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Performance of Different Strains of Shiitake Mushroom *(Lentinus edodes)* on Saw Dust

Nirod Chandra Sarker, Saleh Ahmed, Kamal Hossain, Akhter Jahan and Nur Hohammed Mezbahul Quddus
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Abstract

To determine their performance nine different strains of shiitake mushroom were cultivated on sawdust. A wide variation was found in mycelium growth rate and duration to complete mycelium running. The highest mycelium growth rate (0.35cm/day) was obtained in *Lentinula edodes* (Le)-9, which was statistically similar to Le-6, Le-8, Le-10 and Le-4 and the lowest mycelium growth rate was obtained in Le-2. The minimum time required to complete mycelium running (35.17 days) was observed in Le-9 followed by Le-6 and Le-8 whereas maximum time required to complete mycelium running was found in Le-2. Among the nine strains, Le-10 performed best in the respect of total time required from inoculation to bump formation, primordia initiation and first harvest. Highest number of effective fruiting body (14.33/packet) was recorded in Le-8 which was statistically similar to Le-11 and the lowest number of effective fruiting body was recorded in Le-4. The highest length of stalk (5.50cm), diameter of stalk (1.30cm), diameter of pileus (6.917cm) and thickness of pileus (1.25cm) were observed in Le-5 which was statistically similar to Le-6. Significant variation in biological yield, economical yield and biological efficiency were observed among the selected strains. The highest biological yield, economic yield and biological efficiency were estimated in Le-8 followed by Le-11 and Le-12 whereas Le-4 performed very poorly.

Key words: Shiitake mushroom strains, mycelium, fruiting body, growth and yield.

INTRODUCTION

Shiitake mushroom *(Lentinula edodes)* is the second most popular edible mushroom (Chang, 1999 and Chiu *et al.*, 1999). It is highly prized in the Orient for its flavor and reputed edible and medicinal value. Many strains of shiitake mushroom are available in the world which are extensively cultivated. The strains of this valuable mushroom vary widely, particularly in the time required for mycelium colonization, bump formation and fruiting body development. The morphology and productivity of shiitake mushroom vary according to the strains based on the influence of environmental factors (Triratana and Tantikanjana, 1987). The mycelium growth in the vegetative phase involves producing quality fruiting bodies in the reproductive phase. A spawn run of different strains is of ultimate importance for adjusting the reproductive phase. For one strain, 60 days is sufficient to mature, whereas this time would be insufficient for another strain (Miles and Chang, 1989). Substrate selectivity, growth (some strains may produce pre-mature fruiting), quality (shape, size, thickness, color, flavored and aroma etc.) and yield are also strain related (Chen, 2001). Many studies have been carried out in the world to improve the quality and increase the production of *L. edodes*.

But, the production of this mushroom is fairly new in Bangladesh. The present study was undertaken to evaluate the performance of different strains of shiitake mushroom in order to identify the best strain that can be highly productive and suitable for culture conditions in Bangladesh.

MATERIALS AND METHODS

The experiment was conducted at National Mushroom Development and Extension Centre, Savar, Dhaka during October 2008 to April 2009 to study the performance of vegetative and reproductive growth and yield of nine strains of shiitake mushroom. The strains were designated as Le-2, Le-4, Le-5, Le-6, Le-8, Le-9, Le-10, Le-11 and Le-12.

Preparation of pure culture: Pure culture of different strains were prepared on potato dextrose agar (PDA) medium containing 200g peeled and sliced potato, 20g dextrose and 20g per liter agar. The medium was poured into glass Petri dishes (90mm diameter) at 15 ml/dish. The medium in Petri dish was sterilized in an autoclave for 20 minutes at 120 ^{0}C under 1 kg/cm^2 pressure. After sterilization and solidification, the plates were inoculated separately with the inocula of the strains. After inoculation, the plates were covered with cellophane paper. All operations were done under sterile condition in a clean bench. The inoculated Petri dishes were incubated in a growth chamber at 22 ± 2 ^{0}C and 70% redative humidity (RH). After completion of the whitish mycelium, this culture was used for inoculation of mother culture.

Preparation of mother culture: Medium of mother culture was prepared by mixing sawdust and wheat bran at the ratio of 2:1. Calcium carbonate was added at the rate of 0.2% of the mixture. The moisture level of the mixture was maintained at 65%. Polypropylene bags of 25cm × 17cm size were filled with 250g of the above prepared mixture and packed tightly. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the volume of the bag was made for space to put the inoculums. The neck was plugged with cotton and covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for one hour at 120 ^{0}C under 1 kg/cm^2 pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. Individually, a piece of stock PDA culture medium containing mycelium of different strains of shiitake mushroom was placed aseptically in the hole of mother culture packet and the packet was again plugged as mentioned above. The inoculated packets were placed on a rack in the laboratory at 22 ± 2 ^{0}C temperature for incubation. The substrate of the mother culture was colonized by the growth of whitish mycelium within 15-20 days after inoculation. The fully colonized packets were used for spawning.

Preparation of spawn packets: Saw dust was used as a main substrate and wheat bran, rice husk were used as supplements. For each 500g spawn packet sun dried saw dust, wheat bran and rice husk were mixed together at 160, 80 and 10g respectively. Water was added to adjust moisture content at 65% and $CaCO_3$ was mixed at the rate of 0.2% of the mixture. Polypropylene bags of 25cm × 17 cm size were filled with 500g of substrate

mixture and packets were tied, plugged and covered as mentioned above. The packets were sterilized in an autoclave for 1 hour at 120 °C under 1 kg/cm^2 pressure. After sterilization the packets were cooled and transferred into an inoculation chamber. The packets were inoculated separately with the mother culture of the strains to be tested at the rate of two teaspoonfuls per packet. The inoculated packets were incubated at 22 ± 2 °C.

Mycelial colonization and bump formation: During incubation period, whitish mycelia started to grow in the substrate. All the strains showed optimal mycelial growth at 22 ± 2 °C temperature and 60-70% relative humidity. After full colonization of spawn packets, a thick mycelial coat formed on the outer surface of colonized substrate. Clumps of mycelia appeared as blister like bumps of various sizes on the surface of the mycelial coat in each packet. Bumping usually started when colonization of white mycelia changed to brown.

Cultivation conditions for fruiting: After mycelium maturation and pigmentation of the tip of bump, all the packets were opened on the top side and soaked in water for 15-20 minutes. Then the packets were placed separately on the cemented floor of air conditioned culture house. The temperature, relative humidity and light were maintained at 18-22 °C, 60-70 % and 10-20 lux, respectively. Sufficient water was applied per day and proper aeration was taken in culture house for the release CO_2 and supply of O_2 as required for development of the primordia and fruiting body.

Biological yield in gram per packet was recorded by weighing the whole fruiting bodies without removing the lower hard and dirty portion whereas economic yield was recorded after removing lower hard and dirty portion of fruiting bodies and biological efficiency (%) was determined by the following formula:

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

Experimental design: The experiment was laid out in a completely randomized design with six replications (packets). Data on mycelium growth rate, duration to complete mycelium running, time required for bump formation, days required to primordia initiation, days required to first harvest, number of effective fruiting body, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, biological yield and economical yield were recorded. Data were analyzed using MSTAT-c computer program. Means were compared following Duncan's multiple range test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Mycelium growth rate and duration to complete mycelium running: Mycelium growth rate (MGR) in spawn packet ranged from 0.23 to 0.35 cm/day among the strains. The MGR of Le-4, Le-6, Le-8, Le-9 and Le-10 was statistically similar but significantly

higher as compared to other strains. The lowest MGR was observed in Le-2, which was statistically similar to Le-5. Duration to complete mycelium running (DCMR) in packet ranged from 35.17 to 51.83 days in the selected strains. The DCMR in Le-9 Le-6 and Le-8 was statistically similar but significantly lower as compared other strains. The highest DCMR was found in Le-2 which was followed by Le-5, Le-12 and Le-11 (Table 1). The duration to complete mycelium running of different strains of shiitake mushroom on saw dust varied greatly. This result is more or less similar to the findings of Miles and Chang (1989), who found that 60 days is sufficient to mature for one strain, whereas this time would be insufficient for another strain.

Duration to bump formation: Maximum of 98.33 days was required by Le-2, which was followed by Le-5. The strain Le-10 required only 65.83 day to forms bumps, which was followed of Le-4 and Le-11. The duration required for bump formation under last three strains was statistically similar but significantly higher as compared to Le-2, Le-5, Le-6 and Le-12 (Table 1). In present study, it was observed that shiitake mushrooms took 65.83 to 98.33 days for bump formation during incubation period. This result supports the findings of Kawai *et al.* (1997) who reported that 60 to 90 days is required for incubation period of shiitake mushroom.

Table 1. Effect of different strains of shiitake mushroom on mycelium growth and bump formation

Strains	Mycelium growth rate in spawn packet (cm/day)	Duration to complete mycelium running in spawn packet (days)	Time required for bump formation in spawn packet (days)
Le-2	0.23 b	51.83 a	98.33 a
Le-4	0.31 a	38.83 c	67.67 cd
Le-5	0.24 b	50.50 ab	95.00 a
Le-6	0.34 a	36.50 cd	77.50 b
Le-8	0.33 a	37.17 cd	74.83.bc
Le-9	0.35 a	35.17 d	73.83 bc
Le-10	0.31 a	38.83 c	65.83 d
Le-11	0.26 b	48.00 b	70.50 bcd
Le-12	0.26 b	48.33 b	76.33 b
CV (%)	8.97	6.62	7.37

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

Days required for primordia initiation and first harvest: Among the strains, a wide variation was observed in the duration from inoculation to primordia initiation and first harvest. Days required for primordia initiation ranged 69.83-103.5 days and for first harvest ranged 78.67-111.2 days. The minimum days for primordial initiation and first harvest was observed in Le-10, which was statistically similar to Le-11, and Le-4. Both the parameters in Le-2 and Le-5 were statistically similar but significantly higher as compared to other strains. Variations in days for primordial initiation and first harvest observed in four strains were not significantly different (Table 2).

Effective fruiting body: Strains Le-8 and Le-11 yielded 14.33 and 13.50 effective fruiting bodies per spawn, which were statistically similar and significantly higher as compared to other stains. The lowest number of effective fruiting bodies of 3.83 per spawn packet was recorded from Le-4, which was statistically similar to Le-5, Le-6, Le-9 and Le-10. Strains Le-2 and Le-12 yielded 8.67 and 8.83 effective fruiting bodies per spawn packet (Table 2).

Length and diameter of stalk: Length and diameter of stalk of fruiting bodies ranged from 3.583 - 5.500 cm and 0.750 - 1.300 cm, respectively among different strains. The highest stalk length was found in Le-5, which was statistically similar to Le-4, Le-6, Le-9 and Le-12. The lowest stalk length was found in Le-11, which was statistically similar to the stalk length recorded from Le-10, Le-8 and Le-2. Strain Le-5 also yielded maximum stalk diameter, which was followed by Le-4 and Le-6. This difference was not significant. The minimum stalk diameter was found in Le-5, which was statistically similar to the strains Le-11, but significantly different from the diameter recorded from Le-9, Le-8 and Le-2 (Table 2).

Diameter and thickness of pileus: Diameter and thickness of pileus of fruiting bodies produced by different shiitake strains ranged 4.750-6.917 cm and 0.833-1.250 cm, respectively. The highest diameter and thickness of pileus were recorded from Le-5, which was statistically similar to Le-6 and the lowest diameter and thickness of pileus (0.833 cm) were recorded from Le-11 (Table 2).

Table 2. Effect of different strains of shiitake mushroom on yield contributing characters (1st Flush)

Strains	Days required to primordia initiation	Days required for 1st flush	Number of effective fruiting body/spawn	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Le-2	103.5 a	111.2 a	8.67 b	4.17 cde	0.900 cd	5.667 bcd	0.933 cde
Le-4	72.33 de	80.83 cd	3.83 c	4.917 abc	1.17 abc	5.750 bcd	1.083 abc
Le-5	100.3 a	109.5 a	4.17 c	5.50 a	1.30 a	6.917 a	1.250 a
Le-6	76.00 bcd	86.17 bc	5.50 bc	5.25 ab	1.15 ab	6.250 ab	1.183 ab
Le-8	78.50 bc	85.50 bc	14.33 a	3.83 de	0.77 d	4.917 cd	0.8167 e
Le-9	78.00 bcd	86.33 bc	4.83 c	4.58a-d	0.92 cd	5.917 abc	1.017 b-e
Le-10	69.83 e	78.67 d	7.50 bc	4.33b-e	0.98 bc	5.833 bc	0.983 b-e
Le-11	74.50 cde	81.83 cd	13.50 a	3.58 e	0.75 d	4.75 d	0.833 de
Le-12	80.67 b	88.00 b	8.83 b	4.75a-d	1.00 bc	5.917 abc	1.050 a-e
CV (%)	5.71	5.14	37.06	16.68	17.28	14.53	17.21

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

Biological and economical yield and Biological efficiency: Performance of different strains, in respect of biological and economical yield of shiitake mushroom strains are presented in Fig. 1. The highest biological yield were found in Le-8 which was followed by Le-11, Le-12, Le-10 and Le-2. The lowest yield was recorded from Le-4, which was followed by Le-9, Le-5 and Le-6. The trends in biological efficiency of different strains (Fig. 2) were the same as found in biological and economic yield. The result is in line

with the report of Przybylowicz and Donoghue (1990) who found the biological efficiency of shiitake mushroom to vary between 50 and 80% for 2-5 harvests. The strain Le-4 performed very poorly.

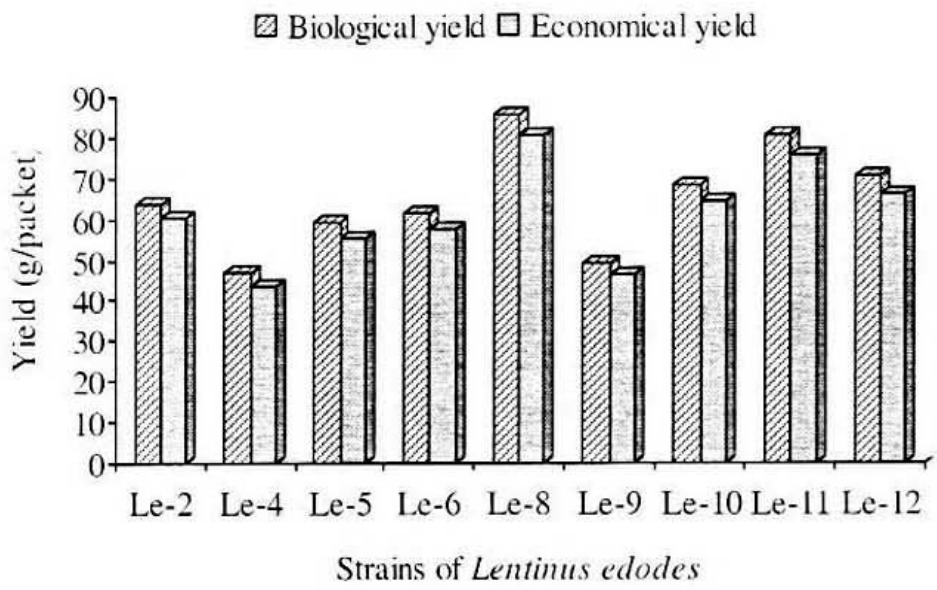

Fig. 1. Effect of different strains of *Lentinus edodes* on biological and economical yield

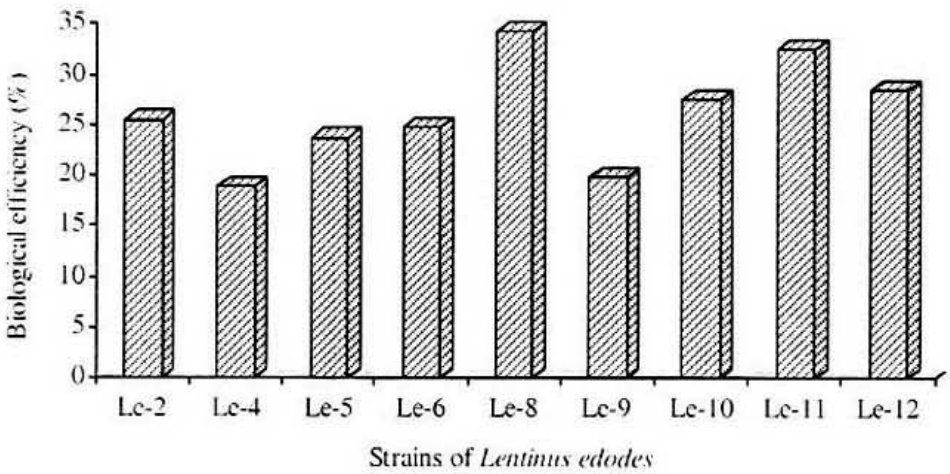

Fig. 2. Effect of different strains of *Lentinus edodes* on biological efficiency

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Antimicrobial Activity of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer Upon Human Pathogenic Bacteria

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Abstract

In vitro antimicrobial activity was examined for the aqueous extract of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer in nutrient agar (NA), Mueller-Hinton (MH) and Mc Conkey agar (MC) medium following the filter paper disc and agar well diffusion methods. The microorganisms studied include human pathogenic forms of *Klebsiella, Staphylococus, Pseudomonas, Citrobacter, Proteus, Enterobacteriaceae, Salmonella sp.* and *Escherecia coli.* The paper disc study was performed against a standard tetracycline (TE, 30 μg) disc. The result of growth inhibitory effect of the well surpassed that of the filter disc form of the test extract. The *Proteus sp* (diameter of growth inhibition zone, 28 mm) was found to be most vulnerable towards the extract in case of NA medium and the *Klebsiella sp.* the least (10 mm). In case of MH agar, the test organisms varied in their extent of susceptibility towards the extract. The extract showed its best effect towards the pathogenic microorganisms used in case of Mc Conkey agar medium. Here, the mostly affected organism was the *Proteus sp.* in both the agar well (19 mm) and the filter disc (16 mm) methods and the least affected one was the *E.coli* (0 mm).

Key words: Antimicrobial action, pathogenic microorganisms, aqueous extract, *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer.

INTRODUCTION

Antibacterial activity is the ability of a substance to inhibit or kill bacterial cells. Different types of antibiotics and chemotherapeutic agents are being used in the treatment of one form of disease or the other. Most of these antibiotics were originally derived from micro-organisms while the chemotherapeutic agents are from plants. Since humans and fungi share common microbial antagonists such as *Escherichia coli, Staphylococcus, Pseudomonas* and *Salmonella sp.*, humans can benefit from the natural defensive strategies of fungi that produce antibiotics to fight infection from microorganisms. Mushrooms, the edible fungi, being used from the Paleolithic time as a food item, have been implicated to have immune enhancing and antibiotic property (Benedict *et al.*, 1972, Collins *et al.*, 1997, Kupra *et al.*, 1979, Suzuki *et al.*, 1990).

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Some mushrooms and their active components are target-specific in their antibiotic properties, whereas others have broader effects. With an increasing number of bacteria developing resistance to commercial antibiotics, extracts and derivatives from mushrooms hold great promise for novel medicine in modern times (Chihara *et al.*, 1992, Hobbs *et al.*, 1986 and Mizuno *et al.*, 1995).

Although mushrooms have been used as a culinary item from the pre-historic time for centuries, they have been prescribed for treatment of diseases such as gastro-intestinal disorder, bleeding, high blood pressure and various types of bacterial infections (Stametes, 1993 and Brodie, 1998). In Bangladesh, mushroom, as a nutraceutical, have recently been introduced and the local populace here is embracing it due to its health-giving properties. The climatic condition of Bangladesh favors mostly the cultivation of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer and it is found here in large extent. The present research topics is bestowed to establish that the health-promoting antimicrobial effects of our native mushroom, *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer, are not just exaggeration and the healing property of it is not based only on the superstitious belief but are authentic. Therefore, this study was primarily undertaken to confirm the acclaimed antibacterial properties of *Pleurotus ostreatus*, generally known as the oyster mushroom.

MATERIALS AND METHODS

Sample Preparation and Extraction: The fruit bodies of the mushroom *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer were collected from the National Mushrooom Development and Extension Center, Department of Agricultural Extension, Ministry of Agriculture, Government of the People's Republic of Bangladesh. These were sun-dried and made into a coarse powder by grinding. 30 g of air-dried powder was added to 600 ml of distilled water followed by boiling on slow heat for 2 h. The plant residues was further purified by filtration through Whatmann No. 1 filter paper (Atata *et al.*, 2003). The filtrate was then centrifuged at 5000g for 10 min. The supernatant was collected and this procedure had been repeated twice. After 6 h, the supernatant collected at an interval of every 2 hours had been pooled together and concentrated to make the final volume one-fourth of the original volume. It was then autoclaved at 121^0C and at 15 lbs pressure and stored at 4^0C.

Sterility Proofing of the Extracts: The extract was tested for sterility after Millipore filtration by introducing 2ml of this supposed sterile extract into 10ml of sterile nutrient broth. Incubation was done at 37^0c for 24 hours. A sterile extract was indicated by absence of turbidity or clearness of the broth after the incubation period (Ronald, 1995).

Bacterial Strains: *In vitro* antimicrobial activity was examined for aqueous extract of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer. Microorganisms had been collected from Bangladesh Institute for Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM). The microorganisms studied include *Klebsiella*,

Staphylococus, Pseudomonas, Citrobacter, Proteus, Enterobacteriaceae, Salmonella and E. coli. All the microorganisms were maintained at 4^0C on nutrient agar slants.

Determination of the Minimum Inhibitory Concentration (MIC) of the Extract: The standard concentration of the extract used was 20 mg/ml. Sterile distilled water was used as the diluents. *Pleurotus ostreatus* extract with activity at this concentration was regarded as having antimicrobial property while with no activity at this concentration was disregarded.

