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Bangladesh Journal of Mushroom

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Bangladesh Journal of Mushroom

Volume 9 **Number 1** **June 2015**

Contents

Bangladesh J. Mushroom. 9(1): 1-7. 2015 (June)

Molecular Characterization of Six Oyster Mushroom Species through RAPD Marker

Ruhul Amin, Akhter Jahan Kakon, Nirod Chandra Sarker and Md. Bazlul Karim Choudhury[1]
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

The experiment was conducted to study genetic diversity and molecular relationship among the selected species of oyster mushrooms (*Pleurotus*). Genomic DNA was amplified by the RAPD technique with 5 sorts of arbitrary 10-base oligonucleotide primers. All five primers were efficient to amplify the genomic DNA. The number of amplified bands was variable depending on the primers or the species. Five primers generated 143 bands from the six different oyster mushroom species. The primer OPA-03 amplified the highest number of bands (45) and the primer OPA-05 amplified the lowest number of bands (15). The primer OPA-02 and OPA-03 produced 11 and 16 polymorphic bands and showed the highest level of polymorph 42% and 35% respectively. The maximum linkage distance (33.0) was found in P2 vs. P4 species pair while the minimum linkage distance (28.0) was found in P3 vs. P5 species pair. Six different species segregated into two main clusters at linkage distance 46. P1, P2, P3 and P5 grouped in cluster 1(C_1) and P4 and P6 grouped in cluster 2 (C_2). RAPD results suggested that among six species, P3 and P5 is very close to P2 also P1 and P4 also close to P6, while P1, P2, P3and P5 were quite different as compared to P4 and P6.

Keywords: *Pleurotus*, RAPD, genomic DNA, PCR, Molecular relationship.

INTRODUCTION

The oyster mushrooms (*Pleurotus spp.*) are the second most important cultivated mushroom in the world. The genus consists of a number of species and these edible species have high commercial importance. Various molecular genetics tools have been introduced for the verification of fungi, such as restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), and small subunit ribosomal DNA (SSU rDNA), and internal transcribed spacer (ITS) sequence analyses. Among the molecular approaches, random amplified polymorphic DNA (RAPD) had been first developed to detect polymorphisms between organisms despite the absence of sequence information, to produce genetic markers, and to construct genetic maps (Williams *et al.*, 1990). The random amplified polymorphic DNA (RAPD) is a convenient method to detect genetic diversity (Marino *et al.*, 2003; Sunagawa and Magae, 2005) This method has been particularly successful when applied to check the strains of mushrooms with different origins (Lopandic *et al.*, 2005). Therefore, RAPD has been adapted in various fungal species and is increasingly popular due to simple, rapid, and low cost method for detecting genetic diversity (Alam *et al.*, 2010). The aim of this study was to

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

investigate molecular genomic polymorphism among the selected species of *Pleurotus* by RAPD analysis.

MATERIALS AND METHODS

Mushroom Species: Six indigenous species of *Pleurotus* were collected from culture house of Mushroom Development Institute, Savar, Dhaka.

DNA Extraction: Finally 500µl of sterilized distilled water was added. DNA concentration was measured using spectrophotometer.

DNA Extraction: In the present study, modified method of Aljanabi *et al.* (1999) has been used to isolate the total genomic DNA from mushroom. DNA of three different mushrooms was grinded in extraction buffer (200 mM Tris-HCl-pH 8.5, 250 mM NaCl, 25 mM EDTA, 20% CTAB, 0.5% SDS) with a mortar pestle. The lysates were incubated at 65^{0}C for 40 min in water bath and centrifuge 30 min at 10,000 rpm. DNA was precipitated from the supernatant by adding equal volumes of iso propanol and resultant pellet was washed with 70% ethanol. The DNA palette was air dried and dissolved in 50 µl TE buffer. DNA quantification was performed and a dilution of 50ng/µl was used in downstream application.

RAPD Analysis: Genomic DNA was amplified by the RAPD technique (Williams *et al.*, 1990) in which 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; were used to produced 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^{0}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 trans illuminator. 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). To estimate the similarity and genetics distance among different species, cluster analysis based on Nei's unweighted pair-group with arithmetic average (UPGMA) was performed using the 'statistica' software and a dendrogram was constructed.

RESULTS AND DISCUSSION

Five arbitrary 10-base oligonucleotide primers were used to amplify segments of genomic DNA for the selected species of *Pleurotus*.

Table. 1. RAPD primers with corresponding bands scored,their size range, number of monomorphic and polymorphic bands, polymorphism and number of band per species

Primer codes	Size ranges (bp)	Total no. of bands	Total monomorphic bands	Total polymorphic bands	Polymor-phism (%)	No. of bands per species
OPA-01		21	15	6	28	-
OPA-02		26	15	11	42	-
OPA-03		45	29	16	35	-
OPA-04		36	26	10	27	-
OPA-05		15	12	3	2	-
Total		143	97	46	134	-

Table. 2. Summary of linkage distances (based on new statistica) values for different species pairs of oyster mushroom P1, *Pleurotus ostreatus*; P2, *Pleurotus djamor*; P3, *Pleurotus citrinopileatus*; P4, *Pleurotus geesteranus*; P5, *Pleurotus cystidiosus*; P6, *Pleurotus eryngii*

	P1	P2	P3	P4	P5	P6
P1	00.0	40.0	38.0	35.0	40.0	50.0
P2	40.0	00.0	36.0	51.0	40.0	46.0
P3	38.0	36.0	00.0	49.0	28.0	44.0
P4	35.0	51.0	49.0	00.0	45.0	33.0
P5	40.0	40.0	28.0	45.0	00.0	44.0
P6	50.0	46.0	44.0	33.0	44.0	00.0

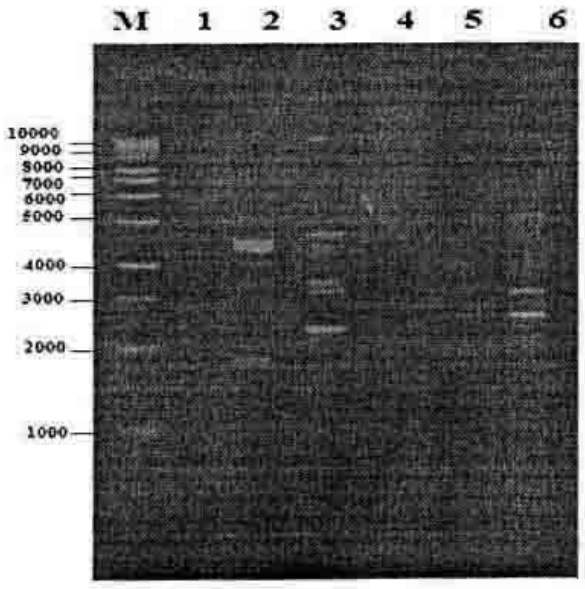

Fig. 1. Random amplification of polymorphic DNA profiles in different species of *Pleurotus* with primer OPA-1. M, molecular size marker (1 kb DNA ladder); lane 1, *Pleurotus ostreatus*; lane 2, *Pleurotus djamor*; lane 3, *Pleurotus citrinopileatus*; lane 4, *Pleurotus geesteranus*; lane 5, *Pleurotus cystidiosus*; lane 6, *Pleurotus eryngii*.

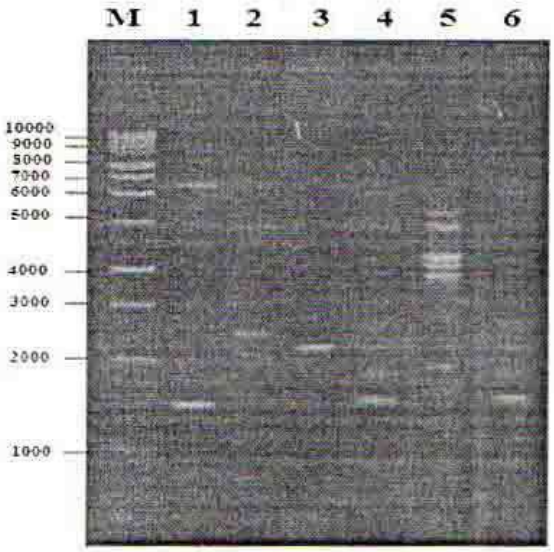

Fig. 2. Random amplification of polymorphic DNA profiles in different species of *Pleurotus* with primer OPA-2. M, molecular size marker (1 kb DNA ladder); lane 1, *Pleurotus ostreatus*; lane 2, *Pleurotus djamor*; lane 3, *Pleurotus citrinopileatus*; lane 4, *Pleurotus geesteranus*; lane 5, *Pleurotus cystidiosus*; lane 6, *Pleurotus eryngii*.

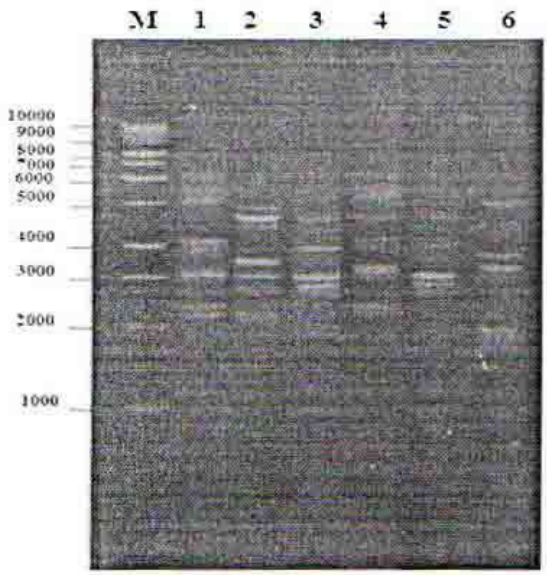

Fig. 3. Random amplification of polymorphic DNA profiles in different species of *Pleurotus* with primer OPA-3. M, molecular size marker (1 kb DNA ladder); lane 1, *Pleurotus ostreatus*; lane 2, *Pleurotus djamor*; lane 3, *Pleurotus citrinopileatus*; lane 4, *Pleurotus geesteranus*; lane 5, *Pleurotus cystidiosus*; lane 6, *Pleurotus eryngii*.

Five primers generated 143 bands from the six different oyster mushroom species using the Thermal Cycler (Genius, Techne) and 1.4% agarose gel electrophoresis. Five primers, the total number of bands (143) varied from 15 to 45 (Table 1). The primer OPA-03 amplified the highest number of bands (45) and the primer OPA-05 amplified the lowest number of bands (15). Out of the 143 bands, 97 bands were monomorphic bands and 46 bands were polymorphic bands. The primer OPA-01 produced the 6 polymorphic bands.

Thus it showed higher level of polymorphism (28%). The primer OPA-02 and OPA-03 produced 11 and 16 polymorphic bands respectively and showed 42% and 35% polymorphism followed by OPA-4 and OPA-5 produced10 and 3 polymorphic bands where level of polymorphism was 27% and 2% respectively. The values of pair-wise comparisons of linkage distances analyzed by using computer software "Statistica" between varieties were computed from combined data for the eight primers, ranged from 28.0 to 51.0 (Table. 2). The highest linkage distance (33.0) was found in P2 vs. P4 species pair. Comparatively higher distance was observed between P1 vs. P6 (50); P3 vs. P4 (49); P4 vs. P5 (45) species pairs than the other species combination. The lowest linkage distance (28.0) was found in P3 vs. P5 species pair. Dendrogram based on linkage distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the six different species into two main clusters at linkage distance 46. P1, P2, P3 and P5 grouped in cluster 1(C_1).

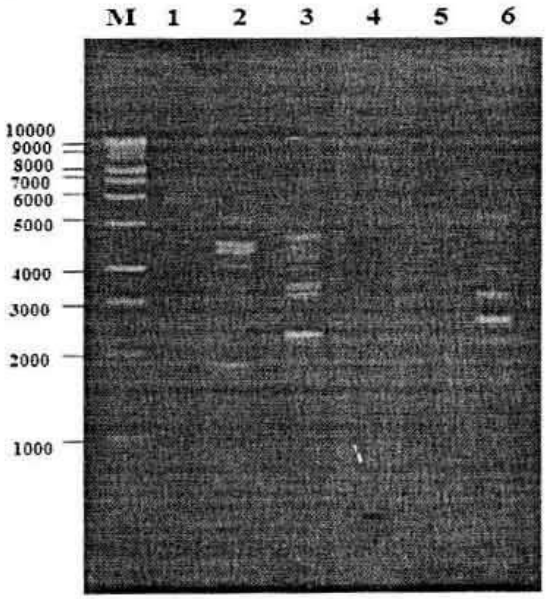

Fig. 4. Random amplification of polymorphic DNA profiles in different species of *Pleurotus* with primer OPA-4. M, molecular size marker (1 kb DNA ladder); lane 1, *Pleurotus ostreatus*; lane 2, *Pleurotus djamor*; lane 3, *Pleurotus citrinopileatus*; lane 4, *Pleurotus geesteranus*; lane 5, *Pleurotus cystidiosus*; lane 6, *Pleurotus eryngii*.

Again P4 and P6 grouped in cluster 2 (C_2) (figure 6). In cluster 1, P2, P3 and P5 formed sub cluster 1(SC_1) and P4 and P6 formed sub cluster 2(SC_2). Again, between the species of sub cluster 1, P3 and P5 grouped together with lower linkage distance of (27). RAPD-PCR generated distinct multiple products showing considerable variability among the tested species. The number of amplified bands varied depending on the primers used or the species tested. The DNA polymorphisms showed the same characteristics in the replication tests. Therefore, if same primers are used for the screening of DNA polymorphisms, it could be possible to distinguish genetically different species of *Pleurotus*. To maximize the specificity of the polymorphic patterns, a combined dendrogram was constructed using the RAPD-PCR amplified bands obtained from the five RAPD primers. Three putative groups among the 6 species of *Pleurotus* were obtained by cluster analysis based on banding patterns and size of amplified products

(Fig.6). RAPD results suggested that among six species, P3 and P5 is very close to P2 also P1, P4 also close to P6 while P1 , P2 ,P3,P5 were quite different as compared to P4 and P6.

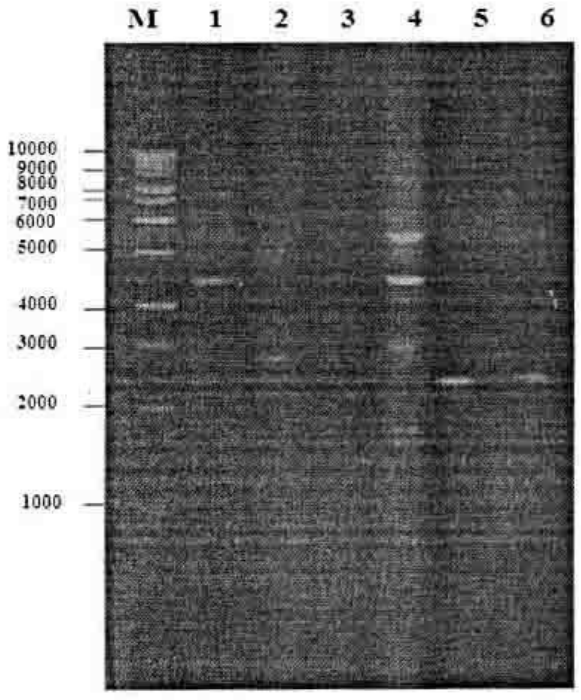

Fig. 5. Random amplification of polymorphic DNA profiles in different species of *Pleurotus* with primer OPA-5. M, molecular size marker (1 kb DNA ladder); lane 1, *Pleurotus ostreatus*; lane 2, *Pleurotus djamor*; lane 3, *Pleurotus citrinopileatus*; lane 4, *Pleurotus geesteranus*; lane 5, *Pleurotus cystidiosus*; lane 6, *Pleurotus eryngii*.

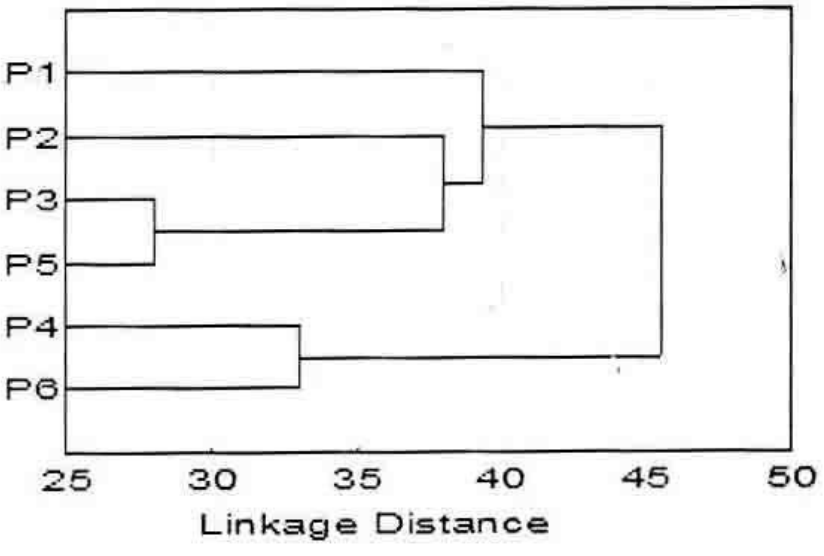

Fig. 6. Dendrogram constructed based on the random amplification of polymorphic DNA markers of *Pleurotus* species determined by the average linkage cluster. P1, *Pleurotus ostreatus*; P2, *Pleurotus djamor*; P3, *Pleurotus citrinopileatus*; P4, *Pleurotus geesteranus*; P5, *Pleurotus cystidiosus*; P6, *Pleurotus eryngii*.

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Bangladesh J. Mushroom. **9**(1): 9-16. 2015 (June)

Effect of Mycelia Growth Duration on Yield and Yield Attributes of Pink Oyster Mushroom

Nirod Chandra Sarker, Manirul Shaheen, Tahera Binte Mujib and Akhter Jahan Kakon
Mushroom Development Institute, Savar, Dhaka, Bangladesh

Abstract

The experiment was conducted to evaluate the effect of different culture ages in multiple phases on the mycelial growth rate, yield and yield attributes of commercially important pink oyster mushroom. Minimum days (7.0 days) required for completion of mycelial running in PDA media (sub culture) which was done just after completion of stock culture (0 days). Spawn packet using mother culture which was prepared by this subculture required 17 days for completion (T_2 treatment). Maximum days (13 days) required for completion of mycelial running in PDA media which was done by using stock culture 15 days after its completion. Spawn packet using mother culture which was prepared by the subculture required 28 days for completion. On the other hand when spawn packet prepared by using sub mother culture which was prepared directly from tissue mother culture (without subculture in PDA media) required lowest days for completion (16 days). The highest yield (98.32g), biological efficiency (51.75%), number of effective fruiting body (20.50), diameter of stalk & pileus (0.93,7.21), lowest days (1.83) requirement to pin head initiation and lowest days (3.83) requirement from opening to first harvest were obtained in T_2 treatment, followed by T_3 (5 days aged stock culture). The lowest yield (59.50g), highest days (6.83) requirement to pin head initiation, highest days (8.83) requirement from opening to first harvest were obtained in T_1 treatment, where spawn packet was prepared by using tissue mother directly.

Key words: Vigour, Pink oyster, Preservation, Pure culture, Age, Subculture.

