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Effect of Spawn Rate on Growth and Yield of Maple Oyster Mushroom

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Abstract

The objective of the study aimed to compare the yield effect of pearl oyster mushroom by different spawn rates. The oyster mushroom (*Pleurotus ostreatus*) was cultivated on mixture of pasteurized sawdust and rice straw as substrates using sawdust based spawn as mother culture at different rates (10%, 15%, 20%, 25%, 30%, 35% and 40%). Different parameters such as number of fruiting bodies, number of effective fruiting bodies, length and diameter of stalk, diameter and thickness of pileus, yield, biological efficiency and contamination rate were evaluated for each spawn rate with four replicate. The result of this study indicates, the highest number of fruiting body (40.00), highest number of effective fruiting body (34.00), maximum yield (235.00 g/packet with 74.60% biological efficiency), the highest length of stalk (4.43cm), and the highest diameter of pileus (6.38cm) was observed in 40% spawn rate. The lowest number (11.00) of fruiting bodies, the lowest number (10.00) of effective fruiting bodies, the lowest yield (110.0g) and biological efficiency (34.92%) was found in 10% spawn rate. The highest contamination rate (83.33%) was found in 10% spawn rate and the lowest contamination rate (16.67%) was found in 40% spawn rate.

Keywords: Pasteurization, Rates, Sawdust, Spawning, Spawn, Mother culture.

INTRODUCTION

The global food and nutritional security of growing population is a great challenge, which looks for new crop as a source of food and nutrition. In this context, mushroom cultivation helps to address the issue of nutritional security and also provides solution for proper recycling of agro wastes. Oyster Mushroom *(Pleurotus spp.)* cultivation has increased tremendously throughout the world during the last few decades (Chang 1999 and Royse 2002). *Pleurotus spp.* are also known as oyster mushroom or dhingri or abalone mushroom, these are the second most important mushroom after button mushroom all over the world.

Spawn comprises mycelium of the mushroom and a supporting medium which provides nutrition to the fungus during its growth. The propagating material used by the mushroom growers for planting beds is called spawn (Pathak *et al.*, 2000). Growing medium of the mushroom is generally known as substrate. Oyster mushroom can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, bagasse etc. However, an ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth (Ashraf *et al.*, 2013). The mushroom mycelia requires specific nutrients for its growth and the addition of supplements increases mushroom yield by providing specific nutrients for the mycelium growth. Hence good growth and better yield of mushroom can be achieved when different substrates are supplemented (Josephine, 2015). Spawning is the method of mixing the spawn/ inocula with substrate. In practice, spawning rates vary among commercial mushroom farms. Some growers spawn at rates as low and others will spawn at a rate as high. Increasing the spawning rate increases mushroom production and reduces the time needed for spawn growth; this is especially true when the spawn is mixed-spawned. So, the present study was undertaken on the effect of spawn rate

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on growth, development and yield of oyster mushroom *Pleurotus ostreatus* with a target to find out the optimum spawn rate for getting early and high yield crop with short duration.

MATERIALS AND METHODS

The experiment was conducted in the culture house of Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh from May 2015 to July 2015. In this experiment pasteurized sawdust and rice straw were used as substrate for the cultivation of pearl oyster mushroom. The fixed amount of substrate (sawdust: rice straw = 1:1) mixed with different ratio of mother culture was used as treatments. The treatments were T_1 = 10%, T_2 =15%, T_3 = 20%, T_4 =25%, T_5 = 30% T_6 = 35% and T_7=40%. One strain of *Pleurotus ostreatus*, namely PO10 was used as test materials which is also known as pearl oyster mushroom.

Preparation of spawn packets which were used as mother culture: Sawdust spawn packets of 500 g size were prepared, inoculated and incubated following the procedure that developed and explained by Sarker *et al.* (2007). After completion of mycelium running, spawn packets were used as mother culture.

Preparation of substrate: The substrate was prepared by MDI developed pasteurization method. At first the straw was chopped to 4-5 cm length. Ten kg sawdust and ten kg rice straw with 17 litre water were mixed together. Then the mixture was poured (3-4 kg/ bag) in net's bag. The bags were kept in a rack of MDI developed sterilization cum chamber at 60-70^{0}C for one hour. There after the bags were kept in same place for 16-22 hours to get cool slowly. After about 16-22 hours the prepared straw and sawdust mixture was ready for preparation of spawn packets. The moisture level of the substrate was 65%.

Preparation of spawn packets: Pasteurized substrate and sawdust based spawn packet were mixed thoroughly without supplementation according to treatments. The polypropylene bags were filled with the mixture ie substrate mixture was poured into polypropylene bags according to spawn rate. Then their mouths were plugged by inserting absorbent cotton with the help of plastic neck. The neck of the bag was prepared by using heat resistant plastic pipe. The prepared packets were incubated in culture house at 25-30^{0}C. Thorough spawning of the substrate was also followed in which the spawn was thoroughly mixed with the wet substrate before bagging.

Experimental condition: The packets were kept in a dark room at 25^{0}C for incubation. When colonization of mycelium was completed, the spawn packets were taken to culture house and were opened by 'D' shaped cut on the shoulder and removed the sheet. The relative humidity and temperature of the culture house were maintained at 80-90% and 20-25^{0}C respectively by spraying water. Water was sprayed 4-5 times per day. Diffused light, about 200 lux and proper ventilation in culture house were maintained. After harvesting of mushroom, the residues were removed from the packet and temperature and relative humidity were maintained as before. The yield was obtained from single, double and third flush in the harvest period.Yield in g/packet was recorded by weighing all the fruiting bodies in a packet after removing the lower dirty portion. Biological efficiency was calculated according to the formula:

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

Data collection and statistical analysis: The experiment was laid out following completely randomized design (CRD) with 4 replications. Data on number of fruiting bodies, number of effective fruiting bodies, length and diameter of stalk, diameter and thickness of pileus, yield, biological efficiency and contamination rate were recorded and analyzed following Gomez and Gomez (1984)

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

RESULTS AND DISCUSSION

Number of fruiting body: Number of fruiting bodies under different treatments differed significantly (Table 1). The highest number of fruiting bodies (40.00) was found in T_7 followed by T_6 (35.00) which were statistically different to other treatments. The lowest number (11.00) of fruiting bodies was found in T_1 which was statistically differ to other treatments. This result is partially supported by Shelly *et al.* (2010) who observed that the number of fruiting body of *Pleurotus ostreatus* 30.25/packet on paddy straw substrate. Moonmoon *et al.* (2012) observed that the number of fruiting body of PO2 on rice straw based substrates 33.75/500g packet. Pradeep *et al.* (2018) observed that maximum number of fruiting bodies (28.33) was observed in 6% spawn rate of wheat (control) which was statistically higher than all other spawn rate. Minimum number of fruiting bodies (22.66) was observed in 5% spawn rate of maize which was statistically lower than all other spawn rate.

Number of effective fruiting body: Number of effective fruiting bodies under different treatments differed significantly (Table 1). The highest number of effective fruiting bodies (34.00) was found in T_7 which was followed by T_6 (26.00). The lowest number (10.00) of effective fruiting bodies was found in T_1 which was statistically differing to other treatments.

Yield/ Packet (g): Significant variation was observed in yield under different treatments (Table 1). The highest yield (235.00g) was found in T_7 followed by T_6 (200.00g). The lowest yield was found in T_1 (110.0g). Yield was counted in the harvest period third flush. This result is partially supported by Khan *et al.* (2012) who reported that yield of oyster mushroom in rice straw substrate ranged from 106.00g – 534.50g and also reported yield increased with increasing the amount of rice straw. Shelly *et al.* (2010) observed that the total yield of *Pleurotus ostreatus* 176.30g/packet on paddy straw substrate.

Table 1. Effect of spawn rate on yield and yield related attributes of maple oyster mushroom

Treatments	Total number of fruiting body	Total number of effective fruiting body	Total yield (g)	Biological efficiency (%)
T_1= 10%	11.00g	10.00e	110.00f	34.92f
T_2= 15%	19.00f	14.00d	144.00e	45.71e
T_3= 20%	23.00e	16.00d	152.00d	48.25d
T_4= 25%	27.00d	22.00c	184.00c	58.41c
T_5= 30%	30.00c	24.00bc	187.00c	59.36c
T_6= 35%	35.00b	26.00b	200.00b	63.49b
T_7= 40%	40.00a	34.00a	235.00a	74.60a
CV (%)	**6.91**	**8.24**	**2.23**	**2.23**

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

It is also apparent from the results that increasing spawn rate quantities in increases in mushroom production. These results were in contrast with the results obtained by Pradeep *et al.* (2018). The results indicated that maximum yield (550.00 g/kg dry substrates with 55.00% biological efficiency.) was observed in 6% spawn rate of paddy which was statistically higher than all other spawn rates. It was followed with 6% spawn rate of bajra (540.00 g/kg dry substrates with 54.00%.

