ISSN 1995-0683

# Bangladesh Journal of Mushroom

Volume 1 Number 2 December 2007

National Mushroom Development & Extension Centre
Department of Agricultural Extension
Ministry of Agriculture
Sobhanbag, Savar, Dhaka-1340
Bangladesh

**Published by :** **Sheikh Md. Ruhul Amin**
Project Director
Mushroom Development Project
National Mushroom Development and Extension Centre
Department of Agricultural Extension, Ministry of Agriculture
Sobhanbag, Savar, Dhaka.

**Printed by :** **Bersha (Pvt) Ltd.**
8/3, Babupura, Nilkhet, Dhaka-1205
Phone: 8617158, 8611433

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

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**Volume 1** **Number 2** **December 2007**

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# Bangladesh Journal of Mushroom

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*Books:*

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# Bangladesh Journal of Mushroom

**Volume 1 Number 2 December 2007**

## Contents

Bangladesh J. Mushroom. 1(2): 1-7, 2007 (December)

# Nutritional Analysis of Dietary Mushroom- *Pleurotus florida* Eger and *Pleurotus sajor-caju* (Fr.) Singer

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## Abstract

Mushroom cultivation has been started recently in Bangladesh. People in Bangladesh are still not very aware of the nutritional and medicinal importance of mushrooms. In this study, the nutritional values of two species of mushroom (*Pleurotus sajor-caju* and *Pleurotus florida*) which are very popular among the mushrooms cultivated in Bangladesh have been determined. These mushrooms are rich in proteins (20-25%) and fibers (22-23% in dry sample) and contain a lower amount of lipid (4 to 4.5%). The carbohydrate contents range from 39 to 43% (on the basis of dry weight). *Pleurotus sajor-caju* and *Pleurotus florida* are also rich in mineral contents (total ash content is 8-9.5%). The pileus and gills are protein-rich and stipe is fiber-rich. The moisture content of mushrooms ranges from 87 to 87.5%. Data of this study suggest that mushrooms are protein and fiber rich food.

**Key words:** Protein, lipid, fiber, carbohydrate minerals and oyster mushrooms.

## 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, 1996). There is a common saying that "medicines and foods have a common origin" (Kaul, 2001). Dietary mushrooms provide a wide variety of medicinal properties and they are active against certain life-threatening diseases. Major medicinal properties attributed to mushrooms include anticancer, antibiotic, antiviral activities, immune response stimulating effects and blood lipid lowering effects. Mushrooms of *Pleurotus* species are also rich in medicinal values. *Pleurotus florida* has antioxidant and antitumor activities in experimental animals (Nayana and Janardhanan, 2000 and Manpreet *et al.* 2004) *Pleurotus sajor-caju* inhibits hypertensive effects through its active ingredients which affect the renin- angiotensin system (Chang, 1996), *P. ostreatus* possesses antitumor activity (Yoshioka *et al.*, 1985) and hypoglycaemic effects in experimentally induced diabetic rats (Chorvathova *et al.*, 1993). *Pleurotus* species are very much effective in reducing harmful plasma lipids (Nuhu Alam *et al.*, 2007) and thus reduce the chance of atherosclerosis and other cardiovascular and artery- related disorders. These medicinal properties might be due to the presence of

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some important components in dietary mushrooms. Nutritional analysis of several mushroom species of different origins had been carried out in many laboratories in abroad. But nutritional values of locally cultivated mushrooms remain speculative. Moreover nutritional composition is affected by many factors; these include differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting, specific portion of the fruiting bodies used for analysis, time interval between harvest and measurement methods (Benjamin, 1995). People in Bangladesh are still not very aware of nutritional and medicinal importance of mushrooms. The history of mushroom cultivation is very recent in Bangladesh. Only some species of mushrooms are now cultivated in this country and among these *Pleurotus sajor-caju* and *Pleurotus florida* are popular and widely accepted after *Pleurotus ostreatus* (Ruhul Amin *et al.*, 2007). Thus one of the objectives of the present study was to analyze the nutritional values of selected oyster mushroom.

## MATERIALS AND METHODS

This study was carried out in the Department of Biochemistry and Molecular Biology, Jahangirnagar University and 'Quality Control and Quality Assurance' laboratory of National Mushroom Development and Extension Centre, Savar, Dhaka as well as the laboratory of Food Technology Division, Bangladesh Atomic Energy Commission, Savar, Dhaka from March to November, 2007. Mushroom was cultivated and harvested in culture house of National Mushroom Development and Extension Centre.

**Moisture Analysis:** Twenty gram of fresh mushroom was weighed into a weighed moisture box (*A&D company ltd. N 92; P1011656, Japan*) and dried in an oven at 100-105$^0$C and cooled in a dessicator. The process of heating and cooling was repeated till a constant weight was achieved. The moisture content was calculated as following equation:

Moisture (%) = (Initial weight- final weight) × 100 /Weight of sample
(Raghuramulu *et al.*, 2003)

**Determination of Total Protein:** Five gram of grinded mushroom was taken with 50 ml of 0.1N NaOH and boiled for 30 minutes. The solution was cooled in room temperature and centrifuged at 1000g by a table centrifuge machine (*DIGISYSTEM: DSC-200T; Taiwan*)The supernatant was collected and total protein content was measured according to the method of Lowry *et al.* (1951). For the determination of protein content from fresh mushroom, 5g was taken with 50ml phosphate buffer and homogenized with a tissue homogenizer (*Polytron: PT 1200*). Five ml of homogenized was taken with 50 ml of 0.1N NaOH and protein content was determined as mentioned above.

**Determination of Total Lipid:** Total lipid was determined by slight modified method of Folch *et al.* (1957). Five gram of grinded mushroom was suspended in 50 ml of chloroform: methanol (2:1 v/v) mixture then mixed thoroughly and let stand for 3 days. The solution was filtrated and centrifuged at 1000g by a table centrifuge machine. The

upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid. For the determination of total lipid from fresh mushroom, 5g was taken with 50ml phosphate buffer and homogenized with a tissue homogenizer. Five ml of homogenized was taken with 50 ml of chloroform: methanol (2:1 v/v) mixture and lipid content was determined as mentioned above.

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

**Determination of Total Ash:** One gram of the sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5-6 hours at 600$^0$C. It was then cooled in a dessicator and weighed. To ensure completion of ashing, the crucible was then heated in the muffle furnace for 1h, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or grayish white in color. Then total ash was calculated as following equation:

Ash content (g/100g sample) = Wt of ash × 100 / Wt of sample taken
(*Raghuramulu et al.*, 2003)

**Total Carbohydrate Estimation:** The content of the available carbohydrate was determined by the following equation:

Carbohydrate (g/100g sample) =
100 – [(Moisture + Fat + Protein + Ash + Crude Fiber) g/100g]
(Raghuramulu *et al.*, 2003)

**Mineral Analysis:** Total ash was taken for the analysis of mineral contents. Two ml of conc. $HNO_3$ was added to the ash and heated for 2 minutes. One drop of hydrogen peroxide was added into the solution to remove turbidity. The solution was then transferred into a volumetric flask and total volume was made 50ml by adding deionized water. This was then used to analyze the contents of calcium (Ca), iron (Fe), manganese

(Mn), magnesium (mg), zinc (Zn), Selenium (Se) and arsenic (As) by flame and graphite method with atomic absorption spectrophotometer (*PERKIN ELMER: AS 80*).

## RESULTS AND DISCUSSION

Several nutritional parameters were measured for both the fresh and dry mushrooms. Table 1 shows the nutritional parameters of fresh mushrooms and Table 2 shows the nutritional parameters of dry mushrooms. The moisture contents of *Pleurotus sajor-caju and P. florida* were found about 87.0 and 87.5% respectively. Hundred gram of fresh *P. sajor-caju* contains 3-3.6 g of proteins, 0.52-0.62 g of lipids, 2.8-3.1 g of fiber and 5.0-5.3 g of carbohydrates. In case of fresh *P. florida* these are as follows: 2.5-2.75 g of proteins, 0.5-0.6 g of lipids, 2.9-3.1 g of fiber and 5.0-5.6 g of carbohydrates.

The protein, lipid, fiber and carbohydrate contents in 100g of dried *P. sajor-caju* were found as 23-26 g, 4.2-4.6 g, 22.-23.6g and 37-41.5 g respectively. 100g of dried *P. florida* contains 19-22g of proteins, 4-4.6 g of lipids, 22-24.6 g of fiber and 40-45 g of carbohydrates. The protein content of *P. sajor-caju* was found 10-15% greater than that of *P. florida* (difference is significant). Also a total fat content is slightly greater in *P. sajor caju* and *P. florida* contains more amounts of carbohydrates and fibers than *P. sajor-caju,* but these difference is not significant. Mushrooms are also rich in mineral contents. The total ash content found in fresh *P. sajor-caju* and *P. florida* were 1-1.2 g and 1.1-1.2 g respectively. In case of dry sample these were 8-8.6 g and 8.6- 9.5 g respectively (Table 2).

**Table 1. Nutrient contents of fresh oyster mushrooms (g/100g)**

| Mushroom Species | Moisture (%) | Protein | Lipid | Fiber | Ash | Carbohydrate |
|---|---|---|---|---|---|---|
| *P. sajor-caju* | $87.0\pm 0.4^a$ | $3.26\pm0.33^a$ | $0.57\pm0.05^a$ | $2.97\pm0.17^a$ | $1.1\pm0.1^a$ | $5.09\pm0.19^a$ |
| *P. florida* | $87.5 \pm .35^a$ | $2.6\pm0.13^b$ | $0.54\pm0.07^a$ | $3.0 \pm 0.12^a$ | $1.13\pm.07^a$ | $5.24\pm0.4^a$ |

Results show mean ± SEM of 5 trials. Values in the same column that do not share a common superscript are significantly different at P<0.05 (one way ANOVA then LSD post hoc comparison)

**Table 2. Nutrient contents of dried oyster mushrooms (g/100g)**

| Mushroom Species | Protein | Lipid | Fiber | Ash | Carbohydrate |
|---|---|---|---|---|---|
| *P. sajor-caju* | $24.63\pm1.51^a$ | $4.41\pm 0.2^a$ | $22.87\pm0.8^a$ | $8.28\pm0.29^a$ | $39.82\pm1.73^a$ |
| *P. florida* | $20.56\pm 1.45^b$ | $4.30\pm0.29^a$ | $23.29\pm1.3^a$ | $9.02\pm0.48^a$ | $42.83\pm 2.54^a$ |

Results show mean ± SEM of 5 trials. Values in the same column that do not share a common superscript are significantly different at P<0.05 (one way ANOVA then LSD post hoc comparison)

Table 3 shows the contents of some important minerals.100g of dried *P. sajor-caju* contains Ca (22.15mg), Fe (33.45mg), Mg (20.22mg), Mn (2.87mg), Zn (20.9mg), Se (25µg) and As (95µg). And 100g of *P. florida* contains Ca (33.7mg), Fe (43.2mg), Mg (13.4mg), Mn (2.7mg), Zn (16mg), Se (13.2µg) and As (83 µg). The total ash content was

found slightly higher in *P. florida* (not significant). The contents of calcium and iron were significantly greater in *P. florida*. And *P. sajor-caju* contained greater amounts of zinc, magnesium and selenium (significant). The variation in manganese and arsenic content in these two species does not vary significantly. The findings in nutritional analysis of this study are comparable to the reports by Chang, (1980) and Bano and Rajarathnam, (1982).

**Table 3. Mineral contents of dried oyster mushrooms (mg / 100g)**

| Elements | *P. sajor-caju* | *P. florida* |
|---|---|---|
| Calcium (Ca) | $22.15\pm2.3^{a}$ | $33.7\pm1.9^{b}$ |
| Iron (Fe) | $33.45\pm3.8^{a}$ | $43.2\pm4.0^{b}$ |
| Zinc (Zn) | $20.9\pm1.5^{a}$ | $16\pm0.9^{b}$ |
| Magnesium (Mg) | $20.22\pm1.2^{a}$ | $13.4\pm3.1^{b}$ |
| Manganese (Mn) | $2.87\pm0.5^{a}$ | $2.7\pm0.3^{a}$ |
| Selenium (Se) | $0.025\pm0.004^{a}$ | $0.0132\pm0.003^{b}$ |
| Arsenic (As) | $0.095\pm0.02^{a}$ | $0.083\pm0.009^{a}$ |

Results show mean ± SEM of 3 trials. Values in the same row that do not share a common superscript are significantly different at $P<0.05$ (one way ANOVA then LSD post hoc comparison)

Figure 1 and 2 show the variation of nutritional parameters among the different parts of mushrooms. From each figure it can be shown that pileus and gills are richer in protein (about 50-60%), lipid (30-60%) and ash content (5-10%) than stipe. On the other hand the stipe is richer in fiber (40-50%) and carbohydrate content (10-15%). The variation in protein, fat and fiber is significant.

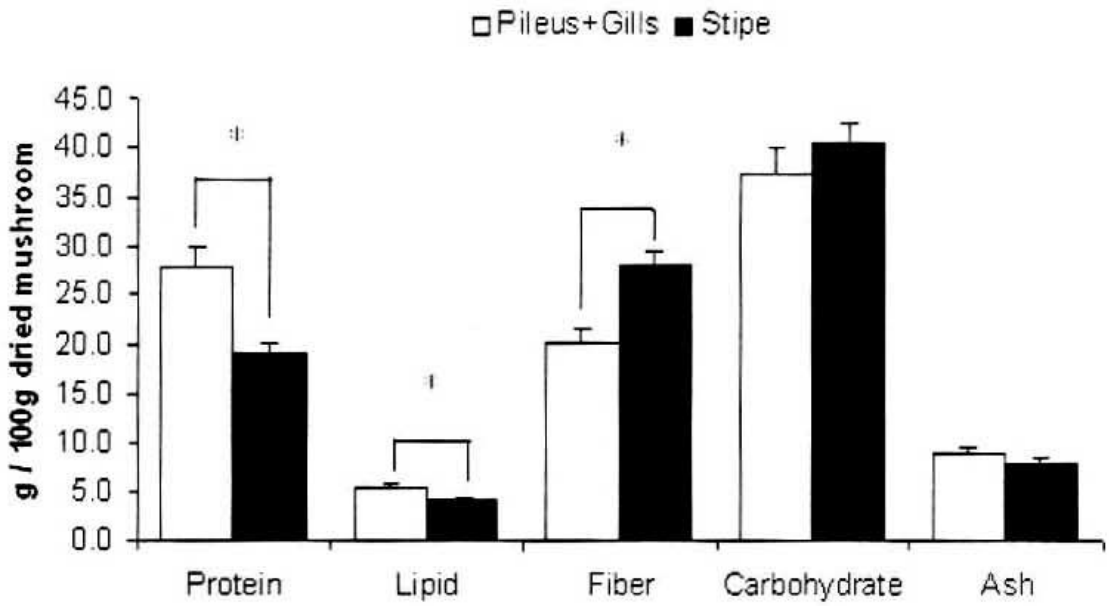


**Fig. 1. Nutritional variation in different parts of *Pleurotus sajor-caju*: Bars show mean + SEM of 5 trials. Data was analyzed by one way ANOVA and then post hoc LSD test. * indicates difference is significant at $P\leq0.05$ level**

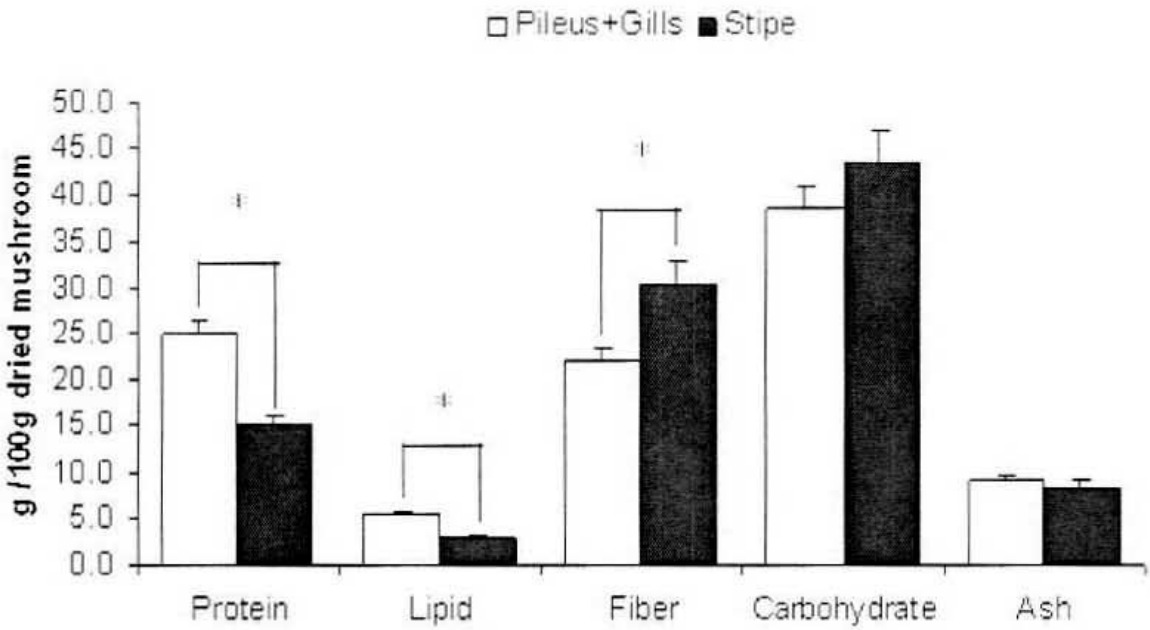


**Fig. 2. Nutritional variation in different parts of *Pleurotus florida*: Bars show mean + SEM of 5 trials. Data was analyzed by one way ANOVA and then post hoc LSD test. * indicates difference is significant at P≤0.05level**

These data suggest that dietary oyster mushrooms are good source of protein and fiber. Mushrooms are also rich in mineral or ash content but the fat or lipid content is very low. This composition of mushrooms makes them foods with high nutritional and medicinal values. Protein is an important nutritional component and protein deficiency is the world's most serious human nutritional problem, especially in third world countries like Bangladesh. So mushroom is a promising food that may overcome protein-energy malnutrition problem in the third world. The protein, fiber, mineral, carbohydrate and fat contents of oyster mushroom might make it an ideal vegetable for diabetic, cancer and heart patients.

## ACKNOWLEDGEMENT

We would like to thank Md. Shamsul Islam and Md. Afjal Hossain (Food Technology Division, Bangladesh Atomic Energy Commission, Savar, Dhaka) and Mafroz Ahmed Basunia (National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh) for their support in this study.