INTRODUCTION

Mushroom cultivation for food and medicine is a well established profitable in many countries of the world (Jong, 1989; Trinci, 1992). Successful mushroom production depends upon the proper maintenance of pure culture, spawn capable of providing higher yields, excellent flavour, palatable texture, colour and resistance to pest and diseases. Maintenance of vigour and genetic characteristics of a pure strain in form of a culture is the main objective of strain preservation. There are various methods of maintenance and preservation of mushroom culture but sub culturing or periodic transfer on a culture media is a common method in our country. Pure culture age is also an important factor to maintain the vigour of the desired strain. Degeneration of culture or spawn refers to the loss of desired traits leading to slow development, poor rate of survival and low level of productivity (Chang and Miles, 1989). So, the aim of the study was to determine the optimum age of pure/ stock culture for the production of vigorous mycelium in spawn packet within short time and higher yield.

MATERIALS AND METHODS

This experiment was conducted in the tissue culture laboratory of Mushroom Development Institute during March to September 2012. Different culture ages in multiple phases were used in cultivation of *Pleurotus djmor* (Pop). The inocula were collected from the germplasm centre of National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka.

Preparation of synthetic (PDA) media: The PDA media was prepared by 25g of dextrose and 250g, potato mixed with 20g agar at pH 6.5. The mixture was boiled on gas burner until the agar dissolved. The media was poured into petri plate at 20 ml/plates and sterilized in an autoclave for 20 minutes at 120^0C under 1.5 kg/cm^2 pressure. After sterilization and solidification, the plates were inoculated with the inocula of testing mushroom species. Then plates were transferred in incubation room for mycelium running at 20-25^{0}C temperature. Different stages of pure culture were used for preparing sub culture, mother culture and spawn packet (According to treatment combination).

Comparison of growth rates of mushroom mycelia on culture media: Obtained quantity of mushroom mycelia were expanded through same tissue culture techniques. Pieces (0.5 cm in diameter) from the culture plates of each of the treatment were transferred with the help of a cork borer aseptically on fresh media plates. For each treatment four replications were done. Then plates were transferred in incubation room for mycelium running at 20-25^{0}C temperature. The growth of mushroom mycelia on these replica plates was recorded daily with help of a transparent ruler.

Preparation of mother culture: Mother culture was prepared by mixing sawdust and wheat bran at the ratio of 2:1. Calcium carbonate was used at the rate of 0.2% of the mixture. The moisture level of the mixture was maintained at 60% by adding fresh water. Polypropylene bags of 18 cm × 25 cm size were filled with 300 g of the above prepared mixture and packed tightly. The neck of the bag was prepared by using heat resistant plastic neck. A hole of about 2/3 deep of the volume of the bag was made at the center with a sharp end stick for space to put inoculums. The neck was plugged with cotton and covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for one hour at 120^0C under 1.5 kg/cm^2 pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. A piece of pure culture medium containing mycelium of pink oyster mushroom variety at different phase according to treatments was placed aseptically in the hole of mother culture packet and the packet was again plugged as mentioned before. Then the inoculated packets were placed on a wooden rack in the laboratory at 25 ± 2^{0}C temperature for incubation. The medium of the mother culture was colonized by the strains as manifested by white colony growth of mycelium. The fully colonized packets were used for spawning.

Spawn packets preparation: The substrate of spawn packets were prepared using sawdust and wheat bran mixture at the ratio of 2:1 (dry weight/weight basis). Water was added to make the moisture content at 60% and $CaCO_3$ was added at the rate of 0.2%

(w/w) of the total mixture to maintain the pH level at 6.5 to 7.0. Polypropylene bags of 18cm x 25 cm were filled with 500 g of prepared substrate. The packets were tied, plugged with absorbent cotton and covered with brown paper. Then the packets were sterilized in an autoclave for 2.0 hour at 121^0C under 1.5 kg/cm^2 pressure. After sterilization the packets were cooled and transferred to an inoculation chamber and inoculated with the mother culture of test materials at the rate of one teaspoonful per packet. The inoculated packets were placed on a still rack at 25 ± 2^0C temperature for incubation.

Experimental condition: The packets were kept in a dark room at 25^0C for incubation. When colonization of mycelium was completed, the spawn packets were taken to a culture house and were opened by 'D' shaped cut on the shoulder and removed the sheet. The relative humidity and temperature of the culture house were maintained at 80-90% and 20-25^0C respectively by spraying water. Diffused light, about 200 lux and proper ventilation in culture house were maintained. After harvesting of mushroom, the residues were removed from the packet and temperature and relative humidity were maintained as before. The yield was obtained from single, flush in the harvest period. Yield in g/packet was recorded by weighing all the fruiting bodies in a packet after removing the lower dirty portion. The biological efficiency was determined using the following formula:

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

Data collection and statistical analysis: The experiment was laid out following completely randomized design (CRD) with 4 replications. Data on duration of mycelia completion at different phase, days required to pinhead initiation, days required from opening to first harvest, number of fruiting bodies, size of fruit body , yield, and biological efficiency were recorded and analyzed following Gomez and Gomez (1984) using MSTAT-C computer program. Means separation were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULT AND DISCUSSION

Duration of mycelial completion at different phase (days): Duration of mycelia completion in different phase of culture is shown in Table 1.

Days to complete mycelium running in Stock/ pure culture: 10 days required for the completion of mycelium running when tissue of *Pleurotus djmor* was directly inoculated in PDA media.

Days to complete mycelium running in secondary pure culture: Minimum days (7.0days) required for completion of mycelium running was observed when tissue completes and 0 days age then sub-culture was done in PDA media. Maximum days required for completion of mycelium running was observed when tissue complete and 5 days age then

sub-culture was done in PDA media which was followed by tissue complete and 15 days age then sub-culture.

Days to complete mycelium running in tertiary pure culture: 11 days required for the completion of mycelium running when 10 days age sub-culture tissue was subculture in PDA media.

Days to complete mycelium running in quaternary pure culture**:** 8 days required for the completion of mycelium running when 10 days age sub.sub culture tissue was subculture in PDA media.

Days to complete mycelium running in master mother culture: Minimum days (32.0days) required for completion of mycelium running was observed when tissue completes and 15 days age then sub-culture was done in PDA media. Maximum days (37) required for completion of mycelium running was observed when tissues complete and 10 days age then sub-culture was done in PDA media.

Days to complete mycelium running in secondary (sub mother) mother culture: 27 days required for the completion of mycelium running when sub mother directly prepared to tissue mother in sawdust based media.

Days to complete mycelium running in tertiary (sub. sub mother) mother culture: 24 days required for the completion of mycelium running when sub.sub mother prepared to sub mother in sawdust based media.

Days to complete mycelium running in spawn packet: Minimum days (27.0days) required for completion of mycelium running was observed when tissue completes and 0 days age then sub-culture was done in PDA media. Maximum days (38) required for completion of mycelium running was observed when tissues complete and 15 days age then sub-culture was done in PDA media.

Days required to pin head initiation: The minimum days (1.83) required to pin head initiation was observed from the treatment when tissue complete and 0 days age then sub-culture was done in PDA media. The maximum days (6.83) required to pin head initiation was observed when mother culture directly prepared to tissue ie. pure culture without subculturing (Table 2).

Days required to first harvest: The minimum days (3.25days) required from opening to first harvest was observed from the treatment when tissue complete and 15 days age then sub-culture was done in PDA media then prepared mother culture and spawn packet respectively .The maximum days (8.83 days) required from opening to first harvest were observed when tissue ie pure culture complete then directly prepared mother culture no sub culture was done and spawn packet respectively (Table 2).

Number of fruiting body: The number of fruiting body under different treatment combinations varied significantly (Table 2). The highest number (29.08) of fruiting body was observed from the treatment T_3 followed by T_2, T_4 and T_7. The lowest number (14.17) of fruit body was observed from the treatment T_1.

Table 1. Effect of different age of culture in multiple phases on mycelial growth rate of *Pleurotus djamor* mushroom (pop)

Treatments	Days to completion of mycelium running on PDA media	Days to complete mycelium running in sub tissue	Days to complete mycelium running in sub to sub tissue	Days to complete mycelium running in sub.sub to sub tissue	Days to complete mycelium running in mother culture	Days to complete mycelium running in sub mother	Days to complete mycelium running in sub sub mother	Days to complete mycelium running in spawn packet
T_1	10.00	--	--	--	23.00	--	--	27.00
T_2	--	7.00	--	--	16.00	--	--	17.00
T_3	--	13.00	--	--	18.00	--	--	20.00
T_4	--	11.00	--	--	20.00	--	--	25.00
T_5	--	13.00	--	--	22.00	--	--	28.00
T_6	--	--	11.00	--	23.00	--	--	26.00
T_7	--	--	--	8.00	--		--	17.00
T_8	--	--	--	--		17.00	--	16.00
T_9	--	--	--	--	--	--	14.00	20.00

In a column, means followed by a common letter are not significantly different at 5% level by DMRT. T_1= Tissue to mother, T_2= Tissue complete to 0 days sub, T_3= Tissue complete to 5 days sub, T_4= Tissue complete to 10 days sub, T_5= Tissue complete to 15 days sub, T_6= 10 days sub to sub, T_7= 10 days sub.sub to sub, T_8= Tissue mother to sub mother, T_9= Sub mother to sub.sub mother.

Table 2. Effect of different age of culture in multiple phases on yield and yield attributes of *Pleurotus djamor* mushroom (pop)

Treatments	Days required to pin head initiation	Days required opening to harvest	Number of fruit body	Number of effective fruit body	Diameter of stalk (cm)	Diameter of pileus (cm)	Length of stalk (cm)	Thickness of pileus (cm)	Yield per packet (g)	Biological efficiency (%)
T_1	6.83a	8.83a	14.17g	10.50e	0.87b	7.17a	1.88d	0.62cd	59.50f	31.32e
T_2	1.83f	3.83e	25.58b	20.50a	0.93a	7.21a	2.28b	0.65bc	98.32a	51.75a
T_3	2.83cd	4.67bc	29.08a	18.33b	0.91a	6.63b	1.92cd	0.69ab	91.42b	48.11b
T_4	3.00bc	4.50bc	25.00b	20.92a	0.82bc	6.04ef	2.00c	0.52e	76.75d	40.39d
T_5	1.58g	3.25f	23.33c	18.83b	0.68f	6.13def	1.73e	0.59d	82.75c	43.55c
T_6	1.83f	3.92de	20.75d	16.00c	0.77de	6.33c	1.94cd	0.53e	71.63e	37.70d
T_7	3.08b	4.92b	24.75b	20.00a	0.72ef	6.21cde	1.56f	0.41f	76.67d	40.35d
T_8	2.67d	4.67bc	17.50e	13.00d	0.78cd	6.25cd	1.95cd	0.73a	76.50d	40.26d
T_9	2.42e	4.25cd	16.17f	10.25e	0.83b	5.96f	2.48a	0.61cd	59.67f	31.40e
CV%	**4.12**	**5.72**	**3.71**	**4.66**	**4.15**	**1.76**	**3.49**	**3.89**	**3.44**	**4.37**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT. T_1= Tissue to mother, T_2= Tissue complete to 0 days sub, T_3= Tissue complete to 5 days sub, T_4= Tissue complete to 10 days sub, T_5= Tissue complete to 15 days sub, T_6= 10 days sub to sub, T_7= 10 days sub.sub to sub, T_8= Tissue mother to sub mother, T_9= Sub mother to sub.sub mother.

Number of effective fruit body: The number of effective fruiting body under different treatment combinations varied significantly (Table 2). The highest number (20.92) of effective fruiting body was observed from the treatment T_4 which was statistically significant to the treatment T_2 and T_7. The lowest number (10.25) of fruit body was observed from the treatment T_9 which was statistically significant to the treatment T_1.

Size of fruiting body: The diameter of stalk ranged from 0.68 to 0.93 cm with significant difference (Table 2). The highest diameter (0.93 cm) of stalk was found in T_2 which was statistically significant to the treatment T_3. The lowest diameter (0.68 cm) of stalk was found from the treatment T_9.

The diameter of pileus ranged from 5.96 cm to 7.21 cm with significant difference among the treatments (Table 2). The highest diameter (7.21 cm) of pileus was found in T_2 which was statistically significant to the treatment T1 and the lowest diameter 5.96 cm) of pileus was found in treatment T_9 which was statistically significant to the treatment T_4 and T_5.

The length of stalk ranged from 1.56 to 2.48 cm with significant difference (Table 2). The highest length (2.48 cm) of stalk was found in T_9 followed the treatment T_2. The lowest length (1.56 cm) of stalk was found from the treatment T_7.

The thickness of pileus in different species differed significantly and ranged from 0.41 cm to 0.73cm (Table 2). The highest thickness (0.73 cm) of pileus was found in the treatment T_8 which was statistically significant to the treatment T_3. The lowest thickness (0.41 cm) of pileus was found in the treatment T_7.

Yield / Packet (g): Significant variation was observed in yield under different treatment (Table 2). The highest yield (98.32g) was found in T_2 followed by T_3 and the lowest yield (59.50 g) was found in T_1 which was statistically significant to the treatment T_9.

Biological efficiency (%): Significant variation was observed on biological efficiency (Table 2). The highest biological efficiency (51.75%) was found in T_2 and the lowest biological efficiency (31.32%) was found in T_1 which was statistically significant to the treatment T_9.

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

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

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

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Effect of *Ganoderma lucidum* on Hypertension and Lipid Profile Status of Randomly Selected Male Volunteers

Syed Sakhawet Hussain[1], Md. Bazlul Karim Choudhury[2], Akhter Jahan Kakon, Khodeja Khatun[3], Rashida Begum[4] and M. Badrul Alam[5]
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

For evaluating the effect of *Ganoderma lucidum* on hypertension and lipid profile status of randomly selected male volunteers, the present study was conducted in Nationl Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka. Total 1.5 grams of dried *Ganoderma lucidum* powder as capsule form was taken by the subjects in three divided doses for three months. The finding of the study showed a non significant small (1.84%) reduction of systolic blood pressure (116.86 ± 2.06 and 114.70 ± 1.61, p = 0.263). There was also non significant small (0.91%) reduction of diastolic blood pressure (75.78 ± 1.16 and 75.09 ± 1.32, p = 0.628). Considering lipid profile status it was observed that, three months consumption of *Ganoderma lucidum* causes a significant (8.49%) reduction of total cholesterol (166.39 ± 5.49 and 151.25 ± 6.25, p = 0.010), a non-significant (11.50%) reduction of TG (167.25 ± 15.97 and 148.01 ± 16.44, p = 0.056), a significant (16.91%) elevation of HDL-C (30.80 ± 1.04 and 36.01 ± 1.31, p = 0.000), and a significant (13.49%) reduction of LDL-C (100.41 ± 5.04 and 86.86 ± 5.60, p = 0.007). Findings of the study suggest that regular consumption of *Ganoderma lucidum* has a positive impact on reducing blood pressure and it also causes sufficient improvement of lipid profile status.

Key words: Blood pressre, Lipid profile, Reishi mushroom.

INTRODUCTION

Reishi (*Ganoderma lucidum*) is a wood-degrading basidiomycetes with numerous pharmacological effects. It produces a large reservoir of bioactive compounds; thus far, more than 400 different compounds have been identified (Shiao, 2003), making this fungus a virtual cellular 'factory' for biologically useful compounds. The most important pharmacologically active constituents of *G. lucidum* are triterpenoids and polysaccharides. Triterpenoids have been reported to possess anti-hypertensive, hypocholesterolemic effects (Boh *et al.*, 2007; Bao *et al.*, 2002).

Ganoderma lucidum is a traditional Chinese medicine product known to the layman as the "herb of immortality". Its pharmacological activities are widely recognized, as indicated by its inclusion in the American Herbal Pharmacopoeia and Therapeutic Compendium (Sanodiya *et al.*, 2009). Some authors have suggested antifungal, anti-

[1] Department of Biochemistry, Parkview Medical College, Sylhet, Bangladesh; [2] Department of Biochemistry, Manikganj Medical College, Manikganj, Bangladesh; [3] Department of Radiology & Imaging, Rajshahi Medical College, Rajshahi, Bangladesh; [4] Department of Biochemistry, Bangladesh Medical College, Dhaka, Bangladesh, [5] Department of Cardiovascular Surgery, National Institute of Cardiovascular Disease, Dhaka, Bangladesh.

inflammatory, antitumor, antiviral, antibacterial, hepatoprotective, antidiabetic, hypolipedemic, antithrombotic and hypotensive activities for reishi (Ajith and Kainoor, 2007; Raquel *et al.*, 2008).

It has been shown that *G. lucidum* reduce blood pressure after only 10 days of consumption. In mild cases the normalization of blood pressure is between 25 and 120 days. One study with 53 patients with an average blood pressure of 165.5 (systolic) over 106.4 (diastolic) had 136.6/92.8 after 6 months, well within the WHO guide lines for healthy blood pressure (Kanmatsue *et al.*, 1985; Yearul, 1988). *G. lucidum* is considered to be a natural medicine that promotes longevity and maintains the vitality of human beings. The fruiting bodies, mycelia, and spores of *G. lucidum* have recently received more and more attention not only as home remedies but also as new drug sources (Gao *et al.*, 2004).

Reishi mushroom has been widely researched and hence it has been a part of many publications as it is already evident from the literature cited above. On the basis of many such evidences, there are many products in the market which are sold primarily as neutraceuticals. Some of them are marketed as dietary supplements and very widely used by consumers in combination with coffee and tea. Hence further research on *Ganoderma* for validating its usage is a demand of the present day (Nahata, 2013).

Systemic Blood pressure (BP) rises with age, and the incidence of cardiovascular disease (particularly stroke and coronary artery disease) is closely related to average BP at all ages, even when BP readings are within the so-called 'normal range'. Optimal systolic BP is <120 mmHg and Diastolic BPis <80 mmHg, Normal BP range is 130/85 mmHg and Hypertension is divided in three grade. Grade 1 (mild) systolic 140–159 mmHg, diastolic 90–99 mmHg, Grade 2 (moderate) systolic 160–179 mmHg, diastolic 100–109 mmHg and Grade 3 (severe) systolic ≥180 mmHg, diastolic >110 mmHg (Newby *et al.*, 2014).

Various alternative attempts to prevent or control hypertension have been investigated in view of the serious consequences of this condition and the side effects of anti-hypertensive drugs. *Ganoderma lucidum*, a traditional medicine that has been used for centuries is one of the approaches to achieve this goal. In a human clinical study conducted in Japan, *Ganoderma lucidum* extract was shown to have blood pressure lowering effect on patients with essential hypertension and not exhibited any side effects on patients with essential or borderline hypertension during six months oral intake(Kanmatsue *et al.*, 1985).

Dyslipidaemia is characterized by elevated levels of small dense low-density lipoprotein (LDL) cholesterol and triglycerides, and a low level of high-density lipoprotein (HDL) cholesterol. Dyslipidaemia is a component of 'metabolic syndrome' and is much more common in patients who are obese. It is a metabolic disorder which is often associated with diabetes mellitus (Pearson and McCrimmon, 2014).

Previous studies have suggested that *Ganoderma lucidum* has antioxidant effects and possibly beneficial effects on blood pressure, plasma lipids, but these have not been confirmed in subjects with hypertension and/or dyslipidaemia. The objective of the current study was to assess the net effect of antihypertensive and lipid profile responses to therapy with *Ganoderma lucidum* in patients with elevated blood pressure and/or dyslipidaemia in a controlled cross-over trial.

MATERIALS AND MATHODS

The experiment was conducted in the Laboratory of Strengthening Mushroom Development Project, National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka.

Total 46 randomly selected healthy male subjects aged from 32.to 63 years reside at Savar (Dhaka) were considered in the study. The subjects were clarified about the study and after getting their written consent showing willingness to participate in the study they were included. The details history was taken from the subjects which included age, occupation, educational status, marital status, family history and drug history.

Any acute or chronic disease or medication, mal-absorption during the study and any addiction except cigarette smoking were excluded from the study. None of the female subjects were included.

