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Editor-in-Chief
Bangladesh Journal of Mushroom
and
Programme Director
Mushroom Development & Extension Programme
National Mushroom Development and Extension Centre
Sobhanbag, Savar, Dhaka
E-mail: bjm_namdec07@yahoo.com
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Bangladesh J. Mushroom. 8(1): 1-6. 2014 (June)

Yield Performance of Monkey Head Mushroom on Sawdust

Nirod Chandra Sarker, Akhter Jahan Kakon, Md. Bazlul Karim Choudhury[1], Khan Mohammad Shamsuzzaman, Bimal Chandra Dey and Tahera Binte Mujib
National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

Hericium erinaceus a Chinese edible and medicinal mushroom was grown under local conditions in Bangladesh using the available lignocellulosic wastes as growing media. Different growth and yield parameters of two strains of this mushroom were determined in winter seasons. Also, two amount of substrates were used to estimate the yield. The incubation time for the tested strain and amount of substrates ranged from 21 to 45 days. The highest fresh (128.00g/packet) and dry (13.17g/packet) yield as well as biological efficiency (69.18%) were found in He 2 and 300g substrate. The lowest fresh (64.50g)) and dry (6.64g/packet) yield as well as biological efficiency (34.86%) were obtained when He 1 cultivated on 500g sawdust. Cultivation of *H. erinaceus* in Bangladesh is a very important achievement, since this mushroom type is highly prized for their nutritive and medicinal benefits.

Key words: Sawdust, Yield, Growth, Edible.

INTRODUCTION

Hericium erinaceus is an edible mushroom with medicinal values, which is also known as Lion's Mane Mushroom or Hedgehog Mushroom. This mushroom is a basidiomycete fungus taxonomically belonging to the family Hericiaceae, order Hericiales, division Homobasidiomycetes, class Hymenomycetes (Ko *et al.*, 2005). Its fruiting body is called houtou in Chinese due to its shape resembling monkey's head, and has been used as an edible and medicinal fungus in China and other oriental countries and areas for many years (Jia *et al.*, 2004). This mushroom is rich in some physiologically important components, especially β-glucan polysaccharides, which are responsible for anti-cancer, immuno-modulating, hypolipidemic, antioxidant and neuro-protective activities. *H. erinaceus* has also been reported to have anti-microbial, anti-hypertensive, antidiabetic, wound healing properties among other therapeutic potentials. Eisenhut and Fritz (1995) stated that, sawdust proved an effective, economic substrate for *H. erinaceus* growing. The present study was designed to evaluate the growth and yield performance of this mushroom under Bangladesh condition.

MATERIALS AND METHODS

The experiment was conducted in tissue culture laboratory and the culture house of National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka,

[1] Department of Biochemistry, Faridpur Medical College, Faridpur, Bangladesh.

Bangladesh from October to February 2014. In this experiment the treatments which consisted of two packets size i.e. amount of substrate (300g and 500g sawdust) and two strains (He 1and He 2) for the cultivation of monkey head mushroom.

Preparation of mother culture: To prepare mother culture of the test mushroom strain sawdust and wheat bran were mixed together at 2:1 (v/v) and supplemented with $CaCO_3$ at 0.2% (w/w) of the mixture. The moisture level of the mixed substrate was maintained at 65% with tap water. The substrate was poured into polypropylene bags (18 cm× 25 cm) at 300g/bag. The substrate in bags was sterilized in an autoclave for 1 h at 121^0C under 1.1 kg/cm^2 pressures and allowed to cool for 24h. Pure cultures of two strains were grown on potato dextrose agar (PDA) following hyphal tip method. A piece of the PDA culture of each strains containing mycelium was placed aseptically in the opening of the mother culture packets. The inoculated packets were placed on a rack in the laboratory at 22 ± 2 ^{0}C for incubation. The substrate of the mother culture was covered by whitish mycelium of two strains within 15-20 days after inoculation. The fully colonized packets were used for spawning.

Preparation of spawn packets: The spawn packets were prepared separately according to individual treatment. The substrate mixture was poured into 18cm × 25cm polypropylene bags at 300g/bag and 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 two strains at the rate of two tea spoonful per packet. The inoculated packets were incubated at 22 ± 2^0C.

Cultural condition: After completion of mycelium running, spawn packets were transferred to the culture house for fruiting body initiation without opening. The fruit body developed at the top of the packet. Room temperature was 20 to 25^0C and relative humidity 85 - 90%. Water was sprayed 4-5 times per day to maintain the temperature and relative humidity. The yield was obtained from double flush in the harvest period.

Data collection and analysis: The experiment was laid out following completely randomized design (CRD) with four replications. Data on days to complete mycelium running, days required from opening to first harvest, days required from inoculation to first harvest, total days for harvest, length and diameter of stalk, diameter and thickness of pileus, fresh and dry yield per packet, biological efficiency were recorded and analyzed following standard methods using MSTAT-C computer program. Means were computed following DMRT using the same computer program.

RESULT AND DISCUSSION

Days required to complete mycelium running: Days required to complete mycelium running in spawn packet was significantly influenced by substrate and strains (Table 1).

Among the treatments, mycelium running was faster in He 2 and 300g bag size than others. This variation might be strain related, amount of substrates etc. The highest days (45.00 days) required to complete mycelium running when He 1 cultivated on sawdust in 500g bag size which was statistically dissimilar to other treatments. The lowest days (21.00 days) required when He 2 cultivated in 300g bag size. This result is supported by the findings of Hassan *et al.* (2007) who reported that spawn run time for *Hericium* mushroom ranged from 37-46 days. Pawlak *et al.* (2003) also reported differences in the growth of mycelium of different strains.

Days required from opening to first harvest: Days required from opening to first harvest was significantly influenced by amount of substrate and strain and ranged from 5.00 to 8.00 days (Table 1). The highest days (8.00) required from opening to first harvest was observed from He 1 with 300g bag size. The lowest (5.00) days required from opening to first harvest was observed from He 2 with 500g bag size.

Days required from inoculation to first harvest: Days required from inoculation to first harvest was significantly influenced by substrate and strain and it was ranged from 27 to 52 days (Table 1). The highest (52.00) days required from opening to first harvest was from He 1 with 500g substrate. The lowest (27.00) days required from opening to first harvest was found from He 2 with 300g substrate.

Total days for harvest: The maximum days (69.00 days) required for harvest was obtained from the treatment combination of strain He 1 with 500 g substrate which was dissimilar to other treatment combination. The minimum days required for first harvest (54.00 days) was obtained from the treatment combination strain He 2 300 g substrate (Table 1). Fast mycelium growth in the cultivation substrate is a desirable characteristic in mushroom cultivation because it reduces the risk of contamination with competitive microorganisms and speeds up harvesting (Eisenhut and Fritz, 1991; Oei, 2003).

Table1. Effect of amount of substrates on growth of two strains of *Hericium* mushroom

Treatments	Days to complete mycelium running	Days required from opening to first harvest	Days required from inoculation to first harvest	Total days for harvest
He 1 (300g)	23.00c	8.00a	31.00c	59.00c
He 2 (300g)	21.00d	6.00c	27.00d	54.00d
He 1 (500g)	45.00a	7.00b	52.00a	69.00a
He 2 (500g)	43.00b	5.00d	48.00b	64.00b
CV (%)	**3.91**	**8.71**	**3.27**	**2.10**

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

Diameter of stalk (DS): The diameter of stalk ranged from 4.50 to 4.75 cm but no significant difference was found. The highest (4.75cm) diameter of stalk was found in He 2 with 300g substrate which was statistically similar to other treatments. The lowest diameter (4.50cm) of stalk was found where He 1 cultivated on 500g substrate (Table 2).

Diameter of pileus (DP): The diameter of pileus was statistically significant and it was ranged from 6.55 -7.45 cm. The highest diameter of pileus (7.45 cm) was found from the treatment combination of strain He 2 with 300 g substrate and the lowest diameter of pileus (6.55 cm) was found from strain He 1 with 500 g substrate (Table 2).

Thickness of pileus (TP): The thickness of pileus in two strains as well as substrate differed significantly and ranged from 2.50 cm to 4.50 cm. The highest (4.50cm) thickness of pileus was observed in He 2 with 300g which was statistically dissimilar with other treatments. The lowest (2.50cm) thickness of pileus was observed where He 1 cultivated on 500g substrate (Table 2).

Fresh yield/packet (g): The fresh yield differed significantly and ranged from 64.50 to 128.00g (Fig. 1). The highest (128.00g) yield was found in He 2 and 300g substrate while it was lowest (64.50g) when He 1 cultivated on 500g sawdust (Fig. 1). This result is similar with Hassan *et al.*, 2007 who found the yield of *H. erinaceus* grown on the different media ranged from 122g-184g /1kg wet media.

Dry yield/packet (g): The dry yield was found highly significant by the effect of strains and substrates. The highest yield (13.17g/packet) was observed in He 2 mushroom and 300g substrate. The lowest (6.64g/packet) yield was observed in He 1 with 500g sawdust (Fig. 1).

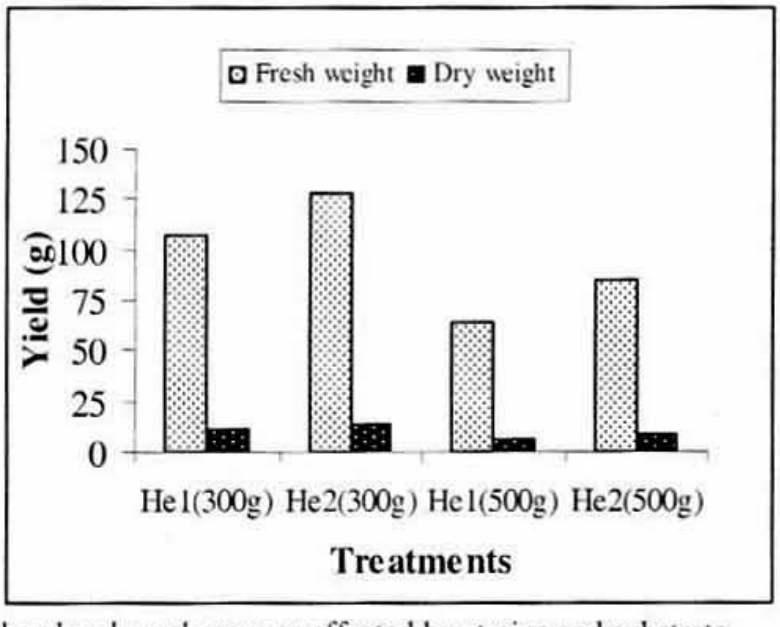

Fig. 1: Yield of monkey head mushroom as affected by strains and substrate.

Biological efficiency: Biological efficiency was also statistically significant. In this study the highest (69.18%) biological efficiency was found in He 2 with 300g substrate while the lowest (34.86%) biological efficiency was observed from He 1 and 500g sawdust (Table 2). This result is similar with Hassan *et al.*, 2007 who found the biological efficiency of *H. erinaceus* grown on the different media ranged from 33.9–50.3%.

Table 2: Effect of amount of substrates on yield attributes of two strains of *Hericium* mushroom

Treatments	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Biological efficiency (%)
He 1 (300g)	4.65a	6.95ab	2.95c	57.93b
He 2 (300g)	4.75a	7.45a	4.50a	69.18a
He 1 (500g)	4.50a	6.55b	2.50d	34.86d
He 2 (500g)	4.60a	7.00ab	4.00b	45.85c
CV (%)	**5.51**	**5.74**	**5.41**	**4.21**

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

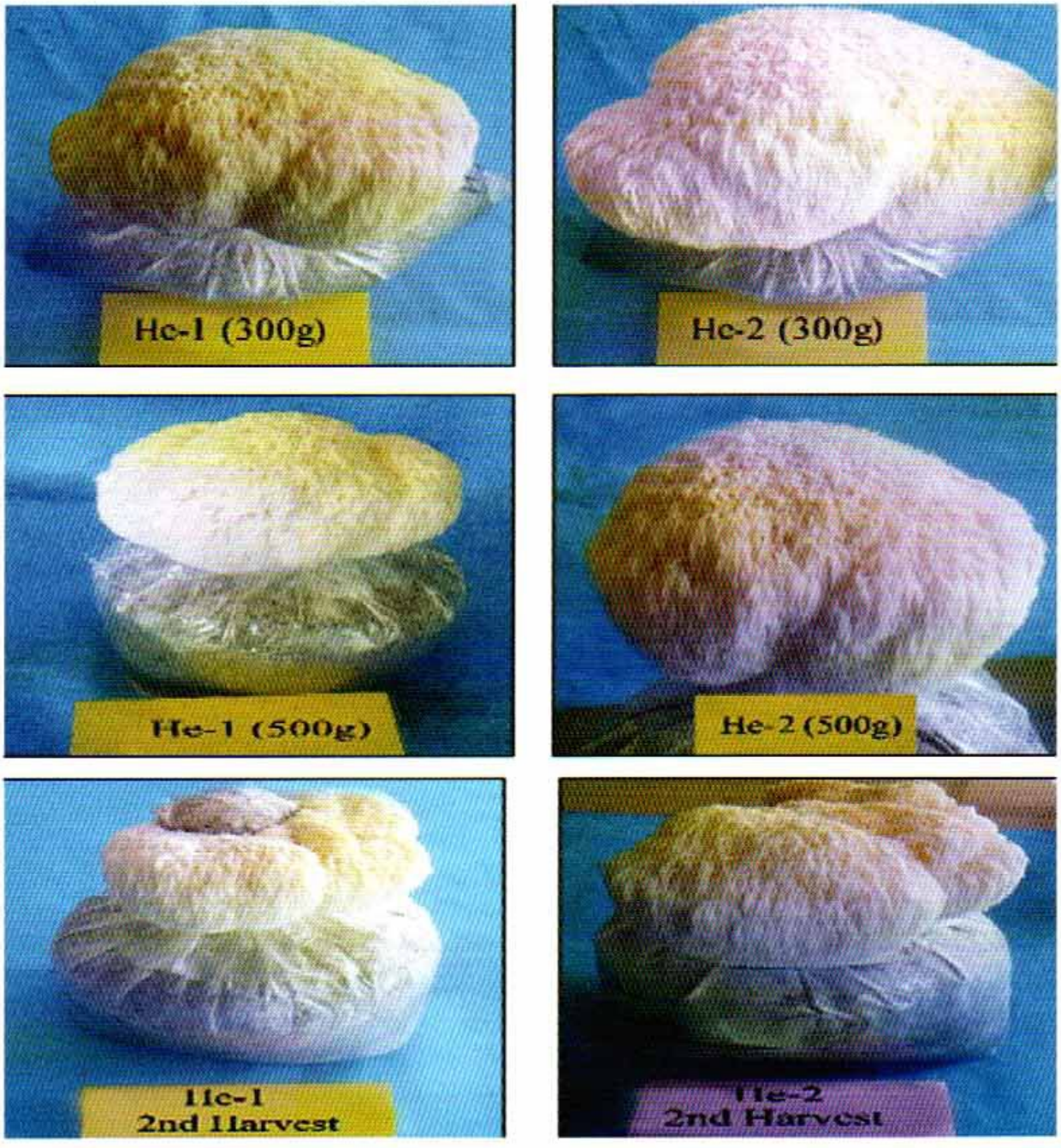

Fig. 1. Two strains of *Hericium* mushroom in different size of spawn packet.

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Comparative Classical Taxonomy of Cultivated *Pleurotus cystidiosus*, *Pleurotus djamor* and *Pleurotus eryngii* in Bangladesh

Zeenia Afsary, Nuhu Alam, Nirod Chandra Sarker, Md. Bazlul Karim Choudhury[1] and Akhter Jahan Kakon

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

Abstract

This study was performed to the macro and micro-morphological variation in three different species of oyster mushrooms i.e. *Pleurotus cystidiosus*, *Pleurotus djamor* and *Pleurotus eryngii*. The studied species showed a wide range of variation. Pileus shape was flabelliform, convex and convex at first but tapering to downward in *P. djamor*, *P. eryngii* and *P. cystidiosus*, respectively. Pileus color of *P. cystidiosus*, *P. djamor* and *P. eryngii* were navajo white, misty rose and sandy brown. Pileus margin were laciniate and sinuate. Stipe texture was fibrous in *P. djamor*, while smooth in *P. cystidiosus* and *P. eryngii*. Stipe color was misty rose, navajo white and blanched almond. Descending gill attachment was recorded in selected species. Gill spacing showed crowded and close. Highest diameter (8.5 cm) and thickness (1.7 cm) of pileus were found in *P. cystidiosus* and *P. eryngii*, while lowest diameter (7.5 cm) and thickness (0.6 cm) of pileus were found in *P. eryngii* and *P. djamor*, respectively. Highest length (5.9 cm) and diameter (2.4 cm) of stipe was recorded in *P. eryngii* and lowest length (1.5 cm) and diameter (0.9 cm) was found in *P. djamor*. Leucosporae and rhodosporae spore print was observed. Hymebophral trama was irregular. Basidia were 2-spored in *P. djamor* and *P. cystidiosus*, while 3-spored in *P. eryngii*. Cylindrical basidiospore was present in *P. djamor* and *P. cystidiosus*, while ovate in *P. eryngii*. Cheilocystidia absent in *P. cystidiosus* and present in other two species. Therefore, variation key was formed, which is used to identify and classify species.

Key words: Macro and micro morphology, *Pleurotus cystidiosus*, *Pleurotus djamor*, *Pleurotus eryngii*, Taxonomic key.

INTRODUCTION

The genus *Pleurotus* (Fr.) P. Kumm belongs to the family Pleurotaceae and order Agaricales (Kirk *et al.*, 2008). Mushrooms of *Pleurotus* spp. are commnly known as oyster mushrooms. They are the second most popular mushrooms after button mushroom all over the world (Adejoye *et al.*, 2006; Alam *et al.*, 2009). The systematic position of *Pleurotus* spp. has been much debated (Singer and Digilio, 1951). The *Pleurotus* spp. is considered one of the most important genus of edible mushrooms currently known for the great variety in their morphology of individuals (Lopes, 1999). Taxonomically coremiopleurotus is represented by *P. cystidiosus*, which was earlier documented from India by Natarajan and Raaman (1984). There is utmost need of advance technique used for species identification beyond morphological and physiological criteria, because these characteristics are highly influenced by cultivating conditions (Staniaszek *et al.*, 2002;

[1] Department of Biochemistry, Faridpur Medical College, Faridpur, Bangladesh.

Khan *et al.*, 2011). A taxonomic key is a method used to classify and identify objects and organisms. It shows a series of choices about the characteristics of different organisms. A user picks from these choices and ultimately finds the identity of a specimen. This study were conducted in considering three species and their taxonomic position or taxonomic history was evaluated by making a taxonomic key and finally came to an conclusion that they belongs to the genus *Pleurotus* and their species identity.

MATERIALS AND METHODS

Fruiting bodies of *Pleurotus cystidiosus, Pleurotus djamor* and *Pleurotus eryngii* (Fig. 1) were obtained from the National Mushroom Development and Extension Centre (NAMDEC), Department of Agricultural Extension, Ministry of Agriculture, Savar, Dhaka-1340, for the comparative morphometric analysis. This experiment was carried out at the laboratory of NAMDEC and Mycology, Plant Pathology and Crop Protection Laboratory, Department of Botany, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

Methods for collection, preservation and description of *Pleurotus* spp. have been followed as per standard techniques (Atri *et al.*, 2012). Pileus shape, diameter, margin, texture, color, gill attachment to stipe, gill spacing, stipe length, diameter, color, texture were described macroscopically on fresh specimens. Spore color is one of the simplest aids for the identification of mushrooms with gills. To determine the color of the spores it is necessary to make a spore print. A spore print is made by cutting off the stem of a mushroom just beneath the cap and placing the cap, gills down, on a piece of paper, covering it with an inverted glass or any other vessel that will keep the air moist and quiet around the cap. Microscopic characters were studied from free hand sections mounted in 5% KOH, stained with 1% Congo red. Colors in description are based on Methuen Handbook of Colors (Kornerup and Wanscher, 1978). After observing all the morphological characters, the carpophore tissue was taken from the point of junction of stipe with the pileus and sterilized by dipping in 1 % sodium hypochlorite for few minutes. After thoroughly washing in sterilized distilled water the fungal tissue was inoculated on potato dextrose agar medium. The optimum temperature for mycelium growth was 25 ± 1°C. Mycelial color, growth rate, odor and microscopic structures of both the aerial and the submerged mycelium were observed weekly up to 6 weeks according to Nobles (1965).

Fig. 1. Fruiting bodies of A, *Pleurotus cystidioses*; B, *Pleurotus djamor*; C, *Pleurotus eryngii*.

RESULTS AND DISCUSSION

Taxonomy of *Pleurotus* (Fr.) P. Kummer (1871): Basidiomes usually large, fleshy, solitary to imbricate, flabellate to dimidiate, glabrous to tomentose, white, cream, gray, pink, brown, more rarely blue, yellow or lilac. Stem short, solid, eccentric to lateral, rarely sub central. Lamellae decurrent, sometimes anastomosing to the stem, light-colored, thin to broad, margin entire. Veil present or absent at margin of pileus or forming an annular zone on the stem. Spore print white, cream, pinkish or lilac. Spores cylindrical to subcylindrical, thin-walled, hyaline, not amyloid or dextrinoid, without germ pore. Cheilocystidia absent or poorly developed, disappearing early, thin-walled, clavate or mucronate. Subhymenium well developed hymenophoral trama irregular. Pileipellis frequently poorly developed, with parallel radial hyphae, sometimes pigmented. Hyphal system monomitic or dimitic, gelatinous tissue usually absent, clamps connection present and ligni colors.

The genus *Pleurotus* does not have clear distinctive characters as occurs with other genera. The hyphal system may be monomitic or dimitic without binding hyphae. This character might confuse the boundaries with other related genera such as *Lentinus* (Stankovičová, 1973). There exists a clear delimitation in *Pleurotus* between species with a monomitic hyphal system, thin or thick-walled generative hyphae, such as *P. cystidiosus* and dimitic ones, such as *P. djamor*. All *Pleurotus* species have thin-walled, smooth, cylindrical to subcylindrical spores. They may be small < 8 µm, medium 8-12 µm and large >13 µm long (Cormer, 1981).

