent kit. All the tests were carried out as early as possible.

Fresh fruiting bodies of *Pleurotus ostreatus* were collected from culture house of National Mushroom Development and Extension Centre (NAMDEC). They were dried with hot air oven at 40^0C for 48 hours at moisture level 4-5%, then pulverized and poured into capsule shells which contain 500 mg powder.

Mushroom capsules containing 500 mg *Pleurotus ostreatus* powder in each were supplied to take two capsules three times daily, so that each subject took 3 gm mushroom powder daily. After the end of three months the subjects were re-evaluated and all the investigations were repeated.

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

The present study was performed to observe the weight reducing effect of *Pleurotus ostreatus* on diabetic obeys females. A total 26 subjects were included in the study. Their mean age (years ± SE) was 43.46 ± 2.04, ranging from 28 to 65 and mean (± SE) height (meter) of the subjects was 1.49 ± 0.01 ranging from 1.38 to 1.62. The mean (± SE) fasting plasma glucose (FPG) (mmol/l) was 10.63 ± 0.74, ranging from 7.0 to 19.8 and mean (± SE) plasma creatinine level (mg/dl) was 0.68 ± 0.04 ranging from 0.4 to 1.3 (Table 1).

Table 1. Evaluation of age, fasting plasma glucose and creatinine level of the subjects

Parameter	N	Mean ± SE	Range
Age (years)	26	43.46 ± 2.04	28 to 65
Height (meter)	26	1.49 ± 0.01	1.38 to 1.62
FPG (mmol/l)	26	10.63 ± 0.74	7.0 to 19.8
Creatinine (mg/dl)	26	0.68 ± 0.04	0.4 to 1.3

It was observed in the study that, the mean ± SE body weight before and 3 months after mushroom supplement was 63.38 ± 1.32 and 63.05 ± 1.28 respectively (Fig. 1). There was statistically non-significant small reduction of body weight ($p = 0.238$) observed after three months mushroom treatment.

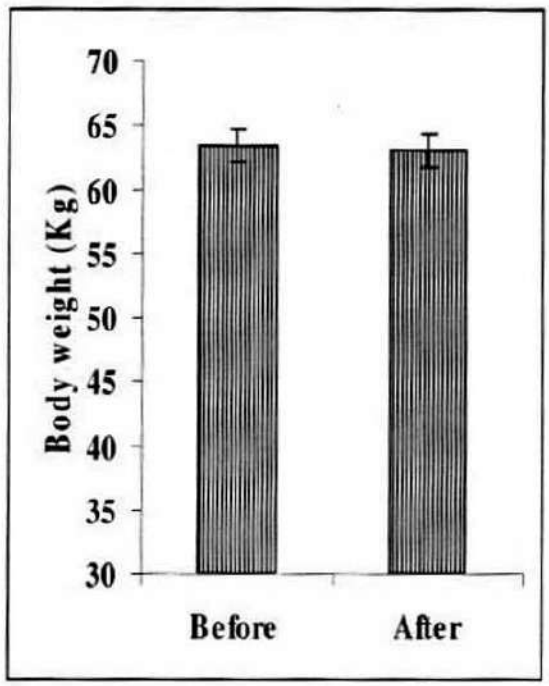

Fig. 1. Mean (± SE) body weight before and 3 months after mushroom supplementation.

The mean ± SE body mass index (BMI) before and three months after mushroom treatment was 28.18 ± 0.48 and 28.03 ± 0.47 respectively. No significant mean difference of BMI ($p = 0.224$) observed. But here also small reducing tendency of BMI was observed after the end of three months mushroom supplementation (Fig. 2).

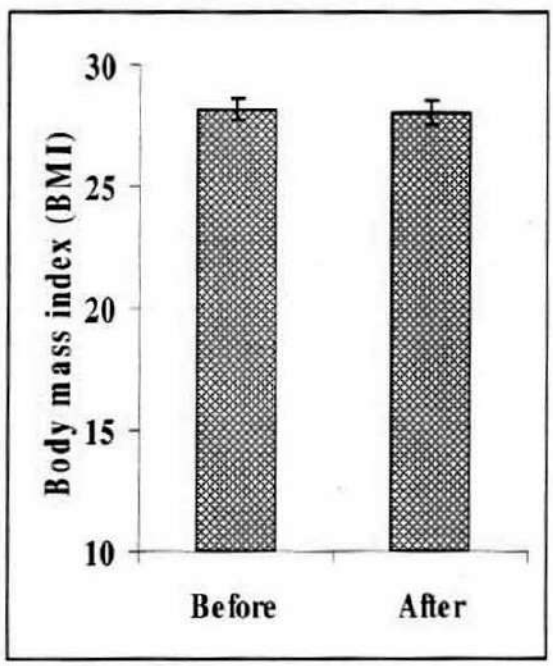

Fig. 2. Mean (± SE) body mass index (BMI) before and 3 months after mushroom supplementation.

Mushrooms contain a lot of different bioactive components, which mainly include triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, amino acids, soluble proteins, oleic acid, anergosterol peroxide and inorganic ions like Mg, Ca, Zn, Mn, Fe, Cu, Ge etc. These compounds reduce TG accumulation significantly and ultimately may able to reduce the body weight (Iksoo *et al.*, 2010). Some other observations showed that feeding of *Plerotus ostreatus, Pleurotus sajor-caju* and *Pleurotus florida* mushroom reduced body weight significantly in hyper-cholesterolemic rats by 17.36%, 23.37% and 24.13% respectively (Lee *et al.*, 2009).

In a study Amin *et al.* (2012) observed that 5% powder of *Ganoderma lucidum* adminstration significantly reduced body weight in obeys rats. Besides this, other mushroom like Yamabushitake (*Hericium erinaceus*) also reduces body weight significantly in high-fat diet fed mice (Shirakawa *et al.*, 2010). Our study is not fully agreement with these studies but it is mentionable that all of the studies were conducted on animal model, whose metabolic system might be different from human.

Choudhury *et al.* (2008) showed no significant change of BMI of the subjects before and 1 month after supplementation of 5 gm of mushroom powder 3 times per day and commended that BMI might not be changed due to very short interval between the two observations, as reduction of body weight depends on time and other dietary modulation that were not strictly maintained in the study. Current study is fully agreement with previous one, although the duration of the current study period is more than the previous one (3 months in contrast with 1 month) but the dose of mushroom consumption was less (3 gm/day in contrast with 15 gm/day). In another study Choudhury *et al.* (2012) observed the same type of findings in diabetic obeys male volunteers. These findings are also supported by others, who observed *Pleurotus tuber-regium* and *Termitomycetes clypeatus* feeding reduced body weight in hypercholesterolemic rats slightly but not significantly (Oyetayo, 2006).

Although findings of this 3 months trial with supplementation of 3 gms *Pleurotus ostreatus* powder per day showed a non significant change of body weight of obeys diabetic female but it provides a clue that body weight might be reduced by increasing the dose and duration of mushroom consumption. So, it is assumed that regular intake of considerable amount of *Pleurotus ostreatus* may able to reduce obesity of diabetic person and hance take part on improving diabetes.

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Bangladesh J. Mushroom. 8(2): 45-57. 2014 (December)

Seasonal Effect on the Performance of Different Shiitake Mushroom Strains Available in Bangladesh

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Abstract

Shiitake *(Lentinus edodes)*, is worldwide one of the most widely cultivated mushrooms and it is the second most popular edible mushroom. Many strains of shiitake mushroom are available in the world which is extensively cultivated. The morphology and productivity of shiitake mushroom vary according to the strains based on the influence of environmental factors. Temperature and relative humidity are the main factors for successful growing of shiitake mushroom. In this study twenty three strains of shiitake were grown in four seasons (autumn, late autumn, winter and spring) to select the suitable strain and best season for production of shiitake mushroom in Bangladesh. The temperature (in ^{0}C) and relative humidity (% RH) of culture house in each season, and parameters of mushroom production were recorded. Le 8 gave the highest yield (191.00 g) and highest biological efficiency (109.10 %) in winter season. The strain Le 8 also gave highest yield (145.00 g) and highest biological efficiency (83.29 %) in autumn season. Le 16 gave the highest yield (175.00 g) and highest biological efficiency (100.30%) in late autumn season which was statistically similar to the strain Le 8 and second highest yield (171.80 g) in winter season. Le 12 gave second highest yield (99.00 g) and biological efficiency (56.57 %) in autumn season which was statistically similar to Le 11. Le 1 gave the highest yield (163.50 g) and highest biological efficiency (93.43 %) in spring season but no yield was obtained in autumn season. Regarding Le 13 there was no production of fruit body at any season in Bangladesh.

Keywords: Season, Strain Temperature.

INTRODUCTION

Lentinus edodes, a mushroom primarily of temperate climate, is indigenous in the Far East. It has been cultivated for centuries in China, Korea, Japan, Singapore, Thailand and other Asian countries (Ciesla, 2002). The word 'shiitake' was originally derived from Japanese words: *shii* which means oak and *take* which means mushroom, reflecting the importance of oak wood as the natural host of the fungus (Davis 1993; Royse 2001). There are many commercial strains of shiitake selected and propagated for their adaptability to different log species, the time taken to fruit after inoculation and the size, colour, taste and shape of the mushroom (Stamets, 1993). Shiitake mycelium growth and mushroom development are clearly influenced by environmental factors. Moisture and temperature are the two most important factors (Kaul, 1997) although the log itself plays an important role in buffering the environmental factors and protecting the fungi from extremes. The availability of nutrients, either as drawn from the substrate or provided as a supplement in supplied water, is also important. Different strains respond differently to forcing and produce mushrooms with different characteristics (McCoy and Bruhn, 2005).

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Different strains are often categorized by fruiting temperature requirements. Przybylowicz and Donoghue (1988) indicate that temperature has a strong influence on survival, growth rate, time of fruiting, yield and the shape of the mushroom produced. The optimum temperature for mycelial development of *Lentinula edodes* is ranged from 24-28^{0}C reported by Tokimoto and Komatsu 1982 or 15-24^{0}C (Sabota 2007). The temperature required for fruiting ranges from 5 to 30^{0}C (Sabota 2007), while the optimum range is from 10 to 25^{0}C (Przybylowicz and Donoghue 1988). However, the optimum temperature for fruiting may vary with the particular strain used for cultivation. For example, cold weather strains will fruit when temperatures are between 7 and 15^{0}C (Przybylowicz and Donoghue 1988). In contrast, warm weather strains will fruit when temperatures are between 10 and 28^{0}C (Sabota 2007) and wide-range fruiting strains will produce fruiting body between 10 and 27^{0}C. Based primarily on the Chinese system, strains are classified into four categories according to their fruiting temperatures. Faced with massive imports, the Japanese developed a number of new shiitake strains with large and thick basidiocarps (Watanabe, 2001). Performance and stability of superior strains are both important. Experienced growers know the potential problems of strain attenuation. For example repeated subcultures and prolonged storage of the stock culture may result in smaller fruiting bodies and lower yield. Shiitake strains vary widely, particularly in fruiting temperature and mycelial maturation (early or late; shorter or longer production time). Substrate selectivity, growth rate (some fast strains may produce pre-mature fruiting), quality (shape, size, thickness, color, flavor and aroma, etc.), yield and ecological adaptability to extreme temperature (usually cold tolerance) are also strain-related.