Biological efficiency (%): The highest biological efficiency (74.60%) was found in T_7 followed by T_6 (63.49%) and the lowest biological efficiency was found in T_1(34.92%) (Table1). As spawn rate increased in substrate, substrate utilization increased. Fan *et al.*, (2000) carried out the studies with 2.5-25% spawn rates, 25% spawn rate appeared superior, but recommended 10% spawn rate in view of the process economics. The first fructification occurred after 20-23 days of inoculation and the biological efficiency reached about 90-97% after 50-60 days.

Contamination rate (%): There was a significant difference in precent contamination rate, which ranged from 16.67% to 83.33% (Fig 1) by green mould and others bacteria. The highest contamination rate (83.33%) was found in T_1 which was statistically similar to T_2 (83.33%) and the lowest contamination rate (16.67%) was found in T_7 which was followed by (28.57%) T_6. A mushroom farmer must be able to identify and eradicate these microbial contaminants which could affect mushroom yield. This could be achieved by proper sterilization and incorporation of appropriate antibacterial agents into the medium used for mycelial propagation of these mushrooms. They also believed that higher spawning rate in substrate reduces the contamination rate. It is concluded that contamination was increased with decreasing the amount of spawning rate in substrates of pearl oyster mushroom.

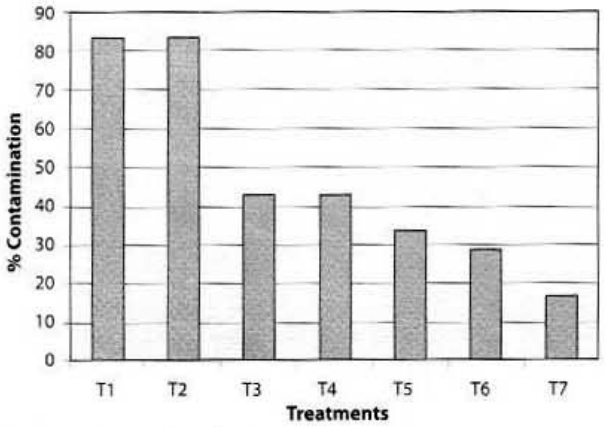

Fig. 1. Effect of spawn rates on contamination

Size of fruiting body: The length of stalk ranged from 3.26 to 4.43 cm with significant difference (Table 2). The highest length of stalk was found in T_7 (4.43cm) which was statistically similar to treatment T_4 (4.23cm). The lowest length of stalk was found in T_3 (3.26cm). The diameter of stalk was also significant and ranged from 0.60 to 0.80cm (Table 2). The highest diameter of stalk was found in T_6 (0.80) while the lowest diameter of stalk (0.60cm) was found in T_1.

The diameter of pileus ranged from 5.38 cm to 6.38 cm with significant difference among the treatments (Table 2). The highest diameter of pileus (6.38cm) was found in T_7which was statistically similar to treatment T_4 and the lowest diameter of pileus (5.38 cm) was found in T_1.The thickness of pileus in different treatments differed significantly and ranged from 0.43cm to 0.91cm (Table 2).

Table 2. Effect of spawn rate on size of fruiting body of maple oyster mushroom

Treatments	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus
T_1= 10%	3.95b	0.60d	5.38d	0.43f
T_2=15%	3.45c	0.73b	6.15ab	0.53e
T_3=20%	3.26c	0.68c	5.88bc	0.63d
T_4=25%	4.23a	0.63d	6.38a	0.70c
T_5=30%	3.75b	0.76ab	5.95bc	0.75bc
T_6=35%	3.80b	0.80a	5.63cd	0.80b
T_7=40%	4.43a	0.72b	6.38a	0.91a
CV (%)	**4.23**	**4.23**	**3.76**	**6.18**

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

The highest thickness was found in T_7 (0.91cm) which was statistically different to T_1 other treatments. The lowest thickness of pileus (0.43cm) was found in T_1 which was followed by T_2.

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Proximate Composition of Golden Oyster (*Pleurotus citrinopileatus*) Mushroom Available at MDI

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Abstract

Mushroom is an important food item in the modern society as it is a highly nutritious food that produced from the agro-industrial wastage. It possesses flavors, delicious taste and nutrient. Edible mushrooms provide a good supplement to the diet including proteins, carbohydrates, minerals and vitamins. The present study was to evaluate the nutritional of different strains of Golden oyster (*Pleurotus citrinopileatus*) mushroom. Nutritional status of these strain were slightly differed from each other. Moisture content of most of the test mushrooms ranged from 90.32 to 92.28%. The highest protein, carbohydrate and fibre content were found in PY-1 (34.22g/100g), PO96-1 (30.12g/100g) and PO96-2 (12.78g/100g) respectively. The lowest amount of lipid (3.92g/100g) and ash (19.43g/100g) were observed in PY-1.

Keywords: Mushroom, Nutrition, Analysis.

INTRODUCTION

Golden oyster mushroom is definitely an impressing edible mushroom species especially due to its distinctive taste and delightful color. This particular species has additional therapeutic value and is actually susceptible to further research. It contains useful antitumor polysaccharides (Zhang *et al.*, 1994 and Wang *et al.*, 2005) and it has antioxidant activities (Hu *et al.*, 2006). This mushroom enhances immunity and delay aging (Wang *et al.*, 2001). It is delicious in taste and rich in nutrients (Ghosh *et al.*, 1991). Edible mushrooms provide a good supplement to the diet including proteins, carbohydrates, valuable salts and vitamins, in addition to meat and vegetables. Mushrooms can substantiate the sufferings from malnutrition to some extent as they provide much nutritional support in a short time (Gupta, 1996). They are recognized as one of the important food items for their significant roles in human health, nutrition and diseases (Chang and Buswell, 1996). Due to low caloric value and high protein and mineral contents, it is recommended for heart patients.

Mushrooms play the significant impact on human welfare (Chang, 1999). Further more, it has a huge domestic and foreign market. Mushroom cultivation will be very profitable entrepreneurship. It can provide self employment opportunity to large number of unemployed youth, farmers and specially the woman entrepreneurs. Females can do this type of business in their houses to generate much money and improve their socio- economic conditions. Mushroom-related industry based on utilization of the lignocellulosic waste materials those are abundant in rural and urban areas can have positive global impacts on long-term food nutrition, health, environmental conservation and regeneration, and economic and social change.

MATTERIALS AND METHODS

This study was carried out in quality control and quality assurance (QCQA) laboratory of Mushroom Development Institute (MDI), Savar, Dhaka, Bangladesh. Four different strains of Golden oyster mushrooms i.e., PY-1, PY-2, PO96-1and PO96-2 were obtained from MDI.

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Determination of moisture: Five gram of fresh mushroom was weighed into a weighed moisture box (A&D company ltd. N 92; P1011656; Japan) and then it was dried in oven at 100-105°C and cooled in desiccators. The process of heating and cooling was repeated till a constant weight was achieved. The moisture content was calculated as following equation (Raghuramulu *et al.*, 2003):

Moisture (%) = initial weight- final weight/ weight of sample × 100.

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

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

Determination of crude fibre: Ten gram of moisture and fat-free sample was taken in a beaker and 200ml of boiling 0.255N H_2SO_4 wasadded. 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°C and weighed (We) in an electric balance (*KEY1: JY-2003; China*). The crucible was heated in a muffle furnace (*Nebertherm: Mod-L9/11/c6; Germany*) at 600°C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber.

Crude fibre (g/100g sample) = [100-(moisture + fat)] × (We-Wa) / Wt. of sample (*Raghuramulu et al.*, 2003).

Determination of total ash: One gram of the sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5-6 hours at 600°C. It was then cooled in a desiccator 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).

Determination of total carbohydrate: The content of the available carbohydrate was determined by the following equation (Raghuramalu *et al.*, 2003):

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

RESULTS AND DISCUSSION

Moisture content of fresh golden oyster mushroom and total protein, lipid, fibre, ash and carbohydrate contents of different strains of golden oyster mushroom (dry) i.e., PY-1, PY-2, PO96-1 and PO96-2 have been presented in Table 1.

Moisture content: Moisture content of most of the test mushrooms ranged from 90.32 to 92.28%. The highest moisture content was found in PY-1 (92.28%). The two different strains PY-2 and PO96-2 were also observed different moisture content 91.67% and 91.03% respectively. The lowest moisture content was found in PO96-1 (90.32%).