Key to the species

1(a). Pileus convex at first tapering to down ward, grey to brown cracked or punctiform squamules, laciniate margin, spore print leucosporae.....2

1(b). Pileus flabelliform, glabrous, sinuate margin, spore print rhodosporae .....*P.djamor*

2(a). Pileus with punctiform squamules, grey to brown, basidia 2-spored, cheilocystididia absent, basidiospoes cylimndrical.....*P.cystidiosus*

2(b). Pileus cracked, sandy brown, basidia 3-spored, cheilocystidia present, basidiospores ovate.....*P.eryngii*

***Pleurotus cystidiosus*:** Basidiome solitary to imbricate Pileus 13–17 × 8.5–9.5 cm, pleurotoid, surface grey to brown with numerous punctiform squamules formed by surface cracking, more numerous toward the margin; margin entire, slightly to much festooned, hardly involute. Stipe grey tobrown. Lamellae white when fresh, yellow when dry, 4–10 mm broad, thinner toward the stipe, decurrent, and forming a pseudo reticulum. Odor is agreeable, fungal edibility is good (Table 1 and 2).

Spores hyaline, cylindrical-oblong, thin-walled, smooth. Basidia are 2-, 3- spores and cheilocystidia absent. Pleurocystidia are granular contents, clavate, hymenophoral trama formed by generative, thin-walled, clamped hyphae (Fig. 2).

Table 1. Qualitative characters of *Pleurotus cystidiosus*, *Pleurotus djamor* and *Pleurotus eryngii*

Characters	Oyster mushrooms		
	P. cystidiosus	*P. djamor*	*P. eryngii*
Pileus shape	Pileus convex at first tapering to downward	flabelliform	Convex depressed
Pileus color	Navajo white	Misty rose	Sandy brown
Pileus texture	With numerous punctiform squamules	smooth	cracked
Pileus margin	laciniate	sinuate	laciniate
Stipe color	Navajo white	Misty rose	Blanched almond
Stipe texture	smooth	fibrous	smooth
Gill attachment tostipe	descending	descending	descending
Gill spacing	crowded	crowded	close

Table 2. Quantitative characters of *Pleurotus cystidiosus*, *Pleurotus djamor* and *Pleurotus eryngii*

Name of species	Pileus diameter (cm)	Thickness of pileus (cm)	Stipe length (cm)	Stipe diameter (cm)
P. cystidiosus	8.5	1	3.2	0.9
P. djamor	8.3	0.6	1.5	0.9
P. eryngii	7.5	1.7	5.9	2.4

Cultural characters: Mycelium hyaline, filamentous, dense, whitish near the inoculum, growth radial, well adhered to the substrate. At first, a large amount of hyaline, aerial mycelium was observed, more whitish later, they later appeared away from it advancing mycelium with hyaline, clamped, thin-walled, regularly branched hyphae.

***Pleurotus djamor*:** Pileus pink, 25-40 × 23-40 mm, spathulate to flabelliform brown when dry; glabrous, smooth to touch; margin sinuate, frequently lobate and imbricate in old specimen. Lamellae broad, decurrent, crowded, smooth, margin entire. Stipe 5-6 mm long × 3-4 mm diameter, lateral, smooth, rarely absent. Spore print white. Odor not distinct. Good edibility. Sipe solid, firm, fibrous, misty rose color. Spores cylindrical hyaline, not dextrinoid or amyloid, thin-walled. Basidia club-shaped. Cheilocystidia present, Hymenophoral trama dimitic, with sharp-pointed skeletal hyphae and clamped generative hyphae (Fig. 3).

Cultural characters: Mycelium hyaline, filamentous, radial growth, well adhered to substrate. Margin irregular, undulate. Against the light, an unequal refraction was

observed, causing a relief aspect. Odor not distinct. The mycelium did not cover the Petri plates in 6 wk. Pinkish zones were observed in some strains.

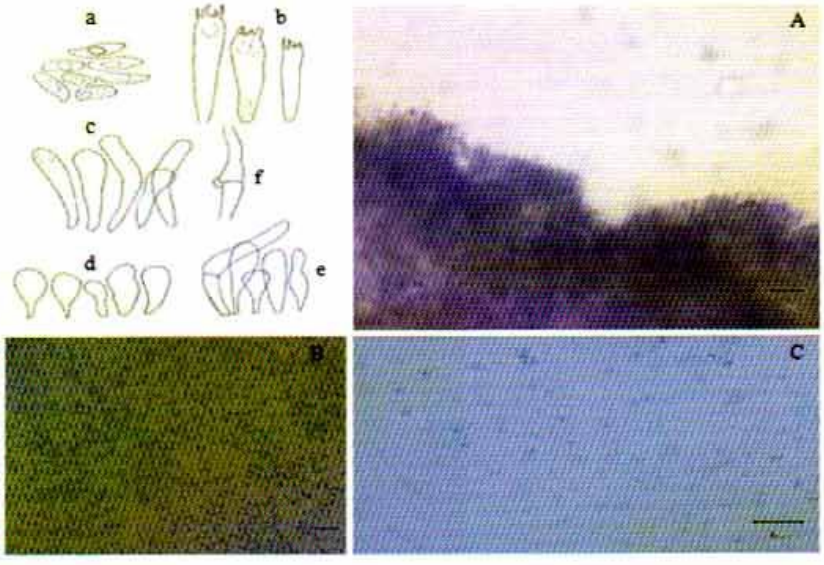

Fig. 2. A. a, basidiospores; b, basidia; c, pleurocystidia; d, cheilocystidia; e, pileocystidia; f, hypha with clamp connection; B. spore length and spore diameter; C. germinating spore of *Pleurotus cystidiosus*

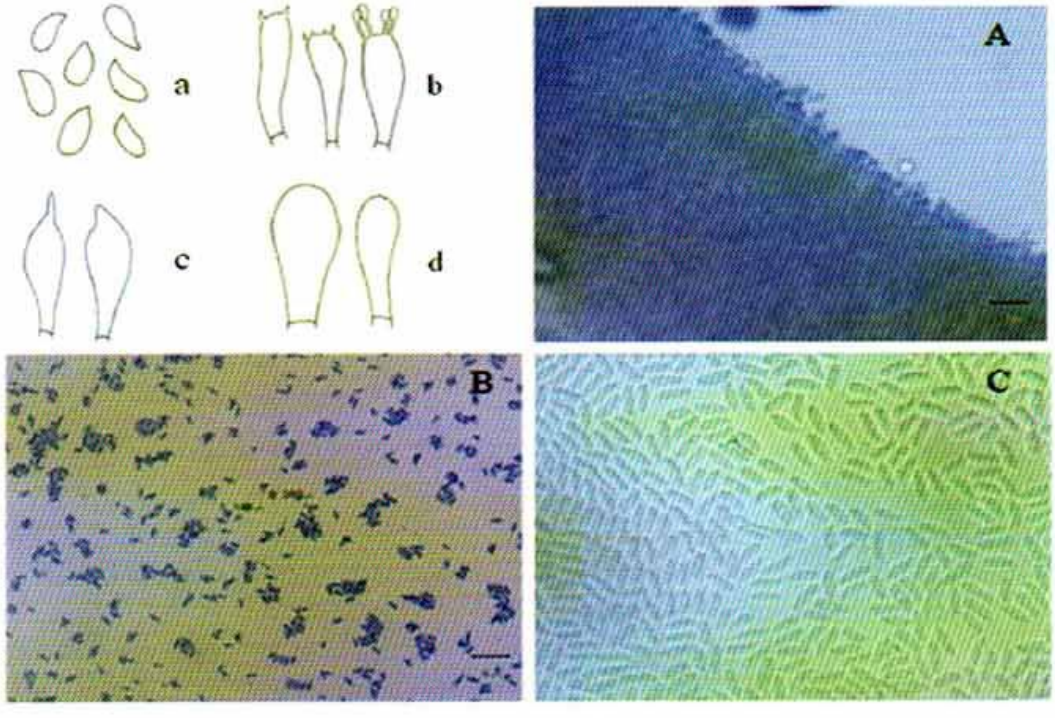

Fig. 3. A. a, basidiospores; b, basidia; c, cheilocystidia; d, pleurocystidia; B. spore length and diameter; C. germinating spore of *Pleurotus djamor*

***Pleurotus eryngii*:** *Pleurotus eryngii* is by far the best tasting Oyster mushroom, well deserving of the title, the King Oyster. Pileus usually 4-15 cm in diameter, convex at first then expanded and depressed downward sandy brown, cuticle glabrous, margin turned down ward. Gills subdistant, decurrent, whitish to cinerous. Stipe usually 4-7 cm, whitish, stuffed, baase fusiform. Flesh white, tough, odor mild. Hymenophoral trama usually

irregular, clamp present, subhymenium well fored, Basidia 3-spored, 4-spored, spore print leucosporae, Pleurocystidia none (Fig. 4).

Cultural characters: Whitish, longitudinally radial at first, sometimes rhizomorphic, soon thickening and becoming cottony in age.

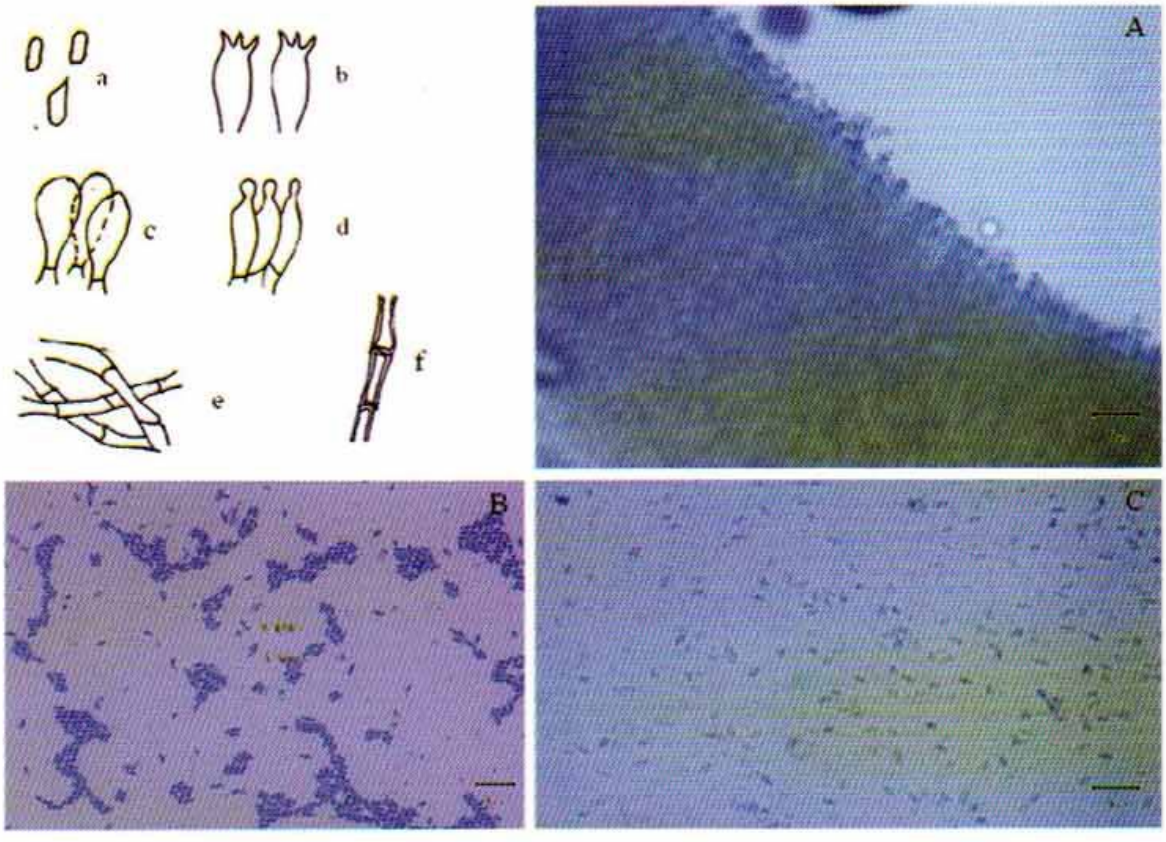

Fig. 4. A. a, basidiospores; b, basidia; c, pleurocystidia ; d, cheilocystidia; e, hyphae of context; f, hyphae of stem; B. spore length and spore diameter; C. germinating spore of *Pleurotus eryngii*.

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Effect of Drying Methods on Quality of Oyster, Shiitake and Milky Mushrooms

Syeda Tashnim[1], Nirod Chandra Sarker, Md. Mustafizur Rahman[2], Mahbuba Moonmoon[3], Md. Mominul Hoque and Abdus Salam Khan
National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

The experiment was conducted at National Mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka from April 2010 to March 2011 to study the performance of different drying methods and their effect on quality of oyster, shiitake and milky mushroom. The experiment consists of two factors such as Factor A- four different drying methods; Gas burner, White polypropylene covered tunnel, Open sundry and Indirect dryer. Factor B- three species of mushroom: oyster, shiitake and milky. The results revealed that treatment of white polypropylene covered tunnel showed the best performance in terms of reducing the maximum weight and containing minimum moisture. The different drying methods also influenced the colour of mushroom. For oyster mushroom, the best colour: upper part of pileus- tan (gray), lower part of pileus-corn silk (light yellow) and stipe (stalk) – corn silk (light yellow) / wheat was found under the treatment of white polypropylene covered tunnel dryer. For shiitake mushroom the best colour: upper part of pileus – saddle brown (dark brown), lower part of pileus – moccasin (light brown) and stipe – tan (gray) was obtained from the treatment of open sundry and indirect dryer. For white milky mushroom the best colour: upper part of pileus and stipe – wheat colour, lower part of pileus – corn silk (light yellow) was found under the treatment of white polypropylene covered tunnel dryer, open sundry and indirect dryer. However the Indirect dryer may also be used for drying of mushroom.

Keywords: Drier, Moisture, Weight, Colour, Mushroom, Quality, Drying method.

INTRODUCTION

Mushrooms are reproductive structure of edible and medicinal fungi that belong to classes Ascomycetes and Basidiomycetes (Pathak *et al.*, 1998). There are about 2000 species of edible mushroom of which only 20 are commercially cultivated (Pathak *et al.*, 1998). Among them Oyster, Milky, Straw, Shiitake and Reishi mushrooms are usually cultivated in our country. The shelf life of mushrooms is very short. It is highly perishable as it contains 85% water. To store the mushrooms for longer time canning, freezing and drying processes are employed (Kim, 2004). But drying is more suitable for long term storage in our country. Drying is a thermo-physical action and its dynamic principles are governed by heat and mass transfer laws inside and outside the product. The weight of the product is reduced to the extent of $1/4^{th}$ to $1/9^{th}$ of its original fresh weight (Sethi, 2007). Drying of mushrooms and biological products is a widely applied process for different purposes

[1] M.S. Student, Sher-e-Bangla Agricultural University, Dhaka-1207; [2] Planning Wing, Ministry of Agriculture, Secretariate, Segunbagicha, Dhaka, [2]Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh.

such as increasing shelf life, reducing packaging costs, lowering shipping wastes, encapsulating flavours, making food available during off-season, adding value by changing the phase structure of the native material and maintaining nutritional value. However, the ever-rising cost of electricity and natural fuels coupled with growing concern about their availability in both the short and long terms, has resulted in growing interest in the use of renewable resources especially solar energy; in both direct and indirect forms (Adaramola *et al.*, 2004; Koua *et al.*, 2009).

Several types of dryers and drying methods, each better suited for a particular situation are commercially used to remove moisture from a wide variety of mushrooms (Oei, 2005). Different types of drying processes are as follows: Open sundry, gas burner, electric dryer, indirect sundry, sun drying in polypropylene covered tunnel, drying room etc. Among of them gas burner, white polypropylene covered tunnel, open sundry and indirect dryer were chosen for this experiment, because of their low cost and easy mechanism so that rural farmers can use them properly. The consumer simply dehydrates the material and uses for different purposes. Mushrooms available only during seasons with the help of drying process they can be preserved for all seasons. The biological forces acting upon foods are minimized. Spoilage of foods is easily controlled in drying process. Considering the above facts the present investigation was carried out to evaluate the performance of different drying methods for each of the mushroom species and to determine the colour quality of mushrooms dried in different methods.

MATERIALS AND METHODS

The investigation was carried out at National Mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka from April 2010 to March 2011 to study the performance of different drying methods. The mushrooms were collected from the centre and different drying equipments were installed at the yard of the centre.

Species of mushrooms: Three different mushroom species like oyster mushroom (*Pleurotus ostreatus*), shiitake mushroom (*Lentinula edodes)* and milky mushroom (*Calocybe indica*) were collected in a fresh condition from the centre.

Description of different driers: Four different driers were used in this research work. Among of them the Gas burner was installed in the laboratory and other three driers- White polypropylene covered tunnel, Indirect drier and open sun drier were installed at the yard of the culture house of NAMDEC.

Gas burner: The gas burner was an iron made frame which is rectangular in shape. At the lower portion of the burner, a round iron pipe having several small holes is adjusted through which gas is blown (Fig. 1). Over the gas pipe there was a steel sheet which becomes heated when the burner starts burning. Three iron net trays were placed over the steel sheet according to the frame one after another by maintaining some distance. The collected fresh mushrooms were cut, spited and spread over the trays (Fig. 1). Each of the trays contained the same amount of mushroom. As the gas burner produces the maximum

amount of heat so it requires minimum duration of time and it's about three hours to dried the mushroom.

Fig. 1. Fresh mushrooms were dried on gas burner.

Fig. 2. White polypropylene covered tunnel drier.

White polypropylene covered tunnel: The frame of the tunnel was made of bamboo slices and covered with white polypropylene. The tunnel was about 2.13m long, 1.52m width and 0.914m high. Two opening portion was covered with white mosquito net. Black cloth was used as a base material in the tunnel (Fig. 2). Fresh collected mushroom's were cut, splited and spread over the base material in three replications. As it depends on the sun light, it was used for three days to complete the drying process.

Open sundry: It is the most traditional method. Steel trays were used in open sunlight (Oei, 2005). The trays were placed at the yard of NAMDEC. Freshly collected mushroom's were cut, splited and spread over the tray. As the drying process fully depends on the sun light, it required three days to complete the process.

Indirect drier: The drier was made of iron and tin. The structure was about 1.82m high and 1.06m width. Four stands of the drier were made of iron and the drying panel was covered with tin with a ventilation system at the top (Fig. 3). Inside of the panel three steel net trays were placed where freshly collected mushrooms were cut, spited and spread over (Fig. 4). Another iron rod made frame was adjusted at lower part of the panel. Inside of the frame a black cloth was added and the whole frame was covered with transparent polyethylene paper leaving both upper and lower opening end. The opening end of the frame was adjusted at the lower part of the panel (Oei, 2005). The black cloth absorbed the sun light, produced heat and blown it to the panel and thus increase the temperature inside the panel. As this drying process depends on the sun light, mushrooms were dried here for three days to complete the drying.

Fig. 3. Indirect drier

Fig. 4. Inside the panel

Description of used materials: Total 2400g for each of oyster, shiitake and milky mushrooms were used in four drying methods as well as in four treatments. For every drying process 600g of each of the mushroom species were used at a time in three replications. Each of the replication contained 200g of mushrooms. The procedures were closed when the mushrooms become crispy, light in weight and fade in colour. The mushroom was weighted for several times – firstly, at fresh condition, after every two hours of drying and finally at dried condition. For weight, digital weight machine (low profile weighing scale, Kemdy) was used at the laboratory of NAMDEC. The moisture percentage (%) was taken for two times- at fresh condition and at dried condition. The moisture percentage (%) was collected by using moisture analyzer (A&D company Ltd N92: P1011656, Japan) at the laboratory of NAMDEC.

Data recording: Data were recorded on the following parameters from the mushrooms during the experiment. The details of data recording are given below on individual mushroom species basis.

Oyster mushroom: For Gas burner, three trays were used in the burner and each of the trays contained 200g of oyster mushroom. The total drying time was 2.5 hours. The temperature and relative humidity (RH) inside the dryer were noted down for every half an hour. For white polypropylene covered tunnel, three replications were used in the white polypropylene covered tunnel and each of the replication contained 200g of oyster mushroom. The total drying time was 12 hours. The temperature and relative humidity (RH) inside the dryer were noted down for every an hour. For open sundry, three trays were used in the open sun light and each of the tray contained 200g of oyster mushroom. The total drying time was 14 hours. The temperature and relative humidity (RH) inside the dryer were noted down for every an hour. For indirect drier, three replications were used in the indirect drier panel and each of the replication contained 200g of oyster mushroom. The total drying time was 13 hours. The temperature and relative humidity (RH) inside the dryer were noted down for every an hour.

Shiitake mushroom: For Gas Burner, three trays were used in the burner and each of the trays contained 200g of shiitake mushroom. The total drying time was 3 hours. The temperature and relative humidity (RH) inside the dryer were noted down for every half an hour. For white polypropylene covered tunnel, three replications were used in the white pp covered tunnel and each of the replication contained 200g of shiitake mushroom. The total drying time was 13 hours. The temperature and relative humidity (RH) inside the dryer were noted down for every an hour. For open sundry, three trays were used in the open sun light and each of the tray contained 200g of shiitake mushroom. The total drying time was 14 hours. The temperature and relative humidity (RH) inside the dryer were noted down for every an hour. For indirect drier, three replications were used in the indirect drier panel and each of the replication contained 200g of shiitake mushroom. The total drying time was 13 hours. The temperature and relative humidity (RH) inside the dryer were noted down for every an hour.