Lentinus edodes, no doubt, is the most important specialty mushroom. Despite increasing interest in growing this species, successful cultivation is a challenge. Appropriate strains must be used particularly in fruiting temperature and mycelial maturation. Strains vary greatly not only in fruiting temperature and the time required for spawn maturation but also other traits. Close attention should be given to crucial stages during transition from intricate vegetative phase to reproductive phase. In order to cultivate shiitake mushroom, it is important to first understand the biology, environmental and nutritional requirements of shiitake. The present study was undertaken to evaluate the influence of seasonal variation in different strains of shiitake mushroom available at National mushroom Development and Extension Centre (NAMDEC) and to identify the best strain and season that can be highly productive and suitable for culture conditions in Bangladesh.

MATERIALS AND METHODS

An experiment in completely randomized design with 4 replications was carried out at the National Mushroom Development and Extension Centre, Savar, Dhaka during the period from June 2011 to April 2012. The first factor was different season and second factor was different strains of shiitake mushroom available in Bangladesh. Four different seasons namely autumn (August to October), late autumn (October to December), winter (December to February) and spring (February to April) and twenty three strains of shiitake mushroom viz. Le 1, Le 2, Le 3, Le 4, Le 5, Le 5(H), Le 6, Le 8, Le 9, Le 10, Le

10(H), Le 11, Le 12, Le 13 Le 14, Le 15, Le 16, Le 17, Le 18, Le 19, Le 20, Le 21, Le JR, were used in the experiment.

Preparation of pure culture: Pure culture of twenty three strains were prepared on potato dextrose agar (PDA) medium containing 200g peeled and sliced potato, 20g dextrose and 20 g agar per liter. The medium was poured into test tube at 10 ml/tube. The medium in test tube was sterilized in an autoclave for 20 minutes at 121^0C under 1.5 kg/cm^2 pressure. After sterilization and solidification, the plates were inoculated separately with the inoculants of above mentioned 23 strains. After inoculation, the tubes were covered with cotton plug. All operations were done under sterile condition in a clean bench. The inoculated tubes were incubated in a growth chamber at 22 ± 2^0C. After completion of the whitish mycelium, this culture was used for inoculation of mother culture.

Preparation of mother culture: To prepare mother culture of test mushroom sawdust and wheat bran mixed together at the ratio of 2:1 (v/v). Water was added to adjust moisture content at 65% and $CaCO_3$ was mixed at the rate of 0.2% of the mixture. 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 pipe. A hole of about 2/3 deep of the volume of the bag was made for space to put the 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 (1) hour at 121^0C under 1.5 kg/cm^2 pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. Individually, a piece of stock PDA culture of each strain containing mycelium was placed aseptically in the hole of mother culture packet and the packet was again plugged as mentioned above. The inoculated packets were placed on a rack in the laboratory at 22 ± 2^0C temperatures for incubation. The substrate of the mother culture was colonized by the growth of whitish mycelium within 15-20 days after inoculation. The fully colonized packets were used for spawning.

Preparation of spawn packet: The substrate of spawn packets were prepared using sawdust and wheat bran mixture at the ratio of 2:1. Water was added to make the moisture level about 65% and $CaCo_3$ was added at 0.2% (w/w) of the mixture. 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 the mother culture of the twenty three strains at the rate of two tea spoonful per packet. The inoculated packets were incubated at 22 ± 2^0C.

Mycelial colonization and bump formation: During incubation period, whitish mycelia started to grow in the substrate. All the strains showed optimal mycelial growth at 22 ± 2^0C temperatures and 60-70% relative humidity under control condition. After full

colonization of 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 colonization of white mycelia changed to brown.

Cultivation conditions for fruiting: After mycelium maturation and bump formation, all the packets were fully opened by removing the polypropylene bag. Then the packets were placed separately on the rack of culture house under natural condition. Temperature, relative humidity was recorded season wise and light was maintained at 10-20 lux. Temperature of culture house was recorded at autumn (25.8^0C-32.0^0C), late autumn (19.6^0C-29.1^0C), winter (14.6^0C-25.9^0C) and spring (21.9^0C-32.8^0C). Similarly relative humidity was recorded at autumn (82.35% - 84%), late autumn (77.20% - 82.35%), winter (72.50% - 78.45%) and spring (68.50% -79.55%). Water was applied per day as required and proper aeration was maintained in culture house for the release of excess CO_2 and supply of sufficient O_2 as required for the development of primordia and fruiting body.

Data collection and analysis: Data were recorded on time required for bump formation, time required from opening to first harvest (TROFH), time required for harvesting (TRH), number of fruiting body (NFB), number of effective fruiting body (NEFB), length of stalk (LS), diameter of stalk (DS), diameter of pileus (DP), thickness of pileus (TP), yield (g), biological efficiency (BE %).

Yield was recorded after removing lower hard and dirty portion of fruiting bodies and biological efficiency (%) was determined by the following formula:

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

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

RESULTS AND DISCUSSION

Time required for bump formation (days): Time required for bump formation in different seasons ranged from 88.00-108.00, 81.00-121.00, 84.00-138.00 and 75.25-170.00 days, respectively (Table 1). The strain Le 10(H) developed bump earlier (75.25 days) in spring season. On the other hand, Le 8 required highest (170.00 days) duration to form bump in same season compare to other seasons and strains. The strain Le 8 developed bump earlier in autumn season followed by winter and late autumn while developed bump late in spring season.

Table1. Time required for bump formation of different strains of shiitake mushroom in different growing season

Strains	Time required for bump formation (days)			
	Autumn	Late autumn	Winter	Spring
Le 1	--	109.00bc	98.00hi	120.50d
Le 2	--	81.00i	114.00de	135.50c
Le 3	--	90.00h	91.50jk	90.00l
Le 4	--	97.00fg	96.00ij	106.80hi
Le 5	--	121.00a	121.00c	95.50k
Le 5 (H)	--	-----	130.00b	110.00fg
Le 6	--	90.00h	115.30de	122.00d
Le 8	88.00b	108.00bc	107.00fg	170.00a
Le 9	--	94.00gh	103.00gh	119.80d
Le 10	--	100.00ef	103.00gh	86.00m
Le 10 (H)	--	-----	90.00k	75.25n
Le 11	106.50a	92.25h	105.00g	115.00e
Le 12	108.00a	100.30ef	107.00fg	105.00i
Le 13	--	-----	-----	-----
Le 14	--	90.00h	138.00a	113.50e
Le 15	--	89.50h	112.00ef	109.00fgh
Le 16	108.00a	94.00gh	91.00jk	100.80j
Le 17	--	106.00cd	102.00gh	107.50ghi
Le 18	--	103.00de	104.00g	96.50k
Le 19	--	-----	84.00l	88.00lm
Le 20	108.00a	112.00b	119.00cd	152.80b
Le 21	--	89.50h	90.50jk	119.50d
Le JR	--	101.00ef	114.00de	110.80f
CV (%)	2.45	2.97	3.45	1.63

Time required from opening to first harvest (days): The time required from opening to first harvest in different strains of shitake mushroom was non significant in autumn season but highly significant in other seasons. The duration from opening of spawn packet to first harvest in different seasons ranged 5.0-6.0, 4.0-11.0, 4.25-13.50 and 3.25-14.50 days respectively. The strain Le 12 required maximum days (14.50 days) from opening to first harvest in spring season while Le 8 required minimum days (3.25 days) in same season (Table 2).

Time required for harvest (days): Time required for harvest was highly significant in different strains of shiitake mushroom under four seasons. Time required for harvest in autumn, late autumn, winter and spring ranged from 93.00 -114.00, 92.00-131.00, 95.00-147.00 and 94.00-173.30 days, respectively (Table 2). The strain Le 8 required the maximum time (173.30 days) in spring season and the strain Le 2 required minimum time (92.00 days) in late autumn season.

Table 2. Time required from opening to first harvest and time required for harvest of different strains of shiitake mushroom in different growing season

Strains	Time required from opening to first harvest (days)				Time required for harvest (days)			
	Autumn	L. Autumn	Winter	Spring	Autumn	L. Autumn	Winter	Spring
Le 1	--	7.00cdef	13.50a	5.00efg	--	116.00b	111.50hi	125.50e
Le 2	--	11.00a	7.00efg	6.25cdef	--	92.00i	121.00ef	141.80c
Le 3	--	8.00bcde	7.00efg	8.25c	--	98.00fgh	98.50kl	98.25k
Le 4	--	10.00ab	7.00efg	6.25cdef	--	107.00de	103.00jk	113.00h
Le 5	--	10.00ab	10.00bc	7.00cde	--	131.00a	131.00c	102.50j
Le 5H	--	-----	7.50def	6.00def	--	-----	137.50b	116.00gh
Le 6	--	8.00bcde	4.75gh	7.75cd	--	98.00fgh	120.00efg	129.80d
Le 8	5.00a	5.00fg	5.00gh	3.25g	93.00b	113.00bc	112.00hi	173.30a
Le 9	--	8.00bcde	6.00fgh	4.75fg	--	102.00ef	109.00ij	124.50e
Le 10	--	10.00ab	11.00b	8.00cd	--	110.00cd	114.00ghi	94.00l
Le10H	--	-----	7.00efg	7.00cde	--	-----	97.00kl	82.25m
Le 11	6.00a	6.00defg	8.00cdef	8.25c	112.50a	98.25fgh	113.00hi	123.30e
Le 12	5.75a	5.00fg	9.00bcde	14.50a	113.80a	105.30de	116.00fgh	119.50f
Le 13	--	-----	-----	-----	----	-----	-----	-----
Le 14	--	6.00defg	9.00bcde	6.00def	--	96.00ghi	147.00a	119.50f
Le 15	--	8.25bcd	9.00bcde	6.00def	--	97.75fgh	121.00ef	115.00gh
Le 16	5.00a	5.75efg	4.25h	7.25cd	112.80a	99.75fg	95.25l	108.00i
Le 17	--	9.00abc	6.50fgh	7.00cde	--	115.00b	108.50ij	114.50gh
Le 18	--	4.00g	7.00efg	7.00cde	--	107.00de	111.00hi	103.50j
Le 19	--	-----	11.00b	11.00b	--	-----	95.00l	99.00k
Le 20	6.00a	4.00g	9.50bcd	6.75cdef	114.00a	116.00b	108.50ij	159.50b
Le 21	--	5.00fg	6.50fgh	5.00efg	--	94.50hi	97.00kl	124.50e
Le JR	--	6.00defg	10.00bc	6.25cdef	--	107.00de	124.00de	117.00fg
CV (%)	**22.19**	**19.97**	**18.03**	**17.38**	**1.68**	**3.00**	**3.75**	**1.70**

Number of total fruiting body: Number of total fruiting body in four seasons ranged from 14.00–55.00, 1.50-55.00, 1.75-52.00 and 2.0-40.00 per packet respectively. Their differences were highly significant. The highest number (55.00) of fruiting body was recorded from the strain Le 12 in late autumn season and the lowest number (1.50) of fruiting body was recorded from the strain Le 9 in same season. The second highest number (52.00) of fruiting body was recorded from the strain Le 11 in winter season (Table 3).