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Bangladesh J. Mushroom. 1(2): 9-20, 2007 (December)

# Performance of Different Substrates on the Growth and Yield of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer

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## Abstract

The experiment was carried out during January to April 2003 to find out the performance of different cheap agricultural and household by-products, grasses and weeds as substrate available in Bangladesh. Mycelium growth rate and time required to complete mycelium running in spawn packet varied significantly in different substrates. The minimum duration to complete mycelium running was 17.75 days in waste paper, which differed significantly from that in all other substrates. Significant variation was found in duration from stimulation to primordia initiation, primordia initiation to first harvest and stimulation to first harvest in different substrates. The minimum duration required from stimulation to first harvest was observed in sugarcane bagasse (6.75 days), which was statistically identical to that in waste paper, wheat straw and sawdust (7.00 days). The number of fruiting body was positively correlated with biological efficiency, biological yield and economic yield of oyster mushroom. The number of fruiting body grown on different substrates differed significantly and the highest number of fruiting body per packet (183.25) was recorded on waste paper, which was significantly higher as compared to all other substrates. The lowest number of fruiting body (19.25) was observed in water hyacinth. Significant variation in biological efficiency, biological yield and economic yield of oyster mushroom were observed in different substrates. The highest economic yield (225.43 g/packet) was estimated from the waste paper followed by wheat straw (215.72 g/packet). The economic yield on sugarcane bagasse was 191.98 g/packet, which was statistically identical to that grown on rice straw (183.28 g/packet), kash (182.93 g/packet) and ulu (175.15 g/packet). The economic yield on sawdust was 160.40 g/packet, which was statistically identical to that on ulu. The lowest economic yield was observed in water hyacinth (33.59 g/packet). No fruiting body and economic yield were obtained from para and nepier grasses. Performances of the substrates were compared based on benefit cost ratio (BCR). The highest BCR (6.50) was estimated when wheat straw was used as substrate followed by sugarcane bagasse (5.90), waste paper (5.65), rice straw (5.58) and kash (5.25). The lowest BCR was obtained from water hyacinth (1.05) followed by ulu (4.74) and sawdust (4.90).

**Key word:** Different substrates, growth, yield and *Pleurotus ostreatus*.

## INTRODUCTION

The substrate is an important item for growing of oyster mushroom. Bano and Srivastava (1962) reported that maize cobs, straw of all cereals, waste paper, wood shaving, saw dust, vegetable waste and food industry waste are sufficient to meet the growth requirements of most of the species of *Pleurotus* under cultivation. A lot of work has been done in the world on the suitability of various substrates for *Pleurotus* production. *Pleurotus sajor-caju* and *P. flabellatus* are found to grow on various substrates, namely

rice straw, wheat straw, ragi straw, maize cob, waste cotton, banana pseudostem and waste paper (Jandaik, 1974, Bano *et al.*, 1978 and Sivaprakasan *et al.*, 1979). Thilagavathi *et al.* (1991) observed maximum yield of *P. sajor-caju* from banana pseudostem. Similarly, various workers have suggested different substrates for *Pleurotus* cultivation. Some of them are jower straw and groundnut pod (Khandar *et al.*, 1991), wheat straw (Gupta and Langar, 1988), rubber wood waste and sawdust of rubber (Mathew *et al.*, 1991), oil palm mesocarp waste (Babu and Nair, 1991), *Eupatorium odoratum* and Water hyacinth (Das *et al.*, 1988), fermented coffee pulp (Upadhyay and Sohi, 1988) for different species of *Pleurotus*. But in Bangladesh limited works on substrate have been done. Only Ahmed (1998) studied the suitability of *P. sajor-caju* on saw dusts of mango, jackfruit, koroi, sugarcane bagasse, jute stick, rice straw, waste cotton and maize cobs. No work is done on the performance of *P. ostreatus* on the agricultural by-products, wastes, grasses or weeds as substrates available in Bangladesh though Ruhul Amin (2002) found that the *Pleurotus ostreatus* is the best performing oyster mushroom in Bangladesh.

The National Mushroom Development and Extension Centre (NAMDEC), Savar grows oyster mushroom using sawdust. But, sawdust in our country has been becoming scarce due to its use in huge amount in developing poultry industries and its price is also increasing day by day. In the mid of 80$^{th}$, some wild grasses were successfully used for mushroom cultivation in the laboratory of Fujian Agricultural University (Xia, 1997). Therefore, it is necessary to identify the alternative suitable substrates for mushroom production that will be easily available, low cost and more yielding. Considering the facts the present experiment was undertaken to find out the effect of different substrates on the growth and yield of *Pleurotus ostreatus*.

## MATERIALS AND METHODS

The experiment was conducted at NAMDEC, Savar and Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during January to April 2003.

**Preparation of pure culture:** Pure culture of *Pleurotus ostreatus* was prepared on Potato Dextrose Agar (PDA) medium containing infusion of 200g of peeled and sliced potato, 20g of dextrose and 18g of agar. The mixture was boiled on gas burner until the agar dissolved. 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 with the inocula of the fungus. Pieces of inner tissues of stalks were used as inocula. A fresh and full grown sporophore of oyster mushroom was surface-sterilized with 70% ethanol by rubbing cotton soaked in alcohol. The stalk was peeled from outside. Tissues were collected from inner region of stalk of the sporophore. The tissues were cut into small pieces and placed on the solidified Petri dish containing PDA. After inoculation, the PDA plates were covered with cellophane paper. All operations were done under sterile condition in a clean bench. The inoculated Petri dishes were transferred to a growth chamber maintaining temperature at 27$^0$C and incubated for 8 days. This eight days old PDA culture was used for inoculation of mother culture.

**Preparation of mother culture**: Mother culture was prepared by mixing sawdust and wheat bran at the ratio of 2:1. Calcium carbonate was used at the rate of 0.2% of the mixture. The moisture level of the mixture was maintained at 65% by adding tap water. Polypropylene bags of 18 X 25 cm size were filled with 200 g of the above prepared mixture and packed tightly. The neck of the bag was prepared by using heat resistant plastic neck. A hole of about 2/3 deep of the volume of the bag was made at the center with a sharp end stick for space to put inoculum. The neck was plugged with cotton and covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for one hour at 120°C under 1 kg/cm$^2$ pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. A piece of pure PDA culture medium containing mycelium of oyster mushroom was placed aseptically in the hole of mother culture packet and the packet was again plugged as mentioned before. Then the inoculated packets were placed on a wooden rack in the laboratory at 25 ± 2°C temperature for incubation. The medium of the mother culture was colonized by the fungus as manifested by white colony growth of mycelium within 15-16 days of inoculation. The fully colonized packets were used for spawning.

**Preparation of substrates and spawn packets:** Ten different substrates namely, sawdust, rice straw (*Oyrza sativa*), water hyacinth (*Echhornia crassipes*), wheat straw (*Triticum aestivum*), sugarcane bagasse (*Saccharum officinarum*), ulu (*Imperata cylindrica*), kash (*Saccharum spontaneum*), waste paper, para grass (*Brachiaria mutica*) and nepier grass (*Pennisetum typhoides*) were tested in the experiment. The plant materials were cut into small pieces (0.5- 1 cm) and mixed preparing with nutrient materials, wheat bran at the ratio of 2:1. 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 of 22.5 cm X 30 cm size were filled with 500 g of substrate mixture. The procedure of packet preparation, plugging, sterilization and incubation were the same as mentioned under the section describing preparation of mother culture. Each spawn packet was inoculated with the mother culture at the rate of two teaspoonfuls per packet. After inoculation, the packets were incubated in the laboratory at about 25°C temperature. Growth of mycelium in the spawn packets was completed within about 18 to 61 days.

**Cultivation of *Pleurotus ostreatus*:** The brown paper, rubber bands, cotton plug and plastic neck of the spawn packets were removed and the mouths of polypropylene bags were wrapped and tied with rubber bands. Two ends, opposite to each other of the upper position of the plastic bag were opened by removing the plastic sheet with a scalpel making 'D' shape opening. The opened surface of the substrate was scraped slightly with a teaspoon for removing the thin whitish mycelial layer. The spawn packets were soaked in water for 15 minutes and inverted for another 15 minutes to remove excess water. The packets were placed separately side by side on the rack of Mushroom Culture House. The relative humidity and temperature of culture house were maintained at 70-80% and 20-30°C respectively by watering twice daily. Diffused daylight and proper ventilation in culture house were maintained for fruiting body development of *Pleurotus ostreatus*. The mushroom fruiting body was harvested by gentle twisting before the mushroom showed any splitting on the edges. After first harvest the scrapping and soaking were done again

as done during first opening of the packet for subsequent harvests. Watering and other operations were done as mentioned before.

**Data collection and statistical analysis:** The experiment was laid out following completely randomized design (CRD) with four replications. Data on mycelium growth rate, days to complete mycelium running, stimulation to primordial initiation, primordial initiation to harvest, stimulation to harvest, total harvest, yield of mushroom, dimension of sporophores, and some other parameters 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

**Mycelium growth rate**: Mycelium growth rate (MGR) in spawn packet ranged from 0.30 to 0.92 cm/day. The highest MGR was observed on wheat straw, which was statistically similar to waste paper. The lowest MGR was recorded on para grass, which was not significantly different with nepier grass and water hyacinth. Mycelium running rate on waste paper, kash, ulu and rice straw was statistically similar but significantly higher as compared to sawdust, water hyacinth, para grass and nepier grass (Table 1).

The presence of right proportion of alpha-cellulose, hemi-cellulose and lignin was the probable cause of higher rate of mycelium running in wheat straw and waste paper substrates. The substrates nepier grass, para grass and water hyacinth gave lower MGR which might be due to the presence of different kinds of polyphenolic substances in them as suggested by Wang (1982) and low content of cellulose (Gohl, 1993). Suitable C:N ratio might be responsible for the higher mycelial growth in wheat straw, waste paper, rice straw, ulu and kash. Quimio and Sardsud (1981) supported the results, who found that the optimum carbon/nitrogen ratio for mycelial growth of *P. ostreatus* was ranged from 40:1 to 90:1.

**Days to complete mycelium running**: Days to complete mycelium running in spawn packet ranged from 17.75 to 60.50 days on different substrates (Table 1). Significantly the lowest days to complete mycelium running was recorded on waste paper. Days to complete the mycelium running on sawdust, rice straw and ulu were statistically similar but insignificantly lower as compared to other substrates. Maximum days were required to complete mycelium running on nepier grass, which was followed by para grass, water hyacinth and kash. Their effect on mycelium running time was significantly different. The mycelium running time on kash, wheat straw and sugarcane bagasse was also statistically similar (Table 1).

The appreciable days to complete mycelium running of oyster mushroom on different substrates might be due to variations in their chemical composition and C:N ratio as reported by Bhatti *et al.* (1987). The results recorded on waste paper, sawdust, rice straw,

wheat straw, ulu and kash were more or less similar to the findings of Shah *et al.* (2004). He reported that the spawn running took 16-25 days after inoculation. Similar results were also reported by Tan (1981). Baysal *et al.* (2003) found the fastest spawn running (15.8 days) in waste paper as substrate.

**Table 1. Effect of different substrates on mycelial growth of oyster mushroom**

| Substrates | Mycelium growth rate in spawn packet (cm/day) | Time required to complete mycelium running in spawn packet (day) |
|---|---|---|
| Sawdust | 0.54 d | 23.25 e |
| Rice straw | 0.73 bc | 23.75 e |
| Water hyacinth | 0.39 e | 47.00 c |
| Wheat straw | 0.92 a | 27.50 d |
| Sugarcane bagasse | 0.64 cd | 28.25 d |
| Ulu | 0.73 bc | 24.50 e |
| Kash | 0.72 bc | 28.25 d |
| Waste paper | 0.83 ab | 17.75 f |
| Para grass | 0.30 e | 55.75 b |
| Nepier grass | 0.38 e | 60.50 a |
| CV (%) | 11.60 | 5.47 |

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

**Day required from stimulation to primordia initiation**: Appreciable variation was found in duration from stimulation to primordia initiation on different substrates tested in the present experiment (Table 2). Days required from stimulation to primordia initiation (DRSPI) were minimum on waste paper, sawdust, sugarcane bagasse and wheat straw ranged 3-4 and is not significantly different. The highest DRSPI was found on water hyacinth followed by ulu, kash and rice straw. The effect of the two substrates on this parameter was significantly different. The DRSPI on other substrate ranged 5.00-5.25 and are statistically similar. Nepier and para grasses did not produced any primordia (Table 2). Kothandaraman *et al.* (1989) also reported similar results, who found that in 3 common species of *Pleurotus* (*P. sajor-caju, P. citrinopileatus* and *P. florida*) small fruiting bodies appeared 4.00 days after spawn running in rubber tree sawdust.

The DRSPI in some substrates is in agreement with the findings of other investigators. Shah *et al.* (2004) found that the spawn heads appeared 6 days after the spawn running. Ahmed (1998) stated that *P. ostreatus* completed spawn running in 17-20 days on different substrates and time for pinheads formation was noted at 23-27 days. Murugesan *et al.* (1995) observed that the longest time was taken to reach the pinhead stage on water hyacinth that was in agreement to the present study.

**Days required from primordia initiation to harvesting:** Duration required from primordia initiation to harvesting (DRPIH) varied 3.00-4.25 on different substrates (Table 2). The lowest DRPIH of 3 days was observed on rice straw, wheat straw, ulu and kash.

The highest DRPIH was observed on water hyacinth which was followed by sawdust and waste paper. The findings of the present experiment are in conformity with Shah *et al.* (2004) who found that fruiting bodies of oyster mushroom became suitable for harvest within 3-6 day of primordia initiation in the spawn packet. Ahmed (1998) and Quimio (1976 and 1978) also reported the similar results.

**Table 2. Time required for fruiting body formation as influenced by different substrates**

| Substrate | Days required | | | |
|---|---|---|---|---|
| | from stimulation to primordia initiation | from primordia initiation to first harvest | from stimulation to first harvest | for total harvest |
| Sawdust | 3.00 d | 4.00 ab | 7.00 cd | 51.75 bc |
| Rice straw | 5.00 bc | 3.00 c | 8.00 bc | 49.75 c |
| Water hyacinth | 7.25 a | 4.25 a | 11.50 a | 22.25 d |
| Wheat straw | 4.00 cd | 3.00 c | 7.00 cd | 52.00 bc |
| Sugarcane bagasse | 3.00 d | 3.75 b | 6.75 d | 59.25 a |
| Ulu | 5.25 b | 3.00 c | 8.25 b | 48.50 c |
| Kash | 5.00 bc | 3.00 c | 8.00 bc | 52.50 bc |
| Waste paper | 3.00 d | 4.00 ab | 7.00 cd | 55.75 ab |
| Para grass | - | - | - | - |
| Nepier grass | - | - | - | - |
| CV (%) | 16.90 | 7.14 | 8.33 | 5.77 |

- = Fruiting body was not produced. In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

**Days required from stimulation to first harvesting:** The duration from stimulation to first harvest on sawdust, wheat straw, sugarcane bagasse and waste paper was 7.00, 7.00, 6.75 and 7.00, respectively. The maximum of 11.50 days were required from stimulation to first harvest on water hyacinth which was significantly higher as compared to other materials. On other three materials the parameters ranged 8.00-8.25 days (Table 2). The results of present experiment are in agreement with the findings of Bugarski *et al.* (1994), who found that the first fruit occurred on different days depending on substrates. Baysal *et al.* (2003) also reported almost similar results.

**Days required for total harvest:** Duration required for total harvest after stimulation ranged 22.25-55.75 days (Table 2). Significantly the highest duration for total harvest was recorded on sugarcane bagasse, which was statistically similar to waste paper. The lowest value of the parameter was found on water hyacinth, on which two flushes of fruiting body were harvested. On other effective materials the values of parameter were not significantly different.

**Number and weight of fruiting body and length and diameter of stalk and pileus:** Maximum number of fruiting body was recorded on waste paper which was significantly

higher as compared to all other materials. But the lowest weight of fruiting body was found on this material. Water hyacinth yielded lowest number of fruiting bodies having minimum stalk length, but gave highest weight of fruiting bodies (Table 3). It indicated that waste paper produced large number of small size fruiting bodies. Though the size was small, the yield was higher due to higher number of fruiting body on waste paper. On the other hand, water hyacinth gave lower yield due to lower number of fruiting bodies. Number and weight of fruiting bodies per packet on sawdust, rice straw, wheat straw, sugarcane bagasse, ulu and kash ranged 110.50-145.00, 1.33-1.59 g and 0.95-1.59 cm, respectively. But no appreciable relationships were found among the three parameters (Table 3). Diameter of stalk, diameter of pileus and thickness of pileus of fruiting bodies produced on eight different materials ranged 0.56-0.60 cm, 4.23-5.06 cm and 0.52-6.60 cm, respectively. The differences in these three parameters under different materials were not significant (Table 3).

The fruiting body of oyster mushroom was harvested in six flushes. The maximum NFB was obtained from the first flush then the second, third and so on for almost all the treatment except waste paper and water hyacinth. In waste paper the NFB was sharply decreased in the 2$^{nd}$ flush and stopped after 5$^{th}$ flush and in water hyacinth it was stopped after the 2$^{nd}$ flush (Fig. 1). The lack of nutrient in the substrates in successive flushes might be the probable cause of declining the number of fruiting body. The hemicelluloses and cellulose of substrates decreased with the increase of time after inoculation (Adamovic *et al.*, 1996) that might be the possible cause of lower number of fruiting body and yield in the later flushes.

**Table 3. Effect of different substrates on some yield attributes of oyster mushroom grown on different substrates**

| Treatments | Number of fruiting body/pkt | Weight of fruiting body/pkt | Length of stalk (cm/stalk) | Diameter of stalk (cm/stalk) | Diameter of pileus (cm/pileus) | Thickness of pileus (cm/pileus) |
|---|---|---|---|---|---|---|
| Sawdust | 119.00 de | 1.36 bcd | 1.59 ab | 0.59 a | 5.26 a | 0.62 a |
| Rice straw | 126.50 cd | 1.45 bcd | 1.43 abc | 0.60 a | 4.63 a | 0.60 a |
| Water hyacinth | 19.25 f | 1.79 a | 0.95 d | 0.51 a | 4.23 a | 0.54 a |
| Wheat straw | 136.50 bc | 1.58 ab | 1.64 a | 0.62 a | 5.06 a | 0.55 a |
| Sugarcane bagasse | 145.00 b | 1.33 cd | 1.39 abc | 0.64 a | 4.58 a | 0.62 a |
| Ulu | 110.50 e | 1.59 ab | 1.13 cd | 0.61 a | 4.75 a | 0.55 a |
| Kash | 124.25 cd | 1.48 bc | 1.32 bc | 0.56 a | 4.59 a | 0.52 a |
| Waste paper | 183.25 a | 1.23 d | 1.69 a | 0.60 a | 4.80 a | 0.53 a |
| Para grass | - | - | - | - | - | - |
| Nepier grass | - | - | - | - | - | - |
| CV (%) | 7.36 | 10.30 | 14.03 | 11.02 | 9.45 | 9.51 |

- = No fruiting body was formed on these materials. In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

**Biological efficiency and yield, economic yield, dry weight and benefit cost ratio:** Biological efficiency and yield and economic yield of oyster mushroom grown on different substrates ranged 20.89-145.66 %, 36.56-254.90 g/packet and 33.59-225.43 g/packet, respectively. The effects of waste paper and wheat straw on these three parameters were statistically similar but significantly higher as compared to other substrates. The parameters under rice straw, sugarcane bagasse, ulu and kash were also statistically similar and significantly higher as compared to only water hyacinth. The lowest biological efficiency and yield and economic yield were recorded from the packet containing water hyacinth, which was followed by sawdust. The effect of the two materials on these parameters was not significantly different. The highest dry weight/packet was obtained from waste paper, which was followed by wheat straw and sugarcane bagasse. Dry weight (g/packet) under rice straw, ulu and kash was statistically similar but significantly higher as compared to sawdust and water hyacinth. The effect of the two substrates on this parameter was significantly different (Table 4).