Study design: At the beginning of study, subjects were evaluated for health status. Fasting blood sample was collected for analysis. Mushroom capsules which contain 500 mg *Ganoderma lucidum* powder in each were supplied to take one capsule three times daily, so that each subject took 1.5 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.

Collection of blood sample: 10 ml fasting blood sample was collected from median cubital vein with all aseptic precaution. Immediately after collecting blood it was poured into a clean test tube. The test tube then kept in a test tube holder. After half an hour blood was centrifuged by 3000 rpm for 5 minutes. Serum was separated which were transferred into two eppendorf containing 1 ml in each. All the tests were carried out as early as possible.

Preparation of Mushroom capsule: Fresh fruiting body of *Ganoderma lucidum* was collected from culture house of National Mushroom Development and Extension Centre (NAMDEC). Collected mushrooms were sun dried at moisture level 4-5% then grinded and pour into capsule shells which contain 500 mg powder. Prepared capsules were preserved into moisture free glass container which was ready to dispense.

Measurement of blood pressure: Blood pressure was measured using sphygmomanometer by trained physician. Cuff of the sphygmomanometer was binded on upper two third of the right arm of the subjects. Bell of the stethoscope was placed over bifurcation of right brachial artery, hand pump was used to block the brachial artery and reading of systolic and diastolic blood pressure was taken according to changes of corrotcoffs sound.

Biochemical Analysis: Serum total cholesterol (TC), triglyceride (TG), HDL-C and LDL-C were investigated during the procedure. All the biochemical parameters for the measurement of lipid profile were estimated by semi-auto analyzer (3000 evaluation) using the available reagent kit.

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

RÉSULTS AND DISCUSSION

Study of blood pressure (BP): The Mean (± SE) systolic blood pressure (mmHg) before and after three months mushroom treatment were 116.86 ± 2.06 and 114.70 ± 1.61, respectively (Table 1). A non-significant reduction of systolic blood pressure ($p = 0.263$), 1.84% between the two periods was observed. Considering diastolic blood pressure (mmHg), the mean (± SE) before mushroom supplementation was 75.78 ± 1.16 and after mushroom supplementation it was 75.09 ± 1.32 (Table 1). Here also a non-significant reduction of mean difference observed between the two periods ($p = 0.628$), 0.91%.

Table 1. Showing the blood pressure status of the subjects

Parameter	Number of subjects (n)	Values		p	%
		Pre Treatment (mean ± SE)	Post Treatment (mean ± SE)		
Systolic BP	46	116.86 ± 2.06	114.70 ± 1.61	0.263	1.84% reduction
Diastoic BP	46	75.78 ± 1.16	75.09 ± 1.32	0.628	0.91% reduction

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

Study of serum total cholesterol (TC): The mean (± SE) total cholesterol (TC) (mg/dl) before- and after- supplementation was 166.39 ± 5.49 and 151.25 ± 6.25, respectively. A significant ($p = 0.010$) 8.49% reduction of TC was observed (Table 2).

Study of serum triglyceride (TG): The mean (± SE) TG (mg/dl) before- and 3 months after- supplementation of Reishi mushroom was 167.25 ± 15.97 and 148.01 ± 16.44,

respectively. In this case, a non-significant (p = 0.056) 11.50% reduction of TG was observed (Table 2).

Table 2. Showing the lipid profile status of the subjects

Parameter	Number of subjects (n)	Values		p	%
		Pre Treatment (mean ± SE)	Post Treatment (mean ± SE)		
Cholesterol (mg/dl)	46	166.39 ± 5.49	151.25 ± 6.25	0.010	8.49% reduction
Triglyceride (mg/dl)	46	167.25 ± 15.97	148.01 ± 16.44	0.056	
HDL-C (mg/dl)	46	30.80 ± 1.04	36.01 ± 1.31	0.000	
LDL-C (mg/dl)	46	100.41 ± 5.04	86.86 ± 5.60	0.007	

Results show mean ± SE. Data were analyzed by Student's Paired 't' test. Means were significantly different at $p < 0.05$ at 95% confidence limit (HDL-C = High density lipoprotein and LDL-C = Low density lipoprotein).

Study of serum high density lipoprotein cholesterol (HDL-C): The mean (± SE) plasma high density lipoprotein (HDL-C) (mg/dl) before- and 3 months after-supplementation of *Ganoderma lucidum* was 30.80 ± 1.04 and 36.01 ± 1.31, respectively. A significant (p = 0.000), 16.91% elevation of HDL-C was observed (Table 2).

Study of serum low density lipoprotein cholesterol (LDL-C): The mean (± SE) serum low density lipoprotein (LDL-C) (mg/dl) before- and 3 months after- supplementation of *Ganoderma lucidum* was 100.41 ± 5.04 and 86.86 ± 5.60, respectively. A Significant (p = 0.007), 13.49% reduction of LDL-C was observed (Table 2).

Considering the above findings on blood pressure and lipid profile, it is observable that *Ganoderma lucidum* causes reduction of blood pressure and improves lipid profile status. But how does *Ganoderma* help on high blood pressure and dyslipidaemia? Within any population, blood pressure values occur within a continuum, and are determined by mechanical, hormonal and environmental factors. Any definition of hypertension therefore utilizes arbitrary threshold values within this continuum. The triterpenoids in *Ganoderma lucidum* effectively clears blood vessel blockage by reducing accumulated fatty substances such as cholesterol and triglycerides. The adenosine in *Ganoderma lucidum* may help to dissolve thrombus and helps to remove blood vessel blockage. This herb makes the blood flows smoother thus reducing the stress on the heart. The most important point is *Ganoderma lucidum* is a natural herb that being used traditionally for thousand years with no reports of any adverse effects.

Our liver produces most of the circulating cholesterols and triglycerides in order to allow essential biochemical activities, such as energy provision and cell metabolism. Unfortunately, due to our diet (increased meat consumption) and lack of exercise, there is an excess amount of cholesterol and triglycerides in our body, resulting in the development of hyperlipidemia. In 2004, Swiss researches used an animal testing model

on hyperlipidemic mini-pigs and found that *Ganoderma* effectively reduced total cholesterol by 20% and levels of bad cholesterol (LDL) by up to 27%. In 2005, the same Swiss research team discovered that the cholesterol-lowering mechanism of *Ganoderma* was a result of *Ganoderma* triterpenoids (ganoderic alcohol and ganoderic acid) inhibiting the bio-synthesis of cholesterols in the liver of the mini-pigs. This means *Ganoderma* triterpenoids could prevent the liver from producing an excess of cholesterol. Based on an analysis of *Ganoderma*, these effective *Ganoderma* triterpenoids are found to be rich in Yung Kien *Ganoderma* (belonging to a strain of G. tsugae).

Ganoderma lucidum can effectively stretch coronary artery, increase coronary vessel blood flow, and improve circulation in cardiac muscle capillaries, therefore increasing the supply of oxygen and energy to cardiac muscle. Hence *G. lucidum* mushroom guards the heart from shortage of blood supply, and is ideal for both curing and preventing heart related ailments. It reduces the level of blood cholesterol and triglycerides in a hypertensive patient. If one is suffering from cholesterol, the magic medicine will reduce cholesterol in arterial wall and soften the blood vessel to avoid further damage. It also partially improves blood circulation making the body and heart less prone to a heart attack and a stroke. *Ganoderma lucidum* has been proven reduce blood pressure upon consumption. It reduces stress since it encourages blood flow and reduces oxygen consumption to the vessels of the heart. For centuries eastern physicians have carried out constant researches that lead to one fact.

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Mineral Content of Fifteen Oyster Mushroom Strains Available at Mushroom Development Institute

Akhter Jahan Kakon, Md. Bazlul Karim Choudhury[1], Tasnima Mahjabin, Tahera Binte Mujib, and Manirul Shaheen
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

At present different oyster mushroom genotypes are cultivating in Bangladesh. Among them the oyster mushrooms are important because of their enrichment with proteins and minerals. Thus the study was conducted to determine the contents of 7 minerals (Ca, Fe, Mo, Co, Cu, Se, Zn) in fifteen different oyster mushroom genotypes. The total ash content ranged from 6g to 11 g/100g dry wt. The highest (11g/100g) ash content was obtained in Po2 mushroom and the lowest (6g/100g) was found in Po9, Py2 and Psc1 mushroom. Fibre content ranged from 20.4g to 38.2 g/100g dry wt. The highest (38.2/100g) fibre content was obtained in Po4 mushroom and the lowest (20.4g/100g) was found in Po2, mushroom Content of calcium (Ca), Iron (Fe), Cobalt (Co) Copper (Cu), Selenium (Se), Zinc (Zn) and Molybdenum (Mo) are ranged from 11.14mg – 26.00mg, 6.95mg – 16.83mg, 0.028mg - 0.119mg, 0.45 mg – 0.78 mg, 0.002 -0.085, 2.00 – 10.05 and 0.008 – 0.082 /100g, respectively. The highest (16.83mg/100g) iron content obtained in Po10 mushroom and lowest (6.95mg/100g) was found in Po3. The highest (26.00mg/100g) calcium content was obtained in Po1 mushroom and the lowest concentration (11.14mg/100g) was found in Psc1 mushroom. The highest (0.119mg/100g) cobalt was found in Po2 mushroom and lowest (0.028 mg/100g) was found in Po8 mushroom. Considering molybdenum content, the highest (0.082mg/100g) was found in Po2 mushroom and the lowest (0.008mg/100g) was found in Py2. The highest (0.78mg/100g) cupper was found in Po9 mushroom and lowest (0.45mg/100g) was found in POP2 mushroom. The highest (10.05mg/100g) Zinc was found in Po10 mushroom and lowest (2.00mg/100g) was found in Ws mushroom. The highest (0.082mg/100g) Selenium was found in Po4 mushroom and lowest (0.008 mg/100g) was found in Po5 mushroom.

Key words: *Pleurotus spp,* Minerals, Ash.

INTRODUCTION

Minerals are essential for human beings. It is necessary for metabolic function in human body. Minute amounts of minerals are requiring for metabolic function but due to its deficiency body cannot maintain its proper function. Mineral deficiency is a common feature at rural area in Bangladesh. Due to iron deficiency, People suffers from anemia. Calcium is a macro mineral for all age group. Inadequate calcium intake causes osteoporosis in old age and rickets in childhood. Cobalt and molybdenum also helps metabolic function, cellular activities, and normal brain development of human body. So supplementation of mineral rich foods is essential for the country like Bangladesh. Ongoing oyster mushroom cultivation and its intake can mitigate mineral deficiency for

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

all ages. Most of the edible mushrooms are protein rich and excellent source of fibers, vitamins and minerals (Cheung, 1996; Mattila *et al.*, 2002; Barros *et al.*, 2007; Ouzouni *et al.*, 2009; Manjunathan and kaviyarasan, 2011). Mushrooms are consumed not only for nutritive but also as medicinal purpose (Agrahar *et al.*, 2005). Mushrooms are also reported as therapeutic food. Since the different oyster mushroom genotypes may taken as minerals and nutrients supplement in human diet, its content in different mushroom is a valuable information to categorize them. This is why the experiment was conducted to find out minerals content of some edible oyster mushroom genotypes.

MATERIALS AND METHODS

Fifteen genotype of oyster mushroom namely, *Pleurotus ostreatus* (Po1, Po2, P03, Po4, Po5, Po6, Po8, Po9, Po10, Po11), *Pleurotus ostreatus* var. white snow (Ws), *Pleurotus djamour* (pop2), *Plerotus sajor cajo* (Psc-1, Psc-2), *Pleurotus citrinopileatus* (Py2) were selected for mineral analysis. The genotypes were cultivated using mixed sawdust as substrate. Analysis of these mushroom were performed in the quality control and quality assurance laboratory of the NAMDEC during June to August 2012.

Determination of total ash: One gram of dry oyster mushroom 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 600^0C. Then the sample containing crucible were cooled and kept in a desiccators. Then total ash was calculated as following equation (Raghuramulu *et al.*, 2003):

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

Determination of minerals: Total ash was used for analysis of mineral contents. Two ml of conc. HNO_3 was added to the ash and placed it for two hours. One drop of hydrogen peroxide (H_2O_2) was added to the solution to remove turbidity. The solution was then transferred to a volumetric flask and total volume was made 100 ml by adding de-ionized water.

One ml of standard solution (Normal or any other) was taken into a volumetric flask and total volume was made100 ml by adding de-ionized water and shaken it properly. This solution is called secondary stock solution for the particular minarets. Standard solution of the mineral was prepared as per the instruction of the AAS. The standard was then used to analyze the contents of calcium (Ca), iron (Fe) zinc (Zn) and cobalt (Co), by flame method and selenium (Se) by graphite furnace method with Atomic Absorption Spectrophotometer (AAS 240,Varian) and Graphite furnace (GTA 120, Varian).

RESULTS AND DISCUSSION

Total ash content: Among 15 mushroom genotypes, total ash content was varied from 6 to 11 g /100 g of dried mushroom. The highest (11 g/100g) ash content was obtained in

Po2 mushroom and the lowest (6g/100g) was found in Po9, Py2 and Psc-1 mushroom. The findings is supported by Ahmed *et al.* 2009 who reported that the total ash content of *Pleurotus florida* in different substrate was found in the ranged from 6 to 8.9g/100g.

Table 1. Total ash and mineral content of oyster mushroom (*Pleurotus spp.*) genotypes and strains

Mushroom genotype	Total ash content g/100g	Fibre g/100g	Mineral content (mg/100g dry weight)						
			Iron	Calcium	Zinc	Cobalt	Coppe r	Selenium	Molybdenum
Po1	9	26.1	15.27	26.00	8.44	0.064	0.71	0.029	0.0098
Po2	11	20.4	10.47	23.87	3.94	0.119	0.70	0.036	0.082
Po3	7	26.2	6.95	12.30	5.93	0.060	0.71	0.048	0.0099
Po4	8	36.1	7.48	12.21	8.90	0.058	0.58	0.085	0.011
Po5	8	38.2	12.24	13.34	9.93	0.050	0.62	0.002	0.010
Po6	7	22.3	11.16	13.36	9.35	0.056	0.49	0.035	0.010
Po8	7	24.6	13.79	15.00	7.27	0.028	0.57	0.028	0.011
Po9	6	28.4	11.47	15.41	6.37	0.083	0.78	0.062	0.012
Po10	9	22.1	16.83	12.10	10.05	0.097	0.64	0.049	0.014
Po11	9	28.3	13.41	14.13	6.21	0.086	0.67	0.056	0.013
Ws	8	26.0	13.49	17.11	2.00	0.080	0.68	0.053	0.014
Py-2	6	24.7	13.92	16.49	3.78	0.097	0.59	0.031	0.008
Pop-2	8	28.0	13.7	21.09	2.28	0.052	0.45	0.049	0.011
Psc-1	6	26.2	12.13	11.14	3.45	0.094	0.58	0.025	0.010
Psc-2	7	25.8	12.37	21.50	7.77	0.068	0.50	0.027	0.071

Fibre content: Among 15 mushroom genotypes, fibre content was varied from 20.4 to 38.2 g /100 g of dried mushroom. The highest (38.2 g/100g) fibre content was obtained in Po5 mushroom and the lowest (20.4g/100g) was found in Po2 mushroom. The findings is supported by Ahmed *et al.* 2009 who reported that the total ash content of *Pleurotus florida* in different substrate was found in the range from 6 to 8.9 g/100g.

Mineral contents: Iron content was ranged from 6.95 - 16.83 mg/100g dry wt. basis among 15 genotypes of oyster mushroom. Highest (16.83 mg/100g) of iron content obtained by Po10 mushroom and lowest (6.95mg/100g) one was found in Po3 mushroom which is more or less similar with Ahmed *et al.* (2009) who showed that *Pleurotus florida* content 13.06mg/100g of iron on dry wt basis. Here it is notable that this content of iron can fulfill the daily requirement of children adults both male and female. So it is notable that oyster mushroom can fulfill the demand of non hem iron. Among the 15 oyster mushroom the highest (26.0 mg/100g) amount of calcium was obtained in Po1 mushroom which is supported by Mallikarjuna *et al.* (2012) who reported that *Pleurotus florida* and *Pleotus djamor* contains calcium ranged from 8. 27 and 34.2 mg/100g respectively. And the lowest content was found in 11.14 mg/100g in Psc-1 mushroom. Zinc content was ranged from 2.0 to 10.05 mg/100g dry wt. basis. The highest zinc (10.05 mg/100g) content was found in Po10 mushroom and the lowest (2.0 mg/100g) one was found in Ws

mushroom. Cobalt content was ranged from 0.028 to 0.119 mg/100g dry wt. basis. The highest cobalt (0.119 mg/100g) content was found in Po2 mushroom and lowest (0.028 mg/100g) one was found in Po8 mushroom. Copper content was ranged from 0.45 to 0.78 mg/100g dry wt. basis. The highest copper (0.78 mg/100g) content was found in Po9 mushroom and lowest (0.45 mg/100g) one was found in Pop2 mushroom. Selenium content was ranged from 0.002 to 0.085 mg/100g dry wt. basis. The highest selenium (0.085 mg/100g) content was found in Po4 mushroom and lowest (0.002 mg/100g) one was found in Po5 mushroom. In the case of molybdenum the highest content (0.082g/100g) was found in Po2 mushroom and the lowest (0.008g/100g) content was found in Py2 mushroom.

The present findings indicated that the oyster mushroom genotypes under analysis can supply recommended dietary allowances (RDA) of iron, calcium cobalt and molybdenum for children and adults. So intake of adequate amount of oyster mushroom can help to mitigate all the necessary minerals in human body.

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Yield Attributes and Molecular Analysis through RAPD Markers of *Pleurotus geesteranus* Strains Available at Mushroom Development Institute in Bangladesh

Md. Bazlul Karim Choudhury[1], Akhter Jahan Kakon, Nirod Chandra Sarker, Ruhul Amin and Md. Mominul Hoque
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

The purpose of this study was to investigate the growth and yield performances as well as molecular genomic polymorphisms among the strains of *Pleurotus geesteranus* available at Mushroom Development Institute in Bangladesh through RAPD analysis. Significant variation was observed in yield and yield related attributes. The highest yield (122.8g) and number of fruiting body (14.55) was found in Pg2 which was followed by Pg1. The lowest yield (100.9g) and number of fruiting body (11.38) was found in Pg3. The highest length of stalk (2.92 cm) was found in Pg-1 and the lowest (2.36 cm) was found in Pg-2. In case of RAPD analysis, the maximum (100%) polymorphism was produced by the primer OPA-06 and OPA-08. The dendrogram based on similarity matrix differentiated the species into two distinct clusters C_1 and C_2. Cluster C_1 constituted strain Pg-3 whether cluster C_2 comprises the other strains. The highest linkage distance (42.0) was recorded between the strain Pg-3 and Pg-4. The lowest linkage distance (16.0) was recorded between strain Pg-1 and Pg-2.

Key words: *Pleurotus geesteranus*, RAPD markers, Polymorphism, Linkage distance.

INTRODUCTION

Mushrooms are very important high potential non-green crop in Bangladesh. It can play an important role in improving the nutritional status of the population. It can also helps greatly in improvement of employment opportunities and empowerment of woman populations in this country. Mushrooms are recognized as an alternative source of good quality protein and are capable of producing the highest quantities of protein per unit area and time from the worthless agro wastes (Chadha and Sharma, 1995). Mushrooms are not only sources of nutrients but also have been reported as therapeutic and functional foods. Mushroom of *Pleurotus* spp. are also rich in medicinal component and useful in preventing diseases such as hypertension, hypercholesterolemia (Khatun *et al.*, 2007 and Choudhury *et al.*, 2008), hyperglycemia and different types of cancer (Nayana and Janardhanan, 2000).