Number of effective fruiting body: Number of effective fruiting body in different strains of shiitake mushroom in different seasons was highly significant. Number of effective fruiting body in autumn, late autumn, winter and spring ranged from 3.25–39.50, 1.25-36.25, 1.50-37.00 and 1.00-32.25 per packet respectively. The highest number (39.50) of effective fruiting body was recorded from the strain Le 12 followed by Le 11 in winter season and the lowest number (1.00) of effective fruiting body was recorded from the strain Le 20 in spring season (Table 3).

Table 3. Number of total fruiting body and number of effective fruiting body of different strains of shiitake mushroom in different growing season

Strains	Number of total fruiting body				Number of effective fruiting body			
	Autumn	L.autumn	Winter	Spring	Autumn	L. autumn	Winter	Spring
Le 1	--	32.25d	23.75d	32.75c	--	26.50b	16.50e	27.75b
Le 2	--	5.50ij	28.50c	11.00g	--	3.00gh	22.50d	9.25e
Le 3	--	5.25ij	9.75f-h	17.00e	--	2.50gh	6.75hijk	12.00d
Le 4	--	5.25ij	9.25f-h	8.75hi	--	4.75gh	7.25ghij	5.75gh
Le 5	--	5.00ij	18.25e	7.75ij	--	5.00g	12.75f	6.75fg
Le 5H	--	-----	6.00g-i	6.00jk	--	-----	4.25jklm	4.25hi
Le 6	--	6.00hi	10.00f-h	10.25gh	--	4.00gh	8.00ghi	7.50fg
Le 8	40.50b	50.50b	45.25b	40.00a	35.00b	37.50a	35.75ab	32.25a
Le 9	--	1.50j	1.75i	4.50kl	--	1.25h	1.50m	3.25ij
Le 10	--	20.25f	31.00c	14.25f	--	18.75c	29.25c	8.25ef
Le10H	--	-----	4.00i	4.25klm	--	-----	3.50lm	4.00i
Le 11	14.00d	19.50f	52.00a	18.00e	5.25d	15.25d	37.00a	13.50d
Le 12	55.00a	45.50c	47.50b	36.00b	39.50a	35.75a	36.75ab	28.50b
Le 13	--	-----	-----	-----	--	-----	-----	-----
Le 14	--	12.00g	6.50f-i	7.25ij	--	6.00fg	6.25ijkl	3.00ij
Le 15	--	4.50ij	5.25hi	3.00lmn	--	4.25gh	3.50klm	2.50ijk
Le 16	21.00c	55.00a	44.00b	21.75d	15.75c	36.25a	33.75b	18.75c
Le 17	--	25.75e	27.75c	7.50ij	--	18.00cd	20.50d	6.00g
Le 18	--	1.75j	2.75i	2.25mn	--	1.25h	2.25m	1.75jk
Le 19	--	-----	3.75i	3.00lmn	--	-----	2.75m	2.75ij
Le 20	4.00e	12.75g	4.25i	2.00n	3.25d	11.25e	3.75klm	1.00k
Le 21	--	10.00g	11.25f	6.25jk	--	9.00ef	9.50gh	3.50ij
Le JR	--	9.50gh	10.75fg	7.75ij	--	8.75ef	10.00fg	7.25fg
CV (%)	**6.58**	**14.41**	**16.23**	**11.42**	**10.72**	**17.23**	**14.43**	**11.89**

Length of stalk (cm): The length of stalk in different strains of shiitake mushroom under four seasons was highly significant. Ranges of length of stalk under different strains with different seasons were 4.25-7.25, 2.45-9.50, 3.15-6.18 and 3.15-7.65cm respectively. Most of the strains produced shorter stalk in winter season compare to other seasons. On the other hand, the length of stalk gradually increased with the increase of temperature in three seasons. The strain Le 20 produced longest (7.25 cm) stalk when grow in autumn season and the strain Le 1 produced shortest stalk (2.45 cm) in late autumn season (Table 4).

Diameter of stalk (cm): Differences in diameter of stalk in twenty three strains of shiitake mushroom in four seasons were 1% level of significant. The maximum stalk diameter 2.70cm of Le 5(H) was found in spring season whereas lowest diameter of 0.75 cm was obtained from the strain Le 8 in same season (Table 4).

Table 4. Length and diameter of stalk of different strains of shiitake mushroom in different growing season

Strains	Length of stalk (cm)				Diameter of stalk (cm)			
	Autumn	L.autumn	Winter	Spring	Autumn	L.autumn	Winter	Spring
Le 1	--	2.45j	3.38ij	3.18j	--	1.28e-i	1.08g	1.25ef
Le 2	--	3.80i	4.75cdef	5.15e	--	1.58cde	1.28fg	1.08fg
Le 3	--	4.12hi	5.00bcd	4.80f	--	1.50def	1.25fg	1.33e
Le 4	--	5.78c	5.17bcd	6.30c	--	1.93bc	2.00cd	2.08b
Le 5	--	4.20ghi	4.08efghi	4.75f	--	1.45d-h	1.83cde	0.88ghi
Le 5H	--	------	4.75cdef	7.65a	--	-------	1.35fg	2.70a
Le 6	--	4.95defg	4.63defg	4.18gh	--	1.80bcd	2.48ab	0.95ghi
Le 8	6.28b	4.58efghi	3.15j	4.05hi	1.07bc	1.65cd	1.25fg	0.75i
Le 9	--	4.85defgh	3.80ghij	5.50d	--	2.55a	2.48ab	1.60cd
Le 10	--	5.43cd	5.07bcd	5.50d	--	1.28e-i	1.15g	0.93ghi
Le10H	--	------	4.03efghi	4.45fg	--	------	1.15g	1.63c
Le 11	4.63cd	4.53fghi	4.88bcde	4.50fg	1.18ab	1.65bcd	1.28fg	1.60cd
Le 12	4.25d	4.25ghi	4.88bcde	3.15j	0.90c	0.95i	1.43efg	1.55cd
Le 13	--	-------	-----	-----	--	-------	-----	-----
Le 14	--	4.55fghi	5.53abc	7.23b	--	1.20f-i	1.53efg	2.13b
Le 15	--	9.50a	4.98bcd	5.13f	--	2.00b	1.83cde	1.03gh
Le 16	4.88c	5.35cde	3.70hij	4.63f	1.38a	1.13ghi	1.20fg	1.40de
Le 17	--	3.88i	4.05efghi	6.00c	--	1.07i	1.23fg	1.65c
Le 18	--	4.05hi	5.70ab	6.03c	--	1.10hi	2.15bc	2.10b
Le 19	--	------	4.58defgh	3.75i	--	------	2.65a	2.63a
Le 20	7.25a	8.20b	6.18a	5.23de	1.10bc	1.10hi	2.00cd	1.95b
Le 21	--	5.15cdef	3.80ghij	3.33j	--	1.50def	1.65def	1.65c
Le JR	--	3.80i	3.98fghij	4.00hi	--	1.48d-g	1.53efg	0.85hi
CV (%)	**5.84**	**10.80**	**11.77**	**4.53**	**12.20**	**15.27**	**17.59**	**8.81**

Diameter of pileus (cm): The diameter of pileus in different strains in autumn, late autumn, winter and spring season ranged from 4.25-6.68, 2.48-9.50, 5.05-10.38 and 5.30-10.80 cm respectively with highly significant difference. The highest diameter of pileus (10.80 cm) was obtained from the strain Le 18 in spring season and the lowest diameter of pileus (2.48cm) was obtained from the strain Le 18 in late autumn season (Table 5).

Thickness of pileus (cm): The thickness of pileus in different strains of shiitake mushroom as well as different seasons differed significantly and ranged from 0.80-1.40, 0.88-1.44, 1.05-2.50 and 0.65-2.40cm respectively. The highest thickness of pileus (2.50 cm) was obtained from the strain Le 9 in winter season and the lowest thickness of pileus (0.65 cm) was obtained from the strain Le 8 in spring season (Table 5).

Table 5. Diameter and thickness of pileus of different strains of shiitake mushroom in different growing season

Strains	Diameter of pileus (cm)				Thickness of pileus (cm)			
	Autumn	L.Autumn	Winter	Spring	Autumn	L.Autumn	Winter	Spring
Le 1	--	5.00hi	5.70fg	6.86hi	--	1.00hi	1.28ghij	1.15jk
Le 2	--	9.50a	6.68def	6.98h	--	1.88bcd	1.23hij	1.50gh
Le 3	--	9.13a	5.75fg	7.33g	--	1.08ghi	1.05j	1.30hij
Le 4	--	7.13bcd	7.65bcd	8.68d	--	2.05ab	2.25ab	1.78def
Le 5	--	5.23ghi	7.75bcd	6.65hij	--	1.58def	1.60efg	1.10jkl
Le 5H	--	-------	7.88bcd	7.88ef	--	------	1.45fghi	2.40a
Le 6	--	7.90b	8.50bc	6.58ijk	--	2.13ab	1.60efg	0.98kl
Le 8	5.85c	6.40def	5.95efg	4.63m	0.90bc	1.28fgh	1.13ij	0.65m
Le 9	--	7.38bc	10.38a	9.00cd	--	2.30a	2.50a	1.68efg
Le 10	--	6.58cdef	6.00efg	7.50g	--	1.25fgh	1.23hij	1.08jkl
Le10H	--	------	5.05g	7.60fg	--	------	1.15hij	1.60fg
Le 11	6.18b	6.38df	5.13g	7.40g	0.95bc	1.35e-h	1.25hij	1.23j
Le 12	4.25e	4.45i	7.45cd	5.30l	1.05b	0.88i	1.33ghij	1.28ij
Le 13	--	------	-----	-----	--	------	-----	-----
Le 14	--	6.88cde	6.70def	9.15c	--	1.90bcd	1.48fgh	1.48ghi
Le 15	--	6.88cde	7.58bcd	8.20e	--	1.63cde	1.85cde	0.90l
Le 16	5.45d	6.45def	6.08efg	6.25k	1.40a	1.30e-h	1.23hij	1.08jkl
Le 17	--	5.83fgh	5.45g	9.05c	--	1.08ghi	1.08j	2.05bc
Le 18	--	2.48j	10.05a	10.80a	--	1.93bc	2.10bc	2.13b
Le 19	--	------	10.25a	9.78b	--	------	1.85cde	1.85cde
Le 20	6.68a	5.15hi	8.65b	8.18e	0.80c	1.40efg	2.05bcd	1.95bcd
Le 21	--	6.43def	7.05de	7.60fg	--	1.08ghi	1.75def	1.58fg
Le JR	--	6.05efg	7.03de	6.38jk	--	1.28fgh	1.35ghij	0.93kl
LSD	0.17	0.79	1.03	0.33	0.19	0.30	0.29	0.205
CV (%)	**4.78**	**8.80**	**10.14**	**3.10**	**12.40**	**14.52**	**13.23**	**9.96**

Yield (g): The yield was highly significant in different strains of shiitake mushroom in different seasons. Total 23 strains were grown in different season. Among the strains, maximum number of strain produces fruit body in winter and spring season and minimum in autumn season. Out of 23 strain only five strain produce fruit body in autumn season sixteen in late autumn and twenty two in winter as well as spring season. Most of the strains gave higher yield in winter season compare to other seasons while yield performance was poor in autumn season (Table 6). Yield of different strains of shiitake mushroom in autumn, late autumn, winter and spring season ranged from 42.25-145.80g, 18.75-175.50g, 191.00-40.50g and 39.50-163.50g, respectively. The highest yield (191.00g) obtained from the strain Le 8 in winter season and the lowest yield (18.75g) gave the strain Le 18 in late autumn season. Each strain gave different yield on season based. Le 1 gave highest yield (163.50g) in spring season while no yield (0.0 g) obtained in autumn season. Among the strains four strains were commercially cultivated round the year and yield variation was observed in four seasons. Le 8, Le 11 and Le 12 gave highest yield in winter season but Le 16 gave highest yield in late autumn. On the other hand Le 8 and Le 12 gave lowest yield in spring season as well as Le 11 and Le 16 gave lowest yield in autumn season.