Milky mushroom: For Gas Burner, three trays were used in the burner and each of the trays contained 200g of milky mushroom (white). The total drying time was 3 hours. The

temperature and relative humidity (RH) inside the dryer were noted down for every half an hour. For white polypropylene covered tunnel, three replications were used in the white pp covered tunnel and each of the replication contained 200g of milky mushroom (white). The total drying time was 12 hours. The temperature and relative humidity (RH) inside the dryer were noted down for every an hour. For open sundry, three trays were used in the open sun light and each of the tray contained 200g of milky mushroom (white). The total drying time was 14 hours. The temperature and relative humidity (RH) inside the dryer were noted down for every an hour. For indirect drier, three replications were used in the indirect drier panel and each of the replication contained 200g of milky mushroom (white). The total drying time was 13 hours. The temperature and relative humidity (RH) inside the dryer were noted down for every an hour.

Colour analysis: For Oyster mushroom the colour of upper part of pileus, lower part of pileus and stipe of the randomly selected samples were visibly checked with the colour chart. Several colours were found like peru- brown, moccasin- light brown, wheat colour, tan- dark gray, corn silk- light yellow etc. For shiitake mushroom the colour of upper part of pileus, lower part of pileus and stipe of the randomly selected samples were visibly checked with the colour chart. Several colours were found like peru- brown, moccasin- light brown, wheat colour, tan- dark gray, corn silk- light yellow, saddle brown- deep brown. For White Milky mushroom the colour of upper part of pileus, lower part of pileus and stipe of the randomly selected sample of white milky mushroom were visibly checked with the colour chart and the following colours were found: moccasin- light brown, wheat colour, corn silk- light yellow.

Statistical Analysis: Data were statistically analyzed following the CRD with MSTAT-C computer programme. Means were computed following Duncun's Multiple Range Test (DMRT) using the same computer programme.

RESULTS AND DISCUSSION

Total drying time: Drying time of oyster mushroom for the gas burner required the least of time (2.5 hrs) and the maximum time (14 hrs) was required for open sundry method. For shiitake mushroom, the required time for drying under the gas burner was the shortest period of time (3 hrs) and the maximum drying time (14 hrs) was required under open sundry method. For milky mushroom, the gas burner required the least of time (3 hrs) and the maximum time of drying (14 hrs) was required for open sundry method (Table 1).

Increasing the moisture absorption resulted in a considerable decrease in drying time (Soysal, 2004). The treatment of gas burner took the minimum time to dry as it produces higher amount of heat. Heat that produced within a short time may not absorb the moisture from the mushrooms uniformly and might be responsible for darkening. Oei (2005) suggested that the mushrooms could become toasted at high temperatures, longer drying at low temperature is safer than faster drying at high temperature. It was found that the heat inside the white pp covered tunnel and inside the panel were higher than the ambient temperature during the day light (Bukola *et al.*, 2005). The tunnel and the panel

retained the heat for a long constant which may absorb moisture uniformly. Heat is generated by absorption of solar radiation on the product itself as well as on the internal surface of the drying chamber (Jayaraman and Dasgupta, 1990).

Table 1. Influence by drying methods on drying time (hr) of oyster, shiitake and milky white mushroom

Treatment	Drying time (hr)		
	Oyster	Shiitake	Milky
Gas burner	2.50b	3.00b	3.00c
White pp covered tunnel	12.00a	13.00a	12.00b
Open sundry	14.00a	14.00a	14.00a
Indirect dryer	13.00a	13.00a	13.00ab
CV (%)	**11.87**	**8.14**	**8.58**

Means with in the column and rows, under a parameter, having a common letter do not differ significantly (P=0.05).

Dry weight (g) of mushroom: The minimum dry weight of oyster mushroom was found under the treatments of white polypropylene covered tunnel and indirect dryer (25.33g). The maximum dry weight (28.67g) was found under the treatment of open sun light. Dry weight of shiitake mushroom that found under different dryers ranged from 26.33g to 29.00g. Dry weight of shiitake mushroom was the minimum under the treatment of white polypropylene covered tunnel (26.33g) and the maximum dry weight (29.00g) was found in the treatment of open sun light. Dry weight of milky mushroom under different dryers ranged from 26.67g to 28.67g. The minimum dry weight (26.67g) of white milky mushroom was found in the treatment of white polypropylene covered tunnel and the maximum dry weight (28.67g) was found under the treatment of indirect dryer (Table 2).

Table 2. Influence by drying methods on the dry weight (g) of oyster, shiitake and milky white mushroom

Treatment	Dry weight (g)		
	Oyster	Shiitake	Milky
Gas burner	26.33ab	27.67a	28.33a
White pp covered tunnel	25.33b	26.33a	26.67a
Open sundry	28.67a	29.00a	28.33a
Indirect dryer	25.33b	26.66a	28.67a
CV (%)	**5.78**	**6.07**	**6.27**

Means with in the column and rows, under a parameter, having a common letter do not differ significantly (P = 0.05).

In this study, treatment of white polypropylene covered tunnel and indirect dryer was the best because by these treatments dry weight of oyster, shiitake and white milky mushroom became lower. In white polypropylene covered tunnel and indirect dryer, the sun light was absorbed, fixed and increased the heat inside. The increasing heat vaporized the moisture as well as dry weight of mushroom (Oei, 2005). Study also showed that the

comparatively poor result was come from the treatment of open sundry might be due to the low heat at open place.

Final moisture (%) of mushroom: For oyster mushroom the minimum moisture (7.01%) was found in treatments of white polypropylene covered tunnel and the maximum moisture (8.26%) was found under the treatment of open sun light. The minimum moisture (7.01%) of shiitake mushroom was found under the treatment of indirect dryer and the maximum moisture (7.88%) was found under treatments of open sun light. For milky mushroom the minimum moisture (7.14%) was found under the treatment of white polypropylene covered tunnel and the maximum moisture (7.55%) was found under the treatment of open sun light and indirect dryer (Table 3).

Table 3. Influence by drying methods on the final moisture (%) of oyster, shiitake and milky white mushroom

Treatment	Final moisture (%)		
	Oyster	Shiitake	Milky
Gas burner	7.77a	7.68a	7.21a
White pp covered tunnel	7.01a	7.79a	7.14a
Open sundry	8.26a	7.88a	7.55a
Indirect dryer	7.68a	7.01a	7.55a
CV (%)	**18.22**	**18.28**	**15.43**

Means with in the column and rows, under a parameter, having a common letter do not differ significantly (P=0.05).

The present study indicated that the treatment of white polypropylene covered tunnel and indirect dryer was the best because under these treatment the maximum amount of moisture was reduced. Temperature and relative humidity (RH) are inversely related (Bala *et al.*, 2009) When temperature increases, relative humidity (%) decreases (Fig. 6). Under the treatment of white pp covered tunnel and indirect dryer the decreasing RH may causes absorption of moisture from the mushrooms. Mushroom losses lower amount of moisture under open sundry may be due to the presence of higher relative humidity. The moisture content of sun dried mushroom is higher and therefore they can be kept for a shorter period of time than the artificially dried ones (Oei, 2005).

Colour of dried mushroom: For oyster mushroom different types of colour were found under 4 dryers like Peru (deep brown), Tan (gray), Moccasin (light brown), wheat colour, corn silk (light yellow) etc. Among of 4 treatments 3 showed the same colour. For three treatments the upper part of pileus colour was tan, lower part of pileus colour was moccasin and stipe colour was wheat (Table 4).

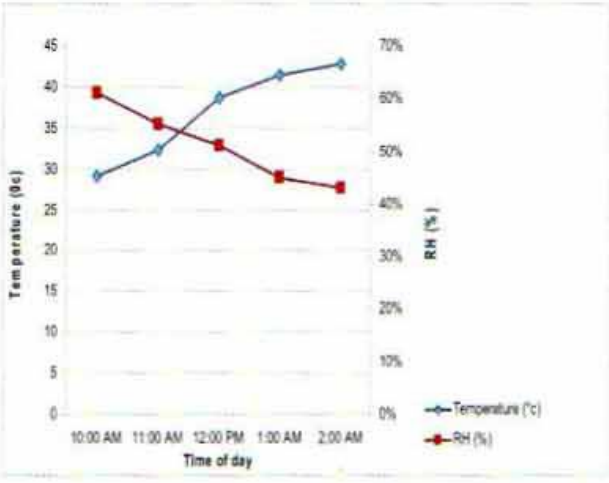

Fig. 5. Relative humidity decreases with the increase of temperature inside the white pp covered tunnel.

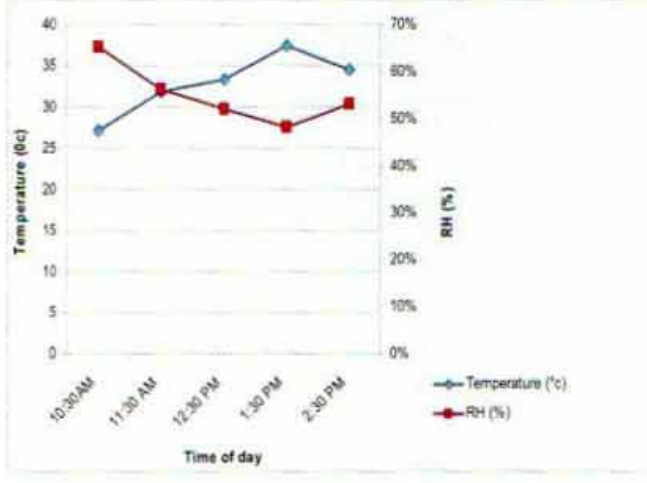

Fig. 6. Relative humidity decreases with the increase of temperature and again increase with the decreases of temperature at open place.

Table 4. Influence by drying methods on the colour of oyster mushroom

Treatment	Upper part of pileus	Lower part of pileus	Stipe
Gas burner	Peru	Moccasin	Wheat
White pp covered tunnel	Tan	Corn silk	Corn silk/ Wheat
Open sundry	Tan	Moccasin	Wheat
Indirect dryer	Tan	Moccasin	Wheat

Peru is visibly like deep brown; Corn silk is visibly like light yellow; Tan is visibly like gray; Wheat is visibly like wheat colour; Moccasin is visibly like light brown.

This study showed that the treatment of white pp covered tunnel drier was the best (upper part of pileus - gray, lower part of pileus - light brown and stipe –wheat) for oyster mushroom. According to Oei (2005) mushroom do not need to be crisp to the touch after drying; they should still be slightly flexible. Generally fresh oyster mushroom is grayish white in colour and dried mushroom shows lighter colour (Kulsheshtha *et al.*, 2009). The quality of sun dried mushrooms is generally less than that of artificially dried ones (Oei, 2005) contain higher nutrition than dark coloured dried mushroom. The dark colour indicates excess burning which reduces the food value. So the lower result (deep brown) came from the treatment of gas burner.

For shiitake mushroom different types of colour were found under 4 dryers like Saddle brown (dark brown), Peru (deep brown), Tan (gray), Moccasin (light brown), wheat (wheat colour), corn silk (light yellow) etc. 2 treatments showed the same colour. For the treatment of open sundry and indirect dryer the upper part of pileus colour was saddle brown, lower part of pileus was moccasin and stipe colour was tan (Table 5).

Table 5. Influence by drying methods on the colour of shiitake mushroom

Treatment	Upper part of pileus	Lower part of pileus	Stipe
Gas burner	Tan	Moccasin	Peru
White pp covered tunnel	Saddle brown	Corn silk	Wheat
Open sundry	Saddle brown	Moccasin	Tan
Indirect dryer	Saddle brown	Moccasin	Tan

Peru is visibly like deep brown; Corn silk is visibly like light yellow; Tan is visibly like gray; Wheat is visibly like wheat colour; Moccasin is visibly like light brown; Saddle brown is visibly like dark brown.

This study found that treatment of open sundry and indirect dryer were the best (upper part of pileus - dark brown, lower part of pileus - light brown and stipe –gray) for shiitake mushroom. Generally fresh shiitake mushroom is brown in colour (Kulsheshtha *et al.*, 2009) so it might be deep or dark brown after drying.

For milky mushroom different types of colour were found under 4 dryers like Moccasin (light brown), wheat (wheat colour), corn silk (light yellow) etc. 3 treatments showed the same colour. For the treatment of white pp covered tunnel, open sundry and indirect dryer the upper part of pileus, lower part of pileus and stipe were wheat, corn silk and wheat in colour respectively (Table 6).

Table 6. Influence by drying methods on the colour of milky white mushroom

Treatment	Upper part of pileus	Lower part of pileus	Stipe
Gas burner	Moccasin	Wheat	Burly wood
White pp covered tunnel	Wheat	Corn silk	Wheat
Open sundry	Wheat	Corn silk	Wheat
Indirect dryer	Wheat	Corn silk	Wheat

Corn silk is visibly like light yellow; Burly wood is visibly like burly colour; Wheat is visibly like wheat colour; Moccasin is visibly like light brown.

Thi present study indicated that the treatment of white pp covered tunnel, open sundry and indirect dryer were showing the same result (upper part of pileus and stipe – wheat colour, lower part of pileus - light yellow) for milky white mushroom . Generally fresh milky mushroom is milky white in colour and dried mushroom become lighter (Kulsheshtha *et al.*, 2009) after drying.

It was observed from the results that white polypropylene covered tunnel and indirect dryer showed the best performance in terms of reducing maximum moisture and getting the minimum dry weight with quality, whereas gas burner was responsible for excess burning and open sundry for improper drying and unhygienic condition. The standard drying time for oyster mushroom (12 hrs), for shiitake mushroom (13 hrs) and for white milky mushroom (12 hrs) was found under white polypropylene covered tunnel. For oyster mushroom the minimum dry weight (25.33g), for shiitake mushroom, the minimum dry weight (26.33g), for white milky mushroom, the minimum dry weight

(26.67g) was found under white polypropylene covered tunnel. The minimum final moisture for oyster (7.01%), shiitake (7.01%) and white milky mushroom (7.14%) was found under white polypropylene covered tunnel, indirect dryer and white polypropylene covered tunnel, respectively.

The colour evaluation also showed that for oyster mushroom the best colour (upper part of pileus - gray, lower part of pileus - light yellow and stipe – light yellow /wheat) was found under white polypropylene covered tunnel. For shiitake mushroom, the best colour (upper part of pileus - dark brown, lower part of pileus - light brown and stipe – gray) was found under open sundry and indirect dryer. For white milky mushroom, the best colour (upper part of pileus and stipe –wheat colour, lower part of pileus - light yellow) was found under white polypropylene covered tunnel, open sundry and indirect drier.

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Influence on Glycemic Status of Hypertensive Non-diabetic Females by *Pleurotus ostreatus*

Md. Bazlul Karim Choudhury[1], Sheikh Md. Ruhul Amin[2], Akhter Jahan Kakon, Tahera Binte Mujib, Syed Sakhawat Hussain[3] and M. Shahabuddin Kabir Choudhuri[4]

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

Abstract

The study was carried out in the National Mushroom Development and Extension Center (NAMDEC) with the active supervision of the Department of Pharmacy Jahangirnagar University, Savar, Dhaka. Fasting plasma glucose, glycated hemoglobin (HbA1c) and plasma creatinine level of hypertensive non-diabetic female volunteers were estimated before and three months after administration of three grams *Pleurotus ostreatus* mushroom capsule daily. The feeding of mushroom capsule showed non significant mean difference of plasma creatinine between the two periods of observation ($p = 0.174$). There was significant 14.08% reduction of fasting plasma glucose (5.61 ± 0.24 and 4.82 ± 0.37, $p = 0.003$). Considering the glycated haemoglobin (HbA1c), there observed a non-significant 3.69% reduction (4.33 ± 0.26 and 4.17 ± 0.27, $p = 0.340$) after mushroom treatment. Findings of the study suggest that without interfering kidney function, *Pleurotus ostreatus* improves glycemic status of non-diabetic hypertensive females.

Key words: Female, Non-diabetes, *Pleurotus ostreatus,* Plasma glucose, HbA1c.

INTRODUCTION

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

Considering these situations, it is worth mentioning that mushrooms could play an important role in improving the health and nutritional status of the population. It is now established that mushrooms are good source of high quality proteins and minerals (Pathak *et al.,* 1998). Mushrooms are not only sources of nutrients but also have been reported as therapeutic foods. Mushroom of *Pleurotus* species are also rich in medicinal values and useful in preventing disease such as hypertension, hypercholesterolemia (Khatun *et al.,*

[1] Department of Biochemistry, Faridpur Medical College, Faridpur, Bangladesh; [2] Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh; [3] Department of Biochemistry, North East Medical college, Sylhet; [4] Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh.

2007; Choudhury *et al.*, 2008) hyperglycemia and different types of cancer (Nayana and Janardhanan, 2000).

Today mushrooms are considered as a type of functional food which can ameliorate and prevent diabetes and its complications (Milner, 2000; Perera and Li, 2011). Advances in medicine and developments in understanding the disease characteristics have given rise to novel therapies to fight diabetes and related complications (Lo and Wasser, 2011 and Smith *et al.*, 2012). At present few clinical drugs are available for diabetes, and those that are available usually have adverse side effects (Purnell, 2008; Cheng and Fantus, 2005). Thus, more efficacious and safer anti-hyperglycemic agents are needed. Therefore, research and development into novel drugs for diabetes has been in great demand (Choi *et al.*, 2011 and Avogaro, 2012). Many therapeutic strategies from natural products with plant origins have been developed as supportive methods for preventing and controlling diabetes (Goyal *et al.*, 2008 and Huseini *et al.*, 2012). Currently, there is renewed interest in the plant based medicines and functional foods for modulating physiological effects and in the prevention and cure of diabetes.

Mushrooms can produce several bioactive metabolites that can directly act upon glucose metabolism and related biochemical pathways. On the other hand they are important as nutritional foods to provide complete defense against external, internal stressors and inflammatory processes, and indirectly help in prevention and amelioration of diabetes (Lo and Wasser, 2011; Da-Silva *et al.*, 2012). Thus, incorporation of mushrooms as a daily food or as a supplement, containing many nutrients such as vanadium (Poucheret *et al.*, 1998) and bioactive substances, can assist in maintaining more normal cellular and immune function which helps in normalizing blood glucose levels (Zhou and Han, 2008; Han and Liu, 2009; Guo *et al.*, 2010; Brennan *et al.*, 2012).

Few previous studies support that *Pleurotus ostreatus* improves glycemic status of diabetes (Khatun *et al.*, 2007; Choudhury *et al.*, 2008 and 2011), hence it is important to find out whether *Pleurotus ostreatus* has any anti-hyperglycemic activity on normoglycemic subjects or not. In a study supporting the ameliorative effect of *Pleurotus ostreatus* on glycemic status of non-diabetic hypertensive male volunteers (Choudhury *et al.*, 2012). On the other hand it was also important to find out the sex variation in effectiveness of mushrooms. The aim of the study was to evaluate the effect of *Pleurotus ostreatus* on glycemic status of hypertensive non-diabetic females.

MATERIALS AND METHODS

Under supervision of Department of Pharmacy, Jahangirnagar University, the study was conducted in the Laboratory of Strengthening Mushroom Development Project, National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka. A total 14 hypertensive (systolic BP ranged from 120-155 mmHg and diastolic BP ranged from 80-100 mmHg) non-diabetic female volunteers aged (years) from 29 to 62 were included. After getting informed written consent, the details history was taken from the subjects including age, sex, occupation, educational status, marital status, family history

and drug history. Any acute or chronic disease or medications were excluded from the study.

Systolic BP $\geq$ 140 mmHg and/or Diastolic BP $\geq$ 90 mmHg were considered as hypertension. Fasting plasma glucose < 7 mmol/L were considered as non-diabetes.

Eight to ten hours fasting blood sample was collected for analysis. Mushroom capsules were supplied to take two capsules three times daily. Each capsule contains 500 mg *Pleurotus ostreatus* powder, so that each subject took 3 gms mushroom powder daily. After three months the subjects were evaluated and all the investigation procedures were repeated. If any drug previously getting by the subjects, it was continued.

Ten ml of blood sample was collected from median cubital vein with all aseptic precaution from the subjects. It was immediately poured into test tube containing fluoride and EDTA. The test tube then gently shaken so that anti coagulant and fluoride mix with the blood properly. Then it was centrifuged by 3000 rpm for 5 minutes. Plasma was separated which were transferred into two eppendorf containing 1 ml in each. All the tests were carried out as early as possible.

The fresh fruiting body of *Pleurotus ostreatus* was collected from the culture house of NAMDEC. They were dried using an electric drier at moisture level 4-5%. Then grinded and pour into capsule shell which contains 500 mg powder. Prepared capsules were preserved into moisture free glass containers for dispense.

Both systolic and diastolic blood pressure was measured following standard procedure using sphygmomanometer by a trained physician. Mean of duplicate measurements was taken. Plasma creatinine was estimated to detect renal impairment by using 'alkaline picrate' method. Plasma glucose level was estimated by enzymatic 'Glucose oxidase method'. Glycated hemoglobin was estimated by a photometric method using 'Stanbio reagent kit'. Analysis was done by semi auto biochemical analyzer 3000 evaluation using commercially available reagent kit. All the tests were carried out as early as possible.

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 mean ± SE plasma creatinine (mg/dl) before and three months after mushroom treatment was 0.73 ± 0.05 and 0.08 ± 0.04 ranged from 0.4 - 1.1 and 0.5 - 1.0 respectively. There was no statistically significant mean difference ($p = 0.174$) between the two periods of observation (Fig. 1). This finding suggests that there was no harmful effect on kidney functions among, the mushroom-fed females.

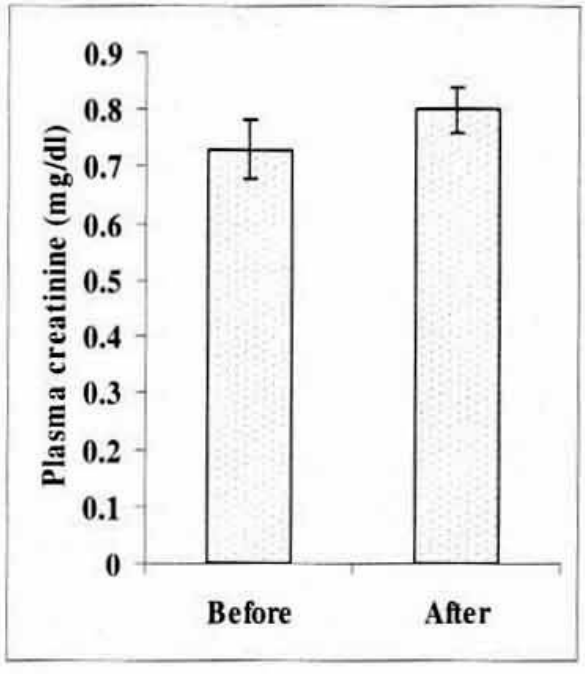

Fig. 1. Mean (± SE) plasma creatinine before and 3 months after mushroom supplementation.