Study of genetic and phenotypic diversity is necessary to distinguish genotypes of *P. geesteranus* when seeking traits of interest and to identify strains with high yield potential. Various molecular genetic tools have been introduced for the verification of mushrooms, such as RFLP, RAPD, and SSU rDNA and ITS sequence analyses. Among

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

the molecular approaches, the random amplification of polymorphic DNA (RAPD) had been first developed to detect polymorphisms between organisms despite the absence of sequence information, to produce genetic markers, and to construct genetic maps (Williams *et al.*, 1990). RAPD has been adapted in various fungal species and is increasingly popular due to simple, rapid, and low cost method for detecting genetic diversity (Alam *et al.*, 2009; Alam *et al.*, 2010). A number of strains of the mushroom are available in Bangladesh, though all of them are not performing well round the year. The purpose of this study was to investigate the performance of different strains of *Pleurotus geesteranus* in order to identify the best strain that can be highly productive and suitable for the cultivation in Bangladesh and to investigate molecular genomic polymorphism among the selected strains of *P. geesteranus* through RAPD analysis.

MATERIALS AND METHOD

This experiment was conducted at the Mushroom Development Institute, Savar, Dhaka, Bangladesh. In this experiment four different strains of *P. geesteranus viz.* Pg-1, Pg-2, Pg-3 and Pg-4 were selected and grown in the culture house of the institute.

Spawn packet preparation, inoculation and incubation: Spawn packets (500g size) were prepared by using sawdust as substrate, inoculated and incubated following the procedure that developed and explained by Sarker *et al.* (2007). Data for morphological traits and yields were recorded.

Experimental design, data collection and analysis: The experiment was laid out in completely randomized design (CRD) with 4 replications. Data on yield (g/packet), number of fruiting body/packet, number of effective fruiting body/packet, length and diameter of stalk, diameter and thickness of pileus were collected and analyzed following Gomez and Gomez (1984) using MSTAT-C computer programme. Means were separated by Duncan's Multiple Range Test (DMRT) using the same computer programme.

DNA extraction and RAPD analysis: In the present investigation, modified method of Aljanabi *et al.* (1999) has been used to isolate the total genomic DNA from mushroom. It was grinded in extraction buffer (200 mM Tris-HCl-pH 8.5, 250 mM NaCl, 25 mM EDTA, 20% CTAB, 0.5% SDS) with a mortar pestle. The lysates were incubated at 65^{0}C for 40 min in water bath and centrifuge 30 min at 10,000 rpm. DNA was precipitated from the supernatant by adding equal volumes of iso-propanol and resultant pellet was washed with 70% ethanol. The DNA palette was air dried and dissolved in 50 µl TE (Tris HCL 1M and EDTA 0.5M in Sdd H_2O) buffer. DNA quantification was performed and a dilution of 50ng/µl was used in downstream application.

Genomic DNA was amplified by the RAPD technique (Williams *et al.*, 1990) in which ten sorts of arbitrary 10-base oligonucleotide primers (Operon technologies Inc.) such as 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, OPA-10 and OPA-15,

TTCCGAACCC;. RAPD-PCR reaction was performed using a thermal cycler with an initial denaturation stage of 5 min at 94^{0}C, followed by 40 cycles of denaturation for 1 min at 94^{0}C, annealing for 1 min at 36^{0}C, extension for 2 min at 72^{0}C and a final extension for 10 min at 72^{0}C.

Gel electrophoresis and RAPD data scoring: RAPD products were electrophoresed on 1.4% agarose gel in 1X TBE buffer for 1.15 hr at 100 V with 1kb DNA ladder as a size marker and then stained while agitated in an EtBr solution (0.5% µg/ml). The stained gels were photographed using gel documentation system. The amplification product generated by each RAPD primer was scored as '1' or '0' for presence or absence of specific allele respectively. To estimate the similarity and genetics distance among different species, cluster analysis based on Nei's unweighted pair-group with arithmetic average (UPGMA) was performed using the 'statistica' software and a dendrogram was constructed.

RESULTS AND DISCUSSION

The results of yield and yield related attributes of different strains of *P. geesteranus* mushroom are differed significantly (Table 1).

Yield (g/packet): Significant variation was observed in yield (Table 1). The highest yield (122.8g) was found in Pg-2 which was followed by Pg-1 (112.9g). The lowest yield (100.9g) was found in Pg-3.

Total number of fruiting bodies and number of effective fruiting bodies: The number of fruiting bodies and effective fruiting bodies (NEFB) in different treatments differed significantly (Table 1). The highest number of fruiting bodies (14.55) was found in Pg-2 and the lowest (11.38) was found in Pg-3. The highest NEFB (11.80) was found in Pg-4 followed by Pg-2 (11.55). The lowest NEFB (8.60) was found in Pg-3.

Table 1. Yield and yield related attributes of *P. geesteranus* mushroom strains

Mushroom strains	Yield (g/packet)	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)	Diameter of Stalk (cm)	Diameter of Pileus (cm)	Thickness of Pileus (cm)
Pg-1	112.9 b	11.94 b	9.0 b	2.92 a	1.03 ab	5.90 c	0.51 b
Pg-2	122.8 a	14.55 a	11.55 a	2.36 c	0.92 b	5.77 c	0.48 b
Pg-3	100.9 d	11.38 b	8.60 b	2.51 bc	1.13 a	7.30 a	0.61 a
Pg-4	110.0 c	14.38 a	11.80 a	2.83 ab	0.85 c	6.65 b	0.48 b
CV (%)	**1.33**	**4.77**	**5.21**	**8.20**	**8.93**	**4.89**	**5.05**

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

Length and diameter of stalk (cm): The length of stalk (LS) ranged from 2.36 to 2.92cm with significant difference (Table 1). The highest length of stalk (2.92 cm) was

found in Pg-1 and the lowest (2.36 cm) was found in Pg-2. The diameter of stalk (DS) differed significantly and ranged from 0.85 cm to 1.13 cm (Table 1). The highest diameter of stalk (1.13 cm) was found in Pg-3 and the lowest (0.85 cm) was found in Pg-4.

Diameter and thickness of pileus (cm): The diameter of pileus (DP) ranged from 5.77 to 7.30 cm with significant difference among the treatments (Table 1). The highest diameter (7.30 cm) was found in Pg-3 and the lowest (5.77 cm) was found in Pg-2. The highest thickness of pileus (0.61 cm) was found in Pg-3.

RAPD analysis: The genomic DNA of four strains was analyzed using 10 decamer random primers. All the primers except OPA05 gave different bands. The number of bands (Table 2) and banding pattern (Fig. 1-3) were variable depending upon the primer and type of species tested and it ranged from 6 to 37 in counting. The maximum (100%) polymorphism was produced by the primer OPA06 and OPA08.

Table 2. RAPD primers with corresponding bands scored and their size ranges in selected mushrooms

Primer	Size ranges (bp)	Total number of bands scored	Number of polymorphic bands	Polymorpihism (%)
OPA-01	500-5000	24	23	95.83
OPA-02	100-4000	32	28	87.5
OPA-03	100-5000	35	29	82.85
OPA-04	350-5500	26	22	84.61
OPA-06	2500-4000	6	6	100
OPA-07	1200-5500	15	13	86.66
OPA-08	700-4000	14	14	100
OPA-10	500-5500	37	35	94.56

The dendrogram (Fig. 4) based on similarity matrix differentiated the species into two distinct clusters C_1 and C_2. Cluster C_1 constituted strain Pg-3. Cluster C_2 subdivided into sub-cluster SC_1 and SC_2. Sub-cluster SC_1 belonged the strain Pg-4 and sub-cluster SC_2 comprised the strains Pg-1 and Pg-2 (Fig. 4). The genetic similarities (Table 3) among the strains ranged from 16 to 42%. The highest linkage distance (42.0) was recorded between the strain Pg-3 and Pg-4. The lowest linkage distance (16.0) was recorded between strain Pg-1 and Pg-2.

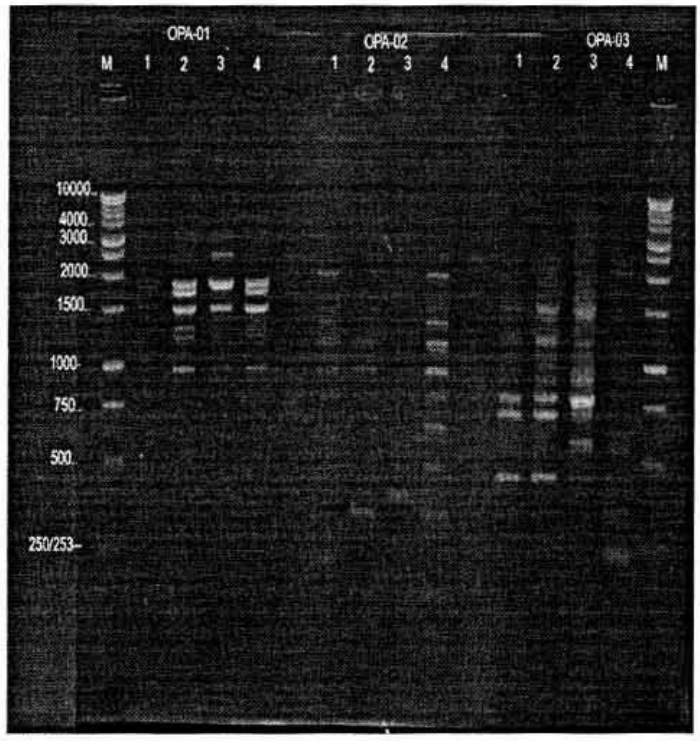

Fig. 1. RAPD profile of *Pleurutus geesteranus*. M = DNA ladder, 1 = pg-1, 2 = pg-2, 3 = pg-3, 4 = pg-4.

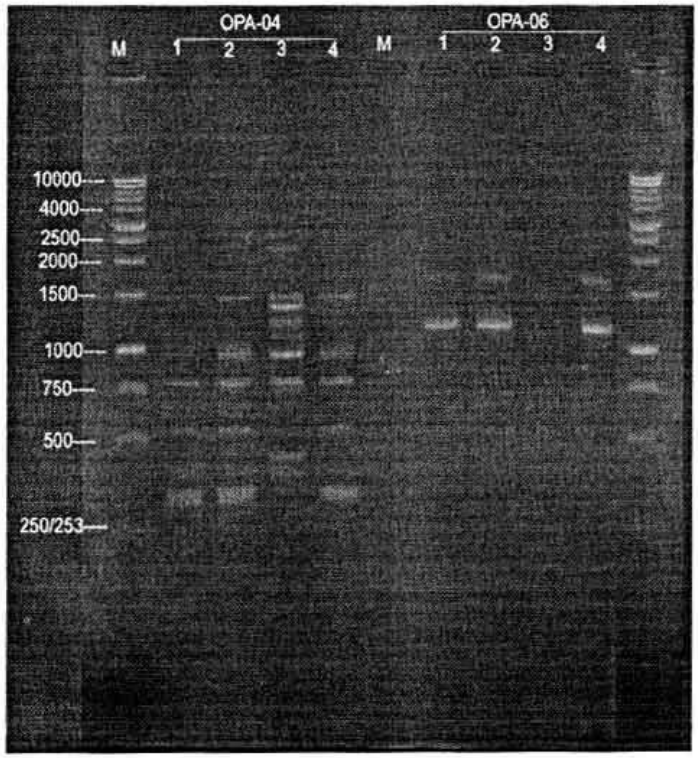

Fig. 2. RAPD profile of *Pleurutus geesteranus*. M = DNA ladder, 1 = pg-1, 2 = pg-2, 3 = pg-3, 4 = pg-4.

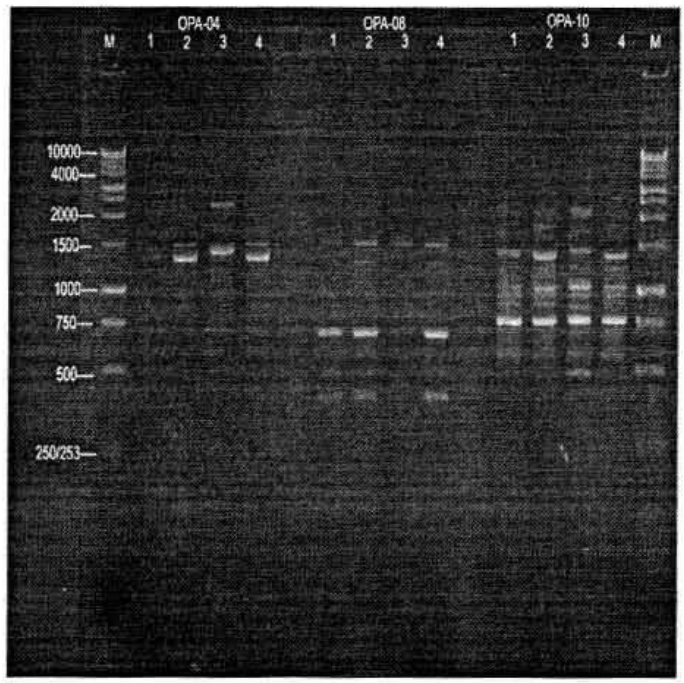

Fig. 3. RAPD profile of *Pleurutus geesteranus*. M = DNA ladder, 1 = pg-1, 2 = pg-2, 3 = pg-3, 4 = pg-4.

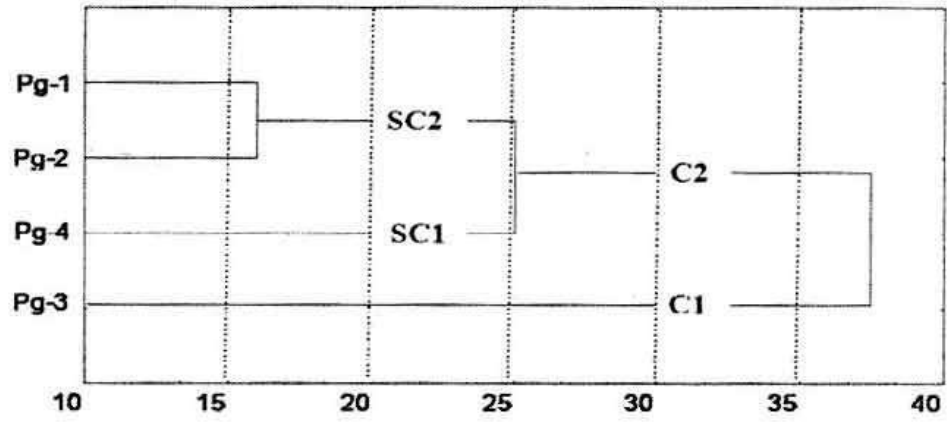

Fig. 4. Cluster analysis by unweighted pair group method of arithmetic means (UPGMA) of four strains of *P. geesteranus* mushroom.

Table 3. Summary of linkage distances for different pairs of selected mushrooms by RAPD markers

Strains	Pg-1	Pg-2	Pg-3	Pg-4
Pg-1	0	16.0	41.0	27.0
Pg-2	16.0	0	29.0	23.0
pg-3	41.0	29.0	0	42.0
Pg-4	41.0	23.0	42.0	0

The random and genomic wide nature of the RAPD technique is best to indicate over all genetic relatedness/dissimilarity than the morphological analysis (Alam, 2011 and Ravash

et al., 2009). The different primers produced different number of bands in PCR. This variation in the number of bands may be due to the sequence of primer, availability of annealing sites in the genome and template quality (Alam *et al.*, 2009 and Kernodle *et al.*, 1993). The polymorphism produced by nine RAPD primers may be due to the base substitution, insertion and deletion or collection of genetic material from different sources (Chopra, 2005 and Jusuf, 2010). The genetic make up is correlated with environmental heterogeneity (Alam *et al.*, 2010 and Nevo, 1998). The results depicted that, there is strong correlation between molecular and morphological criteria (Zervakis *et al.*, 2004). The current study demonstrated that, the RAPD analysis is useful for characterization, genetic diversity and identifying relationships among the mushroom strains.

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Yield Performance of Oyster Mushroom Using Waste Paper

Manirul Shaheen, Nirod Chandra Sarker, Mahbuba Moonmoon[1], Abdus Salam Khan and Tahera Binte Mujib
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

To evaluate the waste paper as substrate the comparative trial on growth and yield of eight species of *Pleurotus* viz. *Pleurotus ostreatus* (Po_2) = V_1, *Pleurotus florida* (Flo) = V_2, *Pleurotus ostreatus var. white snow* (Ws) = V_3, *Pleurotus ostreatus var. high king51* (HK 51) = V_4, *Pleurotus sajor-caju* (Psc 1) = V_5, *Pleurotus sajor-caju* (Psc 3) = V_6, *Pleurotus djamor* (Pop) = V_7, *Pleurotus salmoneostraminious* (Pss) = V_8 were grown on two different substrates sawdust (T_0), and waste paper (T_1). Each of the substrate was supplemented with 0.1% calcium carbonate ($CaCO_3$), wheat-bran 30% dry basis and about 65% water was added. The highest average number of fruiting body (NFB) was produced in V_8 using sawdust (53.50) followed by sawdust substrate and V_7 (52.50). The highest average number of effective fruiting body (NEFB) was produced in sawdust substrate and V_7 (29.00) followed by sawdust substrate and V_8 (28.50). *P. florida* produce the longest stalk (7.00 cm) on paper substrate and V_2 which was statistically similar to the treatment V_1 when cultivated in sawdust substrate. The shortest stalk (1.15 cm) was found in V_7 in sawdust substrate. The maximum stalk diameter (2.66 cm) was found V_7 in paper substrate which was similar to the V_4 in paper substrate. The lowest diameter (0.42cm) was found in V_8 and paper substrate. Yield per packet was found higher in V_2 (126.0g) from paper substrate. The lowest yield was found in V_6 (43.73g) from sawdust substrate followed by V_4 (44.33g) from sawdust substrate.

Key Words: Oyster, strain, paper, sawdust, yield.

INTRODUCTION

Mushrooms are fleshy, spore-bearing reproductive structures of fungi grown on organic substrates and for a long time, have played an important role as a human food due to its nutritional and medicinal properties (Etich *et al.*, 2013).Oyster mushroom can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, bagasse etc. (Hassan *et al.*, 2011). This has been reported to influence its growth, yield and composition (Iqbal *et al.*, 2005 and Kimenju *et al.*, 2009). However, an ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth (Khare *et al.*, 2010). Oyster mushroom cultivation can play an important role in managing organic wastes whose disposal has become a problem.

In Bangladesh, about 30 million tones of agricultural wastes like sawdust, paddy straw, wheat straw, and sugarcane bagasse are available (Ahmed, 2001), which can be used for mushroom cultivation. Total waste generation in Khulna city is calculated at 520 tons/day where paper waste forms almost 9.5% of this total waste (Alamgir *et al.*, 2005). But the performances of these agro-wastes have not yet properly been investigated in the climatic

[1] Horticulture wing, Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh.

conditions of Bangladesh for the specific species of oyster mushroom. The main motive of this study is to introduce a sustainable management process for paper waste recycling which is beneficial in economic consideration and defend the working environment from its harmful effect.

MATERIALS AND METHODS

The experiment was conducted in the laboratory and culture house of Mushroom Development Institute, Savar, Dhaka during October to December 2014 to study the effect of two substrates such as sawdust and waste paper on the growth and yield of eight selected oyster mushroom varieties. Eight different oyster mushroom varieties such as *Pleurotus ostreatus* (Po2) = V_1, *Pleurotus florida* (Flo) = V_2, *Pleurotus ostreatus var.* white snow (WS) = V_3, *Pleurotus highking 51* (HK-51) = V_4, *Pleurotus sajor-caju* (Psc) = V_5, *Pleurotus sajor-caju* (Psc3) = V_6, *Pleurotus djamor* (Pop) = V_7, *Pleurotus salmoneo-stramineus* (Pss) = V_8 were grown on two different substrates sawdust (T_0) and waste paper (T_1).