The highest benefit cost ratio of 6.51 was obtained with wheat straw, which was followed by sugarcane bagasse, waste paper, rice straw and kash. The lowest benefit-cost ratio of 1.05 was achieved with water hyacinth, which was followed by ulu and sawdust (Table 4).

**Table 4. Effect of different substrates on the biological efficiency, biological and economic yield of oyster mushroom grown on different substrates**

| Substrate | Biological efficiency (%) | Biological Yield (g/packet) | Economic yield (g/packet) | Dry yield (g/packet) | Benefit cost ratio |
|---|---|---|---|---|---|
| Sawdust | 102.76 c | 179.83 c | 160.40 c | 20.41 d | 4.90 |
| Rice straw | 115.66 b | 202.40 b | 183.28 b | 23.35 c | 5.58 |
| Water hyacinth | 20.89 d | 36.56 d | 33.59 d | 4.28 e | 1.05 |
| Wheat straw | 136.89 a | 239.55 a | 215.72 a | 28.39 ab | 6.51 |
| Sugarcane bagasse | 121.30 b | 212.28 b | 191.98 b | 27.07 b | 5.90 |
| Ulu | 111.03 bc | 194.30 bc | 175.15 bc | 23.30 c | 4.74 |
| Kash | 115.75 b | 202.56 b | 182.93 b | 23.09 c | 5.25 |
| Waste paper | 145.66 a | 254.90 a | 225.43 a | 29.98 a | 5.65 |
| Para grass | - | - | - | - | - |
| Nepier grass | - | - | - | - | - |
| CV (%) | 7.43 | 7.43 | 6.98 | 7.00 | |

- = No fruiting body was formed in the treatments. In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

In the present study maximum yield was obtained from waste paper supplemented with wheat bran. Similar results were obtained by Baysal *et al.* (2003). They found the fastest spawn running (mycelia development) (15.8 days), days to pin head formation (21.4 days) and days to fruit body formation (25.6 days), and the highest yield (350.2 g/packet) on waste paper substrate supplemented with 20% rice husk (w/w).

In the present study, higher yield was obtained from kash (182.93 g/packet) and ulu (175.15 g/packet) as compared to sawdust (160.40 g/packet). Yuexin (1997) also found similar results, who reported that the mushroom yield was 20-30% higher on grasses than obtained on sawdust.

The maximum economic yield was harvested in the 1st flush than the 2nd, 3rd, 4th, 5th and 6th flushes (Fig. 2). In the 1st harvest the highest economic yield was observed in waste paper that were greatly differed from all other substrates. The economic yield on waste paper decreased rapidly in the successive flushes and no fruiting body and yield was obtained after 5th flush. Bhatti *et al.* (1987) observed similar trend in yield and reported that the first flush gave the highest yield in all the treatments and there was a progressive decrease in the yield of successive flushes. Ramesh and Ansari (1987) also reported similar flushing pattern. The hemicellulose and cellulose of substrates decreased with the increase of time after inoculation (Adamovic *et al.*, 1996) that might be the possible cause of lower number of fruiting body and yield in the later flushes.

Ahmed (1998) observed the benefit-cost ratio of 73.20, 23.78 and 16.23 in case of *P. sajor-caju* on sugarcane bagasse, mango sawdust and rice straw respectively. The cause of these variations between the results of the two studies might be due to consideration of other costs involved in the production of oyster mushroom. To maximize profit from mushroom production, the increases in the use of wheat straw, sugarcane bagasse and waste paper and decreases in the use of labor must be adjusted to reach the optimum level.

Relationship between yield attributes and economic yield: A positive linear relationship was observed between number of fruiting body and economic yield per packet (Fig. 3). It was observed that the equation $y = 1.2272x + 23.145$ gave a good fit to the data and the value of co-efficient of determination ($R^2 = 0.9264$) showed that the fitted regression line had a significant regression co-efficient. So, it indicated that economic yield per packet increased as the number of fruiting body increased. When the data of economic yield per packet regressed against biological efficiency, a linear relationship was obtained between them (Fig. 4). The equation $y = 1.5582x + 1.6211$ gave a very good fit of the data and the co-efficient of determination ($R^2 = 0.9993$) showed that the best fitted regression line had a significant regression co-efficient. It indicated that the economic yield per packet increased with the increase of biological efficiency.

From the results it can be concluded that among the substrates waste paper was most suitable for its early return with minimum maintenance cost. The economic yield was also positively correlated with mycelium running rate ($r = 0.79^{**}$), number of fruiting bodies ($r = 0.94^{**}$) and biological yield ($r = 0.99^{**}$). Highly significant positive correlation was observed between economic yield and dry yield ($r = 0.994^{**}$).

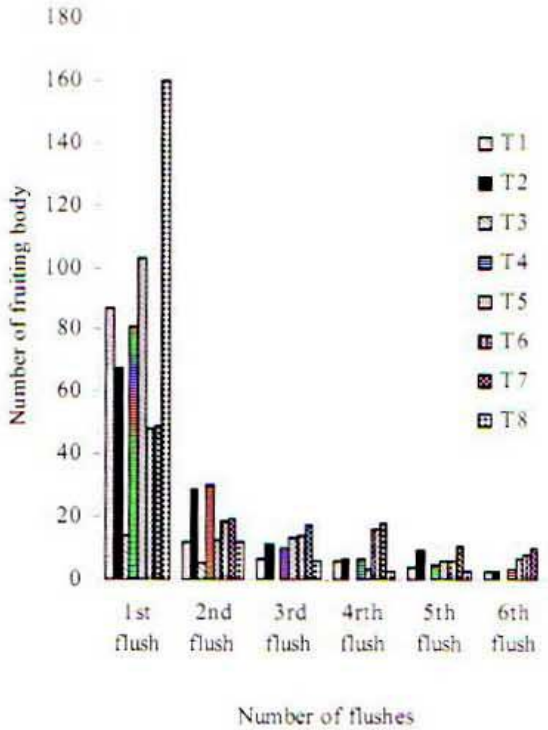


**Fig. 1. Flush wise number of fruiting body of oyster mushroom as influenced by different substrates (T1 = Sawdust, T2 = Rice straw, T3 = Water hyacinth, T4 = Wheat straw, T5 = Sugarcane bagasse, T6 = Ulu, T7 = Kash, T8 = Waste paper)**

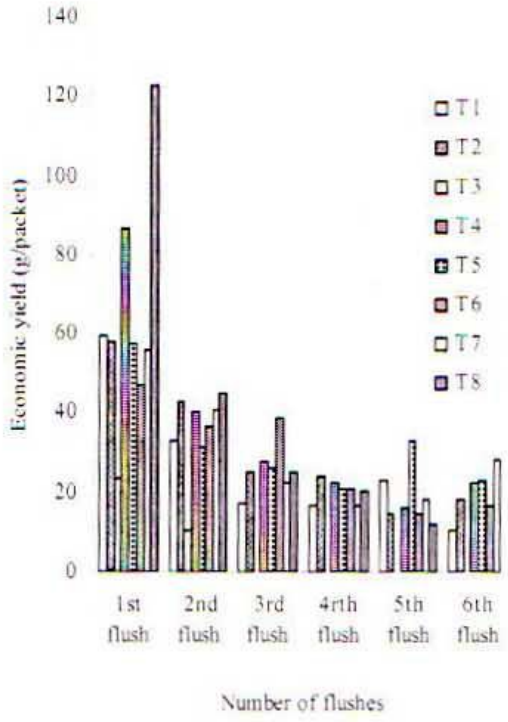


**Fig. 2. Flush wise economic yield of oyster mushroom as influenced by different substrates. (T1 = Sawdust, T2 = Rice straw, T3 = Water hyacinth, T4 = Wheat straw, T5 = Sugarcane bagasse, T6 = Ulu, T7 = Kash, T8 = Waste paper)**

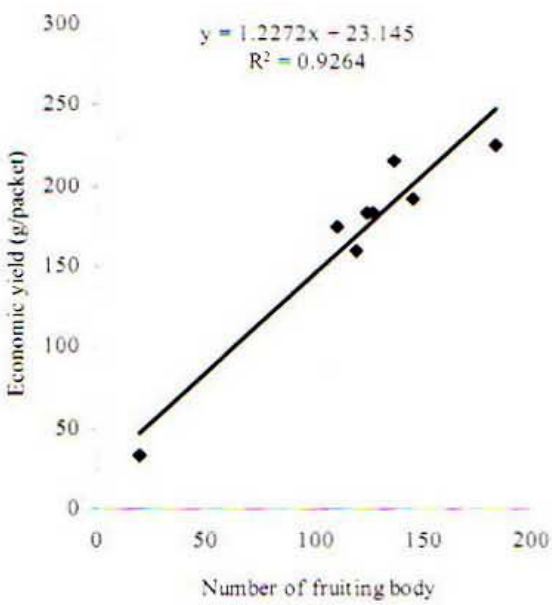


**Fig. 3. Functional relationship between number of fruiting body and economic yield of oyster mushroom influenced by different substrates**

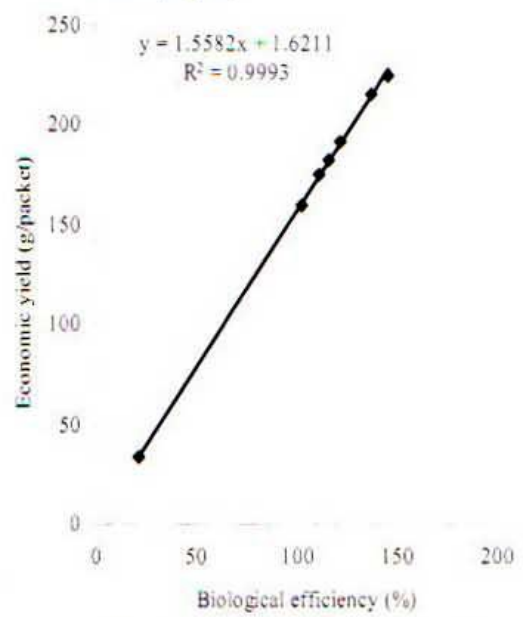


**Fig. 4. Functional relationship between biological efficiency and economic yield of oyster mushroom influenced by different substrates**

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Bangladesh J. Mushroom. 1(2): 21-25, 2007 (December)

# Effect of Different Substrates on the Growth and Yield of Five Selected Oyster Mushrooms

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**Abstract**

An experiment was conducted at the Laboratory and Culture house of Mushroom Development and Extension Centre, Savar Dhaka during the period of April to September, 2002 to study the effect of substrates on the growth and yield of five selected oyster mushroom varieties. Five different oyster mushroom varieties *Pleurotus ostreatus* ($PO_2$), *Pleurotus ostreatus* (Florida), *Pleurotus ostreatus* (HK51), *Pleurotus sajor-caju* (PSC) and *Pleurotus sajor-caju* (Grey) were grown on four different substrates sawdust: wheat bran: rice husk = 8:4:1 ($S_1$), sugarcane bagasse: saw dust: wheat bran = 4:2:1 ($S_2$), wheat straw: saw dust : wheat bran = 4:2:1 ($S_3$) and rice straw: saw dust: wheat bran = 4:2:1 ($S_4$). From the study it was evident that among the four substrates used in the study sugarcane bagasse: saw dust: wheat bran = 4:2:1 was most suitable for the growth and yield of oyster mushroom variety *Pleurotus ostreatus* (HK51).

**Key words**: Suitable substrate, growth, yield and oyster mushrooms.

## INTRODUCTION

Mushrooms are large reproductive structures of edible fungi and have been considered as a special kind of food since earliest time. Mushrooms are very nutritious food and are rich in protein, vitamins and minerals and poor in calorie and cholesterol (Pathak *et al.*, 1998; Chandha and Sharma 1995; Chang and Miles, 1988 and Bano and Rajarathnam, 1986). Mushrooms display certain medicinal properties like anti-cancerous, anti-cholesterol and anti-tumor activities and are useful against diabetes, ulcer and lung diseases (Quimio, 1976). There are about 2000 different species of edible mushroom in the world. From these species about 80 have been grown experimentally, 20 cultivated commercially and 4-5 produced on industrial scale throughout the world (Chang and Miles, 1988). Among the mushrooms oyster mushrooms, *(Pleurotus* spp.) rank second among the important cultivated mushrooms in the world (Chang and Miles, 1988). In our country, 10 different species of oyster mushroom are available and 5 of them perform better compared to others. In Bangladesh, about 30 million tones of agricultural wastes like saw dust, paddy straw, wheat straw, and sugarcane bagasse are available (Ahmed, 2001), which can be used for mushroom cultivation. But the performances of these agro-wastes have not yet properly been investigated in the climatic conditions of Bangladesh for the specific species of oyster mushroom.

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So, it is necessary to specify the suitable substrates available in our country for the specific species of oyster mushroom to increase the yield and quality. Therefore the present study was undertaken to identify the suitable substrate for specific species of oyster mushroom available in Bangladesh.

## MATERIALS AND METHODS

The experiment was conducted at the Laboratory and Culture House of Mushroom Development and Extension Center, Savar, Dhaka during the period of April to September, 2002 to study the effect of substrates on the growth and yield of five selected oyster mushroom varieties. Five different oyster mushroom varieties *Pleurotus ostreatus* ($PO_2$)=$V_1$, *Pleurotus ostreatus* (Florida)=$V_2$, *Pleurotus ostreatus* (HK51)=$V_3$, *Pleurotus sajor-caju* (PSC)=$V_4$ and *Pleurotus sajor-caju* (Grey)=$V_5$ were grown on four different substrates sawdust: wheat bran: rice husk = 8:4:1 ($S_1$), sugarcane bagasse: saw dust: wheat bran = 4:2:1 ($S_2$), wheat straw: saw dust : wheat bran = 4:2:1 ($S_3$) and rice straw: saw dust: wheat bran = 4:2:1 ($S_4$). Each of the substrate was supplemented with 0.1% calcium carbonate ($CaCO_3$) and about 65% water was added. In case of substrate $S_3$ and $S_4$, the dry straw were chopped into small pieces of 2.5 cm. In case of substrate $S_2$, the sugarcane bagasse was collected from local sugarcane crusher in the Savar market and it was chopped into very small piece of about 1 cm or less. Before use, it was dried well in the sun for 2-3 days. Then, all the substrates were prepared, following the general procedure of substrate preparation as are followed in National Mushroom Development and Extension Centre, Savar, Dhaka. The experiment was laid out in Completely Randomized Design (Factorial). Data on time required for primordia initiation, number of primordia initiation, number of effective fruiting bodies, time required for harvesting, biological yield and economical yield were collected and statistically analyzed for ANOVA. Means were separated by Duncan's Multiple Range Test (DMRT).

## RESULTS AND DISCUSSION

**Time of primordia initiation**: Appreciable variation was found in the duration from stimulation to primordia initiation of 5 different species of oyster mushroom on different substrates except $S_4$ (Table 1). Days required from stimulation to primordia initiation (DRSPI) was minimum in $S_2$ (3.15 days) followed by $S_3$ (3.27 days) and $S_1$ (3.67 days). The maximum DRSPI was recorded in $S_4$ (4.00 days) (Table 1). No significant difference was observed in DRSPI among five selected varieties in $S_4$ substrate, whereas it differed significantly in other substrates for each varieties. The minimum DRSPI was observed in $V_5$ variety (3.32 days) and the maximum DRSPI was recorded in $V_3$ variety. The results varied with the findings of Patra and Pani (1995) who found that oyster mushroom took 4-8 days for initiation of fruiting bodies but in the present study, it was ranged from 2.84 to 4.08 days. The difference among the findings may be due to the difference in cultural environment, substrates or the varieties.

**Number of primordia initiation**: The number of primordia on different varieties in different substrates differed significantly (Table 2). The highest number of average

primordia was initiated in $S_2$ (51.8). There was no significant difference among the varieties in $S_3$ that meant that all the five varieties produced more or less same number of primordia. Considering the overall performance, $V_1$ produced the highest number of primordia in all the substrates.

**Number of effective fruiting body**: The highest average number of effective fruiting body (NEFB) was produced in $S_2$ (41.1) followed by $S_1$ (35.8) (Table 3). The lowest average NEFB was observed in $S_4$ (30.3). Ahmed (2001) also reported similar number of primordia initiated in sawdust (22.95-39.55), in sugarcane bagasse (35.55) and in rice straw (20.00). The highest NEFB was recorded in $V_1$ variety (38.5); whereas, the lowest number was recorded in $V_2$ variety (31.3).

**Table 1. Interaction effect of V×S in time for primordia initiation (days)**

| Variety (V) | Substrate (S) | | | | |
|---|---|---|---|---|---|
| | Saw dust ($S_1$) | Sugarcane bagasse ($S_2$) | Wheat straw ($S_3$) | Rice straw ($S_4$) | Variety mean |
| $V_1$ | 3.75a | 3.00bc | 3.25b | 3.97a | 3.49 |
| $V_2$ | 3.96a | 2.97bc | 3.49a | 4.08a | 3.63 |
| $V_3$ | 3.76a | 3.83a | 3.29ab | 3.92a | 3.70 |
| $V_4$ | 3.54b | 3.10b | 3.20b | 4.05a | 3.47 |
| $V_5$ | 3.33c | 2.85c | 3.15b | 3.95a | 3.32 |
| Substrate mean | 3.67 | 3.15 | 3.28 | 4.00 | 3.52 |

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

**Table 2. Interaction effect of V×S for number of primodia initiation**

| Variety (V) | Substrate (S) | | | | |
|---|---|---|---|---|---|
| | Saw dust ($S_1$) | Sugarcane bagasse ($S_2$) | Wheat straw ($S_3$) | Rice straw ($S_4$) | Variety mean |
| $V_1$ | 53.7a | 57.3a | 45.3a | 41.7ab | 49.5 |
| $V_2$ | 44.3b | 53.3c | 44.3a | 38.0bc | 42.5 |
| $V_3$ | 52.0a | 56.0a | 46.0a | 41.1abc | 48.8 |
| $V_4$ | 40.3c | 52.0b | 42.3a | 37.7c | 43.1 |
| $V_5$ | 46.0b | 50.3b | 45.0a | 43.3a | 46.2 |
| Substrate mean | 47.3 | 51.8 | 44.6 | 40.4 | 46.0 |

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

**Harvesting time**: The duration of stimulation or scratching of the spawn packet to the formation of complete fruiting bodies were found significantly different among the varieties in 4 substrates which was called time of harvest or duration of harvest. This duration is important for growing mushroom commercially. The lowest harvesting duration was found in $S_2$ (5.9 days) and the highest was in $S_4$ (5.42 days) (Table 4). On the basis of overall performance, $V_1$ showed better due to shorter duration of harvesting time in all the substrates. $V_5$ showed the longest duration in case of $S_1$, $S_3$ and $S_4$ but it was shortest in $S_2$. A similar result was reported by Ahmed (2001) and Patra and Pani (1995).