The mean ± SE of fasting plasma glucose (mmol/L) before and 3 months after mushroom treatment was 5.61 ± 0.24 and 4.82 ± 0.37, ranged from 3.7 - 6.9 and 3.5 - 6.6 respectively (Fig. 2). A significant 14.08% reduction of plasma glucose was observed ($p = 0.003$) between the two periods.

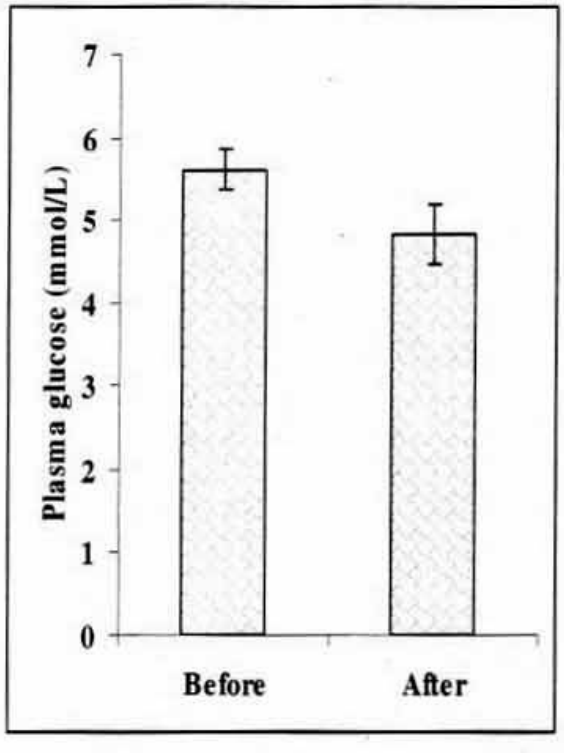

Fig. 2. Mean (± SE) fasting plasma glucose before and 3 months after mushroom supplementation.

The mean ± SE HbA1c (%) before and 3 months after mushroom treatment were 4.33 ± 0.26 and 4.17 ± 0.27, ranged from 3.1- 6.2 and 3.1 – 6.1 respectively. A non-significant 3.69% reduction of HbA1c ($p = 0.340$) was observed (Fig. 3).

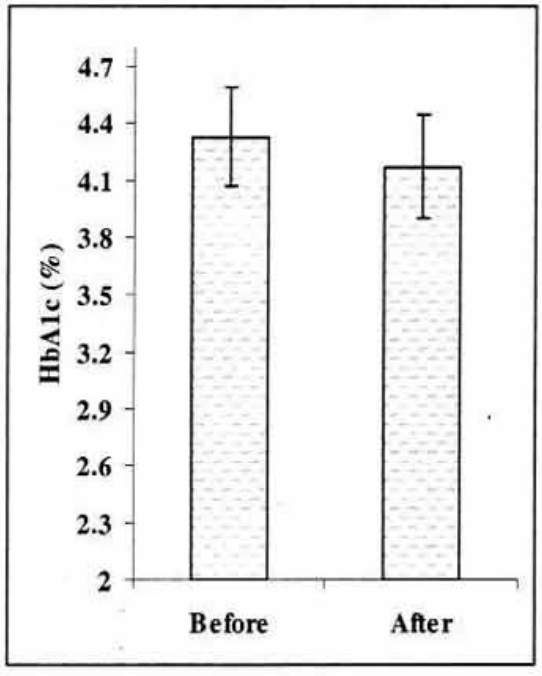

Fig. 3. Mean (± SE) HbA1c before and 3 months after mushroom treatment.

Influence of *Pleurotus ostreatus* on blood glucose level in hypertensive non-diabetic female subjects has been evaluated in this clinical trial of 14 subjects. It was observed that blood glucose was reduced significantly after 3 months consumption of *Pleurotus ostreatus* mushroom. There was also reducing impact on HbA1c in non-diabetic females. The significant fall in fasting blood glucose and some reduction of HbA1c may be attributed to the hypoglycemic potential of the *Pleurotus ostreatus* mushroom supplement. Although it was reported that mushroom significantly reduced blood glucose level in diabetic subjects (Khatun *et al.*, 2007 and Choudhury *et al.*, 2008) and reduced glycated hemoglobin in streptozotocin diabetic mice (Swanston-Flatt *et al.*, 1989), but there is lacking of data on the effect of oyster mushroom on non-diabetes. This author observed in his previous study that oyster mushroom has the ability to reduce fasting plasma glucose and HbA1c within normal physiological range in hypertensive non-diabetic male volunteers (Choudhury *et al.*, 2012). Current study agrees the previous one and it is applicable for female sex.

Water soluble extracts from *Lentinus edodes, Pleurotus ostreatus* and *Phellinus linteus* showed blood glucose and triglyceride (TG) lowering effects in the streptozotocin-induced diabetic model (Kim *et al.*, 1997 and Kim *et al.*, 2001). Such results suggest that these mushrooms have potential preventive and therapeutic action on diabetes mellitus. In a study Krishna *et al.* (2009) assess the hypoglycaemic effect of *Pleurotus ostreatus* in alloxan diabetic rats and a dose dependent decrease in blood glucose level was obtained with *Pleurotus ostreatus* at doses of 250, 500 and 1000 mg/kg. Raphel *et al.* (2002) also reported a decrease in blood glucose level after administration of *Phyllanthus amarus*

extract. Current study is also supportive with them. But further studies with large sample size are needed. In conclusion, the findings of the study throw light on the potential use of *Pleurotus ostreatus* for better glycemic control and better quality of life.

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Relationship among Cultivated Strains of *Pleurotus florida* Based on RAPD

Shohana Nusrat[1], Nuhu Alam[1], Md. Bazlul Karim Choudhury[2], Akhter Jahan Kakon, Ruhul Amin and Nirod Chandra Sarker
National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

This experiment was conducted to screening the suitable conditions for mycelial growth and phylogenetic relationship of the selected strains of *Pleurotus florida*. Suitable temperature for the mycelial growth was obtained at 25°C and minimum mycelial growth was found at 10°C. This mushroom has a broad pH range for its mycelial growth and most favorable growth was observed at pH 6. In case of culture media, results indicated that mushroom complete glucose peptone, and yeast malt extract media were the favorable, while Hennerberg and Hoppkins were the unfavorable media. Dextrin was the best and xylose was the less effective carbon sources. Results revealed that inorganic nitrogen sources were the less effective for the mycelial growth of *P. florida*. Investigation of genetic diversity is necessary to confidently identify the strains. Five strains of *P. florida* were also analyzed by RAPD with 20 arbitrary primers. Twelve primers were efficient to amplify the genomic DNA. The number of amplified bands was variable depending on the primers or the strains. The size of polymorphic fragments was obtained in the range of 0.2 to 2.1 kb.

Key words: Mycelial growth, *Pleurotus florida*, Phylogenetic relation, RAPD.

INTRODUCTION

Pleurotus florida is a commercially important edible mushroom and it is widespread in various geographical regions of Bangladesh and south-east Asia. It has very good abilities to grow at a wide range of temperatures utilizing various lignocelluloses, so that is becoming more popular throughout the world. *P. florida* is a good source of dietary fiber and other valuable nutrients (Alam *et al.*, 2008a). This mushroom contained a number of biologically active compounds with therapeutic activities such as modulation of the immune system, hypoglycemic and antithrombotic activities, decreasing blood lipid concentrations, prevention of high blood pressure and atherosclerosis (Alam *et al.*, 2009a). It has a tetrapolar system of sexual compatibility and a well defined haplo dikaryotic life cycle. Two compatible monokaryotic hyphae fuse and produce a dikaryotic mycelium in which the two parental nuclei remain independent throughout the vegetative growth. Commercial strains of mushrooms sometimes decline in their performance of production or reduction in the yield due to several consecutive subcultures. Biological efficiency can be sometimes raised by optimization of cultural conditions, such as combining different substrates and/or adding nutritional supplements (Watling, 1992). Alam *et al.* (2010) reported that mycelium cultivation is enhanced by different

[1] Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh; [2] Department of Biochemistry, Faridpur Medical College, Faridpur, Bangladesh.

environmental and nutritional factors as well as propagation of mycelium is an earlier and essential step to cultivate fruiting bodies of mushrooms.

Evaluation of genetic and phenotypic diversity is necessary to distinguish genotypes of *P. florida* when seeking traits of interest and to identify strains with high yield potential. Various molecular genetic tools have been introduced for the verification of fungi, such as RFLP, RAPD, and SSU rDNA and ITS sequence analyses. RAPD was particularly successfully when applied for the verification of *P. florida* strains from various hosts with a wide range of geographical origins (Zervakis *et al.*, 2001). The aim of this study was to investigate molecular genomic polymorphism among the selected strains of *P. florida* RAPD analysis. Experiments also explored the physical and chemical conditions for the mycelial growth.

MATERIALS AND METHODS

Mushroom strains: Five cultivated strains of *P. florida* such as PF-1, PF-2, PF-3, PF-4 and PF-5 were obtained from the National Mushroom Development and Extension Centre (NAMDEC), Department of Agricultural Extension, Ministry of Agriculture, Savar, Dhaka. Pure cultures were deposited in Mycology, Plant Pathology and Plant Protection laboratory, Department of Botany, Jahangirnagar University, Savar, Dhaka.

Temperature and pH: Six different temperatures such as 10, 15, 20, 25, 30 and 35 °C were used to find out the optimum temperature for the mycelial growth of *P. florida*. A 5 mm diameter agar plug removed from 10 days of old culture and placed in the centre of PDA plate. The medium was adjusted to pH 6 and incubated for 10 days at the temperature 10, 15, 20, 25, 30 and 35^{0}C. In case of pH, the medium was adjusted to pH 4, 5, 6, 7, 8 and 9 with the addition of 1 N NaOH or HCl before autoclave and incubated for 10 days at 25°C temperature. The measurement of mycelial growth was performed according to the methods described by Alam *et al.* (2010).

Culture media: Ten different culture media (Czapek dox, glucose peptone, glucose tryptone, Hamada, Hennerberg, Hoppkins, Lilly, mushroom complete, potato dextrose agar and yeast malt extract) were used to investigate the mycelial growth of *P. florida*. The compositions of culture media were prepared according to described method (Alam *et al.*, 2009b). The media were adjusted to pH 6 before autoclave.

Carbon and nitrogen sources: This experiments were performed on the basal medium (0.05g $MgSO_4$, 0.46g KH_2PO_4, 1.0g K_2HPO_4, 120µg thiamine-HCl, 20g agar and 1 litter of distilled water) supplemented with each of 10 carbon sources (Dextrin, Fructose, Galactose, Glucose, Lactose, Maltose, Mannose, Sorbitol, Sucrose and Xylose) and ten nitrogen sources (Alanine, Ammonium acetate, Ammonium phosphate, Arginine, Calcium nitrate, Glycine, Histidine, Methionine, Potassium nitrate and Urea). To evaluate the most favorable carbon and nitrogen sources for the mycelial growth, each carbon source with 5g of peptone was added to the basal medium separately at the concentration of 0.1 M/1000 ml and mixed thoroughly. Each nitrogen source with 20g of glucose was

supplemented to the basal medium at the concentration of 0.02 M (Alam *et al.*, 2009b). In both cases, the basal medium was adjusted to pH 6 before autoclaving.

DNA extraction: Genomic DNA was extracted according to the procedure of Lee and Taylor (1990) with some modifications as follows. Fresh mycelia were collected from the 10 days old culture on PDA medium and were frozen with liquid nitrogen. Frozen mycelia were grounded with sterilized mortar-pestle and kept in 1.5 ml micro tube. As extraction buffer, equal amount of 50 mM Tris-HCl (pH 7.5), 50 mM EDTA (pH 8) and 1% sarkosyl was added to the micro tube and incubated at 65°C for 30 min. After incubation, same amount of PCI (25 ml phenol: 24 ml chloroform: 1 ml isoamyl-alcohol) was added, vortexed and centrifuged at 4°C, 10 min, 12000 rpm. After wards, only supernatant of upper part was taken in 1.5 ml micro tube, added 1000 µl of 99.9% alcohol and centrifuged at 4°C, 5 min, 12000 rpm. In this case, supernatant was removed, added 500 µl of 70% alcohol with precipitated DNA, vortexed and centrifuged at 4°C, 5 min, 12000 rpm. Again supernatant was removed and waited until residual alcohol evaporated. Finally 500 µl of sterilized distilled water was added. DNA concentration was measured using spectrophotometer (Cubero *et al.*, 1999).

RAPD analysis: Genomic DNA was amplified by the RAPD technique (Williams *et al.*, 1990) in which 20 sorts of arbitrary 10-base oligonucleotide primers (Operon Technologies Inc.) i.e. OPA-01, 5'CAGGCCCTTC3'; OPA-02, TGCCGAGCTG; OPA-03, AGTCAGCCAC; OPA-04, AATCGGGCTG; OPA-05, AGGGGTCTTG; OPA-06, GGTCCCTGAC; OPA-07, GAAACGGGTG; OPA-08, GTGACGTAGG; OPA-09, GGGTAACGCC; OPA-10, GTGATCGCAG; OPA-11, CAATCGCCGT; OPA-12, TGCGCGATAG; OPA-13, CAGCACCCAC; OPA-14, TCTGTGCTGG; OPA-15, TTCCGAACCC; OPA-16, AGCCAGCGAA; OPA-17, GACCGCTTGT; OPA-18, AGGTGACCGT; OPA-19, CAAACGTCGG; OPA-20, 5'GTTGCGATCC3' were used to produce amplified fragments. RAPD-PCR reaction was performed using a thermal cycler with an initial denaturation stage of 5 minutes at 94^{0}C, followed by 35 cycles of denaturation for 1 minute at 94^{0}C, annealing for 1 minute at 36°C, extension for 2 minutes at 72^{0}C and a final extension for 7 minutes at 72^{0}C. RAPD products were electrophoresed on 1.4% agarose gel in 1 × TAE buffer for 1.15 hour at 100V, with a 1kb DNA ladder as a size marker and then stained while agitated in an EtBr solution (0.5% µg/ml). The stained gels were visualized and photographed using a UV transilluminator. RAPD bands were recorded as present (1) or absent (0) to generate the data matrix. The similarity coefficients (S) were calculated between isolates across bands for all primers using the formula S = 2Nxy/ (Nx + Ny), where Nx and Ny are the number of bands shared by the two strains (Nei and Li, 1979).

RESULTS AND DISCUSSION

Effect of temperature and pH: A temperature range of 10-35^{0}C was considered to find out the most suitable one. The highest average mycelial growth (86.82 mm) was recorded at 25^{0}C and lowest (9.33 mm) at 10^{0}C temperature. Almost similar mycelial growth was observed at the temperature of 15^{0}C and 35^{0}C (Table 1). In case of maximum mycelial

growth, there are no significant difference between the temperature of 15°C and 35°C. Therefore, experimental results indicated that optimum temperature range was 20 - 30°C for the mycelial growth of *P. florida*. The findings of this study are comparable to the previous study of Alam *et al.* (2008b), he reported that 30°C is the optimum temperature for the mycelial growth of *P. adiposa*.

Table 1. Effect of temperature on the mycelial growth in different strains of *Pleurotus florida*

Strains	Mycelial growth (mm)*					
	10°C	15°C	20°C	25°C	30°C	35°C
PF-1	9.08±1.0	45.67±1.4	62.50±1.0	87.00±0.0	63.92±5.3	24.67±1.1
PF-2	8.83±0.8	48.58±4.4	66.67±0.8	87.00±0.0	76.67±0.4	65.42±6.5
PF-3	6.25±0.5	35.58±1.4	67.42±0.5	86.08±0.5	67.00±0.0	22.42±6.2
PF-4	10.58±1.4	41.58±5.1	58.83±1.4	87.00±0.0	75.83±0.6	75.83±0.6
PF-5	11.92±1.2	36.33±3.5	70.92±1.2	87.00±0.0	66.25±0.3	41.08±5.3
Mean	9.33±0.9	41.55±3.2	65.21±0.9	86.82±0.1	69.93±1.3	45.88±3.9

*Mean of 3 replications

pH value at the range of 4-9 was studied to find out the suitable culture conditions for the mycelial growth. The highest radial growth of mycelium was found at pH 6 (Table 2). There was no significant variation between the ranges of pH 6-7 on the mycelial growth. This result is agreeable with the data collection from the study of Hur (2008). He studied that the cultural characteristics and log-mediated cultivation of *P. linteus* and found that the pH value 6 was the best. Present results indicated that *P. florida* grew well at acidic or neutral or alkaline conditions.

Table 2. Effect of pH on the mycelial growth in different strains of *Pleurotus florida*

Strains	Mycelial growth (mm)*					
	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
PF-1	44.17±4.5	76.25±0.4	87.00±0.0	81.17±1.4	76.78±0.4	57.33±2.5
PF-2	61.75±2.2	76.50±0.4	86.83±0.3	82.58±0.3	76.67±0.4	67.00±0.0
PF-3	49.33±1.6	66.58±0.3	86.83±0.3	77.42±5.0	74.92±0.2	59.25±0.9
PF-4	34.75±1.2	65.92±0.3	87.00±0.0	83.78±2.8	74.33±2.0	52.89±1.6
PF-5	35.25±0.7	56.92±0.2	86.75±0.5	79.67±4.2	77.00±0.0	37.22±0.7
Mean	44.45±2.1	68.43±0.3	86.88±0.2	80.92±2.7	75.94±0.6	54.74±1.1

*Mean of 3 replications.

Effect of culture media: Ten different culture media were used to find out the optimum mycelial growth of the selected strains of *P. florida*. According to the mycelial growth results indicated that mushroom complete, glucose peptone, and yeast malt extract, media were the most favorable, while Hennerberg and Hoppkins were the unfavorable media for the vegetative growth of *P. florida*. Average highest and lowest mycelial growth was recorded in mushroom complete (86.58 mm) and Hennerberg (32.02 mm), respectively (Table 3). Hur (2008) reported that the excellent mycelial growth of *P. linteus* was found in mushroom complete medium which is almost similar to our findings.

Effect of carbon and nitrogen sources: Among the ten different carbon sources, dextrin was found to be the best for the mycelial propagation of *P. florida* and followed by fructose, mannose, sucrose and maltose. On the other hand xylose and galactose were the most unfavorable carbon sources (Table 4). This result is similar to that of Shim *et al.* (2003). Ten different nitrogen sources were assayed to find out vegetative growth conditions of *P. florida*. Among the nitrogen sources, arginine was found to be the best and followed by ammonium acetate and urea. Mycelial growth was totally absent in alanine, however, lowest mycelial growth were recorded in histidine and methionine (Table 5). Shim *et al.* (2005) reported that glycine was the most favorable nitrogen source, which result is not similar to our findings. They also clarified that histidine was the most unfavorable nitrogen sources for the mycelial growth of *M. procera*, which is similar to our result. In general organic nitrogen sources are more effective then inorganic nitrogen sources.

Table 3. Effect of culture media on the mycelial growth in different strains of *Pleurotus florida*

Strains	Mycelial growth (mm)*									
	CZA	GLP	GLT	HAM	HEN	HOP	LIL	MUC	PDA	YEM
PF-1	42.58±2.6	86.67±0.4	86.25±0.6	80.42±0.6	34.42±1.3	42.92±3.2	68.75±1.6	87.00±0.0	80.58±1.2	87.00±0.0
PF-2	42.08±1.1	87.00±0.0	85.08±1.5	83.17±2.3	33.08±2.4	35.33±0.5	71.67±1.2	87.00±0.0	76.89±3.0	84.33±1.8
PF-3	39.33±2.7	86.08±0.6	83.50±1.6	81.08±1.0	25.92±1.8	30.33±0.8	64.83±4.0	84.92±1.0	80.17±1.0	87.00±0.0
PF-4	40.17±3.2	86.50±0.4	85.89±1.2	81.67±0.7	38.67±1.4	42.75±3.0	69.75±0.3	87.00±0.0	82.50±3.6	86.58±0.8
PF-5	38.92±0.7	86.58±0.5	81.92±1.9	77.92±1.4	28.00±4.5	33.75±0.8	59.00±3.6	87.00±0.0	86.75±0.5	87.00±0.0
Mean	40.61±2.6	86.57±0.4	84.42±1.4	80.85±1.2	32.02±2.3	37.02±1.7	67.6±2.2	86.58±0.2	81.38±1.9	86.38±0.5

*Mean of 3 replications. CZA: Czapek Dox, GLP: Glucose peptone, GLT: Glucose tryptone, HAM: Hamada, HEN: Hennerberg, HOP: Hoppkins, LIL: Lilly, MUC: Mushroom complete, PDA: Potato dextrose agar and YEM: Yeast-malt extract.

Table 4. Effect of carbon sources on the mycelial growth in different strains of *Pleurotus florida*

Strain	Mycelial growth (mm)*									
	Dex	Fru	Gal	Glu	Lac	Mal	Man	Sor	Suc	Xyl
PF-1	72.10±5.5	70.20±8.5	21.30±1.6	35.60±2.0	49.30±3.7	50.40±3.7	66.30±6.4	42.90±6.0	39.70±2.2	13.30±2.1
PF-2	65.80±2.9	71.30±8.0	13.00±0.5	32.80±1.2	35.40±1.7	45.30±2.1	74.30±3.8	37.80±2.6	61.00±3.3	8.30±0.8
PF-3	71.40±3.3	50.00±4.0	13.50±2.1	30.10±2.4	35.30±0.3	47.70±5.0	53.3±10.6	40.50±1.5	38.10±1.9	8.60±1.5
PF-4	76.30±6.4	63.80±5.0	22.80±0.3	45.80±4.4	32.80±1.3	61.40±5.0	71.00±3.8	42.80±3.9	64.80±4.5	10.70±0.6
PF-5	62.40±3.7	42.60±6.5	19.90±3.1	24.50±0.8	24.10±0.8	52.6±14.6	34.70±1.9	40.50±3.5	58.50±9.6	6.10±0.2
Mean	69.6±4.4	59.57±6.3	18.10±1.5	33.76±2.2	35.38±1.6	51.48±6.1	54.52±5.3	40.9±3.5	52.42±4.3	9.4±1.0

*Mean of 3 replications, Dex: Dextrin, Fru: Fructose, Gal: Galactose, Glu: Glucose, Lac: Lactose, Mal: Maltose, Man: Mannose, Sor: Sorbitol, Suc: Sucrose and Xyl: Xylose.