Spawn packets preparation: Each of the substrate was supplemented with 0.2% calcium carbonate ($CaCO_3$), wheat bran 30% (dry basis) and water was added.

The substrate of spawn packets were prepared using sawdust and wheat bran mixture at the ratio of 2:1 (dry weight/weight basis). Water was added to make the moisture content at 60% and $CaCO_3$ was added at the rate of 0.2% (w/w) of the total mixture to maintain the pH level at 6.5 to 7.0. Polypropylene bags of 18cm x 25 cm were filled with 500 g of prepared substrate. The packets were tied, plugged with absorbent cotton and covered with brown paper. Then the packets were sterilized in an autoclave for 2.0 hour at 121^{0}C under 1.5 kg/cm^2 pressure. After sterilization the packets were cooled and transferred to an inoculation chamber and inoculated with the mother culture of test materials at the rate of one teaspoonful per packet. The inoculated packets were placed on a still rack at 25 ± 2^{0}C temperature for incubation. In case of paper substrate, the dry papers were chopped into small pieces of 2.5 cm and about 50% water was added. The above mentioned procedure was follows for spawn packet preparation.

Experimental condition: The packets were kept in a dark room at 25^{0}C for incubation. When colonization of mycelium was completed, the spawn packets were transferred into the culture house and were opened by 'D' shaped cut on the shoulder and removed the sheet. The relative humidity and temperature of the culture house were maintained at 80-90% and 20-25^{0}C respectively by spraying water. Diffused light, about 200 lux and proper ventilation in culture house were maintained. After harvesting of mushroom, the residues were removed from the packet as well as temperature and relative humidity were maintained as before. The yield was obtained from single, flush in the harvest period. Yield in g/packet was recorded by weighing all the fruiting bodies in a packet after removing the lower dirty portion. The biological efficiency was determined using the following formula:

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

Data collection and statistical analysis: The experiment was laid out following completely randomized design (CRD) with 4 replications. Data on total number of fruiting bodies, number of non-effective fruiting bodies, number of effective fruiting bodies, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, yield, and biological efficiency were recorded and analyzed following Gomez and Gomez (1984) using MSTAT-C computer program. Means separation were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Total number of fruiting body: The highest average number of fruiting body (NFB) was produced in sawdust substrate and varietyV_8 (53.50) followed by sawdust substrate and V_7 (52.50) (Table 1). The lowest average NFB was observed in paper sabustrate and V_5 (4.00). Ahmed (2001) also reported similar number of primordia initiated in sawdust substrate (22.95-39.55), in sugarcane bagasse (35.55) and in rice straw (20.00).

Number of non effective fruiting body: The highest average number of non effective fruiting body (NEFB) was produced in sawdust substrate and V_8 (25.00) followed by sawdust substrate and V_7 (23.50) (Table 1). The lowest average NEFB was observed in paper substrate and V_5 (0.00).

Number of effective fruiting body: The highest average number of effective fruiting body (EFB) was produced in sawdust substrate and variety V_7 (29.00) followed by sawdust substrate and treatment V_8 (28.50) (Table 1). The lowest average EFB was observed in waste paper substrate and treatment V_5 (4.00). Ahmed (2001) also reported similar number of primordia initiated in sawdust (22.95-39.55), in sugarcane bagasse (35.55) and in rice straw (20.00).

Length and diameter of stalk: The Length and diameter of stalk was significantly different. Flo (V_2) strain produces the longest stalk (7.00 cm) on paper sabustrate which was statistically similar to V_1 when cultivated in sawdust substrate. The shortest stalk (1.15 cm) was found in V_7 and sawdust substrate which was statistically similar to V_8 and sawdust substrate. The maximum stalk diameter (2.66 cm) was found in V_7 and paper substrate which was similar to the V_4 and same substrate. The lowest diameter (0.42cm) was found in V_8 and paper substrate which was statistically similar to V_8 and sawdust substrate.

Diameter and thickness of pileus: The highest diameter of pileus (10.41 cm) was found from V_5 with paper substrate followed by V_1 with sawdust substrate and V_4 with paper substrate. The minimum diameter of pileus (4.77 cm) was found from V_7 with sawdust substrate followed by the V_8 with sawdust substrate (Table1).

Table1. Interaction effect of Variety × Substrate on yield attributing characters of different oyster mushroom variety

Variety	Number of fruit body	Number of non effective fruit body	Number of effective fruit body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
			Sawdust (T_0)				
V_1	8.44 i	1.65k	7.78f	6.22b	1.27h	10.22ab	1.14d
V_2	10.13h	2.50i	7.63f	6.13bc	1.18hi	8.63d	1.04e
V_3	20.20f	8.50e	11.70e	5.70d	1.56fg	9.35c	1.04e
V_4	8.17i	2.33ij	5.83h	4.17f	2.00c	9.17c	1.28a
V_5	29.83c	13.25c	16.58b	3.28h	1.03i	7.00h	0.77h
V_6	8.55i	2.00jk	6.55g	2.71i	0.80j	6.09i	0.68i
V_7	52.50b	23.50b	29.00a	1.15k	0.73j	4.77k	0.56j
V_8	53.50 a	25.00a	28.50a	1.75j	0.65j	5.55j	0.35k
			Waste Paper (T_1)				
V_1	7.50i	2.08j	5.42h	5.93c	1.73e	8.42de	1.24ab
V_2	25.00d	8.63e	16.38b	7.00a	1.50g	8.31e	0.99f
V_3	19.40f	7.75f	11.65e	3.63g	1.76d	9.31c	1.19c
V_4	12.00g	4.67h	7.33f	5.37e	2.30b	10.09b	1.13d
V_5	4.00j	0.001	4.00i	6.00c	1.60fg	10.41a	1.20bc
V_6	19.22f	7.11g	12.11e	6.01c	1.62efg	8.63d	1.28a
V_7	22.91e	9.18d	13.73d	2.82i	2.66a	7.97f	0.92g
V_8	24.23d	8.690e	15.54c	3.23h	0.42k	7.52g	0.55j
CV	3.37	3.40	2.90	3.07	6.30	2.09	3.12

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT. *Pleurotus ostreatus* (Po_2) = V_1, *Pleurotus florida* (Flo) = V_2, *Pleurotus ostreatus var. white snow* (WS)=V_3, *Pleurotus ostreatus var. high king51* (HK 51)=V_4, *Pleurotus sajor-caju* (Psc 1) = V_5, *Pleurotus sajor-caju* (Psc 3) =V_6, *Pleurotus djamor* (PoP) = V_7, *Pleurotus salmoneostraminious* (Pss) = V_8

Table 2. Effect of different variety and substrate on yield of oyster mushroom

Variety (V)	Substrate	
	Sawdust (T_0)	Paper (T_1)
V_1	63.00j	75.33h
V_2	66.13i	126.0a
V_3	85.80f	92.00d
V_4	45.33l	63.50j
V_5	81.75g	58.00k
V_6	43.73.l	89.22e
V_7	108.8b	85.27f
V_8	104.0c	80.38g
Mean	1.73	1.73

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT. *Pleurotus ostreatus* (Po_2) = V_1, *Pleurotus florida* (Flo) = V_2, *Pleurotus ostreatus var. white snow* (WS)=V_3, *Pleurotus ostreatus var. high king51* (HK 51)=V_4, *Pleurotus sajor-caju* (Psc 1) = V_5, *Pleurotus sajor-caju* (Psc 3) =V_6, *Pleurotus djamor* (PoP) = V_7, *Pleurotus salmoneostraminious* (Pss) = V_8

Yield and biological efficiency: Both yield and biological efficiency was significantly difference. The maximam yield and biological efficiency per packet (Table 2) were found in V_2 (126.0g and 56.00%) from paper substrate followed by V_7 (108.8 g and 54.40%) from sawdust substrate. The lowest yield and biological efficiency was found in V_6 (43.73g and 21.87%) from sawdust substrate followed by V_4 (44.33g and 22.67%) from sawdust substrate. The performances of eight (8) varieties significantly differed in each substrate.

The present study indicate that *P. florida* species given higher yield when cultivated on paper substrate but other strain did not given best yield when cultivated on paper substrate. *Pleurotus florida* is a commercially important edible mushroom and it is wide spread in various geographical region of Bangladesh and south-east Asia. It has very good abilities to grow at a wide range of temperatures utilizing various lignocelluloses, so that more popular throughout the world. *P. florida* is a good source of dietary fiber and other valuable nutrients (Alam *et al*., 2008). 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*., 2009). Alam *et al.* (2010) reported that mycelium cultivation is enhanced by different environmental and nutritional factors as well as propagation of mycelium ia an earlier and essential step to cultivate fruiting bodies of mushroom. Similar results were reported by Badsha *et al.* (1994), Sivaprakasam (1986) and Rajarathnam *et al* (1983). Considering all the factors V_2 (*Pleurotus florida*) performed best on T_1 (waste paper) substrate.

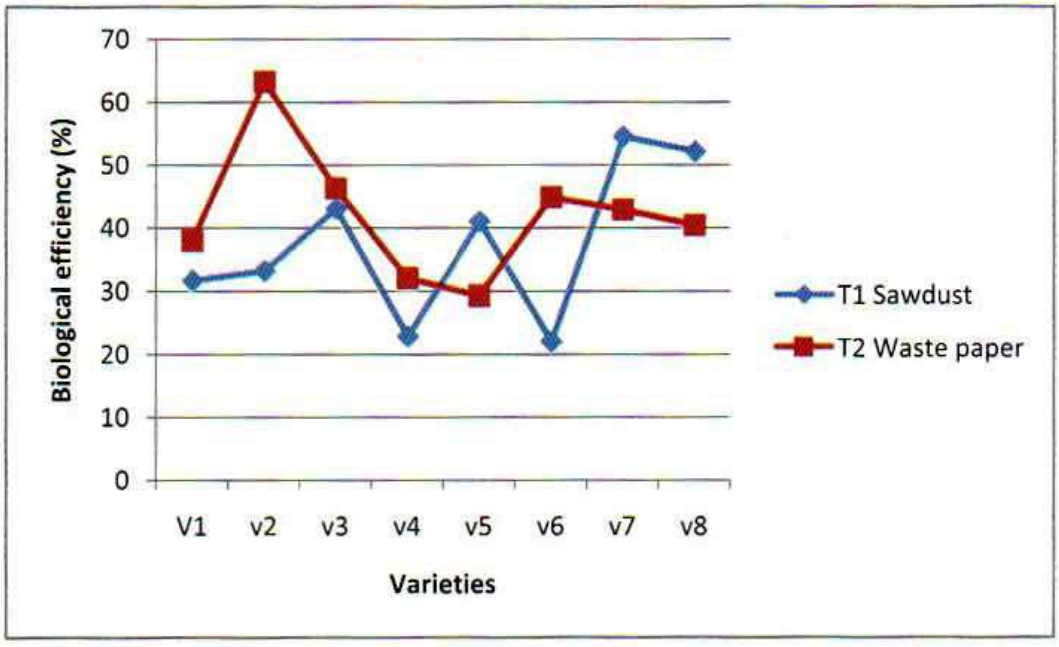

Fig.1. Biological Efficiency of different variety of oyster mushroom.

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Microbiological Assessment of Mushroom Capsules as Food Supplement Produced by Bangladeshi Mushroom Traders

Niroid Chandra Sarker, Akhter Jahan Kakon, Ruhul Amin, and Md. Bazlul Karim Choudhury[1]

Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

The present study was to evaluate the microbiological quality of mushroom capsules or mushroom enriched capsules. The results of total viable count (TVC), among the 18 analyzed capsule samples sample-7 contains higher microbial load (log_{10} value 6.301 or 2.0×10^6 cfu/g) and the lowest microbial load (log_{10} value 2.477 or 3.0×10^2 cfu/g) was found in powder-12. In case of total coliform, 66.66% of analyzed capsule samples were acceptable and can be consumed directly. The capsule sample 8 contains the highest (>1100 MPN/g) coliform bacteria. About 94.44% of the samples were acceptable in view to fecal coliform. Moreover, all the capsule samples except the sample 2 were free from *Escherichia coli* contamination. In case of *salmonella spp.* the presence of this bacterium was higher than that of the tolal coliform, fecal coliform and *Escherichia coli.*

Key words: Microbes, Mushroom capsules, Food supplement.

INTRODUCTION

Mushroom is now believed as the same origin of nutrients and medicinal properties throughout the world. In Bangladesh, the demand as well as its consumptions is increasing day by day. Mushrooms are now being available in fresh pack, processed pack (pickle, halua, chutney, cake, biscuits etc), ready to eat food (fry, crispy, susliks, noodles etc), powdered form, capsule form, canned products as well as mushroom enriched cosmetics in most upazilla level, town's shopping malls and mega shops of the country. Usually small entrepreneurs are producing and marketing these mushroom based products and the consumers take it as dietary or food supplements.

Disease causing microorganisms can get into the food supply. Food borne infections and illnesses is a major international health problem with consequent economic reduction. It is a major cause of illness and death worldwide (Adak *et al.*, 2005). According to Clarence *et al.* (2009), food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by micro organisms present in food. *Escherichia coli*, particularly serotype O157:H7 and various serotypes of *Salmonella* sp. including *S. enteritidis* have been reportedly responsible for food borne epidemics in various

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

countries, emphasizing the importance of the pathogen as a food safety concern (Altekruse *et al.*, 1997; Todd, 1997).

Controlled production practices and manufacturing help reduce the risk of food contamination. According to Amin *et al.* (2013), harmful or pathogenic organisms like coliform, fecal coliform, *E. coli* and *Salmonella* sp. were comparatively higher in powder mushrooms than those of processed or preserved mushroom products. Improvements in food security will bring significant socioeconomic benefits by checking microbial status (kell *et al.,* 2011). However, the present investigation was done to determine the present microbial status of mushroom capsules or mushroom enriched capsules as well as to improve food security or food safety in Bangladesh.

MATERIALS AND METHODS

The present study was to evaluate the microbiological quality of mushroom capsules or mushroom enriched capsules. The experiment was conducted at microbiology laboratory of Mushroom Development Institute, Sobhanbagh, Savar, Dhaka.

Collection of Samples: About 18 samples of mushroom capsules were collected from local sales centre at Savar, Dhaka.

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

Presumptive test of total coliform (TC), fecal coliform (FC) and *E. coli*: 25 g sample was weighed and added into 225 ml of Butterfield's phosphate-buffered water and decimal dilutions were prepared. Number of dilutions was prepared depending on anticipated coliform density. All suspensions were shaked 25 times in arc for 30 cm or vortex mix for 7 second. 1 ml of each dilution was transferred to 3 McCartney bottles containing LST broth and inverted Durham's tubes. Inoculated McCartney bottles were incubated at 37^{0}C for 24h. Gas production were examined and recorded in tubes. Gas-negative tubes were re-incubated for an additional 24 h and examined (BAM, 1998).

Confirmed test for coliforms: A loop-full of suspension was transferred into a tube of Brilliant Green Bile Broth (BGBB) from each gas positive Lauryl Sulphate Broth (LST) tube and pellicle was being avoided if present. BGBB tubes were incubated at 37^{0}C and examined for gas production at 24h. Then Most Probable Number (MPN) of coliforms

was calculated based on proportion of confirmed gas positive tubes for 3 consecutive dilutions using MPN charts.

Confirmed test for fecal coliforms and *E. coli*: From each gas positive LST tube of the presumptive test, a loopful of each suspension was transferred to tube containing BGBB and EC broth. Inoculated tubes were incubated at 44^0C for 24 h and examined for gas production. When negative, reincubated and examined again at 48h. Observing the gas production, the number of fecal coliform and *E. coli* were calculated using MPN charts (BAM, 1998).

Isolation of Salmonella: Twenty five grams (25g) sample was weighed and homogenated in 225 ml Buffered Peptone water and incubated at 35^0C for 24 h. Three (03) mm loop full (10µl) of incubated broth medium was streaked on bismuth sulfite (BS) agar and xylose lysine desoxycholate (XLD) agar, and incubated plates for 24h at 37^0C. The presence of colonies were examined that may be Salmonella.

Identification of Salmonella

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

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

Simmons citrate agar test: Containing growth from unclassified TSI agar slant was inoculated into the medium using needle by streaking slant and stabbing butt. It was incubated 96h at 37^0C. The results were considered positive when color of the medium changed from green to blue and when there was no or very little growth and no change of color, the result was considered as negative. Most cultures of Salmonella were citrate-positive.

RESULTS AND DISCUSSION

The results of total viable count (TVC), among the 18 analyzed capsule samples (Fig. 1), sample-7 contains higher microbial load ($\log_{10}$ value 6.301 or 2.0×10^6 cfu/g) and the lowest microbial load ($\log_{10}$ value 2.477 or 3.0×10^2 cfu/g) was found in powder-12. According to the Bangladesh standard (BDS) 1829:2010, the acceptable microbial load is 1000 cfu/g and the fig.1 shows that about 55% tested samples were acceptable in quality.

In case of total coliform, 66.66% of analyzed capsule samples (Table 1) were acceptable and can be consumed directly. The capsule sample 8 contains the highest (>1100 MPN/g) coliform bacteria. The remaining 33.33% samples were

not acceptable due to high microbial load. On the other hand, the tested capsule samples were nearly free from fecal coliform contamination. About 94.44% of the samples were acceptable in view to fecal coliform. Only the capsule sample 2 contains 7.2 MPN/g.

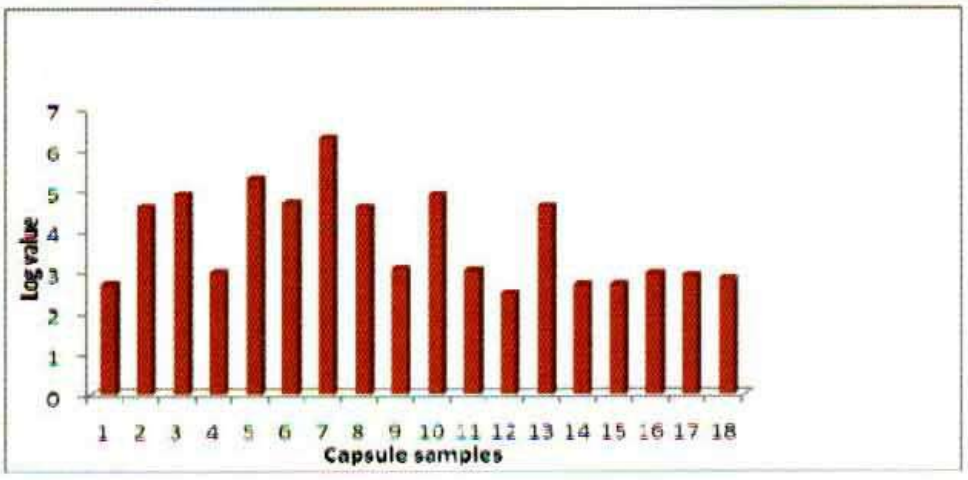

Fig. 1. Total viable count (Log value) of mushroom capsule samples.

Moreover, all the capsule samples except the sample 2 were free from *Escherichia coli* contamination. In case of *salmonella spp.* the presence of this bacterium was higher than that of the tolal coliform, fecal coliform and *Escherichia coli.* Among the capsule sample About 61% was free from *salmonella spp.* Contamination and rest of the samples were contaminated by *Salmonella* sp.

