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Books:

Gomez, K. A. & Gomez, A. A. 1984. **Statistical Procedures of Agricultural Research,** 2nd ed., John Wiley and Sons, Singapore. p. 21.

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Dr. Nirod Chandra Sarker
Editor-in-Chief
Bangladesh Journal of Mushroom
and
Deputy Director
Mushroom Development Institute
Sobhanbag, Savar, Dhaka
E-mail: bjm_namdec07@yahoo.com
Fax: 880-2-7710646/02224445646

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Effect of Different Water Sources on Yield of Tree Oyster Mushroom

Nirod Chandra Sarker, Akhter Jahan Kakon, Mohammad Mizanur Rahman[1], Ruhul Amin and Md. Bazlul Karim Choudhury[2]
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

An experiment was conducted to determine the effect of different water sources containing shallow tube well water, deep tube well water, pond water, arsenic contaminated water, sea water, iron rich water, deionized water, distilled water, Vodra river's water, Rupsha river's water on yield and yield attributes of tree oyster mushroom (*Pleurotus ostreatus*) during November 2012 to May 2013. The highest yield and biological efficiency (137.0g and 78.29%) were recorded in T_7 (DI water) which was statistically similar to T_9 (Vodra river's water) (132.00g and 75.86%). The lowest yield and biological efficiency (30.75g and 17.56%) were recorded in T_5 (sea water). The highest number of total fruiting bodies (10.25) was found in T_9 (Vodra river's water) which was statistically similar to T_6 and the lowest (5.78) was in T_3 (pond water) which was statistically similar to T_1. The highest number of total effective fruiting bodies (4.31) was found in T_6 (Iron rich water) which was statistically similar to T_{10}, T_9, T_4, and T_1 whereas the lowest (2.00) was in T_5 (sea water). The length of stalk ranged from 2.50 to 4.00cm. The highest length of stalk (4.00 cm) was found in T_3 (pond water) and the lowest (2.50 cm) was in T_5. The diameter of stalk, pileus and thickness of pileus ranged from 0.27 to 0.83 cm, 6.50 to 11.50 cm and 0.60 to 0.80 cm respectively. Therefore, it may be concluded that except sea water and arsenic contaminated water all water sources are suitable for oyster mushroom cultivation.

Keywords: Water source, Yield, Sawdust, Spawn packet, Mother culture.

INTRODUCTION

Mushrooms of *Pleurotus spp.* are commonly known as oyster mushrooms under the class basidiomycetes is cultivated and consumed by 97%, of which *Pleurotus ostreatus* alone accounts for 61%. Water is one of the main factors that influence the success in mushroom growth. Nutrients are transported from the mycelium to the fruiting bodies by a steady moisture flow (Oei and Nieuwenhuijzen, 2005). High moisture content in the substrate will result in difficult breathing for the mycelium inhibiting perspiration, rendering the development of fruiting body impossible, even with elevated inoculum amounts or number of holes in mushroom cultivation packages, resulting in the development of non-desired organisms such as bacteria and nematodes (Urben, 2004). Low moisture content will result in the death of the fruiting body. The optimum moisture content for growth and substrate utilization depends upon the organism and the substrate used for cultivation. Increasing moisture level is believed to reduce the porosity of the substrate, thus limiting oxygen transfer. For this reason, the use of high moisture content limited the growth within the whole substrate, resulting in surface growth (Patel *et al.,* 2009). According to Chang and Miles (2004), the appropriate moisture in the substrate should encompass a range between 50% and 75% in the substrate, enabling the satisfactory growth of *Pleurotus spp.* Water sources also an important factor. Tap water

[1] Horticulture Centre, Balaghata, Banderban, Bangladesh; [2] Manikganj Medical College, Manikganj, Bangladesh.

is commonly used by the mushroom grower's. According to Meji'a and Alberto (2013), tap water was added up to 70% of final moisture. Water contaminated with heavy metals like mercury, lead and copper can cause undesirable flavor to the product and be a source of human contamination. In our country different water sources like tubewell water, saline water, deep tubewell water etc. are used by the mushroom growers. Mushroom cultivation has a special relevance to Bangladesh, because sawdust and other materials are available to the farmers. So, mushroom production could keep great importance on our economy as a whole. However, the research on the effect of different water sources on the production of oyster mushroom had not been well established. Therefore, the present experiment was undertaken to determine the most suitable water source for better growth of mushroom.