Biological Efficiency (%): Differences in this parameter under those four seasons were highly significant. Biological efficiency was higher in winter season in maximum strain. Biological efficiency gradually increased with the decrease of temperature in winter season. The highest biological efficiency (109.10%) was obtained from the strain Le 8 grow in winter season and the lowest biological efficiency (10.72%) was obtained from the strain Le 18 in late autumn (Table 6).

Table 6: Yield performance and biological efficiency (%) of different strains of shiitake mushroom in different growing season

Strains	Yield(g)				Biological efficiency (%)			
	Autumn	L.Autumn	Winter	Spring	Autumn	L.Autumn	Winter	Spring
Le 1	-----	83.00g	105.50f	163.50a	--	47.43g	60.28f	93.43a
Le 2	-----	53.00h	151.00cd	80.00h	--	30.28h	85.78d	45.72h
Le 3	-----	57.75h	50.75j	146.00b	--	33.00h	29.00j	83.43b
Le 4	-----	88.75	120.00e	108.80e	--	50.72fg	68.57e	62.14e
Le 5	-----	82.75fg	122.50e	66.00i	--	47.28g	70.00e	37.71i
Le 5H		-----	50.00j	95.00f	--	-----	28.57j	54.28f
Le 6	-----	90.00g	115.50ef	64.00i	--	51.43fg	66.00ef	36.57i
Le 8	145.80a	172.00a	191.00a	123.80c	83.29a	98.28a	109.10a	70.71c
Le 9	-----	31.50i	88.25g	64.00i		18.00i	50.43g	36.57i
Le 10	-----	121.80bc	162.50bc	144.30b		69.57bc	92.85bc	82.43b
Le10H	-----	-----	40.50j	41.25k		-----	23.14j	23.57k
Le 11	97.25b	126.50b	151.80cd	120.00d	55.57b	72.28b	88.21cd	68.57d
Le 12	99.00b	110.30de	148.50d	94.00f	56.57b	63.00de	84.85d	53.71f
Le 13	-----	-----	----	-----	---	-----	-----	-----
Le 14	-----	125.50b	63.50i	97.75f	--	71.71b	36.29i	55.85f
Le 15	-----	107.50e	70.25hi	57.75j	--	61.43e	40.14hi	33.00j
Le 16	85.25c	175.50a	171.80b	110.00e	48.71c	100.30a	98.14b	62.85e
Le 17	-----	115.50cd	152.50cd	95.50f	--	66.00cd	87.14cd	54.57f
Le 18	-----	18.75j	86.50g	80.00h	--	10.72j	49.43g	45.72h
Le 19	-----	-----	83.75g	87.50g	--	-----	47.86g	50.00g
Le 20	45.25d	115.00cd	77.00gh	64.75i	24.15d	65.71cd	44.00gh	37.00i
Le 21	-----	96.00f	105.30f	39.50k	--	54.85f	60.14f	22.57k
Le JR	-----	90.00fg	88.50g	86.50g	--	51.43fg	50.57g	49.43g
LSD	4.97	6.99	11.48	3.65	2.84	3.99	6.14	2.08
CV (%)	**3.51**	**5.05**	**7.46**	**2.80**	**3.51**	**5.05**	**7.29**	**2.80**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT
----- = Not produce fruit body.

Results of the present experiment reveal that there are appreciable variations in growth and yield contributing attributes with the variation of season (temperature, humidity) and different strains of shiitake mushroom. In terms of yield and yield attributes *Le-8, Le-11, Le-12 and Le-16* produces fruit body round the year. Performance of the strain Le-1 was better in spring season than other seasons. Among the seasons autumn suitable for mycelium running late autumn, winter and spring suitable for cultivation. Many other

investigators also found variations in effect of temperature, moisture, strain on growth, yield and yield contributing characters of shiitake mushroom.

Many strains of shiitake mushroom are available in the world which is extensively cultivated. The strains of this valuable mushroom vary widely, particularly in the time required for mycelium colonization, bump formation and fruiting body development. The morphology and productivity of shiitake mushroom vary according to the strains based on the influence of environmental factors (Triratana andTantikanjana, 1987).

Under natural condition, fruiting occurs primarily in spring and autumn due to seasonal rains and temperature changes (Leatham, 1982). While, in forced fruiting, mushrooms can be produced more frequently and even during winter and summer by carefully managing temperature and humidity conditions (Anderson and Marcouiller, 1990).

Different strains are often categorized by fruiting temperature requirements. Shiitake from strains of cool season, wide range and warm season will generally fruit at log temperatures between 5 and 20^{0}C, 10 and 27^{0}C, and 10 and 30^{0}C, respectively. Productivity, appearance, mushroom size and length of time it takes to fruit will also differ as a result of different strain employed (Sabota, 1998). Whilst, requirement of LMC for fruiting is over 40%, however, higher LMC is favourable (Leatham, 1982). During formation of primordial fruit bodies, the environment is managed to ensure the right number of pins form and begin to grow out. Recommended moisture content of logs (LMC) varies at this stage of development. Komatsu and Tokimoto (1982 cited in Przybylowicz and Donoghue 1988) and Tokimoto *et al.* (1980) recommend a range of LMC from 35 to 65% with optimum LMC between 55 and 65%.

All shiitake strains show optimal mycelial growth at 25^{0}C (Chen, 2000). The duration of spawn run is usually 1-4 months depending on strains and methodology (Oei, 1996). There is an optimum temperature for mycelial growth above and below which growth is restricted (Przybylowicz and Donoghue, 1988; Miles and Chang, 1997). This may be due to the effect of temperature on enzyme activity and the resulting changes in rates of chemical processes (Miles and Chang, 1997).

Clumps of mycelia appear as blister- or popcorn-like bumps of various sizes on the surface of the mycelial coat in most strains. This usually begins when colonization of white mycelia covers the entire substrate in the bag, or sometimes earlier. Primordia are produced at the tips of some of these bumps. However, most bumps are aborted and never develop into fruiting bodies. Time of bump formation varies with strains, substrate and temperature. Usually bumps form 10 days faster at 25^{0}C than at 15^{0}C (Miles and Chang, 1989). Fluctuation of temperature and high CO_2 concentration encourage bump formation. Lower the CO_2 in the bag, when bumps become too numerous by cutting slits on the bag. In any case, some aeration should be provided when bumps are formed.

As the shiitake mycelium spread through a log it secretes exoenzymes that degrade the dead wood in order to obtain nutrients (Andrade *et al.* 2008). The production of

mushroom fruiting bodies (sporophores) starts when the logs are fully colonized. Under natural conditions heavy rains and an associated drop in temperature stimulates mushroom production (Shiomi *et al.*, 2007).

There is an optimum temperature for mycelial growth above and below which growth is restricted (Przybylowicz and Donoghue, 1988; Miles and Chang, 1997). This may be due to the effect of temperature on enzyme activity and the resulting changes in rates of chemical processes (Miles and Chang, 1997). Temperature also plays an important role in the initiation and development of fruiting bodies and a sudden shift in temperature may be required to induce fruiting of mushrooms (Komatsu, 1961, cited in Przybylowicz and Donoghue, 1988).

Sarker *et al.* (2009), observed that duration to complete mycelium running of different strains of shiitake mushroom on sawdust varied greatly. Mycelium growth rate (MGR) in spawn packet ranged from 0.23 to 0.35 cm/day among the strains. He also observed that shiitake mushrooms took 65.83 to 98.33 days for bump formation during incubation period. This result supports the findings of Kawai *et al.* (1997) who reported that 60 to 90 days is required for incubation period of shiitake mushroom. Sarker *et al.* (2009) also reported that among the strains, a wide variation was observed in the duration from inoculation to primordia initiation and first harvest. Days required for primordia initiation ranged 69.83-103.5 days and for first harvest ranged 78.67-111.2 days. The highest biological yield was found in Le-8 which was followed by Le-11, Le-12, Le-10 and Le-2. The lowest yield was recorded from Le-4, which was followed by Le-9, Le-5 and Le-6. The result is in line with the report of Przybylowicz and Donoghue (1990) who found the biological efficiency of shiitake mushroom to vary between 50 and 80% for 2-5 harvests. The strain Le-4 performed very poorly.

Temperature also affects mushroom shape of *Lentinula edodes* (Ohira *et al.*, 1982, cited in Przybylowicz and Donoghue, 1988). Mushrooms developed under higher temperatures tend to form long stems and thin cap, whereas those cultivated under cooler temperature have short stems and thick caps (Tokimoto and Komatsu, 1978).

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Bangladesh J. Mushroom. 8(2): 59-68. 2014 (December)

Taxonomic Study of Seven Wild Edible Mushrooms to the Genus *Agaricus* in Bangladesh

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ABSTRACT

A survey was conducted to collect mushrooms from ethnic habitat in Rangamati hilly area including forest, fields and homestead garden of tribal village during March 2012 to June2014. Hilly area of Rangamati is a harbours of diversified mycoflora in rainy summer to late autumn. Seven species of mushrooms belonging to family Agaricaceae viz., *Agaricus abruptibulbous A. augustus, A. arvensis, A. campestris, A. nivescense A. croceolutescens, A. haematosarcus are* taxonomically investigated. Among the species five species of *Agaricus* viz. *Agaricus abruptibulbous A. augustus, A. arvensis, A. campestris, A. nivescense* are consumed by ethnic people of Rangamati. The main characteristics of this genus are free gills, chocolate brown spores, the presence of a veil and a central stalk that separates easily from the cap. In this study detailed descriptions of these seven species provided along with microscopic details.

Key words: *Agaricus*, Agaricaceae, Taxonomy.