The antimicrobial activity of the extract was determined using filter paper disc and agar well diffusion methods of Stoke and Ridgway (1980) using nutrient agar (NA), Mueller-Hinton(MH) and McConkey agar media. The sterile distilled water used in the dilution of solid mushroom extracts was used as the control. The inhibitory zone produced was measured in millimeters. A control experiment was set up by either soaking disc of filter paper with sterile distilled water or by adding drops of this diluent into the agar well on the plates. Negative results were regarded as those in which no zone of inhibition was observed. All the tests were carried out in triplicates and their means recorded. The MIC was determined according to the method of Unaeze (1987).

Determination of the Comparative Antimicrobial Effect of the Extract and a Standard Antibiotic: A standard tetracycline disc (TE, 30 μg) was used to compare the antimicrobial effect of it against that of the test extract. The concentration of the extract in case of filter disc and agar well diffusion method was 10 μg and 45μL, respectively. All the tests were carried out in triplicates and their means recorded.

RESULTS AND DISCUSSION

In case of Nutrient Agar (NA) medium: Almost all the microorganisms showed zone of growth inhibition in NA medium. The effect of standard TE disc was followed by that of the agar well and filter disc of the *Pleurotus ostreatus* extract. TE showed the most potent action against *Citrobacter sp.* (diameter of the zone of inhibition 28 mm) and the least against *Klebsiella sp.* (10 mm). This feature was also found similar in case of agar well diffusion method (6mm). However, the *Staphylococcus sp.* was found to be most susceptible towards the concentration of the extract used in well (16mm). The least inhibitory effect of the extract (0 mm) for *Klebsiella* was also found to be same in case of filter disc method of the extract. Here, the most susceptible microorganism detected was the *Proteus sp* (14 mm).

In case of Mueller-Hinton (MH) agar medium: Though most of the test organisms showed vulnerability towards the TE disc, they exhibited anomaly in case of well diffusion and filter disc methods. Here, the growth of the *Salmonella* was most affected (20 mm) by the TE whereas towards the test extract, they were insensitive (0 mm in both cases). However, the insensitivity pattern of *Klebsiella, Proteus* and *Enterobacteriaceae* towards the both forms of the test extract strictly correlated with that of TE.

Table 1. Comparative growth inhibitory effect of the *Pleurotus ostreatus* extract and Tetracycline (TE) disc on Nutrient agar medium (measured by the zone diameter, mm)

Name of Bacteria	Tetracycline (30 μgm)	Well (45 μl)	Disk (10 μgm)
Klebsiella sp.	10	6	0
Staphyococcus sp.	25	18	8
Citrobacter sp.	28	14	8.5
Proteus sp.	18	13	6.5
Pseudomonas sp.	16	14	14
Enterobacteraceae sp.	12	15	12
Salmonella sp.	19	13	10
Escherechia coli	18	11	7

Table 2. Comparative growth inhibitory effect of the *Pleurotus ostreatus* extract and Tetracycline (TE) disc on Mueller-Hinton (MH) agar medium (measured by the zone diameter, mm)

Name of Bacteria	Tetracycline (30 μgm)	Well (45 μl)	Disk (10 μgm)
Klebsiella sp.	0	0	0
Staphyococcus sp.	20	0	0
Citrobacter sp.	15	8	6
Proteus sp.	13	0	0
Pseudomonas sp.	19	10	7
Enterobacteraceae sp.	0	0	0
Salmonella sp.	14	8	0
Escherechia coli	10	11	9

In case of MacConkey agar medium: The extract of the *Pleurotus ostreatus* showed its best effect towards the pathogenic microorganisms used in case of Mc Conkey agar medium. Here, the mostly affected organism was the *Proteus* in both the agar well (19 mm) and the filter disc (16 mm) methods. However, the least affected one was the *Eschrechia coli* in case of TE and also the two types of method. Other organisms showed average inhibition against TE and the two types of method.

Table 3. Comparative growth inhibitory effect of the *Pleurotus ostreatus* extract and Tetracycline (TE) disc on MacConkey agar medium (measured by the zone diameter, mm)

Name of Bacteria	Tetracycline (30μgm)	Well (45μl)	Disk (10μgm)
Klebsiella sp.	11	12	7
Staphyococcus sp.	6	11	8
Citrobacter sp.	27	13	9
Proteus sp.	26	10	7
Pseudomonas sp.	28	19	16
Enterobacteraceae sp.	0	11	9
Salmonella sp.	30	12	8
Escherechia coli	0	10	7

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Effect of Culture Media and Environmental Factors on the Mycelial Growth of *Grifola frondosa*

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Abstract

The effects of culture media, pH, temperature, light and carbon sources on the mycelial growth of *Grifola frondosa* were investigated. The best mycelial growth (0.3375 cm/day) was observed on potato dextrose agar medium while the least growth (0.1200 cm/day) was recorded on cane juice yeast agar medium. The optimum temperature for the best mycelial growth of this mushroom was 24^0-27^0C. The highest mycelial growth (0.2775 cm/day) was recorded in pH 5.0 while it was the lowest in pH 9.0. The best mycelial growth and density of mycelium were found in absence of light (0 lux). Among the five carbon sources, fructose was the best for mycelial growth of *Grifola frondosa*.

Key words: *Grifola frondosa*, culture media, pH, temperature, light, carbon sources and mycelial growth.

INTRODUCTION

Grifola frondosa commonly known as maitake mushroom is a Basidiomycete fungus belonging to the order *Aphyllopherales* in the family *Polyporaceae* (Loncoff, 1981). It is a very effective medicinal mushroom. It has anti-tumor (Hobbs, 1996), anti-viral, blood pressure regulating (Kabir *et al.*, 1987), immuno-stimulating and antioxidant (Zhang *et al.*, 2002) activities. Mycelia of *G. frondosa* are white, longitudinally linear, eventually thick cottony on enriched agar media. The mycelial growth depends on culture media, pH, temperature, nutrient element and some environmental factors (Calam, 1971). Growth medium is the most important factor because it supplies necessary nutrient for the growth of mycelium. Mycelium production of most Basiodiomycetes is affected by the pH of culture medium and temperature. Mushroom can be grown on different carbon sources and usually, potato dextrose agar (PDA), malt extract agar (MEA), malt extract yeast agar (MEYA) and potato dextrose yeast agar (PDYA) media are used as culture media for mycelial growth of mushroom. It is very important to evaluate the factors that enhance the growth of *Grifola frondosa*. So the aim of this study was to find out the suitable culture media, nutritional requirements and environmental factors that enhance the optimum mycelial growth of the mushroom.

MATERIALS AND METHODS

This experiment was conducted in the Tissue Culture Laboratory of National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka. The inocula of *Grifola frondosa* were collected from the germplasm center of NAMDEC.

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Culture media: Eleven different culture media such as potato dextrose agar (PDA), potato dextrose yeast agar (PDYA), malt extract agar (MEA), malt extract yeast agar (MEYA), yeast extract agar (YEA), cane juice agar (CJA), Cane juice yeast agar (CJYA), wheat extract agar (WEA), wheat extract yeast agar (WEYA), Sawdust extract agar (SEA) and Corn extract agar (COEA) medium were used to determine the best medium for mycelial growth of *Grifola frondosa*. Constituents of different culture media were maintained according to Moonmoon *et al.* (2008). The basal components of each media were mixed with agar and the mixture was boiled until the agar dissolved. Chloramphenicol BP 0.5% (100 ±5µl) was aseptically added to the medium and then cooled to prevent bacterial contamination. The media was adjusted to pH 6.5 and poured into petri plates at 20 ml/plates. The media were autoclaved for 20 minutes at 121° C temperature and 1kg/cm^2 pressure and cooled at normal temperature. Then equals size portions of inocula were transferred from the culture grown on PDA to place in the centre of petri plates of each media. Then the inoculated petri dishes were incubated at 21^0 to 25° C temperature.

pH: The selected PDA medium was adjusted to 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0 pH level by adding 1N HCl or NaOH before autoclaving.

Temperature and light: To determine a suitable growth temperature the inoculated plates were incubated at bellow 8° C, 10-13° C, 17-23° C, 24-27° C, 28-31° C, and 35° C separately. In case of light effect, the inoculated plates were incubated at different light intensity viz: 0 lux (continuous dark), 40 to 60 lux, alternation of light (40-60 lux) and dark (0 lux),120 to 150 lux, alternation of light (120-150 lux) and dark (0 lux), 250 to 300 lux, alternation of light (250-300 lux) and dark (0 lux) as well as alternation of light (200-280 lux) and dark (0 lux) separately at 21^0 to 25° C.

Carbon source: Five different carbon sources such as glucose (pH 6.63), maltose (pH 6.65), sucrose (pH 6.68), fructose (pH 6.50) and lactose (pH 6.47) were used to investigate the mycelial growth of *Grifola frondosa*. Two percent of each carbon source was mixed separately with 2% agar and boiled until the agar dissolved. The media was poured into petri plates at 20 ml/plates. After sterilization and solidification, the plates were inoculated with the same size of inocula of *G. frondosa*. Then the plates were incubated at 21^0 to 25° C for mycelial growth.

Data collection and analysis: The experiment was laid out following completely randomized design (CRD) with 4 replications. Data on mycelium growth rate (cm/day) was recorded according to the method and formula described by Moonmoon *et al.*, (2008). The collected data were analyzed following Gomez and Gomez (1984) and using MSTAT-C computer program. Means were separated following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Effect of culture media: The effect of different culture media on mycelial growth is shown in Table 1. Significantly the highest mycelial growth (0.3375 cm/day) was

observed in PDA medium followed by MEA which was statistically similar to YEA and WEYA. The lowest mycelial growth (0.1200 cm/day) was recorded in CJYA. Imtiaj *et al.* (2007) reported that MYA was the best culture medium for mycelial growth of *Grifola frondosa*. Other media like malt extract yeast agar, malt extract yeast peptone agar or dog food agar have also been successfully used to grow *Grifola frondosa* (Stramets 2000). PDA has been widely reported to support mycelial growth of mushroom (Fasidi, 1996, Huang, 1993 and Oso, 1977).

Table 1. Effect of different culture media on mycelial growth of *Grifola frondosa*

Culture Media	Mycelial growth (cm/day)
Potato dextrose agar (PDA)	0.3375 a
Potato dextrose yeast agar (PDYA)	0.2075 cd
Malt extract agar (MEA)	0.2800 b
Malt extract yeast agar (MEYA)	0.1700 d
Yeast extract agar (YEA)	0.2725 b
Wheat extract agar (WEA)	0.2225 c
Wheat extract yeast agar (WEYA)	0.2775 b
Cane juice agar (CJA)	0.1925 cd
Cane juice yeast agar (CJYA)	0.1200 e
Sawdust extract agar (SEA)	0.1775 cd
Corn extract agar (COEA)	0.2075 cd
CV (%)	12.33

In a column, means bearing same letter do not differ significantly at 5% level by DMRT.

Table 2. Effect of pH of culture media on mycelial growth of *Grifola frondosa*

pH level	Mycelial growth (cm/day)
3.5	0.1200 de
4.0	0.1350 cd
4.5	0.1825 bc
5.0	0.2775 a
5.5	0.2150 b
6.0	0.1825 bc
6.5	0.1325 c-e
7.0	0.1900 b
7.5	0.1050 de
8.0	0.1350 cd
8.5	0.0950 de
9.0	0.0825 e
CV (%)	16.47

In a column, means bearing same letter do not differ significantly at 5% level by DMRT

Effect of pH: The pH ranges of 3.5 to 9.0 were evaluated to determine the suitable pH for mycelial growth of *Grifola frondosa*. The highest mycelial growth (0.2775 cm/day) was recorded at pH 5.0. This result is similar to Imtiaj *et al.* (2007) who reported that *G.*

frondosa grew well at pH 5. The lowest mycelial growth was observed at pH 9.0 which was followed by 8.5, 7.5 and 3.5. Shim *et al.* (1997) observed that the most favourable and unfavourable pH of *G. umbellate* was 4 and 9 respectively. Stamets (2000) stated that *G. frondosa* grew well at pH ranges of 5.5 to 6.8 that were nearly similar to the present findings.

Effect of temperature: The significant effect of temperature was observed on mycelial growth of *Grifola frondosa* (Table 3). The maximum mycelial growth (0.1900 cm/day) was observed at 24^0-27^0 C which was statistically similar to 17^0-23^0 C. A poor mycelial growth (0.0425 cm/day) was found at bellow 8^0 C and no mycelial growth was found at 35^0 C. The result is completely similar to Imtiaj *et al.* (2007) who reported that mycelial growth of *G. frondosa* was maximum at 25^0 C and no growth was observed at 35^0 C.

Effect of light: The effect of different light intensity on mycelial growth is shown in Table 4. The highest mycelial growth (0.2325 cm/day) was found in absence of light (0 lux) which was followed by alternation of light (120-180 lux) and dark (0 lux) as well as alternation of light (250-300lux) and dark (0 lux) condition. In absence of light (0 lux) the density of mycelium was more vigorous than other treatment. Similar findings was reported by Khandaker *et al.* (2008), who reported that absence of light gave best mycelial growth of *Pleurotus citrinopileatus.*

Table 3. Effect of temperature on mycelial growth of *Grifola frondosa*

Temperature (^{0}C)	Mycelial growth (cm/day)
Bellow 8	0.0425 c
10-13	0.0875 bc
17-23	0.1500 a
24-27	0.1900 a
28-31	0.0950 b
35	- - - -
CV (%)	22.45

In a column, means bearing same letter do not differ significantly at 5% level by DMRT

Table 4. Effect of light on mycelial growth of *Grifola frondosa*

Light	Mycelial growth rate (cm/day)	Mycelium density
0 Lux	0.2325 a	A
40- 60 Lux	0.1050 d	D
Alternation of light (40-60 lux) and dark (0 lux)	0.1700 bc	C-D
120-180 Lux	0.1300 cd	C
Alternation of light (120-180 lux) and dark (0 lux)	0.2050 ab	D
250-300 Lux	0.1400 cd	D
Alternation of light (250-300 lux) and dark (0 lux)	0.2125 ab	D
CV (%)	13.74	

In a column, means bearing same letter do not differ significantly at 5% level by DMRT.
A, B, C and D indicate mycelial densities: where, A > B > C > D.

Effect of carbon source: The highest mycelial growth (0.1475 cm/day) was found in fructose which was followed by maltose, glucose and lactose. Imtiaj *et al.* (2007) observed that xylose was the most suitable carbon source for mycelial propagation of *G. frondosa*. Shim *et al.* (1997) reported that *G. umbellate* responded to maximum carbon sources except salicin, cellobiose and lactose.

Table 5. Effect of carbon source on mycelial growth of *Grifola frondosa*

Carbon source	Carbon (%)	Mycelial growth (cm/day)
Glucose	40.0	0.1050 ab
Maltose	42.1	0.1075 ab
Sucrose	42.1	0.0900 b
Fructose	40.0	0.1475 a
Lactose	42.1	0.1000 ab
CV (%)		22.08

In a column, means bearing same letter do not differ significantly at 5% level by DMRT.

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Investigation on the Nutritional Composition of Common Edible and Medicinal Mushrooms Cultivated in Bangladesh

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Abstract

Several species other than oyster mushrooms are being cultivated in Bangladesh. The nutrient contents of eleven edible and medicinal mushroom species were investigated and a significant variation was observed among the species. The total protein, total carbohydrate, total lipid, crude fiber and total mineral (ash) content of each mushroom was studied on dry weight basis. The protein, carbohydrate, total lipid, crude fiber, and total mineral contents were ranged from 18g to 38g, 9g to 50g; 1g to 12g; 8g to 52g and 5g to 12g per 100g of the mushroom species. Also these mushrooms contain some important minerals like, Ca, Fe, Zn, P and Mg among which the content of P was highest. The metabolizable energy content of these mushroom species was also calculated and it was found that the energy content of these mushroom ranged from 150Kcal/100kg to 300 Kcal/100kg of mushroom. This study suggests that these mushrooms may be used as low energy, healthy food stuff with protein supplementing properties.

Key words: Mushroom, protein, lipid, fiber, carbohydrates and minerals.

INTRODUCTION

Mushroom is being widely used as food and food supplements from ancient times. They are increasingly being recognized as one of the important food items for their significant roles in human health, nutrition and diseases (Chang, 1996a). Although the history of mushroom cultivation is very recent in Bangladesh but mushroom consumption is being increase rapidly in this country. In Bangladesh, at present oyster mushrooms (*Pleurotus spp.*) are mainly cultivated for their growing suitability nutritional and medicinal importance (Hossain *et al.*, 2003, Khan *et al.*, 2008a and Alam *et al.*, 2009) and suitability in Bangladeshi condition (Amin *et al.*, 2007), mushrooms of other species are also being initiated to cultivate. Some of these are popular worldwide for their nutritional and medicinal importance. For example, shiitake mushroom (*Lentinus edodes*) is very much popular in all over the world for their nutritional value, taste and medicinal importance (Wasser, 2005). This mushroom has anti-tumor, antihypertensive, hypocholesterolemic and antibacterial activities (Chihara, 1970, Kabir *et al.*, 1987, Hirasawa, 1999 and Kim *et al.*, 1999). Reishi mushroom (*Ganoderma lucidum*) is the most popular medicinal mushroom. It is known as the 'mushroom of immortality' or 'longevity mushroom' (Kaul, 2001). The beta-glucan polysaccharide of this mushroom has potential application

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in immune surveillance and chemoprevention of cancer (Chang, 1996b). *Ganoderma lucidum* has anti microbial and anti-HIV effects (Yoon, 1994 and Kim, *et al.*, 1994). The hepatic and renal protective effects of *G. lucidum* in mice were also evaluated (Shieh, *et al.*, 2001). Shaggy mushroom (*Coprinus comatus*) is anti-cancerous (Wu, *et al.*, 2003), hypoglycemic (Bailey, *et al.*, 1984) and antioxidant (Badalyan, *et al.*, 2003). Button mushroom (*Agaricus bisporous*) is the most popular and mostly consumed all over the world for their edibility, taste and medicinal importance (Kaul, 2001 and Chang & Miles, 1989). Straw mushrooms (*Volveriela volvacea*), ear mushroom (*Auricularia spp.*) are also important both nutritionally and medicinally (Kaul, 2001 and Chang & Miles, 1989). These medicinal values of these mushrooms are due to their nutritional or chemical composition. However nutritional composition is affected by many factors including differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting, specific portion of the fruiting bodies used for analysis, time interval between harvest and measurement methods ((Kaul, 2001, Chang & Miles, 1989 and Khan *et al.*, 2008b). Although many scientific researches have been conducted to determine the nutritional composition of different mushrooms in different culture conditions, it should require further research works to investigate the nutritional composition of mushrooms cultivated in Bangladesh, which are important both nutritionally and medicinally. The objective of this work was to evaluate the nutritional composition of popular mushrooms which are newly cultivated in Bangladesh to increase the awareness of people about the significance of this important food item.

MATERIALS AND METHODS

Eleven mushroom species such as *Agaricus bisporous, Pleurotus citrinopleatus, Pleurotus eryngii, Coprinus. comatus, Hypsizygous ulmarius, Agrocybe aegerita, Auricularia polytricha, Volveriella volvacea, Lentinus edodes, Ganoderma lucidum* and *Coriolus versicolor* were selected in this study for investigation. Mushroom was cultivated on saw dust (and compost, in case of button mushroom) fruiting bodies of these mushrooms were harvested and taken for nutritional analysis. Fresh mushroom was taken for the determination of moisture and then mushroom was dried for the estimation of total protein, total lipid, crude fiber, total mineral (ash) and total carbohydrates.

Moisture determination: Moisture of fresh mushrooms were determined by conventional method using automatic moisture analyzer (Manufactured by Japan).

Determination of total protein: Five gram of grinded mushroom was taken with 50ml of 0.1N NaOH and boiled for 30 minutes. The solution was cooled in room temperature and centrifuged at 1000rpm by a table centrifuge machine (*DIGISYSTEM: DSC-200T; Taiwan*). The supernatant was collected and total protein content was measured according to the Biuret method (Raghuramulu *et al.*, 2003).

Determination of total lipid: Total lipid was determined by slight modified method of Folch *et al.*, 1957. Five gram of grinded mushroom was suspended in 50ml of chloroform:methanol (2:1 v/v) mixture then mixed thoroughly and let stand for 3 days.

The solution was filtrated and centrifuged at 1000 rpm. The upper methanol layer was collected by Pasteur pipette and the crude lipid extract was determined by evaporating the chloroform.

Determination of crude fiber: Moisture and fat free sample was treated with 0.255N H_2SO_4 and 0.313N NaOH and washed with ethanol and ether. It was then transferred to a crucible, dried overnight at 80-100^{0}C and weighed (W_1) in an analytical balance. The crucible was heated in a muffle furnace at 600^{0}C for 6 hours, cooled and weighed again (W_2). The difference in the weights (W_1-W_2) represents the weight of crude fiber.

$$\text{Crude fiber (g/100g)} = \frac{[100-(\text{moisture} + \text{fat})] \times (W_1-W_2)}{\text{Weight of mushroom (dried)}}$$

Determination of total ash: One gram of the sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 6 hours at 600^{0}C. It was then cooled in a desiccators and weighed. Then total ash was calculated as following equation:

$$\text{Ash content (g/100g)} = \frac{\text{Weight of ash} \times 100}{\text{Weight of dried sample taken}}$$

Determination of total carbohydrate: The content of the available carbohydrate was determined by the following equation.

Total Carbohydrate (g/100g sample) =
[100 – (Total Lipid + Total Protein + Ash + Crude Fiber)]

Determination of mineral contents: Total ash was taken for the analysis of mineral contents. 2ml of concentrate HNO_3 was added to the ash and heated for 2 minutes. One drop of hydrogen peroxide was added into the solution to remove turbidity. The solution was then transferred into a volumetric flask and total volume was made 50ml by adding deionized water. This was then used to analyze the contents of calcium (Ca), iron (Fe), phosphorus (P), magnesium (Mg) and zinc (Zn) by flame photometry with atomic absorption spectrophotometer (*AAS 249, Varian*).