MATERIALS AND METHOD

Experimental site: This experiment was conducted at the National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka, Bangladesh during November 2012 to May 2013.

Treatments: In this experiment ten different water sources were used as treatments. The treatments were T_1 = Shallow tube well water, T_2 = Deep tube well water, T_3 = Pond water, T_4 = Arsenic contaminated water, T_5 = Sea water (3.5% salinity), T_6 = Iron rich water, T_7 = Deionized water (DI), T_8 = Distilled water, T_9 = Vodra river's water, T_{10} = Rupsha river's water. Tree oyster mushroom (*Pleurotus ostreatus*), namely PO2 used as test materials.

Spawn packet preparation, inoculation and incubation: Sawdust spawn packets of 500 g size were prepared, inoculated and incubated following the procedure that developed and explained by Sarker *et al.* (2007).

Experimental condition: The packets were kept in a dark room at 25°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 25-30°C respectively by spraying water. Water was sprayed 3-4 times per day according to treatments. 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:

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

Experimental design, 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, number of flush ie. yield of 1st, 2nd, 3rd flush, total yield, average yield and biological efficiency were recorded and analyzed following Gomez and Gomez (1984) using MSTAT-C computer program. Means separation were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Yield (g): Significant difference was observed on yield of oyster mushroom bed in different treatments and it was ranged from 30.75 to 137.00 g/packet (Table 1). The highest yield (137.0 g/packet) was obtained from DI water (T_7) which was statistically similar to Vodra river's water (132.80 g/packet) while the lowest yield (30.75 g/packet) was observed in sea water (T_5). Moonmoon *et al.* (2012) observed that the yield of *pleurotus ostreatus* on sawdust based substrate 194.30 g/packet in summer season using tap water/normal water. Khan *et al.* (2013) also observed that the yield of *pleurotus ostreatus* on sawdust based substrate was188.00 g/packet.

Yield of first flush/harvest (g): Significant difference was observed in YFH among the different water sources (Table 1). The maximum YFH (59.75 g/packet) was recorded in iron rich water which was statistically similar to DI water and it was minimum (0.0 g/packet) in sea water. Moonmoon *et al.* (2013) reported that tree oyster mushroom (PO2) gave 60.33 g/packet in 1st flush using normal water. Sarker *et al.* (2014) also reported that tree oyster mushroom (PO2) gave 87.00 g/packet in 1st flush using normal water on sawdust based substrate.

Yield of second flush (g): Significant difference was observed in YSF among the different water sources (Table 1). The maximum YSF (53.25g/packet) was recorded in Rupsha river's water which was statistically similar to arsenic contaminated water (53.00 g/packet) and shallow tube well water (50.00 g/packet). The minimum yield was (11.75g/packet) obtained from sea water. It is very important that the second flush play an important role to give yield in all treatments.

Table 1. Effect of different water sources on yield of tree oyster mushroom

Treatment (different water sources)	Yield (g)				
	1st harvest	2nd harvest	3rd harvest	Total yield	Average yield
T_1	45.00c	50.00a	14.75f	109.80c	36.50c
T_2	24.00e	39.00b	14.00f	77.25f	25.75f
T_3	40.00d	33.25c	13.50f	86.75e	28.75e
T_4	24.00e	53.00a	42.25c	119.30b	39.75b
T_5	0.0f	11.75e	19.00e	30.75g	10.50g
T_6	59.75a	42.25b	14.50f	116.50b	38.75bc
T_7	57.00a	24.75d	55.25b	137.00a	46.00a
T_8	48.75b	35.25c	10.00g	94.00d	31.25d
T_9	39.25d	35.00c	58.50a	132.80a	44.25a
T_{10}	42.25cd	53.25a	22.25d	117.80b	39.25b
CV (%)	**6.75**	**6.02**	**8.30**	**4.53**	**4.71**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT. T_1 = Shallow tubewell water, T_2 = Deep tubewell water, T_3 = Pond water, T_4 = Arsenic contaminated water, T_5 = Sea water (salinity 3.5%), T_6 = Iron rich water, T_7 = DI water, T_8 = Distilled water, T_9 = Vodra river's water, T_{10} = Rupsha river's water.