Table 1. The results of Mushroom capsule of coliform, fecal coliform, *E. coli* and salmonella

Sample No.	Total Coliform (MPN/g)	Fecal Coliform (MPN/g)	*Escherichia coli* (MPN/g)	*Salmonella* spp.
1	3.6	<3.0	<3.0	Absent
2	150	7.2	14	Present
3	20	<3.0	<3.0	Present
4	<3.0	<3.0	<3.0	Absent
5	<3.0	<3.0	<3.0	Absent
6	<3.0	<3.0	<3.0	Present
7	<3.0	<3.0	<3.0	Absent
8	>1100	3.6	<3.0	Present
9	3.6	<3.0	<3.0	Present
10	9.2	<3.0	<3.0	Present
11	<3.0	<3.0	<3.0	Absent
12	<3.0	<3.0	<3.0	Absent
13	<3.0	<3.0	<3.0	Present
14	<3.0	<3.0	<3.0	Absent
15	<3.0	<3.0	<3.0	Absent
16	<3.0	<3.0	<3.0	Absent
17	<3.0	<3.0	<3.0	Absent
18	<3.0	<3.0	<3.0	Absent

The capsule samples were made from various mushroom powder and plant derived powder. According to Amin *et al.* (2013), it was found that powder form mushrooms were more susceptible to contamination than other processed mushroom based products like pickle, halua etc. It may be due to the lack of suitable moisture level, proper drying method, hygienic condition, packaging and over all handling process. Burton (1989) found that bacterial counts were consistently lower depending on processing and storage conditions. Kakon *et al.* (2013) showed that powder mushroom could be contamination free from the above pathogenic microbes treated by irradiation at the doses of 10 kilo Gray. Thus it can be concluded that the raw materials of mushroom capsules i.e. mushroom powder as well as overall handling process should be hygienic and contamination free to improve our food security and quality.

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Effect of Shiitake Mushroom (*Lentinus edodes*) on Some Hepatic Dysfunction Marker Present on Blood in Male Volunteers of Bangladesh

Md. Bazlul Karim Choudhury[1], Nirod Chandra Sarker, Md. Erfan Reza[2], Rajib Ahmed[1] and G H M Shahidul Haque[3]
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

The study was conducted in the laboratory of National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka, during the period of June 2013 to February 2014 to investigate the effect of shiitake mushroom (*Lentinus edodes*) on the plasma level of hepatocellular enzymes like Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma Glutamyl Transferase (GGT), Alkaline Phosphatase (ALP) and Lactate Dehydroginase (LDH) the common liver function tests (LFTs). Mushrooms were supplemented as capsule form which contains 500mg *Lentinus edodes* powder in each. Subjects were getting 3 shiitake mushroom capsules daily. Feeding of 1.5 grams shiitake mushroom powder daily for 3 months significantly reduced the plasma levels of ALT ($p = 0.006$), AST ($p = 0.000$) and ALP ($p = 0.020$) of male subjects. There were also nonsignificant small reduction of plasma GGT ($p = 0.193$) and LDH ($p = 0.167$). Findings of the study suggest that shiitake mushroom may able to improve hepatocellular functions of males volunteers and hence the human subjects.

Key words: *Lentinus edodes*, ALT, AST, GGT, ALP, LDH.

INTRODUCTION

Medicinal mushrooms have an established history of use in traditional oriental therapies. Historically, hot-water-soluble fractions from medicinal mushrooms were used as medicine in the Far East, where knowledge and practice of mushroom use primarily originated (Wasser, 2002; Hobbs, 2000). Mushrooms such as *Ganoderma lucidum*, *Lentinus edodes*, *Inonotus obliquus*, and many others have been collected and used for hundreds of years in Korea, China, Japan, and eastern Russia (Wasser, 2002). It is well known that shiitake mushrooms contain proteins, fats, carbohydrates, soluble fiber, vitamins, and minerals. In addition, shiitake's key ingredient found in the fruiting body is a polysaccharide called lentinan. This mushroom is used for hepatitis, cancer and building the immune response.

Shiitake mushrooms are a functional food because that contains natural bioactive substances, such as β-glucan and eritadenine. Using a shiitake mushroom-enriched diet to

[1] Department of Biochemistry, Manikganj Medical College, Manikganj, Bangladesh [2] Department of Biochemistry, Rajshahi Medical College, Rajshahi, Bangladesh. [3] Department of ENT and Head Neck Surgery, Manikganj Medical College, Manikganj, Bangladesh.

lower plasma lipid has been widely reported (Kabir and Kimura, 1989; Takashima *et al.*, 1973; Chen *et al.*, 2008; Fukushima *et al.*, 2001). More recently, the role of shiitake mushrooms in preventing body weight gain has been reported but its mechanism was largely unknown (Handayani *et al.*, 2011).

Dyslipidemia is elevation of plasma cholesterol, triglycerides (TGs), or both, or a low high-density lipoprotein level that contributes to the development of atherosclerosis. Causes may be primary (genetic) or secondary. Diagnosis is by measuring plasma levels of total cholesterol, TGs, and individual lipoproteins. Treatment involves dietary changes, exercise, and lipid-lowering drugs. Shiitake mushrooms contain many chemical compounds that protect DNA from oxidative damage, which is partly why they are so beneficial. Lentinan, for example, heals chromosome damage caused by anticancer treatments. Eritadenine substances help reduce cholesterol levels and support cardiovascular health. Researchers at Shizuoka University in Japan found that eritadenine supplementation significantly decreased plasma cholesterol concentration (Sugiyama *et al.*, 1995).

Certain components of the shiitake mushroom have hypolipidaemic (fat-reducing) effects, such as eritadenine and β-glucan, a soluble dietary fiber that's also found in barley, rye and oats. Studies have reported that β -glucan can increase satiety, reduce food intake, delay nutrition absorption and reduce plasma lipid (fat) levels.

A 2011 study published in the *Journal of Obesity* examined the effects of shiitake mushrooms on plasma lipid profiles, fat dispositions, energy efficiency and body fat index. Rats were fed a high-fat diet for a six-week period. Researchers found significant effects of dietary intervention on body weight gain. Rats on a high dose of shiitake mushroom diet (which involved adding mushroom powder to a high-fat diet) had 35 percent lower body weight gains than rats on low and medium shiitake mushroom diets. Rats on the high dose shiitake mushroom diet also had significantly lower total fat masses and had a trend of lower fat accumulation. The researchers concluded by suggesting that shiitake mushrooms can help prevent body weight gain, fat deposition and plasma triacylglycerol when added to a high-fat diet. This encourages an effort to pursue human studies that examine the efficacy of shiitake mushrooms for the prevention and treatment of obesity and related metabolic disorders (Handayani *et al.*, 2011).

Previous studies have suggested that shiitake mushrooms has protect DNA from oxidative damage and possibly beneficial effects on preventing body weight gain, but these have not been confirmed in subjects with dyslipidaemia. On the other hand blood lipid status and liver functions run hand by hand. The objective of the current study was to assess the effect of shiitake mushroom on some hepatic dysfunction marker present on blood in a controlled cross-over trial.

MATERIALS AND METHODS

The study was conducted in the laboratory of National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka, during the period of June 2013 to February 2014.

Subjects: Total 26 adult male volunteers aged (years) from 25 to 69 were selected in the study.

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

Exclusion criteria: Patients suffering from any acute or chronic illness, malabsorption, and alcoholism were excluded. Also during the study period any acute or chronic disease, were excluded.

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

Study design: At the beginning of study, health status was evaluated. Fasting blood sample was collected from the subjects for analysis. Mushroom powder was supplied as capsule form. Subjects took one capsules three times daily. Each capsule contained 500 mg shitake mushroom powder, so that each subject took 1.5 gm mushroom powder daily. After 3 months the subjects were re-evaluated and all the investigation procedures were repeated.

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

Biochemical analysis: As hepatic dysfunction marker, estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and lactate dehydroginase (LDH) were done from the obtained plasma sample. Analysis was done by semi auto biochemical analyzer 3000 evaluation using the available reagent kit.

Statistical analysis: 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. The recorded characteristics of the subjects were analyzed by using computer software, SPSS package programme.

RESULTS AND DISCUSSION

Study of plasma alanine aminotransferase (ALT): The mean (± SE) ALT (U/L) before- and after supplementation of mushroom capsules was 28.23 ± 2.15 and 21.10 ± 1.11, respectively. A significant (p = 0.006) reduction of ALT was observed after 3 months (Table 1).

Study of plasma aspartate aminotransferase (AST): The mean (± SE) plasma aspartate aminotransferase (AST) (U/L) before and after mushroom supplementation was 29.96 ± 1.20 and 23.24 ± 0.87, respectively. Here a significant (p = 0.000) reduction of AST was observed (Table 1).

Study of plasma gamma glutamyl transferase (GGT): The mean (± SE) plasma gamma glutamyl transferase (GGT) (U/L) before and after mushroom supplementation was 15.34 ± 0.72 and 13.78 ± 0.67, respectively. A non-significant (p = 0.193), reduction of GGT was observed (Table 1).

Table 1. Evaluation of serum ALT, AST and GGT before and after supplementation of mushroom capsule

Name of hepatocellular marker	Number of subjects (n)	Period of observation	Mean ± SE (U/L)	p
ALT	26	Before mushroom	28.23 ± 2.15	0.006
		After Mushroom	21.10 ± 1.11	
AST	26	Before mushroom	29.96 ± 1.20	0.000
		After Mushroom	23.24 ± 0.87	
GGT	26	Before mushroom	15.34 ± 0.72	0.193
		After Mushroom	13.78 ± 0.67	

Results show mean ± SE. Data were analyzed by Pair t test. Means were significantly different at $p<0.05$ at 95% confidence limit. (ALT = Alanine Aminotransferase, AST = Aspartate Aminotransferase and GGT = Gamma glutamyl transferase).

Study of plasma alkaline phosphatase (ALP): The mean (± SE) plasma alkaline phosphatase (ALP) (U/L) before- and after mushroom supplementation was 107.88 ± 2.66 and 99.28 ± 2.93, respectively. A significant (p = 0.020) reduction of ALP was observed (Table 2).

Study of plasma lactate dehydroginase (LDH): The mean (± SE) plasma lactate dehydrogenate (LDH) (U/L) before and after supplementation of mushroom capsule was 220.69 ± 4.49 and 211.48 ± 5.11, respectively. A non-significant (p = 0.167) reduction of LDH was observed here (Table 2).

Table 2. Evaluation of serum ALP and LDH before and after supplementation of mushroom capsule

Name of hepatocellular marker	Number of subjects (n)	Period of observation	Mean ± SE (U/L)	p
ALP	26	Before mushroom	107.88 ± 2.66	0.020
		After Mushroom	99.28 ± 2.93	
LDH	26	Before mushroom	220.69 ± 4.49	0.167
		After Mushroom	211.48 ± 5.11	

Results show mean ± SE. Data were analyzed by Pair t test. Means were significantly different at $p<0.05$ at 95% confidence limit. (ALP = Alkaline phosphatase, LDH = Lactate dehydroginase).

In this study it was observed that supplementation of a considerable amount (1.5 grams per day) of dried shiitake mushroom powder for 3 months significantly reduced plasma ALT, AST and ALP. Also there observed a nonsignificant small reduction of plasma GGT and LDH. These observations suggesting the beneficial effect of shiitake mushroom on the liver.

Liver is the vital organ for lipid metabolism, on the other hand dyslipidaemia is one of the most important factor for hepatic parenchymal diseases. Shiitake mushroom have been shown to have health benefits including lowering plasma lipids and preventing body weight gain. However, their underlying mechanisms are largely unknown.

Shiitake naturally contains many important contributions to human nutrition and health. Among these are proteins that are built by a total of 18 different amino acids, including essential amino acids, lipids (primarily linoleic acid), carbohydrates, fiber, minerals, vitamins B1, B2, B3, and B12 (in trace amounts), vitamin C, and ergosterol, and the D2 provitamin. Each of these nutritional factors has been isolated from fruiting bodies (i.e., the mushroom) and mycelia, which may contribute in improving lipid profile status as well as improves liver functions.

Modern research indicates that polysaccharides are the main chemical components related to the bioactivity and pharmacological properties of shiitake (Hearst *et al.*, 2009; Rao *et al.*, 2009; Li *et al.*, 2009; Turło *et al.*, 2010). In particular, one of the most medically significant compounds isolated from the shiitake mushroom is lentinan, a polysaccharide that has a mean molecular mass of 500 kDa. Lentinan activates macrophage T-lymphocytes and other immune effector cells that in turn modulate the release of cytokines. This molecular mechanism may account for the indirect antitumor and antimicrobial properties of this polysaccharide. Other compounds with biological activity are lentinacin and lentysine, which each have been reported to show hypocholesterolemic and hypoglycaemic effects.

In this study it is evident that shiitake mushroom is able to improve liver functions. Considering the previous observations now a day it is well established that shiitake

mushroom improves lipid profile status. In a study it was identified that a shiitake mushroom-enriched diet in rats fed a high fat diet (HFD) significantly lowered plasma triacyl glycerol (TAG) and fat deposition by −55% and −35%, respectively, compared to HFD alone (Handayani *et al.*, 2011). It has also been identified that a high dose mushroom (HD-M) enriched diet significantly increased the ratio of faecal fat to faecal weight by +58% compared to HFD alone (Handayani *et al.*, 2012). The β-glucan of Shiitake mushrooms was possibly assisting faecal fat exclusion through the effects of β-glucan viscosity (Guo *et al.*, 2004; Kudo *et al.*, 2007).

Another biological component of Shiitake mushrooms, namely eritadenine, has been reported to have a plasma lipid lowering effect (Takashima *et al.*, 1974; Shimada *et al.*, 2003). Eritadenine has been reported to be ten times as effective in improving dyslipidaemia as clofibrate (Takashima *et al.*, 1973). Eritadenine is effective in lowering dyslipidaemia by decreasing the concentration of phosphatidylcholine (PC) and increasing the concentration of phosphatidyl ethanolamine (PE) in the liver (Sugiyama *et al.*, 1995; Walkey *et al.*, 1998). PC is an important phospholipid for lipoprotein assembly and secretion from the liver (Cole *et al.*, 2012). Adding eritadenine to the rat diet significantly decreased the level of plasma TAG (Takashima *et al.*, 1974; Sugiyama *et al.*, 1995).

Its effect upon cholesterol may also come from its fiber. Fiber is divided into two general categories water soluble and water insoluble. Soluble fiber lowers cholesterol. The proteins contain all of the essential amino acids, and most commonly occurring nonessential amino acids and amides. The fatty acids are largely unsaturated, and shiitake are rich in vitamins and minerals. Key therapeutic substances are glucans, a major constituent of the cell walls. Shiitake yields Lentinan. shiitake's historical usage is centered around cancer, high cholesterol, diseases of the liver, such as hepatitis, fatty liver and cirrhosis, and general immune response support.

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Comparison of Morphological Feature of *Ganoderma lucidum, Pleurotus pulmonarius* and *Pleurotus florida*

Nirod Chandra Sarker, Zeenia Afsary, Akhter Jahan Kakon and Nuhu Alam[1]
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

This investigation was initiated to study comparative morphology of three different mushrooms *Ganoderma lucidum, Pleurotus pulmonarius and Pleurotus florida* belonging to two different genus *Ganoderma* and *Pleurotus* as well as variation among three strains of *Ganoderma lucidum* on the basis of classical taxonomy. Basidiocarp of *Ganoderma lucidum* is stipitate, porus pileate, stipe color reddish brown, pileus dimitic to reniform, primary margin whitish, fewly zonate, with a lucid and laccate crust. Pore surface yellowish cream. Stipe central to lateral, sometimes several stipes joined in fascicles, cylindrical, compressed to irregular 3-5 cm long, 10-12 mm thick near the apex, red brown laccate often brighter than pileal surface. Hyphal system trimitic, generative hyphae with clamp connections. Cystidia absent, basidia clavate, 4-spored, sterigmata sometimes aurcuate. Spores ellipsoidal, truncated at one end when matured, brown, double walled, outer wall smooth , hyaline, inner wall rough thick walled. Three different strain Gl 2, Gl 4, Gl 6 showed considerable variation in shape, size, color of pileus, stipe and spores. Gl 4 showed the highest pileus diameter 8.4 cm while the pileus diameter of Gl 6 was lowest 4.2 cm. Thickness of pileus was same for all. Maximum stipe length 6.6 cm was for Gl-6 and minimum stipe length 1.9 cm for Gl 4. Stipe diameter was highest for Gl 6 and lowest for Gl 2. Only Gl 2 had two types of spores obovate and globose, wheather Gl 4 and Gl 6 had three types of spores attaltoid, obovate and globose. For only Gl 6 stipes were joined in fascicles but for two other strains stipes were not joined in fascicles. While pileus of *P.pulmonarius and P. florida* nonporous pileate, pileus color is ivory for *P. florida* and whitish to grayish for *P.pulmonarius* pileus shape umblicate for *P.pulmonarius* while flabelliform for *P. florida*. Pileus texture smooth for both species. Crenate margin was found for *P. florida*, and sinuate for *P.pulmonarius*. Pileus texture was fibrous and smooth for *P. florida* and *P.pulmonarius* respectively. Two different stipe colour gainsboro and old lace were observed for *P. florida* and *P.pulmonarius*.Gill attachment was descending for both species. Gill spacing was crowded for *P.pulmonarius* and distant for *P. florida*. Pileus diameter for *P.pulmonarius* and *P. florida* were 10.5 and 5.5 respectively. Stipe length was highest for *P.pulmonarius* and lowest for *P. florida*. Thickness of pileus was approximately similar 5mm and 8 mm respectively.The highest stipe length was4.5 cm for *P.pulmonarius* and the lowest 3.6 cm for *P. florida*. Hymenophoral trama was regular for *P. florida* and irregular for *P.pulmonarius*. Basidiospores cylindrical-oblong, hyaline, amyloid for *P. florida* while spores cylindrical hyaline, thin-walled for *P.pulmonarius*. Basidia club shaped, 3-spored for *P.pulmonarius* and basidia 2-spored for *P. Florida*.

Keywords: *Ganoderma, Pleurotus pulmonarius, Pleurotus florida*, Taxonomy.

INTRODUCTION

Most of the basidiomycetes fungi belongs to the order Agaricales whose fruiting bodies are commonly called mushrooms (Alexopolus and Mims, 1977). Mushrooms are cultured worldwide for their taste, nutritional attributes and potential application in industries

[1] Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

(Mata *et al.*, 2005). Edible mushrooms are nutritionally endowed fungi mostly basidiomycetes which grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials (Stamets, 2000; Lindequist *et al.*, 2005; Iwalokun *et al.*, 2007).

Ganoderma is largest genus in order aphyllophorales with more than 300 species. It is known to cause root or butt rot of the hardwood trees, and also known as medicinally important mushroom in the Asian continent. Traditionally, genus *Ganoderma* has been classified on morphological characteristics of fruit body such as size and color, and stipe attachment patterns (Steyaert, 1972; Corner, 1983). According to Ryvarden (1995) variation of shape and size of basidiospore, colour of pileus and stipe change with age, pore size, hyphal system, size and shape of apical pilear cells (cutis/dermal elements),variation of basidiospore size and shape are valuable in judging morphological variation. In nature, however, the morphological variation appears to be affected by environmental conditions during basidiocarp development. Furthermore, maturation of basidiocarps requires somewhat long periods that are affected by environmental factors to morphogenesis. Because most samples were generated to singletons which were affected by different environmental factors each other, the taxonomy of these fungi by morphological characteristics is very confuse.