Determination of metabolizable energy content: Metabolizable energy was calculated as the following formula:

ME (Kcal /100g) = [(3.5 X CP) + (8.5 X CF) + (3.5 X NFE)] (Chang and Miles, 1989).
Where, ME = Metabolic Energy; CP = % Crude Protein; CF = % Crude Fat; NFE = % Nitrogen Free Extract (carbohydrate)

Statistical analysis: Statistical analysis was done by following method used by Gomes and Gomes, 1984. Computer package program, MS Excel and SPSS were also used for determination of analysis of variemel and means were separated and ranked by DMRI.

RESULTS AND DISCUSSION

The nutrient contents of the tested mushroom spp. were given in Table 1. Moisture content of most of the test mushrooms ranged from 85 to 91%, whereas, it was 78%, 70% and 58% for *Lentinus edodes*. *Ganoderma lucidum* and *Coriolus versicolor* relatively. According to Kurtzman (1997), the moisture content of most of the edible mushrooms ranges from 85-94% but Chang and Miles (1989) reported the moisture content ranged from 70-94% and for tough edible mushroom, 50-75%. So, the present findings were fully in agreement with the reported values.

Table1. Nutrient contents of different species of mushrooms

Mushroom Spp	Moisture (%)	Dry weight basis (g/100g of mushroom)				
		Protein	Lipid	Carbo-hydrate	Fiber	Ash
A.bisporous	89.4±1.2^{a}	37.6±2.1^{a}	10.0±0.5^{a}	23.6±2.4^{c}	21.2±1.4^{d}	7.8±0.6^{b}
P.citrinopleatus	88.0±1.9^{a}	30.3±1.3^{b}	3.4±0.5^{c,d}	33.1±3.7^{b}	22.2±1.9^{d}	11.0±0.8^{a}
P. eryngii	85.0±3.4^{b}	23.5±2.2^{c}	11.9±0.8^{a}	29.9±1.9^{b}	25.8±2.1^{c,d}	8.9±0.7^{a,b}
C. comatus	90.5±0.9^{a}	24.2±1.4^{c}	7.3±0.4^{b}	32.1±2.8^{b}	27.9±1.8^{c}	8.5±1.1^{a,b}
H. ulmarius	87.1±2.1^{a,b}	31.3±2.2^{b}	1.0±0.2^{e}	49.9±4.5^{a}	8.6±1.1^{e}	9.2±0.8^{a,b}
A. aegerita	91.4±1.1^{a}	27.6±2.0^{b,c}	5.4±0.8^{b,c}	28.7±1.8^{b,c}	26.7±2.3^{c,d}	11.6±1.1^{a}
A. polytricha	85.0±2.7^{b}	18.3±1.9^{d}	2.0±0.6^{d,e}	18.9±2.2^{d}	50.0±3.4^{a}	10.8±0.7^{a,b}
V. volvacea	89.1±0.8^{a}	20.0±1.8^{c,d}	10.8±1.3^{a}	42.0±1.0^{a,b}	15.2±4.7^{d,e}	12.0±1.0^{a}
L. edodes	78.5±2.1^{c}	27.0±1.6^{b,c}	7.2±1.1^{b}	21.7±2.1^{c,d}	38.3±1.4^{b}	5.8±0.6^{b}
G. lucidum	70.2±2.2^{d}	26.4±2.9^{b,c}	2.7±0.4^{d,e}	12.8±1.1^{e}	51.5±4.5^{a}	6.5±0.8^{b}
C. versicolor	58.2±4.5^{e}	26.0±3.1^{b,c}	3.7±0.4^{c,d}	9.2±1.4^{e}	50.1±2.1^{a}	11.0±0.3^{a}

Results are mean±SEM (n =5). Values in a column with different superscript are significantly different at P≤0.05.

Means were ranked but not separated by list significant value (LSD), so, LSD value must be calculed and documeted in the table.

The total protein, total lipid, carbohydrate, crude fiber and ash content in *Agaricus bisporus* were found 37.6g, 10g, 23.6g, 21.2g and 7.8g per 100g of dried mushroom respectively. This result is nearly similar to the reported value of Chang and Miles (1989), however they reported less fiber content (12%). This variable may be due to compost composition.

In *Pleurotus citrinopleatus*, the total protein, total lipid, carbohydrate, crude fiber and ash content was found 30.3g, 3.4g, 33.1g, 22.1g and 11g respectively; in *Pleurotus eryngii*, 23.5g, 11.9g, 29.9g, 25.8g and 8.9g per 100g of dried mushroom respectively. These results were very much similar to the nutritional composition of other *Pleurotus* mushrooms (Chang & Miles, 1989 and Khan *et al.*, 2008b). In 100g of dried *Coprinus*

comatus, the total protein, total lipid, carbohydrate, crude fiber and ash content was found 24.2g, 7.3g, 32.1g, 27.9g and 8.5g respectively.

The total protein, total lipid, carbohydrate, crude fiber and ash content in *Hypsizygous ulmarius*, was found 31.1g, 1.0g, 49.9g, 8.6g and 9.2g; in *Agrocybe aegerita*, 27.6g, 5.4g, 28.7g, 26.7g and 11.6g; in *Auricularia polytricha*, 18.3g, 2.0g, 18.9g, 50g and 10.8g per 100g of dried mushroom respectively. These reports suggest that *Hypsizygous ulmarius* is a protein rich mushroom like *Agaricus bisporus* and *Auricularia polytricha* is fiber rich but poor in protein content.

In 100g of dried *Volveriella volvacea*, the total protein, total lipid, carbohydrate, crude fiber and ash content was found 20g, 10.8g, 42g, 15.2g and 10.8g respectively. These results were nearly similar to the findings of Chang and Miles (1989), but protein content is less in this study, however similar to the report of Belewu and Belewu (2005).

In *Ganoderma lucidum*, the total protein, total lipid, carbohydrate, crude fiber and ash content was found 26.4g, 2.7g, 12.8g, 51.5g and 6.5g per 100g of dried mushroom respectively. The total protein, total lipid, carbohydrate, crude fiber and ash content in *Coriolus versicolor* was found 26g, 3.7g, 9.2g, 50.1g and 11g per 100g of dried mushroom respectively. According to Chang and Miles, 1989 and Kaul, 2001 the high fiber or polysaccharide content of these mushrooms might be responsible for their several medicinal importances.

The contents of some important minerals are given in Table 2. All the varieties of mushroom, tested here contain considerable amount of minerals, among which phosphorus (P) [650-1750 mg/100g] content is much higher than calcium (Ca) [15-60mg/100g, however it was found 275mg in *C. versicolor*], iron (Fe) [15-70mg/100g], zinc (Zn) and magnesium (Mg) [10-30mg/100g]. All the data presented in her are dry weight basis. Findings of this study is very much similar to the data reported by (Kaul, 2001 and Chang & Miles, 1989). They reported that about 70% of minerals in mushrooms are phosphorus, potassium (K) and sodium (Na), however, in this study determination of Na and K was not possible.

The metabolizable energy content of each of the 11 mushroom species were shown in Fig. 1, which ranges from 150Kcal/ 100kg to 300 Kcal/ 100kg of mushroom (dry weight basis). *A. bisporous*, *P. eryngii* and *H. ulmarius* mushroom spp. with higher metabolizable energy content, whereas *A. polytricha*, *G. lucidum* and *C. versicolor* contain lower metabolizable energy. Energy content of other mushrooms fall between these groups and these values were statistically significant.

From the present finding it was concluded that the test mushrooms were protein and fiber rich with low fat content. The ash (total minerals) content was also considerable and the carbohydrate content was less than other food from plant origin. These nutrient contents made mushroom as a low energy, healthy foodstuff and these mushroom may also be used as protein supplementary diet.

Table 2. Mineral content of mushroom (mg/100g)

Mushroom	Fe	Ca	Zn	Mg	P
A.bisporous	18.5	44	24.6	31.4	1289
P.citrinopleatus	29.9	21.5	28.8	23	1543
P. eryngii	37.1	17.5	23	17.7	1602
C. comatus	69	16	23.1	16	983
H. ulmarius	25.7	23.5	27.9	18.9	1045
A. aegerita	27.3	16.5	26.1	22.6	1432
A. polytricha	29.3	55	13.2	26	1281
V. volvacea	49.2	23	26.4	15	1740
L. edodes	18	22	23.4	17	875
G. lucidum	53.5	27.5	14.7	16.8	685
C. versicolor	15.4	275.3	11.1	14	1007

Results are mean (n =3).

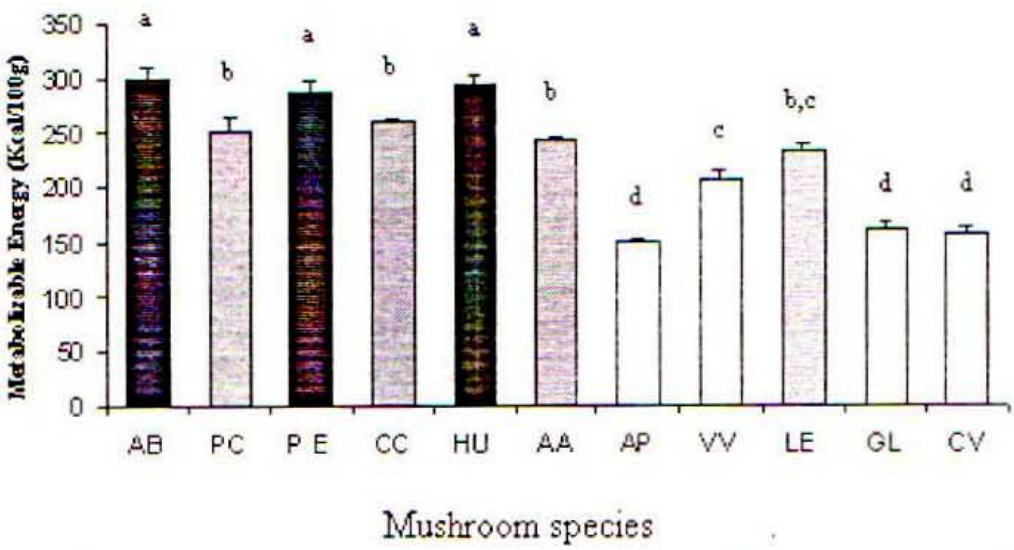

Fig. 1. Metabolizable energy content (Kcal/100g) of mushroom. Results shown in bar diagram are mean±SEM (n=5). Bars with different superscript are significantly different at P≤0.05. AB: *A.bisporous*, PC: *P.citrinopleatus*, PE: *P. eryngii*, CC: *C. comatus*, HU: *H. ulmarius*, AA: *A. aegerita*, AP: *A. polytricha*, VV: *V. volvacea*, LE: *L. edodes*, GL: *G. lucidum*, CV: *C. versicolor*.

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Neuropharmacologic study of *Agaricus bisporus* (Lange) Singer

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Abstract

Neuropharmacological effect of water extract of *Agaricus bisporus* (Button mushroom) was studied using laboratory mice. Spontaneous motor activity, hole cross test, open field test, elevated plus maze test and infrared thermometry test were carried out. Treatment with water extract of *Agaricus bisporus* showed somewhat depressing feature but this depressing effect was not statistically significant.

Key words: Neuropharmacological effect, *Agaricus bisporus.*

INTRODUCTION

Agaricus bisporus (Lange) Singer, commonly known as the button mushroom, is the most popular mushroom and contributes to about 40% of the global mushroom production. The predominating interest of the world populace towards it is due especially to its appealing texture, good taste and nutrient content. Although mushrooms have been used as a culinary item from the pre-historic time for centuries, they have been implicated in traditional medicine having anti-inflammatory, analgesic, homeostatic, diuretic, antibiotic and antitumor effects (Stametes, 1993, Koyama *et al.*, 1997, Brodie, 1998). Investigations into the effects of *Agaricus sp.*onto the autonomic and central nervous system have pointed towards mild sedative to depressing and delirium effect as well as the ameliorating action upon Alzheimer's disease (Eugester, 1967). The active components involved in these processes have been supposed to be β-D-glucan (Ukawa *et al.*, 2000). The present experiment has been aimed at determining the neuropharmacologic effect of the water extract of *Agaricus bisporus* (Button mushroom) following some established experimental models.

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MATERIALS AND METHODS

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

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

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

Spontaneous Motor Activity Test (Locomotor activity test): For this experiment, the sand displacement method of Siegmund and Wolf (1952) was employed. The amount of displaced sand particles (of a particular size), through the wire netting, due to the spontaneous motor activity of the animals, was recorded with 5 minutes interval for a period of 1 hour, and, 15 minutes interval after 1 hour to 6 hour.

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

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

Elevated Plus Maze Test: A modified version of the procedure described by Lister *et al.* (1987) had been used. The maze consisted of two open arms (30 x 5 x 0.5 cm) and two closed arms (30 x 5 x 15 cm) with an open roof, arranged so that two pairs of identical arms were opposite to each other. Arms emerged from a central platform (5 x 5 cm), and the entire apparatus was raised to a height of 50 cm above the floor level. The maze was

constructed from black Plexi glass. Button extract had been administered to the mice and placed individually in the center of the maze, facing one of the open arms. The number of entries into both the open or closed arms and the amount of time spent in the open arms was recorded. Each test lasted for 5 min and each mouse was tested only once. The apparatus was cleaned between each test. The water extract was administered orally 30 min before the test in a volume of 10 ml/kg body weight. All tests were conducted between 08:00 and 14:00.

Infrared Thermometry: The back skin temperature of each mouse was measured using a standard infrared thermometer. Back temperature had been found to be close to and consistent with rectal temperature and the obtained temperatures at these sites were almost constant. These results of back skin temperature obtained using a convenient and non-invasive infrared thermometer were safer and less stressful to the animal subjects, compared to standard rectal temperature measurements (Saegusa *et al.*, 2003).

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

RESULTS AND DISCUSSION

Spontaneous Motor Activity Test: Testing experimental drug using intact animal is considered to be the best method for investigating the action of drug on central nervous system. A common technique, employed by the ethologists in evaluating CNS effects of a sample is by observing its effect on the spontaneous motor activity of animals. In its broadest sense, motor activity refers to the whole repertoire of unconditioned behavior and in its narrowest sense, it refers to the whole body locomotor activity, such as running or walking. The experimental data is presented in Fig. 1. It was observed that the administration of extract increased motor activity for a period of 21-25 min after administration. However after that animal receiving extract did become more sedate and displaced fewer sand particles.

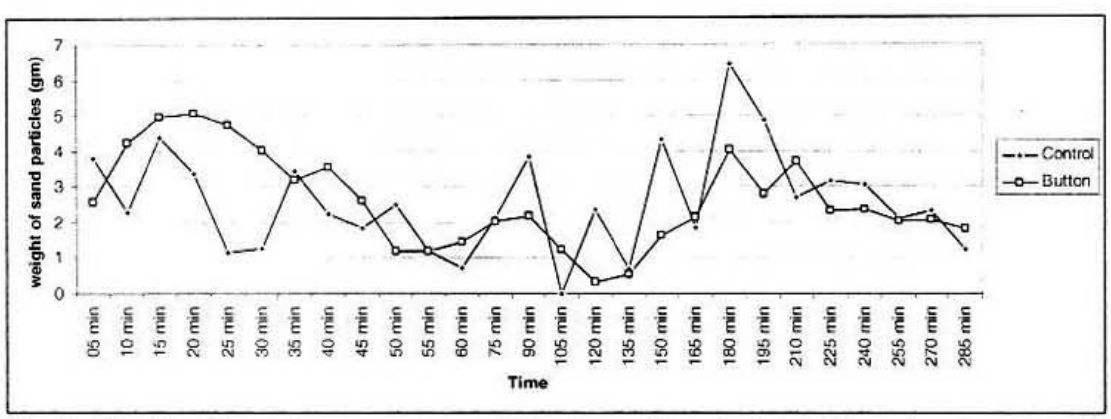

Fig. 1. Results of spontaneous motor activity test, after administration of water extract of *Agaricus bisporus* (20 ml/kg body weight)

Hole Cross: The next test performed was hole cross test. This test was designed to evaluate effects on the exploratory behavior. In order to further investigate the effects of the drug on the exploratory behavior of the treated animals, this test was performed. Result of this test are given in Fig. 2 indicating that administration of test extract reduced the exploratory activity of the treated animals and the animals showed less interest in crossing the hole, in comparison to that of the control animals.

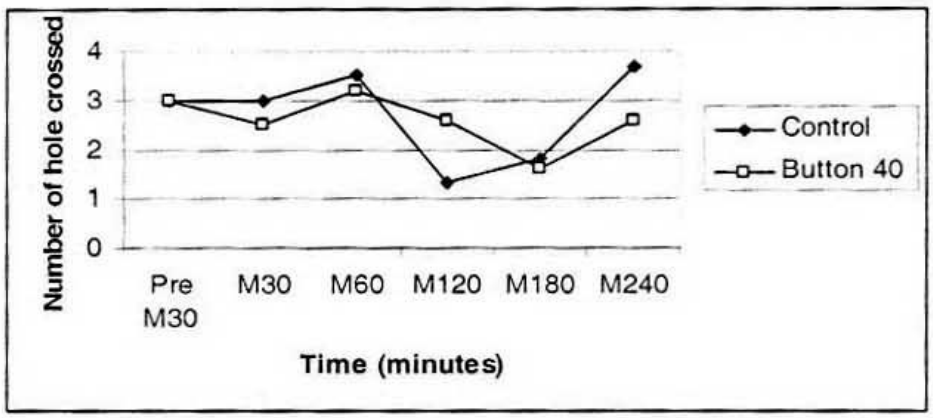

Fig. 2. Graphical Presentation on hole cross test after administration of water extract of *Agaricus bisporus* (40 ml/ kg body wt)

Open Field Test: It has been experimentally proven that, in the absence of a special task to perform, the behavior of a given animal tends to maintain the inner activation level which is, at times, inconsistent with the actual level of activation of the animals. In order to get as accurate a picture as possible, on the effect of the drug on exploration, the open field test was performed. Test sample treated animal showed reduced standing up tendency in the open field test. However the effect was not significant (Fig. 3). There was no significant change in the extract treated group with control group in the dose of 20 ml and 40 ml per kg body weight. Water extract of *Agaricus bisporus* treated group showed reduced tendency of emotional defecation and movement as indicated by the result presented in the Fig. 4 and Fig. 5 respectively.

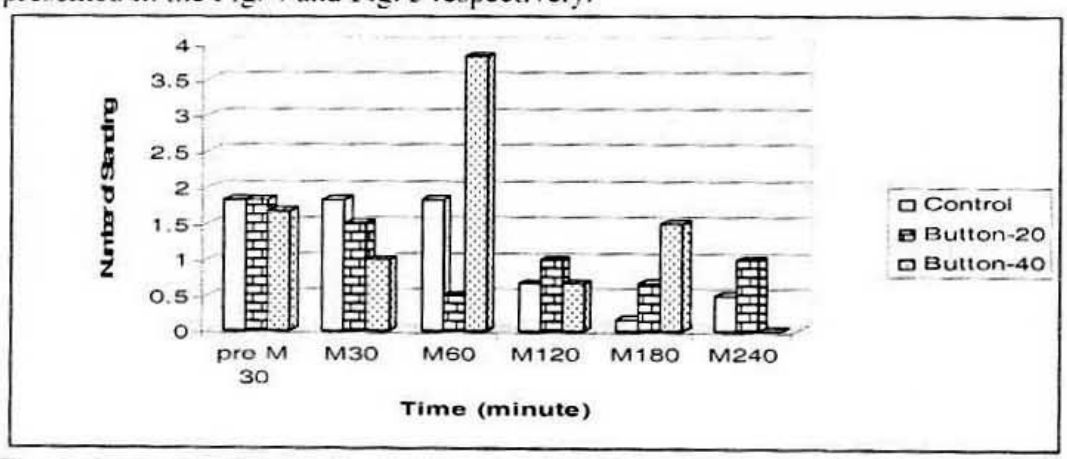

Fig. 3. Graphical presentation on open field test (standing tendency) after administration of water extract of *Agaricus bisporus*

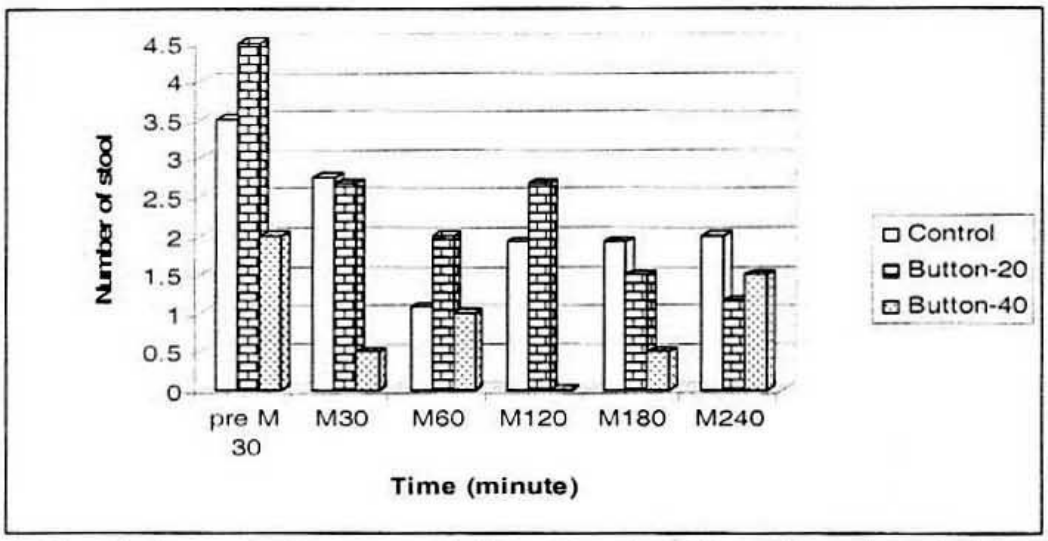

Fig. 4. Graphical presentation on open field test (emotional defecation) after administration of water extract of *Agaricus bisporus*

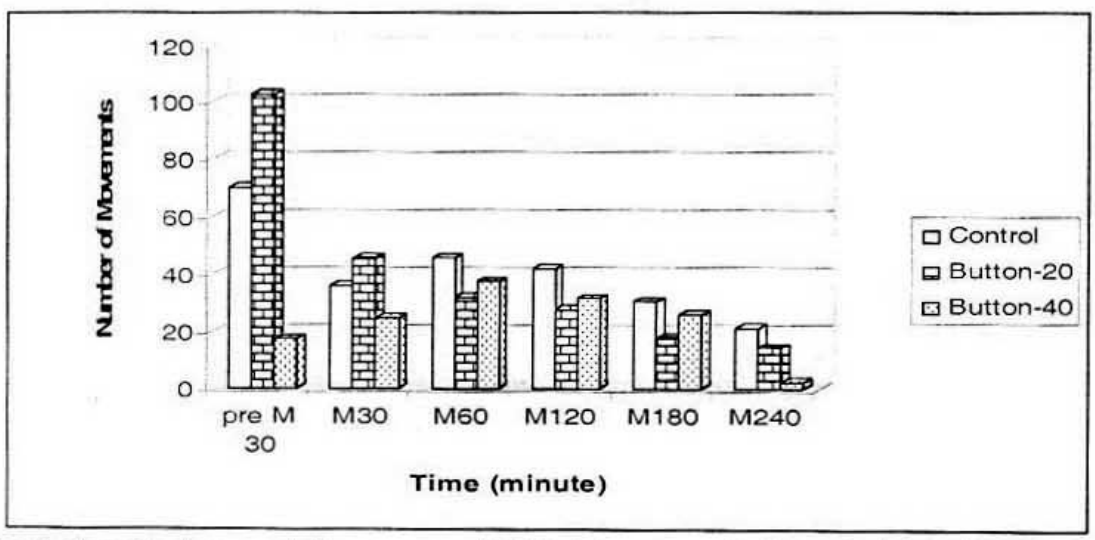

Fig. 5. Graphical presentation on open field test (movement) after administration of water extract of *Agaricus bisporus*

Elevated Plus Maze: The role of water extract of *Agaricus bisporus* in anxiety was also investigated. For this the elevated plus maze test was utilized, a classical animal model considered to be the most popular test to search for new benzodiazepine like anxiolytic agents (Pellow *et al.*, 1985. Rodgers *et al.*, 1997). The results showed (Table 1) that the animals treated with *Agaricus bisporus* water extract at a dose of 40 ml/kg body weight decreased the number of entries in the open arms and the time of permanence, suggesting an increase of the natural animal aversion to the open arms, indicating an anxiogenic effect.