Yield of third flush (g): Significant difference was observed in YTF among the different water sources (Table 1). The maximum YTF (58.50 g/packet) was recorded in Vodra river's water which was followed by DI water (55.25 g/packet). The minimum yield was (13.50 g/packet) obtained from pond water which was statistically similar to deep tube well water (14.00 g/packet) and shallow tube well water (14.75 g/packet).

Average yield (g): Significant difference was observed in average yield among the different water sources (Table 1). The maximum AVY (46.00 g/packet) was recorded in iron rich water which was statistically similar to DI water and it was minimum (0.0 g/packet) in sea water.

Total number of fruiting body/packet (TNFB): The total number of fruiting body was significantly difference. The highest TNFB (10.25) was observed when Vodra river's water was spraying for cultivation of mushroom which was statistically similar to iron rich water (9.8) and the lowest number of fruiting bodies (5.88) was found when shallow tube well water was used which was statistically similar to pond water (5.78) (Table 2). Moonmoon *et al.* (2013) observed that the number of fruiting body of PO2 on sawdust based substrates 14.25 and Shaheen *et al.* (2015) also observed that the number of fruiting body of PO2 on sawdust based substrates 8.44. In case of 1st, 2nd and 3rd flushes the highest NFB (4.5, 4.88, 3.00) was observed respectively when Iron rich water and Vodra river's water were used for cultivation of mushroom.

Number of effective fruiting body/packet (TNEFB): The number of effective fruiting body was significantly difference. The highest TENFB (4.31) was observed when iron rich water was spraying for cultivation of mushroom which was statistically similar to Vodra river's water, Rupsha river's water, shallow tube well and arsenic contaminated water. The lowest number of fruiting bodies (2.00) was found when sea water was used (Table 2). Moonmoon *et al.* (2013) observed that the number of fruiting body of PO2 on sawdust based substrates 14.25 and Shaheen *et al.* (2015) also observed that the number of fruiting body of PO2 on sawdust based substrates 8.44. In case of 1st, 2nd and 3rd flushes the highest TNEFB (1.69, 1.63, 1.75) was observed respectively when iron rich water and arsenic contaminated water which was statistically similar to Vodra river's water were used for cultivation of mushroom.

Table 2. Effect of different water sources on yield attributing characters of tree oyster mushroom

Treatments (different water sources	Number of fruiting body				Effective number of fruiting body			
	Ist harvest	2nd harvest	3rd harvest	Total	Ist harvest	2nd harvest	3rd harvest	Total
T_1	2.38c	2.50e	1.00f	5.88f	1.30b	1.63a	1.00d	3.93a
T_2	2.25c	2.58e	1.55e	6.38e	1.00c	1.00c	1.00d	3.00b
T_3	2.08c	2.45e	1.25f	5.78f	1.00c	1.00c	1.00d	3.00b
T_4	1.70d	3.50bc	1.88cd	7.08d	1.00c	1.30b	1.75a	4.05a
T_5	0.0e	1.08f	1.18f	2.25g	.000d	1.00c	1.00d	2.00c
T_6	4.5a	2.93d	2.38b	9.80a	1.69a	1.63a	1.00d	4.31a
T_7	3.43b	3.38c	1.88cd	8.68bc	1.50ab	.000d	1.43b	2.93b
T_8	3.13b	3.50bc	1.68de	8.30c	1.00c	1.00c	1.00d	3.00b
T_9	2.38c	4.88a	3.00a	10.25a	1.38b	1.00c	1.63a	4.00a
T_{10}	3.38b	3.70b	2.03c	9.15b	1.33b	1.58a	1.25c	4.15a
CV (%)	**8.50**	**6.32**	**10.18**	**4.61**	**13.72**	**15.50**	**9.52**	**7.50**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT. T_1 = Shallow tubewell water, T_2 = Deep tubewell water, T_3 = Pond water, T_4 = Arsenic contaminated water, T_5 = Sea water (salinity 3.5%), T_6 = Iron rich water, T_7 = DI water, T_8 = Distilled water, T_9 = Vodra river's water, T_{10} = Rupsha river's water.