INTRODUCTION

Taxonomic study of macro fungi is essential to understand the biodiversity and conservation process. *Agaricus* is a genus of mushrooms containing both edible and non edible mushrooms, with possibly over 300 members worldwide (Bas 1991, Capelli 1984). Most species are non toxic (Calvano-Bado 2001). The genus includes the common button mushroom *Agaricus bisporus* the dominant cultivated mushrooms of the West and the field mushroom *Agaricus campestris*. Members of *Agaricus* are characterized by having a fleshy cap or pileus, from the underside of which grow a number of radiating plates or gills on which the necked spores are produced. They are distinguished from other members of their family, Agaricaceae, by their chocolate-brown spores. Members of *Agaricus* also have a stem or stipe, which elevates it above the object on which the mushroom grows, or substrate, and a partial veil, which protects the developing gills and later forms a ring or annulus on the stalk. For many years, members of the genus *Agaricus* were given the generic name *Psalliota*, and this can still be seen in older books on mushrooms. All proposals to conserve *Agaricus* against *Psalliota* or vice versa have so far been considered superfluous. The genus contains the most widely consumed and best-known mushroom today but in our country this mushroom is inadequate, expensive because low temperature and high humidity required for cultivation. Hilly area of Rangamati is a harbours of diversified mycoflora. So the present study was undertaken to identify and to select suitable species of the genius for commercial cultivation in Bangladesh.

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MATERIALS AND METHODS

Mushroom samples were collected from different location of Rangamati hilly area in Bangladesh. These mushrooms were photographed in their natural habitat and macroscopic details were recorded. The collections were studied for their macroscopic and microscopic details up to species level after reference to David Norman Pegler (1977). For working out color codes and terms were coded according to Ridgway (1912). Micromorphological features were recorded with the aid of a light microscope: Olympus CX 41 based on the dry samples mounted in a mixture of 3–5 % KOH and Congo Red, lactophenol, glycerin, cotton blue and Melzer's reagent. Measurements of basidiospores are recorded. Basidiospores are measured in side view. Specimens were deposited in the Mycological Herbarium of Mushroom Development Institute, Savar, Dhaka. Part of each collection is preserved in wet form in 4% formalin solution in glass jars and dry specimens in the Herbarium. Crystal of naphthalene balls have been used to protect dried specimens against insect infestations.

RESULTS AND DISCUSSION

Agaricus abruptibulbus:

Pileus: 4-10 cm diam., ovate when young, gradually convex to planoconvex; surface dry, with few silky scales, white, slowly becoming pale yellow then pale yellow to light yellow with maturity and / or after bruising; margin with veilar remnants. **Lamellulae:** free, edge wavy, white when young, gradually pinkish then dark brown with age, narrow, crowded, with lamellulae of three lengths; edge white, floccose. **Stipe:** 5-10 cm × 4–12 mm, mostly cylindric with an abruptly-bulbous base, concolorous with pileus or yellowish white to pale yellow after maturity, surface dry, annulus white, of double membrane, split into a star shape around the stipe. **Annulus:** superior, membranous, large, white, striate above, squamulose below. Context thick in pileus and pithy in stipe, white then slowly becoming pinkish, turning yellow with KOH and unchanging in FeSO4.**Odour:** has a flavor of anise. **Spore print:** White. **Basidiospores:** 5–6.3–7.6 × 3.5–3.6–4 µm, smooth, ellipsoid to oblong, slightly wider towards the hilum, one or two guttulate, dark brown to grey-brown at maturity; thick walled. **Basidia:** 14–18 × 5–6 µm, 4-spored, clavate; sterigmata 1–1.6 × 0.2 µm. Lamellar edge fertile, composed of basidia, Pleurocystidia, and marginal cell; cheilocystidia present but not clearly distinguished, clavate to subclavate; marginal cell 8–12 × 4–6 µm, cylindrical to narrowly clavate. **Subhymenium:** 14–17 µm thick, cellular, composed of irregularly swollen inamyloid cells. Hymenial trama hyphal, of parallel to interwoven pattern; hyphae septate, branched, to 8.5 µm wide. **Pileipellis:** thick, of erect to interwoven pattern; hyphae regularly septate, constricted at septa, branched, clamp connections absent. **Stipitipellis:** hyphal, regularly septate with rounded apex. Stipe trama thick, pseudoparenchymatous, divided into several chambers towards centre, cells irregular. **Etymology**: The specific epithet named due to continuous bulbous base. **Specimen examined**: Haza chhori, Rangamati, from dead jackfruit tree, ARMT 032, August 16, 2013.

Fig.1. *Agaricus abruptibulbus*. A. Basidiocarp B; Longitudinal section of Basidiocarp, C Basidia. D. Basidiospore; E. Pleurocystidia.

Agaricus abruptibulbus is one of the rare species found in the forested areas of Rangamati hill district and commonly known as 'gash owl' (wood mushroom). It is also reported from two states (Maharashtra and Uttarakhand) of India (Bilgrami *et al.*, 1991; Vishwakarma *et al.*, 2012). Taxonomically, it is placed under sect. *Arvenses* of the genus *Agaricus*. *Agaricus arvensis* Schaeff. and *A. sylvicola* (Vittad.) Peck resemble in macro- and micromorphological characters with this species. But, the earlier two differ from the latter one by their continuous bulbous base (without any margin) as stated by Miller and Miller (2006) and Bessette *et al.*, (1997). Moreover, *A. arvensis* distinguishes from the present species by its more robust (pileus 80–200 mm diam., stipe 50–200 × 10–30 mm) stature, larger (7–9.2 × 4.4–5.5 µm) spores, as after Miller and Miller (2006), and presence of clamp connections in hyphal elements of pileipellis (Wasser 2000), whereas, *A. sylvicola* has shorter (5–6.8 × 3.5–4.4 µm) spores, as after Miller and Miller (2006). In spite of having the morphological similarities, these three species are now phylogenetically well established with the aid of molecular taxonomy (Geml and Royse, 2002).

Similar species: *Agaricus silvicola* is very similar in appearance and also grows in woodlands, but it may be distinguished by the lack of an abruptly bulbous base. *Agaricus arvensis* has a more robust stature, lacks the bulbous base, and grows in grassy open areas like meadows and fields. It has larger spores than *A.abruptibulbus*.

***Agaricus augustus*:**

Pileus: 4-8.5 cm diam., fleshy, at first almost globose, soon convex then plane or slightly depressed, occasionally with a low, broad imbo; surface dry, soon breaking upto numerous, more or less concentric, small, sepia to connamon, fibrillose squamules on a pale background, remaining entire brown, up to 7 mm broad, moderately crowded with lamellulae of six lenghts; edge pale. The cap shape is hemispherical during the so-called button stage, and then expands, becoming convex and finally flat, with a diameter of up to 22 cm. The cap cuticle is dry, and densely covered with concentrically arranged, brown-coloured scales on a white to yellow background.The flesh is thick, firm and white and may discolour yellow when bruised. **Lamellae:** free, pale pinkish buff then vinaceous, finally blackish brown, up to 7 mm broad, modreately crowded with lamellulae of six

lenghts; edge pale. The gills are not attached to the stem — they are free. Immature specimens bear a delicate white partial veil with darker-coloured warts, extending from the stem to the cap periphery. **Stipe:** is clavate up to 20 cm tall and 4 cm thick. In mature specimens, the partial veil is torn and left behind as a pendulous ring adorning the stem. Above the ring, the stem is white to yellow and smooth. Below, it is covered with numerous small scales. Its flesh is thick, white and sometimes has a narrow central hollow. The stem base extends deeply into the substrate. **Annulus:** superior, membranus, persistant, up to 10 mm wide, floccose fibrillose below. **Odour:** is strong and nutty, of anise or almonds. **Taste:** sweet, **Spore print:** purple-brown. **Basidiopores:** 6.5–8.5 × 4.5–6.5 µm, ovoid to clavate, bearing four sterigmata up to 5 µm long. Lamella-edge sterile. Cheilocystedia abundant as chains of globose, ellipsoid or piriform elements, 6-30 × 5-18 µm thin walled hyaline or brownish; each chain comprising maturity. Pleurocystedia absent.

Fig. 2. ***Agaricus augustus.*** Left. Fresh basidiomata. Right. A. Carpophore B. Basida C. Basidiospore D. Cheilocystidia.

Hymenophoral trama: regular, hyaline, with thin walled hyphae, 3-7 µm diam., inflated to 22 µm diam. Subhymenial layer pseudoparenchymatous, 18-27 µm wide, pileal surface a repent, disrupted epicutis, 100- 180 µm thick, of radial hyphae, 3-12.5 µm diam., constricted at the septa, and containing a brown vacuolar pigment. All hyphae lacking clamp-connections. **Etymology:** The specific epithet *augustus* is thought by some to be a reference to the month of August, when this mushroom is likely to be found. **Specimens examined:** Horticulture Centre Rangamati, on humicolus soil, AJU 40, 18 August 2011.

Agaricus augustus was first named and described by the Swedish botanist / mycologist Elias Magnus Fries (1838), and the name has remained unchanged to this day.The taxonomic details of this specimen matches well with details given for A. *augustus* by Pegler 1977.

Agaricus arvensis:

Pileus: 6-21 cm broad, convex, expanding to plano-convex; margin incurved, decurved at maturity; surface smooth to finely scaled, cracking in dry weather; disc yellowish-buff, shading to a cream-colored margin, bruising yellow slowly, especially when young; flesh white, unchanging or yellowing slightly, thick, firm, but soft in age; odor and taste of anise when fresh. **Lamellae:** free from the stem; crowded; whitish at first, becoming

brown (without a pink stage). **Stipe:** 4-14 cm tall, 1-3.5 cm thick, equal to tapering to an enlarged base, stuffed; surface smooth at the apex, white to cream, sometimes with scattered scales below, occasionally yellowing slowly; veil membranous, upper surface smooth, lower surface, with cream to buff, cottony patches arranged in a gear-tooth pattern, forming a membranous, superior, **Annulus:** skirt-like. **Flesh:** thick and white throughout; not changing color when exposed, or yellowing slightly; flesh in stem base not yellowing. **Odor and Taste:** odor sweet (reminiscent of anise or almonds) when young and fresh, becoming less distinctive; taste pleasant. **Chemical Reactions:** cap yellow with KOH. **Spore Print:** Dark brown. **Basidiospores:** 6.5-9.0 X 4.5-6 µm, elliptical, smooth. **Basidia:** 14-16 × 4.5-6 µm, clavate, bearing four sterigmata up to 2.5 µm long. Lamella-edge sterile, with crowded cheilocystida. **Cheilocystidia:** 17-30 × 12-16 µm, piriform to inflated clavate, hyaline, thin-walled. Pleurocystedia absent. **Hymenophoral trama:** regular, fairly narrow, with thin-walled hyphae, 2-8 µm diam. **Subhymenial layer:** pseudoparenchymatous. Pileal surface a disrupted, repent epicutis of chains of hyaline, thin-walled elements, 27-50 × 2-9 µm, often with reddish brown, homogenous vacular pigment, and a fine grandular, encrusting pigment. All hyphae lacking clamp-connections. **Ecology:** saprobic; growing alone, scattered, or gregariously in grassy places, lawns, fields, and so on; summer and fall; widely distributed in study area.