**Table 3. Interaction effect of V×S for number of effecting fruiting bodies**

| Variety (V) | Substrate (S) | | | | |
|---|---|---|---|---|---|
| | Saw dust ($S_1$) | Sugarcane bagasse ($S_2$) | Wheat straw ($S_3$) | Rice straw ($S_4$) | Variety mean |
| $V_1$ | 41.3 a | 46.3 a | 34.3 a | 32.0 a | 38.5 |
| $V_2$ | 31.7 c | 35.0 c | 32.0 ab | 26.3 b | 31.3 |
| $V_3$ | 37.7 b | 41.7 b | 34.0 a | 30.0 a | 35.8 |
| $V_4$ | 31.0 c | 40.0 b | 32.7 ab | 30.3 a | 33.5 |
| $V_5$ | 37.3 b | 42.7 b | 30.3 b | 33.0 a | 35.8 |
| Substrate mean | 35.8 | 41.1 | 32.7 | 30.3 | 35.0 |

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

**Yield**: Both biological yield (BY) (Table 5) and economic yield (EY) (Table 6) were found higher in $S_2$ (95.1g and 89.9g, respectively) followed by $S_3$ (78.8g and 71.7g). The lowest yield was found in $S_4$ (60.6g and 53.9g). The performances of 5 varieties significantly differed in each substrate. Among the varieties, $V_1$ gave significantly higher BY and EY in different substrates. The average BY and EY of $V_1$ in different substrates were 81.2g and 74.8g respectively which was about 20% higher than that of the lowest yielding variety $V_2$. Similar results were reported by Badsha *et al.* (1994), Sivaprakasam (1986) and Rajarathnam *et al* (1983). Considering all the factors variety $V_1$ (*Pleurotus ostreatus*, HK51) performed best on $S_2$ (sugarcane bagasse) substrate.

**Table 4. Interaction effect of V×S in time for harvesting (days)**

| Variety (V) | Substrate (S) | | | | |
|---|---|---|---|---|---|
| | Saw dust ($S_1$) | Sugarcane bagasse ($S_2$) | Wheat straw ($S_3$) | Rice straw ($S_4$) | Variety mean |
| $V_1$ | 6.01b | 5.86ab | 6.167b | 6.39bc | 6.11 |
| $V_2$ | 6.14b | 6.00a | 6.49a | 6.49a | 6.46b |
| $V_3$ | 6.08b | 5.89ab | 6.14b | 6.26c | 6.10 |
| $V_4$ | 6.04b | 5.95ab | 6.30ab | 6.74a | 6.26 |
| $V_5$ | 6.58a | 5.80b | 6.32ab | 6.88a | 6.40 |
| Substrate mean | 6.17 | 5.90 | 6.28 | 6.55 | 6.23 |

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

**Table 5. Interaction effect of V×S for biological yield (g)**

| Variety (V) | Substrate (S) | | | | |
|---|---|---|---|---|---|
| | Saw dust ($S_1$) | Sugarcane bagasse ($S_2$) | Wheat straw ($S_3$) | Rice straw ($S_4$) | Variety mean |
| $V_1$ | 71.7 a | 103.3 a | 86.7 a | 63.0 a | 81.2 |
| $V_2$ | 60.0 c | 89.7 c | 66.7 d | 50.0 b | 66.6 |
| $V_3$ | 67.3 ab | 97.0 b | 81.7 b | 64.0 a | 77.5 |
| $V_4$ | 68.3 ab | 92.0 c | 82.0 b | 61.0 a | 75.8 |
| $V_5$ | 65.3 b | 93.7 bc | 77.0 c | 65.0 a | 75.3 |
| Substrate mean | 66.5 | 95.1 | 78.8 | 60.6 | 75.3 |

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

**Table 6. Interaction effect of V×S for economical yield (g)**

| Variety (V) | Substrate (S) | | | | |
|---|---|---|---|---|---|
| | Saw dust ($S_1$) | Sugarcane bagasse ($S_2$) | Wheat straw ($S_3$) | Rice straw ($S_4$) | Variety mean |
| $V_1$ | 66.7 a | 95.0 a | 80.0 a | 57.7 ab | 74.8 |
| $V_2$ | 52.3 d | 83.3 d | 60.7 d | 43.0 c | 59.8 |
| $V_3$ | 59.0 c | 93.0 ab | 72.7 bc | 57.0 ab | 79.4 |
| $V_4$ | 63.0 b | 90.7 bc | 74.7 b | 54.0 b | 70.6 |
| $V_5$ | 57.0 c | 87.7 c | 70.7 c | 58.0 a | 68.3 |
| Substrate mean | 59.6 | 89.9 | 71.1 | 53.9 | 68.8 |

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

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Bangladesh J. Mushroom. 1(2): 27-31, 2007 (December)

# Effect of Polar and Non-polar Extracts of Oyster Mushrooms on the Growth of Human Pathogenic and Non-pathogenic Fungi

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**Abstract**

Ethanol, methanol and petroleum ether extracts of *Pleurotus ostreatus* and *P. sajar-caju* were used to determine the antifungal activity against three non-pathogenic fungi, i.e. *Aspergillus niger, Penicillum notatum* and *Mucor racemosus* and one human pathogenic fungal species, that is *Epidermophytes floccosum*. The growth of *E. floccosum* was suppressed by 55% and the growth of the three non-pathogenic fungal species were not inhibited.

**Key words:** Antifungal activity, ethanol, methanol, petroleum ether, extracts and oyster mushrooms.

## INTRODUCTION

*Pleurotus ostreatus* and *Pleurotus sajar-caju* are widely cultivated mushrooms in Bangladesh. Together they also rank second among the most important cultivated mushrooms in the world (Bano and Rajarathnam, 1986 and Chang and Miles, 1988). Mushrooms are considered to be natural nutraceuticals and are cultivated for dietary consumption and medicinal purposes. Many edible mushrooms possess enriched proteins and some medicinal properties such as antibacterial, antifungal and antiviral (Periasamy, 2005). Oyster mushroom contains 19-35% protein on dry weight basis as compared to 7.3% in rice, 13.2% in wheat and 25.2% in milk (Chang and Miles, 1988). It contains 4.0% fat having good quantity unsaturated fatty acids which are essential in our diet (Hossain *et al.*, 2007). It is rich in essential minerals and trace elements (Chandha and Sharma 1995). Oyster mushrooms contain ascorbic acid 92-144 mg, thiamin 1.4-2.2 mg, niacin 60.6-73.3 mg, riboflavin 6.7-9.0 mg, pantothenic acid 21.1-33.3 mg and folic acid 1.2-1.4 mg/100g in dry weight basis (Bano and Rajarathnam, 1986).

Oyster mushrooms also contain some compounds that can act as an antimicrobial agent. Plants and plant parts have been used to relieve human ailments since the dawn of civilization and thus the plants used in traditional medicine have gained special attention as an important source of potentially useful new compounds for the development of anti-infective agents. There have been many studies in different coners of the world on antibacterial and antifungal potential of various plants with interesting and encouraging outcomes (Alam *et al.*, 2002, Ghani *et al.*, 1999, Ibrahim and Osman, 1995). Present study was undertaken to investigate the antifungal activity of the oyster mushroom

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against human pathogenic and non-pathogenic fungi. Some important non-pathogenic fungi such as *Aspergillus niger, Penicillium notatum, Mucor racemosus* and one pathogenic fungus, that is, *E. floccosum* were used in this experiment. Considering the medicinal importance of the oyster mushrooms this study was taken to determine the antifungal activity of ethanol and methanol and petroleum ether extract of oyster mushrooms against some human pathogenic and non-pathogenic fungi.

## MATERIALS AND METHODS

**Collection of fungal species:** Three non-pathogenic fungi i.e. *Aspergillus niger, Penicillium notatum,* and *Mucor racemosus* and one human pathogenic fungal species *Epidermophyton floccosum* were taken for the antifungal activity test of oyster mushrooms. Two species of oyster mushroom i.e., *Pleurotus ostreatus* and *Pleurotus sajar-caju* were collected from National Mushroom Development and Extension Centre, Savar, Dhaka.

**Preparation of mushroom extracts:** The collected oyster mushrooms were sun dried for 5 days and then kept in an oven at 72°C for 48 hours. The dried mushrooms were ground into coarse power with the help of a grinder. About 250g of mushroom powder was dissolved in 1 litre of ethanol, methanol and petroleum ether in cleaned flat-bottomed container with occasional shaking and stirring Mixtures were filtered repeatedly, using cotton cloth and Whitman filter paper. The clear filter was then evaporated to dryness under vacuum in a rotatory evaporator.

**Preparation of media:** PDA medium was used for the culture of the above-mentioned three nonpathogenic fungal species and The Sabouraud medium was used for the culture of the human pathogenic fungus. Sabouraud medium was prepared by mixing 10g peptone, 40g dextrose and 20g microbiological agar in one litre of distilled water. The PDA medium was prepared by using 250g potato, 20g glucose and 20g agar.

**Preparation of solution:** At first 250mg each of methanol, ethanol and petroleum ether extract of *Pleurotus ostreatus* and *Pleurotus sajar-caju* was taken in separate 6 test tubes containing 1 ml distilled water and mixed well. Then each of the 1 ml solution was separately mixed in 250 ml Saboroud media and mixed well. 20 ml melted Sabouraud medium was poured in each 90 mm Petri plate. So the final concentration of the extracted substances in the medium was 1000 ppm. The same procedure was repeated with regard to the PDA medium.

**Antifungal activity test:** Antifungal activity of mushroom extracts was indicated as their percent growth inhibition against fungal species. In this method culture blocks with a diameter of three mm were cut from the edges of the 6 days old culture of the fungal species with the aid of sterilized cork borer and inoculated at the centre of those Petri-plates containing solidified culture medium amended with particular concentrations of extracted substances. In the control set, Petri plates containing culture medium without extracted substances were also inoculated with the same fungal cultures as described

above. Three replications were maintained in each case. All inoculated Petri plates were incubated under white florescent light and 25±2°C temperature. The radial growth of the fungal colonies were measured 2 days after inoculation and continued up to 8 days at 2 days interval using millimeter scale. The percent growth inhibition of each fungal species was calculated by using the formula as given below-

$$\text{Percent growth inhibition} = \frac{C-T}{C} \times 100$$

Where,
C = Growth in control
T = Growth in treatment

## RESULTS AND DISCUSSION

Percent growth inhibition by methanol, ethanol and petroleum ether extracts of *Pleurotus ostreatus* and *Pleurotus sajar-caju* against four fungal species have been presented in Table 1 and Table 2 and Table 3. Four fungal species namely *Aspergillus niger, Penicillium notatum, Mucor racemosus* and *Epidermophyton floccosum* were used as test organisms for antifungal screening of *Pleurotus ostreatus* and *Pleurotus sajar-caju.* A single dose (1000 ppm) of the extracted substances was used against all the four fungal species. Table 1 shows the maximum percent growth inhibition (23%) being recorded in case of methanol extract of *Pleurotus sajar-caju* against *Epidermophyton floccosum* followed by the grow the inhibition (15.78%) of methanol extract of *P. ostreatus* against *Pencillium notatum.* No percent growth inhibition was recorded in case of methanol extract *P. ostreatus* and *P. sajor-caju* against *Aspergillus niger* and *Mucor racemosus.*

Table 2 reveals maximum percent growth inhibition (23%) being recorded in case of ethanol extract of *P. sajar-caju* against *E. floccosum* followed by the growth inhibition (15.78%) of ethanol extract of *P. ostreatus* against *Penicillium notatum.* Ethanol extract of *P. ostreatus* showed 14.6% growth inhibition against *E. floccosum.* No percent growth inhibition was detected in case of ethanol extract of *P. ostreatus* and *P. sajar-caju* against *A. niger* and *M. racemosus.*

Table 3 shows the maximum percent growth inhibition (55%) being recorded in case of petroleum ether extract of *Pleurotus ostreatus* against *E. floccosum.* Petroleum ether extract of *P. sajar-caju* showed a 48% growth inhibition against *E. floccosum,* while 29°41% growth inhibition was recorded in case of petroleum ether extract of *P. ostreatus* against *M. racemosus.* No detectable growth inhibition was observed against *A. niger.* Other studies showed that substances isolated from culture filtrates, fresh mycelia, and dried fruiting bodies of several mushrooms such as *Lentinus edodes, Calocybe indica* and *Pleurotus ostreatus* possessed antifungal properties(Periasamy 2005). Periasamy used several solvents such as acetone, chloroform, ethanol, ethyl acetate and methanol to extract active ingredients from different mushroom species. The maximum inhibition was observed in the dried fruiting bodies of *Pleurotus ostreatus* extracted with the solvent ethyl acetate, followed by *Calocybe indica* against several human pathogenic fungi such

as the dermatophytes or ringworm fungi (Periasamy, 2005). But studies conducted to investigate the effects of mushroom extracts on the growth of non-pathogenic fungi are so far, been almost non-existent. In this regard, the result of the present study deserves attention.

Mushrooms need antibacterial and antifungal compounds to survive in their natural environment. Antimicrobial compounds with more or less strong activities could be isolated from many mushrooms and that they could be of benefit for human. It is evident from the results of the present study that oyster mushroom, *Pleurotus ostreatus* and *Pleurotus sajar-caju* has antifungal activity in one direction. Further study is needed with different doses and pathogenic and nonpathogenic fungal species.

**Table 1. Antifungal activity of methanol extract *Pleurotus ostreatus* and *Pleurotus sajor-caju* against human pathogenic and non-pathogenic fungi**

| Fungi | *P. ostreatuds* | | | | *P. Sajar-caju* | | | |
|---|---|---|---|---|---|---|---|---|
| | Percent growth inhibition* | | | | Percent growth inhibition* | | | |
| | 2nd day | 4th day | 6th day | 8th day | 2nd day | 4th day | 6th day | 8th day |
| *A. niger* | - | - | - | - | - | - | - | - |
| *P. notatum* | 10.25 | 11.5 | 14.22 | 15.78 | - | 4.6 | 5.4 | 7.5 |
| *M. racemosus* | 4.6 | 5.8 | - | - | - | - | - | - |
| *E. floccosum* | 5.6 | 5.6 | 12.5 | 14.6 | 7.5 | 14.6 | 18.4 | 23 |

-Not detected. * mean of three replications.

**Table 2. Antifungal activity of ethanol extract *P. ostreatus* and *P. sajar-caju* against human pathogenic and non-pathogenic fungi**

| Fungi | *P. ostreatuds* | | | | *P. Sajar-caju* | | | |
|---|---|---|---|---|---|---|---|---|
| | Percent growth inhibition* | | | | Percent growth inhibition* | | | |
| | 2nd day | 4th day | 6th day | 8th day | 2nd day | 4th day | 6th day | 8th day |
| *A. niger* | - | - | - | - | - | - | - | - |
| *P. notatum* | 10.25 | 11.5 | 14.22 | 15.78 | - | 3.5 | 5.4 | 7.5 |
| *M. racemosus* | 4.6 | 5.8 | - | - | - | - | - | - |
| *E. floccosum* | 5.6 | 5.6 | 12.5 | 14.6 | 2.8 | 11.5 | 18.4 | 23 |

-Not detected. * mean of three replications.

**Table 3. Antifungal activity of petroleum ether extract *P. ostreatus* and *P. sajar-caju* against human pathogenic and non-pathogenic fungi**

| Fungi | *P. ostreatuds* | | | | *P. Sajar-caju* | | | |
|---|---|---|---|---|---|---|---|---|
| | Percent growth inhibition* | | | | Percent growth inhibition* | | | |
| | 2nd day | 4th day | 6th day | 8th day | 2nd day | 4th day | 6th day | 8th day |
| *A. niger* | - | - | - | - | - | 2 | 4 | - |
| *P. notatum* | 1.55 | 3.5 | 4.12 | 5.26 | 1.03 | 1.5 | 4.12 | 5.26 |
| *M. racemosus* | 1.7 | 14.22 | 26.78 | 29.41 | - | - | - | - |
| *E. floccosum* | 18 | 28 | 35 | 55 | 17.5 | 27.5 | 35 | 48 |

-Not detected. * mean of three replications.

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Bangladesh J. Mushroom. 1(2): 33-37, 2007 (December)

# Detection of Novel Supplements of Paddy Straw Substrate on Oyster Mushroom Cultivation

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## Abstract

Primordia and fruiting body formation and yield of oyster mushroom (*Pleurotus ostreatus*) on paddy straw supplemented with wheat bran (WB), wheat flour (WF), maize powder (MP), rice bran (RB), and their three combination (WB +MP, 1:1), (WB+RB, 1:1), (WB +MP + RB, 1:1:1) and wheat broken(WBr) at six different levels namely 0, 10, 20, 30, 40 and 50% were studied. The minimum time (4.5 days) for primordia initiation was observed in the MP at 20% level and the highest number of effective fruiting bodies (60.75) was obtained in WF at 50% level. The highest biological yield (247.3 g/packet) and economic yield (234.3 g/packet) was recorded at 10% level of (WBr).

**Key words**: Supplements, paddy straw, production and oyster mushroom.

## INTRODUCTION

*Pleurotus ostreastus* is a famous edible mushroom in Bangladesh having excellent flavor and taste. Its cultivation on agricultural wastes is a value-added process, producing mushroom of high market value and reducing the environmental pollution problems associated with waste disposal. The production trends of oyster mushroom are increasing geometrically as it grows on a wider array of forest and agricultural wastes. In recent years, oyster mushroom are being cultivated on substrates such as cotton seed hulls, wheat or oat straw, sawdust, sugarcane baggasse or combination of these materials (Roy and Schisler,1987; Roy and Bahler, 1988; Roy and Zaki, 1991; Roy, 1997). In Bangladesh these raw materials may not be available and/or are available relatively at high prices. Thus farmers are perpetually searching for alternative substrates that may be readily available or cost effective or that may provide higher yield and better mushroom quality. Paddy is the main crop in Bangladesh. It was reported that about 30 million tons of paddy straw is produced annually, which can be used as excellent substrate for mushroom production. Zhang *et al.* (2002) reported that *Pleurotus sp.* is produced more than 10% in rice straws compared to wheat straw under the same condition. Oyster mushrooms have an important feature that is the biological efficiency or conversion rate of paddy straw to mushroom. Its biological efficiency often exceeds 100%.

Various supplements added to the substrate were tested and whether these can boost yields of mushroom (Hadwan *et al.*, 1997). An and Awan (1996) obtained the highest yield with the use of rice straw as the main substrate and 15% rice bran and sugar as

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additive. Yoshida *et al.* (1993) reported the highest yield with substrates (chopped straw or sawdust) mixed with wheat bran, rice bran and bean curd refuse at 45%. Therefore, the purpose of the present work was to find out the suitable supplement of paddy straw for oyster mushroom production and to determine the best level of them.

## MATERIALS AND METHODS

The experiment was conducted at the National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka during the months of January to April 2007. Paddy straw was used as base material. Different nutritive materials such as wheat bran (WB), wheat flour (WF), maize powder (MP), rice bran (RB) and their three combinations such as (WB+MP, 1:1), (WB+RB, 1:1), (WB+MP+RB, 1:1:1) and wheat broken (WBr) were used as supplement. Six different levels such as 0, 10, 20, 30, 40 and 50% were used as treatments.