Size of fruiting body: The length of stalk ranged from 2.50 to 4.0 cm with non -significant difference (Table 3). The highest length of stalk was found in pond water ie T_3 (4.0 cm) which was followed by iron rich water (T_6) and the lowest length of stalk was found in T_5 (2.50). The diameter of stalk also non significant and ranged from 0.27 to 0.83 cm (Table 3).

Table 3. Effect of different water sources on size of fruiting body of tree oyster mushroom

Treatments (different water sources)	Size of fruiting body			
	Length of stalk	Diameter of stalk	Diameter of pileus	Thickness of pileus
T_1	3.50a	0.75a	6.56b	0.80a
T_2	3.00a	0.46a	7.50b	0.70b
T_3	4.00a	0.49a	7.30b	0.60c
T_4	3.05a	0.79a	7.10b	0.70b
T_5	2.50a	0.27a	6.50b	0.70b
T_6	3.60a	0.78a	7.20b	0.70b
T_7	2.80a	0.73a	8.50b	0.70b
T_8	3.50a	0.83a	11.50a	0.60c
T_9	3.20a	0.61a	6.50b	0.70b
T_{10}	3.00a	0.69a	7.60b	0.60c
CV (%)	**5.87**	**4.79**	**2.88**	**3.03**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT. T_1 = Shallow tubewell water, T_2 = Deep tubewell water, T_3 = Pond water, T_4 = Arsenic contaminated water, T_5 = Sea water (salinity 3.5%), T_6 = Iron rich water, T_7 = DI water, T_8 = Distilled water, T_9 = Vodra river's water, T_{10} = Rupsha river's water.

The highest diameter of stalk was found in T_8 (0.83cm) while the lowest diameter of stalk (0.27 cm) was found in sea water (T_5). Moonmoon *et al.* (2013) reported that the length of stalk and diameter of stalk of PO2 on sawdust based substrates was 3.42 and 1.29 respectively.

The diameter of pileus ranged from 6.50 cm to 11.50 cm with significant difference among the treatments (Table 3). The highest diameter of pileus (11.50cm) was found in (T_8) which was statistically identical and the lowest diameter of pileus (6.50cm) was found in sea water (T_5). Moonmoon *et al.* (2013) reported that the diameter of pileus of PO2 on sawdust based substrates was 7.62 cm.

The thickness of pileus in different treatments differed significantly and ranged from 0.60cm to 0.80 cm (Table 3). The highest thickness was found in T_1 (0.80 cm) which was statistically identical to other treatments. The lowest thickness of pileus (0.60 cm) was found in T_3 which was statistically similar to T_8 and T_{10}. Moonmoon *et al.* (2013) observed that the thickness of pileus of PO2 on sawdust based substrates was 0.63 cm.

Biological efficiency (Be%): The highest biological efficiency (78.29%) was found in T_7 followed by T_9 (75.86%) and the lowest biological efficiency was found in T_5 (17.56%) (Fig. 1) whereas Moonmoon *et al.* (2013) was observed that the biological efficiency (30.16%) of *Pleurotus ostreatus* using sawdust based substrate.

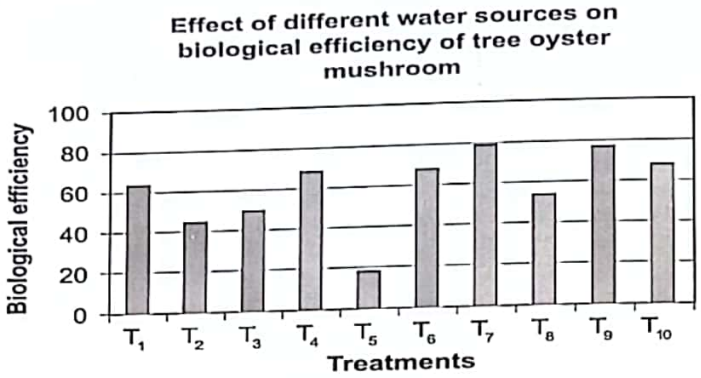

Fig. 1. Effect of different water sources on biological efficiency of tree oyster mushroom.

REFERENCES

Chang, S. T. & Miles, P. G. 2004. Mushrooms: Cultivation, nutritional value medicinal effect, and environmental impact, 2nd ed. Boca Raton, Fl: CRC Press. pp. 2-3.