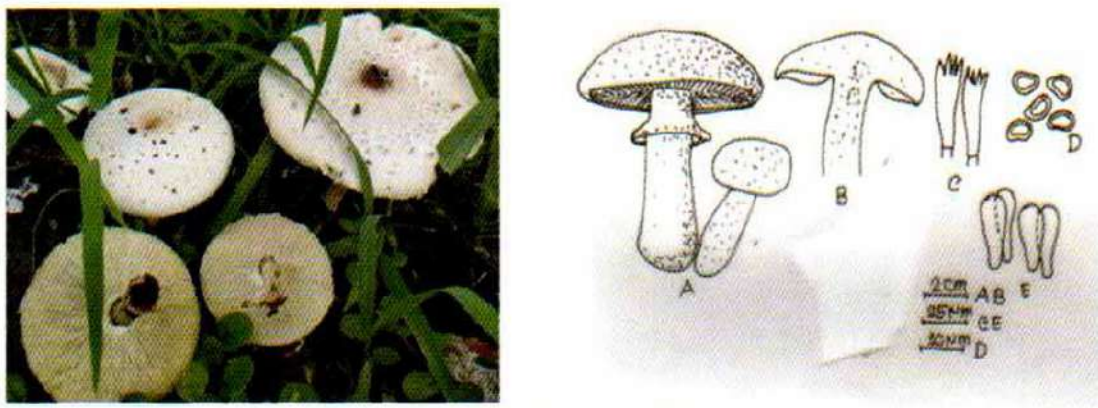

Fig. 3. *Agaricus arvensis*. Left. Fresh basidiomata. Right. A. Carpophore; B Basida.. C. Basidiospore; D. Cheilocystidia.

Specimens examined: Rangamati, Bangladesh, on humicolus soil in open pasture, AJU 41, 18 August 2011.

Agaricus arvensis is a cosmopolitan mushroom commonly known as Horse Mushroom. This is a impressive mushroom, recognized by its preference for grassy areas, its cap colors (pale yellow to whitish, often with pressed-down fibers), its sweetish smell, and its "cogwheeled" ring. The cap will often bruise yellow if rubbed, and the flesh will sometimes turn yellowish on exposure to air--but the flesh in the base of the stem is *not* yellow, which helps distinguish it from other species, it often grows in fields, and sometimes fields have horses in them. The taxonomic details of this specimen matches well with details given for *A. arvensis* as key feature given for this species by Pegler (1977).

Agaricus campestris *L.*

Pileus: 4 to 10 cm in diameter, globose then convexo-campanulate, often broadly umbonate, sometimes slightly depressed, fleshy; surface pure white, discolouring dull ochraceous or buff in dry conditions, particularly at thr disk, smooth and glabrous, at times cracking to appear subsquamulose; margin incurved, appendiculate with velar remnants. Usually the margin remains down-turned or slightly in-rolled even when the cap has expanded fully. **Lamellae:** free from the stipe, deep pink at first, ventricose,

Fig. 4. *Agaricus campestris.* Left. Fresh basidiomatsa, Right. A. Carpophore; B. Basida; C. Basidiospore.

up to 8 mm broad, the free crowded gills turn dark chocolate brown and eventually almost black as the fruitbody matures, covered with a thin white partial veil when in the button stage. **Flesh:** thick and white throughout; not bruising yellow anywhere, even in the base of the stipe; very rarely discoloring a pinkish wine color in wet weather. It's quickly collapsing white ring, and the fact that it does not discolor yellow when bruised. **Stipe:** 4 to 8 cm × 7-15 mm, cylindric or attenuated below, solid; surface smooth, white, slightly discolouring yellowish to the touch. **Annulus:** superior, membranus, white, friable. Context up to 10 mm thick at the disk, fleshy, white, reddening slightly on exposer, of tightly interwoven, thin-walled hyphae, 1.5-5 µm diam., inflated to 20 µm diam. **Spore print:** deep chocolate brown. **Basidiospores:** 6.8-8 x 3.8-5(7.5 × 4.5) µm, ovoid to 23-26 × 6-7 µm, narrowly clavate, bearing four sterigmata up to 5 µm long. Lamellae-edge fertile, cheilocystedia absent. Pleurocystedia absent. **Hymenophoral trama:** regular, pale yellowish brown, with thin-walled hyphae, 2.5-9 µm diam., Subhymenial layer indistinct, 3.5-4 µm wide. Pileal surface a repent epicutis of radial, thin-walled hyphae, 3-9 µm diam. All hyphae lacking clamp connections. **Odor and Taste:** pleasant. **Chemical Reactions:** cap surface not yellowing with KOH. **Habit and Habitat:** saprobic; growing alone, gregariously, or sometimes in fairy rings, in meadows, fields, lawns, and grassy areas; late fall to early winter. it comes up in meadows, fields, and grassy areas, after rains. It is recognized by its habitat, its pink gills (covered up by a thin white membrane when the mushroom is young) which become chocolate brown as the fruit body matures.

Etymology: *campestris*, From Latin campestre, an adjective. Definitions: level, even, flat, of level field; on open plain/field; plain-dwelling. The specific epithet *campestris*, given to this mushroom by Carl Linnaeus in 1753 and unchanged to this day, comes from the Latin word for a field. In the USA this common edible fungus, which is in fact the 'type

species' of the genus *Agaricus*, is more often referred to as the Meadow Mushroom. Gilled mushrooms are often referred to as 'agarics', and in the early days of fungal taxonomy most gilled mushrooms were simply included in one gigantic genus, *Agaricus*. **Specimens examined**: Rangamati, Bangladesh, on humicolus soil, in horticulture Centre, AJU 42, 19 August 2011.

Agaricus nivescens:

Pileus: 4-16 cm diam., globose to convex then applanate or apically truncate; surface white, ochraceous at the centre, smooth and glabrous; margin appendiculate with bright ochraceous squamules. Lamellae free, white then pink, finally dark purplish brown, up to 10 mm wide, crowded with lamellulae of four lenghts. **Stipe:** 6-15 cm × 1.5-2.5 cm, obclavate or short-cylindric with a swollen base up to 3 cm diam., fistulose, surface white, staining ochraceous when touched, glabrous.

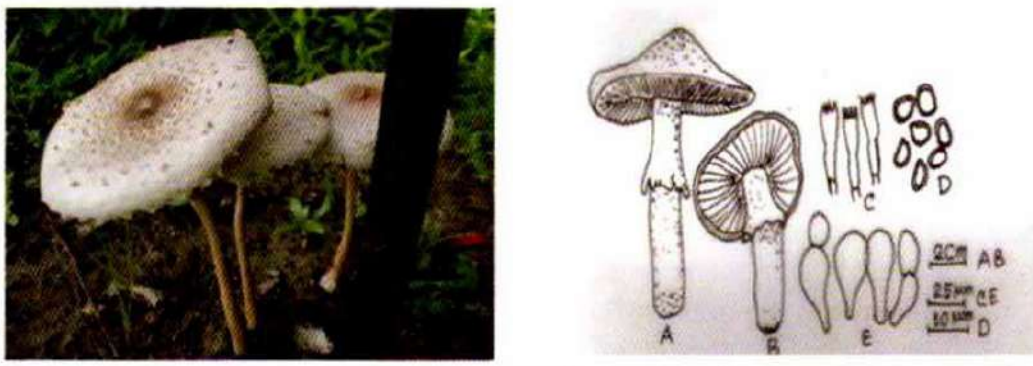

Fig. 5: *Agaricus nivescens*, A. Basodiocarp B. Longitudinal section of Basidiocarp C. Basidia D. Basodiospore E. Cheilocystidia.

Annulus: superior, membranous, white above, with yelloish orange squamules on the underside. Context thick but very thin at the margin, white, consisting of densly interwoven, thin-walled hyphae, 2-6 um diam.**Odour:** plesant. **Basidiospores:** 5.7-3.8-5.3 um, ellipsoid, fuscus, with a fairly thick wall and homogenous contents. **Basidia:** clavate, bearing four sterigmata. Lamella-edge sterile, cheilocytidia crowded but detersile and sometimes difficult to find. Cheilocystidia ovoid or forming short irregular chains, thin-walled, hyaline or brownish. Pleurocystedia absen. **Hymenial trama:** regular, hyaline. Subhymenial layer pseudoparenchymatous. Pileal surface an undifferentiated epicutis of radial hyphae, hyaline, thin-walled, not or only slightly constricted at the septa. All hyphae lacking clamp-connexions. **Specimens examined:** Rangamati Hill tracts, growing in roadside. ARMT 029, August 11, 2012. The taxonomic details of this specimen matches well with details given for *A. nivescens* as key features given for this species by Pegler (1977).

***Agaricus croceolutescens*:**

Pileus: 4-15 cm diam., subglobose to campanulate, truncate at the apex, finally applanate; surface pale raddish brown, darker at the centre, disrupting in adpressed fibrillose squamules on a whitish background; becoming yellowish with age or on drying; context white, becoming yellowish, thick, highly flavored and easily digested, with an agree-able odor. **Lamellae:** free, crowded, broad, at first pallid, becoming slowly grayish-pink, and

finally blackish-brown; spores very broadly ellipsoid, smooth, pale-purplish-brown under the micro-scope, cocoa-brown, thin, ventricose, up to 10 mmbroad. Stipe 3-10 × 1-2 cm, cylindric or obclavate with a slightly swollen base, solid then hollow; surface whitish with ochraceous tints, yellowing on bruising. **Annulus:**of two parts, membranous, persistent, white becoming yellowish when bruised. **Context:** white, discolouring yellow, thick, made up of tightly interwoven hyphae, 2-6 µm diam., inflated to 15 µm diam. Spore 3.5-4.5 × 5.7-7.2 µm, ellipsoid, bistre, with a thickened wall. **Basidia:** 6-7.5 × 20-25 µm, clavate, bearing four sterigmata up to 4 µm long. Lamellae-edge catenulate, thin walled hyaline. Pleurocystidia absent. **Hymenophoral trama:** regular, hyaline, with hyphae 2-6 µm diam. Subhymenial layer pseudoparenchymatous, 7-8 µm wide. Pileal surface a disrupted, repent epicutis comprising chains of cylindric elements, 4-13 × 40-120 µm, thin walled, hyaline or with a brownish vacuolar pigment, All hyphae lacking clamp connections.

Habitat: in rich soil in pastures, fields, and wood borders. This mushroom is commonly found in non cultivated grassy area, in gardens and by roadside verges. The fungus is saprotrophic and terrestrial — it acquires nutrients from decaying dead organic matter and its fruiting bodies occur on humus-rich soil. The species seems adapted to thriving near human activity. In Rangamati, *A. croceolutecens* fruits in rainy summer to autumn. **Etymology:** from latin *croceus* – saffron-coloured, yellow, and *lutescens* – turning yellow, yellowing, yellowish.The specific epithet *croceolutecens* is thought by some to be a reference to the month of August, when this mushroom is likely to be found. **Specimens examined:** Rangamati, Bangladesh, on humicolus soil, in horticulture Centre, AJU 43,18 August 2011.

Fig. 6. *Agaricus croceolutescens* Heinemans & Goss. Left. Fresh basidiomata. Right. A. Carpophore; B. Basida. C. Basidiospore; D. Cheilocystidia

The taxonomic details of this specimen matches well with details given for *A. croceolutecens* by Pegler (1977).