Table 1: Nutrient contents of different strains of golden oyster (*Pleurotus citrinopileatus*) mushrooms

Golden oysterstrains	Moisture (%)	Dry weight basis (g/100g of mushroom)				
		Protein	Carbohydrate	Lipid	Fibre	Ash
PY-1	92.28	34.22	30.05	3.92	12.38	19.43
PY-2	91.67	33.78	28.95	4.58	12.11	20.58
PO96-1	90.32	33.65	30.12	4.50	9.53	22.20
PO96-2	91.03	32.53	29.23	4.28	12.78	21.18

Protein content: The highest protein content was found in PY-1 (34.22g/100g). In case of PY-2 and PO96-1 the protein content were 33.78g and 33.65g respectively. On the other hand, the lowest protein content (32.53g/100g) was found in PO96-2.

Carbohydrate content: Among these strains the highest carbohydrate content was observed in PO96-1 (30.12g/100g) which was followed by PY-1 (30.05g/100g). In case of strain PO96-2, it was contained 29.23g/100g. The lowest carbohydrate content was found in PY-2 (28.95g).

Lipid content: The highest lipid content was found in PY-2 (4.58g/100g) which was followed by PO96-1 (4.50g/100g). PO96-2 was another strain of *P. citrinopileatus* which contained 4.28g/100g lipid. The lowest amount of lipid was observed in PY-1 (3.92g/100g).

Fibre content: The highest fibre content was found in PO96-2 (12.78g/100g). In case of PY-1 and PY-2 the fibre content were 12.38g and 12.11g respectively. The lowest fibre content was observed in PO96-1 (9.53g/100g).

Ash content: The different ash content was found in the strains of *P. citrinopileatus.* The highest ash content was found in PO96-1 (22.20g/100g) which was followed by PO96-2 (21.18g/100g). Another strain PY-2 was contained 20.58g/100g ash. The lowest ash content was found in PY-1 (19.43g/100g).

Moisture content of most of the test mushrooms ranged from 90.32 to 92.28%. The highest moisture content was found in PY-1 (92.28%). The lowest moisture content was found in PO96-1 (90.32%). Moisture content, by itself may not be of any nutritional significance but it influences the nutritional value of mushrooms. Moisture is a variable component in the proximate analysis of the mushrooms and is significantly affected by environmental factors such as temperature and relative humidity during growth and storage as well as by the relative amount of metabolic water which may be produced or utilized during storage (Crisan and Sands, 1978). Crisan and Sands (1978) also reported that moisture

content of fresh mushrooms varies between 85% and 95%. According to Kurtzman (1997), the moisture content of most of the edible mushrooms ranges from 85-94% but Chang and Miles (1989) reported the moisture content ranged from 70-94% and for tough edible mushroom, 50-75%. Afsary *et al.* (2013) observed that the maximum (92.2%) moisture contents were found in *P. citrinopileatus*. Thus, the present findings were fully in agreement with the reported values.

The highest protein content was found in PY-1 (34.22g/100g) and the lowest protein content (32.53g/100g) was found in PO96-2. Protein is the most critical component in the nutritional value and protein deficiency is the most serious human nutritional problem (Kaul, 1983). Protein content of mushrooms has been reported to vary from species to species and even flush to flush within same species and also with cultivation substrate (Rai, 1995).

Among these strains the highest carbohydrate content was observed in PO96-1 (30.12g/100g). The lowest carbohydrate content was found in PY-2 (28.95g). Carbohydrates are the main components of mushrooms apart from water. Total carbohydrate content of *Agaricus bisporus*is about 60%. In *Pleurotus* sp. carbohydrate content is reported in the range of 35 to 51% (Rai, 1995).

The highest lipid content was found in PY-2 (4.58g/100g). The lowest amount of lipid was observed in PY-1 (3.92g/100g). The average fat content of mushrooms is 4-6% of the dry weight. All classes of lipid compounds are represented including relatively large amounts of the essential fatty acids, especially linoleic acid (Rai, 1995).

The highest fiber content was found in PO96-2 (12.78g/100g) and the lowest fiber content was observed in PO96-1 (9.53g/100g).The different ash content was found in the strains of *P. citrinopileatus*. The highest ash content was found in PO96-1 (22.20g/100g) and the lowest ash content was found in PY-1 (19.43g/100g).This result partially supported Yesmin *et al.* (2009) who reported that the fruit bodies of *Pleurotus citrinopileatus* are rich in carbohydrates, proteins and minerals. Hundred grams of dry *Pleurotus citrinopileatus* grown on different substrate contain 23.5-36.91 g carbohydrate, 30.27-36.63 g protein, 20.9-27.06 g fibre, 1.86-5.3 g fat and 5.1-11.8 g ash.

Khan *et al.* (2009) evaluated that in *Pleurotus citrinopleatus*, the total protein, total lipid, carbohydrate, crude fiber and ash content was found 30.3g, 3.4g, 33.1g, 22.1g and 11g respectively. It was shown that these characters were differing or similar with the other works; it may be due to use of different substrates, supplements and overall different environmental conditions.

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Impect of Shiitake Mushroom on Nonfunctional Plasma Enzyme AST, ALT, and GGT which Considered as Hepatic Dysfunction Markers of Healthy Female Subjects

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Abstract

Current study was carried out at National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka, during the period of June 2013 to February 2014 to investigate the impect of shiitake mushroom on nonfunctional plasma enzyme AST, ALT, and GGT which considered as hepatic dysfunction marker of healthy female Subjects. Three grams of dried shiitake mushroom powder were supplemented in 3 divided dosages per day as capsule form for 3 consecutive months. Three months after getting mushroom capsule it was observed that shiitake mushroom significantly reduced plasma ALT (from 24.34 ± 1.92 to 18.15 ± 1.06, p = 0.004), plasma AST (from 25.83 ± 1.10 to 18.35 ± 1.23, p = 0.002) and plasma GGT (from 16.52 ± 0.83 to 12.96 ± 0.77, p = 0.008). Findings from the study are suggestive of improved liver function of healthy females.

Keywords: Shiitake mushroom, ALT, AST, GGT.

INTRODUCTION

Shiitake mushrooms are the second most popular and the third widely cultivated edible mushroom in the world (Chang, 2006). It has been used as a food and medicine for thousands of years. Many compounds have been isolated and their health promotion activities demonstrated (Wasser, 1997; Hobbs, 2000).

Shiitake mushrooms have excellent nutritional value. Their raw fruit bodies include 88 to 92% water, protein, lipids, carbohydrates, vitamins, and minerals. The mushroom is a good source of vitamins, especially provitamin D2. It also contains B vitamins, and minerals include Fe, Mn, K, Ca, Mg, Cd, Cu, P, and Zn. (Mizuno, 1995; Hobbs, 2000). It is the source of several well-studied preparations with proven pharmacological properties. In addition to glycogen-like polysaccharides, (1-4)-,(1-6)-a-D-glucans and antitumor polysaccharides, lentinan, (1-3)-,(1-6)-b-bonded heteroglucans, heterogalactans, heteromannans, and xyloglucans have been identified (Wasser, 1997; Hobbs, 2000). Mushrooms also contain various biologically active compounds such as gallic acid, protocatechuic acid, chlorogenic acid, naringenin, hesperetin, and biochanin-A (Alam *et al.*, 2008; Alam *et al.*, 2010). In addition, shiitake's key ingredient found in the fruiting body is a polysaccharide called lentinan. This mushroom is used for hepatitis, cancer and building the immune response.

Extracts derived from various mushrooms, are known to modulate immune response and stress reduction on poultry (Dalloul et al., 2006; Lee et al., 2010). Various studies have confirmed that the mushrooms can lower blood pressure and free cholesterol in plasma (Hobbs, 2000; Yoon *et al.*, 2011), as well as accelerate the accumulation of lipids in the liver by removing them from circulation, and can prevent cardiovascular disease (Guillamón *et al.*, 2010). Xu *et al.* (2008) reported that the administration of poly-saccharides from *L. edodes* significantly reduced serum total cholesterol, triglyceride, low-density lipoprotein cholesterol, and enhanced serum antioxidant enzyme activity and thymus and liver index in high-fat rats.

For the above mentioned causes shiitake mushrooms are considered as functional food. Functional food is a concept of nutrition, based on the role of reducing the risk of disease. A food will be

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considered as a functional food if it gives one or more benefits or positive effects to the body beyond adequate nutritional effects in a way that is relevant to either improve the stage of health and well-being and/or reduce the risk of disease (Roberfroid, 2000). Considering the previous studies it is assumed that shiitake mushroom is able to improve liver function.