**Spawn packet preparation:** Paddy straw was chopped to 3-4 inch length. On dry weight basis, 0.2% $CaCO_3$ was added with chopped straw and mixed thoroughly. Water was added to make the moisture level of the mixture 65%. Substrates with different supplements (0.5kg) were filled in polypropylene bag (7" × 10" size) and their mouths were plugged by inserting water absorbing cotton with the help of plastic neck. The bags were autoclaved at 121° C and 15 psi for 1 hour. After autoclaving and cooling, the bags were inoculated with the mother culture of oyster mushroom at the rate of 1-2 teaspoonfuls per packet.

**Experimental condition:** The packets were kept in a dark room at 25°C. After completion of mycelium running, spawn packets were opened by D-shaped (1" ×2") cut on the shoulder of the bags and transferred to the culture room at 20-25°C and 70-80% relative humidity. The temperature and relative humidity of mushroom culture house was maintained by spraying water.

**Experimental design:** The experiment was laid out in a Completely Randomized Design (CRD) with 48 treatment combinations and four replications. Data on mycelium growth rate on substrates, days required to primordia initiation, days required for total harvest, number of effective fruiting bodies, biological efficiency and economic yield were collected. Data were analyzed by Duncan's Multiple Range Test (Gomez and Gomez, 1984) using MSTAT-C computer program. Means were computed following DMRT using the same computer program.

## RESULTS AND DISCUSSION

**Days required to primordia initiation:** Significant difference was observed in days required to primordia initiation and it ranged from 4.5 -7.0 days. The lowest time (4.5 days) was observed in the MP at 20% level which was statistically lower as compared to those of other treatments except WB at 10%, MP at 30%, RB at 40%, WB+MP at 30%, RB+MP at 40% and WB+RB+MP at 30% level. The maximum time (7 days) was

observed in WB + MP; 1:1, at 40% level (Table 1). The results are in agreement with the findings of Sarker *et al.* (2007) who found that the pinheads appeared 4.25 days after the spawn running on sawdust supplemented with the combination of Molasses (3%) and Wheat bran (40%) level.

**Table 1. Effect of different supplements and their levels to paddy straw on days to primordia initiation of *Pleurotus ostreatus***

| Level of supplement (%) | Supplements | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Wheat bran (WB) | Wheat flour (WF) | Maize powder (MP) | Rice bran (RB) | Wheat broken (WBr) | WB+MP, 1:1 | RB+MP, 1:1 | WB+RB+MP, 1:1:1 |
| 0 | 6.00a-d | 6.00a-d | 6.00a-d | 6.00a-d | 6.00a-d | 6.00a-d | 6.00a-d | 6.00a-d |
| 10 | 5.50c-e | 5.75b-d | 6.50a-c | 5.75b-d | 6.25a-d | 5.75b-d | 6.25a-d | 6.25a-d |
| 20 | 6.50a-c | 6.75ab | 4.50e | 6.25a-d | 6.25a-d | 5.75b-d | 6.50a-c | 6.00a-d |
| 30 | 5.75b-d | 6.25a-d | 5.50c-e | 6.00a-d | 5.75b-d | 5.50c-e | 6.75ab | 5.50c-e |
| 40 | 6.50a-c | 6.50a-c | 6.50a-c | 5.25de | 6.25a-d | 7.00a | 5.50c-e | 6.00a-d |
| 50 | 5.75b-d | 6.75ab | 6.75ab | 6.25a-d | 6.50a-c | 6.25a-d | 6.00a-d | 6.50a-c |

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

**Days required for total harvesting**: The significant difference was observed in days required for total harvest of fruiting bodies. The minimum (47.25) days were observed in WBr at the level of 50% and maximum (86.00) days in RB at 40% level (Table 2). Ruhul Amin *et al.* (2007) reported similar results and found that 87 days were required for total harvesting on sawdust with combination of (WB+RB;1:1) at 50% level .

**Table 2. Effect of different supplements and their levels to paddy straw on days to total harvest of *Pleurotus ostreatus***

| Level of supplement (%) | Supplements | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Wheat bran (WB) | Wheat flour (WF) | Maize powder (MP) | Rice bran (RB) | Wheat broken (WBr) | WB+MP | RB+MP | WB+RB +MP |
| 0 | 73.25a-g | 73.25a-g | 73.25a-g | 73.25a-g | 73.25a-g | 73.25a-g | 73.25a-g | 73.25a-g |
| 10 | 54.75g-i | 67.00a-i | 76.75a-f | 71.25a-g | 83.25a-c | 68.25a-h | 77.00a-f | 69.75a-h |
| 20 | 69.50a-h | 66.75a-i | 66.25a-i | 76.75a-f | 76.75a-f | 79.50a-d | 84.75a-c | 64.50b-i |
| 30 | 57.75f-i | 67.00a-i | 74.25a-g | 81.25a-d | 67.25a-i | 79.25a-e | 77.25a-f | 81.00a-d |
| 40 | 58.00e-i | 70.25a-g | 76.75a-f | 86.00a | 61.50d-i | 85.50ab | 67.00a-i | 80.75a-d |
| 50 | 49.50h-i | 69.25a-h | 73.50a-g | 75.75a-g | 47.25i | 63.50c-i | 72.00a-g | 73.50a-g |

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

**Number of effective fruiting bodies**: The highest number of effective fruiting bodies (60.75) was obtained in WF at 50% level which was statistically similar to that in WB at 40%, WF at 30 - 40 %, MP at 40 – 50 % , RB, WB+MP , RB+MP at all levels, WBr at 10-20 and 40% level and WB+RB+MP up to 40%. The lowest number of fruiting bodies (23.50) was observed in WB at 10% level (Table 3). The findings of these experiment are in support with the findings of Bugarski *et al.* (1994). Baysal *et al.* (2003) and Ruhul Amin *et al.* (2007) also reported almost similar results.

**Biological yield and economic yield:** Results on the effect of different supplements and their levels to paddy straw on the biological and economic yield of *Pleurotus ostreatus* have been presented in Table 4 and 5. The highest biological yield (247.3) was recorded in 10% level of (WBr) that was statistically similar to WF at 50%, MP at 10 and 30-50%, WBr at 20%, WB+MP at 10-40% level and RB, RB+MP and WB+RB+MP at 10-50% level. The lowest biological yield (132.5) was recorded on paddy straw at 50% level of (WBr) supplement. The trend of biological yield corresponded with different supplements at different level was observed increasing at MP and WF up to 50%, WB + MP and RB+ MP up to 30% level and WB+RB+MP up to 40% but others were observed with decreasing trend. Similar trend was observed in case of economical yield. The highest economic yield (234.3 g/packet) was recorded in 10% level of (WBr) and lowest economical yield (127.3) was recorded on paddy straw at 50% level of (WBr) supplement. (Table 5). Ruhul Amin (2007) reported similar results where the lower biological efficiency and economic yield were obtained in 50% level of (WB + MP, 1:1). Yoshida *et al.* (1993) also reported similar results where the number of effective fruiting bodies was lower, but increased when the substrate was mixed with wheat bran, rice and curd refuse.

**Table 3. Effect of different supplements and their levels to paddy straw on the number of effective fruiting bodies of *Pleurotus ostreatus***

| Level of supplement (%) | Supplements | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Wheat bran (WB) | Wheat flour (WF) | Maize powder (MP) | Rice bran (RB) | Wheat broken (WBr) | WB+MP | RB+MP | WB+RB +MP |
| 0 | 25.50j-k | 25.50j-k | 25.50j-k | 25.50j-k | 25.50j-k | 25.50j-k | 25.50j-k | 25.50j-k |
| 10 | 23.50k | 33.25g-k | 41.00b-k | 43.75a-i | 43.50a-i | 49.75a-h | 45.00a-i | 44.00a-i |
| 20 | 37.50c-k | 32.75h-k | 36.75d-k | 45.50a-i | 43.75a-i | 47.00a-i | 47.50a-i | 45.75a-i |
| 30 | 34.50f-k | 48.50a-i | 39.50b-k | 45.75a-i | 38.25c-k | 52.00a-f | 54.25a-d | 50.25a-h |
| 40 | 46.25a-i | 60.00a | 54.25a-d | 51.00a-g | 47.75a-i | 55.00a-n | 57.00a-b | 46.25a-i |
| 50 | 42.00b-j | 60.75a | 53.25a-e | 47.00a-i | 31.00i-k | 44.50a-i | 51.00a-g | 41.50b-j |

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

**Table 4. Effect of different supplements and their levels to paddy straw on the Biological yield of *Pleurotus ostreatus***

| Level of supplement (%) | Supplements | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Wheat bran (WB) | Wheat flour (WF) | Maize powder (MP) | Rice bran (RB) | Wheat broken (WBr) | WB+MP | RB+MP | WB+RB+MP |
| 0 | 156.3d-h | 156.3d-h | 156.3d-h | 156.3d-h | 56.3d-h | 156.3d-h | 156.3d-h | 156.3d-h |
| 10 | 147.0e-h | 162.3c-h | 189.5a-h | 232.5ab | 247.3a | 194.0a-h | 206.0a-g | 210.8a-f |
| 20 | 170.8b-h | 169.3b-h | 166.8b-h | 181.3a-h | 97.3a-h | 199.8a-h | 213.0a-e | 184.8a-h |
| 30 | 142.5f-h | 177.3b-h | 206.0a-g | 185.0a-h | 73.3b-h | 216.3a-e | 228.8a-c | 218.3a-d |
| 40 | 146.5e-h | 156.5d-h | 212.8a-e | 185.3a-h | 75.8b-h | 198.3a-h | 201.0a-h | 231.5a-c |
| 50 | 137.5gh | 183.3a-h | 224.0a-d | 183.3a-h | 32.5h | 166.8b-h | 190.8a-h | 190.0a-h |

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

**Table 5. Effect of different supplements and their levels to paddy straw on the Economical yield of *Pleurotus ostreatus***

| Level of supplement (%) | Supplements | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Wheat bran (WB) | Wheat flour (WF) | Maize powder (MP) | Rice bran (RB) | Wheat broken (WBr) | WB+MP | RB+MP | WB+RB+MP |
| 0 | 146.8e-h | 146.8e-h | 146.8e-h | 146.8e-h | 146.8e-h | 146.8e-h | 146.8e-h | 146.8e-h |
| 10 | 137.5f-h | 153.0d-h | 215.8a-e | 218.0a-e | 234.3a | 181.5a-h | 195.0a-g | 198.5a-f |
| 20 | 1445e-h | 161.0b-h | 173.3b-h | 170.3b-h | 148.8a-h | 187.0a- | 197.3a-f | 173.0b-h |
| 30 | 135.5g-h | 174.3b-h | 192.0a-g | 173.0b-h | 163.0b-h | 203.0a-e | 213.3a-d | 205.0a-e |
| 40 | 135.8g-h | 145.3e-h | 199.0a-e | 172.5b-h | 162.3b-h | 185.0a-h | 189.8a-g | 220.5a-b |
| 50 | 126.5h | 168.3b-h | 211.0a-d | 170.3b-h | 127.3h | 157.3c-h | 171.3b-h | 173.8b-h |

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

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Bangladesh J. Mushroom. 1(2): 39-45, 2007 (December)

# Effect of Moisture Content in the Substrate on Growth and Yield of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer

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## Abstract

The experiment was carried out during January to May 2004, comprising eight levels of moisture in substrate (45, 50, 55, 60, 65, 70, 75 and 80%). The mycelium growth rate and the time required to complete mycelium running, first harvest and total harvest were significantly influenced by the moisture levels in substrates, whereas the days required from stimulation to primordia initiation and from stimulation to first harvest were not influenced. A wide variation was observed in the number of fruiting body of oyster mushroom due to influence of the moisture levels in substrate. Sixty percent moisture level in substrate produced the maximum number of fruiting body per packet (91.50). The lowest number of fruiting body per packet (22.25) was recorded in 80% moisture level. Weight of fruiting body is influenced by moisture level in substrates but no trend was observed. Biological efficiency was associated with different moisture level in substrate and 60% moisture showed the best biological efficiency (202.50) followed by 55% (192.00). The worst biological efficiency was observed in 80% moisture. Wide variation was found in the biological yield and economic yield among the moisture levels in substrate. The economic yield per packet gradually increased up to 60% moisture. For further increase of moisture level, the economic yield declined. The highest economic yield (324.10 g/packet) was recorded in 60% moisture, which was statistically similar to that in 55% (304.30 g/packet).

**Key words:** Moisture level, substrate, yield and *Pleurotus ostreatus*.

## INTRODUCTION

*Pleurotus ostreatus* grows in nature on dead wood as saprophytes. In this sense sawdust is an ancient and excellent substrate for oyster mushroom production (Ruhul Amin *et al.*, 2007). Moisture content in substrate is a very important factor for the growth, development and yield of oyster mushroom. Appropriate moisture content in substrate, suitable relative humidity and temperature in growing room are necessary for good yield (Nerona and Latiza, 1990). Different moisture levels in different substrates under different environmental conditions have been tested by researchers. Yoshida *et al.* (1993) adjusted the moisture content between 65-70% to either chopped straw or sawdust for *P. ostreatus* production. Kothandaraman *et al.* (1989) soaked rubber tree sawdust in water for 24 hours, then dried to about 70% moisture for *P. sajor-caju, P. citrinopileatus* and *P. florida* production. Visscher (1989) adjusted moisture content at 72-78% in straw substrate amended with rice bran, lucerne meal, soya meal, poultry manure and gypsum

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as supplements for *P. pulmonarius* and *P. columbinus* production. Chung *et al.* (1981) found that the optimum moisture content were 70% for strain 2068, 80% for strains 2093, 2095 and 2091, 75% for other strains of *Pleurotus* and Luis (1999) found that *P. sajor-caju* growing in bags with 60 percent moisture content had the best cap quality, longer fruiting span of 3.5 months and higher yield.

Waste paper is an effective substrate to grow mushroom. Suitable moisture level for the production of oyster mushroom (*P. ostreatus*) on waste paper is yet to be standardized. With this view in mind, the present experiment was undertaken to determine the moisture level suitable for the production of *Pleurotus ostreatus*.

## MATERIALS AND METHODS

The experiment was carried out during January to May 2004. Eight different moisture levels of the substrate were tested in the experiment. Requisite quantity of tap water was added to substrate of each treatment to have 45, 50, 55, 60, 65, 70, 75 and 80% moisture levels. The substrates were allowed sufficient time for soaking of water uniformly as much as possible.

Waste paper was used as the main substrate. Wheat bran was mixed with the waste paper at 2:1 ratio. Calcium carbonate ($CaCO_3$) was added to the mixture at 0.57% (w/w). Before mixing, both waste paper and wheat bran was sun dried; paper was cut into small pieces and thoroughly mixed. The mixture was poured into polypropylene bags at 500 g/bag to prepare spawn packet. The experiment was laid out following completely randomized block design with four replications.

## RESULTS AND DISCUSSION

Mycelium growth rate varied from 0.80 cm to1.05 cm/day and days to complete mycelium running ranged from 16.25 to 19.75 at the moisture level 45 to 80% (w/w) of the substrate. Significantly the highest run rate was recorded at 70% moisture level. The parameter at 45-65% and 75-80% (w/w) was statistically similar. Duration to complete mycelium running in spawn packet decreased with the increase in moisture level up to 70% and increased thereafter. The duration to complete mycelium running at 70% was significantly lowest (Table 1).

At eight different levels of moisture (45-80%) the ranges of duration from stimulation to primordia initiation, from primordia initiation to first harvest and for total harvest were 3.50-4.00, 3.00-3.50, 6.50-7.25 and 30.25-51.75 days, respectively. The effect of moisture on the first three parameters was not significantly different. The maximum duration for total harvest was recorded at 65% moisture. Days for total harvest at 45, 50, 55, 60 and 70% were statistically similar but significantly higher as compared to 75 and 80% moisture at which the parameter is minimal and statistically similar (Table 2).

**Table 1. Effect of different levels of moisture in substrate on mycelial growth of *Pleurotus ostreatus***

| Moisture levels in substrate (%) | Mycelium growth rate in spawn packet (cm/day) | Days to complete mycelium running in spawn packet (day) |
|---|---|---|
| 45 | 0.80 b | 19.75 a |
| 50 | 0.86 b | 18.25 b |
| 55 | 0.85 b | 18.25 b |
| 60 | 0.90 b | 17.50 bc |
| 65 | 0.84 b | 17.25 c |
| 70 | 1.05 a | 16.25 d |
| 75 | 0.86 b | 18.00 bc |
| 80 | 0.88 b | 17.75 bc |
| CV (%) | 7.44 | 3.13 |

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

**Table 2. Time required for fruiting body formation as influenced by different moisture levels in substrate**

| Moisture levels in substrate (%) | Duration for primordia initiation (day) | Duration for primordia initiation to first harvest (day) | Duration from stimulation to first harvest (day) | Duration for total harvest (day) |
|---|---|---|---|---|
| 45 | 4.00 | 3.00 | 7.00 | 42.75 b |
| 50 | 3.50 | 3.00 | 6.50 | 42.25 b |
| 55 | 4.00 | 3.00 | 7.00 | 42.50 b |
| 60 | 3.75 | 3.00 | 6.75 | 43.50 b |
| 65 | 4.00 | 3.25 | 7.25 | 51.75 a |
| 70 | 3.75 | 3.50 | 7.25 | 40.50 b |
| 75 | 4.00 | 3.00 | 7.00 | 30.25 c |
| 80 | 4.00 | 3.00 | 7.00 | 30.50 c |
| CV (%) | 11.17 | 8.73 | 7.89 | 8.48 |

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

The number of fruiting body/packet ranged 22.25-91.50 at 45-80% moisture level in the substrate. The parameter increased up to 60% and decreased thereafter with maximum at 60% and minimum fruiting body was recorded at 80% moisture. At 60% moisture, the number of fruiting body was significantly higher as compared to other levels except 55%. Weight of fruiting body/packet ranged 3.53-4.71 at 45-80% moisture. But differences of weight of fruiting body at different moisture levels were not significant (Table 3). The biological efficiency, biological yield and economic yield at 60% moisture were the highest, which was statistically similar to 55% and significantly higher as compared to other levels of moisture. The lowest biological efficiency, biological and economical yield was recorded at 80%, which was followed by 75, 70 and 65% moisture level. The effects of moisture level at 65-80% moisture on these three yield related parameters were

statistically similar. The two low levels of moisture gave statistically similar biological efficiency and biological and economic yield (Table 3).

Maximum yield was obtained at first flash, which was followed by second, third, fourth and fifth flash. After second flash, production of effective mushroom was reduced abruptly (Fig. 1).