***Agaricus haematosarcus*:**
Pileus: 4-8 cm diam., subglobose thin convex, finally applanate, surface grayish white becoming sordid ochraceous, covered by a cottony layer of velar flocculate; margin appendiculate-floccose. **Lamellae:** free, pink then dark reddish brown, up to 7 mm broad, moderately crowded, with numerous lamellulae. **Stipe:** 6-10 cm × 5-14 mm, cylindric,

hollow, surface whitish to ochraceous, coated below the annulus by a uniform layer of cottony, velar squamules. **Annulus:** superior, fibrillose-floccose, mostly composed of tissue of universal veil, white. Context up to 8 mm thick at the disk, white, immediately reddening on exposure, of loosely interwoven, thin-walled hyphae. **Odour and taste:** not done. Basidiospores 6.5-8.5 × 4.5-5.5 um, ovoid to short ellipsoid, dark fuscous brown, with a thickened wall and irregularly guttulate contents. Basidia 15-17 × 6-8 um, clavate to subcylindric, bearing four short sterigmata. Lamella-edge sterile. Cheilocystedia not found, Pleurocystidia absent. **Hymenophoral trama** regular, hyaline, with thin-walled hyphae 2-5 um wide. Pileal surface a repent epicutis, underlying the universal veil, consisting of radially parallel hyphae. All hyphae lacking clamp-connections. **Specimens examined:** Rangamati Hill tracts, growing in vegetable garden rich in organic matters. ARMT 031, August 10, 2012.

Fig. 7. *Agaricus haematosarcus* Heinem. & Gooss A. Basidiocarp B. Longitudinal section of Basidiocarp C. Basidia D. Basidiospore.

The taxonomic details of this specimen matches well with details given for *A. haematosarcus* as key features given for this species by Pegler (1977).

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Bangladesh J. Mushroom. 8(2): 69-75. 2014 (December)

Influence of *Pleurotus ostreatus* on Kidney Function of Females Suffering from Hypertension

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Abstract

Kidneys are vital organs that filter impurities out of the blood stream; the present study was carried out to evaluate the effect of *Pleurotus ostreatus* on the kidney function of hypertensive females. The study was conducted in the National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar Dhaka. A total 16 subjects free from diabetes were included in the study. 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 change of plasma urea (25.96 ± 3.13 and 32.75 ± 2.60, p = 0.095), creatinine (0.85 ± 0.05 and 0.94 ± 0.07, p = 0.092) and uric acid (4.63 ± 0.35 and 4.57 ± 0.35, p = 0.896). Findings of the study suggest that *Pleurotus ostreatus* mushroom has no harmful effect on renal function of hypertensive females.

Key words: *Pleurotus ostreatus*, Creatinine, Urea, Uric acid.

INTRODUCTION

Mushrooms are tremendous food supplements which comprise a wide range of fungal world. They have been in use not only for consumption, but also for medicinal purposes, since ages. They contain a wide variety of bioactive molecules including terpenoids, steroids, phenols, nucleotides and their derivates, glycoproteins, and polysaccharides (Borchers *et al.*, 1999; Mizuno *et al.*, 1995).

Oyster mushroom (*Pleurotus ostreatus*) are easy to cultivate and common all over the world. It was 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. *Pleurotus ostreatus* has proven positive effects on the human body and spirit.

Pleurotus ostreatus 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

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be richer in another. However, they are generally similar to each other in special food values. 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).

Pleurotus ostreatus 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. It 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 *Pleurotus ostreatus* 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).

Kidneys are the vital organs that filter impurities from the blood, which body then flushes out though the excretory system. Protein is an essential nutrient but its metabolic waste products are harmful for the body. 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, hypertension makes the heart work harder and, over time, can damage blood vessels throughout the body. If renal blood vessels are damaged, they may stop excretion of waste products and extra fluid from the body which ultimately may cause rising of blood pressure and makes the body toxic. Current study was to investigate whether or not the oyster mushroom causes harmful effect on kidney function of females suffering from hypertension.

MATERIALS AND METHODS

The study was conducted in the laboratory of National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka. In this study a total of 16 hypertensive female volunteers (systolic BP $\geq$ 140 mmHg and/or diastolic BP $\geq$ 90 mmHg) free from diabetes (fasting plasma glucose < 7 mmol/l) were included. The age ranges of the subject were 26 to 63 years. After getting the written consent the subjects were included. Persons suffering from renal impairment and other known acute or chronic diseases as well as history of addiction were excluded in the study. If any drugs previously getting by the subjects, they were allowed to continue the medication.

Information including age, sex, occupation, educational status, marital status, family history and drug history were recorded in a preformed data collection form.

At the beginning of the study subjects were evaluated for health status. Using sphygmomanometer by a trained physician both systolic and diastolic blood pressure was measured following standard procedure. 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.

Fresh fruiting bodies of *Pleurotus ostreatus* were harvested from culture house of National Mushroom Development and Extension Centre (NAMDEC). They 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. 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.

Collected data were analyzed using software Statistical Program for Social Science (SPSS), versin 17. 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

Mushrooms of *Pleurotus* species is one of the most famous edible mushroom. They have lot of beneficial effect on health. But it is questionable, whether it has any harmful effect on kidney. The current study was conducted to observe the effect of *Pleurotus ostreatus* on the renal function of hypertensive females.

Adult female subjects suffering from hypertension and free from diabetes were included in the study. The mean age (years) of the subjects were 41.3 ± 2.89, ranges from 26 to 63. The mean fasting plasma glucose (mmol/l) was 5.69 ± 0.65 ranges from 4.1 to 6.8. The mean of systolic and diastolic blood pressure (mmHg) were 140.93 ± 4.33 and 89.06 ± 2.42 ranges from 110 to 170 and 70 to 110 respectively (Table 1).

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

Parameter	N	Mean (± SE)	Range
Age (years)	16	41.3 ± 2.89	26 to 63
FPG (mmol/l)	16	5.69 ± 0.65	4.1 to 6.8
Systolic BP (mmHg)	16	140.93 ± 4.33	110 to 170
Diastolic BP (mmHg)	16	89.06 ± 2.42	70 to 110

It was observed in the study that the mean ± SE plasma urea before and 3 months after mushroom supplement was 25.96 ± 3.13 and 32.75 ± 2.60 respectively (Fig. 1). There was no statistically significant mean difference of plasma urea (p = 0.095) between the two periods.

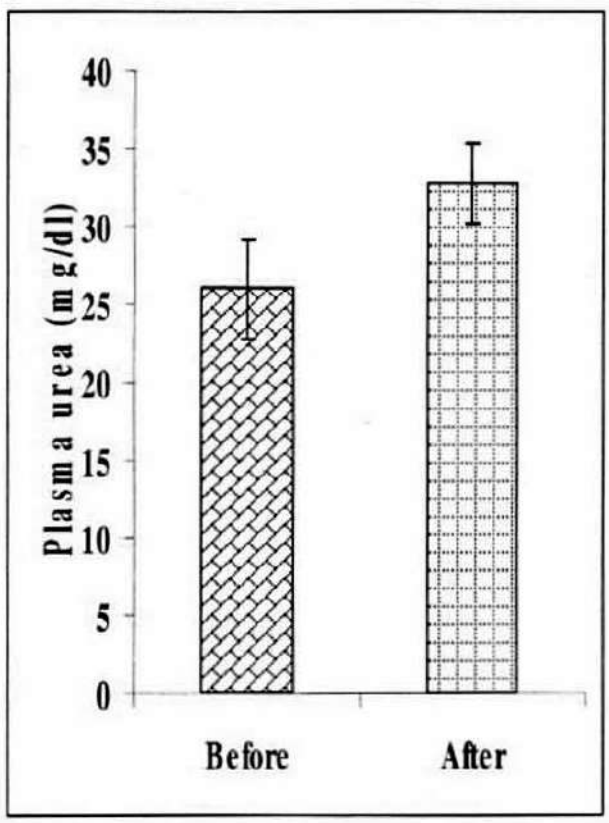

Fig. 1. Mean (± SE) plasma urea before and 3 months after mushroom supplementation.

Considering plasma cretinine, the mean ± SE (mg/dl) before and three months after mushroom treatment was 0.85 ± 0.05 and 0.94 ± 0.07 respectively. Here also a non-significant mean difference (p = 0.092) was observed (Fig. 2).

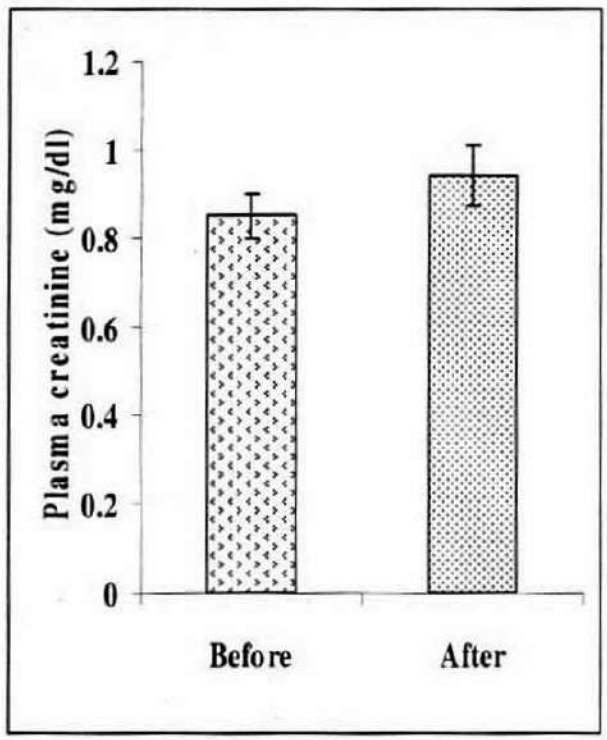

Fig. 2. Mean (± SE) plasma creatinine before and 3 months after mushroom supplementation.

The mean ± SE plasma uric acid (mg/dl) before and 3 months after mushroom treatment were 4.63 ± 0.35 and 4.57 ± 0.35 respectively. No statistically significant mean different was observed ($p = 0.896$), between the two periods (Fig. 3).

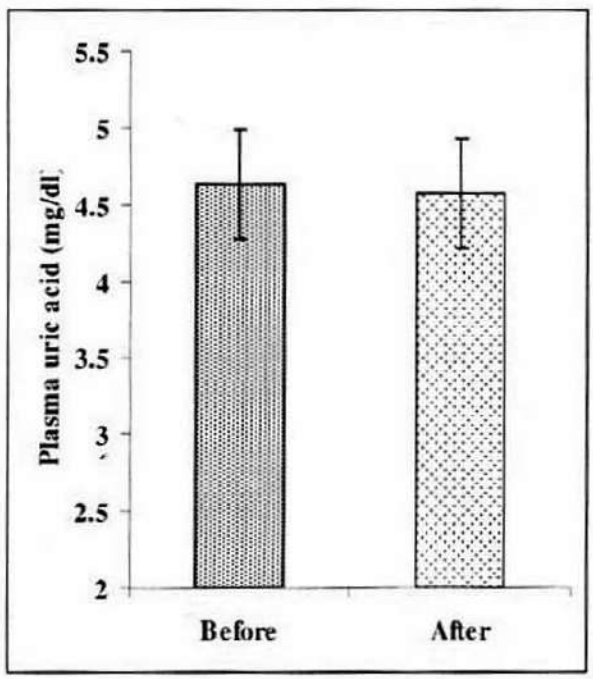

Fig. 3. Mean (± SE) plasma uric acid before and 3 months after mushroom supplementation.