Table 1. Results of elevated plus maze test after administration of water extract of *Agaricus bisporus*

Type	Body wt	Preliminary Min:-30	Hr 1	Hr 2	hr 3	hr 4
Control (n=6)	27.1±4.83	3.167±1.17	1.167±0.40	1.167±0.65	2.00±0.77	2.33±0.67
Button 40 ml/kg bwt female (n=4)	30.2±0.88	2.00±0.91	1.25±0.75	2.00±1.15	4.00±2.16	1.50±0.645
t/p	-0.510 /0.624	0.717 /0.494	-0.107 /0.917	-0.680 /0.516	-1.019 /0.338	0.853 /0.419
95%confidence interval	-17.179 to 10.919	-2.585 to 4.919	-1.874 to 1.708	-3.659 to 1.992	-6.527 to 2.527	-1.420 to 3.087

Infrared Thermometry: The classic CNS depressants and/or tranquillizers have got the ability to produce a state of hypothermia. The viability of the *Agaricus bisporus* water extract in producing a state of hypothermia was experimented in the back skin infrared thermometry test. The results presented in Table 2 indicate that the test sample, *Agaricus* bisporus (40 ml/kg body wt), does not produce discernible hypothermia in treated mice in comparison with both the control and theophylline (10 mg/ kg body wt) group.

Table 2. Results of IR Thermometry test after administration of water extract of *Agaricus bisporus* (40 ml/kg body wt)

Type	Body wt	Preliminary Min30	M30	M60	M120	M180	M240
Control n=10	24.36 ± 0.397	93.82 ± 0.301	94.29 ± 0.32	94.15± 0.26	93.75 ± 0.436	94.23 ± 0.33	92.69 ± 0.42
Button (40 ml/kg body wt) n=10	25.44 ± 0.33	93.16 ± 0.298	94.63 ± 0.199	94.90 ± 0.278	93.55 ± 0.454	94.09 ± 0.30	92.80 ± 0.34
Theophylline (10mg/kg body wt) n=10	24.94 ± 0.33	93.84 ± 0.345	94.96 ± 0.29	95.10± 0.270	94.57 ± 0.36	94.51 ± 0.34	94.58 ± 0.22
t/p value							
Control vs Button	-2.079/ 0.052	1.558 / 0.137	-0.896/ 0.382	-1.962/ 0.065	0.317/ 0.755	0.312/ 0.759	-0.202/ 0.842
Control vs Theophylline	-1.122/ 0.277	-0.044/ 0.966	-1.541/ 0.141	-2.507/ 0.022	-1.441/ 0.167	-0.587/ 0.565	-3.946/ 0.001
Button vs Theophylline	1.063/ 0.302	-1.498/ 0.154	-0.934/ 0.362	-0.513/ 0.614	-1.750/ 0.097	-0.914/ 0.373	-4.375/ 0.000
95% confidence level interval							
Control vs Button	-2.171 to 0.0114	-0.230 to 0.550	-1.137 to 0.457	-1.553 to 0.0529	-1.124 to 1.524	-0.803 to 1.083	-1.255 to 1.035
Control vs Theophylline	-1.666 to 0.506	-0.982 to 0.942	-1.58 to 0.243	-1.746 to -0.154	-2.015 to 0.375	-1.282 to 0.723	-2.896 to -0.884

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Cultivation of Oyster Mushroom (*Pleurotus ostreatus* (Jacquin ex Fr.) Kummer) in a Fully Automated Controlled Agricultural Environment

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Abstract

A fully automated, controlled agricultural environment (CAE) has been developed as part of an integrated waste management system with value-added product generation. Gourmet mushrooms have been cultivated to determine and set up optimum cultivation techniques essential for a steady supply of quality mushrooms, based on which future marketing strategies could be formulated. Reproducible and quantitative results have been obtained for oyster mushrooms grown in three types of substrate media using coffee waste, yard waste and sawdust. Results have been compared with oyster mushrooms produced by a commercial farm in Malaysia. Mycelium run period has been significantly shortened; and both economic yield and biological efficiency (BE) have been increased for the various substrates placed in the controlled agricultural environment. Maximum yield was obtained from substrate made from yard waste giving a biological efficiency of about 120% as opposed to 72% BE obtained from the indoor cultivation by the commercial farm.

Key words: Controlled agricultural environment, oyster mushroom, food waste, agricultural waste.

INTRODUCTION

Mushrooms have long been used to fight disease and provide nutrient-rich diet for human beings. During the last 32 years or so, worldwide mushroom production has increased about 18 fold (Chang, 1999). Oyster mushroom production, in particular, has literally exploded in the international market. Increasing trends in mushroom production as well as consumer demand for exotic and speciality mushrooms (such as oyster, shitake, matsutake, morel, etc.) worldwide point towards a diverse and growing international mushroom market. One of the major challenges faced by the mushroom industry in many countries lies in producing consistently high quality speciality mushrooms to meet consumers' demands. Mushrooms fruit in response to unique sets of conditions involving nutrition (substrate), temperature, pH, relative humidity, light and carbon dioxide (Stamets and Chilton, 1983). Growing parameters for mushrooms vary with every species and optimizing the environmental ranges for each stage in the mushroom's life cycle is crucial for a cultivator in order to maximize fruitbody production in a precise and deliberate fashion ensuring the desired quality. In addition, food safety is another major concern with today's consumers who want and expect food that is not laden with harmful chemicals.

An effective way to address the above concerns is through controlled agricultural environments (CAE) for the commercial cultivation of gourmet and medicinal

mushrooms. In CAE, customized growth chambers are designed around specific mushroom species and local climatic and environmental factors for high quality mushroom cultivation. CAE also allows safe, biological control over insects and disease thereby reducing and eliminating the need for harmful chemicals. CAEs, however, have certain drawbacks in terms of its construction and maintenance costs as well as high energy demand for heating and cooling.

A market study has revealed that there is an ever-increasing demand for gourmet and medicinal mushrooms in the local as well as international market. In the context of Singapore, there are only two mushroom farms that are currently in operation producing mainly shiitake, oyster and portobello mushrooms. In order to meet greater consumer demand, significant amounts of oyster mushrooms are imported mainly from Malaysia, primarily due to its geographical proximity and the mushrooms' limited shelf life. Cultivating these and other exotic gourmet mushrooms locally in a cost-effective way will provide better control over growing conditions and product efficacy, and at the same time open the option of making fresh mushrooms available to the local market at a similar or even lower price. In addition, the entire project can be integrated into Singapore's waste recycling and waste management plan over the future years.

The current project was, thus, undertaken to set up a mushroom-growing (and marketing) facility to meet the increasing demand of gourmet crop in Singapore. The aim was to see whether locally-grown produce could compete with imported goods in terms of cost, taste and quality. Oyster mushrooms were chosen for the project, mainly because of their popularity, versatility and reasonable market price. Oyster mushrooms, just like any other mushroom, require specific temperature, humidity, lighting and air circulating conditions that change throughout their production cycle. All of these would need to be incorporated into the design and construction of the fully-automated controlled environment/ growth chambers.

MATERIALS AND METHODS

According to the scope of this R&D project, a 40-feet metal container was chosen to house the controlled agricultural environment (CAE) where temperature, humidity, light, and air circulation would be monitored and maintained via an automated control system. Fig. 1 shows the floor plan and elevation of the CAE container indicating the basic system layouts. The system is divided into three separate environmental areas, the control room (room 1 in Fig. 1), spawn room (room 2), and the fruiting/grow room (room 3). For most commercial CAEs designed to produce mushrooms, an inoculation room is included in the basic layout wherein the growing media are treated/sterilized, inoculated with the mushroom spawn, and then bagged. For the present study however, inoculation was conducted outside the CAE unit. One of our in-campus laboratories, equipped with autoclaving machine and laminar flow hood, was utilized for media sterilization and spawn inoculation. The inoculated bags were then moved to the spawn room of the CAE unit where the mycelium continues to run through the substrate medium under conditions of total darkness or minimal lighting. Temperature and humidity parameters required in

the spawn room to facilitate the vegetative growth of the specific mycelium and the consequent colonization of the grow bags were controlled by an air conditioner and an overhead sprinkler system, respectively. Once the grow bags were colonized, they were moved into the adjacent grow room to complete their cycle. Temperature, humidity, light and carbon dioxide levels can be quite different in the grow room depending on the same or different species cultivated. These environmental parameters in the grow room were adjusted to initiate "pinhead" and consequent fruitbody formations, allowing up to six harvests from the bags before they were removed and replaced with fresh grow bags. Again, air conditioners and a high pressure, atomizing mist system were used in conjunction with a programmable logic controller (PLC) to maintain the desired humidity in the grow room, which is discussed in the following paragraphs.

Automatic control is essential to maintain a reasonable environment in the CAE unit. On a summer or winter day with varying amounts of sunlight and clouds, the temperature and/or humidity can fluctuate considerably; close supervision would be required for an otherwise system (such as the one adopted by the Malaysian mushroom growing farm that served as the "benchmark" for the work considered herein) that operates on manual controls to maintain the environmental parameters. The automated control system developed herein consists of the following major components:

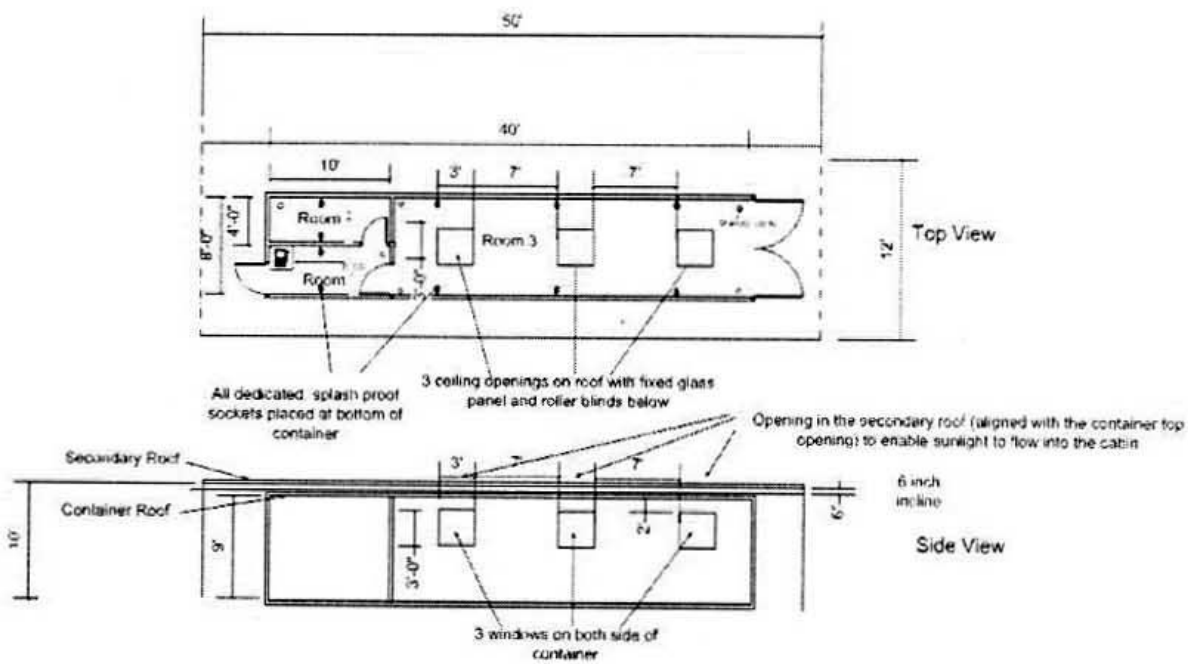


Fig. 1. Basic system layouts of the customized CAE

Split air conditioner (AC) with three units: One unit was mounted to serve the spawn room while the two remaining units were used in the grow room for controlling the room temperatures (Fig. 2b). Each unit was independently controlled via independent sensors and control devices. The compressor of the air conditioning system was located outside of the CAE container.

Temperature and humidity sensors: Two sensors were installed in the larger grow room and two in the small spawn room to sense the temperature and humidity in these environments. The sensors were mounted on the walls and encased within individual aspirated boxes such that these remained well shaded from direct sunlight, not obstructed by the grow bags and shelving units, and had constant airflow over them letting the room's air through the box and over the sensor. The box was painted white to avoid any absorbtion of heat from sunlight and/or electrical light (s) thus allowing accurate readings of the air temperature. Each sensor was connected via cable connectors to the main control panel of the automation system, as indicated in Fig. 2(b).

High pressure atomizing mist sprinkler system: A central overhead pipe, with atomizing nozzles spaced at about 2-feet intervals and suspended at the ceiling level, ran the lengths of the spawn room and the grow room. Incoming municipal water from an external PUB line was designed to enter a high pressure pump via a filtering unit and the pressurized water was then forced into the central overhead sprinkler pipe. Two solenoid valves were used to regulate the flow of the pressurized water into the two rooms so that they could be operated either simultaneously or independently for increasing the humidity in the two rooms. The solenoid valves and the water pump system were connected to the main controller (Fig. 2b).

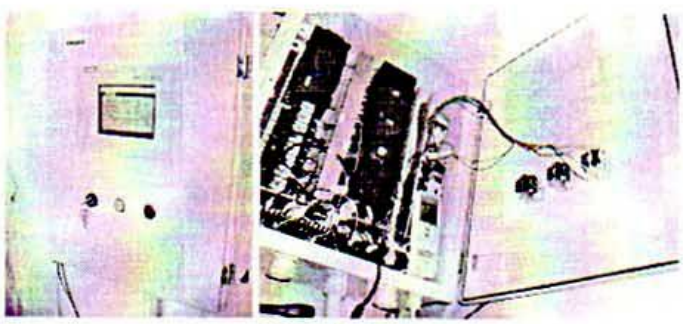
Lighting: Many cultivated mushrooms require light for pinhead initiation and proper development of the fruitbody (Stamets and Chilton, 1983). Consequently, it is important to equip the growing room with a lighting system that provides even illumination to all areas and levels. Three fluorescent light fixtures were mounted horizontally on the ceiling above the central isle of the grow room and their operating switch connected to the PLC for turning them on or off for a desired period of time. The three openings (Fig. 1), with fixed glass panels and attached roller blinds below, on the grow room roof also aided in the control of natural sunlight during fruit body formation.

Air circulation: Fresh air serves many important functions in mushroom culture, primarily by supplying oxygen to the growing mushrooms and carrying away CO2. This becomes very crucial, particularly during fruitbody formation. In the present study, mechanical ventilation was implemented via two exhaust fans to suck in outside air entering one end of the grow room while another exhaust fan pushes inside air out the other end of the grow room through motorized inlet louvers. All fans were adequately screened to prevent the entry of flies and other insects into the grow room and mounted about 1 foot above the floor level of the grow room (since CO2 is heavier than air and settles near the ground level). The size of the exhaust fans were selected based on a rough estimate of the total volume of air exchange per minute in the greenhouse. Accordingly, the fans were programmed to switch on and off via their connection to the PLC.

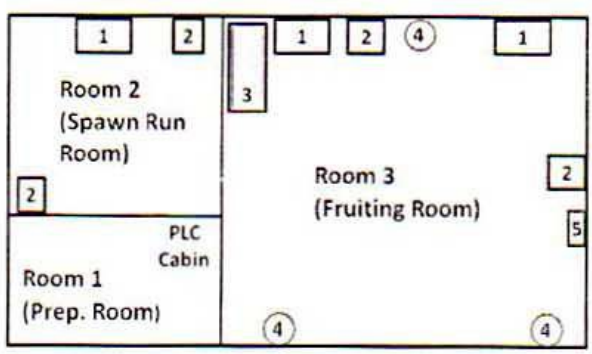
Programmable logic controller (PLC): Automation of the artificial environment in this project was done through a PLC. Fig. 2(a) and 2(b) display the panel overview of the PLC and the layout of the devices connected to it, respectively. A touch screen was used as the Man-Machine Interface (MMI) between the user & the main controller. The system had two modes of operation – manual and automatic. In the automatic mode, the screen

displayed instantaneous readings of the temperature & humidity values within the spawn room and the grow room. With real-time clock setting, a user was allowed to enter minimum and maximum values for temperature & relative humidity (within the specified temperature range of the air conditioners) and set the time for switching on the lights as well as the exhaust fans. For example, when the displayed current temperature (as read by the analogue card module of the PLC from signal transmitted by the temperature sensors via sensor cables) exceeded the maximum user-specified value, the air conditioner would be switched on. It switched off when the room temperature fell below the minimum specified value. Similarly, the water spray mist was activated when displayed present humidity (as detected by the humidity sensors) fell below the minimum specified value, and deactivated when humidity level increased beyond the maximum specified value. Pump timer specified the duration for which the misting continued each time the sprinkler system was switched "ON". Any faulty occurrence would be displayed as an "alarm" on the display screen including probable source (s) of error.

The entire CAE unit with complete installation of the devices, shelving units, wirings, and software and hardware installation was monitored for a continuous, one-month trial run period to ensure that adequate sensing and controlling of the environmental parameters were being achieved successfully without interruption. Cultivation of oyster mushrooms was then carried out on recycled food and agricultural wastes under the CAE conditions.



(a) Panel overview



(b) Device locations in the system

Fig. 2. Programmable Logic Controller

Substrates used for the growing media: Used coffee grounds from the school's canteens and garden wastes from Singapore Polo Club were collected and mixed with sawdust and other supplements in various proportions to formulate the growing media. A Malaysian mushroom production farm that supplied oyster mushrooms to Singapore was contacted for the supply of oyster mushroom spawn, sawdust, rye bran and limestone. The commercial farm uses 100% sawdust supplemented with 10% rye bran and 1% limestone as the growing media to cultivate oyster mushroom indoors where manual spray of water is used to maintain humidity and/or control ambient temperature. The substrate prepared by this farm served as the "reference" medium against which the performance of six other substrate media would be investigated under the CAE developed herein. The average yield from the same commercial farm (about 180g/1kg substrate bag) would again be used as the benchmark yield value for comparison of all substrate media used in the present study. Three major types of substrates were used. Clean and animal free crushed oyster shells, a popular source of calcium carbonate to act as a pH buffer, was added to each of these three substrates to study its influence on growth and yield of mushrooms .Table 1 lists the different compositions of the substrate medium that were used for growing oyster mushrooms under CAE conditions.

Table 1. Summary of different types of substrates used as growing medium

Substrate type	Substrate composition	Measured pH value	Total number of bags prepared
CW	50% used coffee ground, 50% sawdust, 10% rye bran, 1% limestone	5.7-6.2	20
CWO	50% used coffee ground, 50% sawdust, 10% rye bran, 1% limestone, 1% ground oyster shell	5.9-6.3	20
YW	50% yard waste, 50% sawdust, 10% rye bran, 1% limestone	5.6-6.8	20
YWO	50% yard waste, 50% sawdust, 10% rye bran, 1% limestone, 1% ground oyster shell	6.3-6.6	20
SD	100% sawdust, 10% rye bran, 1% limestone	5.7-6.8	20
SDO	100% sawdust, 10% rye bran, 1% limestone, 1% ground oyster shell	5.9-6.5	20
CNTRL	Commercial mushroom farm prepared (100% sawdust, 10% rye bran, 1% limestone) and supplied	5.5-6.0[1]	20

[1] These control bags were prepared and supplied by the commercial farm as 1-day inoculated bags (1 kg weight) with an approximate estimated range of pH value between 5.5 and 6.0 was stated.