The liver is an essential organ of the body that performs over 500 vital multipurpose functions. These include removing waste products and foreign substances from the bloodstream, regulating blood sugar levels, and creating essential nutrients. But the main duty of the liver is to filter the blood coming from the digestive tract, before passing it to the rest of the body. The liver also detoxifies chemicals and metabolizes drugs. As it does so, the liver secretes bile that ends up back in the intestines. The liver also makes proteins important for blood clotting and other functions. Eating healthy food is one of the best way to avoid liver disease. Taking excessive fats can make it difficult for the liver to function and lead to fatty liver disease.

Various types of test can perform to check liver functions. Among them some enzymes persisting in the blood are able to determine how well the liver is working. They include ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), GGT (Gamma-glutamyl Transferase), ALP (Alkaline Phosphatase), Bilirubin etc. Elevated blood level of ALT, AST and GGT checks for liver damage. The aim of the study was to evaluate the effect of shiitake mushroom on hepatic markers as ALT, AST and GGT of healthy female subjects.

MATERIALS AND METHODS

This study was carried out at National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka. during the period of June 2013 to February 2014. A total 34 adult female subjects aged (years) from 22 to 67 were selected in the study.

The subjects were clarified about the study and after getting their written consent showing willingness to participate in the study they were included. The details history was taken from the subjects which included age, sex, occupation, educational status, marital status, family history and drug history. Patients suffering from any acute or chronic illness, malabsorption, and alcoholism were excluded. During the study period any acute or chronic illness, were excluded.

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

At the beginning of study, health status of the subjects was evaluated. Fasting blood sample was collected from the subjects for analysis. Subjects took two capsules prepared with shiitake mushroom powder three times daily. Each capsule contained 500 mg shitake mushroom powder, so that each subject took 3 gm mushroom powder daily. After 3 months the subjects were re-evaluated and all the investigation procedures were repeated.

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

Estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) were done from the obtained plasma sample. Analysis was done by semi auto biochemical analyzer 3000 evaluation using the available reagent kit. Results were expressed as mean ± SE. Paired Student's 't' test was used to see the level of significance. 95% confidence limit was taken as level of significance. The recorded characteristics of the subjects were analyzed by using computer software, SPSS package programme.

RESULTS AND DISCUSSION

Mean age (years) of the subjects was 42.3, ranged from 22 -67. All the subjects were non diabetic and their mean fasting plasma glucose (FPG) was 5.8 mmol/l, ranged from 3.9 mmol/l-6.8 mmol/l. The subjects were free from renal failure and mean of their serum creatinine level was 0.75 mg/dl, ranged from 0.61-1.13 mg/dl (Table 1).

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

Parameter	n	Mean	From	To
Age	34	42.3 Years	22 Years	67 Years
Fasting plasma glucose	34	5.8 mmol/l	3.8 mmol/l	6.8 mmol/l
Plasma creatinine	34	0.75 mg/dl	0.61 mg/dl	1.13 mg/dl

The mean (± SE) plasma ALT (U/L) before- and after supplementation of shiitake mushroom capsules was 24.34 ± 1.92 and 18.15 ± 1.06, respectively. A significant ($p = 0.004$) reduction of ALT was observed after 3 months (Fig. 1).

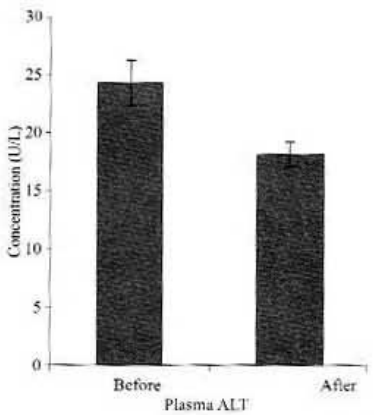

Fig. 1. Mean (± SE) plasma alanine aminotransferase (ALT) before and 3 months after mushroom supplementation.

The mean (± SE) plasma aspartate aminotransferase (AST) (U/L) before and after mushroom treatment was 25.83 ± 1.10 and 18.35 ± 1.23, respectively. Here a significant ($p = 0.002$) reduction of AST was observed (Fig. 2).

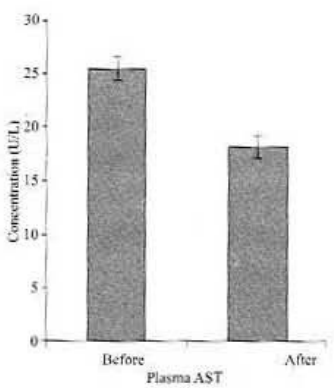

Fig. 2. Mean (± SE) plasma aspartate aminotransferase (AST) before and 3 months after mushroom supplementation.

The mean (± SE) plasma gamma glutamyl transferase (GGT) (U/L) before and after mushroom capsule supplementation was 16.52 ± 0.83 and 12.96 ± 0.77, respectively. A significant ($p = 0.008$), reduction of GGT was observed (Fig. 3).

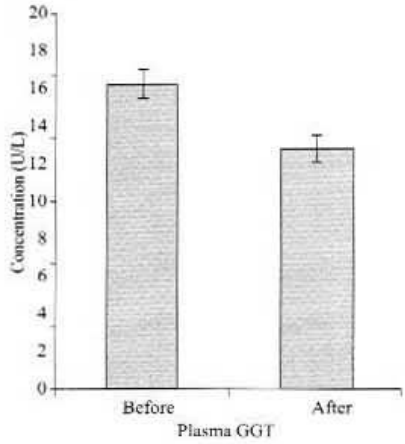

Fig. 3. Mean (± SE) plasma gamma glutamyl transferase (GGT) before and 3 months after mushroom supplementation.

Supplementation of 3 grams of dried shiitake mushroom powder per day for 3 months significantly reduced plasma ALT, AST and GGT was observed in the study. This observation is suggestive of beneficial effect of shiitake mushroom on the liver function. It is well known that Liver is the most important organ for lipid metabolism. Again persistent dyslipidemia is injurious for the liver. Shiitake mushroom have been shown to have health benefits including lowering plasma lipids and preventing body weight gain. However, their underlying mechanisms are largely unknown.

Previous studies have suggested that shiitake mushrooms has protect DNA from oxidative damage and possibly beneficial effects on preventing body weight gain, Shiitake mushrooms contain many chemical compounds that protect DNA from oxidative damage, which is partly why they are so beneficial. On the other hand blood lipid status and liver functions run hand by hand.

In a study Choudhury *et al.* (2015) observed that feeding of 1.5 grams shiitake mushroom powder daily for 3 months significantly reduced the plasma levels of ALT ($p = 0.006$), AST ($p = 0.000$) and ALP ($p = 0.020$) of male subjects. There were also nonsignificant small reduction of plasma GGT ($p = 0.193$) and LDH ($p = 0.167$). Findings of the study suggest that shiitake mushroom may able to improve hepatocellular functions of males volunteers and hence the human subjects.

Certain components of Shiitake mushroom have hypolipidaemic effect, such as eritadenine and β-glucan. Eritadenine, one of the biological components of Shiitake mushrooms, has been reported to have a plasma lipid lowering effect (Chen and Seviour, 2007; Shimada, *et al.*, 2003). Eritadenine has been reported to be ten times as effective in improving dyslipidaemia as clofibrate (Takashima *et al.*, 1973). Eritadenine is effective in lowering dyslipidaemia by decreasing the concentration of phosphatidylcholine (PC) and increasing the concentration of phosphatidylethanolamine (PE) in the liver (Sugiyama *et al.*, 1995; Walkey *et al.*, 1998). PC is an important phospholipid for lipoprotein assembly and secretion from the liver (Cole, *et al.*, 1912). β-glucan from Shiitake mushroom is a primarily soluble dietary fibre (Chen and Seviour, 2007). Studies have reported that β-glucan can increase satiety, reduce food intake,

delay nutrition absorption, and reduce plasma lipid levels (Shimada *et al.*, 2003; Sugiyama *et al.*, 1995; Walkey *et al.*, 1998).

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

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Effect of Different Substrates, Duration of Pasteurization and Size of Substrate Packet on Severity of Contamination in Substrate Packet

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Abstract

Different substrates, such as rice straw, waste paper, mango sawdust and combination of these three substrates were used to observe the contamination level considering size of packets and the duration of traditional steam pasteurization without inoculation of mushroom seed. It was revealed that, waste paper was not contaminated. Contamination rate was higher in mixed substrate packets; steam pasteurization of substrates for three hours was effective to minimize the contamination. Contamination was high in large size packets (1000g substrate) in comparison with smaller one (500g). Considering oyster mushroom it was observed that three hours pasteurization of the substrates for small size packet (500g) were ideal for less contamination.