Gomez, K. A. & Gomez, A. A. 1984. Statistical Procedures for Agricultural Research. Second edn. John Wiley and Sons. Inc. New York. pp. 304-307.

Khan, A.S., Sarker, N. C., Hoque, M. M., Mahjabin, T., Moonmoon, M., Shamuszzaman, K., & Amin, M. S. 2013. Yield performance of oyster mushroom variety at different locations in Bangladesh. *Bangladesh J. Mushroom.* 7(1): 73 - 82.

Mejı´a, S.J., Alberto´, E., 2013. Heat treatment of wheat straw byimmersion in hot water decreases mushroom yield in Pleurotus ostreatus. *Ver. Iberoam. Micol.* **30**(2): 125 –129 (in Spanish).

Moonmoon, M., Kakon, A. J., Hoque, M. M., Sarker, H. K., Huda,N., Nahar, K., Mujib, T. B. & Khan, A. S. 2013. Yield performance of oyster mushroom variety at different locations in Bangladesh. *Bangladesh J. Mushroom.* 7(1): 27 - 34.

Moonmoon, M., Mahjabin, T., Sarker, N. C., Khan, A. S., Rahman, T.& Kakon, A. J. 2012. Performance of oyster mushroom variety on rice straw and sawdust in summer season. *Bangladesh J. Mushroom.* **6**(2): 35 - 40.

Oei, P. & Nieuwenhuijzen, B. V. 2005. Small-scale Mushroom Culti-vation: Oyster, Shiitake and Wood Ear Mushrooms. Agromisa Foundation and CTA, Wageningen p.37.

Patel, S. J., Onkarappa, R., & Gurumurthy, S. 2009. Pretreatment studies on wheat straw and rice straw. *Internet Journal of Microbiology.* 7(1): 1 - 4.

Sarker, N. C., Hossain, M. M., Sultana, N., Mian, I. H., Karim, A. J. M. S. & Amin, S. M. R. 2007. Performance of different Substrates on the Growth and Yield of *Pleurotus ostreatus* (jacquin ex Fr.) Kummer. *Bangladesh J. Mushroom.* **1**(2): 9 - 20.

Sarker, N. C., Shamsuzaman, K. M., Kakon, A. J. & Choudhury, B. K. 2014. Performance of hybrid strains of oyster mushroom developed at Mushroom Development Institute. *Bangladesh J. Mushroom.* **8**(2): 19 - 22.

Shaheen, M., Sarker, N. C., Moonmoon, M., Khan, A.S., & Mujib, T.B. 2015. Yield performance of oyster mushroom using waste paper. *Bangladesh J. Mushroom.* **9**(1): 37-42.

Urben, A. F. 2004. Produc¸a˜o de cogumelos por meio de tecnologiachinesa modificada. Embrapa Recursos Gene´ticos e Biotecnologia, Brası´lia (in Portuguese).187p.

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Effect of Opening Pattern and Placement of Spawn Packet on Bump Initiation and Yield of Shiitake Mushroom (*Lentinus edodes*)

Mohammad Golam Mohsin[1], Md. Aminul Hoque[2], Nirod Chandra Sarker and Akhter Jahan Kakon
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

The experiment was conducted to find out the appropriate opening pattern and suitable strain. The experiments consists of eleven types of opening pattern (top open place on floor, top open place on rack, total open and covered with polypropylene bag place on floor, total open and covered with polypropylene bag place on rack, only cotton plug open and place on floor, no open and place on floor, only cotton plug open and place on rack, no open and place on rack (control), total open and place on rack, total open and place on floor, no open and place on culture house floor and two strains (Le 8, Le 16) of shiitake mushroom were used as treatment. Time required for bump formation, time required for bump formation after treatment, time required from opening to first harvest, time required for harvest was found lowest from the treatment combination Le 16 with T_5 (only cotton plug open and place on floor) and those parameters were highest except time required from opening to harvest in the treatment T_8 (where spawn packets were no open and place on rack i.e. control) with the strain Le 8. Yield attributes of two strains such as diameter and thickness of pileus were significantly higher in T_9 when packets were total open and place on rack with Le 16 and diameter of stalk was higher in treatment T_5 where only cotton plug open and place on floor with Le 8. The highest length of stalk (1.88 cm) was found from the treatment combination of Le 16 with T_2 (Top open and place on rack). The highest number (62.00) of fruiting body, the highest number (37.25) of effective fruiting body, highest yield (193.00g) and highest biological efficiency (110.30%) were recorded from the strain Le 16 with treatment T_5 (Only cotton plug open and place on floor) and those parameters were lowest from the treatment combination of Le 8 with T_{10} (Total open and place on floor). In general, performance of Le 16 was better than Le 8 in terms of yield and yield contributing characters. Strain Le 16 and treatment T_5 i.e. only cotton plug open and place on floor may be recommended to grow shiitake mushroom under Bangladesh conditions.