The various types of substrates were mixed with water in an electric mixer to form a homogenous mixture at about 70% moisture content resulting in a pH range of about 5.6 to 6.8. About1 kg of mixture was bagged into autoclavable plastic bags, sealed with plastic caps and autoclaved at 121°C at 15psi for about 2 hours. Twenty numbers of bags were prepared from each type of the substrate mix, as stated in Table 1, and labelled accordingly. The autoclaved bags were then cooled to room temperature and inoculated with oyster mushroom spawn (about 5% by weight of solid substrate) and finally moved

to the spawn room in the CAE unit the same day. Once completely colonized by white, cottony mycelium (considered as 100% mycelium run), the bags were moved to the growing room for consequent fruiting flushes to occur. Picture1 illustrates some pictures of the CAE unit at different stages of the cultivation.

RESULTS AND DISCUSSION

During the spawn run for oyster mushroom, temperature and humidity levels were maintained at about 26-28°C and 90% relative humidity, respectively. Number of fresh air exchange was zero and incubation took place in total darkness. Almost all bags took about 19-22 days for complete colonization. Fig. 3 shows a comparison for average time taken to complete mycelium run by each type of substrate bags. Mycelium growth rate in the spawn bags ranged from 0.533 cm/day to 1.417 cm/day. The highest growth rate was recorded on yard waste without oyster shell (YW) substrate while 100% sawdust with oyster shell (SDO) substrate showed the lowest growth rate. In general, it was noted that addition of oyster shell to each type of substrate media slowed down the mycelium run rates, with the effect being most significant for media consisting of yard wastes (between YD and YDO), as depicted in Fig. 4. Yield of the media had also been slightly affected by the addition of oyster shell, as discussed later. The interesting thing to note from Fig. 3 is the fact that all substrate bags prepared in the present work had completed 100% mycelium run between 19-22 days whereas the reference bags required an average of about 33 days to complete mycelium run. Also, increased daily growth rates of mycelium have been observed for all test bags as compared to that of the reference bags. This is most likely, due to the better adjustments of the humidity, temperature and lighting conditions within the spawn run room.

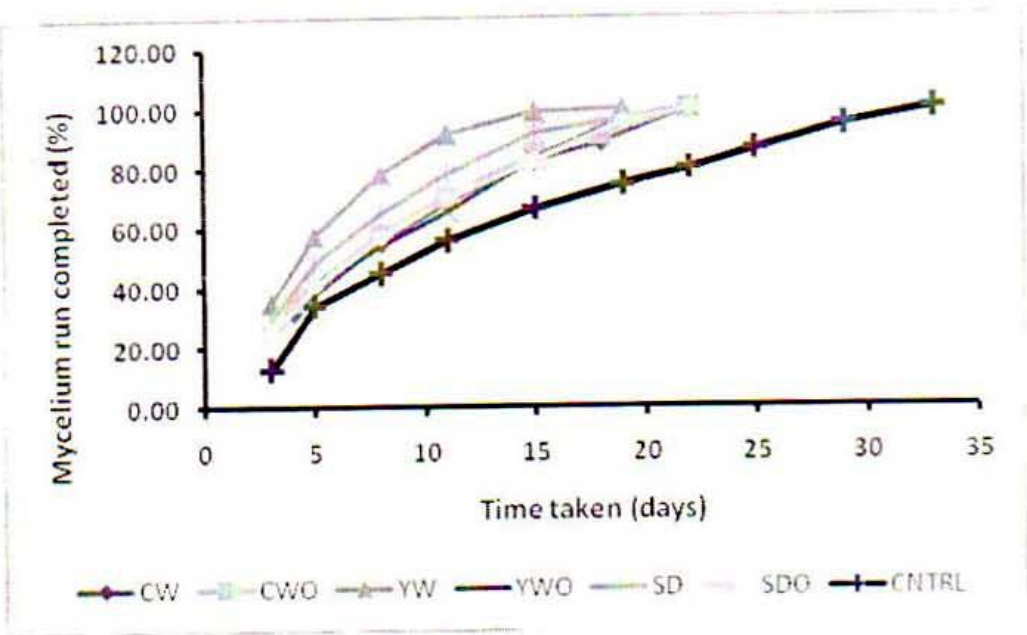


Fig. 3. Growth rate of mycelium run in various substrates. See Table 1 for composition of the different substrate type

Air temperature was reduced to about 16-18°C while the relative humidity increased to about 95% for the next 4 days to promote pinhead initiation within the grow room. Exhaust fans were switched on to allow air exchange every 15 minutes and diffused natural light was allowed to enter for 12 hours/day during this period. The highest days to primordial initiation was recorded to be about 10 days on the CNTRL bags. The lowest days (about 4 days) to primordial initiation was observed to take place on the substrate YW bags. Once pinheads were formed, harvest periods varied between 6-13 days for most fruiting bags, which agree well with similar findings reported in the literature (Amin *et al.*, 2007). The total number of harvests varied between 3 to 6 and the duration required for total harvest after pinhead formulation ranged over approximately 40-70 days for the different bags tested. In general, CNTRL bags yielded maximum number of flushes over a period of about 70 days.

Mushroom strains vary in their ability to convert substrate materials into mushrooms as measured by a simple formula known as the Biological Efficiency, BE (Stamets, 2003), which states that 1 kg of fresh mushrooms grown from 1 kg of dry substrate is 100% BE. Mushroom yields that are substantially higher than 100% BE are possible and some growers have been able to achieve an exceptional 250% BE with oyster and a few other varieties (Stamets, 2000). However, an average to good grower should operate within 75-125% range. In the present work, the highest BE of about 119.6% was obtained for the substrate type YW while substrate type SDO resulted in the minimum B.E of about 56.9%. Except substrate type CWO (175g/bag), all other bags containing the various substrates produced mushroom yields that exceeded the benchmark production value of 180g/kg, that is, 180g/bag. Addition of oyster shell reduced the total yield and BE of all the substrate media (Fig. 4). Table 2 summarizes the results obtained from the cultivation work carried herein.

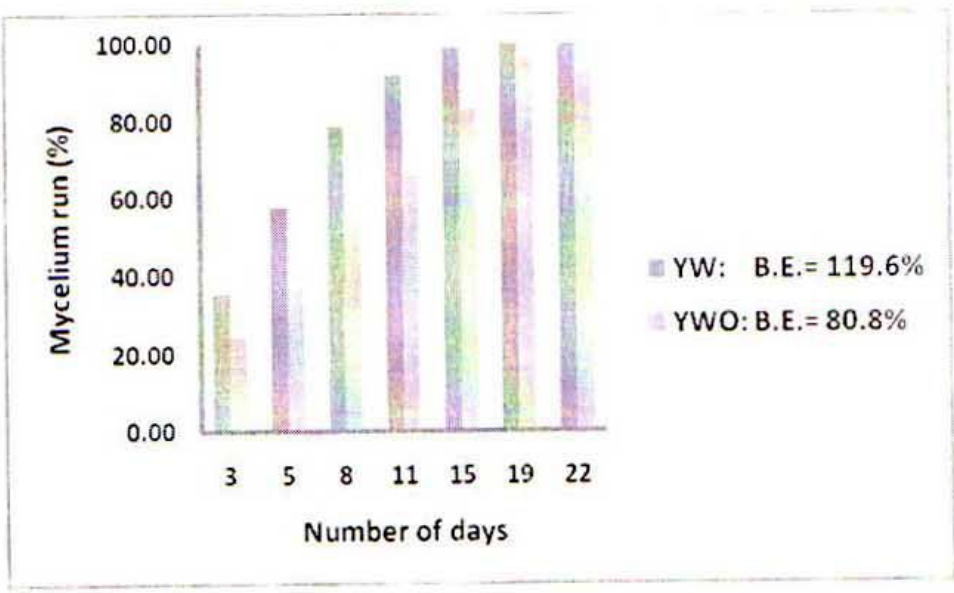


Fig. 4. Effect of oyster shell on growth rate and yield of oyster mushrooms grown on yard waste substrate

Table 2. Summary of test results

Substrate type	Maximum average number of days taken to complete mycelium run	Maximum time (days) between mycelium run and fruiting	Maximum yield (g) per bag	Biological efficiency (average)
CW	22	5	276	84.5
CWO	22	8	175	70.0
YW	19	6	339	119.6
YWO	22	8	287	80.8
SD	22	7	327	65.0
SDO	22	9	263	56.9
CNTRL	33	10	281	73.4
CNTRL[2]	28-36	7-14	180	72.0

[2] These are 1 kg bags (100% sawdust, 10% rye bran, 1% limestone and about 70-75% water) prepared, inoculated and grown in the commercial mushroom growing farm.

Comparing results from one day inoculated bags supplied by the commercial farm, the same composition of substrates performed better in the controlled agricultural environment , as reflected from their maximum yield of 281g (>180g) per bag and BE of 73.4% (>72.0%) with regards to the corresponding benchmark values. In addition, the appearance, texture and quality of the fruited mushrooms in terms of taste and flavour turned out better as a result of a "taste test" conducted AMONG the students, staff and canteen employees of Republic Polytechnic as well as people from external organizations. There is room for further improving the BE by optimizing the substrate compositions.

From the work done in this project and the results presented, it may be concluded that Controlled agricultural environment, in general, can contribute significantly to the drive to maximize food availability and product quality while keeping prices down. This is particularly true for its application in terms of mushroom cultivation, especially if the CAE is designed carefully and, in this case, properly integrated with a waste management program. Although they cannot compete with some open field commodity crops, CAEs can increase yields for more specialist products (like gourmet and medicinal mushrooms) many fold – for example, by allowing the possibility of year-long cropping in countries where seasonal climate limits the outdoor growing-season to less than half of the year. In addition, pests and diseases can be excluded from the CAEs, allowing crops to be grown without the need for artificial pesticides or fungicides. The project has thus far been a resounding success, with delicious, large, succulent gourmet mushrooms being produced from organic waste material.

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Bangladesh J. Mushroom. 3(1): 47-52, 2009 (June)

Effect of Media and Environmental Factors on Mycelial Growth of *Boletus edulis*, *Morchella esculenta* and *Pleurotus geesternaus*

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Abstract

Boletus edulis, *Morchella esculenta* and *Pleurotus geesternaus* were cultured on different media under different environmental conditions such as temperature, pH, and light as well as different carbon sources to determine the optimum culture conditions for mycelium growth. Maximum mycelial growth (0.38cm/day) of *Boletus edulis* was observed on Malt Extract Agar medium and the lowest (0.16 cm/day) on Yeast Agar medium. In case of *Morchella esculenta* and *Pleurotus geesternaus*, Potato Dextrose Agar medium showed the best performance. Minimum days required for completion of mycelium growth was 9.25 days in *Boletus edulis*, 6.75 days in *Morchella esculenta* and 9.25 days in *Pleurotus geesternaus*. The highest mycelium growth (0.45 cm/day) of *Boletus edulis* was found in pH 7.0 and the lowest (0.39 cm/day) was observed in pH 4.5. No significant difference was observed in *Morchella esculenta* when cultured on different pH levels. In case of *Pleurotus geesternaus* the highest mycelium growth (0.40 cm/day) was recorded in pH 7.5 whereas the poorest growth was at pH 4.5. Among the carbon sources, significant variation was not found for mycelium growth of *Boletus edulis* whereas maltose was highly significant for *Morchella esculenta*. Sucrose was best for mycelium growth of *Pleurotus geesternaus*. With respect to temperature, 30^{0}C proved to be most suitable for mycelium growth of *Boletus edulis* and *Pleurotus geesternaus* and 20^{0}C for *Morchella esculenta* whereas 10^{0}C was not suitable for the mushrooms. In case of light, the highest mycelium growth of *Boletus edulis* and *Pleurotus geesternaus* were observed in 0 lux, while 200-300 lux was most suitable for *Morchella esculenta*.

Key word: Media, mycelial growth, light, temperature, carbon source, pH.

INTRODUCTION

The potential of mushroom as fungal protein and as a source of medicinal compounds make the production of mycelium an attractive prospect. Mushroom mycelium is used for medicinal and therapeutic purposes; mycelial biomass powder can be used to formulate various types of health tablets and capsules (Chen and Xiu, 2001). The biological efficiency of mushroom depends on the development of mycelia in the first cultural stage. Healthy and active mycelial growth plays crucial roles in protecting themselves against several stress factors (Herderio *et al.*, 2006). The growth of mycelium depends on different factors such as media, pH, temperature, nutrient element and some environmental factors (Calam, 1971). Medium is important because it supplies necessary nutrient for the growth of mushroom mycelium. Culture media permit acceleration of mycelial growth, ensure quality and year round production (Chang, 2001). Mushroom mycelium is significantly influenced by various environmental factors such as light, temperature, pH, carbon sources etc. pH has great effects on nutrition and morphological development of mushrooms (Chang and Miles, 1998). Generally the growth of fungi is

reduced at very strong acidic and alkaline pH. Different agar media such as Potato Dextrose Agar, Malt Extract agar, Lamberts Agar, Compost Extract Agar are mostly used for the growth of mycelium (Pathak *et al.*, 1998).

Boeletus edulis is a valuable edible mushroom with medicinal importance. It has high nutritive value, anti-tumor activity and hypolipidemic effects (Manzi *et al.*, 2001.) The flavor, texture and test of *Boeletus edulis* mushroom are excellent. The mycelia of *Morcella esculenta* and *Pleurotus geesternaus* are used in food industry and enzyme production

Mycelial growth requires short time in comparison with fruiting bodies development. So the purpose of this study was to determine the suitable medium and environmental factors for the production of good mycelia within short period of time in laboratory conditions.

MATERIALS AND METHODS

This experiment was conducted in the tissue culture laboratory of National Mushroom Development and Extension Centre (NAMDEC) during the period of January to May-2009. Different mushroom species such as *Pleurotus geesternaus*, *Morcella esculenta*, *Boletus edulis* and different synthetic media such as Potato Dextrose Agar, Yeast Extract Agar, Malt Extract Agar, Wheat Extract Agar and Potato Dextrose Yeast Agar were used in this study. The inocula were collected from the germplasm centre of NAMDEC.

Preparation of synthetic media: The basal components of each medium were mixed with 20g of dextrose and 18g of agar. The mixture was boiled on gas burner until the agar dissolved. Then the pH level was adjusted at 6.5. The media was poured into Petri dishes (90mm diameter) at 20 ml/plate and sterilized in an autoclave for 20 minutes at 120 ^{0}C temperature and 1 kg/cm^{2} pressure. After sterilization and solidification, the plates were inoculated with the inocula of selected mushroom species. Plates were then transferred in incubation room for mycelium running at suitable temperature. Average mycelial growth and duration of completion of mycelium running of each replication were calculated.

pH: In order to screen the optimum pH value for mycelial growth, *Boletus edulis* was cultured on Malt Extract Agar, *Morchella esculent* and *Pleurotus geesternaus* on Potato Dextrose Agar. The media were adjusted to different pH values at 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 with the addition of 1N HCl or 1N NaOH before pouring into Petri plates and autoclaving. The Petri plates were inoculated with the inocula of above three mushrooms. Then the plates were transferred into incubation room for mycelium running. Average mycelial growth and duration of completion of mycelium running of each replication were calculated.

Light: To evaluate the suitable light for mycelial growth of *Boletus edulis* on Malt Extract Agar and *Morchella esculenta* and *Pleurotus geesternaus* on Potato Dextrose Agar, the inoculated plates were placed at four different light conditions such as dark, (0 lux) 40-60 lux, 100-200 lux, 200-300 lux.

Temperature: The inoculated plates of *Boletus edulis* on Malt Extract Agar and *Morchella esculenta* and *Pleurotus geesternaus* on Potato Dextrose Agar were placed at five different temperature values such as 10^0 C, 15^0 C, 20^0 C, 25^0 C and 30^0 C, with the pH value of each medium being 6.5.

Carbon compounds: Selected carbon sources were glucose, fructose, maltose, lactose and sucrose. Eighteen grams of each of the carbon sources was incorporated into the basal medium and distilled water was added to make 1 liter.

Data collection and statistical analysis: The experiment was laid out following completely randomized design (CRD) with four replications. Data on mycelium growth rate and days to complete mycelium running were recorded. Data were analyzed following MSTST-c computer programme. Means were computed following Duncan's Multiple Range Test (DMRT) using the same computer programme.

RESULTS AND DISCUSSION

Effect of culture media: The effect of mycelial growth rate in five different culture media is shown in Table 1. Significantly the highest mycelium growth (0.38 cm/day) of *Boletus edulis* was observed in Malt Extract Agar media which is statistically similar (0.37cm/day) to Potato Dextrose Agar media and the lowest mycelium growth rate (0.16 cm/day) was found in Yeast Extract medium. In case of *Morchella esculenta*, the highest mycelium growth (0.67 cm/day) was observed in Potato Dextrose Agar media, which was statistically similar to Malt Extract Agar media (0.59 cm/day) and the lowest mycelium growth rate (0.38 cm/day) was found in Yeast Extract Agar media. This result is similar to Erbil (2008) and Kalm & Kalyoncu (2008) The highest mycelium growth of *Pleurotus geesternaus* was observed in Potato Dextrose Agar media (0.41 cm/day) and this medium has been demonstrated to be highly supportive of mycelial growth of mushrooms by several authors such as Jeffers & Mertin (1996). The lowest mycelium growth rate (0.29 cm/day) was found in Yeast Extract Agar media.

Table 1. Effect of mycelial growth (cm/day) on different media

Media	Mushroom species		
	Boletus edulis	*Morchella esculenta*	*Pleurotus geesternaus*
Malt Extract Agar	0.38a	0.59b	0.33b
Potato Dextrose Agar	0.37a	0.67a	0.41a
Wheat Extract Agar	0.31b	5375c	0.31bc
Yeast Extract Agar	0.16c	0.38d	0.29bc
Potato Dextrose Yeast Agar	0.30b	0.50c	0.27c

Days required for completion of mycelium running: The days required to the completion of mycelium running in different media is shown in Table 2. The lowest time (9.25days) required for the completion of mycelium running of *Boletus edulis* was observed in Malt Extract Agar media which was statically similar to Potato Dextrose

Agar media (9.75days) and highest time (13.75days) was observed in Yeast Extract Agar media. In case of *Morchella esculenta*, the lowest time (6.75days) required for the completion of mycelium running was observed in PDA media and highest time (13.75days) was observed in Yeast Extract Agar media. In case of *Pleurotus geesternaus* lowest time (9.25days) required for the completion of mycelium running was observed in Potato Dextrose Agar media and the highest time (13.25days) was observed in yeast media.

Table 2. Effect of days to complete mycelial running on different media

Media	Mushroom species		
	Boletus edulis	*Morchella esculenta*	*Pleurotus geesternaus*
Malt Extract Agar	9.25c	8.50bc	12.25a
Potato Dextrose Agar	9.75bc	6.75c	9.25b
Wheat Extract Agar	12.50a	11.25a	12.25a
Yeast Extract Agar	13.75a	11.25a	13.25a
Potato Dextrose Yeast Agar	12.00ab	9.50ab	11.50ab

Effect of pH on mycelial growth: Significantly the highest mycelium growth of *Boletus edulis* was observed in pH 7.0 (0.45cm/day) and pH 7.5 (0.45 cm/day), which is statistically similar to all the pH ranges except pH 4.5. The lowest pH range for the growth of mycelium of *Boletus edulis* was observed in pH 4.5. In case of *Morchella esculenta* the highest pH range (0.17 cm/day) was observed in pH 7.0, pH 6.0 and pH 7.5 which are statistically similar. The lowest pH range (0.12 cm/day) was observed in pH 4.5. In case *Pleurotus geesternaus* the highest pH range (0.40 cm/day) was observed in pH 7.5 and pH 7.0 which are statistically similar. The lowest pH range (0.18cm/day) for the growth of mycelium of *Pleurotus geesternaus* was observed in pH 4.5. These findings proved that these three mushrooms prefer alkaline pH which is similar to the report of Chang and Miles (1987), who separately obtained very good mycelium growth of *Auricularia auricula* , *Flammuina velutipes* and *Volborila volvaceaat* at pH values of 7.5, 8.0 and 7.5 respectively.

Effect of carbon sources: The experiment showed that maltose was the best carbon source (0.47cm/day) for *Morchella esculenta*; this result is similar to that of Erbil (2008). In the case of *Pleurotus geesternaus*, sucrose showed the best performance (0.39 cm/day) as carbon source. In the case of *Boletus edulis* maltose showed the best performance (0.26 cm/day) for mycelial growths. This result is similar to Shim *et al.* (2005) who showed that maltose, dextrin, sucrose and mannose were effective carbon sources for the growth of mushroom mycelium.

In all three cases, lactose showed the lowest performance as carbon source for mycelium growth which is similar to Imtiaz *et al.* (2007). They reported that lactose, galactose and sorbitol are the most unfavorable carbon sources for mycelium growth.