Table 5. Effect of nitrogen sources on the mycelial growth in different strains of *Pleurotus florida*

Strain	Mycelial growth (mm)*									
s	Amp	Arg	Ama	Ala	Can	Gly	His	Met	Pon	Ure
PF-1	31.25±2.1	61.89±2.9	53.75±1.6	-	21.92±5.4	42.25±4.4	21.25±1.3	25.00±0.6	37.33±0.7	47.58±5.0
PF-2	32.67±0.3	54.25±12.7	55.92±1.8	-	21.00±0.5	37.08±2.4	18.83±0.8	21.67±0.3	34.22±1.5	38.50±5.0
PF-3	26.83±1.3	48.08±10.8	52.33±3.2	-	24.42±3.8	36.92±5.7	17.58±1.0	22.58±0.3	34.25±1.9	33.92±1.3
PF-4	42.50±2.1	65.58±1.5	51.33±0.7	-	41.08±3.8	43.08±2.8	20.58±1.0	25.08±0.8	31.50±1.1	66.08±3.8
PF-5	34.75±1.5	58.17±3.3	53.33±2.3	-	33.00±1.1	42.25±1.7	18.50±1.0	16.42±0.3	33.00±1.1	47.50±1.0
Mean	33.6±1.5	57.59±6.3	53.33±1.9	-	28.28±2.9	40.31±3.7	19.35±1.0	22.15±0.5	34.06±1.3	46.72±3.2

*Mean of 5 replications. Ala: Alanine, Ama: Ammonium acetate, Amp: Ammonium phosphate, Arg: Arginine, Can: Calcium nitrate, Gly: Glycine, His: Histidine, Met: Methionine, Pon: Potassium nitrate and Ure: Urea.

Table 6. DNA bands in different strains of *Pleurotus florida* by RAPD assay on 10 base OPA primers

Primers	DNA band (kb)	Strain of *Pleurotus florida*				
		1	2	3	4	5
OPA-01	1.7	+	-	-	-	+
	1.4	+	-	-	-	-
	1.2	+	+	+	+	+
OPA-02	2.0	+	+	-	-	+
	1.5	+	-	-	-	+
	0.9	-	+	+	+	+
	0.6	+	+	-	-	+
OPA-03	1.0	+	+	-	-	-
	0.8	+	+	+	+	-
	0.7	-	+	+	+	-
	0.4	+	+	+	+	+
OPA-05	1.5	+	-	-	-	-
	0.7	+	-	-	-	+
	0.2	-	+	+	+	+
OPA-07	1.5	+	+	+	+	+
	1.3	+	+	+	+	-
	1.0	-	+	+	+	+
	0.6	-	+	+	+	+
OPA-09	2.1	+	-	-	-	-
	1.3	+	+	-	-	+
	1.0	+	+	+	+	-
	0.9	+	+	+	+	-
OPA-10	1.2	-	-	-	-	-
	1.0	-	+	+	+	-
	0.7	+	+	+	+	-
	0.5	-	-	-	-	+
OPA-11	1.7	-	-	-	-	+
	1.2	+	-	-	-	-
	0.6	-	+	+	+	+
	0.3	-	+	+	+	+
OPA-12	1.5	+	-	-	-	-
	0.7	+	-	-	-	-
	0.2	-	+	+	+	+
OPA-13	1.7	+	-	-	-	-
	1.1	+	+	+	+	+
	0.9	+	+	+	+	+
	0.5	-	+	+	+	-
OPA-18	1.1	-	-	-	+	+
	0.8	-	-	-	-	+
	0.6	-	+	+	+	-
	0.2	+	+	+	+	-
OPA-20	1.5	+	-	-	-	-
	0.8	+	+	+	+	+
	0.4	-	+	+	+	+

Lane 1, PF-1; 2, PF-2; 3, PF-3; 4, PF-4; 5, PF-5, - indicate absence of DNA band, + indicate presence of DNA band.

RAPD analysis: Twenty primers were used to amplify the segments of DNA in five different strains of *P. florida*. Among the 20 primers, 12 primers, OPA-01, OPA-02,

OPA-3, OPA-05, OPA-07, OPA-09, OPA-10, OPA-11, OPA-12, OPA-13, OPA-18 and OPA-20 were found to be efficient for amplifying the genomic DNA (Table 6). These 12 primers showed significant band profiles on the tested strains and high possibilities to screening of each strain (Fig. 1, 2, 3 4, 5 and 6). The size of these polymorphic fragments was in the range of 0.2 to 2.1 kb. Polymorphism of DNA bands showed the same characters in the replication tests. Therefore, if a certain strain is tested for DNA polymorphisms using the same primers, it could be identified whether the strain is the similar or not by consulting Table 6. Similar results have been reported by Ro *et al.* (2007) in the phylogenetic classification of some strains of *Pleurotus eryngii* and *Lentinus edodes* mushrooms respectively.

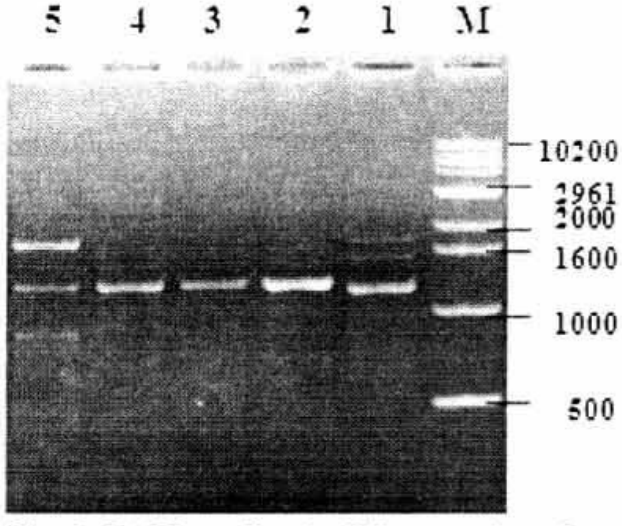

Fig. 1. RAPD profiles in different strains of *Pleurotus florida* with primer OPA-1. M, molecular size marker (1 kb DNA ladder); lane 1, PF-1; 2, PF-2; 3, PF-3; 4, PF-4; 5, PF-5.

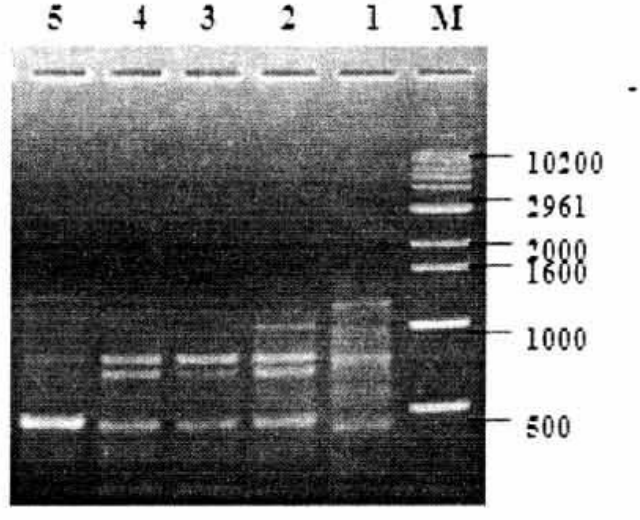

Fig. 2. RAPD profiles in different strains of *Pleurotus florida* with primer OPA-3. M, molecular size marker (1 kb DNA ladder); lane 1, PF-1; 2, PF-2; 3, PF-3; 4, PF-4; 5, PF-5.

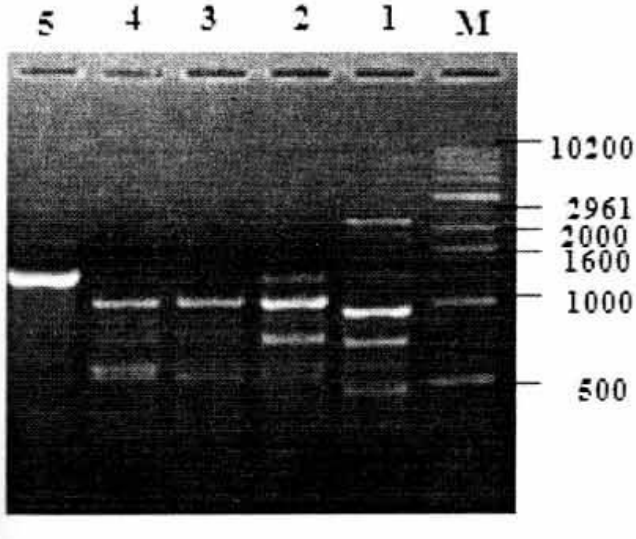

Fig. 3. RAPD profiles in different strains of *Pleurotus florida* with primer OPA-9. M, molecular size marker (1 kb DNA ladder); lane 1, PF-1; 2, PF-2; 3, PF-3; 4, PF-4; 5, PF-5

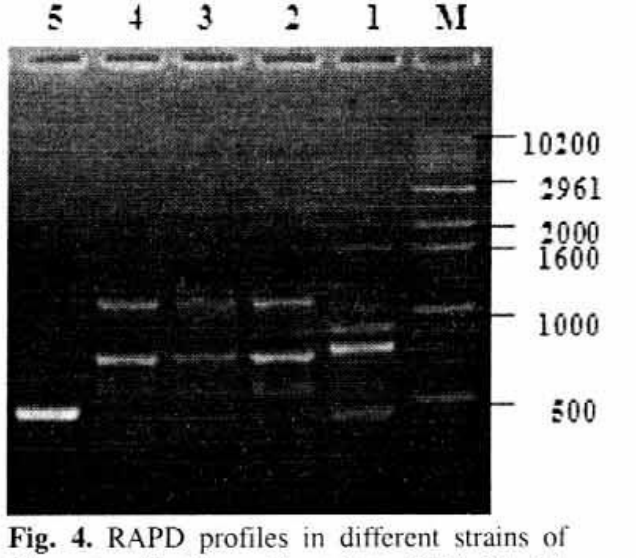

Fig. 4. RAPD profiles in different strains of *Pleurotus florida* with primer OPA-10. M, molecular size marker (1 kb DNA ladder); lane 1, PF-1; 2, PF-2; 3, PF-3; 4, PF-4; 5, PF-5

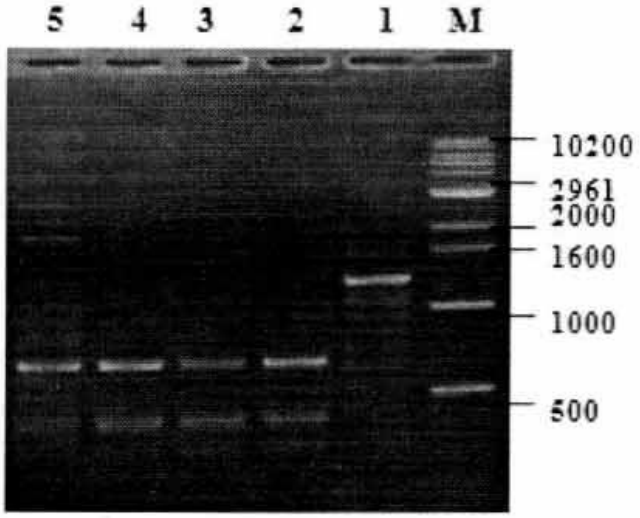

Fig. 5. RAPD profiles in different strains of *Pleurotus florida* with primer OPA-11. M, molecular size marker (1 kb DNA ladder); lane 1, PF-1; 2, PF-2; 3, PF-3; 4, PF-4; 5, PF-5.

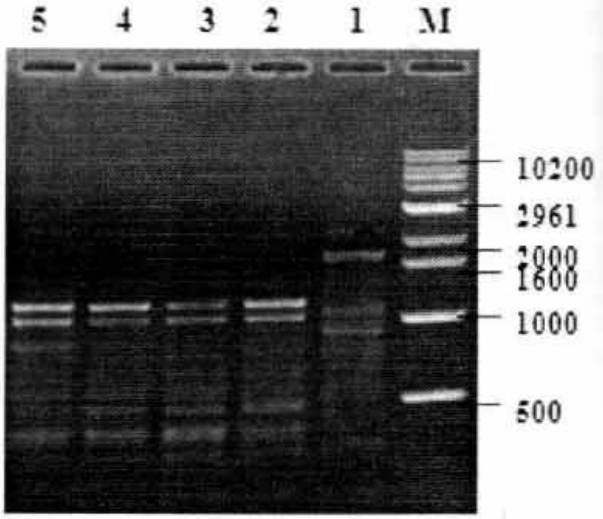

Fig. 6. RAPD profiles in different strains of *Pleurotus florida* with primer OPA-13. M, molecular size marker (1 kb DNA ladder); lane 1, PF-1; 2, PF-2; 3, PF-3; 4, PF-4; 5, PF-5.

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

Manirul shaheen, Mahbuba Moonmoon[1], Abdus Salam Khan, Taufiqur Rahman[2] and Md. Mominul Hoque
National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

Six different mother culture media viz. T_1= rice straw + wheat bran (3:1), T_2= rice straw, T_3= wheat grain, T_4= paddy grain, T_5= saw dust and T_6= rice husk + wheat bran (3:1) were used to find out their effect on growth and yield of straw mushroom (*Volvariella volvaceae).* Minimum days (9.00 days) required to completion of mycelium running and the highest yield (935.30g/bed) were obtained from T_4 treatment where paddy grain used as media of mother culture. In treatment T_5 no mycelium was running and no yield was obtained. Maximum days (24.50 days) required to completion of mycelium running and the lowest yield (494.00 g/bed) were obtained from T_2 treatment. The highest contamination rate (100%) was found in T_5 and the lowest contamination rate (16.67%) was found in T_1 treatment.

Key words: Mother culture, Growth, Yield, *Volvariella volvaceae.*

INTRODUCTION

Paddy straw mushroom (*Volvariella volvaceae*) is one of the popular variety among the consumers because of its distinct flavor, pleasant tastes, higher protein content and shorter cropping duration compared to other cultivated mushrooms. It is one of the eminent edible mushrooms in Bangladesh and it is very much preferable for its attractive fruiting bodies as well as unique taste (Sarker *et al.*, 2012). Recently, these mushrooms have become a common edible mushroom in Bangladesh. Straw Mushrooms can be produced in large quantities in a short time, and it provides more protein per unit area than any other crop (Gupta, 1986). The edible straw mushroom is a fungus of the tropics and subtropics and has been traditionally cultivated in rice straw for many years in China and South East Asian countries. In 1971, cotton wastes were first introduced as heating material for growing the straw mushroom (Yau and Chang, 1972), and in 1973, cotton wastes had completely replaced the traditional paddy straw to grow the straw mushroom (Chang, 1974). A number of agricultural and industrial by-products and nonconventional plants including cotton waste, cotton seed hulls, coffee by-products, citronella bagasse, tobacco medium, waste tea leaf, pea nut shells, lawn grass, water hyacinth, and apple pomace have been successfully utilized by different workers for growing oyster and paddy straw mushroom (Miller 1987, Singh *et al.*, 1989, Leon *et al.*, 1983, Cho *et al.*, 1981, Tolentino, 1986, Upadhyay and Sohi, 1988). Rice straw is widely used as the

[1] Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh; [2] Department of Agricultural Extension, Bheramara, Kushtia.

substrate for the commercial cultivation of *Volvariella volvacea,* in Bangladesh. This substrate is available, cheap and has no proper utilization (Amin *et al.*, 2010). Paddy straw is the material freely available in Bangladesh and therefore cultivation of this mushroom is ideal in rural area where paddy straw is abundant after each paddy harvest and it can also provide additional income to the farmers. Straw alone is not sufficient as a composting material as it contains a little quantity nutrients and has a slow rate of decomposition (Anon, 1983). Therefore, straw mushrooms presently are grown in some other materials such as cotton waste, sugarcane bagasse, dried banana leaves, wheat bran, rice bran etc (Chang & Hayes, 1978). Unlike the oyster mushroom, the straw mushroom is highly sensitive to the climatic conditions and their fluctuation. The unavailable of mother culture is the common problem to cultivate of this mushroom. To overcome these problems, alternative mother culture is necessary.

Therefore, the present experiment was conducted to identify the suitable alternate mother culture media for the cultivation of paddy straw mushroom under Bangladesh conditions.

MATERIALS AND METHODS

The experiment was carried out from May to August 2013 at National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar, Dhaka. The strain VV-5 of *Volvariella volvaceae* was obtained from the culture house of NAMDEC.

Preparation of PDA media: Pure culture was prepared on PDA medium (20g glucose, 200g potato, 20g agar and 1 litter of distilled water) and sterilized in an autoclave for 20 minutes at 120^0C temperature under 1.5 kg/cm^2 pressure. A 5 mm diameter agar plug inoculums was removed from 10 days old culture grown on PDA petri dish and incubated at 30^0C temperature.

Preparation of mother culture (straw + wheat bran): Mother culture was prepared by chopping the rice straw into 3-4 inch size and mixed with wheat bran at the ratio of 3:1 with equal amount of water. Polypropylene bags of 7" × 10" size were filled with 300g of the above mentioned mixture and packed tightly. The necks of the bags were heat resistant plastic. A hole of about 2/3 deep of the volume of the bags was made at centre with a sharp end stick for space to put inoculum. The neck was plugged with cotton and covered with brown paper and tied with rubber. The packets were sterilized in an autoclave for one hour at 120^0C temperature under 1.5 kg/cm^2 pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. Pieces of PDA culture medium containing mycelium of above mentioned strains were placed aseptically in the hole of mother culture packet and again plugged as mentioned before. Then the inoculated packets were placed in the culture house at room temperature (30 ± 4^0 C).

Preparation of mother culture (straw): Mother culture was prepared by chopping the rice straw into 3-4 inch size with equal amount of water. Polypropylene bags of 7" × 10" size were filled with 300g of straw and packed tightly. The necks of the bags were heat

resistant plastic. A hole of about 2/3 deep of the volume of the bags was made at centre with a sharp end stick for space to put inoculums. The neck was plugged with cotton and covered with brown paper and tied with rubber bands. The packets were sterilization, inoculation and incubation in the same process.

Preparation of mother culture (wheat): Mother culture was prepared by mixing boiled wheat and calcium carbonate. For 300g mother, calcium carbonate was used at the rate of 0.5g per packet. Polypropylene bags of 7" × 10" size were filled with 300 g of the above mentioned mixture and packed tightly. The packets were sterilization, inoculation and incubation in the same process

Preparation of mother culture (sawdust): Mother culture was prepared by mixing sawdust and wheat bran at the ratio of 2:1. Calcium carbonate was used at the rate of 0.2% of the mixture. The moisture level of the mixture was maintained at 65% by adding tap water. Polypropylene bags of 7" × 10" size were filled with 300g of the above mentioned mixture and packed tightly. Then the packets were sterilization, inoculation and incubation in the same process.

Preparation of mother culture (rice husk + wheat bran): Mother culture was prepared by rice husk and mixed with wheat bran at the ratio of 3:1 with equal amount of water. Polypropylene bags of 7" × 10" size were filled with 300g of the above mentioned mixture and packed tightly. Then the packets were sterilization, inoculation and incubation in the same process.

Preparation of substrate: Rice straw was used as substrate and chopped into 4-5 cm lengths and poured into a net bag. Then the net bag with straw was treated with hot water at 60^0 C for one hour and allowed to drain out the excess water by hanging the bag for 20 hours.

Preparation of bed Transparent polythene sheet was placed in the incubation room and then both end open box was placed on the sheet. The size of box was 100 cm (length) × 30 cm (width) × 30 cm (height). The 4 cm of first layer was filled with hot water treated rice straw then spawning with mother culture according to treatments and 4 cm covered with treated rice straw and again spawning with mother culture and 3 cm covered with treated rice straw and again spawning with mother culture covered with 1 cm pasteurized straw.

RESULTS AND DISCUSSION

Days required to completion of mycelium running in mother culture: Time required to completion of mycelium running in mother culture was found significant and ranged from 9.00 to 24.50 days. The highest days (24.50 days) required to completion of mycelium running in the mother of straw substrate and the lowest days (9.00 days) required to completion of mycelium running in the mother of paddy grain. On the other hand when the mother culture prepared from sawdust no mycelium running was found in

the packet. Chen *et al.* (1973) and Chua *et al.* (1973) reported that *Volvariella volvaceae* mycelia grows very well on wide range of lignocelluloses waste such as banana leaves, sawdust, wheat bran, sugarcane baggage, waste tea dust, cotton waste, oil palm bunch wastes but their mean mycelia growth are comparably low in some these wastes. Similar results were shown by the present study. Substrates and supplements structure is an important factor for the growth of the mycelium as it should be suitable for penetration of the mycelium.

Days required to completion of mycelium running in bed: Days required to completion mycelium running in bed was found significant among the different treatments and ranged from 5.25 to 8.50 days (Table 1). The highest days (8.50 days) required to completion of mycelium running was found in the treatment T_6 followed by T_2 and T_3 and the lowest days (5.25 days) required to completion of mycelium running in the Treatment T_1 which was statistically similar to treatment T_4.