This study showed that there is no significant change of plasma urea, creatinine and uric acid after three months treatment of mushroom capsule. 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.

It is now established that *Pleurotus ostreatus* is rich in high quality protein and this value is 19 to 39 gm in 100 gm dried matter (Breene, 1990). Urea and creatinine are protein metabolites. So, these two parameters can rise within normal range in increasing protein intake. This study showed there is non-significant small elevation of urea and creatinine level within physiological limit in the blood, which don't indicative of renal impairment. On the other hand, non significant small reduction 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.

The previous study shows *Pleurotus ostreatus* had non significant changes of palsma urea, creatinine and uric acid of hypertensive diabetic and non diabetic male volunteers (Choudhury *et al.*, 2011 and Choudhury *et al.*, 2013). Current study is fully agreement with the previous observations and it is applicable for females also. 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). Current findings are supported by all of these findings.

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Bangladesh J. Mushroom. 8(2): 77-81. 2014 (December)

Yield Performance of Different Reishi Mushroom Strains Available in Bangladesh

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Abstract

The study was conducted to compare growth, yield and yield attribute of different reishi mushroom (*Ganoderma lucidum*) strains at Mushroom Development Institute, Savar, Dhaka, during the period of March to July 2014. Variations of different parameters related to yield and yield attributes were recorded. The lowest period (16 days) required to complete mycelium running was observed in Gl 8 and the highest (31.99 days) in Gl 1. Minimum time requirement (days) after opening of spawn packet for development of different stages of the mushroom were, primordia 4.58, antler 9.33, conk 14.00, mature first harvesting 29.00 in Gl 7, and maximum were observed in Gl 2 which were 21.67, 28,50, 36,57, 46.17 respectively (in 500g packet of substrate. Among the strains the highest (number) fruiting body 3.25 and yield (g) 29.25 was obtained from Gl 9. And the lowest number (1) was obtained from Gl 8 but lowest yield (12.25) from Gl 1.

Key words: Growth, Strain, Yield, *Ganoderma.*

INTRODUCTION

Ganoderma lucidum is one of the most famous traditional Chinese medicinal herbs, is used as a healthy food and medicine in Far East for more than 2000 years (Fang and Zhong, 2002). Lingzhi or Reishi contains various chemical substances, including more than 119 different types of triterpenes and several types of polysaccharides (Hsieh and Yang, 2004). All parts of reishi mushroom, such as spore, mycelium and basidiocarp are used for health purpose and always prepared either as hot water extracts, concentrates or in powdered form (Smith, *et al.,* 2002). The production of wild *Ganoderma* is inadequate, also it is unsafe to consume as insects and snakes can make the mushroom poisonous; thus its cultivation is essential to meet the demand. In this connection, the techniques of cultivation have been developed and described during the last 15-20 years. In Bangladesh, Reishi mushroom cultivation is getting popularity and 9 strains of the mushroom are being cultivated for about 5-10 years. But all the strains are not performing well in respect of growth parameters and yield. So present study was undertaken to determine the growth and yield performance of the strains to make a suggestion for the mushroom growers.

MATERIALS AND METHODS

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This experiment in completely randomized design with 4 replications was conducted in the culture house of Mushroom Development Institute (MDI), Savar, Dhaka during the period from March to July 2014. Nine reishi mushroom strains such as GI 1, GI 2, GI 3, GI 4, GI 5, GI 6, GI 7, GI 8 and GI 9 were used in this study. The inocula were collected of the germplasm centre of MDI.

Spawn Packet Preparation: The substrates of spawn packets were prepared by using mixed sawdust and wheat bran at the ratio of 2:1. Water was added to make the moisture level about 65% and $CaCO_3$ was added at 0.2% (w/w) of the mixture. All the materials were mixed thoroughly. Polypropylene bags (18 cm × 25 cm) were filled with 500 g of substrate mixture. A neck of the bag was made using heat resistant plastic tube. A hole of about 2/3 deep of the volume of the bag was made at the centre of the bag with a sharp end stick. The neck of the bag was plugged with cotton and covered with a brown paper. The spawn packets were autoclaved at 121^0C and 1.1 kg/cm^2 pressure for 2 hours. After autoclaving and cooling, the packets were inoculated separately with the mother culture of above mentioned tested strains at one teaspoonful per packet. The mother culture carefully poured into the hole of the spawn packet. Then the packets were incubated in the incubation room for mycelial growth.

Opening of the Spawn Packets: After completion of mycelium running, spawn packets were opened following side opening method. In side opening methods, square sized (1cm×1cm) opening was cut on the single side at the middle abdomen of the spawn packets. Then the packets were placed in rack for cultivation.

Experimental Condition: During incubation, the inoculated packets were kept in almost dark at about 25^0C temperature and transferred to the culture room at 28-32^0C temperature, 85-95% relative humidity and 80-200 lux light. Water was sprayed 3-5 times per day and proper aeration was maintained in culture house to facilitate the development of the fruiting bodies. The mushroom was harvested from single flush in the harvest period. The biological efficiency was determined for each packet using the following formula:

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

Data Collection and Data Analysis: Data on days of mycelial completion, days required from opening to primordial stage, antler stage, conk formation, days required from opening to first harvest, number of fruiting bodies, length and diameter of stalk, diameter and thickness of pileus, yield of mushroom (g/packet) were recorded. Biological efficiency of the substrates was computed using standard method. Collected data were analyzed following Gomez and Gomez (1984) using MSTAT-C computer program. Means separation were done following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULT AND DISCUSSION

Completion of mycelium (days): Significant variation was observed in duration of mycelia completion in different strains of reishi mushroom (Table 1). Maximum days (31.99) required for the completion of mycelium running was observed in Gl 1 followed by Gl 9. Minimum days (16.00) required for completion of mycelium running was observed in Gl 8. This result is supported by the study of same author (Kakon, *et al.*, 2009) who observed the lowest mycelial growth rate in Gl 1, Gl 2 and Gl 3.

Days from opening to primordial stage: Considerable variation was found from opening to primordial stage (4.58- 21.67 days). The minimum duration (4.58 days) was required in Gl 7 which was statistically different to other strains. The maximum duration (21.67 days) was required from opening to primordia initiation stage in Gl 2 which was followed by Gl 3, Gl 4, Gl 5 and Gl 8 (Table 1).

Days required to antler initiation: Considerable variation was found days required to antler initiation among different strains of reishi mushroom and ranged from 9.33- 28.50 days. The minimum duration (9.33 days) was required in Gl 7 which was statistically different to other strains. The maximum duration (28.50 days) was required from opening to antler initiation in Gl 2 which was followed by Gl 8, and Gl 1 (Table 1). This result is supported by Kakon *et al.* (2009) who observed that maximum days required from opening to antler initiation was in Gl 1 and Gl 2.

Table 1. Comparison of growth parameters among nine different strains of reishi mushroom

Strains	Days of mycelial Completion	Days from opening to primordial stage	Days from opening to antler stage	Days from opening to conk formation	Days from opening to first harvest
Gl1	31.99a	9.00c	16.57c	25.75c	41.00c
Gl2	28.00bc	21.67a	28.50a	36.57a	46.17a
Gl3	21.00d	10.32b	13.82e	18.33 f	45.50a
Gl4	27.00c	11.00b	15.17d	19.25e	38.75d
Gl5	21.00d	10.50b	14.33de	18.50ef	43.25b
Gl6	21.00d	9.00c	11.92 f	14.67g	36.83e
Gl7	18.00e	4.58e	9.33g	14.00g	29.00g
Gl8	16.00f	11.00b	19.00b	29.00b	46.00a
Red	29.00b	6.00d	14.00e	24.00d	35.00f
CV (%)	**3.83**	**3.31**	**4.26**	**3.60**	**2.33**

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

Days required to conk formation: Considerable variation was found days required to conk formation among different strains of reishi mushroom and ranged from 14.00 - 36.57 days. The minimum duration (14.00 days) was required in Gl 7 which was statistically similar to Gl 6. The maximum duration (36.57 days) was required from opening to conk formation in Gl 2 which was followed by Gl 8 and Gl 1 (Table 1). Similar result observed by Kakon *et al.* (2009) who observed that maximum days required from opening to conk formation was in Gl 1 and Gl 2.

Days from opening to first harvest: Significantly the minimum duration (29.00 days) was required from opening to first harvest in Gl 7 followed by red mushroom. The maximum of 46.17 days were required from opening to first harvest in Gl 2 which was statistically similar to Gl 3 and Gl 8 (Table 1). Slow primordial initiation increases harvesting period.

Number of fruiting body: The highest number (4.00) of fruiting body per packet was harvested from red mushroom which was statistically similar to the strain Gl 6. The lowest number (1) of fruiting bodies per packet was harvested from Gl 8 (Table 2).

Length and diameter of stalk: The highest length of stalk (3.67cm) was recorded in Gl 6 while the highest (0.98 cm) diameter of stalk in Gl 2. Significantly the lowest length and diameter of stalk (0.0cm) were recorded from Gl 8 (Table 2).

Table 2. Comparison of yield and yield contributing characters among nine different strains of reishi mushroom

Strains	Number of fruiting body (NFB)	Length of stem (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	yield (g) (1st flush)	Biological efficiency (%)
Gl_1	2.75b	1.78f	0.74c	3.63d	0.68cd	12.25e	7.0
Gl_2	1.58e	2.23de	0.98a	4.96b	0.76c	14.92d	8.52
Gl_3	2.00c	2.28d	0.89b	5.00b	0.78c	17.58c	10.04
Gl_4	1.83d	2.66c	0.85b	5.17b	0.95b	19.67b	11.24
Gl_5	1.58e	1.90f	0.78c	5.17b	0.72cd	15.75d	9.01
Gl_6	3.25a	3.67a	0.63e	4.21c	0.49e	19.33b	11.04
Gl_7	1.33f	2.12e	0.73c	6.79a	0.65d	18.50bc	10.57
Gl_8	1.00g	0.00g	0.00f	5.25b	1.95a	18.75bc	10.71
Red	3.25a	2.95b	0.68d	5.00b	0.55e	29.25a	16.71
CV (%)	**6.16**	**4.17**	**5.03**	**2.55**	**3.41**	**2.81**	**2.71**

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

Diameter and thickness of pileus: The highest pileus diameter (6.79cm) was found in Gl 7 and the lowest diameter (3.63cm) was found in Gl 1. The highest (1.95 cm) and lowest (0.49cm) thickness of pelius was recorded in Gl 8 and Gl 6 respectively (Table 2).

Yield(g): The highest yield of 29.25 g/ packet (fresh weight) of mushroom was recorded from red mushroom which was statistically different to other strains. The lowest yield of 12.25 g mushroom per packet was obtained from Gl 1 (Table 2).

Biological efficiency (BE): The highest biological efficiency (16.71 %) was recorded from red mushroom and the lowest biological efficiency (7.0%) was obtained from Gl 1 (Table 2).

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Bangladesh Journal of Mushroom

Volume 8 **Number 2** **December 2014**

Contents