Keywords: Substrates, Contamination, Oyster mushroom, Pasteurization.

INTRODUCTION

Pleurotus mushrooms, commonly known as oyster mushrooms, grow in the wild in tropical, subtropical and temperate regions and are easily artificially cultivated (Akindahunsi and Oyetayo, 2006). Oyster mushrooms (*Pleurotus* spp.) are characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. Oyster mushroom can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, baggasse etc. (Hassan *et al.*, 2011) and this has been reported to influence its growth, yield and composition (Iqbal *et al.*, 2005; Kimenju *et al.*, 2009; Khare *et al.*, 2010). Crop residues such as grain crop straw are characterized by the predominance of lignocellulose with cellulose, hemicellulose and lignin as the main components (Yildiz *et al.*, 2002; Das and Mukherjee, 2007; Mamiro and Mamiro, 2011; Jonathan *et al.*, 2012). Using such crop residue as a mushroom substrate would subsequently convert them into a more protein-rich biomass and influence the mushroom yields (Mamiro and Mamiro, 2011).

Williams *et al.* (2003) reported that in preferred conditions moulds exhibited fast growth, therefore they can compete for space and nutrients more effectively than the mushrooms, furthermore, they are able to produce toxic secondary compounds, extracellular enzymes as well as volatile organic compounds, which can result in a drastically decrease in production or even entire crops can be wiped out. Normally mushroom famers of Bangladesh harvested mushroom within 30-60 days after incubation (DAI). Therefore, data on contamination severity was recorded during this period of incubation. The substrate packets prepared with only waste paper in any experiment were free from contamination irrespective of stage of data collection. The experiment was conducted to find out the effect of different substrates, duration of pasteurization and size of substrate packet on severity of contamination.

MATERIALS AND METHODS

Preparation of substrate, spawn packet, pasteurization of substrate packet: The experiment was conducted during September to December 2014 in MCH and plant pathology laboratory of SAU. Four types of substrates were used in the experiment. These were rice straw, mango sawdust, waste paper and mixture of the three materials. Rice straw and waste paper were cut into small pieces (4 mm length) and soaked in water for overnight before substrate preparation. After draining the excess water content of the final mixture was adjusted to 65% (w/w) was filled into polyethylene bags at 500 and 1000 g/bag. Mouth of spawn packets were plugged with

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absorbent cotton inserting through plastic neck fixed in the mouth. The packets were pasteurized for 2, 3, 4, 5 and 6 hours with steam in a steel drum. The drum was filled with water at a level of 10.0 to 12.5 cm from the bottom and heated for the selected period. The packets were incubated in the MCH having 25°C ambient temperature and 75% RH. Diffused day light and proper ventilation were maintained in the MCH.

Incubation of spawn packet: After preparation of substrate in polyethylene bags, the packets were pasteurized in a hot water drum. After pasteurization, the spawn packets were placed on iron shelves and incubated in the MCH. The packets were observed regularly with 10 days' interval to record prevalence of contaminating fungi. Data on prevalence (%) of contamination and severity of contamination were recorded and expressed in percentage computed based on total number of packets checked the number of contaminated packets.

Design of experiment: The experiment was laid out in a 4x5x2 factorial experiment in completely randomized design (CRD), with 5 replications. The first factor was substrate with 4 levels (rice straw, saw dust, waste paper and a mixed substrate containing rice straw, saw dust, waste paper), the second factor was duration of pasteurization with 5 levels (2, 3, 4, 5 and 6 hours) and the third factor was size of spawn packet with 2 levels (500g and 1000g).

Data collection and data analysis: Data on severity of contamination were recorded starting from 30 days after incubation (DAI) and continued up to 60 DAI with 10 days' interval. The area of mycelium covered by contaminating fungi in spawn packet was graded based on a 0-5 scale, where 0= 0% coverage, 1=<0-20%, 2=<20-40%, 3=<40-60%, 4=<60-80% and 5=<80-100% coverage by mycelium of contaminating fungi. The severity of infection was calculated using the following formula: Prevalence of contamination (%) = (Sum of the total scoresx100) ÷ (total number of observation x maximum grade in the scale) (Biswas, 2014). Collected data were analyzed according to Gomez and Gomez (1984) using MSTAT-C computer program. Means were compared using LSD test following the same computer package.

RESULTS AND DISCUSSION

Contaminated rice straw Packets: In case of 500g rice straw packets, poor growth with 5-10% contaminants was observed at 60 DAI (Plate 1 A-C). In case of 1000g rice straw packets, intermediate growth with 40% contaminants was observed at 60 DAI (Plate 2A). Abundant growth with 100% contamination was observed at 60 DAI in treatment combination $S_1B_1T_5$ (Plate 2B).

Contaminated saw dust packets: Contaminating myco-flora in substrate packets containing 500g sawdust showed poor growth and the severity was 2% (Plate 3A) at 40 DAI and 8% (Plate 3B) at 60 DAI. Abundant growth of microorganisms, 60% was observed in 1000g sawdust packet (Plate 3C) at 60 DAI.

Contaminated packets of mixed substrates: In case of 2 hours pasteurized 500g mixed packets, 40% contamination was observed at 60 DAI (Plate 4A). In case of 1000g mixed packets, abundant growth of microorganism (60% contamination) was observed at 60 DAI for 2 hours' steam pasteurization bags (Plate 4 B).

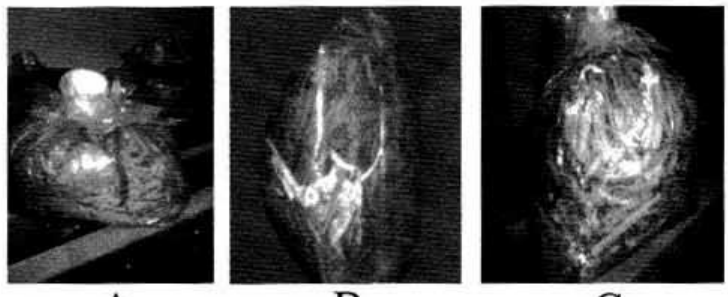
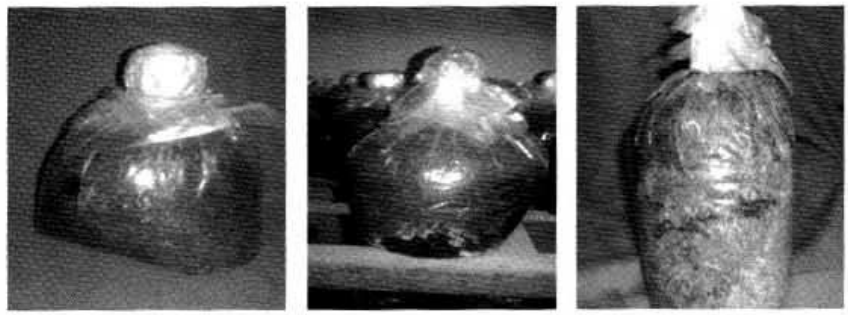


Plate 1. A. Poor growth,5% contamination, **BC** 10% contamination.

A B

Plate 2. Contaminated rice straw packets (1000g): **A.** intermediate growth 40% contaminated at 60 DAI; **B.** abundant growth, 100% contamination.



A B C

Plate 3. Contaminated sawdust packet (500g): A. poor growth, 2% Contaminated at 40 DAI; B. poor growth, 8% at 60 DAI; C. 60% abundant growth 1000 g saw dust packet.

A B

Plate. 4. A. Intermediate growth: 40% Contaminated mixed packets (500g); **B.** Abundant growth: 60% Contaminated mixed packets (1000g) at 60 DAI.

Main effect of different substrates on severity of contamination: The substrate packets prepared with only rice straw, only saw dust and mixed substrate containing rice straw, saw dust and waste paper were contaminated with microflora. The lowest severity of contamination was recorded from sawdust followed by rice straw at all stages of data collection. The maximum contamination was found in mixed substrate (rice straw + sawdust + waste paper) at all days after incubation. The severity of contamination increased gradually with gradual progress of days after incubation (DAI). The effect of rice straw, sawdust and mixed substrate on contamination severity was significantly different (Table 1).