**Table 3. Effect of different moisture levels in substrates on the yield attributes and yield of *Pleurotus ostreatus***

| Moisture levels in substrate (%) | Number of fruiting body/packet | Weight of fruiting body (g) | Biological efficiency (g) | Biological yield (g) | Economic yield (g) |
|---|---|---|---|---|---|
| 45 | 72.50 c | 3.62 | 164.80 d | 288.30 d | 258.90 c |
| 50 | 76.50 bc | 3.60 | 169.60 cd | 296.80 cd | 274.80 bc |
| 55 | 87.25 ab | 3.53 | 192.00 ab | 336.10 ab | 304.30 ab |
| 60 | 91.50 a | 3.58 | 202.50 a | 354.30 a | 324.10 a |
| 65 | 73.75 c | 4.03 | 182.50 bc | 319.40 bc | 293.00 b |
| 70 | 54.75 d | 3.89 | 130.10 e | 227.70 e | 210.20 d |
| 75 | 46.00 d | 3.69 | 100.50 f | 175.10 f | 161.20 e |
| 80 | 22.25 e | 4.71 | 72.61 g | 127.10 g | 104.20 f |
| CV (%) | 12.41 | 15.00 | 7.49 | 7.49 | 8.26 |

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

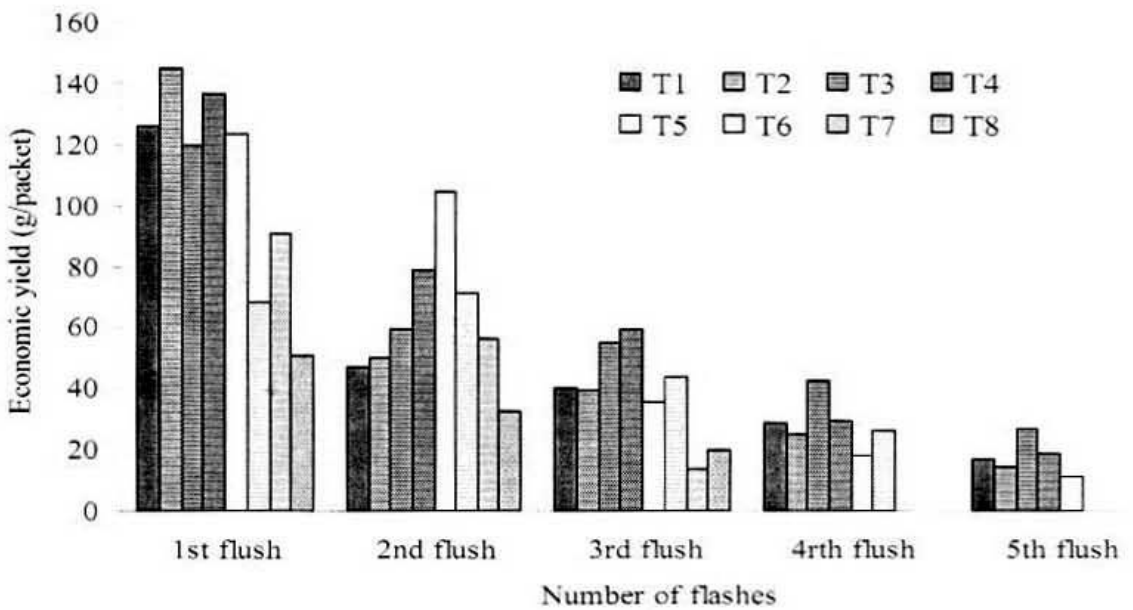


**Fig. 1. Flushwise economic yield of *Pleurotus ostreatus* influenced by different moisture levels in substrate (T1 = Moisture 45%, T2 = Moisture 50%, T3 = Moisture 55%, T4 = Moisture 60%, T5 = Moisture 65%, T6 = Moisture 70%, T7 = Moisture 75%, T8 = Moisture 80%)**

The results of the present experiment are in agreement with the findings of Luis (1999). He reported the yield of *P. sajor-caju* at 60 percent moisture content of the substrate and gave the best cap quality, longer fruiting span of 3.5 months, and higher percent successful bags. But Chung *et al.* (1981) found that the optimum moisture content were 70% for strain 2068, 80% for strain 2083 and 75% for other strains of *Pleurorus ostreatus* used in his study.

Results of the present experiment revealed that maximum mycelium growth rate and minimum day to complete mycelium running were recorded at 70% moisture of the substrate. Highest number of fruiting bodies, biological efficiency, biological and total yield of *Pleurotus ostreatus* were achieved with 60% moisture level. So, it may be concluded that waste paper substrate amended with wheat bran having 60% moisture may give maximum production of *Pleurotus ostreatus.*

**Relationship between economic yield of *Pleurotus ostreatus* with moisture level, number of fruiting body and biological efficiency**

There was highly significant correlation between economic yield and moisture level in substrate. The relationship showed a quadratic equation as $y = -0.3933x^2 + 44.482x - 950.91$ ($R^2 = 0.9535^{**}$). The majority of total variation in economic yield of the mushroom can be explained by this equation. The $R^2$ value indicated that 95.35% of economic yield was attributed to moisture level (Fig. 2). The equation also stated that economic yield was maximum at 60% moisture and it decreased at the rate of 0.3933 g/packet for per unit change of percent moisture level. A significant and linear positive correlation between economic yield and number of fruiting body was recorded and shown in Fig. 3. The regression equation ($y = 3.2725x + 26.997$, $R^2 = 0.9756^{**}$) stated that the economic yield increased gradually at the rate of 3.2725 g per fruiting body. The $R^2$ value indicated that 97.56% economic yield was attributed to number of fruiting body. There existed a linear relationship between economic yield and biological yield estimated as $y = 1.6512x-9.2526$ ($R^2 = 0.9967^{**}$) where $R^2$ value was highly significant (Fig. 4). The relationship indicated that the variation in biological efficiency associated with moisture level in substrate played significant role in controlling economic yield.

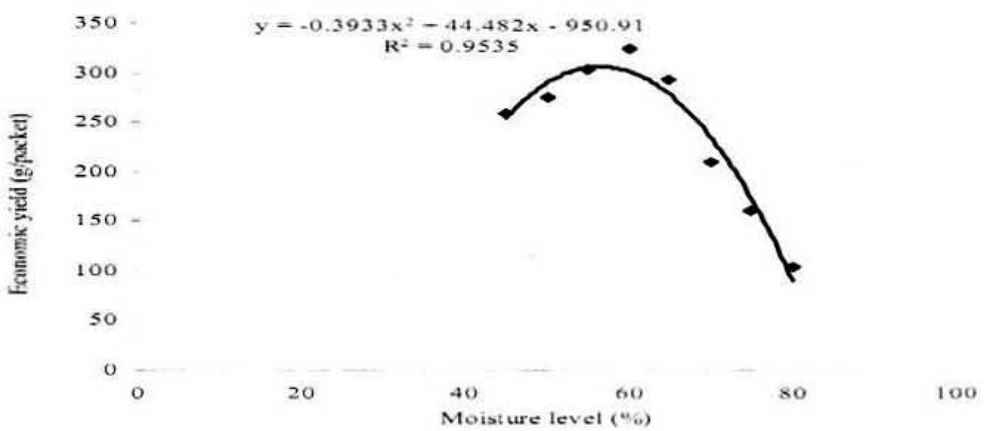


**Fig. 2. Functional relationship between moisture level in substrate and economic yield of *Pleurotus ostreatus***

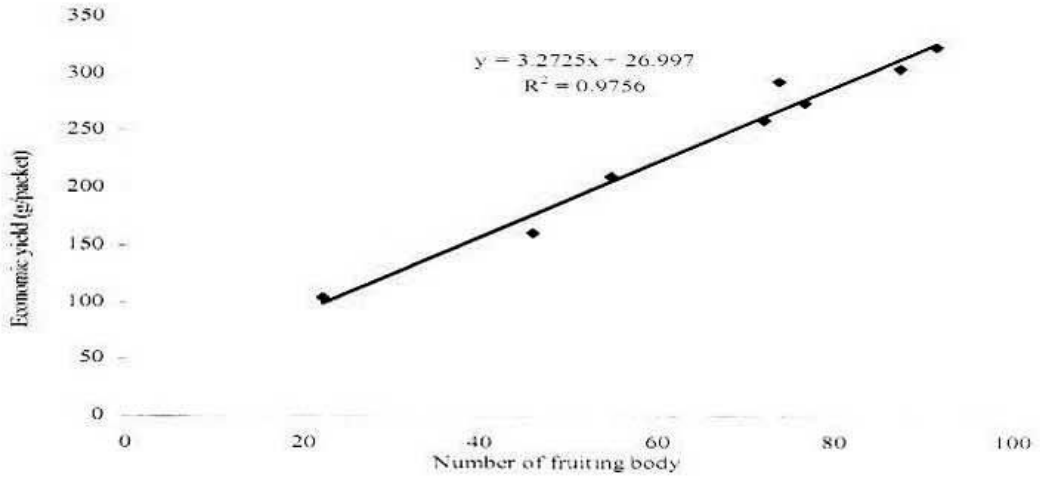


**Fig. 3. Functional relationship between number of fruiting body and economic yield of *Pleurotus ostreatus* influenced by moisture level in substrate**

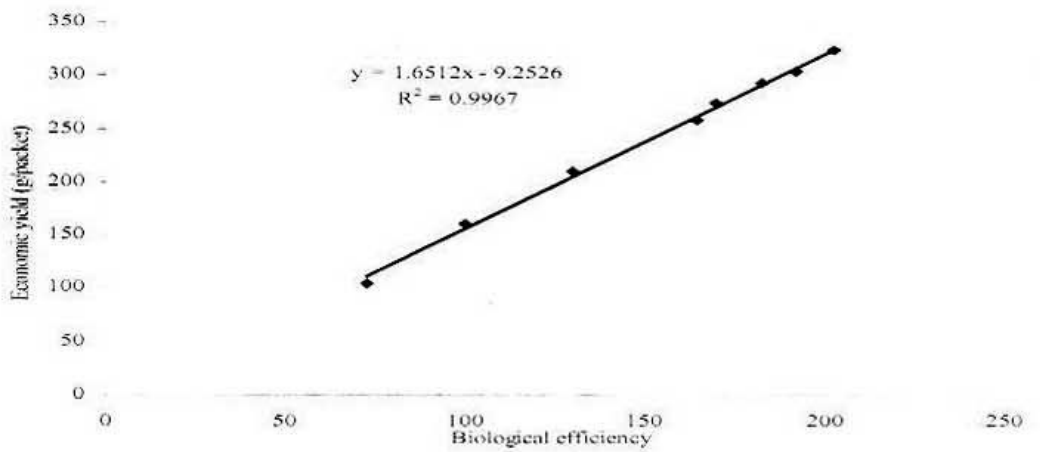


**Fig. 4. Functional relationship between biological efficiency and economic yield of *Pleurotus ostreatus* influenced by moisture level in substrate**

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Bangladesh J. Mushroom. 1(2): 47-50, 2007 (December)

# Influence of Opening Area of Spawn Packet on Growth and Yield of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer

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**Abstract**

The study was performed to select the best opening area of spawn packet for oyster mushroom production. The length of opening area were 1.5cm, 3cm, 4.5cm, 6cm & 7.5cm while the diameters were 1cm, 2cm, 3cm, 4cm & 5cm respectively. Time required from stimulation to primordia initiation, days required for total harvest, number of primordia in 1st flush, number of fruiting bodies, biological yield and economic yield were influenced significantly by opening area of spawn packet. The highest duration for total harvest (73days) and economic yield (167 g/packet) were obtained from 6×1cm² of opening area of spawn packet.

**Key words**: Opening area, spawn packet, growth, yield and *Pleurotus ostreatus*.

## INTRODUCTION

Oyster mushroom (*Pleurotus ostreatus*) is one of the most important mushroom species due to its very good taste, nutritional and medicinal value. This mushroom can be cultivated on a wide range of lignocellulosic materials (Singh and Singh, 2005). Its production technology is relatively simple and materials used as substrate are relatively cheap. But mushroom consumption in many countries, particularly in Bangladesh, is extremely limited (Diana, *et al.* 2006 and Ruhul Amin, 2007). In cultivation of oyster mushroom, several steps are considered such as substrate preparation, inoculation, opening, culturing and harvesting (Zhang, *et al.*, 2002). Among these conditions, opening of spawn packet is one of the most important aspect for fructification. In many countries, different opening systems has been followed for mushroom production. After completion of the spawn-run in the substrate, the polythene or gunny bags are cut open and the coverings are removed from the trays (Chadha and Sharma, 1998).

The bags were opened by removing the plug and the PVC pipe neck, then rolling down the mouth of the bag. Alternatively, the mouth portion might be cut off with a razor blade or the bag might be slit either cris-crossed at four to six places or simply slashed lengthwise. When using blocks instead of bags, the blocks are opened either completely or with only the surface or upper portions exposed (Dutta, 2007). In Bangladesh, most of the mushroom growers are cultivating their mushroom in polypropylene bags and opening the bags at shoulder maintaining no standard area. Therefore, it is necessary to determine the appropriate opening area for mushroom production that will be more yielding which was the main goal of the present study.

## MATERIALS AND METHODS

The research experiment was conducted in the National Mushroom Development and Extension Centre, Savar, Dhaka. Sawdust was used as a main substrate and wheat bran was used as supplement. For each 500g spawn packet, 116.7g sawdust, 58.3g wheat bran and 1g $CaCO_3$ were mixed and moisture was adjusted at 65% by adding water. The mixture was filled into heat tolerant polythene bags of 7"×10" size and their mouth were plugged by inserting water absorbing cotton and covered with brown paper and tied with a placing rubber band. Then bags were autoclaved at 121°C and 15 PSI for 1 hour and then allowed to cool. 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 25days of inoculation, when colonization was completed, the spawn packets were opened with different length and diameters of opening area. The five different length and diameters of opening area viz: 1.5cm, 3cm, 4.5cm, 6cm & 7.5cm and 1cm, 2cm, 3cm, 4cm & 5cm respectively and transferred at culture room. The bags were watered three times in a day during cropping.

The experiment was laid out in Completely Randomized Designs (CRD) with 4 replications. Data on days to primordial initiation, total harvest, yield of mushroom and some yield contributing characters, were collected and analyzed following standard methods (Gomez and Gomez,1984) using MSTAT-C computer Programme.

## RESULTS AND DISCUSSION

The effect of different treatments on days to primordia initiation, days to total harvest, number of primordia in 1st flush, number of fruiting bodies, biological yield and economic yield of oyster mushroom production are depicted in Table 1. Appreciable variation was found in duration from stimulation to primordia initiation on different opening area in spawn packet tested in the present experiment. Days required from stimulation to primordia initiation (DRSPI) were minimum (5.0days) on 3×2cm² and 4.5×4cm² opening area which was not significantly different among the treatment except on 1.5×4, 3×3 and 6×2cm², respectively opening area. The highest DRSPI (6.4days) was found on 3×3cm² opening area. Days required for total harvest after stimulation ranged from 59 to 73 days under different treatments. The lowest time interval (59days) was required to complete total harvest of mushroom under 4.5×3cm² opening area (Table 1). The shortest duration for total harvest was recorded under 4.5×3cm² because no fruiting body grew on the spawn packet after 3rd flush. Highest time interval 73 days to complete total harvest under opening area 6×1 cm² which was significantly higher and similar to other treatments except 4.5×3cm² and 7.5×1 cm² opening area. The maximum number of primordia (81) was found in 6×5 cm² opening area, which was significantly similar to 6×4 cm² opening area. Minimum number of primordia (25.8) was observed on 7.5×2 cm² area which was significantly lower and similar to other treatments except 6×5 cm² and 6×4 cm² opening area. From Table 1, it was observed that there was no significant variation among the twenty five treatments in terms of number of effective fruiting body. In case of number of

fruiting bodies among these treatment groups, the highest number (39.8) was found in 6×1 cm² area and the lowest in 7.5×1 cm² area.

**Table 1. Effect of opening area of spawn packet on growth and yield of *Pleurotus ostreatus***

| Opening area (cm2) | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | Days to primordia initiation | Days to harvest | Number of primordia | Number of effective fruiting body | Biological yield | Economic yield |
| 1.5×1 | 5.4b-d | 62.2a-c | 39.0bc | 29.4a | 132.0b | 125.4b |
| 1.5×2 | 5.4b-d | 61.4 a-c | 35.8c | 29.0a | 152.6ab | 146.8ab |
| 1.5×3 | 5.4b-d | 66.6 a-c | 34.4c | 33.2a | 152.6ab | 148.2ab |
| 1.5×4 | 6.2ab | 68.0 a-c | 42.2bc | 33.0a | 157.4ab | 150.6ab |
| 1.5×5 | 5.4b-d | 65.0 a-c | 40.6bc | 32.0a | 141.4ab | 135.4ab |
| 3×1 | 5.6a-d | 67.4 a-c | 28.6c | 32.2a | 148.2ab | 140.6ab |
| 3×2 | 5.0d | 68.4 a-c | 33.0c | 32.2a | 155.6ab | 149.4ab |
| 3×3 | 6.4a | 67.2 a-c | 32.8c | 36.6a | 134.8b | 128.6ab |
| 3×4 | 5.4b-d | 65.0 a-c | 37.4bc | 30.8a | 153.8ab | 146.8ab |
| 3×5 | 6.0a-c | 68.8 a-c | 28.8c | 29.6a | 144.2ab | 137.8ab |
| 4.5×1 | 5.6a-d | 67.0 a-c | 33.6c | 34.6a | 157.6ab | 150.6ab |
| 4.5×2 | 5.4b-d | 66.8 a-c | 31.8c | 35.4a | 151.4ab | 145.6ab |
| 4.5×3 | 5.2cd | 59.0c | 49.8bc | 39.2a | 159.8ab | 151.8ab |
| 4.5×4 | 5.0d | 69.4a-c | 36.0c | 31.4a | 158.2ab | 151.4ab |
| 4.5×5 | 6.0a-c | 72.0ab | 33.8c | 34.4a | 162.0ab | 153.6ab |
| 6×1 | 5.8a-d | 73.0a | 30.6c | 39.8a | 176.6a | 167.0a |
| 6×2 | 6.2ab | 67.8a-c | 48.8bc | 38.2a | 150.0ab | 143.0ab |
| 6×3 | 5.8a-d | 65.4 a-c | 32.6c | 36.6a | 150.4ab | 143.6ab |
| 6×4 | 5.8a-d | 63.4 a-c | 68.4ab | 39.4a | 135.8b | 127.6ab |
| 6×5 | 6.0a-c | 70.8 a-c | 81.0a | 37.0a | 139.8ab | 133.6ab |
| 7.5×1 | 5.6a-d | 60.2bc | 29.6c | 28.4a | 149.8ab | 141.8ab |
| 7.5×2 | 5.4b-d | 70.6 a-c | 25.8c | 29.8a | 156.0ab | 146.6ab |
| 7.5×3 | 5.6a-d | 62.8 a-c | 28.0c | 30.4a | 156.0ab | 150.2ab |
| 7.5×4 | 5.8a-d | 62.0 a-c | 27.0c | 31.4a | 132.6b | 127.0b |
| 7.5×5 | 5.8a-d | 68.4 a-c | 27.4c | 30.6a | 135.4b | 128.0ab |

Significant variation was observed on biological and economic yield of oyster mushroom grown on different treatments of opening area of spawn packet. The biological yield and economic yield of oyster mushroom ranged 132-176.6 g/ packet and 125- 167 g/packet respectively. The highest result was found in 6×1 cm² opening area. It was revealed that the lowest biological yield (132 g/packet) was obtained from 1.5×1 cm² area which was significantly lower and similar to other treatments except the highest value. The maximum economic yield (167 g/packet) was obtained from that combination where biological yield higher. The highest economic yield was significantly higher and similar to other treatments without 1.5×1 cm² and 7.5×4 cm² opening area. Kothandaraman *et al.* (1989) found in 3 common species of oyster mushroom *(P. sajor-caju, P. citrinopileatus* and *P. florida*) small fruiting bodies appeared 5 days after spawn running in sawdust

substrate. Shah *et al.* (2004) also found that the spawn heads appeared 6 days after the spawn running. Similar results were reported by Ahmed (1998).