Days required to primordial initiation (DRPI): The days required to primordial initiation varied significantly and ranged from 7.50 to 12.75 days (Table 1). Statistically the highest days (12.75 days) required for primordial initiation was found in T_6 treatment followed by T_2 andT_3 which were also statistically similar toT_1 and T_4 treatments. Tripaty *et al.* (2011) reported that there were minimum 5 days and maximum 9 days required for primordia initiation when *Volvariella volvaceae* was cultured on rice bran, wheat bran, rice straw, sawdust, banana leaf and sugarcane baggage supplements. The results are relevant to the findings of Salmones *et al.* (1988) who found that the primordia appeared in 11 to 16 days. Similar findings were also reported by Moonmoon *et al.* (2008).

Days required to first harvest: The days required for first harvest (DRFH) varied significantly and ranged from 9.75 to 13.75 days (Table 1). The lowest days (9.75 days) required for first harvest was found in the treatment T_1 which was statistically similar to T_2 and T_4 and the highest days (13.75 days) were required for first harvest was found in T6. Similar results were reported by Chang and Miles (1987) who found that the days required for first harvest was 8 to 17 days. This little variation may due to different substrates or climates.

Number of effective fruiting body (NEFB): Statistically the number of effective fruiting body varied significantly and ranged from 27.75 to 56.50/bed (Table 1). The highest NEFB (56.50/bed) was observed in T_4 treatment followed by T_1, and the lowest NEFB (27.75/bed) was observed in treatment T_2. Tripaty *et al.* (2011) observed that the average number of effective fruiting body ranged from 40 to 91/bed, when *Volvariella volvaceae* was cultured on rice bran, wheat bran, rice straw, sawdust, banana leaf and sugarcane baggage substrate which was more or less similar with the present study.

Yield/bed (g): Significant variation was observed in yield per bed in different treatments and it was ranged from 494.00 to 935.30g/bed (Table 1). The highest yield (935.30 g/bed) was recorded in the treatment T_4 followed by T_1 (878.00 g/bed), T_6 (687 g/bed) and T_3 (594 g/bed). The lowest yield (494.00g/bed) was recorded in the treatment T_2. Tripaty *et al.* (2011) observed that the yield ranged from 500 to 1360g/bed, when *Volvariella*

volvaceae was cultured on rice bran, wheat bran, rice straw, sawdust, banana leaf and sugarcane baggage substrates. The findings of the present study are corroborated with Moonmoon *et al.* (2008) who reported that *V. volvaceae* gave the highest biological yield (1360 g/bed) when cultivated on wheat bran and rice bran substrates.

Average weight of fruiting bodies: Significantly highest (18.89g) average weight of fruiting bodies was found in T_1 treatment followed by T_2 and T_3. The lowest (16.40g) weight of fruiting bodies was found in T_6 treatment which was statistically similar to T_4.

Table 1. Effect of different media on growth, yield and yield attributes of *Volvariella volvaceae*

Treatments	Days required to completion of mycelium running in mother culture	Days required to mycelium running in bed	Days required to primordial initiation	Days required to first harvest	Number of effective fruiting body/ bed	Yield/ bed (g)	Fresh Weight of each Fruiting Body
T_1	16.50b	5.25c	7.50b	9.75c	46.50b	878.0b	18.89a
T_2	24.50a	6.25b	8.25b	10.75bc	27.75e	494.0e	17.85b
T_3	11.50c	6.25b	8.25b	11.00b	33.50d	594.8d	17.77b
T_4	9.00d	5.25c	7.75b	10.50bc	56.50a	935.3a	16.56c
T_5	0.00	0.00d	0.00c	0.00d	0.00f	0.00f	0.00d
T_6	12.50c	8.50a	12.75 a	13.75a	42.00c	687.0c	16.40c
CV (%)	**8.96**	**11.00**	**7.78**	**7.72**	**4.54**	**1.35**	**4.56**

Means followed by a common letter in a column are not significantly different at 5% level according to DMRT. T_1 = Rice straw + wheat bran (3:1), T_2 = only Rice straw, T_3 = Wheat grain, T_4 = Paddy grain, T_5 = Saw dust (SD), T_6 = Rice husk + wheat bran (3:1).

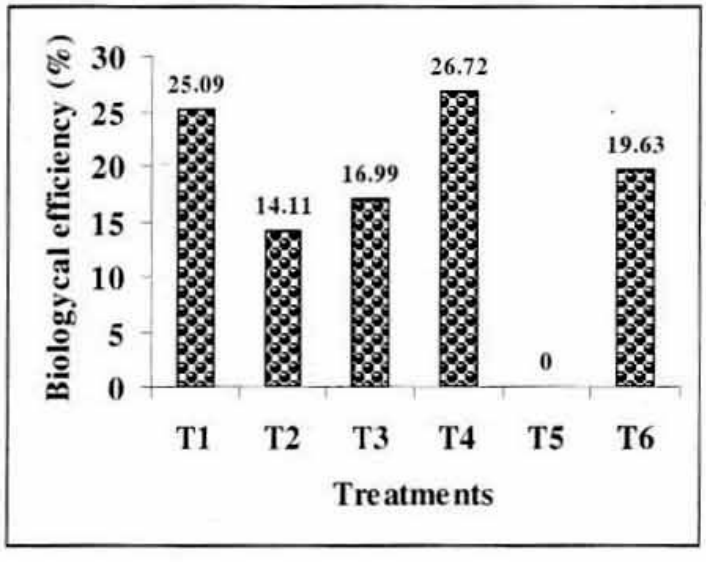

Fig. 1. Biological efficiency of different treatment on yield of *Volvariella volvacea.*

Biological efficiency: Significantly the highest biological efficiency (26.72%) was found in T_4 treatment followed by T_1 (25.09%). The lowest biological efficiency (14.11 %) was found in T_2 treatment (Fig. 1). Bolton and Blair (1982) and Fasidi (1996) reported that

rice husk is good for the production of *V. esculenta* because of its richness in oils and vitamins which are good stimulants for high mushrooms yield.

Contamination rate: Contamination rate of different treatments were also studied. There was a significant difference in percent contamination rate, which ranged from 16.67 to 100% (Fig. 2) by green mould and other bacteria during the period of mother culture. The highest contamination rate (100%) was found in T_5 and the lowest contamination rate (16.67%) was found in T_1. The contamination rates of other media were in between them.

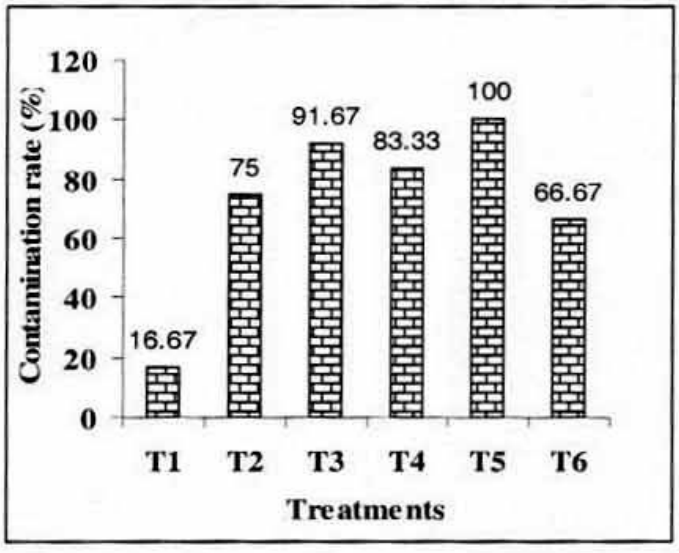

Fig. 2. Contamination rate of different mother culture on yield of *Volvariella volvacea.*

From the present data it was evident that treatment T_1 i.e. Rice straw + wheat bran (3:1) was suitable for *Volvariella volvaceae* mother culture media due to improved growth and less contamination rate.

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Organoleptic Evaluation of Mushroom Fry

Akhter Jahan Kakon, Md. Bazlul Karim Choudhury[1], Md. Kamrul Hassan, Abdus Salam Khan, Ruhul Amin and Tahera Binte Mujib
National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

For developing a standard mushroom fry the organoleptic study was carried out in the National Mushroom Development and Extension Center, Sobhanbag, Savar, Dhaka. The present study was conducted to prepare the fresh mushroom fries as influenced by different pretreatments. Fresh mushroom fries were prepared by frying the fresh mushrooms in oil with use of different pretreatments like B-1= Wheat flour (WF), B-2= Rice flour (RF), B-3= Chick-pea flour (CF), B-4= Anker flour (AF), B-5= Grass-pea flour (GF), B-6= Wheat flour+Rice flour (WF+RF), B-7= Wheat flour+Chick-pea flour (WF+CF), B-8=Wheat flour+Anker flour (WF+AF), B-9=Wheat flour+Grss pea flour (WF+GF), B-10= Wheat flour+Rice flour+Chick-pea flour (WF+RF+CF), B-11=(Control) mixed with equal amounts of all ingredients. Other common ingredients were salt 5.5g, red chili powder 4.00g, turmeric powder 0.5g, black cumin seed 0.5g. Basic sensory methods for food evaluation to measure degree of likeness were adopted for this research. Using a structured questionnaire, 20 male and female panelists independently assessed the samples for colour, appearance, flavor, crispiness, taste and overall acceptability. Considering all the parameters it is observable from the study that B-11 obtained the highest score (3.31±0.64, 79.38%) followed by B-8 (3.18±0.78, 76.21%). The average lowest score in the study was (2.61±0.96, 62.79%) that was obtained by sample B-1. Obtained other scores were B-2 (2.75±0.84, 65.96%), B-3 (2.97±0.88, 71.29), B-4 (2.99±0.80, 72.08%), B-5 (3.11±0.78, 74.63%), B-6 (2.96±0.80, 71.13%), B-7 (2.84±0.80, 68.5%), B-9 (2.93±0.72, 70.54%) and B-10 (3.03±0.68, 72.53%). For preparing mushroom fry the mixture of all kinds of flour B-11 is highly acceptable but for regular household purpose B-8 is most suitable. Preparation of mushroom fry using B-1 ingredients is not so much suitable in this study.

Keywords: Mushrooms, Deep frying, Batter, Organoleptic, Sensory evaluation.

INTRODUCTION

Oyster mushroom is highly perishable and starts deteriorating after few hours depending upon the storage condition. Shelf life of this mushroom varies from one to two days at the ambient temperature due to its high moisture content, delicate texture and unique physiology (Sexena and Rai, 1990). Hence it is necessary to develop suitable post harvest techniques for its prolonged preservation and usage.

Mushrooms have also been reported as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia and cancer (Bobek & Galbavy, 1999; Bobek *et al.*, 1995). Oyster mushroom (*Pleurotus ostreatus*) is one of the most important mushroom species due to its very good taste, nutritional and medicinal value.

[1] Department of Biochemistry, Faridpur Medical College, Faridpur, Bangladesh.

This mushroom can be cultivated on a wide range of lignocellulosic materials (Singh and Singh, 2005). Medical research has also shown that mushrooms contain eritadenine, a substance that helps prevent fat build-up in blood vessels. *Pleurotus spp.* is very much effective in reducing harmful plasma lipids (Alam *et al.*, 2007) and thus reduces the chance of atherosclerosis and other cardiovascular and artery-related disorders. *Pleurotus cystidiosus* is strong antioxidant (Li *et al.*, 2007).

In Bangladesh, fresh mushroom market is largely contribution of marginal and small growers with limited resources have to depend on local market for sale of their produce. Many times grower faces problems of over saturation of market and distress sale at highly non remunerative prices. The retention of fresh mushroom at the level of grower, whole seller, retailer and consumer further results in deterioration in the quality of the produce and economic loss. Presently, long-term preservation of mushrooms by drying, canning and pickling are in vogue (Chandrasekhar *et al.*, 2002). But surplus mushroom production during peak harvesting periods can be checked by adapting appropriate post harvest technology to process surplus mushrooms in the form of novel value added products rather going only for drying or canning. These value added products not only reduce the post harvest losses but also enhance the additional income to the mushroom growers and provide neutraceutical low fat, protein rich food to the consumers (Arumuganathan *et al.*, 2005).

There are many methods for mushroom or food processing. Frying is one of the oldest methods of food preparation. It improves the sensory quality of food by formation of attractive colour, crispy, taste and flavour. In our country mushroom fry is very common and popular item. Frying as a method of culinary treatment influences the characteristics of food products, particularly their nutritional value. A decrease is observed in the level of nutrients such as unsaturated fatty acids, minerals, and vitamins. At the same time, thermal treatment increases the digestibility of proteins, causes starch gelatinization, and produces specific sensory characteristics as a result of the Maillard reaction, as well as texture characteristics specific to the products (Bognar *et al.*, 1998, Kalogeropoulos *et al.*, 2007).

So, it is very necessary to develop easy, economic, available batter composition to obtain quality mushroom fry. The present study deals with the improvement of traditional mushroom fry.

MATERIALS AND METHODS

The experiment was conducted in the Quality Control and Quality Assurance (QCQA) laboratory of National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar, Dhaka, Bangladesh from October to December 2013.

Quality assessment: Once the mushroom fries were prepared, their qualities were assessed periodically in order to determine the best combination of fries by organoleptic

test. The quality parameters included appearance, colour, crispiness, flavor, taste, acceptability, etc.

Sample collection: Fresh oyster mushroom *Pleurotus cystidiosus* (Pcys 2) was collected from NAMDEC culture house and other ingredients were collected from local market.

Raw materials: Wheat flour, Chick-pea flour, Anker flour, Rice flour, Grass-pea flour, Common salt (NaCl), Red chili, Turmeric powder, Black cumin seed, refined Soybean oil ingredients were collected from local market. The different pre-treatments used in preparation of mushroom fries are given below (Table 1 and Table 2). They were designed as sample combination B-1 to B-11.

Treatments: B-1= Wheat flour (WF), B-2= Rice flour (RF), B-3= Chick-pea flour (CF), B-4= Anker flour (AF), B-5= Grass-pea flour (GF), B-6= Wheat flour + Rice flour (WF+RF), B-7= Wheat flour + Chick-pea flour (WF+CF), B-8= Wheat flour + Anker flour (WF+AF), B-9= Wheat flour + Grass-pea flour (WF+GF), B-10= Wheat flour + Rice flour + Chick-pea flour (WF+RF+CF), B-11= Equal mixture of all the treatments (Control).

Table 1. Different treatments

Batter composition	Batter amount (g)	Water (ml)
B-1=Wheat flour (WF)	150	250
B-2=Rice flour (RF)	170	180
B-3=Chick-pea flour (CF)	150	210
B-4=Anker flour (AF)	150	210
B-5=Grass-pea flour (GF)	150	230
B-6=WF+RF	150	200
B-7=WF+CF	150	210
B-8=WF+AF	150	210
B-9=(WF+GF)	150	210
B-10=WF+RF+CF	150	210
B-11=Control		

Mixing ingredients: Whole mushrooms were selected. Batter was made using salt 5.5g, red chili powder 4.00g, turmeric powder 0.5g and black cumin seed 0.5g with different/combine flour150g. They were stirred with water individually in large bowl to make thin paste (Table. 2).

Table 2. Common ingredients used for mushroom fry

Ingredients	Amount (approx)
Oyster Mushroom (pcys 2)	500.00g
Common salt (NaC1)	5.5g
Red chili powder	4.00g
Turmeric powder	0.5g
Black cumin seed	0.5g
Refined soybean oil	1.5litter

Procedure: Selected mushrooms were dipped into batter and coated well, then they were fried few at a time in deep oil heated to 365^{0}C until they became crispy and colored as golden brown. Eleven samples were then fried. It required different time period (3, 4, 7, 3, 4, 12, 4, 8, 5, 6, 8 min respectively) for obtaining the required quality. For frying purpose 1.5L of refined soybean oil was used. After completion of frying the additional amount of oil was drained on tissue paper (Fig. 1 and Fig. 2).

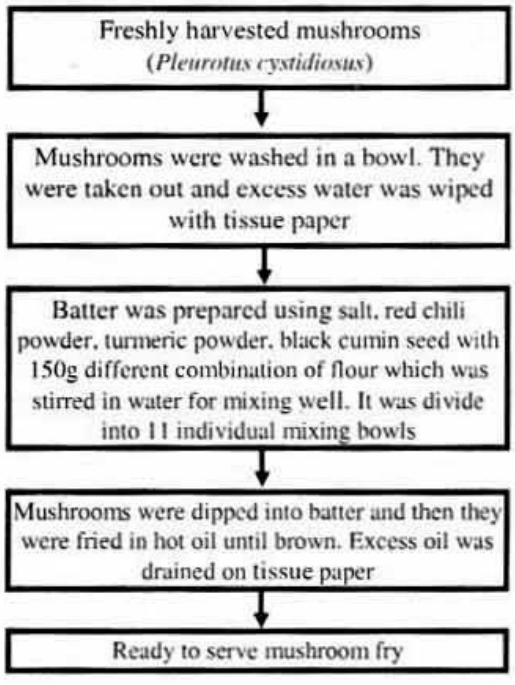

Fig.1. Flow chart for mushroom fries.

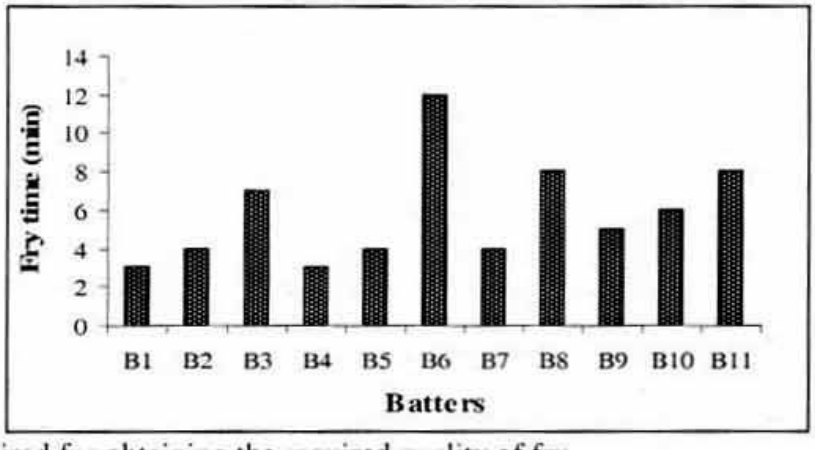

Fig. 2. Time required for obtaining the required quality of fry.

Serving order: Eleven samples were served to each consumer in monadic order. The samples were served coded. The first sample served was removed before the consumer received the second sample. Serving order was randomized so that approximately 50% of the consumers evaluated the "enhanced" sample first and 50% of the consumers evaluated the "non-enhanced" stored sample first.

Sensory / organoleptic evaluation: To carry out sensory evaluation of the mushroom fries, a panel of evaluators was sought by putting up notices for volunteers. It attracted a panel of 20 both male and female untrained panelists but well educated (majority tertiary and above) and well conversant with agricultural products. The panelists were aged 30 to 55 years. Using a well structured questionnaire, the panelists independently tested the mushroom fry prepared from the 11 types of batter and assessed the samples for appearance, taste, flavour and colour. Organoleptic for appearance scores were made like 4 = excellent, 3 = good, 2 = fair, 1 = poor, and overall acceptability in which 4 = highly acceptable, 3 = acceptable, 2 = less acceptable, 1 = not acceptable. Crispiness were made like 5 = crispy, 4 = moderately crispy, 3 = less crispy, 2 = leathery, 1 = not crispy which were supposed to show the degree of likeness. Panelists indicated their rating for each sample by choosing the appropriate numerical score. The evaluation was carried out before lunch (Table 3).

Table 3. Score table of sensory evaluation for mushroom fries

Overall appearance	Colour	Crispiness	Flavour	Taste	Acceptability
4=Excellent√	4=Excellent	5=Crispy√	4=Excellent	4=Excellent	4=Highly acceptable
3=Good	3=Good√	4=Moderate Crispy	3=Good	3=Good√	3=Acceptable√
2=Fair	2=Fair	3=Less Crispy	2=Slightly odorous	2=Fair	2=Less Acceptable
1=poor	1=poor	2=Leathery	1=Bad odour√	1=poor	1=Not Acceptable
		1=Not Crispy			

Statistical analysis: The consumer acceptability of the developed fry was evaluated by a taste-testing panel. The panelist scores were analyzed with SPSS software.

RESULTS AND DISCUSSION

Obtained mushroom fries are shown in Fig. 3. The organoleptic evaluation of fresh mushroom fries was influenced by different pretreatments (Table 4). Higher scores for appearance (85%), colour (83.75%), crispiness (81%), flavour (80%), taste (81.25%) and acceptability (72.5%). The results of organoleptic evaluation of fresh mushroom fries are:

Overall appearance: Results showed that the score for appearance maximum was recorded in sample B-8 (WF + AF). The mean (± SD) of which was 3.4±0.68 and obtained score was 85%. The minimum score was recorded in sample B-1 (WF) and B-2 (RF). The mean (± SD) of both of them was 2.5±0.95 and obtained score was 62.5% (Table 4 and Table 5).

Fig. 3. Prepared mushroom fry.