The fungal genus *Pleurotus* is widely distributed throughout the world and is of particular economic importance as it comprises certain edible species that make up a steadily increasing part of the international mushroom market (Chang, 1984). *Pleurotus* species are characterized by a white spore pint attached to decurrent gills, often with an eccentric (off center) stipe, or no stipe at all. They always grow on wood in nature, usually on dead standing trees or on fallen logs. In nature oyster mushrooms appear in cluster on dead trees from late fall to spring, and are distributed almost all round the world. Four basidiospores form at the end of each basidium on the gill of a fruiting body. Each spore has one nucleus. Spores germinate to become primary mycelia, and then form secondary mycelia by plasmogamy. Secondary mycelia of oyster mushroom can be distinguished by the clamp connections and each cell has two nuclei. Only secondary mycelia can produce fruiting body under the proper conditions. In the basidia of mature fruiting bodies the two nuclei fuse into one, then pass through meiosis, and produce four haploid nuclei. The four haploid nuclei are then made into four new basidiospores.

P. pulmonarius known as grey oyster mushroom or phoenixtail mushroom is very similar to *P. ostreatus*. *P. florida* is a commercially important edible mushroom and it is widespread in various geographical regions of Bangladesh and south-east Asia. 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. Three different species of two different genera were compared according to their morphological variation and identified according to device taxonomic key.

MATERIALS AND METHODS

The study was carried out in Mushroom Development Institute (MDI), Sobhanbag, Savar, Dhaka, Bangladesh. Fruiting bodies of three different genuses such as *Ganoderma lucidum, Pleurotus .pulmonarius,* and *Pleurotus florida* were collected from culture house of MDI. For *Ganoderma lucidum* 3 different strains such as Gl 2, Gl 4, Gl 6 were collected and their fruiting body were studied for the comparative morphometric analysis.

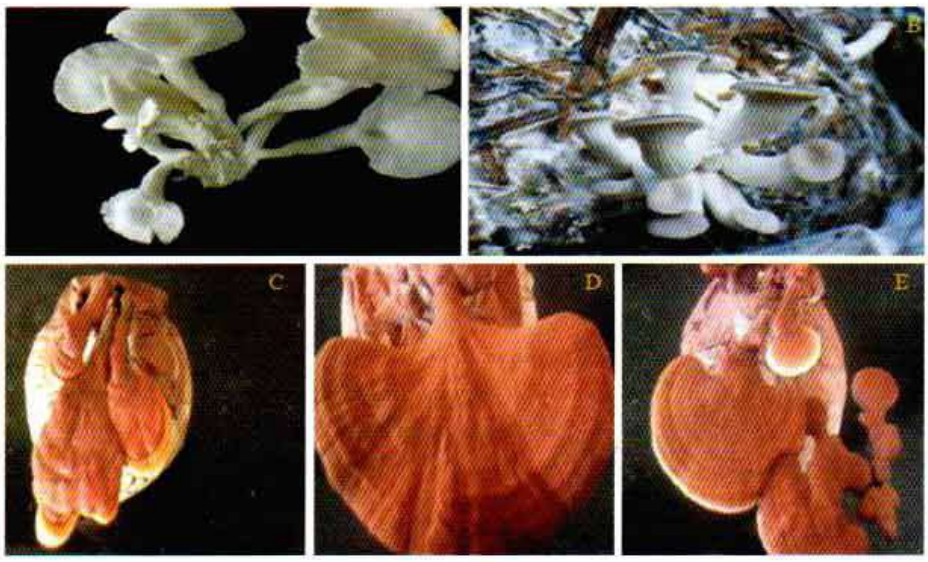

Fig.1. Fruiting bodies of selected mushrooms A. *Pleurotus florida* B. *Pleurotus pulmonarius* and *Ganoderma lucidum* strain C.Gl-2, D.Gl-4, E.Gl-6.

Macromorphology

Specimens were described macroscopically. The description of species combines cultures and fresh specimen. Color names are in according to color index.

Pileus shape/Cap morphology: Generally they were 4 to 9.5 cm, convex, becoming flat or somewhat depressed, kidney shaped to fan shaped ,or nearly circular, somewhat greasy when young and fresh, smooth, pale brown to dark brown, the margin enrolled when young, later wavy, never lined. Cap is expanded, upper of the mushroom, whose surface is the pileus actually pileus is the surface covering of a mushroom.

Pileus diameter (cm): Pileus diameter off our randomly selected fruiting bodies were recorded using a slide caliper.

Pileus margin: Different types of pileus margin were observed in oyster mushroom. Usually they were sinuate, crenate and laciniate.

Pileus texture: Pileus texture was visible with naked eye but sometimes observed through microscope. In general texture was smooth or velvety.

Pileus color: Pileus color was observed with naked eyes. Color varies from species to species. They were usually white, antique white, bisque, linen etc.

Gill attachment to Stipe: Gills are blade like structures on the outside of mushroom caps in agarics. Gill attachments are different in mushroom such as adnate, adnexed, notched, seeding, descending, freecetc. In case of oyster mushroom gill attachment are usually descending.

Gill spacing: Gill spacing was observed in naked eyes. Gill spacing was several types such as crowded, disant, subdistant, close etc. Most of *Pleurotus* species had crowded and only *P. ostreatus* species were close spacing.

Stipe/ Stalk length: Stipe length was recorded using a slide caliper in cm.

Stipe diameter (cm): Diameter of stipe was recorded using slide calipers (3 randomly selected fruiting bodies).

Stipe color: Stipe color was also observed with naked eyes. Stipe color was different from species to species. They were usually white, bisque, cornsilk.

Stipe texture: Stipe texture was visible with naked eyes but sometimes observed through microscope. In general texture was smooth or velvety.

Spore print: Spore color is one of the simplest aids in 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 stalk of a mushroom just beneath the cap and placing the cap, gills down, on a piece of aluminum foil paper in a petriplate, covering it with an inverted glass that will keep the air moist and quiet around the cap.

Spore type observation: Melzer,s solution were added to spore for staining the desired sample to detect whether the spores are amyloid,, inamyloid or dextrinoid.

Micro morphology

With the aid of a compound light microscope the longitudinal section of gill and stripe were observed. Cystidia, basidia, hymenophoral tramal features compiled micro morphology.

Culture characters

Cultures were inoculated in 90 mm petri dishes using PDA medium and incubated in the dark at 25^0C. Aspect, color, growth rate, odor and microscopic structures of both the arial and the submerged mycelium were observed weekly for 6 consecutive weeks according to (Nobles, 1965).

RESULTS AND DISCUSSION

Taxonomy

Key to the genera

1 Basidiocarp porus pileate, pileus rounded or compressed, hyphal system trimitic, cystidia absent, basidia clavate, ,double spherical wall, inner wall ornamented and colored.....*Ganoderma.*

1 Basidiocarp nonporus pileate, pileus convex, hyphal system monomitic or dimitic, cystidia present, basidia ellipsoidal, with,one spherical wall, inner wall not ornamented and not colored.....*Pleurotus.*

Ganoderma

Basidiocarp pileate, stipitate or sessile. Pileus tomentose to glabrous, yellow brown to black etc. glossyor not, often sulcate, with or without a crust. If stipitate rounded or compressed pores small, rarely medium surface white, milky white, pale mustard yellow, ochraceous todark brown etc. Light coloured pores often discolouring often bruised or touched Tubes stratose or not, hyphal system trimitic generative hyphae with clamp connections generative hyphae with clamp connections very rarely seen. Binding hyphae often rarely met, thin to straightly thick walled, often with clamp connections. Usually with conspicuously flagelliform ramuli. Skeletal hyphae present in all species and mostly colored unbranched.Thick walled or long branched and dendritic above. Cystidia absent. Basidia clavate, often very stout and generally not easily seen. Spores with double spherical wall, perisporial wall smooth, hyaline, inner wall ornamented, colored. Terrestrial or lignicolous.

Ganoderma lucidum

Basidiocarp laterally stipitate or eccentric, 4–10.5 × 3– 7 cm, laccate, brittle, stipe color reddish brown, 5–7 cm long and 1cm diameter. Pileus dimitic to reniform 2-8cm long, 1-5cm broad, 18-22µm thick. Upper surface lacccate, sulcate, semidull dark reddish brown. Margin 2mm in thickness, sterile, yellowish to reddish brown. Primary margin whitish, fewly zonate, with a lucid and laccate crust. Pore surface yellowish cream, Blood red to purple red when touched. Pores round, 5-6mm per mm, irregular. Tubes dark brown, stratose or not, 3–5mm long, unstratified, whitish brown. Context 2mm thick, coffee colour, thickening towards the base of the stem. Cutis type thick walled claviform, 22.8–25.8 × 5–5.8µm. Stipe central to lateral, sometimes several stipes joined in fascicles, cylindrical, compressed to irregular 3-5cm long, 10-12mm thick near the apex, red brown laccate often brighter than pileal surface, context double layered upper layer whitish under layer light brown, 8-12mm thick, taste bitter. Hyphal system trimitic, generative hyphae with clamp connections 2.5–3µm thick with clamp connections, hyaline; binding hyphae flexaous 1.5-2.5µm, thick, branched skeletal hyphae straight with transperant lumen, 4-5µm, brown in branched or sometimes rarely unbranched. Cystidia absent. Basidia clavate, 4-spored, sterigmata sometimes aurcuate. Spores mellipsoidal, truncated at one end when matured, brown, double walled, outer wall smooth, hyaline, inner wall rough thick walled.

Key to different strains of *Ganoderma lucidum*

1 Spores attaltoid, globose, obovate.....2
1 Spores obovate and globose.....Gl-2
2 Stipes not joined in fascicles.....Gl-4
2 Stipes joined in fascicles.....Gl-6

Gl 2

Basidiocarp 5.2-8.4cm, woody to corky, sub sessile to laterally stipitate, reniform. Upper Surface laccate, dark reddish, yellowish towards margin, brittle, soft. Margin blunt, rounded, brown white. Pore surface creamish to milky coffee. Two types of spores obovate and globose.

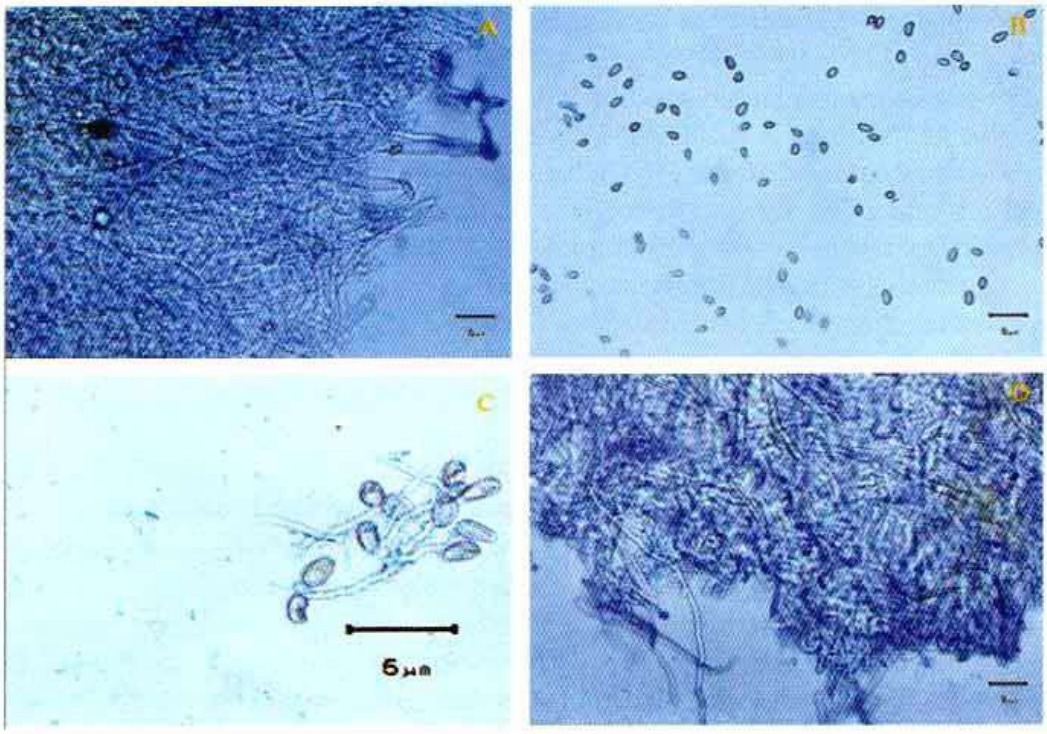

Fig.1. A. gill; B. spore; C. germinating spore D. stipe of srain Gl-2.

Table 1. Pileus diameter, pileus thickness, stipe length, stipe diameter of *Ganoderma* strains

Strain of Ganoderma	Pileus diameter (cm)	Thickness of pileus (cm)	Stipe/Stalk length (cm)	Stipe diameter (cm)
Gl-2	5.3	0.4	4.1	0.6
Gl-4	8.4	0.4	1.9	2.1
Gl-6	4.2	0.4	6.6	1.9

Gl 4

Basidiocarp laterally stipitate or eccentric,7.3-8.4 cm, laccate, brittle, stipe reddish black, 4.1 - 5 cm long.Upper surface radially sulcate, semidull dark reddish brown. Margin thin,

yellowish to brown.Pore surface cream-turning ochraceous. Three types spores attaltoid, globose, obovate.

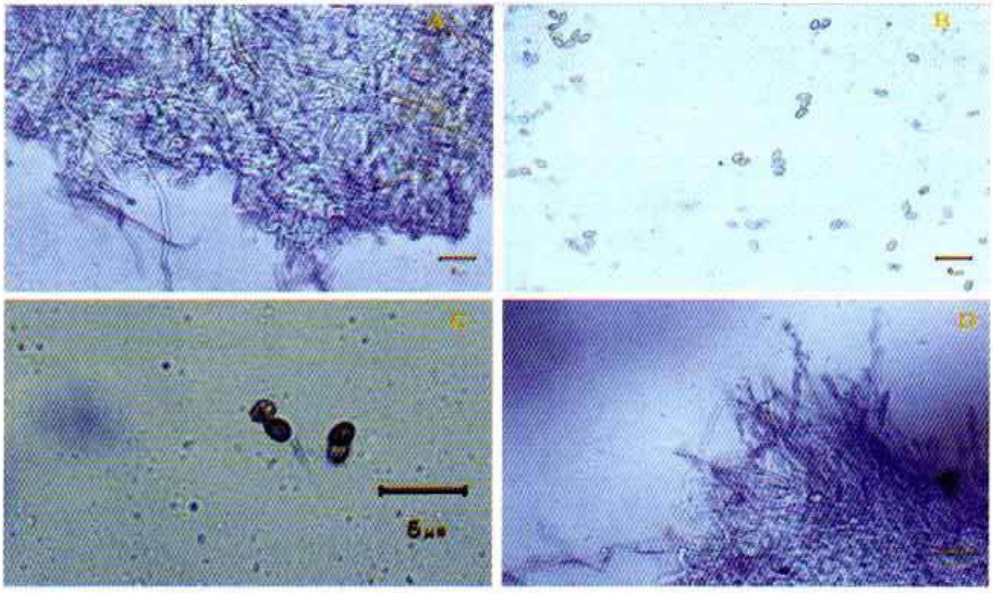

Fig.2. A. gill; B. spore; C. germinating spore D. stipe of srain Gl-4.

Gl 6

Basidiocarp perennial, pileate, stipitate, dimidiate, laccate 4.2cm. Upper surface concentrically sulcate, brown of chestnut. Stipes joined fascicles 6.6cm long. Pore surface creamy white at first later ochraceous to pale brown. Pore round, tube 3mm thick, snuff brown, context shiny, oboovate, attaltoid, globose spores.

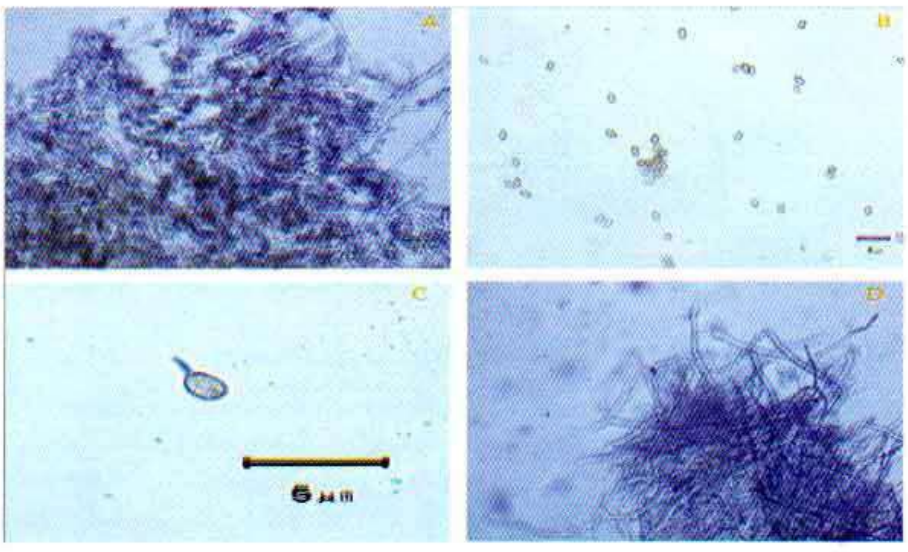

Fig. 3. A. gill; B. spore; C. germinating spore D. stipe of strain Gl-6.

Pleurotus

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 subcentral. 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 present, lignicolous.

Key to the species of *Pleurotus*

1 Pileus flabelliform, whitish to grayish, shiny, gelatinous, pileus margin crenate, gill spacing crowded, stipe color gainsboro, basidia 3-spored, basidiospore cylindrical, hymenophoral trama irregular, cystidia lageniform, ventricose-rostrate, caulocystidia absent, mycelium onomitic.....*P .pulmonarius*

1 Pileus umblicate, ivory, not shiny, not gelatinous, pileus margin sinuate, gill spacing distant stipe color old lace, basidia 2-spored, basidiospores cylindrical-oblong, hymenophoral trama regular cystidia clavate, caulocystidia present, mycelium dimitic.....*P. florida*

Table 2. Comparitive qualitative characters for chracterization of oyster mushroom

Criteria of characterization	*P. florida*	*P. pulmonarius*
Pileus shape	Flabelliform	Umblicate
Pileus color	Ivory	Whitish to grayish
Pileus texture	Smooth	Smooth
Pileus margin	Sinuate	Crenate
Stipe color	Gainsboro	Old lace
Stipe texture	Smooth	Fibrous
Gill attachment to stipe	Descending	Descending
Gill spacing	Distant	Crowded

Table 3. Comparitive quantitive characters of oyster mushroom

Name of species	Pileus diameter (cm)	Thickness of pileus (cm)	Stipe length (cm)	Stipe diameter (cm)
P. florida	10.5	0.8	3.6	1.8
P.pulmonarius	5.5	0.5	4.5	0.7

Pleurotus pulmonarius

Sporophores solitary or caespitose, sessile or laterally stipitate, depeloping horizontionally in overlapping groups, leathery, soft. Pileus 6.0-8.0cm in diameter pileus flabelliform, whitish to grayish, surface smooth, glabrous, shiny, gelatinous. Gill crowded, whitish or creamish, decurrent, edge entire, margin crenulate.Stipe lateral,

eccentric, villose, often attenuated towards the base. Stipe eccentric to lateral, smooth. Spores cylindrical hyaline, thin-walled. Basidia club shaped, 3-spored.Cheilocystidia absent. Numerous basidioles or pleurocystidia. Hymenophoral trama completely irregular, with clamped, sclerified hyphae thin-walled hypha.Context of pileus with generative hyphae thin- and thick-walled. Hyphae of stem generative, thick-walled. Pileipellis a cutis, not well differentiated, with hyphae parallel and radial arranged.