Table 1. Effect of different substrates on severity of contamination in substrate packets recorded 30-60 days after incubation with 10 days' interval

Substrate	Contamination severity (%) at days after inoculation			
	30DAI	40DAI	50DAI	60DAI
Rice straw	6.55b (2.559)	14.07b(3.751)	18.69b(4.323)	21.88b (4.678)
Saw dust	3.28c (1.812)	5.31c (2.304)	8.24c (2.870)	10.49c (3.240)
Mixture of substrates	9.30a (3.050)	21.07a(4.590)	22.66a(4.760)	26.73a (5.170)

Values within the same column with a common letter(s) do not differ significantly at less than 5% level of significance. **Figures within parentheses are squire root transformed values ($\sqrt{(x+0.5)}$), where x=original value.

Iqbal *et al.* (2005) reported 37 days for full colonization completion in exotic strains of *P. ostreatus* on sugarcane bagasse. Mejía and Albertó (2013) reported that the low contamination might have occurred due to quality of a substrate. In present findings, rice straw promoted maximum contamination due to contain more protein and starch content and pore space, which helps to growth and penetration of mycelium of microorganisms. Different substrates have been used to grow *Pleurotus* sp. with BE values varying from 32.10- 79.18% (Dhanda *et al.*, 1994).

Main effect of duration of pasteurization on contamination severity: The main effect of duration of pasteurization on severity of contamination was significant. Irrespective of duration of pasteurization, the severity of contamination increased gradually with increasing DAI. Significantly the lowest severity of contamination was recorded when the substrate packets was pasteurized for 3 hours followed by duration of 4 hours at 30 and 60 DAI. At 40 and 50 DAI, severity of contamination in substrate packets pasteurized for 3 and 4 hours was statistically similar but significantly higher compared other duration of pasteurization. At all stages of data collection significantly the highest severity was found in packets pasteurized for 2 hour followed by 6 hours. So, 3 hours were noted as the best duration for substrate packet pasteurization (Table 2).

Table 2. Effect of duration of pasteurization of substrates with steam on contamination severity of substrate packets recorded up to 60 days after inoculation with 10 days' interval

Duration of pasteurization (hours)	Contamination Severity (%)			
	30DAI	40DAI	50DAI	60DAI
2	6.39a (2.527)	9.98a (3.159)	15.42a (3.926)	18.58a (4.310)
3	2.00d (1.416)	3.47d (1.864)	4.95d(2.224)	5.58e (2.405)
4	2.66c (1.630)	3.74d (1.934)	5.13d (2.266)	6.30d (2.510)
5	3.03b (1.742)	4.56c (2.136)	6.40c (2.530)	8.48c (2.912)
6	3.14b (1.771)	8.08b (2.842)	11.76b(3.429)	12.97b (3.602)

Values within the same column with a common letter(s) do not differ significantly at less than 5% level of significance. **Figures within parentheses are squired root transformed values ($\sqrt{(x+0.5)}$), where x=original value.

Diana *et al.* (2006) recommended disinfection of the substratum before spawning, which should only destroy the competitive fungi and not the useful microorganisms. Chang (2008) reported that the substrates for cultivating edible mushrooms e.g. *Pleurotus ostreatus*, has been reported to require varying degrees of pre-treatment in order to promote growth of the mushroom mycelium to the exclusion of other microorganisms. Sanchez (2010) reported that, substrate

used for the oyster mushroom cultivation do not require sterilization, but only pasteurization, which is less expensive to diminish the damages produced by different pathogens (bacteria, moulds or insect pests) on mushroom development and yield. Staments (1993) commented that pasteurization selectively kills temperature sensitive microorganisms. The population left intact presents little competition to the mushroom mycelia for initial period giving ample opportunity for the mushroom mycelium to colonize. But, Quimio *et al.* (1990) observed that substrate sterilization is not ideal since both beneficial and harmful organisms in the substrate are killed.

Main effect of packet size on severity of contamination: The severity of contamination increased gradually with the progress of days after incubation. Significantly the highest severity of contamination was recorded from 1000g substrate packets and the lowest in 500g packets (Table 3).

Table 3. Effect of spawn packet size on contamination severity

Amount of substrate (g)	Contamination Severity (%)			
	30DAI	40DAI	50DAI	60DAI
500	2.69b (1.642)	4.01b (2.004)	5.99b(2.449)	6.99b (2.645)
1000	3.97a (1.993)	7.67a (2.770)	10.89a (3.301)	13.33a (3.651)

Values within the same column with a common letter(s) do not differ significantly at less than 5% level of significance.**Figures within parentheses are squired root transformed values ($\sqrt{(x+0.5)}$), where, x=original value.

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Effect of Different Substrates Ratio on the Growth and Yield of Oyster Mushroom

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Abstract

The experiment was carried out at the tissue culture laboratory and culture house of Mushroom Development Institute, Savar, Dhaka, during the period from January 2014 to June 2014. The experiment consisted of two varieties, viz. V_1 (*Pleurotus ostreatus*) and V_2 (*Pleurotus djamor*) and nine different substrates ratio: S_1 (25% straw+10% paddy grain (mother culture) + 65% sawdust), S_2 (35% straw + 10% paddy grain (mother culture) + 55% sawdust), S_3 (45% straw + 10% paddy grain mother culture + 45% sawdust), S_4 (55% straw + 10% paddy grain mother culture + 35% sawdust), S_5 (65% straw + 10% paddy grain mother culture + 25% sawdust), S_6 (75% straw + 10% paddy grain mother culture + 15% sawdust), S_7 (85% straw + 10% paddy grain mother culture + 5% sawdust), S_8 (90% straw + 10% paddy grain mother culture), S_9 (90% sawdust + 10% paddy grain mother culture). The experiment was laid out in completely randomized design with three replications. The maximum yield (66.50g), the highest number of fruiting body (16.53), number of effective fruiting body (12.11) were observed in V_1. Significant variation was found in all parameter due to the effect of substrates ratio. The highest number of fruiting body (15.25), number of effective fruiting body (12.11) and highest yield (63.25g) were recorded in S_3.

Keywords: Substrates, Mushroom, Mother culture.

INTRODUCTION

Mushroom substrates may be defined as a kind of ligno-cellulosic material which supports the growth, development and fruiting of mushroom (Chang and Miles, 1988). However, supplementation of the substrates with various materials is recommended prior to spawning for enhancement of the yield of mushrooms. To improve growth and yield of mushroom, various supplements can be added to the substrates (Hadwan *et al.* 1997). It is well known that, mycelium growth and mushroom production both are affected by cellulose, hemicelluloses and lignin proportions along with nitrogen content of the cultivating substrate (Mata and Savoie, 2005).

Substrate plays an important role in the yield and nutrient content of oyster mushroom. The substrates on which mushroom spawn (merely vegetative seed materials) is grown, affects the mushroom production (Klingman, 1950). Oyster mushroom can grow on sawdust, rice and wheat straw, water hyacinth and other agro-waste. Sarker et al. (2007) observed a remarkable variation in nutritional content of oyster mushroom in different substrates. The oyster mushrooms can be cultivated successfully under semi controlled conditions in a small space by using agricultural as well as industrial waste and other refuse as substrate. Kausar and Iqbal (1994) used 5% spawn of Pleurotus (w/w basis) in 15 kg paddy straw, pinheads formed 28 days after spawning. The yield varied from 18.6 to 83.5% based on different nitrogen supplements amended with straw. Cangy and Peerally (1995) used spawning rates 0.75, 1.50, 3.00 and 6.00% of substrate fresh weight for 10 species of Pleurotus. Results showed that 1% spawning rate was found to be adequate when using the smaller bags (yields >16% of spawned substrate weight) at mean temperature 18^{0}C (range 13-23^{0}C).

In Bangladesh oyster mushroom is now widely cultivated in our country because the weather and climate of Bangladesh is suitable for its cultivation and the necessary materials required for oyster mushroom cultivation such as straw, sawdust, wheat bran, water hyacinth, agricultural and industrial waste products etc. are also available and cheap. The present study was planned to

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find out the easiest, economical and practicable methodology of preparation and use of substrate, which may also be helpful to increase the growth and productivity of oyster mushroom. The findings will help and guide the mushroom growers, especially the people interested in the cultivation of oyster mushrooms.

MATERIALS AND METHODS

The experiment was designed out to find out the effect of different substrates ratio on the growth and yield of oyster mushroom and was carried out at the tissue culture laboratory and culture house of Mushroom Development Institute, Savar, Dhaka, during the period from January 2014 to June 2014. The study was laid out in Completely Randomized Design (CRD) with three replications and eighteen treatment combinations. Two oyster Mushroom varieties such as-V_1 (*Pleurotus ostreatus*) and V_2 (*Pleurotus djamor)* were tested on different substrates. Spawn packet of 500g size was prepared by through spawning method and was maintain the definite substrates ratio.