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Bangladesh J. Mushroom. 1(2): 51-56, 2007 (December)

# Impact of Different Substrates on Nutrient Content of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer

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## Abstract

Remarkable difference in nutrient content of oyster mushroom was observed in respect of different substrates. Wide variation was recorded in the protein content of fruiting body. On dry weight basis, the highest protein content (11.63%) was observed in fruiting body grown on sugarcane bagasse. The 2nd highest protein (11.00%) was observed in that grown on wheat straw and water hyacinth. The lowest protein content (7.81%) was observed in that grown on rice straw. Mushrooms are good source of minerals. Maximum of 18400 ppm Ca was found in mushroom which was grown on wheat straw. On other substrates its content varied from 1600 ppm to 18400 ppm. The content of Fe in the mushroom grown on different substrates varied from 92.09 ppm to 118.40 ppm. The highest Fe content was found in waste paper cultured oyster mushroom and lowest on water hyacinth.

**Key words:** Substrates, Protein content, Mineral elements and *Pleurotus ostreatus*.

## INTRODUCTION

Mushrooms are recognized as the alternate source of good quality protein and are capable of producing the highest quantity of protein per unit area and time from the worthless agro-wastes (Chadha and Sharma, 1995). They are the source of extraordinary vigour, power and virility and are used in the preparation of many continental dishes. They are good source of protein, vitamins and minerals (Khan *et al.,* 1981) and contain about 85-95% water, 3% protein, 4% carbohydrates, 0.1% fats and 1% minerals and vitamins (Tewari, 1986). Mushrooms contain appreciable amount of potassium, phosphorus, copper and iron and low level of calcium (Anderson and Feller, 1942). Mushroom protein is intermediate between that of animal and vegetable sources (Kurtzman, 1976) and the amount of niacin, pantothenic acid and biotin is of appreciable level (Subramanian, 1986). The detrimental cholesterol is absent in mushroom but necessary ergosterol is present (Chadha and Sharma, 1995). The present nutritional status of Bangladesh is a matter of great concern. Most of our people suffer from malnutrition. Mushrooms can substantiate the sufferings from malnutrition to some extent as they provide much nutritional support in a short time and provide more protein per unit area than any other crop (Gupta, 1986).

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Mushrooms have medicinal properties like anti-cancer, anti-cholesteral and anti-tumor activities and are useful against diabetes, ulcer and lung diseases (Quimio, 1976). Pharmaceuticals are being produced in Japan from *Lentinus, Coriolus, Schizophyllum* and *Ganoderma*. Cosmetic products and tonic beverages have also been produced in China from *Ganoderma* (Chadha and Sharma, 1995). Considering the above facts, the present studies were undertaken to assess the effect of different substrates on qualitative characteristics especially the nutrient content of *Pleurotus ostreatus*.

## MATERIALS AND METHODS

Eight different substrates, namely, sawdust, rice straw, water hyacinth, wheat straw, sugarcane bagasse, 'ulu', 'kansh' and waste paper were tested in the experiment. Blanket applications of wheat bran at 1/3rd of total dry matter and $CaCO_3$ at 0.57% of total dry matter were mixed thoroughly with each of the substrate materials. The experiment was laid out following completely randomized design (CRD) with four replications.

Chemical analysis of the mushroom fruiting bodies grown on different substrates was performed and contents of protein, P, K, Ca, Mg, S. Na, Cu, B, Fe and Zn were determined.

**Protein content estimation:** Protein content of harvested mushroom was estimated to observe the effect of substrates on quality of mushroom. To estimate the protein content, nitrogen content of mushroom was estimated by "Colorimetric method" described by Linder (1944). Mushroom sample was digested in 'Kjeldahl' digestion flask with salicylic sulfuric acid and digestion catalyst. After digestion, color of the solution was developed with four different reagents. Then absorbance of the solution was measured at 625 nm wavelengths with a Double Beam Spectrophotometer (Model 200-20, HITACHI). The following formula was used for total nitrogen estimation.

$$\text{Total nitrogen} = [Y \times \frac{100\ \text{ml}}{1\ \text{ml}} \times \frac{1}{1000} \times \frac{100}{DW}]\ \%$$

Where, Y = Value calculated from the equation of standard curve
$y = a + bx$
100 ml = Final amount in volumetric flask
1 ml = Amount taken for spectrophotometric measurement
1000 = Conversion of microgram to mg
100 = For conversion of percentage (%)
DW = Dry weight of the sample (0.1g).

Again, Y = Absorbance value of sample x factor x 3.2 x 14
Where, 3.2 = Molarity of $NH_4$, $NH_4$ = 1.6 milimolar, $(NH_4)_2$ = 1.6 X 2 = 3.2 milimolar, 14 = Atomic weight of nitrogen.

Total protein was estimated by multiplying total nitrogen with the factor 6.25

**Mineral content estimation:** Content of different mineral elements in mushroom, such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfer (S), sodium (Na), Copper (Cu), Boron (B), Iron (Fe) and Zinc (Zn) were estimated by "perchloric acid digestion method" as proposed by Yamakawa (1992). Mushroom samples were oven-dried and ground into powder. Then 0.5 g mushroom sample was taken in 100 ml Kjeldahl flask containing 15 ml of nitric acid. The flask was heated gently in a digestion chamber for 30 minutes. The sample was cooled and 10 ml of 60% perchloric acid was added. The flask was heated gently until the color of the solution turned into yellowish green. The sample was cooled at room temperature and 15 ml distilled water was added. The solution was further boiled for few minutes. After cooling the digest was transferred to a 100 ml volumetric flask through a filter paper and adjust the volume up to the mark using distilled water. This was the common digested volume for sample, which was used for determination of the minerals.

**Phosphorus (P):** After digestion of the mushroom sample, the amount of phosphorus was determined by "Venamolybdate colorimetric method" proposed by Yamakawa (1992). The flow chart was followed for determination of phosphorus.

Ten milliliter of digest from the common digested volume was taken in 50 ml volumetric flask. Fifteen milliliter of distilled water was added to the digest. Ten milliliter of Venamolybdate reagent was added to it and the volume was made 50 ml using distilled water and allowed for 5 minutes for color development.

Following the same procedure a blank sample (sample containing no mushroom digest) was made. Then the absorbance of the phosphorus standard series, sample solutions and blank solution were measured at 440 nm of wavelength by using a Double Beam Spectrophotometer (Model 200-20, HITACHI). Finally the amount of phosphorus was calculated from a standard curve and by using the following formula.

$$\text{Phosphorus (ppm) in sample} = (S - B) \times \frac{100\ \text{ml}}{10\ \text{ml}} \times \frac{50\ \text{ml}}{0.5g}$$

Where, S = Concentration of phosphorus for sample
B = Concentration of phosphorus for blank
100 ml = Volume of digest (ml)
10 ml = Aliquot taken from the digest volume (ml)
50 ml = Final volume for spectrophotometer reading
0.5g = Dry weight of sample.

Again, Concentration of phosphorus for sample or blank = Factor x Absorbance
Where, Factor= Average of phosphorus standard series/Average absorbance reading against phosphorus standard series.

Now, Phosphorus (%) = Phosphorus (ppm)/10000

The other minerals content of the substrates were estimated similarly.

## RESULTS AND DISCUSSION

**Protein content**: Protein, the most important constituent of food material, was found in oyster mushroom. The content of protein varied from 7.81-11.63% (w/w) in the mushroom grown on eight different substrates. The highest content of protein was found in mushroom grown on sugarcane bagasse, which was followed by water hyacinth, wheat straw, sawdust, kash, ulu and waste paper. The lowest percentage of protein was recorded in rice straw (Table 1). Chang and Miles (1988) reported that the range of protein in oyster mushroom was 19-35%. Zaman (2004) recorded 7.85-8.81% protein content in oyster mushroom. Findings of the present experiment differed from findings of Chang and Miles (1988) but at par with those of Zaman (2004). Such variations might be due to variation in nutrient status of the substrates. Badshah *et al.* (1994) also supported such opinions and found that the protein content in fruit body is affected by substrates. Hadwan *et al.* (1997) also reported similar results. They reported 9.72-15.07% protein in oyster mushroom. Qin (1989) reported that the crude protein content of the fruiting bodies varied with different substrates. *Pleurotus sajor-caju* contained 41.26% crude protein when cultivated on rice straw and 29% when cultivated on wheat straw.

**Mineral elements**: Minerals are valuable nutrient of human diet. Mushroom contains almost all the minerals essential for human body. It was found that oyster mushroom grown in eight different nutrients contained considerable amount of different minerals (Table 1). The minerals present in the substrate are taken up by the growing mycelium and translocated to the sporophores. As a higher plant, the mineral of highest content is potassium, followed by phosphorus, calcium and magnesium. Bano and Rajarathnam (1982) and Li and Chang (1982) also reported similar trend of mineral in mushroom.

**Table 1. Effect of different substrates on nutrient content of oyster mushroom**

| Treatments | Protein (%) | Mineral elements | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Calcium (ppm) | Fe (ppm) | P (%) | K (%) | Mg (%) | S (%) | Na (%) | Cu (ppm) | B (ppm) | Zn (ppm) |
| Sawdust | 10.25 | 2400 | 118.40 | 0.97 | 1.3 | 0.21 | 0.03 | 0.19 | 3.75 | 65.13 | 30.92 |
| Rice straw | 7.81 | 1600 | 105.25 | 0.53 | 1.34 | 0.12 | 0.016 | 0.12 | 3.75 | 68.13 | 24.74 |
| Water hyacinth | 11.00 | 3300 | 92.09 | 0.52 | 1.34 | 0.13 | 0.024 | 0.17 | 3.75 | 85.17 | 27.83 |
| Wheat straw | 11.00 | 18400 | 105.25 | 0.71 | 1.34 | 0.17 | 0.022 | 1.00 | 7.50 | 82.16 | 60.00 |
| Sugarcane bagasse | 11.63 | 2900 | 105.25 | 0.88 | 1.3 | 0.19 | 0.029 | 0.12 | 7.50 | 50.60 | 43.29 |
| Ulu | 9.81 | 2000 | 105.25 | 0.67 | 1.32 | 0.17 | 0.018 | 0.12 | 9.38 | 61.12 | 30.92 |
| Kash | 10.00 | 2900 | 118.40 | 0.71 | 1.34 | 0.17 | 0.02 | 0.07 | 9.38 | 70.64 | 30.92 |
| Waste paper | 9.69 | 4900 | 98.67 | 0.54 | 1.34 | 0.17 | 0.016 | 0.07 | 3.75 | 71.14 | 27.83 |

Fe=Iron; P= Phosphorus; K= Potassium; Mg= Magnesium; S= Sulfer; Na= Sodium; Cu= Copper; B= Boron; Zn= Zinc.

Maximum of 18400 ppm Ca was estimated in mushroom grown on wheat straw. On other substrates its content varied from 1600 ppm to 18400 ppm (Table 1).The content of Fe in the mushroom grown on different substrates varied from 92.09 ppm to 118.40 ppm. The

highest Fe content was found in mushroom grown on waste paper and the lowest on water hyacinth (Table 1).

The highest percentage of phosphorus (0.97%) was reported in oyster mushroom grown on sawdust and followed by sugarcane bagasse (0.88%), wheat straw (0.71%), Kash (0.71%), Ulu (0.67%), waste paper (0.54%), rice straw (0.53%) and water hyacinth (0.52%), respectively.

The fruit bodies contained minerals such as Ca, K, Mg, S, Na, Cu, B, Fe and Zn. The fruit bodies contained 1.30-1.34% K, 0.16-1.84% Ca, 0.12-0.21% Mg, 0.016-0.029 % S, 0.07-1.00% Na, 3.75-9.38 ppm Cu, 50.60-85.17 ppm B, 92.09-118.40 ppm Fe and 24.74-60.00 ppm Zn. But Chang *et al.* (1981) reported that the fruit bodies of *Pleurotus* contained minerals such as calcium, iron, potassium, sodium and phosphorus in the range of 0.189-0.362, 0.05-0.115,21.3-24.00, 1.432-1.88, 1.58-2.56 and 5.87-8.40 mg/g dry weights of fruit bodies, respectively. Whereas, Ragunathan *et al.* (1996) reported that the fruit bodies contained 0.75-2.45 mg/g of calcium, 5.10-12.2 mg/g of iron, 8.18-18.8 mg/g of potassium, 9.2-14.3 mg/g of phosphorus. Considering the minerals essential for the people of Bangladesh who are suffering from deficiency of Fe, Ca and Zn, wheat straw was the best substrate for oyster mushroom production.

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Bangladesh J. Mushroom. 1(2): 57-62, 2007 (December)

# Study on the Effect of Opening Patterns of Spawn Bag on the Production of *Ganoderma lucidum* (Fr.) Karst

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## Abstract

Different opening patterns of spawn packets exhibited significant variation on yield attributing characters and yield of *Ganoderma lucidum*. The highest value of the diameter of stem, number of pileus diameter and thickness of pileus were recorded from the treatment of single side opening at lower abdomen, double side opening at middle abdomen, single side D-shaped opening at the shoulder of the bag and cutting the bag up to shoulder of the bag whereas the treatment of single side opening at middle abdomen gave moderate result compared with them. Maximum weight of fruiting bodies (22.44 g) was observed in the treatment of single side opening at middle abdomen, whereas the minimum value was 3.5 g was recorded in the treatment of cutting the bag from the bottom of the neck. The highest biological efficiency and fresh and dry yield per packet were also observed in treatment when single side was opened at middle abdomen.

**Key words:** *Ganoderma lucidum*, opening practices, growth and yield.

## INTRODUCTION

*Ganoderma lucidum* is a medicinal mushroom that has been used in the Orient for more than 2000 years (Wagner *et al.*, 2003). It can be potentially beneficial to cancer treatment, viral hepatitis, cardiovascular problems, chronic bronchitis and other infectious diseases (Lin, 2000). *Ganoderma lucidum* has the possibility to generate income for growers (Chen, 2004), especially in Bangladesh where fertile land is scarce and unemployment is a problem. The climatic condition of the country is suitable for *Ganoderma* cultivation throughout 8 months of the year. But the cultivation of this mushroom is almost nil in this country, due to lack of knowledge on its cultivation procedure. This mushroom requires special attention in transitional stages, such as the stage from vegetative phase to reproductive phase when primordia are beginning to form. The most crucial factor during primordia initiation is high relative humidity, preferably 90-95%. Oxygen supply, exposure to diffused dim light and inclusion of calcium in the fruiting substrate are also important (Chen, 2004 and Stamets, 2000). In developed countries, the *Ganoderma* growers provide these factors cautiously as they have the modern facilities and uninterrupted power supply. But in developing countries like Bangladesh, it is difficult to maintain these factors in optimum level as there is no modern infrastructure and uninterrupted electricity supply. In this respect, opening pattern and area may play an important role in maintaining these factors such as the relative humidity which can be considered as the most crucial factor that depends on the opening patterns of spawn packets. Considering these factors, the present study was undertaken to find out the best opening pattern of spawn bag for maximizing the yield and quality of *Ganoderma lucidum*.

## MATERIALS AND METHODS

The experiment was carried out at the Laboratory and Culture house of National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka, during the period of February to October 2007. The single factor experiment included thirteen pattern of opening of the spawn bags viz. $T_1$ : Just open the neck of spawn bag; $T_2$ : Cut the bag from the top of the neck; $T_3$ : Cut the bag from the bottom of the neck; $T_4$ : Cut the bag from the bottom of the neck with increased area; $T_5$ : Cut the bag up to shoulder of the bag; $T_6$ : Single side opening at lower abdomen; $T_7$ : Single side opening at middle abdomen; $T_8$ : Single side opening at upper abdomen; $T_9$ : Single side D-shaped opening at the shoulder of the bag; $T_{10}$ : Double side opening at lower abdomen; $T_{11}$ : Double side opening at middle abdomen; $T_{12}$ : Double side opening at upper abdomen and $T_{13}$ : Double side D-shaped opening at the shoulder of the bag.

The substrate of *Ganoderma lucidum* was prepared by using sawdust and wheat bran at the ratio of 2:1. Calcium carbonate was added at 0.2% of the substrate mixture. Water wad added to make the moisture level about 65% (at 1:1 ratio of the substrate). All the materials were mixed thoroughly and poured into 22.5X30 cm pp bag at the rate of 1 kg per bag. A neck of the bag was prepared by using heat resistant plastic tube. A hole of about 2/3 deep of the volume of the bag was made at the centre of the bag with a sharp-end stick. The neck of the bag was plugged with a piece of cotton and covered with a brown paper.

The packets were sterilized in an autoclave for one hour at $120^0$C and 1 kg/cm$^2$ pressure. After sterilization the packets were cooled and a teaspoonfull of mother culture was poured aseptically in the hole of the packet and placed on a shelf at about $25^0$C temperature for incubation. After completion of mycelium running the packets were opened according to the treatment and transferred to a culture house and watered thrice a day.

Biological yield, in g/1Kg packet, was recorded by weighing the whole fruiting bodies without removing the lower hard and dirty portion and was determined by the following formula:

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

Fresh yield in g/1Kg packet was recorded by weighing all the fruiting bodies in a packet after removing the lower hard and dirty portion.

The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. Each replication includes 2 pieces of spawn bags weighed one kg each. The bags were inoculated in February 2007 and opened on 31 August 2007 according to above mentioned practices and harvested from 20 September to 31 October 2007. The data were statistically analyzed by computer MSTAT-c programme and the means were separated by Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

The growth parameters, yield attributes and yield of *Ganoderma lucidum* were significantly influenced by different opening pattern of spawn packets (Table 1). The minimum days required from the opening to antler initiation (4.25) were observed in treatment $T_7$ where single side was opened at middle abdomen of the packet being significantly lower as compared to $T_1$ and $T_2$ and statistically similar to rest of the treatments. Almost similar trend was observed in days required to conk formation and first harvest.

The numbers of fruiting body per packet in different treatments ranged from 0.50 to 1.75. The highest number of fruiting body per packet (1.75) was observed in $T_{11}$, i.e., when the packets were opened at middle abdomen on both the sides of the packets which was statistically similar to all the treatments except $T_1$, $T_2$, $T_3$, $T_{10}$ and $T_{13}$. The lowest number of fruiting body (0.50) was observed in $T_1$, $T_2$ and $T_3$.