Table 3. Effect of mycelial growth (cm/day) on different pH

PH	Mushroom species		
	Boletus edulis	*Morchella esculenta*	*Pleurotus geesternaus*
4.5	0.39b	0.12a	0.18d
5.0	0.44ab	0.13a	0.20d
5.5	0.42ab	0.16a	0.26c
6.0	0.44a	0.17a	0.33b
6.5	0.41ab	0.16a	0.32b
7.0	0.45a	0.17a	0.39a
7.5	0.45a	0.17a	0.40a
8.0	0.41ab	0.15a	0.34b

Table 4. Effect of mycelial growth (cm/day) on different carbon source

Carbon source	Mushroom species		
	Boletus edulis	*Morchella esculenta*	*Pleurotus Geesternaus*
Glucose	0.22a	0.41b	0.34
Fructose	0.23a	0.44ab	0.34
Lactose	0.21a	0.41b	0.19c
Maltose	0.26a	0.47a	0.30b
Sucrose	0.23a	0.42b	0.39a

Effect of temperature on mycelial growth: Among the three mushroom species the temperature range of 10-30^{0}C was considered to find out the most suitable one. The highest mycelial growth (0.47 cm/day) was found in 30^{0}C in the case of *Boletus edulis*. In case of *Pleurotus geesternaus* the highest mycelial growth (0.39 cm/day) was found in 30^{0}C which is statistically similar with 25^{0}C. This result is supported by Sung *et al.* (1999); they stated that the favorable temperature for mycelial growth was 30^{0}C for *Macrolepiota procea* and *Pleurotus ostreatus*. In the case of *Morchella esculenta* the highest mycelial growth (0.40 cm/day) was found at 20^{0}C. This result is similar to the report of Shim *et al.* (2005); they stated that the mycelial growth of *P. fumosoroseus* was favourable at the temperature of 20 to 25^{0}C. For all three mushrooms, 10^{0}C was found to be not suitable for mycelial growth.

Table 5. Effect of mycelial growth (cm/day) on different temperature

Tempareture	Mushroom species		
	Boletus edulis	*Morchella esculenta*	*Pleurotus Geesternaus*
10^{0}C	0.31d	0.24c	0.18b
15^{0}C	0.39c	0.36ab	0.36a
20^{0}C	0.42bc	0.40a	0.36a
25^{0}C	0.44ab	0.37a	0.39a
30^{0}C	0.47a	0.31b	0.39a

Effect of Light intensity: The effect of different light intensities on the mycelial growth is shown in Table 6. In case of *Boletus edulis* the highest mycelial growth (0.36 cm/day) was found in 0 lux and the lowest mycelial growth (0.26 cm/day) was found in 200-300

lux and 100-200 lux (0.26 cm/day) which is statistically similar. In case of *Pleurotus geesternaus* the highest mycelial growth (0.39 cm/day) was found in 0 Lux and the lowest (0.28 cm/day) was found in (200-300 Lux). Zandrazil (1982) reported that absence of light gave best mycelial growth and yield. In case of *Morchella esculenta* the highest mycelial growth (0.46 cm/day) was found in 200-300 lux and the lowest (0.39 cm/day) was found in 40-60 Lux.

Table 6. Effect of mycelial growth (cm/day) on different Light source

Light	Mushroom species		
	Boletus edulis	*Morchella esculenta*	*Pleurotus geesternaus*
0 Lux	0.36a	0.44ab	0.39a
40-60 Lux	0.28b	0.39b	0.37a
100-200 Lux	0.26b	0.41b	0.28b
200-300 Lux	0.26b	0.46a	0.28b

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Mechanical Detection of Minerals, Heavy Metals and Trace Elements in Processed Mushroom in Relation to Toxicological Aspects

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Abstract

Twenty elements including heavy metals and trace elements were assayed irrespective of their presence in three different processed Oyster mushrooms (*Pleurotus ostreatus*). Freeze drying (FD), Oven drying (OD) and Sun drying (SD) mushrooms were analyzed using Energy Dispersive X-ray microanalyser (E-dx) for the contents of different elements. Among the detected elements K ranked the highest by 2.59, 1.31 and 2.30% in FD, OD and SD samples respectively. Next to potassium, phosphorus (P) contents were 2.20% in F.; 1.15% in O. and 1.74% in S. mushroom samples. The presence of other elements was less then 1% in three different dried mushroom samples. Sn and Al were present in three samples but Cd, Hg, As, Mo, Ni and Co were absent. It was also found that Si was present by 0.06% in OD but in trace level (0.01%) in both of FD and SD. On the other hand Pb and Cr were found in trace level in FD samples but were absent in both of OD and SD. E-dx microanalysis also analyzed that in respect of presence of the maximum content of elements the sample types may be graded in the order of FD, OD and SD. A confirmatory test was also done for Pb, Co, Hg and As (Arsenic) performing AA photometric standard method and was found that presence of As (Arsenic) was below 0.01mg / kg; Pb and Hg were below 0.05 $mgkg^{-1}$ in FD sample. Co(Cobalt) was not detected in any of the samples examined.

Key words: Edible, oyster mushroom, heavy metals, trace elements, minerals and toxicological.

INTRODUCTION

Many mushroom species are known as toxicant heavy metal such as cadmium, lead or mercury accumulators (Svoboda *et al.*, 2002). The heavy accumulation of cadmium, lead and mercury in some edible mushrooms is of great interest when considering human health. Mushrooms are gaining popularity all over the world due to its pleasant aroma, taste and fleshy nature.

The nutritional and medicinal values of mushrooms have long been recognized (Lucas *et al.*, 1957 and Suzuki *et al.*, 1976). The world mushroom production was more than 01 million tones in 1986 (Gupta *et al.*, 1994). Due to its inherent food values, by 2003, world mushroom production had reached 03 million tons (Anon, 2003). Now, mushrooms are being cultivated in more than 100 countries of world, with and estimated total production

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of over 12 million tons. In the back drop of quest to promote mushrooms cultivation, it has been reported by several workers that in case of edible fungi, toxic metals may be incorporated into food chain. Edible mushrooms may contain higher amounts of heavy metals than pants, especially in the vicinity of high ways subject to heavy traffic (Demirbas, 2000, Lepsova and Kral, 1988, Liukkkonen *et al.*, 1986 and Tyler, 1982). The occurrence and distribution of different toxic components in certain mushrooms is not only a theoretical mycological problem, but also has practical environmental and toxicological consideration (Vetter, 1994). According to FAO/WHO (1989; 1993), acceptable weekly intakes of cadmium and lead for adults are 0.42-0.49 and 1.5-1.75 mg, respectively. Some countries have established statutory limits for the metals in edible mushrooms. The limits being 5.0, 2.0 and 10.0 mg kg^{-1} dry matter have been valid in the Czech Republic since 1999 for mercury, cadmium and lead, respectively, in wild-growing mushrooms, while 1.0, 1.0 and 10.0 mg kg^{-1} dry matter are established for cultivated ones. For calculations, usually 300 gm of fresh mushrooms per meal is assumed. Semimetals selenium, arsenic and antimony do not occur in undesirable levels. Present knowledge of metal availability in mushrooms is limited as is knowledge of their bioavailability in man. Thus, consumption of the accumulating species should be restricted. The cultivated species, especially the common mushroom (*Agaricus bisporus*) and oyster mushroom (*Pleurotus ostreatus*) contain only low levels of the trace elements.). Extensive research has been carried out since the 1970's on trace elements (mainly heavy metals) occurrence in mushrooms (higher fungi, macrofungi). Several reviews of heavy metal concentrations in mushrooms have been published (Kalac and Svoboda, 2000, Seeger, 1982 and Vetter, 1994). It has been pointed out that adoptions of some of the processing methods are more efficient operation to decrease level of detrimental elements. Many researchers have reported that after different industrial processes (frozen, dried or fresh); chemical and nutritional characteristics of mushrooms are closely linked to species and processing (Bano and Rajarathnam, 1988, Diez and Justo *et al.*, 1998, Leon *et al.*, 1997 Longvah and Deosthale, 1998 and Manzi, *et al.*, 1999). Very scarce information is available on presence of minerals, toxicant heavy metals and trace elements after processing, preservation and culinary treatments of mushrooms. For this reason, it was felt interesting to evaluate different minerals including major toxicant heavy metal and trace elements in three different dried processed mushrooms particularly in a country like Bangladesh well known for its dense population link to unlimited pollution related adversities including over crowed traffic situation. Literature data dealing mainly with minerals, heavy metals and trace elements in edible mushrooms particular emphasis on toxicological aspects are also reviewed in this article.

MATERIALS AND METHODS

Samples: Common cultivated Oyster mushrooms (*Pleurotus ostreatus*) mushrooms were grown at the Cropping Room of Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, on traditional paddy straw-based substrate, using standard IFST practice. Mature mushrooms were harvested and all visible debris and dirt were removed without washing for cleaning up

the samples. Mushrooms sample were selected for Energy disperse X-ray (Edx). Only first and second flush mushrooms were used: taken mushrooms were in between 5 to 11cm in diameter on an average 8 cm containing 2 - 4 cm stalks. The collected samples were processed by three different drying methods. These were sun dried (30 ± 1°C; consecutive 2 days on a metal sheet; final moisture was 12.5%); Oven dried (55°C; moisture was 5.83%) and Freeze dried (36 hours; moisture was 10.2%). After drying it was powdered using electric blender. The mushroom tissue specimens were then mounted on aluminum stubs and examined using a low vacuum SEM fitted with a backscattered electron detector (BSED) and an energy disperse X-ray spectrometer (HITACHI S-3400N). Scanning Electron Microgram (SEM) of the three processed mushroom particles (tissue) were taken for the identification as specimens. The techniques of energy dispersive X-ray microanalysis (E-dx) were used to quantify the relative presence of selected 20 elements. The intensity of the BSED signal is a function of the mean atomic number of the excited site; therefore, a brighter region in the image modulated by the BSED would indicate the presence of heavier atoms. The different elements in the sample could be unequivocally identified by their characteristic X-ray radiation; showing the peaks of the presence. The results are reported as a relative percentage of the total amount of dominant minerals in our specimens which are considered to be semi quantified. The procedure was followed as recommended by Hershko and Nussinovitch, (1998).

A confirmatory test was done following standard procedure for four heavy metals including As. Determination of heavy metals (Pb, Co, Ni and As) were carried out with SIMADZU model - AA 6401F using air acetylene flame and Varian Atomic Absorption Spectrophotometric (AAS) model Spectra AA-220 was used in hydride generation mode to measure As concentration. The wavelength and slit values, as nm, used for the determination of Pb, Co, Ni and As were: 0.5, 217.0 and 0.2, 240.7 and 0.2, 232.0 and 0.5, 193.7, respectively The detection limit (0.01 mg kg^{-1}) is defined as the concentration value that corresponds to three times the absorbance obtained from 10 replicated measurements of the blank.

Preparation of mushroom sample solution: The sample of mass 0.5 gm was weighed and placed in a porcelain crucible and ashed at 450°C for 15-24 hour, then the ash was dissolved in 2 ml conc. HNO_3, evaporated to dryness, heated again at 450°C for 3 hours and dissolved in 1 ml conc. H_2SO_4, 1 ml conc. HNO_3 and diluted with deionized water up to a volume of 25 ml. All samples were run in duplicate.

Preparation of working As standard: Intermediate As calibration standard (100 µg/l) was prepared from 1mg/ml stock standard As solution.2, 5, 10, 15 and 20 ml aliquots from 100 µg/l As standard solution were taken in separate 100 ml volumetric flasks to prepare 2, 5, 10, 15 and 20 µg/l As working standard solution. 10 ml 1% Potassium Iodide (KI) solution and 10ml conc. HCl then added to each and deionized water was added to each to make up it to the mark. 5ml stock sample solution was taken in 25 ml volumetric flask then 2.5 ml 1% KI solution, 2.5 ml conc. HCl and required volume of deionized water was added to make avolume to 25ml.

Reagent: (a) 0.6% Sodium borohydride in 0.5% NaOH was prepared by dissolving 3g Sodium borohydride in 500ml 0.5% NaOH solution.

Reagent: (b) 250 ml deionized water was taken in a 500 ml plastic reagent bottle and 250 ml HCl was slowly added and carefully mixed well then set up calibration.

Determination of Arsenic (As): Reagent (a), (b) as described above and sample solution were pumped through a peristaltic pump ; three solution mixed together and produced arsine gas which was burnt in air acetylene flames of AAS for decomposition arsine gas and atomization of As. Absorbance of As characteristic radiation was measured in each case by AAS Software. Calibration curve of absorbencies of 2, 5, 10, 15 and 20 μg/l As were adjusted by AAS Software. Absorbance of the software compared mushroom sample solution with the calibration curve and concentration of As(Arsenic) was obtained from it. Determination procedure of Pb, Co and Ni were same as arsenic. The only difference is the use of concerned reagents.

RESULTS AND DISCUSSION

The proximate semi quantitative results of three different dried mushrooms as X-ray spectrum are shown in Fig. 1-3 and also summarized in a tabular form as shown in Table 1. Selected heavy metals, toxic trace elements and the highest elements present in three different processed dry mushrooms are shown in Tables 2 and 3, respectively. Tables 4 and 5, which depicted the ratio between carbon and nitrogen (C: N) of three different dried mushroom powder and the confirmatory-test results for As, Pb, Co and Ni of FD respectively.

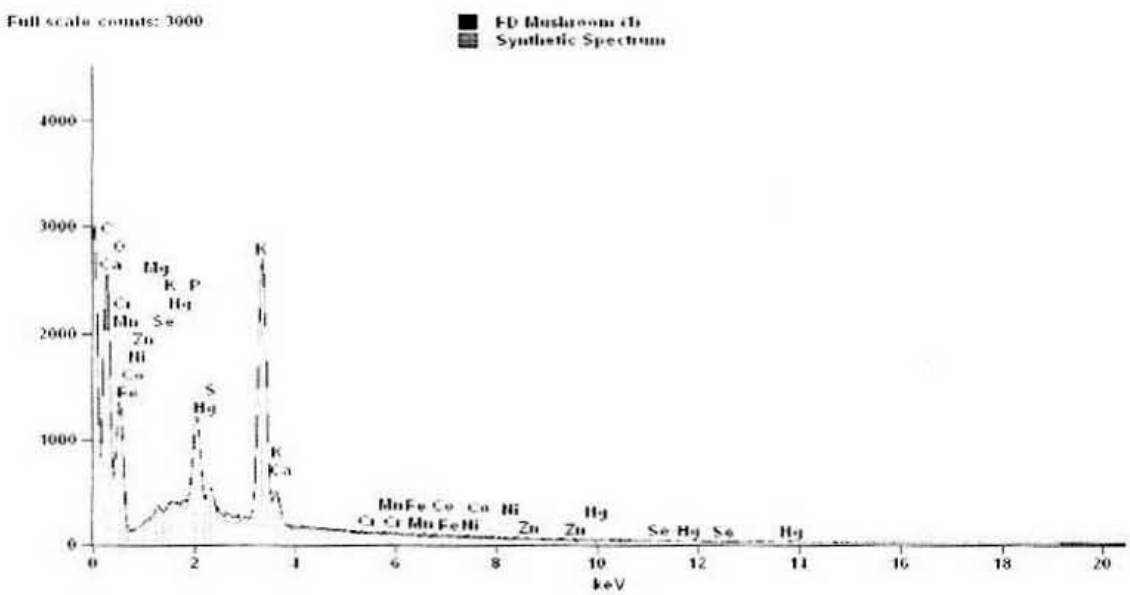

Fig. 1. X-ray spectrum of a Freeze Dried Mushroom particle obtained by E-dx

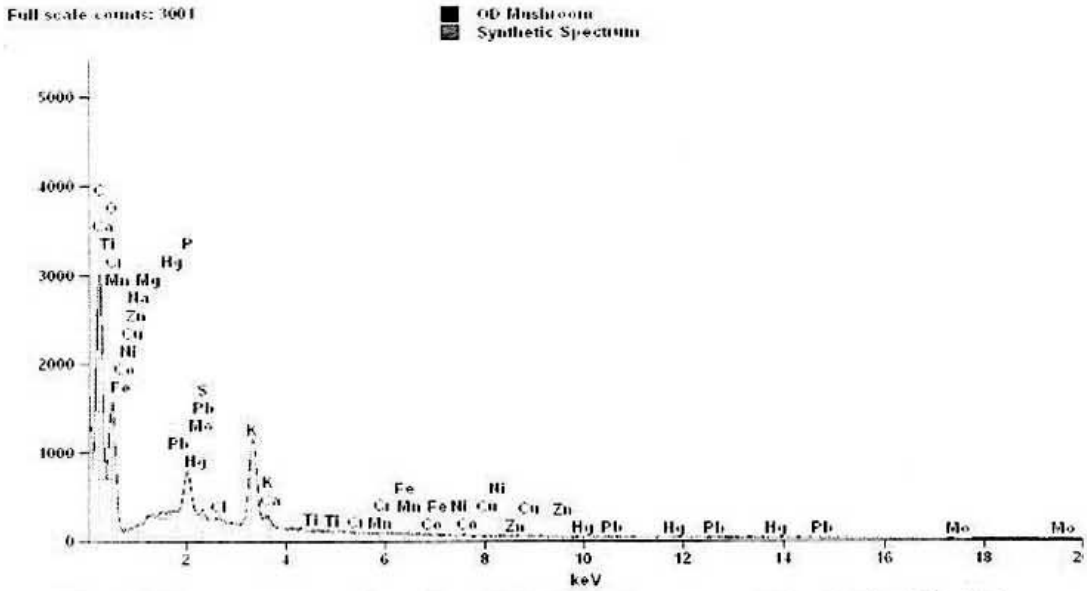

Fig. 2. X-ray spectrum of an Oven Dried Mushroom particle obtained by E-dx

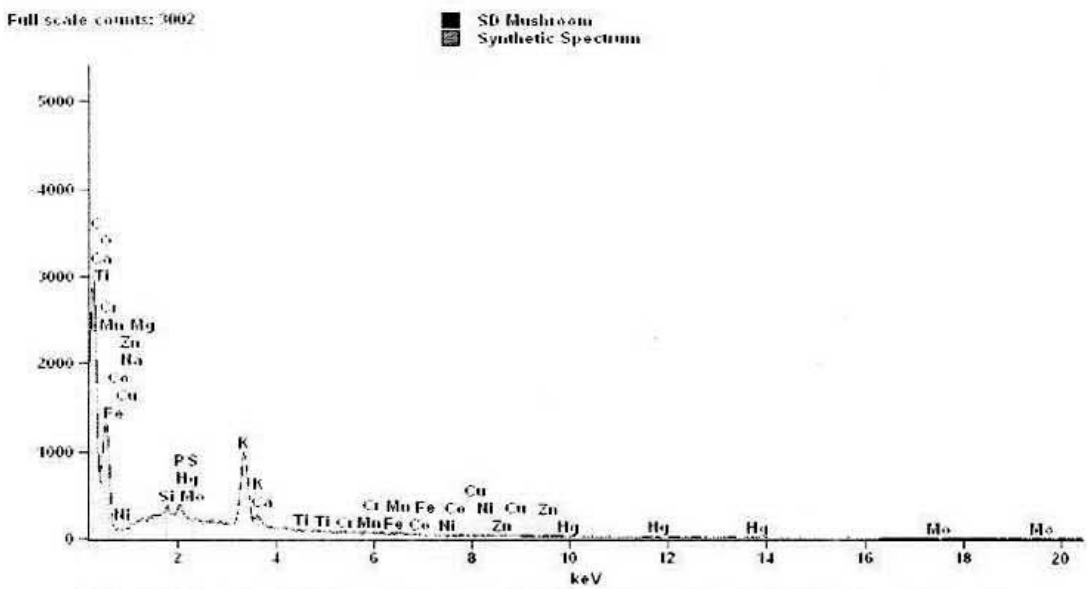

Fig. 3. X-ray spectrum of a Sun Dried Mushroom particle obtained by E-dx

It is evident from Table 1, the levels of K present in Freeze dried (FD), Oven dried (OD) and Sun dried (SD) mushrooms are 2.59, 1.31 and 2.3%, respectively. This percentage shows the relative w/w% among the selected twenty elements. So, it is not the actual quantity of K present in three different dried mushrooms, rather it should be considered as semi-quantified measurement as recognized by some workers (Noran, 2001, Fert and Lottis, 1992).In this experiment K showed the highest peak among three samples (Fig. 1-3). As

observed by Seeger (1982) in a survey of 410 wild-growing species, mushrooms contain potassium between 1.5g and 117g per kg of dry matter. Thus, potassium levels in many mushroom species are considerably higher than those found in foods of plant origin. It was also found that Potassium concentrations within the individual parts of a fruiting body decrease in the following order: cap > stipe > gills or tubes in spores-forming part > spores.

Table 1. Comparison of the selected twenty elements among three different dried mushrooms based on relative weight

Freeze Dried					Oven Dried					Sun Dried				
Elements	Net Counts	Weight %	Weight % Error	Remarks	Elements	Net Counts	Weight %	Weight % Error	Remarks	Elements	Net Counts	Weight %	Weight % Error	Remarks
K	43063	2.59	+/- 0.04	P	K	16383	1.31	+/- 0.04	P	K	36360	2.3	+/- 0.03	P
P	39291	2.2	+/- 0.05	P	F	782	1.15	+/- 0.25	P	F	1392	1.74	+/- 0.25	P
F	596	0.67	+/- 0.22	P	P	8755	0.66	+/- 0.02	P	P	6770	0.41	+/- 0.01	P
S	6849	0.4	+/- 0.02	P	Sn	2487	0.34	+/- 0.09	P	Sn	3208	0.35	+/- 0.08	P
Sn	2831	0.29	+/- 0.08	P	Mg	1144	0.13	+/- 0.01	P	S	2164	0.13	+/- 0.01	P
Mg	2587	0.22	+/- 0.02	P	S	1350	0.1	+/- 0.01	P	Cl	548	0.03	+/- 0.01	P
Se	129	0.08	+/- 0.08	P	Cl	1277	0.1	+/- 0.01	P	Si	426	0.03	+/- 0.01	P
Cl	1155	0.07	+/- 0.01	P	Si	766	0.06	+/- 0.01	P	I	231	0.03	+/- 0.02	T
Fe	330	0.04	+/- 0.02	P	Zn	155	0.05	+/- 0.03	P	Se	31	0.02	+/- 0.06	A
Zn	184	0.04	+/- 0.03	P	Fe	98	0.02	+/- 0.02	A	Fe	188	0.02	+/- 0.02	A
Si	476	0.03	+/- 0.01	P	Cr	95	0.01	+/- 0.01	A	Mg	0	0	---	A
Cr	164	0.02	+/- 0.01	P	Cu	28	0.01	+/- 0.02	A	Zn	0	0	---	A
Co	135	0.02	+/- 0.02	A	Ca	159	0.01	+/- 0.02	A	Cr	29	0	+/- 0.01	A
Cu	84	0.01	+/- 0.03	A	Ni	47	0.01	+/- 0.02	A	Co	0	0	---	A
Na	0	0	---	A	I	93	0.01	+/- 0.03	A	Cu	0	0	---	A
Ca	0	0	---	A	Se	0	0	---	A	Na	0	0	---	A
Mn	0	0	---	A	Co	0	0	---	A	Ca	0	0	---	A
Ni	9	0	+/- 0.02	A	Na	0	0	---	A	Mn	0	0	---	A
Mo	0	0	---	A	Mn	0	0	---	A	Ni	0	0	---	A
I	0	0	---	A	Mo	0	0	---	A	Mo	0	0	---	A

Key: Trace (T) = 0.01% or less than 0.01%;
Present (P) = Weight % above than Trace;
Absent (A) = 0%, weight % of error equivalent to or greater than weight%.
Limitation of this machine: Lowest detection range is 0.01 w/w%.