Table 4. Sensory evaluation of deep fried mushroom prepared from various batter formulations

Treatments /Sample	Overall appearance (%)	Colour (%)	Crispiness (%)	Flavour (%)	Taste (%)	Acceptability (%)	Total score (%)
B-1=WF	62.5	71.25	58	65	65	55	62.79
B-2=RF	62.5	75	67	68.75	65	57.5	65.96
B-3=CF	77.5	78.75	69	70	70	62.5	71.29
B-4=AF	76.25	83.75	65	70	72.5	65	72.08
B-5=GF	75	80	74	75	81.25	62.5	74.63
B-6=WF+RF	73.75	76.25	68	68.75	70	70	71.13
B-7=WF+CF	67.5	76.25	61	76.25	72.5	57.5	68.5
B-8=WF+AF	85	78.75	81	71.25	71.25	70	76.21
B-9=WF+GF	77.5	76.25	62	70	75	62.5	70.54
B-10=WF+RF+CF	75	76.25	72	73.75	72.5	66.25	72.53
B-11=Control	83.75	80	80	80	80	72.5	79.38

Table 5. Obtained score of the individual mushroom fries mean (± SD)

Treatments	Overall appearance	Colour	Crispiness	Flavour	Taste	Acceptability	Total
B-1=WF	2.5±0.95	2.85±0.99	2.9±0.85	2.6±1.05	2.6±1.14	2.2±0.77	2.61±0.96
B-2=RF	2.5±0.95	3±0.73	3.35±1.09	2.75±0.72	2.6±0.99	2.3±0.57	2.75±0.84
B-3=CF	3.1±0.79	3.15±0.75	3.45±0.94	2.8±0.89	2.8±0.95	2.5±0.95	2.97±0.88
B-4=AF	3.05±0.83	3.35±0.49	3.25±0.97	2.8±0.83	2.9±0.79	2.6±0.88	2.99±0.80
B-5=KB	3±0.79	3.2±0.70	3.7±1.03	3±0.92	3.25±0.64	2.5±0.61	3.11±0.78
B-6=WF+RF	2.95±0.69	3.05±0.76	3.4±0.99	2.75±0.72	2.8±0.83	2.8±0.83	2.96±0.80
B-7=WF+CF	2.7±0.80	3.05±0.69	3.05±1.10	3.05±0.69	2.9±0.72	2.3±0.80	2.84±0.80
B-8=WF+AF	3.4±0.68	3.15±0.67	4.05±0.69	2.85±0.81	2.85±0.93	2.8±0.89	3.18±0.78
B-9=WF+GF	3.1±0.64	3.05±0.76	3.1±1.02	2.8±0.83	3±0.56	2.5±0.51	2.93±0.72
B-10=WF+RF+CF	3±0.56	3.05± 0.60	3.6±0.75	2.95±0.60	2.9±0.79	2.65±0.75	3.03±0.68
B-11=Control	3.35±0.59	3.2±0.77	4±0.46	3.2±0.70	3.2±0.62	2.9±0.72	3.31±0.64

Colour: Results showed that the score for color maximum was recorded in sample B-4 (AF). The mean (± SD) of which was 3.35±0.49 and obtained score was 83.75%. The minimum score was recorded in sample B-1 (WF). The mean (± SD) of which was 2.85±0.99 and obtained score was 71.25% (Table 4 and Table 5).

Crispiness: The score crispiness maximum was observed in sample B-8 (WF+AF). The mean (± SD) of which was 4.05±0.69 and obtained score was 81%. Obtained minimum score was recorded in sample B-1(WF). The mean (± SD) of which was 2.9±0.85 and obtained score was 58% (Table 4 and Table 5).

Taste: Results showed that the score for taste maximum was recorded in sample B-5 (GF). The mean (± SD) of which was 3.25±0.64 and obtained score was 81.25%. Obtained minimum score was recorded in sample B-1 (WF) and B-2 (RF). The mean (± SD) of which were 2.6±1.14 and 2.6±0.99, obtained score of both of them was 65% (Table 4 and Table 5).

Flavour: Results showed that the score for flavour maximum was recorded in sample B-11 (Control). The mean (± SD) of which was 3.2±0.70 and obtained score was 80%. Obtained minimum score was recorded in sample B-1 (WF). The mean (± SD) of which was 2.6±1.05 and obtained score was 65% (Table 4 and Table 5).

Acceptability: Results showed that the score for overall acceptability maximum was recorded in sample B-11 (Control). The mean (± SD) of which was 2.9±0.72 and obtained score was 72.5%. The minimum score was recorded in sample B-1 (WF). The mean (± SD) of which was 2.2±0.77 and obtained score was 55% (Table 4 and Table 5).

Considering all the parameters it was observed from the study (mean±SD) that B-11 (control) obtained the highest score (3.31±0.64, 79.38%) followed by B-8 (WF+AF) the value of which were 3.18±0.78, 76.21%. The average lowest score in the study was 2.61±0.96, 62.79% that was obtained by sample B-1 (WF). Obtained other scores were B-2 (RF) 2.75±0.84, 65.96%; B-3 (CF) 2.97±0.88, 71.29; B-4 (AF) 2.99±0.80, 72.08%; B-5 (KF) 3.11±0.78, 74.63%; B-6 (WF+RF) 2.96±0.80, 71.13%; B-7 (WF+CF) 2.84±0.80, 68.5%; B-9 (WF+GF) 2.93±0.72, 70.54% and B-10 (WF+RF+CF) 3.03±0.68, 72.53%. It might be concluded that for preparing mushroom fry the mixture of all kinds of flower (Control) B-11 (WF+RF+CF+AF+GF) is highly acceptable (score 3.31±0.64, 79.38%) but regular collection of all these ingredients for household purpose is difficult. Thus for regular household purpose B-8 (WF+AF) is most suitable (score 3.18±0.78, 76.21%). On the other hand preparation of mushroom fry using B-1 (WF) ingredients was not so much suitable which obtained the lowest score (2.61±0.96, 62.79%) in this organoleptic study.

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Nutritional Status of Reishi Mushroom at Different Stages of Fruiting body

Md. Bazlul Karim Choudhury[1], Akhter Jahan Kakon, Manirul Shaheen, Tahera Binte Mujib, Ruhul Amin and Md. Mominul Hoque
National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh.

Abstract

To differentiate some nutritional contents in different stages of *Ganoderma lucidum*, study was conducted in the National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka. In this study eight developmental stages of reishi mushroom were considered which includes Premature, Antler, Conk, Conk after 10 days, Conk after 20 days, Conk after 30 days, Conk after 40 days and Mature stages. The highest moisture content was observed in 30 days stage (12.85%) and lowest in mature stage (7.33%), lipid content was high in antler (12%) and low in premature (2.8%), fibre was highest in conk after 30 days (23.6) and lowest in antler (2.1%) stages, highest ash content observed in conk after 20 days (7.01%) and lowest was in antler (6.05%) stage. Some important mineral contents such as Calcium, Iron, Zinc, Selenium and Cobalt were also observed. The highest amount of Ca (mg/100g dried sample) was observed in conk after 20 days stage (49.8) and the lowest amount was in antler stage (7.70). Iron content was high in Conk after 20 days stage (1.93) and low in premature stage (1.11). Maximum amount of Zn was observed in antler (1.47) and minimum was in conk after 40 days (0.62) stage. Co content was highest in premature (5.84) and lowest in 40 days (0.43) stage. Considering Selenium, the highest amount (µg/g) was observed in premature (20.8) and lowest amount in conk after 30 days (4.35) stage. From the study it was asumed that all of the stages of *Ganoderma lucidun* were not equeal nutritionally, one stage is reacher in one whereas other stages for another molecules. Obtained findings from the study might be helpful for deciding the proper harvest period of *Ganoderma lucidum* to fulfill the specific nutritional requirement and hence it can take part to overcome nutritional problems.

Key words: Lipid, Fibre, Moisture, Minerals and Different stage of reishi mushrooms.

INTRODUCTION

Ganoderma lucidum, is known as the 'mushroom of immortality' or 'longevity mushroom' (Kaul, 2001). Among cultivated mushrooms, *G. lucidum* is unique in that its pharmaceutical rather than nutritional value is paramount. As part of searching for new chemo preventive and chemotherapeutic agents, hundreds of plant species, including mushrooms, have been evaluated. This has resulted in the isolation of thousands of bioactive molecules that were shown to have antitumor activity from numerous mushroom species, including *Ganoderma* species (Wasser and Weis, 1999; Borchers *et al.*, 2008). Medicinally, reishi is popular for its ability to relieve fatigue, weakness, insomnia, coughs and asthma, because of the fact that it contains several major components including coumarin, sterols, polysaccharides, mannitol and triterpenoids called ganoderic acids. The ganoderic acids present in reishi have the ability to lower

[1] Department of Biochemistry, Faridpur Medical College, Faridpur, Bangladesh.

blood pressure, diabetes mellitus, cancer, and to decrease blood cholesterol level. They also inhibit blood platelets from sticking together as it happens in coronary artery disease. The beta-glucan polysaccharide of this mushroom has potential application in immune surveillance and chemoprevention of cancer (Chang, 1996b). *Ganoderma lucidum* has anti microbial and anti-HIV effects (Yoon, 1994 and Kim, *et al.*, 1994). The hepatic and renal protective effects of *G. lucidum* in mice were also evaluated (Shieh, *et al.*, 2001). In *G. lucidum*, a large number of chemical compounds can be extracted from the fruiting body, mycelia, or spores.

Moreover nutritional composition is affected by many factors; these include differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting, specific portion of the fruiting bodies used for analysis, time interval between harvest and measurement methods (Benjamin, 1995). The objective of this work was to evaluate the nutritional composition of different stages of popular medicinal reishi mushrooms.

MATERIALS AND METHODS

Mushroom was cultivated and harvested in the culture house and the study was carried out in the 'Quality Control and Quality Assurance' laboratory of National Mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka from June 2013 to March 2014.

Treatments: Eight different stages of fruiting body of NAMDEC reishi-1 mushroom species such as premature, antler, conk, 10 days after conk, 20 days after conk, 30 days after conk, 40 days after conk and mature stage were selected in this study for investigation. Mushroom was cultivated on sawdust and fruiting bodies were harvested and taken for nutritional analysis. Fresh mushroom was taken for the determination of moisture and then they were dried for the estimation of lipid, crude fibre, minerals and total ash.

Moisture analysis: Twenty gram of fresh mushroom was weighed into a weighed moisture box (*A&D company ltd. N 92; P1011656, Japan*) and dried in an oven at 100^0C 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 grinded mushroom was taken with 50 ml of 0.1N NaOH and boiled for 30 minutes. The solution was cooled in room temperature and centrifuged at 1000rpm by a table centrifuge machine (*DIGISYSTEM: DSC-200T; Taiwan*). The supernatant was collected and total protein content was measured according to the method of Lowry *et al.* (1951). For the determination of protein content from fresh mushroom, 5g was taken with 50ml phosphate buffer and homogenized with a tissue

homogenizer (*Polytron: PT 1200*). Five ml of homogenized was taken with 50 ml of 0.1N NaOH and protein content was determined as mentioned above.

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 fibre: Ten 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 a dessicator 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 conc. 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 50ml by adding deionized

water. It was then used to analyze the contents of calcium (Ca), iron (Fe), molybdenum (Mo), selenium (se), zinc (Zn) and cobalt (Co) by flame and graphite method with atomic absorption spectrophotometer (*PERKIN ELMER: AS 80*).

RESULTS AND DISCUSSION

Several nutritional parameters were measured. Table 1 shows the nutritional parameters of dry mushrooms.

Moisture content: The moisture contents from different stages of the *Ganoderma lucidum* were varied from 7.333 to 12.83%. The highest moisture contents were observed in the premature stage and lowest in the mature stage fruiting body.

Lipid content: The lipid contents varied in different stages from 2.8 – 12g per 100g of dried *Ganoderma lucidum.* The highest lipid content (12 g/100g) was observed in Antler stages and the lowest lipid content (2.8 g/100g) was observed in Premature stages (Table 1).

Fibre content: The fibre contents in different stages of fruiting body were 2.1 – 23.6g per 100g of dried *Ganoderma lucidum.* The highest fiber content (23.6 g/100g) was observed in 30 days stages and the lowest (2.1 g/100g) was observed in antler stages.

Total ash content: Considering total ash the findings were varied from 6.05 – 7.01% per 100g of dried *Ganoderma lucidum.* The highest amount (7.01%) was observed in 20 days stages and the lowest (6.05 %) was observed in antler stages.

Table 1. Nutrient contents in different stages fruiting body of dried *Ganoderma lucidum* (g/100g)

Different stages of reishi fruiting body	Moisture (%)	Lipid	Fiber	Ash %
Premature	12.83	2.8	11.3	6.50
Antler	10.80	12	2.1	6.05
Conk	11.91	6	12.2	6.12
10 days after conk	12.62	4.9	12.7	6.96
20 days after conk	12.70	6.3	22.8	7.01
30 days after conk	12.85	5.4	23.6	6.83
40 days after conk	10.27	4.8	12.7	6.37
Mature fruit body	7.33	11.6	17.1	6.56

Some minerals: Table 2 shows the contents of some important minerals. Calcium and iron contents were greater in 20 days and 30 days stages respectively, the lowest calcium was observed in antler stage and the lowest iron was showed in premature stage. The contents of zinc were higher in antler and premature stages and the lowest was in 40 days stage. The selenium content was higher in 40 days stages and lowest was in 30 days stage. The cobalt content was higher in premature stage and was lowest in 40 days stage.

Most of the mushrooms contain around 90% water by weight. The remaining 10% consists of 10–40% protein, 2–8% fat, 3–28% carbohydrate, 3–32% fibre, 8–10% ash, and some vitamins and minerals. Among all, potassium, calcium, phosphorus, magnesium, selenium, iron, zinc, and copper accounting for most of the mineral content (Borchers *et al.,* 1999). In a study of the nonvolatile components of *G. lucidum*, it was found that the mushroom contains 1.8% ash, 26–28% carbohydrate, 3–5% crude fat, 59% crude fibre, and 7–8% crude protein (*Mau et al.,* 2001). The protein content of dried *G. lucidum* was found to be around 7–8%, which is lower than that of many other mushrooms (Chang and Buswell, 1996; Mau *et al.,* 2001). The carbohydrate and crude fiber content of the dried reishi mushroom was examined and found to be 26–28% and 59%, respectively (Mau *et al.,* 2001). Elemental analysis of log-cultivated fruit bodies of *G. lucidum* revealed phosphorus, silica, sulfur, potassium, calcium, and magnesium to be their main mineral components. Iron, sodium, zinc, copper, manganese, and strontium were also detected in lower amounts, as were the heavy metals lead, cadmium, and mercury (Chen *et al.*, 1998). Freeze-dried fruit bodies of unidentified *Ganoderma* spp. collected from the wild were reported to have a mineral content of 10.2%, with potassium, calcium, and magnesium as the major components (Chiu *et al.,* 2000). Significantly, no cadmium or mercury was detected in these samples. *G. lucidum* can also contain up to 72 µg/g dry weight of selenium (Falandysz, 2008) and can biotransform 20–30% of inorganic selenium present in the growth substrate into selenium-containing proteins (Du *et al.,* 2008). Findings of the current study is in aggrement with the previous observations with small variations. This variation may be due to variation of these minerals in soil and water of local area which influence in mushroom cultivation.

Table 2. Mineral contents in different stages of dried *Ganoderma lucidum* (mg / 100g)

Different stages of reishi fruiting body	Calcium (Ca)	Iron (Fe)	Zinc (Zn)	Selenium µg/g (Se)	Cobalt (Co)
Premature	9.59	1.11	1.39	20.80	5.84
Antler	7.70	1.20	1.47	18.13	4.86
Conk	35.06	1.38	1.22	5.45	5.70
10 days after conk	38.40	1.66	1.05	12.30	5.09
20 days after conk	49.80	1.93	1.34	13.80	5.62
30 days after conk	42.16	1.87	0.90	4.35	0.52
40 days after conk	25.80	1.48	0.62	4.80	0.43
Mature fruit body	9.49	1.22	1.18	8.36	5.74

Observed data showed that *Ganoderma lucidum* of different stages are good source of lipid and fiber but mature stage contains highest amount of lipid where as 30 days stage contains maximum fiber. Also 20 days stage is the best source of calcium and iron and antler stage is for zinc. The premature stage is the best source of Selenium and Cobalt. *Ganoderma lucidum* are also rich in ash content but it also varies in different stages. Obtained findings from the study might be helpful for deciding the proper harvest period of *Ganoderma lucidum* to fulfill the specific nutritional requirement and hence it can take part to overcome nutritional problems.

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Effect of Spawning Methods and Ratio of Different Ingredient of Substrate on Growth and Yield of Shiitake (Le 16) Mushroom

Akhter Jahan Kakon, Nirod Chandra Sarker, Md. Bazlul Karim Choudhury[1], Tahera Binte Mujib, Abdus Salam Khan and Manirul Shaheen

Abstract

An experiment was conducted to determine the effect of spawning methods and ratio of different ingredient of substrate on growth, yield and yield attributes of shiitake mushroom (*Lentinus edodes*). Spawn packets containing sawdust substrate with through spawning method required shortest duration of 22.00 days to complete mycelium running. The strains required up to 30 days to complete mycelium running on other substrates or spawning methods. The highest days (93.00 days) required for bump formation from the treatment through spawning method and sawdust substrate which was statistically similar to the control treatment. The minimum days required for bump formation (90.00 days), opening to harvest (6.57 days), total days for harvest (96.63 days), diameter (0.76 cm) of stalk, number of total (16.00) and effective fruiting body (10.75) were obtained from the treatment T_5, where packets containing sawdust substrate with three layers spawning. The highest number of fruit body (24.42), number of effective fruiting body (16.25), maximum yield (83.92g) and biological efficiency (45.36%) was observed when paddy grain containing mother culture thoroughly mixed with pasteurized sawdust i. e. through spawning method.

Key words: Grain, Growth, Inoculation, Substrate, Mother culture, Sawdust, Spawning.

INTRODUCTION

The black oak mushroom (*Lentinula edodes*) commonly known as shiitake, is the second most important edible mushroom in the world. This mushroom has been cultivated in Japan and China for about 2000 years (Royse, 2005). In addition, shiitake has also been cultivated in Thailand, Korea and Brazil. Among the countries Japan is the main world producer of this mushroom, reaching production of 7.5 million tones (Smith *et al.*, 2002). The great interest in shiitake's commercialization is due to its unique flavor/taste, nutritive value and medicinal properties (Sugui *et al.*, 2003; Silva *et al.*, 2007). Lack of cultivation technology on locally available substrates and suitable high temperature strains are the reason for its non availability in Bangladesh. Despite sporadic research efforts to standardize its cultivation technology in India (Sharma *et al.*, 2006), it could not reach the commercial level, as available technology was not viable. The major advantages of producing shiitake on sawdust bags compared with producing shiitake on natural logs are: consistent market supply through year round production, increased yields and decreased time required completing a crop cycle. Spawning methods influence the spawn run time for both synthetic and natural logs. Literature regarding the role of methods of spawning the cultivation substrate on mushroom yield is almost lacking. Therefore, the

[1] Department of Biochemistry, Faridpur Medical College, Faridpur, Bangladesh.

present study was undertaken to evaluate different spawning methods on the yield of *Lentinus edodes*.

MATERIALS AND METHODS:

The experiment was conducted in the laboratory and culture house of National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh from October to March 2014. In this experiment rice straw and sawdust were used as substrate for the cultivation of *Lentinus edodes* (Le 16) mushroom. Different amount of pasteurized straw and sawdust as well as two spawning methods were used as treatments. The treatments were T_0= sawdust + inoculation (control), T_1 = sawdust + through spawning, T_2 = sawdust + straw (3:1) with through spawning, T_3 = sawdust + straw (1:1) with through spawning, T_4 = straw + through spawning, T_5 = sawdust + three layer spawning, T_6 = sawdust + straw (3:1) with three layer spawning, T_7 = sawdust + straw (1:1) with three layer spawning, T_8 = straw + three layer spawning

Preparation of mother culture: To prepare mother culture of the test mushroom (Le 16) paddy grains were used as media of mother culture. At first grains collected which was free from diseases and not broken, old, and insect damaged. The grains were thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses, weeds, etc. Washed grains were then soaked in sufficient water for 2-3hours and boiled in a container for 25-45 minutes until slightly cracking. Excess water from the boiled grains was removed by spreading on sieve made of fine wire mesh. The grains were left as such for few hours on the sieve so that the water on surface was evaporated. Then spread on polythene sheet and grains were thoroughly mixed with calcium carbonate at 0.2% so that the pH of the grains was around 7.0 to 7.8. This mixing was done on a smooth surface after wearing gloves.

The substrate was poured into polypropylene bags (18 cm × 25 cm) at 500g /bag. The substrate in bags was sterilized in an autoclave for 2 h at 121^0C under 1.1 kg / cm^2 pressures and allowed to cool for 24 h. Pure cultures of strain Le 16 was grown on potato dextrose agar (PDA) following hyphal tip method. A piece of the PDA culture of Le 16 containing mycelium was placed aseptically in the opening of the mother culture packets. The inoculated packets were placed on a rack in the laboratory at 22 ± 2^0C for incubation. The substrate of the mother culture was covered by whitish mycelium of test strain within 20-25 days after inoculation. The fully colonized packets were used as master mother for inoculation. Then the master mother was poured at 10% in the opening of paddy grain containing mother culture packets and substrate grain was covered by whitish mycelium within 10-15 days after inoculation. The fully colonized packets were used as mother for spawning.

Preparation of substrate: The straw was chopped to 4-5 cm length and then poured (3-4 kg/ bag) into cribriform nylon bag. The bags were submerged in water for sometimes and then drained out the excess water. Sawdust was prepared by pasteurization method. Twenty kg sawdust was mixed with 17 litre of water and 10-12kg mixture was poured

into cribriform nylon bag. The bags were then kept in a rack of pasteurization chamber at 60-65^{0}C for one hour. There after the bags were kept in same place for 18-20 hours to get cool slowly. After about 20 hours the prepared straw and sawdust was spread over polythene sheet in the open place to reduce the moisture level at 63%.

Preparation of spawn packets: The polypropylene bags of 18 cm × 25 cm size were filled with pasteurized straw and sawdust according to treatments. Then the packets were spawning (through and three layers) with the mother culture of Le 16, and their mouths were plugged by inserting absorbent cotton with the help of plastic neck. The neck of the bag was prepared by using heat resistant plastic pipe. Substrates and paddy mother used at the ratio of 2:1 and the substrate mixture was poured into polypropylene bags at 500g / bag. The prepared packets were incubated in culture house at 20-25^{0}C. Thorough spawning of the substrate was also followed in which the spawn was thoroughly mixed with the wet substrate before bagging. In case of layer spawning at first 1/3 wet substrate poured into the bag and mother culture spread on the surface. After complete first layer another two layer done by same technique.

Mycelial colonization and bump formation: During incubation period, whitish mycelium started to grow in the inoculated substrate. The strain showed optimal mycelial growth at 22 ± 2^{0}C and 60-70% relative humidity in the culture house. After full colonization of the spawn packets, a thick mycelial coat formed on the outer surface of colonized substrate. Clumps of mycelia appeared as blister like bumps of various sizes on the surface of the mycelial coat in each packet. Bumping usually started when color of the colonized white mycelia became brown.