The length of stem, diameter of stem, diameter of pileus and thickness of pileus ranged from 3.75-8.25 cm, 0.94-1.73 cm, 4.87-9.81 cm and 0.56-1.18 cm respectively. The highest stem length (8.25 cm) was observed in $T_1$ where the neck of the spawn bag was just opened and it was significantly higher as compared to all other treatments. The highest diameter of stem (1.58 cm) was recorded in $T_6$ treatment which was statistically similar to $T_5$, $T_7$, $T_8$ and $T_9$. The lowest diameter of stem (0.94 cm) was recorded in $T_1$ treatment. The highest diameter of pileus (9.88 cm) was observed in the $T_9$ treatment which was statistically identical to all the treatments except $T_3$. The highest thickness of pileus (1.18 cm) was observed in $T_5$ treatment which was significantly higher as compared to $T_1$, $T_2$, $T_3$ and $T_4$. The lowest thickness of pileus (0.56 cm) was recorded in $T_3$ treatment. However, no appreciable relationship was observed among the four parameters though the effect of opening pattern was observed in all the four qualitative parameters.

Highly significant variation was observed in different treatments in respect of weight of individual fruiting body. The highest weight, 22.44 g was recorded in treatment $T_7$ where a single side was opened at middle abdomen of the spawn packet and no significant difference was observed among the treatment $T_7$, $T_8$, $T_9$, $T_{10}$, $T_{12}$ and $T_{13}$. The highest biological efficiency (8.17%) was also observed in $T_7$.

Wide variation in both fresh and dry yield of *Ganoderma lucidum* was observed in different opening patterns of spawn (Fig. 1). The highest fresh and dry yield per packet was observed in $T_7$ treatment where the bags were opened at single side of middle abdomen of the spawn packets. The minimum fresh and dry yields were recorded in the treatment $T_3$ where the bags were opened by cutting the bag from the bottom of the neck.

**Table 1. Effect of opening pattern of spawn bag on growth and yield contributing characters of reishi mushroom (*Ganoderma lucidum*)**

| Treatment | Days required to antler initiation | Days required to conk formation | Days required to first harvest | Number of fruiting body | Length of stem (cm) | Diameter of stem (cm) | Diameter of pileus (cm) | Thickness of pileus (cm) | Weight of fruiting body (g) | Biological efficiency (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| $T_1$ | 6.125a | 20.75abc | 48.88a | 0.50 c | 8.25 a | 0.94 d | 9.50 a | 0.79 bcd | 8.75 de | 1.09 |
| $T_2$ | 5.250b | 19.88abc | 46.63ab | 0.50 c | 6.25 b | 0.96 d | 7.38 ab | 0.68 cd | 6.38 e | 0.80 |
| $T_3$ | 5.000bc | 22.00ab | 46.50ab | 0.50 c | 4.25 cd | 1.10 bcd | 4.88 c | 0.56 d | 3.50 e | 0.44 |
| $T_4$ | 4.500bc | 20.25abc | 42.75bcde | 1.13 bc | 4.81 bcd | 1.00 cd | 9.25 ab | 0.82 bcd | 14.88 bcd | 4.45 |
| $T_5$ | 4.250c | 18.25bcd | 47.25ab | 1.13 abc | 5.56 bc | 1.37 abc | 8.19 ab | 1.18 a | 12.88 cd | 3.92 |
| $T_6$ | 4.750bc | 18.25bcd | 44.38abcd | 1.25 ab | 5.19 bcd | 1.58 a | 6.69 bc | 0.99 ab | 14.88 bcd | 4.86 |
| $T_7$ | 4.250c | 15.25d | 39.13e | 1.50 ab | 4.38 cd | 1.53 a | 9.69 a | 1.04 ab | 22.44 a | 8.17 |
| $T_8$ | 4.500bc | 18.25bcd | 40.38de | 1.38 ab | 4.69 bcd | 1.42 ab | 9.81 a | 0.99 ab | 20.36 ab | 6.73 |
| $T_9$ | 4.625bc | 18.88abcd | 40.38cde | 1.13 abc | 4.63 bcd | 1.26 abcd | 9.88 a | 0.79 bcd | 17.50 abc | 4.81 |
| $T_{10}$ | 4.375bc | 19.50abc | 40.50cde | 1.00 bc | 5.13 bcd | 1.04 bcd | 9.13 ab | 0.90 abc | 19.00 abc | 4.75 |
| $T_{11}$ | 4.625bc | 17.00cd | 39.88de | 1.75 a | 4.23 cd | 1.10 bcd | 8.79 ab | 0.90 abc | 13.94 cd | 5.79 |
| $T_{12}$ | 4.250c | 16.75cd | 40.75cde | 1.25 ab | 3.75 d | 1.14 bcd | 9.25 ab | 1.03 ab | 17.00 abc | 5.16 |
| $T_{13}$ | 4.250c | 22.50a | 45.13abc | 1.13 bc | 4.34 cd | 1.16 bcd | 8.19 ab | 1.02 ab | 16.88 abc | 4.72 |
| CV (%) | 11.75 | 12.90 | 6.77 | 35.15 | 20.72 | 19.69 | 18.65 | 20.14 | 26.71 | - |

$T_1$ : Just open the neck of spawn bag; $T_2$ : Cut the bag from the top of the neck; $T_3$ : Cut the bag from the bottom of the neck; $T_4$ : Cut the bag from the bottom of the neck with increased area; $T_5$ : Cut the bag up to shoulder of the bag; $T_6$ : Single side opening at lower abdomen; $T_7$ : Single side opening at middle abdomen; $T_8$ : Single side opening at upper abdomen; $T_9$ : Single side D-shaped opening at the shoulder of the bag; $T_{10}$ : Double side opening at lower abdomen; $T_{11}$ : Double side opening at middle abdomen; $T_{12}$ : Double side opening at upper abdomen and $T_{13}$ : Double side D-shaped opening at the shoulder of the bag.

Almost all the positive results were observed in $T_7$ where the bags were opened at single side of middle abdomen. It might be due to preservation of moisture level in optimum condition and exposure to diffused dim light. The packets which were opened at upper part and more area was in exposure, more water was lost and the bag suffered from moisture deficiency (Chen, 2004 and Stamets, 2000) as it was difficult to maintain the conditions as culture house was not totally controlled in indigenous culture house and *Ganoderma* takes a long to give fruiting body. Comparatively high length of stem was observed in $T_1$ treatment, followed by $T_2$ treatment, where there was a chance of accumulation of higher amount of $CO_2$ and lower intensity of light due to presence of long polypropylene neck. Stamets (2000) supported the results. But the overall yield of the experiment was poor which might be due to improper selection of wood species whose sawdust was used in the experiment. Dadwal and Jamaluddin (2004) supported the result and they reported that *Ganoderma lucidum* grows best on *Delonix regia* chips and logs and on other species the results were inferior. So, further experiment should be conducted to screen out the suitable substrate especially the wood species available in Bangladesh.

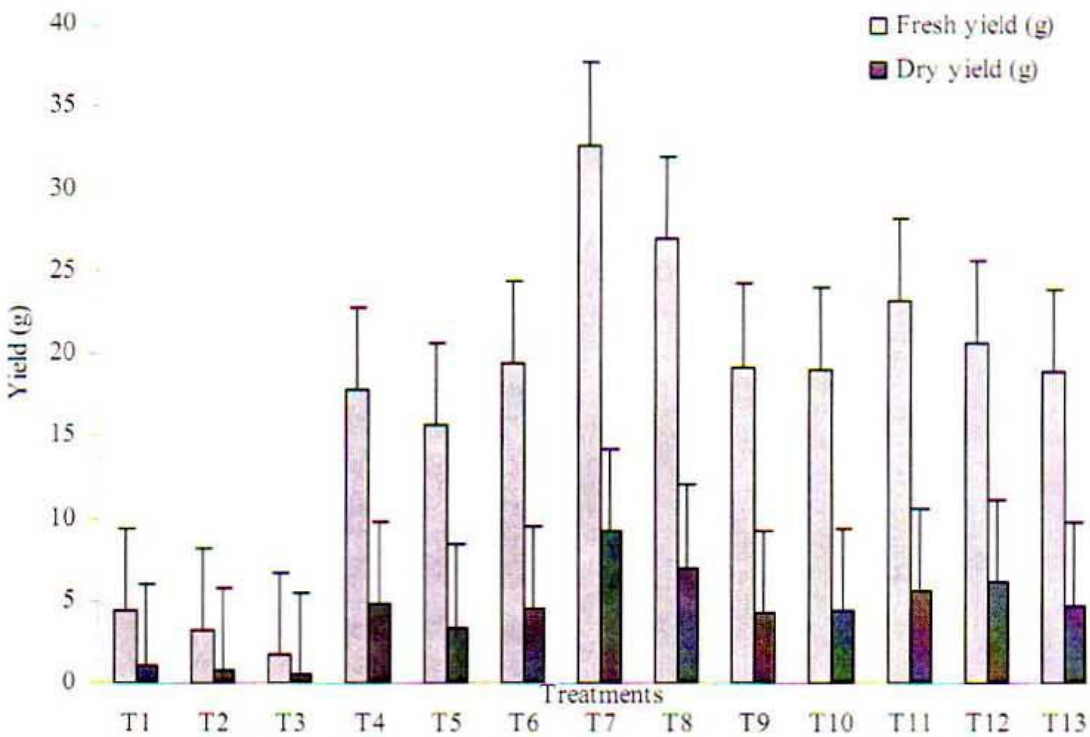


**Fig. 1. Effect of opening pattern of spawn bag on the biological and economic yield of reishi mushroom**

$T_1$ : Just open the neck of spawn bag; $T_2$ : Cut the bag from the top of the neck; $T_3$ : Cut the bag from the bottom of the neck; $T_4$ : Cut the bag from the bottom of the neck with increased area; $T_5$ : Cut the bag up to shoulder of the bag; $T_6$ : Single side opening at lower abdomen; $T_7$ : Single side opening at middle abdomen; $T_8$ : Single side opening at upper abdomen; $T_9$ : Single side D-shaped opening at the shoulder of the bag; $T_{10}$ : Double side opening at lower abdomen; $T_{11}$ : Double side opening at middle abdomen; $T_{12}$ : Double side opening at upper abdomen and $T_{13}$ : Double side D-shaped opening at the shoulder of the bag.

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Bangladesh J. Mushroom. 1(2): 63-66, 2007 (December)

# Performance of Bag Size and Spawning Method on Yield and Yield Attributes of *Agaricus bisporus* (Lange) Singer

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**Abstract**

Different bag size such as 18×25 cm, 23×30 cm, 28×38 cm and 30×46 cm and different spawning method such as top spawning, double layer spawning, triple layer spawning and thorough spawning were used as different treatments. The number of fruiting body, economic yield and biological yield per bag were increased with the increase of bag size. The highest yield per bag was obtained from the bag when it was thoroughly spawned. The highest biological and economic yield, 374.0g and 366.5g per bag respectively, were obtained from 30×46 cm bag when thoroughly spawned.

**Key words:** *Agaricus bisporus*, bag size, spawning method and yield.

## INTRODUCTION

*Agaricus bisporus* is commonly known as white button mushroom and cultivated on composts (Flegg and Wood, 1985). Different types of containers such as earthen pots, wooden trays, bamboo basket, gunny bags and polythene bags are used for its cultivation. Among these containers, polythene bags or compact polybags are the best (Bano and Nagarajan, 1976 and Bhaskaran *et al.*, 1978). The bags are cheaper and disposable and do not pose threat of contamination.

The yield of *Agaricus bisporus* is directly related to the amount of dry matter contained in the compost. The thickness of the compost layer must be at a level determined as suitable for the local climatic condition. It was reported that more mushroom can be grown per square meter on a thick bed of compost than on a thin bed (Vedder, 1978). Ruhul Amin *et.al.* (2007) reported that increasing the compost amount had clear and significant positive effect on biological and economic yield of button mushroom. But the yields did not correspond exactly with the benefit cost ratio (BCR). The compost amount has a positive relation with the bag size. Jain and Singh (1982), Patil and Shinde (1983) and Shandilya (1988) evaluated different methods of spawning like spot spawning, thorough spawning/surface spawning and layer spawning. They showed that thorough spawning is the most useful. Sivaprakasam and Ramaraj (1991) reported that layer spawning on the entire surface increased yield over thorough spawning and spawning of the periphery only. Therefore, it is important to determine which method of spawning is better. So, the purpose of the present study is to determine the suitable bag size and spawning method which are economically more feasible for button mushroom cultivation in Bangladesh.

## MATERIALS AND METHODS

The study was conducted at the National Mushroom Development and Extension Center (NMDEC) Savar, Dhaka during the month of November 2006 to April, 2007.

**Preparation of Compost:** Paddy straw, a cellulose-containing agricultural by-product, was used as the main part of compost. A room with normal air flow where sunlight could not enter directly was chosen for composting. Compost was prepared according to Ruhul Amin *et al.* (2007).

**Experimental materials:** Different sized polypropylene bags such as $B_1$ (18 × 25 cm), $B_2$ (23×30 cm), $B_3$ (28×38 cm), $B_4$ (30×46 cm) and different spawning method such as $T_1$ (top spawning), $T_2$ (double layer spawning) and $T_3$ (triple layer spawning) and $T_4$ (thorough spawning) were used as treatment. The bags were poured by prepared compost followed by above spawning method. Each treatment was replicated 4 times.

**Experimental condition:** The bags were kept in a dark room at $25^0$ C temperatures and 90% relative humidity. After completion of mycelium running, the spawned compost was covered with casing materials, soil and sand at ratio of 3:1. Casing was of 3 cm thickness. After 7 days the temperature was lowered to $16^0$ C with ventilation for pinhead formation. The relative humidity of mushroom culture room was maintained by spraying water. After development of pinhead, the data of yield attributing character and yield were recorded.

**Analysis of data:** The data obtained from different treatments were analyzed (Gomez and Gomez, 1984) using MSTAT-c computer programme. Means were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

## RESULTS AND DISCUSSION

**Days required for primordial initiation (DRPI)**: Minimum DRPI (17.75) was observed in bag size-$B_1$ (18X25cm), which was significantly lower as compare to all other bag sizes. The highest DRPI (21.19) was recorded in bag size $B_3$ (28X23cm) (Table 1). In case of spawning, the minimum DRPI (16.81) was recorded in $T_4$ (thorough spawning) which was statistically similar to triple layer spawning and significantly lower as compared to other two spawning methods (Table 2). The minimum DRPI (15.75) was observed in $B_4T_4$ (30X46cm bag thoroughly spawned) which was significantly lower as compared to all the treatment combinations except $B_1T_3$, $B_1T_4$ and $B_4T_3$ treatment combination. The highest DRPI (25.25) was recorded in $B_4T_1$ treatment combination (Table 3).

**Number of fruiting body (NFB)**: The number of fruiting body was increased with the increase in bag size. The highest number of fruiting body (25.06) was observed in 30X46cm bag, which was statistically similar to $B_3$ and significantly higher to that of other sizes of bag. The highest number of fruiting body (24.31) was recorded in thorough spawning which did not differ significantly with triple layer spawning. In case of

interaction of bag size and spawning method the highest number of fruiting body (34.00) was recorded in $B_3T_4$ which was significantly higher as compared to all other treatment except $B_3T_3$, $B_4T_2$ and $B_4T_4$ (Table 1, 2 and 3).

**Table 1. Effects of bag size on the yield attributes and yield of *Agaricus bisporus***

| Treatment | Days required for primordia initiation | Number of fruiting bodies | Economical yield (g/bag) | Biological yield (g/bag) |
|---|---|---|---|---|
| $B_1$ | 17.75 c | 9.75c | 77.38d | 82.44d |
| $B_2$ | 19.50b | 19.13b | 138.1c | 143.40c |
| $B_3$ | 21.19a | 23.38a | 185.0b | 189.40b |
| $B_4$ | 19.19b | 25.06a | 227.2a | 236.00a |

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

**Table 2. Effects of spawning method on the yield attributes and yield of *Agaricus bisporus***

| Treatment | Days required for primordia initiation | Number of fruiting bodies | Economical yield (g/bag) | Biological yield (g/bag) |
|---|---|---|---|---|
| $T_1$ | 23.50a | 10.00c | 61.50d | 65.50d |
| $T_2$ | 19.88b | 18.25b | 142.8c | 149.0c |
| $T_3$ | 17.44c | 24.75 a | 191.1b | 198.9b |
| $T_4$ | 16.81c | 24.31a | 232.4a | 237.9a |

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

**Table 3. Effects of bag size and spawning method on the yield attributes and yield of *Agaricus bisporus***

| Treatment | Days required for primordia initiation | Number of fruiting bodies | Economical yield (g/bag) | Biological yield (g/bag) |
|---|---|---|---|---|
| $B_1T_1$ | 19.75c | 7.25f | 44.25h | 48.50h |
| $B_1T_2$ | 17.75def | 8.00f | 70.75gh | 78.50gh |
| $B_1T_3$ | 17.00efg | 10.75f | 88.25fg | 92.50fg |
| $B_1T_4$ | 16.50fg | 13.00ef | 106.3f | 110.3f |
| $B_2T_1$ | 22.50b | 13.00ef | 58.00gh | 61.75gh |
| $B_2T_2$ | 19.75c | 19.50d | 146.8e | 154.8e |
| $B_2T_3$ | 18.25de | 25.75c | 174.3e | 178.0e |
| $B_2T_4$ | 17.50def | 18.25de | 173.5e | 179.3e |
| $B_3T_1$ | 26.50a | 9.25f | 65.75gh | 69.25gh |
| $B_3T_2$ | 23.00b | 17.25de | 147.5e | 151.8e |
| $B_3T_3$ | 17.75def | 33.00ab | 243.5c | 248.8c |
| $B_3T_4$ | 17.50def | 34.00a | 283.3b | 288.0b |
| $B_4T_1$ | 25.25a | 10.50f | 78.00fg | 82.50fg |
| $B_4T_2$ | 19.00cd | 28.25bc | 206.0d | 211.0d |
| $B_4T_3$ | 16.75efg | 29.50abc | 258.3bc | 276.5bc |
| $B_4T_4$ | 15.75g | 32.00ab | 366.5a | 374.0a |

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

**Economic and biological yield**: Highly significant variation was deserved among the treatments and their interactions in respect of both economic and biological yield (Table

1, 2 and 3). In bag size of 18X25cm, the economic yield was 77.38g/bag. The yield was significantly increased with the increase in bag size and the highest yield per bag (232.4g) was observed in 30X46cm bag. In case of spawning, the highest economic yield (237g/bag) was observed in thorough spawning which was followed, by triple layer and double layer spawning. During combination effect, the highest biological yield (366.5g/bag) was recorded in 30X46cm bag size when it was thoroughly spawned. Exactly similar trend was observed in case of biological yield.

From the results it is clear that the number of fruiting body and yields were increased with the increase of bag size. This may be due to the presence of higher amount of compost and nutrient materials in larger sizes of bag. Ruhul Amin *et al.*, (2007) reported similar results where both biological and economic yield were increased with the increased amount of compost per bag. The higher yields in thorough spawning were supported by Jain and Singh (1982), Patil and Shinde (1983) and Shandilya (1988).

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# Bangladesh Journal of Mushroom

**Volume 1** **Number 2** **December 2007**

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