Content of P was highest in FD but F was the second highest both in OD and SD respectively. Similarly, it was found that the concentration of S, Sn, Mg, Se, Cl, Fe, Zn, Si and Cr were

present in the descending order in FD. In OD and SD the sequences were as follow (order based on w/w%), Sn> Mg>S> Cl > Si> Zn and Sn> S> Cl > Si >I respectively.

So, elements which are absent may be present below the lowest detection range or absolutely not present. Zn was present in both of FD and OD but found absent in SD samples. Zinc is widespread among living organisms due to its biological significance. Mushrooms are known as zinc accumulators. In sporophore, substrate ratio for Zn ranges from 1 to 10 (Bano *et al.*, 1981).

Although the Se, Fe and Cr was found in FD but was not available in both of OD and SD. But Co, Cu, Na, Ca, Mn, Ni, Mo and I were not found in any of the samples examined. It might be the limitation of using E-dx for measuring Na is that the Na photons would be absorbed by the Helium atmosphere. It was reported earlier that the nutrient content in mushrooms is lower during the second flash. It was also shown that during development of the first flash, in the last four days before harvest, there was depletion in content of almost all the analyzed nutrients in mushrooms, with the electrolytical active minerals K and Na being the only exceptions. The content of other minerals greatly decreased during flash development, with the Ca content in standing out in this respect (Straatsma, 2006). It is calculated that the concentration of K, P, Na, Ca and Mg constitute about 56 to 70% of the total ash content.

As evident from the results presented in Table 2 and as we seen previous records that Sn was found in three samples, Si was present in OD but may be present in traces both in FD and SD. The presence of Pb was not found in OD and SD but might be present in FD as trace level. Content of Co, Ni, As, Mo and Cd were below the tract or fully absent in those three samples. After reviewing 150 original papers dealing with heavy metals accumulation in edible mushrooms, Kalac and Svoboda (2000) were of the opinion that cadmium, mercury and to a lesser extent lead are the metals of toxicological importance. However, their assessment has been difficult due to limited knowledge about their chemical forms and bioavailability in man. In the present study it was found that among those elements highest level of Al was present in OD samples followed by SD then FD. It was also found that As, Cd, Hg and Pb were not detectable in three different dried samples of mushrooms. According to Racz *et al.* (1995) and Sanglimsuwan *et al.* (1993) *Agaricus bisporus* had been very susceptible to increasing content of mercury and to a lesser extent of cadmium in substrate, while the yield of *Pleurotus ostreatus* (oyster mushroom) is not too affected under these conditions. *A. bisporus* uptakes metals from substrate and casing material in the decreasing order: Hg > Zn > Cd and Pb, while those in *P. ostreatus* is in the order Cd > Hg and Zn. Lead is accumulated at minimal levels (Lasota *et al.*, 1990). In *P. ostreatus* bioconcentration factors for cadmium was found to decrease as its concentrations in the substrate increased. Thus, oyster mushroom has probably a regulative mechanism for cadmium intake (Favero *et al.*, 1990).

The results presented in Table 3, indicated that after Freeze drying Co, Cr, Cu, Fe, K, Mg, P, S and Se were present in highest level. In case of Oven dried samples, the elements found were Ca, Cl, Ni, Si and Zn and in sun drying samples only F, I and Sn were also present. These results indicated that due to different operational methods of

drying, there had been significant difference in respect of availability of elements. As mentioned by Manzi *et al.* (2004), the effect of the cooking process in particular, ash content, if compared with the other components shows a higher loss (53%) in dried mushrooms. The effect of the cooking process is generally explained as a decrease (evaporation) in the water content of the raw sample and, consequently, in nutrient concentration. Nevertheless, a decrease in the nutrient amounts can also be hypothesized to be due to interactions between different compounds, chemical reactions and/or thermal degradation. With the aim of understanding whether these reactions really occur, a calculation of the yield, after cooking, of each compound analyzed in their study and has been observed that after cooking 77% weight recovery takes place in respect to raw samples. They also found that recovery of all chemical components of cooked samples, is generally less than 100%, particularly in dried samples.

Table 2. Comparison of heavy metals and trace elements in three different dried mushrooms

Freeze Dried					Oven Dried					Sun Dried				
Heavy Metals	Net Counts	Weight %	Weight % Error	Remarks	Heavy Metals	Net Counts	Weight %	Weight % Error	Remarks	Heavy Metals	Net Counts	Weight %	Weight % Error	Remarks
Si	476	0.03	+/- 0.01	T	Si	766	0.06	+/- 0.01	P	Si	426	0.03	+/- 0.01	T
Cr	164	0.02	+/- 0.01	T	Cr	95	0.01	+/- 0.01	A	Cr	29	0	+/- 0.01	A
Co	135	0.02	+/- 0.02	A	Co	0	0	---	A	Co	0	0	---	A
Ni	9	0	+/- 0.02	A	Ni	47	0.01	+/- 0.02	A	Ni	0	0	---	A
As	0	0	---	A	As	70	0.04	+/- 0.05	A	As	0	0		A
Mo	0	0	---	A	Mo	0	0	---	A	Mo	0	0	---	A
Cd	2	0	+/- 0.04	A	Cd	363	0.05	+/- 0.05	A	Cd	0	0	---	A
Sn	2831	0.29	+/- 0.08	P	Sn	2487	0.34	+/- 0.09	P	Sn	3208	0.35	+/- 0.08	P
Hg	42	0.03	+/- 0.15	A	Hg	98	0.09	+/- 0.14	A	Hg	84	0.06	+/- 0.12	A
Pb	181	0.15	+/- 0.14	T	Pb	0	0	---	A	Pb	0	0	---	A
Al	753	0.05	+/- 0.01	P	Al	856	0.08	+/- 0.01	P	Al	787	0.06	+/- 0.01	P

From Table 4, it is evident that the ratio between Carbon and Nitrogen (C: N) which is considered to be an important factor for growing mushrooms, and in the same species it would not be varied. On the contrary, it was found that in both the cases of FD and SD C:N ratio was nearly 2:1 and 3:1 respectively and in OD it was near about 3:1.The possible explanation for such type of variation could be due to effect of temperature.

Table 5 represents the confirmatory-test results for As, Pb, Co and Ni of FD obtained by AAS. Three different scanned electron micrograms of the mushroom powder particles were also taken as presented in Plates 8a-b (FD); 9a-b (OD) and 10a-b (SD). With these

micrograms it is evident that the surface conditions of those mushrooms of common origin dried but it was not from a specific part or location of a fruit body. An earlier review (Hershko and Nussinovitch, 1998) dealt with electron microgram of *Agaricus bisporus*. Such type of microgram may be employed in quality control for detecting any adulteration with mushroom powder now available as commercial formulation.

Table 3. Chronological presentation of the highest elements in three dried processed mushrooms (based on w/w%)

Freeze Dried	Oven Dried	Sun Dried	Absent
Co	Ca	F	Mn
Cr	Cl	I	Mo
Cu	Ni	Sn	Na
Fe	Si		
K	Zn		
Mg			
P			
S			
Se			

Table 4. C/N Ratio of three Different Dried Mushroom Powder

Ratio	Freeze Dried	Oven Dried	Sun Dried
C : N	47.98 : 18.95[a]	45.04 : 14.88[b]	52.83 : 21.52[c]

[a]C : +/- 0.41, N : +/- 1.95; [b] C : +/- 0.38, N : +/- 1.94; [c] C : +/- 0.43, N : +/- 2.41;

Table 5. Confirmatory test results obtained from AAS in FD

Elements	Result
Arsenic	<0.01 mg/kg
Led	<0.05 mg/kg
Cobalt	Not Detectable
Nickel	<0.05 mg/kg

In a study Seeger (1982) demonstrated that the uptake of metals in mushrooms is in many respects different from that of plants. Reasons for the variations in the concentrations of all metals in mushroom species, the points for consideration should be the distance from polluted-area and age of mycelium. The latter factor seems to be of great importance, but it is very difficult to determine its effect. Knowledge on roles of trace elements in physiology of higher fungi has been limited. Concentrations of the elements in fruiting bodies are generally species-dependent. Substrate composition is also an important factor, but great differences exist in uptake of individual metals (Gast *et al.*, 1988; Michelot *et al.*, 1998; Tyler, 1982). Cadmium, mercury and copper are accumulated in fruiting bodies; levels of zinc and manganese are comparable in the fruiting body and in the relevant substrate, while concentrations of lead and iron are

lower in the fruiting body than in the substrate. The reported bio-concentration factors (Gast *et al.*, 1988; Seeger, 1982; Tyler, 1982) are 50-300 and 30-500 for cadmium and mercury, respectively, while they are only 10^{-1}-10^{-2} for lead.

Age of the fruiting body or its size is of less importance. Some authors report higher metal concentrations in younger fruiting bodies. This is explained by the transport of a metal from mycelium to the fruiting body during the start of fructification. During the increase in mass of the fruiting body the metal concentration decreases. The proportion of metal concentrations from atmospheric depositions seems to be of less importance due to the short lifetime of a fruiting body, which is usually 10-14 days. Kalac and Svoboda (2000) found that metal levels in fruiting bodies of wild growing mushrooms are considerably affected by the age of mycelium and by the interval between the fructifications. The highest metal concentrations are observed in the initial harvest wave of cultivated common mushroom *(Agaricus bisporus).* Knowledge of transport mechanisms of metals from mycelium to the fruiting body has been limited. Mercury transport is likely to be affected by sulphydryl group content in a protein carrier, while cadmium transport has another mechanism (Kojo and Lodenius, 1989). However, it is also evident that washing and peeling of *A. bisporus* decreased concentrations of cadmium, lead, copper and zinc by 30-40% (Zrodlowski, 1995). During bleaching of *A. bisporus* at 95-100°C for 15 min, losses of 45, 36, 23 and 4% were observed for manganese, iron, zinc and copper, respectively (Coskuner and Ozdemir, 1997). They also found that surprisingly, data on changes in trace element concentrations during preservation processes such as drying, freezing or sterilization, and with different culinary treatments are almost absent. Unfortunately, detrimental heavy metals were not determined in that work. In this present study, it has been dealt with detrimental heavy metals and trace elements and it has clearly evidenced that concentration of the minerals were varied on processing adopted.

Plate 1. Top View of a Freeze Dried Mushroom particle obtained by SEM

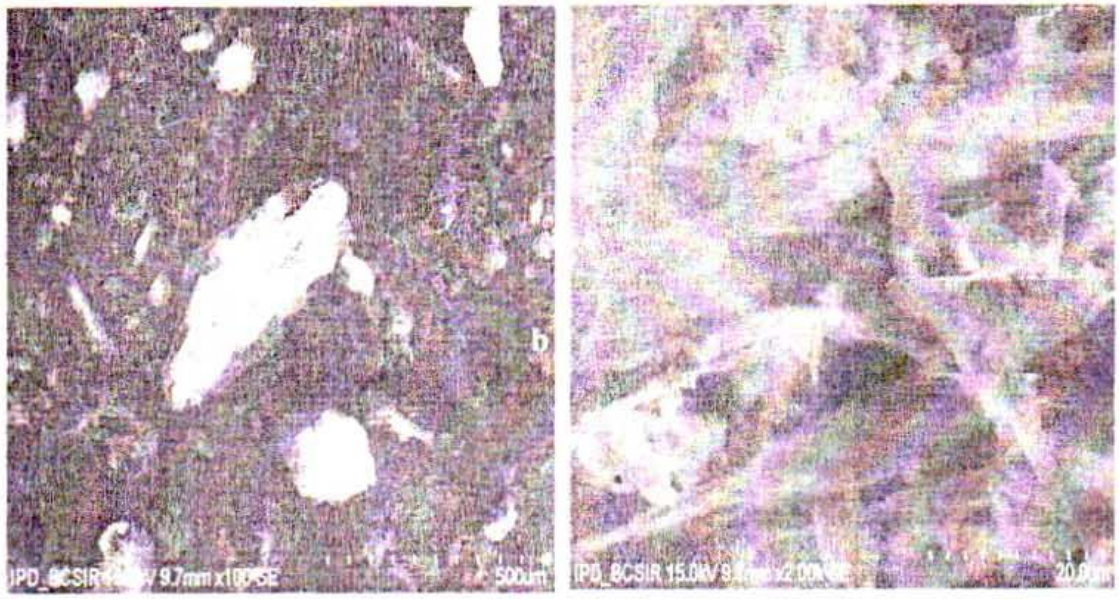

Plate 2. Top View of an Oven Dried Mushroom particle obtained by SEM

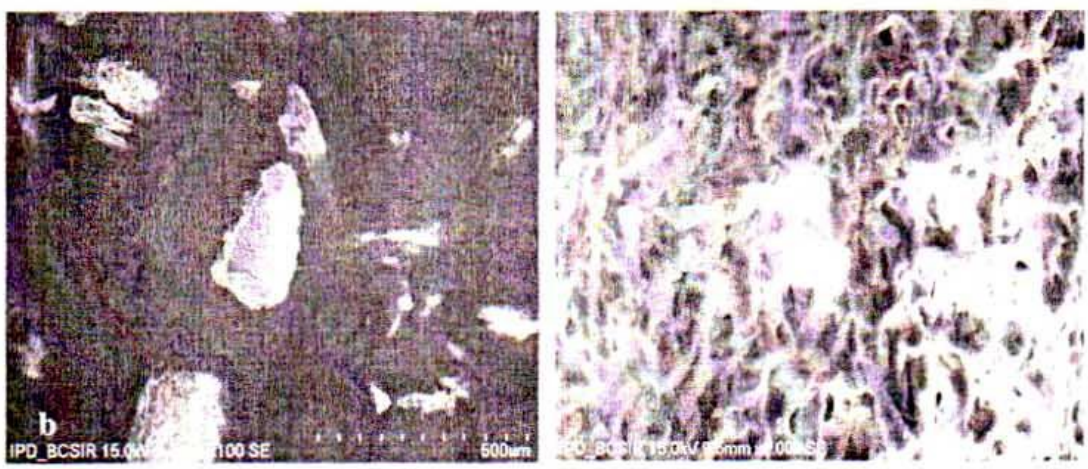

Plate 3. Top View of a Sun Dried Mushroom particle obtained by SEM

CONCLUTION

Energy Dispersive X-ray microanalysis (E-dx) is a nondestructive micro analytical methods by which we can get not only various dimension of picture but also scane the inorganic elements of a particular object. It provides semi-quantified results in relation to w/w% basis, but if run with specific standard it can provide quantitative results. However, we can easily find out the particular elements to consider the nutriceuticals or nutraceuticals properties of mushrooms. It can keep an immense contribution for exporting or importing mushrooms as because many countries have established statutory limits of it's own for the heavy metals and also toxic trace elements in edible mushrooms.

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Comparative Study on the Yield and Yield Related Attributes of Some Newly Introduced Strains of *Pleurotus cystidiosus* with *Pleurotus ostreatus* (PO_2)

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Abstract

Pleurotus ostreatus and four strains of *Pleurotus cystidiosus* were cultivated on sawdust and the yield and yield related attributes were compared with each other. The highest days required from stimulation to primordia initiation was recorded in strains-4 PC_4 followed by strains-2 PC_2, strains-3 PC_3, species-5 PO_2 *Pleurotus ostreatus*) and variety-1 PC_1. Highest number of effective fruiting bodies was found in PC_1 (127) followed by PC_2 (78), PC_4 (73), PC_3 (68) and was lowest in PO_2 (33). Thickness of pileus was highest in PO_2 (0.60cm) followed by PC_2, PC_4, PC_1 and PC_3. The diameter of pileus and the length of stipe ranged from 5.53 to 6.60 cm and 4.70 to 5.26cm respectively without any significant difference. Diameter of stipe was highest in PO_2 (0.83 cm) followed by PC_3, PC_1, PC_4, and PC_2. Highly significant variation was observed in biological yield, economic yield, and biological efficiency. Biological yield (157.0 g/packet), economic yield (152.3 g/packet), dry yield (24.67 g/packet) and biological efficiency (54.13%) were highest in PO_2 followed by PC_4, PC_1, PC_3 and lowest in PC_2.

Key Words: Growth, yield, *Pleurotus cystidiosus, Pleurotus ostreatus.*

INTRODUCTION

Among the edible mushroom species, oyster mushroom (*Pleurotus* spp.) is in the second position in the world and its cultivation has increased rapidly during the last decade (Roye, 2002). Oyster mushroom is widely cultivated in Bangladesh because the weather and climate are very suitable for its cultivation. Besides, different agricultural and industrial wastes such as straw, sawdust, sugarcane bagasse, cotton waste etc. which are used as the base material for mushroom production are available cheaply. Among these base materials sawdust is more available and cheap through out the year. *Pleurotus* spp. grow in nature on dead wood as saprophytes; in this sense sawdust is considered as an excellent substrate for oyster mushroom production (Amin *et al.*, 2007). Moreover, it requires less capital, less number of labour, but provides high yield per unit area and high market price. A number of experiments were carried out to evaluate the yield performance of various species of *Pleurotus* spp. but *Pleurotus cystidiosus* is a newly introduced variety in Bangladesh and no remarkable study has been conducted on it. Therefore, to have a clear understanding about the yield status of such variety, the present study is a modest attempt to examine the yield performance of some newly introduced *Pleurotus cystidiosus* variety in Bangladesh.

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MATERIALS AND METHODS

The study was carried out in the Department of Biochemistry, Sher-e-Bangla Agricultural University and in the culture house of National Mushroom Development and Extension Centre, Savar, Dhaka from January to March, 2008.

One species of *Pleurotus ostreatus* and four strains of *Pleurotus cystidiosus* were selected for the experiment. The substrate used for the experiment was sawdust and wheat bran was used as a supplement. The experiment considered 5 treatments with 3 replications.

V_1 = *Pleurotus cystidiosus* (PC_1)
V_2 = *Pleurotus cystidiosus* (PC_2)
V_3 = *Pleurotus cystidiosus* (PC_3)
V_4 = *Pleurotus cystidiosus* (PC_4)
V_5 = *Pleurotus ostreatus* (PO_2)

Preparation of spawn packets: Sawdust was used as a main substrate and wheat bran was used as supplement. For each 500gm spawn packet, 116.7gm sawdust, 58.23gm wheat bran and 1g $CaCO_3$ were mixed and moisture was adjusted at 65% by adding water. The mixture was filled into heat tolerant polypropylene bags of 7" x 10" size and their mouth were plugged by inserting water absorbing cotton and covered with brown paper and tied with a rubber band. Then the previously prepared bags were autoclaved at 121°C and 15 PSI for 1 hour. Each spawn packet was inoculated with the mother culture at the rate of two teaspoonfuls per packet. Bags were then incubated for mycelium running in the mycelium culture house at 25°C temperature. After 25 days of inoculation, when colonization was complete, the spawn packet was taken to the culture house.

Spawn packet culture in culture house: The spawn packets with mycelium were transferred to the culture house and the brown paper, rubber bands, cotton plug and plastic neck of the spawn packets were removed and the polypropylene bags were wrapped and tied with rubber bands. The plastic bags were opened by 'D' shaped cut on the shoulder side and the sheet was removed. The opened surface of substrate was scraped slightly with a blade for removing the thin whitish mycelial layer. The spawn packets then were then soaked in water for 15 minutes and kept for few minutes to remove excess water. The packets were placed separately side by side on the rack in the culture house.

Cultural condition: The relative humidity was 80-90% and the temperature was maximum 29.5°C and minimum 23°C. The relative humidity (RH %) and the temperature were maintained by watering thrice daily. Diffused day light and proper ventilation in culture house were maintained for fruiting body development of *Pleurotus* spp..

Data collection: The experiment was laid out following Completely Randomized Design (CRD) with 3 replications. Data on days required from stimulation to primordia initiation, approximate number of primordia, number of fruiting body, number of effective fruiting body, average weight of individual fruiting body, length of stipe, diameter of stipe, diameter of pileus, thickness of pileus, colour, biological yield (gm)/packet, economic

yield (gm/packet, dry yield gm/packet) and some other parameters were recorded. The data obtained from the experiment for every parameter were analyzed statistically using MSTAT-C computer programme. The mean values of all the parameters and the variance of each parameter were analyzed by Least Significance Difference Test.

RESULTS AND DISCUSSION

Days Required from Stimulation to Primordia Initiation: Days required from stimulation to primordia initiation ranged from 7 to 8 days without any significant differences (Table 1). The highest days required from stimulation to primordia initiation was found in PC_4 (8 days) followed by PC_2 and PC_3 (7 days) and the lowest days required from stimulation to primordia initiation was found in PC_1 (7 days) preceded by PO_2 (7.33days). The result is similar with Patra and Pani (1995) who reported that oyster mushroom took 4 to 8 days for initiation of primordia.