Substrates ratio: S_1= 25% straw +10% paddy grain (mother culture) +65% sawdust. S_2= 35% straw+10% paddy grain (mother culture) + 55% sawdust. S_3= 45% straw+10% paddy grain (mother culture) + 45% sawdust. S_4= 55% straw+ 10% paddy grain (mother culture) + 35% sawdust. S_5= 65% straw+10% paddy grain (mother culture) +25% sawdust. S_6= 75% straw + 10% paddy grain (mother culture) +15% sawdust. S_7= 85% straw+10% paddy grain (mother culture) + 5% sawdust. S_8= 90% straw+10% paddy grain (mother culture). S_9= 90% sawdust+10% paddy grain (mother culture).

Preparation of pure culture: Pure cultures of two strains were prepared on Potato Dextrose Agar (PDA) medium. Afresh and juvenile stage sporophore of above mentioned mushrooms were collected and surface sterilized with 70% alcohol by rubbing cotton soaked in alcohol. Tissues were collected from inner region of the joint of stalk and pileus. The tissues cut into small pieces and placed on the solidified test tube containing PDA. After inoculation, the tube was covered with cork. All operations were done under sterile condition in a clean bench. The inoculated tubes were kept in a growth chamber maintaining temperature at 20-25^{0}C and incubated 8-15 days until the tubes full of whitish mycelia. Then the pure culture was used for inoculation of master mother culture.

Preparation of mother culture (Paddy grain): To prepare mother culture of test mushroom (Pleurotus ostreatus and Pleurotus djamor) good quality paddy grains were used as media of mother culture. At first 2 kg of grains collected which was free from diseases and not broken, old, and insect damaged. The grains were thoroughly washed in sufficient water three to four times to remove unfilled grain, soil debris, straw particles and other undesirable things. Then washed grains were soaked in sufficient water for 2-3 hours and boiled in a container (saucepan) for 30- 45 minutes until cracking. Excess water from the boiled grains was removed by heating and continuous shaking. When the water removed burner was stopped. Then the boiled grains were kept 1-2 hours for cooling. The cooled grains were thoroughly mixed with sawdust containing master mother culture at 10% rate. This mixing was done the same container after wearing gloves and the mixed grains were poured into polypropylene bags (18cm × 25cm) at 250-300g/bag and their mouths were plugged by inserting absorbing cotton without neck. The bags were kept in rack at room temperature. After 10 to 15 days the mother culture became white due to complete the mycelium running and then it was ready for spawning of spawn packets.

Preparation of substrates: Two different substrates namely, sawdust (SD) and rice straw (RS) were used as media. Both the substrates were prepared by pasteurization method. In case of SD, twenty kg sawdust was mixed with 17 litter of water and 4-5 kg mixture was poured into cribriform nylon bag. In case of RS, the straw was chopped to 4-5 cm length and then poured 4-5 kg into cribriform nylon bag. The bags were submerged in water for sometimes and then drained out the excess water. After that both the bags containing SD and RS were kept in a pasteurization chamber at 60-65^{0}C for one hour. The bags were kept in same place for 18-20 hours to get cool slowly. After 20 hours the prepared sawdust and straw were spread over polythene sheet in open place to reduce moisture 63%. These substrates were ready for spawn packet preparation.

Preparation of spawn packets: According to treatment combination prepared substrates and 10% mother culture were mixed thoroughly and filled into 18cm × 25cm polypropylene bags at 500 g/bag. The mouths of the filled polypropylene bags were plugged by inserting absorbing cotton with the help of plastic neck and rubber band by spawning method.

Mycelium running in spawn packets/ Incubation: The packets were kept at room temperature until the packets become white with the mushroom mycelium. After completion of the mycelium running the rubber band, cotton plug and plastic neck of the mouth of spawn packet were removed and the mouth was wrapped tightly with rubber band. Then these spawn packets were transferred to the culture house.

Opening the packet: Two ends, opposite to each other of the upper position i.e. on shoulder of plastic bag were cut in 'D' shaped with a blade and opened by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a blade for removing the thin whitish mycelial layer.

Cultivation of spawn packet: The packets of each type were placed separately on the rack of culture room. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3-5 times a day. The light around 150-200 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22°C to 25°C. The first primordia appeared 2-4 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

Cultural operation, collection of produced and harvesting of mushroom: After completing the first harvest again the packets were scrapped at the same place where the 'D' shaped cut had been done and then placed in the culture house and water was sprayed regularly. The primordia appeared 9-10 days after first harvest and 7-8 days after second harvest. Water spraying was continued until the mushrooms were ready to be harvested.

Data collection: Days required from pinhead initiation to 1st harvest, number of total fruiting body, number of effective fruiting body/packet, dimension of pileus and stalk, yield were recorded. Yield in g/500g packet was recorded by weighing all the fruiting bodies in a packet after removing the lower hard and dirty portion.

Statistical analysis: The collected data were analyzed statistically following completely randomized design by MSTAT-C computer package programme. The treatment means were compared by Least Significance Differences (LSD), Duncan's Multiple Range Test (DMRT) and regression lines were performed as and when necessary (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Days required from pinhead initiation to 1st harvest: Variety of mushroom showed influence on days required from pinhead initiation to 1st harvest. The lowest time (3.22 days) from pinhead initiation to 1st harvest was in V_1 (*Pleurotus ostreatus*) and the highest time (4.39 days) from pinhead initiation to 1st harvest was observed in the V_2 (*Pleurotus djamor*) (Fig. 1).

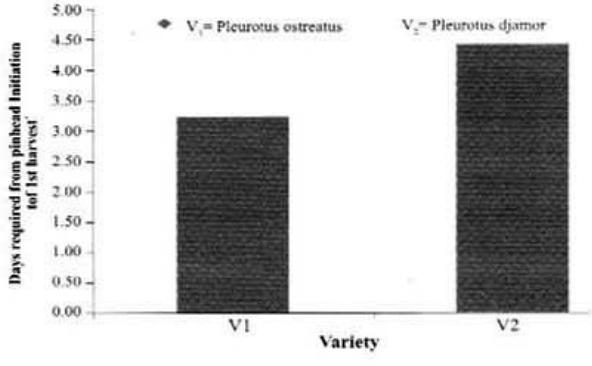

Fig. 1. Effect of variety on Days required from pinhead initiation to 1st harvest of oyster mushroom.

Significant variation was found in days required from pinhead initiation to 1st harvest due to the effect of substrates ratio. The lowest time (3.50 days) from pinhead initiation to 1st harvest was in the treatment S_4, S_5, S_6 and S_7. The highest time (4.50 days) from pinhead initiation of 1st harvest was observed in the treatment S_9 (Fig. 2). The result of the present study keeps in with the findings of several workers (Khan *et al.*, 2001; Dhoke *et al.*, 2001; Royse, 2002 and Kulsum *et al.*,2009). Khan *et al.* (2001) who reported that after spawn running pinhead formation took 7-8 days and fruiting body formed after 3-5 days, sporocarps may be harvested after 10-12 days. Dhoke *et al.* (2001) found significant effect of different agro-wastes on yield of oyster mushroom. The days required for first picking varied from 11.25-12.00 and the final picking complete from 42.25 to 43.50 days depending on different substrates. Royse (2002) found as the spawn rate increased the number of days to production decreased. Kulsum *et al.* (2009) observed that the lowest time from primordial initiation to harvest was 3.2 days due to sawdust supplemented with cow dung and it was 10%.

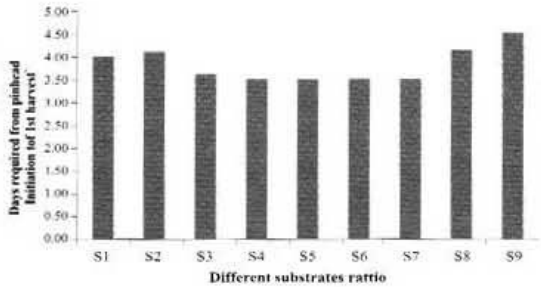

Fig. 2. Effect of substrates ratio on Days required pinhead initiation to 1st harvest of oyster Mushroom.

Number of fruiting body\packet: Variety of mushroom showed influence on number of fruiting body. The highest number of fruiting body (16.53) was observed in V_1 (*Pleurotus ostreatus*) and the lowest average number of fruiting body (5.56) was in the treatment V_2 (*Pleurotus djamor*) (Fig. 3).