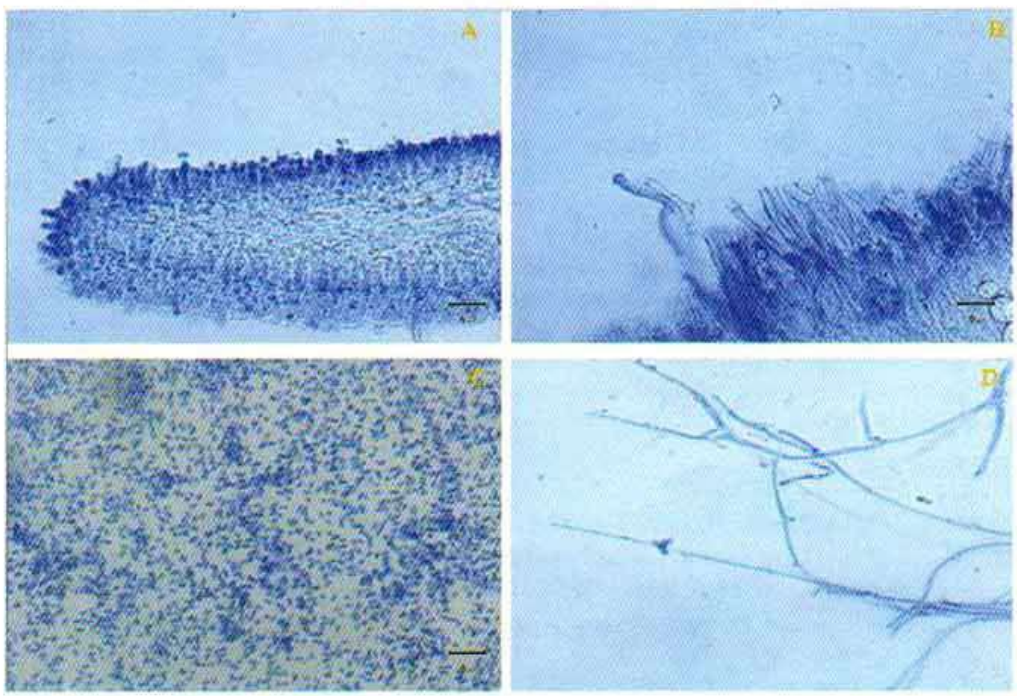

Fig.4. A. gill; B. spore; C. germinating spore D. stipe of *P. pulmonarius.*

Culture characters

Mycelium hyaline, whitish and arachnoid near inoculum. Later more white, cottony, with increasing aerial mycelium. Radial growth was generally observed; sometimes feathery. Margin irregular. Reverse of agar unchanged.Odor of bitter almonds. Mycelium covered Petri plates within 1–2 wk. Advancing mycelium with clamped, thin-walled hyphae, more branched in the center of the colony, with hyphae and near inoculums.

Pleurotus florida

Pileus umblicate pileus color ivory, pileus glabrous, 10.5-12.7cm in diameter, pileus margin sinuate, fleshy, yellowish when dry. Stipe usually lateral, short, sometimes elongated, usually 3.7-5.1cm long, hollow, stipe color gainsboro. Flesh white, Hymenophoral trama regular.Basidiospores cylindrical-oblong, hyaline, amyloid, Cheilocystidia clavate. Spore print leucosporae. Basidia 2-spored.

Cultural characteristics

Cottony, whitish mycelium, often with tufts of dense growth, sometimes with yellowish tones, mycelium monomitic with generative clamped hyphae.

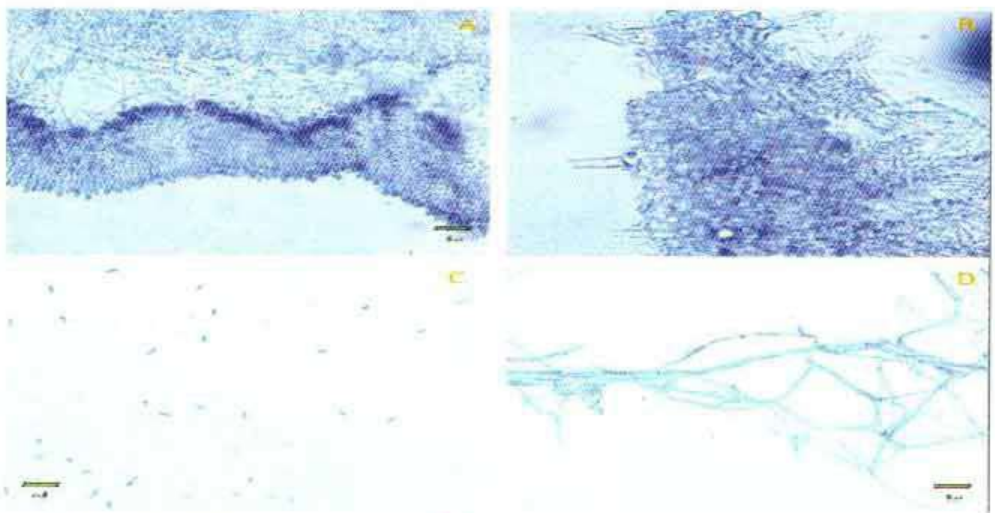

Fig.5. A. gill; B. spore; C. germinating spore D. stipe of *P.florida.*

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Review Article

Nutritional and Medicinal Perspective of Hericium Mushroom

Akhter Jahan Kakon and Md. Bazlul Karim Choudhury[1]
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

Mushrooms are considered as nutritionally functional foods and source of physiologically beneficial medicines. *Hericium erinaceus*, also known as Lion's Mane Mushroom or Hedgehog Mushroom, is an edible fungus, which has a long history of usage in traditional Chinese medicine. It is also called cauliflower mushroom, pom pom mushroom, monkey head mushroom, mountain priest mushroom etc. 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. This review article has overviewed the recent advances in the research and study on *H. erinaceus* and discussed the potential health beneficial activities of this mushroom, with the recognition of bioactive compounds responsible for these medicinal properties.

Keywords: Lion's Mane Mushroom, Hedgehog Mushroom, Immuno-modulation, Neuro-protection.

INTRODUCTION

Hericium erinaceus is a basidiomycete fungus taxonomically belonging to the family Hericiaceae, order Hericiales, division Homobasidiomycetes, class Hymenomycetes (Ko *et al.*, (2005). Native to North America, Europe and Asia, it can be identified by its long spines (greater than 1cm length). It is a good edible and medicinal mushroom, which contains 32 kinds of spices and various bioactive substances with rich nutrition. It is named for its shape, and is literally interpreted as "Monkey Head Mushroom" from Chinese, which could remind as adult monkey with unclear face appearance due to head hair. Its fruiting body is also 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). Gue *et al.*, (2006) mentioned that, *Hericium erinaceum* is a medicinal and edible mushroom with anti-microbial and anti-cancer activities.

Hericium erinaceum has recently attracted considerable attention for its various physiological activities, such as antitumor, antioxidant, hypoglycemic, hypo cholesterolemic and immune stimulating activities; and its various biological compounds such as polysaccharides, extracted from the fruiting bodies and cultured mycelia, and exopolymers produced with submerged culture have been reported as well (Mizuno *et al.*, 1995; Mau *et al.*, 2002; Park, *et al.*, 2002; Wang *et al.*, 2005; Yang *et al.*, 2002; Yang *et*

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

al., 2003). Medicines from the natural sources are usually thought to have fewer side effects. From the ancient times, mushrooms have been recognized as nutritionally functional foods and source of physiologically beneficial medicines. Mushrooms are the manifestation of the common saying, "Medicines and foods have a common origin". Several species of mushrooms under the genus of *Pleurotus, Ganoderma, Cordyceps, Lentinus, Hericium and Grifola* have been reported widely to have anti-cancer, anti-diabetic, anti-hypertensive, anti-microbial, cardio-protective, hepato-protective and other health beneficial roles (Kaul, 2001; Sanodiya *et al.*, 2009; Bisen *et al.*, 2010; Khan *et al.*, 2010; Wani *et al.*, 2010; Lindequist *et al.*, 2010; Khan *et al.*, 2012). This mushroom is mostly found in East Asian countries and has along history of usage in traditional Chinese medicine (Hiwatashi *et al.*, 2010).

DISCUSSION

Nutritional value

Hericium erinaceus is rich in protein, fat, carbohydrates, and crude fiber. Different kinds of vitamins including thiamine, riboflavin and amino acid composition is also more comprehensive. It is rich in almost all types of essential amino acids and various kinds of polysaccharide, polypeptide and unsaturated fatty acids but poor in fat content. It has been proved that *Hericium erinaceus* contains sufficient amount of K, Zn, Fe, Ca, Se, P and small amounts of Na. For health benefit 100g fresh or 10g dry *Hericium erinaceus* mushroom per day is preferred and suggested. Wang *et al.* (1992) investigated the nutritional composition of 5 strains of *Hericium erinaceus* fruiting bodies and observed that it contains highest quantity of crude protein (28.4%) and low crude fat. Zhanxi and Zhanhua, (1999) found that each 100g dried *H. erinaceus* fruit bodies contained 26.3g protein, 4.2g fat, 856mg phosphorus, 18mg iron, 2mg calcium, and a considerable amount of vitamin B complex.

Application Fields

In applicational ground *Hericium* is important in improving inflammation and ulcers, mainly referring to ulcers of gastric and esophageal mucosa, and intestinal flora disorder. This mushroom also important in Pancreatitis, Crohn's disease, Cancer especially

digestive organs (stomach, esophagus, intestines, pancreas). It can be supplied during chemotherapy, to reduce side effects. It causes reduction of overweight and improves different post menopausal disease. This mushroom also able to improve different nervous system diseases like multiple onset neuropathy and alzheimer's disease, anxiety, fear, depression, to promote digestion, hemorrhoids, strengthen the immune system and preventing infection. It is also important in improving metabolism and reducing weight.

Ingredients and Medical Effects

According to the records on Chinese traditional medicine, *Hericium erinaceus* is beneficial for five vital organs like kidney, liver, spleen, heart and stomach, which can improve digestion, strengthen physical condition, restore energy and inhabit cancer. It has been successfully applied in the treatment for gastric cancer, gastritis, gastric ulcer, duodenal ulcer and esophageal cancer. *Hericium erinaceus* especially has great potential on the treatment for digestive system (including esophagus, stomach and intestine).

Well-known effects from some records of many research institutes have been proved that its antimicrobial ingredients can inhibit helicobacter pylori, which could cause gastric ulcer and gastric cancer. These main effects shall be attributable to polysaccharide and polypeptide which can stimulate immune system to recognize and kill cancer cells. In addition, *Hericium erinaceus* could help mucosa recovery.

Japanese scientists found that *Hericium erinaceus* contains erinacine, which enables to stimulate growth factors of nerve cells. And it can be applied to treat nervous system disease and alzheimer disease, and is used to restore peripheral nerve tissue damaged by neuron disease and polyneuropathy, what is more interesting, this science was finally clear according to the report on the effects of *Hericium erinaceus*. It can reduce blood cholesterol and triglyceride levels, regulation of blood lipids, help blood circulation. Thus it is the ideal food for cardiovascular patients. *Hericium erinaceus* contains polysaccharides, polypeptides, and essential fatty substances that can inhibit the synthesis of genetic material in cancer cells, and thus the prevention and treatment of gastrointestinal cancer and other malignancies.

Hericium erinaceus contains a rich variety of amino acids and polysaccharides, to aid digestion, for gastritis, gastric cancer, esophageal cancer, gastric ulcer, duodenal ulcer and other gastrointestinal diseases, the efficacy of eye-catching; *Hericium erinaceus* with improved immunity function can delay aging. For mild insomnia taking *Hericium erinaceus* is a better adjuvant therapy.

Several scientific studies have documented the medicinal or health beneficial effects of fruit bodies and mycelium of *H. erinaceus* mushroom and their chemical extracts. The extracts of *Hericium erinaceus* are available in the market, and the effect can be achieved 18 times than original one after concentration. Doctors usually recommended the combination of high enrichment extracts and powder/tablets as adjuvant treatment for indolent diseases.

Mizuno (1999), reported that, polysaccharides (HEPS) in the fruiting bodies of *H. erinaceus* may have beneficial effects against stomach, esophageal and skin cancers. *H. erinaceus* (its fruiting body, mycelium, and products in the medium) also contains some lower MW pharmaceutical constituents, such as the novel phenols (hercenones A and B) and Y-A-2 which may have chemotherapeutic effects on cancer. Keun *et al.* (2003) studied the hypolipidaemic effect of an exo-biopolymer produced from a submerged mycelial culture of *Hericium erinaceus* in dietary-induced hyperlipidaemic rats. The oral administration of exo-biopolymer, at the dose of 200mg/kg body weight, substantially reduced the plasma total cholesterol (32.9%), low density lipoprotein cholesterol (45.4%), triglyceride (34.3%), phospholipids (18.9%), atherogenic index (58.7%). It increased the plasma high density lipoprotein cholesterol level (31.1%) as compared to the control group. Chyi *et al.* (2005) recorded that, recent studies have determined that *Hericium* spp. mushrooms, may have important physiological functions in humans, including antioxidant activities, the regulation of blood lipid levels and reduction of blood glucose levels.

Mori *et al.* (2010) found that the ethanol extract of H. *erinaceus* potently inhibited platelet aggregation induced by collagen. They identified hericenone B as the active compound against platelet aggregation in human and rabbit platelets.

Mori *et al.* (2008) reported that the ethanol extract of *H. erinaceus* promoted nerve growth factor mRNA expression in a concentration-dependent manner via the activation of the JNK pathway. *H. erinaceus* has been suggested by Mori *et al.* (2011) also to be useful in the prevention or treatment of dementia and cognitive dysfunction, as they found that dietary administration of *H. erinaceus* powder prevented the impairments of spatial short-term and visual recognition memory induced by amyloid β (25–35) peptide in male ICR mice. Nagano *et al.* (2010) investigated the clinical effects of *H. erinaceus* on menopause, depression, sleep quality and indefinite complaints and suggested that intake of this mushroom can reduce the depression and anxiety in female subjects. In another clinical study, a double-blind, parallel-group, placebo-controlled trial was performed by Mori *et al.* (2009) on 50- to 80-year-old Japanese men and women with mild cognitive impairment. Oral administration of *H. erinaceus* powder showed significantly increased scores on the cognitive function scale with no adverse effect in laboratory tests.

Oxidative stresses have been implicated in several degenerative processes, diseases and syndromes, including cancer, cardiovascular complications, neurodegenerative diseases and a wide variety of age-related disorders (Ames *et al.*, 1993 and Khan *et al.*, 2010). For this, food supplements or natural medicines which have antioxidant activities are of special interests. The antioxidant index, as well as free radical scavenging activity, lipid peroxidation inhibitory activities of hot water extract of *H. erinaceus* has been reported by Abdullah *et al.* (2012). Wong *et al.* (2009) found that the mycelium extract of *H. erinaceus* is rich in phenolic content and has potential ferric reducing antioxidant power. The fresh fruit body extract was also found to have the potent 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity. And, oven-dried fruit body extract was excellent in reducing the extent of carotene bleaching. They have also reported that the

total phenolic content and total antioxidant activity in the oven-dried fruit body extract was higher compared to the freeze-dried or fresh fruit body extract. This may be due to the generation and accumulation of Maillard's reaction products, which are known to have antioxidant properties (Wong *et al.*, 2009). In a recent study by Han *et al.* (2013), *H. erinaceus* polysaccharides showed significant antioxidant activity against ischemia reperfusion induced renal oxidative injury damage in experimental animals. In that study, pre-administration of mushroom polysaccharide decreased lipid peroxidation level and increased antioxidant enzyme activities in mice (Han *et al.*, 2013). The antioxidant activity of the endo-polysaccharides from *H. erinaceus* mycelium has also been documented (Zhang *et al.*, 2012).

Medical Research and Use

Hericium erinaceus is a medicinal mushroom. It has been used for centuries in traditional Chinese medicine as a brain tonic and as a treatment for many health conditions. It has long a history of use in traditional Chinese medicine.

In a study conducted on rat showed some compounds in the mushroom, like threitol, D-arabinitol, and palmitic acid, may have antioxidant effects, regulate blood lipid levels and reduce blood glucose levels (Wang *et al.*, 2005). Another study on rats that had suffered brain injury showed that "daily oral administration of *H. erinaceus* could promote the regeneration of injured rat peroneal nerve in the early stage of recovery. (Wong *et al.*, 2012).

More recently and more relevant to human use, is a review of scientific studies, which asserted the medical benefits of the mushroom by saying "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, anti-diabetic, wound healing properties among other therapeutic potentials.(Khan *et al.*, 2013).

Jiang *et al.* (2014) concluded in a scientific review on the therapeutic effects of *H. erinaceus* that it is helpful to various diseases, such as Alzheimer's disease, immunoregulatory, and many types of cancer. A report reveals that pills of this mushroom are used in the treatment of gastric ulcers and esophageal carcinoma (Ying *et al.*, 1987). In a review Abdulla *et al.* (2011) demonstrates the mushroom's wound healing capacities based on following primary researches:

- It stimulates animal nerve cells (Park *et al.*, 2011).
- A double-blind, parallel-group, placebo-controlled trial showed improved cognitive ability in individuals with mild cognitive impairment (Mori *et al.*, 2009).
- *H. erinaceus* stimulates nerve growth factor in an *in vitro* experiment with human astrocytoma cells (Mori *et al.*, 2008), nerve growth factor stimulates by phenol-analogous hericenone (Mizuno, 1999).

- Stimulates myelination in an *in vitro* experiment (Kolotushkina *et al.*, 2003).
- It also regenerates peripheral nerves following crush injury (Wong *et al.*, 2011).

Long-term safety and effects of withdrawal seem to be unknown: After completion of a study Mori (2009) discussed "The subjects of the Yamabushitake group took four 250 mg tablets containing 96 percent of Yamabushitake dry powder three times a day for 16 weeks. After termination of the intake, the subjects were observed for the next four weeks. At weeks eight, 12 and 16 of the trial, the Yamabushitake group showed significantly increased scores on the cognitive function scale compared with the placebo group. The Yamabushitake group's scores increased with the duration of intake, but at week four after the termination of the 16 weeks intake, the scores decreased significantly".

The influence of lion's mane influence on neurological functions may also have other added benefits — making you feel good. In another small clinical study (n = 30), post-menopausal women who consumed lion's mane baked into cookies vs. those without showed less anxiety and depression yet improved in their ability to concentrate (Nagano *et al.*, 2010).

In another small Japanese study with a randomized sample of 30 women, ingesting lion's mane showed that "*H. erinaceus* intake has the possibility to reduce depression and anxiety, and these results suggest a different mechanism from NGF-enhancing action of H. erinaceus" (Nagano *et al.*, 2010).

Due to hormonal fluctuations, women are suffering during menopause because of increased stress. This can have a negative impact on the gastrointestinal tract. Due to its protective effect on mucous and its mucous regenerating effect here supplementation of the Hericium is provento be very successful. Hericium protects but also strengthens the nervous system. Therefore, stress-related fears, anxiety, memory loss and insomnia can be treated well with this medicinal mushroom (Nagano *et al.*, 2010).

CONCLUSION

Mushrooms are the good example of medicinal foods or food supplements. From the ancient times, mushrooms have been recognized as important food items because of their taste, flavor, high nutritional values and several medicinal properties. *H. erinaceus* is a delicious mushroom with potential medicinal importance. This mushroom is mainly consumed in East Asian countries, and most of the research works have been performed in China, Japan and Korea. This study suggests its worldwide research, cultivation and consumption. Daily consumption of this mushroom may keep people away from several life-threatening disorders. However, the worldwide scientific study on *H. erinaceus* is not common. More basic clinical studies are recommended as further research to establish its therapeutic potential against life threatening human disorders.

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Contents