Number of primordia: The number of primordia obtained from 3 flushes in different treatment differed significantly at 1% level of significance (Table 1). The highest number of primordia was observed in PC_1 (212) and the lowest number of primordia was observed in PO_2 (87). The result was approximately similar to Ahmed (1998) who observed that the number of primordia/packet from 6 flushes ranged from 150 to 350 in case of oyster mushroom.

Number of fruiting body: The number of fruiting bodies obtained from 3 flushes in different treatments varied significantly at 1% level of significance and ranged from 61 to 149 (Table 1). The highest number of fruiting bodies was found in PC_1 (149) followed by PC_2 (96), PC_4 (92) and the lowest number of fruiting bodies was found in PO_2 (61) preceded by PC_3 (88). Comparatively similar result was found by Adamovic *et al.*, (1996).

Number of effective fruiting bodies: The number of effective fruiting bodies obtained from 3 flushes in different treatments varied significantly at 1% level of significance (Table 1). Highest number of effective fruiting bodies was found in PC_1 (127) followed by PC_2 (78), PC_4 (73) and PC_3 (68). The lowest number of fruiting bodies was found in PO_2 (33). Comparatively similar result was found by Adamovic *et al.*, (1996).

Weight of individual fruiting bodies (g): Weight of individual fruiting body in different treatments ranged from 1.06 g to 3.66 g and differed significantly at 1% level of significance (Table 1). The weight of individual fruiting body was highest in PO_2 (3.66 g) followed by PC_4 (1.66g), PC_3 (1.50g) and lowest in PC_1 (1.06 g) preceded by PC_2 (1.46g). Ahmed (1998) observed that the weight ranged from 1g to 4g in case of oyster mushroom. The result was approximately similar to that finding.

Thickness of pileus (cm): The thickness of pileus in different treatments differed significantly at 1% level of significance and ranged from 0.41 cm to 0.60 cm (Table 2). The highest thickness was found in PO_2 (0.60cm) and the lowest thickness was found in

PC_3 (0.41 cm). Alam *et al.* (2007) observed that the thickness of pileus ranged from 0.50 to 0.80 cm in case of oyster mushroom.

Table 1. Yield attributes of different varieties of *Pleurotus cystidiosus* and *Pleurotus ostreatus* grown on sawdust (From 3 flushes)

Treatments.	DRSPI (days)	No. of primordia /Packet.	No. of fruiting bodies /Packet.	No. of effective fruiting bodies/Packet.	Weight of individual fruiting bodies(g)
P. cystidiosus (PC_1)	7.00 a	212.0 a	149.0 a	127.0 a	1.06 c
P. cystidiosus (PC_2)	7.66 a	154.0 b	96.00 b	78.00 b	1.46 bc
P. cystidiosus (PC_3)	7.66 a	148.0 c	88.00 c	68.00 c	1.50 bc
P. cystidiosus (PC_4)	8.00 a	148.0 c	92.00 bc	73.00 bc	1.66 b
P. ostreatus (PO_2)	7.33 a	87.00 d	61.00 d	33.00 d	3.66 a
Level of Significance	N/S	**	**	**	**

In a column, means followed by a common letter are not significantly different at 5% level by LSD test. Level of Significance, (**) = 1% ; (N/S) =Not significant.

Diameter of pileus (cm): The diameter of pileus ranged from 5.53 cm to 6.60 cm without any significant difference (Table 2). The highest diameter of pileus was found in PO_2 (6.60cm) followed by PC_4 (6.50cm) and PC_3 (6.10cm) and the lowest diameter of pileus was found in PC_2 (5.53cm) preceded by PC_1 (5.60 cm). The similar result was obtained by Sarker *et al.* (2007a) who observed that the diameter of pileus ranged from 4.00cm to 5.50 cm in case of oyster mushroom.

Diameter of stipe (cm): The diameter of stipe differed significantly at 5% level of significance and ranged from 0.65 cm to 0.83 cm (Table 2). The highest diameter was found in PO_2 (0.83 cm) followed by PC_3 (0.77 cm), PC_1 (0.72 cm), PC_4 (0.72 cm) and the lowest diameter was found in PC_2 (0.65 cm). Sarker *et al.* (2007a) observed that the diameter of stipe was ranged from 0.70cm to 0.88 cm in case of oyster mushroom.

Length of stipe (cm): The length of stipe ranged from 4.70 cm to 5.26 cm without any significant difference (Table 2). The highest length of stipe was found in PC_4 (5.26 cm) followed by PO_2 (5.06cm) and the lowest length of stipe was found in PC_3 (4.70 cm) preceded by PC_1 (4.73 cm) and PC_2 (4.97 cm). More or less similar result was obtained by Alam *et al.* (2007) in case of oyster mushroom.

Biological yield (g/packet): Highly significant variation was observed on biological yield at 1% level of significance. The biological yield from 3 flushes ranged from 128.0 g to 157.0 g/packet (Table 3). The highest biological yield was found in PO_2 (157.0 g/packet) and the lowest biological yield was found in PC_2 (128.0 g/packet). The biological yield of PC_1 (136.0 g/packet) and PC_3 (134.0 g/packet) were statistically similar and significantly lower than PC_4 (146.0 gm/packet) and PO_2 (157.0 gm/packet) but higher than PC_2 (128.0 gm/packet). Alam *et al.* (2007) found that the biological yield of oyster mushroom ranged

from 120.60 g packet to 221.80 g/packet and the result of the experiment was found within that range.

Table 2. Physical properties of different varieties of *Pleurotus cystidiosus* and *Pleurotus ostreatus* grown on sawdust

Treatments.	Thickness of pileus (cm)	Diameter of pileus (cm)	Diameter of stipe(cm)	Length of stipe (cm)
P. cystidiosus (PC_1)	0.46ab	5.60 a	0.72ab	4.73 a
P. cystidiosus (PC_2)	0.54ab	5.53 a	0.65 b	4.97 a
P. cystidiosus (PC_3)	0.41b	6.10 a	0.77ab	4.70 a
P. cystidiosus (PC_4)	0.48ab	6.50 a	0.72ab	5.26 a
P.ostreatus (PO_2)	0.60 a	6.60 a	0.83 a	5.06 a
Level of Significance	**	N/S	*	N/S

In a column, means followed by a common letter are not significantly different at 5% level by LSD test. Level of Significance, (**) = 1% ; (*) = 5%; (N/S) =Not significant.

Table 3. Yield and Biological efficiency of different varieties of *Pleurotus cystidiosus* and *Pleurotus ostreatus* grown on sawdust. (From 3 flushes)

Treatments.	Biological yield g/packet	Economic Yield g/ packet	Dry yield g/packet	Biological Efficiency (%)
P. cystidiosus (PC_1)	136.0c	128.0c	22.67a	46.89 c
P. cystidiosus (PC_2)	128.0d	122.0d	20.67a	44.13 d
P. cystidiosus (PC_3)	134.0c	130.0c	21.67a	46.20 c
P. cystidiosus (PC_4)	146.0b	140.0b	23.67a	50.34 b
P. ostreatus (PO_2)	157.0a	152.3a	24.67a	54.13 a
Level of Significance	**	**	N/S	**

In a column, means followed by a common letter are not significantly different at 5% level by LSD test. Level of Significance, (**) = 1% ; (N/S) =Not significant .

Biological efficiency (%): The biological efficiency obtained from 3 flushes in different treatments varied significantly at 1% level of significance. Highest biological efficiency was found in PO_2 (54.13%) and the lowest biological efficiency was found in PC_2 (44.13%). The biological efficiency of PC_1 (46.89%) and PC_3 (46.20%) were statistically similar and significantly lower than PC_4 (50%) and PO_2 (54.13%) but higher than PC_2 (44.13%). The result was approximately similar to the findings of Alam *et al.* (2007) who observed that the biological efficiency ranged from 45.21% to 125.70% in case of oyster mushroom.

Economic yield (g/ packet): The economic yield obtained from 3 flushes in different treatments varied significantly at 1% level of significance. Economic yield was highest in PO_2 (152.3 gm/packet) followed by PC_4 (140 g/packet), PC_3 (130.0 g/packet) and was lowest in PC_2 (122.0 g/packet) preceded by PC_1 (128.0 g/packet). The result was relevant

with Sarker *et al.* (2007b) who observed the economic yield of oyster mushroom ranged from 100.30 gm to 150.10 gm/packet (from 3 flushes).

Dry yield (g/packet): The dry yield obtained from 3 flushes in different treatments ranged from 20.67g to 24.67 g/packet without any significant difference (Table 3). The highest dry yield was obtained in PO_2 (24.67g/packet) followed by PC_4 (23.67g/packet) and PC_1 (22.67g/packet) and the lowest dry yield was obtained in PC_2 (20.67g/packet) preceded by PC_3 (21.67 g/packet). The result was agreed with Alam *et al.* (2007) who reported that the dry yield of oyster mushroom ranged from 13.16 m to 26.04 m/packet (from 3 flushes).

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Performance of Poplar Mushroom (*Agrocybe aegerita*) on Different Substrates and Supplements

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Abstract

Gorjan sawdust, mango sawdust, acacia sawdust, teak sawdust, chamble sawdust, mara sawdust, mahogany sawdust, rain tree sawdust, mixed saw dust, cotton waste, paddy straw and paper waste were tested as substrates of poplar mushroom. The highest mycelium growth rate (0.36 cm/day) was observed in paddy straw though the days required to complete mycelium running was lowest in cotton wastes (33.50 days). The minimum time required for primordia initiation was observed in cotton wastes (8.5 days). The number of fruiting body and biological yield per packet was highest in mango sawdust. Wheat barn (WB), rice barn (RB) and maize powder (MP) and their combinations WB+RB (1:1), RB+MP (1:1), WB+MP (1:1) and WB+RB+MP (1:1:1) were tested as supplemented at the rate of 10% and 20% level to mixed sawdust. The shortest time (11.00 days) was required for completion of mycelium running in substrate supplemented with WB+RB (1:1) at 20 % level. The highest number of effective fruiting bodies (30.25) and highest biological yield (187.00 g/packet) was recorded in WB+RB (1:1) at 20% level.

Key words: *Agrocybe aegerita*, substrates, supplements, biological yield.

INTRODUCTION

Poplar Mushroom (*Agrocybe aegerita)* is a widely distributed, edible basidiomycete occurring on broad leaved trees. Production of this mushroom is relatively easy on sawdust medium, and its texture and flavor are excellent. It is cultivated on low cost substrates based mainly on agricultural and forest wastes (Nicolini *et al.*, 1987 and Philippoussis *et al.*, 2001). A wide geographical distribution has been reported: although rare in North America, it is found in Europe and Asia; it seems to prefer warm or mild climates (Wating, 1992). *A. aegerita* is a popular edible mushroom in southern Europe, especially in Italy (Pioppino mushroom), where it is also commercially cultured (Stamets, 2000). *Agrocybe aegerita* has gained wide popularity as an effective health food and has become one of the valuable mushrooms. In the last few years various ligno-cellulosic residues have been proposed as substrates for the growth of mushrooms (Donini, 2005). This cultivation underlines the current economically viable biotechnological methods for the conversion of waste plant residues from forests and agriculture (Wood and Smith, 1987). The cultivation of *Agrocybe aegerita* on agricultural wastes which are produced in large quantities not only reduces disposal problems caused by residue accumulation but also provides an economically acceptable alternative for the production of high quality

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food and fodder through production of proteins which might contribute significantly to the increase of farmers' income (Philippoussis *et al.*, 2000). Various basic raw materials or nutritive supplements such as the straw of various cereals, cotton residues, sugar-cane residues, sawdust, fruit pulp and peel, citric residues, banana leaves and coffee pulp have been tried to cultivate or to increase the productivity of this mushroom (Olivier *et al.*, 1999 and Peng, 1996). Lignocellulosic materials are generally low in protein content, insufficient for the cultivation of mushrooms, which requires nitrogen, carbon, phosphate and potassium. According to Moda *et al.* (2005) the supplementation of the substrate is commonly used to raise productiveness, which is evaluated by the biological efficiency. Among the most used cultivation supplements, cereal barns are sources of organic nitrogen, necessary for the growth of the mycelium mass, which may interfere in productiveness and biological efficiency of the fungus. This paper aimed to evaluate the cultivation of *Agrocybe aegerita* in different substrates (mango, garjon, accacia, teak, mahogany, chamble, mara, rain tree, mixed sawdust, paddy straw, cotton wastes and maize stalks) supplemented with different bran for the growth and yield.

MATERIALS AND METHODS

The inoculum of *Agrocybe aegerita* was obtained from the Tissue Culture Laboratory of the National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka. Twelve different substrates namely, gorjan sawdust, mango sawdust, acacia sawdust, teak sawdust, chamble sawdust, mara sawdust, mahogany sawdust, rain tree sawdust, mixed saw dust, cotton waste, paddy straw and paper waste were tested in the experiment. Three different supplements namely, wheat barn (WB), rice barn (RB) and maize powder (MP) and their combinations WB+RB (1:1), RB+MP (1:1), WB+MP (1:1) and WB+RB+MP (1:1:1) at the rate of 10% and 20% level were also tested in the experiment. The plant materials to be used as substrate were cut into small pieces (0.5-1 cm) and mixed with wheat bran at the rate of 30% for the study of performance of the substrates and other supplements were added to the mixed sawdust according to the treatments for the study the performance of supplement and their levels. Water was added to make the moisture content 65% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Polypropylene bags with a size of 25 cm × 17 cm were filled with 500g of substrate mixture. Their mouths were plugged by water absorbing cotton and covered with brown paper and tied with a rubber band. The bags were autoclaved at 121^0C and 1 kg/cm^2 pressure for 1 hour and then allowed to cool down. Each spawn packet was inoculated with the mother culture at the rate of two teaspoonfuls per packet. Bags were then incubated for mycelium running at 22 ± 2^0C temperature.

Experimental condition: The packets were kept in a dark room at 22 ± 2^0C temperature. Mycelium growth rate on each type of substrates and supplements was recorded in every two day interval. After completion of mycelium running, spawn packets were opened by topside opening and then the spawn packets were soaked in water for 15 minutes and inversed to remove excess water for another 15 minutes and transferred to the culture room at 25-30^{0}C temperature and 70-80% relative humidity; then the packets were placed

separately side by side on the floor of the culture house. The temperature and relative humidity of mushroom culture house was maintained by spraying water.

Analytical methods: This experiment was laid out in a completely randomized design with four replications. Data were analyzed following Gomez and Gomez, 1984 using MSTAT-c computer programme. Means were computed following Duncan's Multiple Range Test (DMRT) using the same computer programme.

RESULTS AND DISCUSSION

Effect of substrates on growth and yield of poplar mushroom: Mycelium growth rate in spawn packets varied remarkably on different types of substrate used (Table 1). The highest growth rate (0.36 cm/day) was observed in paddy straw which was significantly higher as compared to all the treatments. The growth rate of mycelium on mango sawdust was 0.312 cm/day which was statistically similar to chamblee sawdust (0.30 cm/day), mixed sawdust (0.30 cm/day), gorjan sawdust (0.27 cm/day) and cotton waste (0.27 cm/day). The lowest growth rate of mycelium (0.170 cm/day) was observed in rain tree sawdust which did not differ from that on mahogany sawdust (0.20 cm/day) and mara sawdus (0.20 cm/day).

Days to complete mycelium running was lowest in cotton wastes (33.50 days) which was statistically similar to mango sawdust (36.50 days). The highest time required for mycelium running was recorded in mahogany and rain tree sawdust (53.00 days) (Table 1).

Table 1. Effect of different substrates on the mycelium growth of poplar mushroom

Substrate	Mycelium growth rate (cm/day)	Days to complete mycelium running
Gorjan sawdust	0.27 b-d	41.00 d
Mango Sawdust	0.31 b	36.50 g
Acacia sawdust	0.25 c-d	39.00 f
Teak sawdust	0.24 de	40.00 e
Chamble sawdust	0.30 bc	50.00 c
Mara sawdust	0.20 ef	52.00 b
Mahogany sawdust	0.20 ef	53.00 a
Rain tree sawdust	0.17 f	53.00 a
Mixed saw dust	0.30 bc	37.00 h
Cotton waste	0.27 b-d	33.50 g
Paddy straw	0.36 a	41.00 d
Paper waste	0.22 de	41.00 d
CV (%)	12.10	0.95

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

Remarkable difference was recorded in stimulation to primordia initiation in different substrates (Table 2). The minimum time required for primordia initiation was observed in cotton wastes (8.5 days) which was statistically similar to all the treatments except mahogany sawdust (47.75 days), rain tree sawdust (29.50 days), mara sawdust (26.00

days) and paddy straw (26.00 days). The longest time (47.75 days) was required for the appearance of primordia in mahogany sawdust which was significantly higher as compared to all the treatments.

The highest number of fruiting body (20.50) was produced in mango sawdust which was statistically similar to chamble sawdust (18.75), cotton waste (18.50), accacia sawdust (16.50) and mixed sawdust (14.00) while the lowest number of fruiting body (3.75) was produced in mara sawdust (Table 2).

The highest stipe length of fruiting body (9.00 cm) was observed in mixed sawdust which did not differ significantly with that of paddy straw (8.87 cm). The lowest stipe length (5.00 cm) was found in paper wastes (Table 2).

The effect of substrates on the diameter of pileus and length of stalk of fruiting bodies was remarkable (Table 2). The highest pileus diameter (5.82 cm) was observed in mixed sawdust which was followed by paddy straw (5.52 cm) and mango sawdust (5.22 cm) and the lowest diameter of pileus (3.17 cm) was recorded in mahogany sawdust.

Considerable variation was found in yield of poplar mushroom in different substrates (Table 2). The highest biological yield (129.50 g/packet) was found in mango sawdust based substrate which was significantly higher as compared to other substrates. The lowest biological yield was recorded in mahogany sawdust (19.75 g) which did not differ significantly from that in rain tree sawdust (24.50 g).

Table 2. Effect of different substrates on growth, yield and morphological characteristics of poplar mushroom

Substrate	Days to primordia initiation	Number of Effective Fruiting body	Total yield (g) (TY)	Stipe length (cm)	Pelius diameter (cm)
Garjon sawdust	16.50 c	12.75 b-d	57.75 de	6.45 cd	3.97 c-e
Mango Sawdust	11.75 c	20.50 a	129.50 a	7.07 c	5.22 a-c
Accacia awdust	15.25 c	16.50 a-c	50.25 e	5.57 de	3.95 c-e
Teak sawdust	12.50 c	7.00 de	35.75 f	5.87 de	4.25 c-e
Chamble sawdust	14.75 c	18.75 ab	107.3 b	5.52 de	3.75 de
Mara sawdust	26.00 b	3.75 e	37.25 f	5.45 e	4.17 c-e
Mahogany sawdust	47.75 a	5.25 e	19.75 g	5.75 de	3.17 e
Rain tree sawdust	29.50 b	5.25 e	24.50 g	5.60 de	3.20 e
Mixed saw dust	11.50 c	14.00 a-c	76.00 c	9.00 a	5.82 a
Cotton waste	8.50 c	18.50 ab	61.00 d	7.95 b	3.70 de
Paddy straw	26.00 b	13.50 bc	51.50 e	8.87 a	5.52 ab
Paper waste	10.75 c	10.0 c-e	34.75 f	5.00 e	4.55 b-d
CV (%)	18.85	34.00	9.76	9.24	19.01

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

Effect of different supplements to mixed sawdust on growth and yield of poplar mushroom: Significant difference was observed in time required for primordia initiation and it ranged from 11.00 to 23.50 days. The lowest time (11.00 days) was recorded in the

WB+RB (1:1) at 20 % level which was statistically lower as compared to that of other treatments except WB+RB (1:1) at 10% and RB at 20%. The maximum time (23.50 days) was required in MP at 10% level (Table 3).

The highest number of effective fruiting bodies (30.25) was recorded in WB+RB (1:1) at 20% level which was statistically similar to treatments RB+MP (1:1) at 20%, WB+MP at 20% and MP at 20% level. The lowest number of fruiting bodies (10.50) was observed in RB at 10% level (Table 3). Yoshida *et al.* (1993) also reported similar results where the number of effective fruiting bodies increased when the substrate was mixed with wheat bran, rice bran and curd refuse.

The highest biological yield (187.00 g/packet) was recorded in WB+RB (1:1) at 20% level which was significantly higher as compared to all the treatments. The lowest biological yield (41.50 g/packet) was observed in WB+RB+MP (1:1:1) at 10% level (Table 3).

Table 3. Effect of different supplement on the growth and yield of poplar mushroom

Supplements	Days to primordia initiation		Number of fruiting body		Biological yield (g/packet)	
	Supplement		Supplement		Supplement	
	10%	20%	10%	20%	10%	20%
Wheat barn (WB)	19.25 bc	15.75 de	13.25 fg	22.50 b-e	112.50 f	175.00 b
Rice barn (RB)	14.50 d-f	13.00 e-g	10.50 g	14.50 fg	77.50 h	103.80 f
Maize powder (MP)	23.50 a	21.25 ab	17.75 d-g	24.25 a-d	90.25 g	135.50 de
WB+RB 1:1	11.75 fg	11.00 g	20.25 b-f	30.25 a	125.8 e	187.00 a
RB+MP 1:1	21.50 ab	19.00 bc	15.00 e-g	26.00 ab	78.50 h	141.80 cd
WB+MP 1:1	21.50 ab	16.75 cd	17.75 c-g	25.50 a-c	101.00 fg	148.50 c
WB+RB+MP 1:1:1	23.00 a	22.00 ab	10.75 g	11.75 g	41.50 j	57.50 i
C. V.	7.47		13.79		5.34	

In two columns of the same parameter, means followed by a common letter are not significantly different at 5% level by DMRT

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