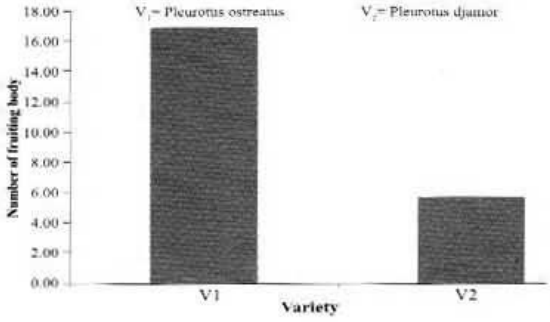

Fig. 3. Effect of variety on number of fruiting bodyof oyster Mushroom.

There was significant variation in number of fruiting body due to substrates ratio. The highest number of fruiting body (15.25) was observed in the treatment S_3 (45% straw+10% paddy grain (mother culture) +45% sawdust), which was statistically similar with S_2 and S_1 and the lowest number of fruiting body (7.26) was in the treatment S_6 (75% straw+10% paddy grain mother culture) +15% Sawdust) (Fig. 4). The other treatments significantly varied in terms of number of fruiting body. The result of the present findings keeps in with the findings of Yoshida *et al.*, 1993; Amin, 2004; Sarker, 2004 and Kulsum *et al.*,2009. Yoshida *et al.* (1993) reported that the number of fruiting bodies was lower but increased when the substrates was mixed with different supplements. Amin (2004) reported that the number of primordia grown on different substrates differed significantly. Sarker (2004) found that the number of primordia increased

with the levels of supplement and continued up to a certain range and decline thereafter. Kulsum *et al.* (2009) observed that the highest average number of fruiting body/packet was 60.42 due to sawdust supplemented with cowdung which was 10%.

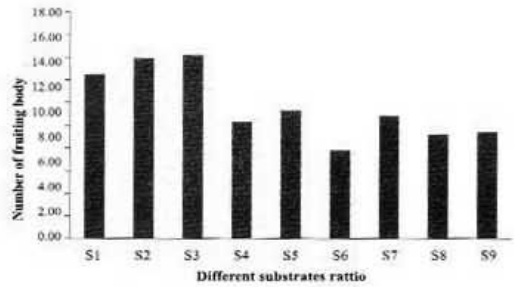

Fig. 4. Effect of Different substrates ratio on number of fruiting body of oyster Mushroom.

Number of effective fruiting body/packet: Number of effective fruiting body affected due to the varieties. The highest number of effective fruiting body (12.11) was obtained from V_1 treatment. The lowest number of effective fruiting body (5.50) was obtained from V_2 treatment (Fig. 5).

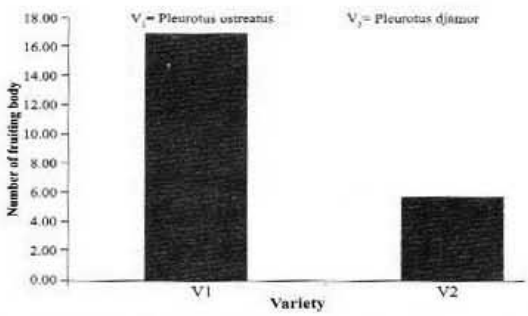

Fig. 5. Effect of variety on number of effective fruiting body of oyster Mushroom.

Significant variation was observed highest number of effective fruiting body due to substrates ratio. The maximum number of effective fruiting body (11.63) was observed in the treatment S_3, and the lowest number of effective fruiting body (5.88) was in the treatment S_9 (90% sawdust+10% paddy grain mother). The other treatments significantly varied in terms of number of effective fruiting body (Fig. 6).

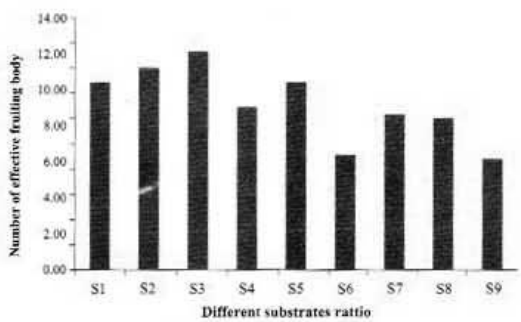

Fig. 6. Effect of Different substrates ratio on number of effective fruiting body of oyster Mushroom.

Length of stalk: A significant variation in the length of stalk was found among the varieties. The longest stalk length (4.04 cm) was obtained from V_1 treatment and the shortest stalk length (2.15 cm) was obtained from V_2 treatment. Length of stalk had significant variation due to the

substrates ratio. The maximum (3.94 cm) length of stalk was recorded from S_3, while S_6 gave the minimum (2.04 cm) length of stalk (Table 1).

Diameter of stalk: A significant variation in the diameter of stalk was found among the varieties. The largest stalk diameter (1.14 cm) was obtained from V_1 treatment, and the shortest stalk diameter (0.72 cm) was obtained from V_2 treatment (Table 1). Stalk diameter differed significantly due to the substrates ratio. The highest (1.06 cm) diameter of stalk was recorded from S_3, which was statistically similar with S_4 treatment. While S_7 (85% straw+10% paddy grain mother culture +5% sawdust) gave the minimum (0.78) diameter of stalk (Table 1).

Length of pileus: A significant variation in the length of pileus was found among the varieties. The longest pileus length (7.72 cm) was obtained from V_1 treatment and the shortest pileus length (6.05 cm) was obtained from V_2 treatment. Length of pileus had significant variation due to the substrates ratio. The maximum (7.32 cm) length of pileus was recorded from S_3, which was statistically similar with S_1 while S_6 gave the minimum (6.34 cm) length of pileus (Table 1).

Diameter of pileus: A variation in the diameter of pileus was found among the varieties. The largest pileus diameter (9.02cm) was obtained from V_1 treatment, and the shortest pileus diameter (5.31cm) was obtained from V_2 treatment (Table 1). Pileus diameter differed significantly due to the substrates ratio. The highest (10.41cm) diameter of pileus was recorded from S_3, while S_6 gave the minimum (4.65) diameter of pileus (Table 1).

Thickness of pileus: A variation in the Thickness of pileus was found among the varieties. The maximum thickness of pileus (5.54 cm) was obtained from V_2 treatment, and the minimum thickness of pileus (0.87 cm) was obtained from V_1 treatment (Table 1). Thickness of pileus differed significantly due to the substrates ratio. The highest (5.66 cm) Thickness of pileus was recorded from S_3, While S_7 gave the minimum (0.78 cm) thickness of pileus (Table 1).

Table 1. Effect of variety and substrates ratio on size of fruiting body and yield of oyster mushroom

Treatment	Length of stalk(cm)	Diameter of stalk(cm)	Length of pileus (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)
Variety						
V_1	4.04	1.14	7.72	9.02	0.87	66.50
V_2	2.15	0.72	6.05	5.31	5.54	33.17
Substrates ratio						
S_1	2.96 b	0.88 abc	7.29a	4.98 ab	4.89d	54.50 b
S_2	3.23 ab	0.86 abc	6.84abc	5.27a	4.84d	62.88a
S_3	3.94 a	1.06a	7.32a	5.66a	10.41a	63.25a
S_4	3.28 ab	1.05a	7.01ab	4.54b	4.93cd	44.50c
S_5	3.06 b	1.03ab	6.58bc	5.03ab	5.77c	58.50ab
S_6	2.04 c	0.82bc	6.34c	0.87c	4.65d	35.00d
S_7	2.89 b	0.78c	6.67bc	0.78c	9.34b	45.50c
S_8	2.98 b	0.89abc	7.00ab	0.79c	9.94ab	42.88c
S_9	3.49 ab	1.00ab	6.96ab	0.95c	9.70ab	41.50c
LSD (0.05)	0.72	0.20	0.53	0.66	0.84	4.59
CV (%)	14.26	16.87	16.58	7.22	12.55	5.65

In a column, means followed by a common letter are not significantly differed of 5% level by DMRT. V_1 = *Pleurotus ostreatus*. V_2 =*Pleurotus djamor*.

Yield (g/packet): A variation in the yield was found among the varieties. The maximum yield of mushroom (66.50g/ plant) was obtained from V_1 treatment, and the minimum yield (33.17g) was obtained from V_2 treatment (Table 1).

Significant variation was observed yield due to substrates ratio. The highest yield (63.25 g) was recorded under treatment S_3 and the lowest yield was recorded under S_1 (35.00 g). The other treatments varied significantly as in terms of yield (Table 1). Chowdhury *et al.* (1998) examined the effects of adding different supplements to substrates for growing oyster mushrooms (*Pleurotus sajor-caju*) and found adding 5% supplements gave the highest yield of oyster mushroom. Baysal *et al.* (2003) found the highest yield of oyster mushroom (*Pleurotus ostreatus*) with the substrate composed of 20% rice husk in weight.

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