Cultivation for fruiting body: After mycelium maturation and bump formation, all the packets were fully opened, and placed separately on the rack in the culture house. Temperature, relative humidity and light intensity of the culture house were maintained at 18-22^{0}C, 60-70% and 10-20 lux, respectively. Sufficient water was sprayed every day and proper aeration was maintained in culture house for the release excess CO_2 and supply of sufficient O_2 as required for the development of primordia and fruiting bodies.

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

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

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

RESULTS AND DISCUSSION

Days to complete mycelium running: Days to complete mycelium running (DMR) on spawning method and ratio of different ingredient of substrate ranged 22.00-30.00 and significantly different (Table 1). The highest DMR (30.00 days) was recorded fromT_6 treatment where sawdust + straw used as substrate at 3:1 ratio with three layer spawning which was statistically similar to control treatment where spawn packets containing sawdust substrate and followed inoculation method as well as T_5 treatment where spawn packets prepared with sawdust and three layer spawning method. The lowest DMR (22.00 days) was observed in T_1 treatment where packets containing sawdust substrate with through spawning method. Kakon *et al.* (2013) observed the lowest DMR when paddy grain thoroughly mixed with pasteurized sawdust that is thorough spawning method.

Days to bump formation: Days required for bump formation (DBF) in different treatments ranged 90.00-93.00 and significantly different. The highest days (93.00 days) required for bump formation from the treatment T_1 through spawning method and sawdust substrate which was statistically similar to the control treatment. The lowest days required for bump formation (90.00 days) from the treatment T_5 where packets containing sawdust substrate with three layers spawning (Table 1). This result partially supported by Kakon *et al.* (2013) who observed sawdust required the highest duration to form bump in inoculation method and paddy grain containing mother culture and through spawning method developed bump earlier.

Days required from opening to harvest: The duration from opening of spawn packet to first harvest in different treatments ranged from 6.57-10.50. Significantly the highest DOH (10.50) was recorded in control treatment. The minimum days (6.57 days) required from opening to harvest (DOH) in T_5 treatment where sawdust used as substrate with three layers spawning which was statistically similar to T_6 and T_1 (Table 1). This result partially supported by Kakon *et al.* (2013) who observed significantly the highest DOH was recorded in sawdust with inoculation method.

Total days for harvest: The minimum days to harvest (96.63 days) was recorded from sawdust and three layer spawning method (T_5) which was statistically similar to the treatment T_1. Effect of those treatments on DH was significantly different. Significantly the highest duration (102.00 days) observed in T_0 treatment (control) which was statistically similar to the treatment T_6 i.e. three layer spawning with sawdust: straw (3:1) (Table 1).

Length and diameter of stalk: The length of stalk was not significantly different**.** The longest stalk (4.07 cm) produced from the treatment T_1 (through spawning + sawdust) which was statistically similar to others treatment. The shortest stalk (3.72 cm) was found in T_5 (three layer spawning + sawdust). The maximum stalk diameter (1.40 cm) was found in sawdust which was statistically different to other treatments. The lowest

diameter of stalk (0.76 cm) was recorded from the treatment T_5 (three layer spawning + sawdust) (Table 2).

Diameter and thickness of pileus: The diameter of pileus ranged 5.75-6.96 cm and its thickness varied from 0.94 to 1.27 cm. The highest diameter of pileus was found in T_5 treatment (three layer spawning + sawdust) which was statistically similar to the treatment T_1 (through spawning + sawdust). The minimum diameter of pileus (5.60 cm) was recorded from T_0 i.e. control treatment. Significantly the highest thickness of pileus was found in T_6 (three layer spawning + sawdust: straw at 3:1 ratio) treatment and the lowest in T_0 i.e. control treatment (Table 2).

Table 1: Effect of spawning method and ratio of different ingredient of substrate on growth of shiitake mushroom

Treatments	Days to complete mycelium running	Days to bump formation	Days required from opening to harvest	Total days for harvest
T_0	28.50a	92.10ab	10.50a	102.00a
T_1	22.00b	93.00a	7.25b	98.00b
T_2	--	--	--	--
T_3	--	--	--	--
T_4	--	--	--	--
T_5	28.00a	90.00c	6.57b	96.63b
T_6	30.00a	91.50b	6.57b	101.3a
T_7	--	--	--	--
T_8	--	--	--	--
CV (%)	**4.05**	**0.85**	**8.36**	**1.05**

In a column do not differ significantly at 5 % level according to DMRT. T_0 = Sawdust + inoculation (control), T_1= Sawdust + through spawning, T_2= Sawdust +straw (3:1) with through spawning, T_3=Sawdust +straw (1:1) with through spawning, T_4= Straw + through spawning, T_5=Sawdust+ three layer spawning, T_6= Sawdust + straw (3:1) with three layer spawning, T_7= Sawdust + straw (1:1) with three layer spawning, T_8= Straw + three layer spawning.

Table 2. Effect of spawning method and ratio of different ingredient of substrate on size of fruit of shiitake mushroom

Treatments	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_0	3.90 a	0.80 b	5.60 b	0.94 c
T_1	4.07 a	1.40 a	6.57 a	1.08 b
T_2	---	---	---	---
T_3	--	--	--	--
T_4	--	--	--	--
T_5	3.72 a	0.76 b	6.96 a	1.09 b
T_6	3.57 a	0.81 b	5.75 b	1.27 a
T_7	--	--	--	--
T_8	--	--	--	--
CV (%)	**8.72**	**6.55**	**7.57**	**6.69**

In a column do not differ significantly at 5 % level according to DMRT. T_0 = Sawdust + inoculation (control), T_1= Sawdust + through spawning, T_2= Sawdust +straw (3:1) with through spawning, T_3=Sawdust +straw (1:1) with through spawning, T_4= Straw + through spawning, T_5=Sawdust+ three layer spawning, T_6= Sawdust + straw (3:1) with three layer spawning, T_7= Sawdust + straw (1:1) with three layer spawning, T_8= Straw + three layer spawning.

Number of total and effective fruiting body: Number of total fruiting body ranged from 16.00-24.42 per packet and significantly different. The lowest number of fruiting body (16.00) was found from the treatment T_5 (three layer spawning + sawdust) and the highest number (24.43) was found T_1 (through spawning + sawdust) treatment. Number of effective fruiting body ranged from 10.75-16.25. Significantly the highest (16.25) number of effective fruiting body was recorded from T_1 treatment followed by T_0 and T_6 treatment. The lowest number of effective fruiting body (10.75) was found from the treatment T_5 (three layer spawning + sawdust) (Table 3).

Yield per packet and biological efficiency: The yield of fruiting body per packet ranged from 65.00-83.92g and biological efficiency ranged from 35.14-45.36%. The maximum yield (83.92g) and biological efficiency (45.36%) were recorded from the treatment T_1 (through spawning + sawdust). The minimum yield (65.00g) and biological efficiency (35.14%) were recorded from the treatment T_6 i.e. three layer spawning + substrate. Here sawdust : straw (3:1) (Table 3).

Table 3. Effect of spawning method and ratio of different ingredient of substrate on yield attributes and biological efficiency of shiitake mushroom

Treatments	Number of fruiting body	Number of effective fruiting body	Yield (g)	Biological efficiency (%)
T_0	20.60b	13.00b	67.00bc	36.22bc
T_1	24.4a	16.25a	83.92a	45.36a
T_2	--	--	--	--
T_3	--	--	--	--
T_4	--	--	--	--
T_5	16.00c	10.75c	71.50b	38.65b
T_6	18.75b	12.50b	65.00c	35.14c
T_7	--	--	--	--
T_8	--	--	--	--
CV (%)	**7.63**	**7.77**	**4.41**	**4.41**

In a column do not differ significantly at 5 % level according to DMRT. T_0 = Sawdust + inoculation (control), T_1= Sawdust + through spawning,T_2= Sawdust +straw (3:1) with through spawning, T_3=Sawdust +straw (1:1) with through spawning, T_4= Straw + through spawning, T_5=Sawdust+ three layer spawning, T_6= Sawdust + straw (3:1) with three layer spawning, T_7= Sawdust + straw (1:1) with three layer spawning, T_8= Straw + three layer spawning.

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Effect of Spawn Rate on Growth and Yield of Shiitake Mushroom

Nirod Chandra Sarker, Akhter Jahan Kakon, Manirul Shaheen, Ruhul Amin, Tahera Binte Mujib and Md. Bazlul Karim Choudhury[1]
National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

In this experiment pasteurized sawdust was used as substrate for the cultivation of *Lentinus edodes* mushroom. Different spawn rates were used as treatments. The maximum days (28.00 days) required to complete mycelium running was obtained from the treatment combination strain Le 16 with the treatment T_4 (4:1) and the minimum days (16.00 days) required to complete mycelium running was obtained from the treatment combination of strain Le 8 with T_1 (1:1). The maximum days (111.00 days) required for bump formation and total days (118.00 days) required for harvest were obtained from the treatment combination of strain Le 8 with T_4 (4:1). The minimum days (72.00 days) required for bump formation and total days (76.00 days) required for harvest were obtained from the treatment combination of strain Le 16 with T_1 (1:1). Strain Le 8 produced the longest stalk (5.12 cm) with T_4 and Le 16 produced the shortest stalk (3.08 cm) with T_2. The maximum stalk diameter (1.10 cm) was found from the treatment combination of Le 16 with the treatment T_4 (4:1) and the lowest diameter of stalk (0.76 cm) was recorded from the treatment combination of Le 8 with T_1 (1:1). The highest number of fruiting body and effective fruiting body was recorded from the treatment combination of Le 16 with the treatment T_2 (2:1). The lowest number (3.00) of fruiting body and number of effective fruiting body, yield (18.00 g) and biological efficiency (9.47%) were recorded from the treatment combination of Le 8 with the treatment T_4 (4:1). The maximum yield (120.80g) and biological efficiency (63.59%) were recorded from the treatment combination of Le 16 with the treatment T_1 (1:1).

Key words: Pasteurization, Ratio, Sawdust, Spawning, Grain, Mother culture.

INTRODUCTION

Shiitake mushroom globally a well known cultivated species is yet to find a place in Bangladesh. The success of commercial cultivation of this mushroom is largely depending on improved cultivation technique on locally available resources. This mushroom has been valued for its unique taste and flavor as well as a medicinal tonic. For all these reasons the demand for shiitake has greatly increased in recent years, followed by an increase in production. With new production methods it is anticipated that there will be a continuous expansion of production in the world (Farr, 1983; Chang and Miles, 1989). In an attempt to develop a more efficient and dependable method for the production of shiitake mushrooms, it has recently been tried to produce it on supplemented sawdust. Indoor cultivation on supplemented sawdust substrates provides a more rapid and controlled method of cultivation than the present one using the traditional outdoor logs method. Spawn is mixed with the substrate by hand or machine shaking i.e.

[1] Department of Biochemistry, Faridpur Medical College, Faridpur, Bangladesh.

through spawning method is rapid and economic method for shiitake cultivation. Spawn rate influence the spawning method and production cost. In general, an increase in the amount of spawn used (spawn rate) will increase production rate of both synthetic and natural logs. This relationship does not hold, however, for top spawning of synthetic logs. Since mycelium must grow from the top of the substrate to the bottom before spawn run is complete, an increase in the amount of spawn will not increase the growth rate of the mycelium. Thus, substrate near the bottom of the bag may not be colonized for as long as 20 to 30 days. Increasing the spawn rate for other spawning methods usually results in an increase in the number of inoculums points in the substrates.

The study reported here was conducted to determine the appropriate spawn rate on spawn run time and yield of *Lentinus edodes* mushroom.

MATERIALS AND METHODS

The experiment was conducted in the culture house of National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh from November 2013 to April 2014. In this experiment pasteurized sawdust was used as substrate for the cultivation of *Lentinus edodes* mushroom. Different spawn rates were used as treatments. The treatments were T_1 = substrate + mother culture (1:1), T_2 = substrate + mother culture (2:1), T_3 = substrate + mother culture (3:1), T_4= substrate + mother culture (4:1). Two strains of shiitake mushroom (*Lentinus edodes*), namely Le 8 and Le 16 were used as test materials.

Preparation of mother culture: To prepare mother culture of the test mushroom (Le 8, Le 16) paddy grains were used as media of mother culture. At first grains collected which was free from diseases and not broken, old, and insect damaged. The grains were thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses, weeds, etc. Washed grains were then soaked in sufficient water for 2-3 hours and boiled in a container for 25-45 minutes until slightly cracking. Excess water from the boiled grains was removed by spreading on sieve made of fine wire mesh. The grains were left as such for few hours on the sieve so that the water on surface was evaporated. Then spread on polythene sheet and grains were thoroughly mixed with calcium carbonate at 0.2% so that the pH of the grains was around 7.0 to 7.8. This mixing was done on a smooth surface after wearing gloves.

The substrate was poured into polypropylene bags (18 cm × 25 cm) at 500 g/bag. The substrate in bags was sterilized in an autoclave for 2 h at 121^0C under 1.1 kg / cm^2 pressures and allowed to cool for 24 h. Pure culture of test strain (Le 8, Le 16) was grown on potato dextrose agar (PDA) following hyphal tip method. A piece of the PDA culture of Le-16 containing mycelium was placed aseptically in the opening of the mother culture packets. The inoculated packets were placed on a rack in the laboratory at 22 ± 2^0C for incubation. The substrate of the mother culture was covered by whitish mycelium of test strain within 20-25 days after inoculation. The fully colonized packets were used as master mother for inoculation. Then the master mother was poured at 10% in the opening of paddy grain containing mother culture packets and substrate grain was covered by

whitish mycelium within 10-15 days after inoculation. The fully colonized packets were used as mother for spawning.

Preparation of substrate: The sawdust was prepared by pasteurization method. Twenty kg sawdust was mixed with 17 litre of water then 10-12kg mixture was poured into cribriform nylon bag. The bags were kept in a rack of pasteurization chamber at 60-65^{0}C for one hour. There after the bags were kept in same place for 18-20 hours to get cool slowly. After about 20 hours the sawdust was spread over polythene sheet in the open place to reduce the moisture level at 63%.

Preparation of spawn packets: The polypropylene bags of 18 cm × 25 cm size were filled with pasteurized sawdust and mother culture according to treatments. Pasteurized sawdust and paddy grain containing mother mixed thoroughly without supplementation. Then their mouths were plugged by inserting absorbent cotton with the help of plastic neck. The neck of the bag was prepared by using heat resistant plastic pipe. Substrate mixture was poured into polypropylene bags at 500g / bag. The prepared packets were incubated in culture house at 20-25^{0}C. Thorough spawning of the substrate was also followed in which the spawn was thoroughly mixed with the wet substrate before bagging.

Mycelial colonization and bump formation: During incubation period, whitish mycelium started to grow in the inoculated substrate. The strain showed optimal mycelial growth at 22 ± 2^{0}C and 60-70% relative humidity in the culture house. After full colonization of the spawn packets, a thick mycelial coat formed on the outer surface of colonized substrate. Clumps of mycelia appeared as blister like bumps of various sizes on the surface of the mycelial coat in each packet. Bumping usually started when color of the colonized white mycelia became brown.

Cultivation for fruiting body: After mycelium maturation and bump formation, all the packets were fully opened, and placed separately on the rack in the culture house. Temperature, relative humidity and light intensity of the culture house were maintained at 18-22^{0}C, 60-70% and 10-20 lux, respectively. Sufficient water was sprayed every day and proper aeration was maintained in culture house for the release excess CO_2 and supply of sufficient O_2 as required for the development of primordia and fruiting bodies.

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

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

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

RESULTS AND DISCUSSION

Days to complete mycelium running: The maximum days required to complete mycelium running (28.00 days) was obtained from the treatment combination strain Le 16 with the treatment T_4 (4:1) which was statistically similar to the treatment combination of Le 8 with T_4 (4:1). The minimum days required to complete mycelium running (16.00 days) was obtained from the treatment combination of strain Le 8 with T_1 (1:1) (Table 1).

Days to bump formation: The maximum days (111.00 days) required for bump formation was obtained from the treatment combination of strain Le 8 with T_4 (4:1) followed by Le 8 with T_3 (3:1) and Le 16 with T_4 (4:1) treatment combination. The minimum days required for bump formation (72.00 days) was obtained from the treatment combination of strain Le 16 with T_1 (1:1).

Days required from opening to first harvest: The maximum days (8.00 days) required from opening to first harvest was obtained from the treatment combination of strain Le 8 with T_4 (4:1) followed by the treatment combination of Le 8 with T_2 (2:1). The minimum days (4.00 days) required from opening to first harvest was obtained from the treatment combination strain Le 16 with T_1 (1:1) and T_2 (2:1) (Table 1).

Total days for harvest: The maximum days (118.00 days) required for harvest was obtained from the treatment combination of strain Le 8 with the treatment T_4 (4:1) followed by the treatment combination of Le 8 with T_3 (3:1) and Le 16 with T_4 (4:1). The minimum days (76.00 days) required for harvest was obtained from the treatment combination of strain Le 16 with T_1 (1:1) (Table 1).

Table 1 Effect of different spawn rates on growth of two strains of shiitake mushroom

Treatments (Substrates: mother culture)	Days to complete mycelium running	Days to bump formation	Days required from opening to harvest	Total days for harvest
Strain (Le-8)				
T_1 =(1:1)	16.00e	87.00d	6.20e	93.20d
T_2=(2:1)(control)	20.00d	90.00c	7.50b	97.50c
T_3=(3:1)	23.00bc	96.00b	6.60d	102.6b
T_4=(4:1)	27.00a	110.00a	8.00a	118.00a
Strain (Le-16)				
T_1 =(1:1)	17.00e	72.00g	4.00g	76.00g
T_2 =(2:1)(control)	21.00cd	77.00f	4.00g	81.00f
T_3 =(3:1)	24.00b	80.00e	5.00f	85.00e
T_4 =(4:1)	28.00a	97.00b	7.00c	104.0b
CV (%)	**7.89**	**2.06**	**2.17**	**1.84**

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

Length and diameter of stalk: The Length and diameter of stalk was significantly different. Strain Le 8 produced the longest stalk (5.12 cm) on T_4 (4:1) treatment which was statistically similar to the treatment combination of Le 16 with T_4 (4:1). The shortest stalk (3.08 cm) was found in Le 16 with T_2 (2:1) treatment combination which was statistically similar to Le 8 with T_1 (1:1), Le 16 with T_1 (1:1) and T_3 (3:1) treatment combination. The maximum stalk diameter (1.10 cm) was found from the treatment combination of Le 16 with the treatment T_4 (4:1) followed by Le 8 with the treatment T_4 (4:1). The lowest diameter of stalk (0.76 cm) was recorded from the treatment combination of Le 8 with T_1 (1:1) which was statistically similar to Le 8 with T_2 and Le 16 with T_2 (2:1) treatment combination (Table 2).

Diameter and thickness of pileus: The highest diameter of pileus (9.50 cm) was found from the treatment combination of Le 8 with T4 (4:1) followed by the treatment combination of Le 16 with T_4 (4:1). The minimum diameter of pileus (5.68 cm) was recorded from the treatment combination of Le 16 with T_2 (2:1). Significantly the highest thickness of pileus (1.50 cm) was found from the treatment combination of Le 8 with T_4 (4:1). The lowest thickness of pileus (0.91 cm) was found from the treatment combination of Le 16 with T_2 (2:1) (Table 2).

Table 2. Effect of different spawn rates on size of fruit body of two strains of shiitake mushroom

Treatments(Substrates: mother culture)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Strain (Le 8)				
T_1=(1:1)	3.53d	0.76e	6.74cde	1.34b
T_2=(2:1)(control)	4.13c	0.78e	7.25c	1.05c
T_3=(3:1)	4.43bc	0.94c	7.10cd	1.28b
T_4=(4:1)	5.12a	1.00b	9.50a	1.50a
Strain (Le 16)				
T_1=(1:1)	3.12d	0.88d	6.17ef	0.98cd
T_2=(2:1)(control)	3.08d	0.77e	5.68f	0.91d
T_3=(3:1)	3.50d	0.93c	6.54de	0.98cd
T_4=(4:1)	4.75ab	1.10 a	8.50b	1.30b
CV (%)	**7.49**	**2.88**	**6.05**	**6.49**

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

Number of total and effective fruiting body: The highest number of fruiting body (21.42) was recorded from the treatment combination of Le 16 with the treatment T_2 (2:1) followed the same strain with T_1 (1:1). The lowest number (3.00) of fruiting body was found from the treatment combination of Le 8 with the treatment T_4 (4:1). The highest number of effective fruiting body (16.00) was recorded from the treatment combination of Le 16 with the treatment T_2 (2:1) which was statistically similar to the same strain with T_1 (1:1). The lowest number of effective fruiting body (2.00) was found from the treatment combination of Le 8 with the treatment T_4 (2:1) (Table 3).

Yield per packet and biological efficiency: Yield per packet and biological efficiency was significantly difference. The maximum yield (120.80g) and biological efficiency (63.59%) were recorded from the treatment combination of Le 16 with the treatment T_1 (1:1). The minimum yield (18.00 g) and biological efficiency (9.47%) were recorded from the treatment combination of Le 8 with the treatment T_4 (4:1) (Table 3).

Table 3. Effect of different spawn rates on yield attributes and yields of two strains of shiitake mushroom

Treatments(Substrates: mother culture)	Number of fruiting body	Number of effective fruiting body	Yield per packet (g)	Biological efficiency (%)
Strain (Le-8)				
T_1=(1:1)	18.58c	13.57b	60.25e	31.71e
T_2=(2:1)(control)	12.00d	9.50c	70.57c	37.14c
T_3=(3:1)	7.67e	5.75e	50.00f	26.32f
T_4=(4:1)	3.00g	2.00g	18.00h	9.47h
Strain (Le-16)				
T_1=(1:1)	20.00b	15.75a	120.80a	63.59a
T_2=(2:1)(control)	21.42a	16.00a	112.50b	59.21b
T_3=(3:1)	11.67d	8.67d	64.67d	34.04d
T_4=(4:1)	4.00f	2.50f	25.00g	13.16g
CV (%)	**3.36**	**3.13**	**4.48**	**4.48**

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

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