Keywords: Opening pattern, Placement, Bump, Yield, *Lentinus edodes*.

INTRODUCTION

Shiitake (*Lentinus edodes)* is an edible mushroom commonly used as food in Asian countries, and also a traditional Chinese medicine (Lin *et al.*, 2008) which is cultivated on a large scale in many countries (Poppe and Hofte, 1995; Chang and Miles, 2004). It can be produced commercially in Latin America for the world market. Its cultivation in Latin America started during the early 1980's, and several attempts for its commercial cultivation have been carried out in Guatemala, Colombia, Mexico, Argentina and Brazil (Martínez-Carrera, 2002).It is the second most popular edible mushroom (Chang, 1999 and Chiu *et al.*, 1999). The production system of this mushroom is quite different from other edible mushrooms. Many strains of shiitake mushroom are available in the world which is extensively cultivated. The strains of this valuable mushroom vary widely, particularly in the time required for mycelium colonization, bump formation and fruiting body development. The mycelium growth in the vegetative phase involves producing quality fruiting bodies in the reproductive phase. A spawn run of different strains is of ultimate importance for adjusting the reproductive phase. To shift the mycelium growth stage to reproductive stage for the formation of bump as well as fruiting body generally some kinds of stimuli are needed. These stimuli can be initiated by some management practices like opening and placement of spawn packets during incubation. Opening is a process to remove cotton plug or total polypropylene bag of the sawdust bags with a sharp blade. On the other hand, placement means the spawn packets put in different locations like rack, floor etc.

This process stimulates the formation of blister like bumps. Among these conditions, opening of spawn packet is the most important aspect for early bump initiation and fructification. For bump initiation stage low temperature and high CO2 concentration is required. Time of bump formation

[1] Department of Agriculture Studies, Nabajug College, Dhamrai, Dhaka, Bangladesh; [2] Department of Agronomy and Agricultural Extension, University of Rajshahi, Rajshahi, Bangladesh.

varies with strains, substrate and temperature. Usually bumps form 10 days faster at 25°C than at 15°C (Miles and Chang, 1989). Fluctuation of temperature and high CO_2 concentration encourage bump formation. Lower the CO_2 in the bag, when bumps become too numerous by cutting slits on the bag. In any case, some aeration should be provided when bumps are formed. In many countries different opening system 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 & Sharma, 1998). If the bag opening is too early or too late, the crop may be failed. It is reported that a time period of 60 to 90 days is necessary for incubation of spawn packet (Kawai *et al.*, 1997 and Iizuka, 1997). The production of shiitake mushroom varies depending on the opening pattern of spawn packet. Many growers produce shiitake mushroom with the opening of the bag partially or fully. Fan *et al.* (2005) suggested opening the bag at the places where primordia have formed. It will give higher yield of quality mushroom; but it is time consuming and laborious task. However, Ramkumar *et al.* (2010) suggested cutting the top portion of polypropylene bag after browning of shiitake packet. Many studies have been carried out in the world to improve the quality and increase the production of *Lentinus edodes*. But, the production of this mushroom is fairly new in Bangladesh. Considering the above facts, the present study was under taken to determine the suitable opening pattern and placement of spawn packet on early bump initiation and yield of shiitake mushroom.

MATERIALS AND METHODS

The experiment was conducted at the tissue culture laboratory and culture house of National Mushroom Development and Extension Centre, Savar, Dhaka, during the period from September 2012 to February 2013. Eleven different types of opening pattern such as top open place on floor (T_1), top open place on rack (T_2), total open and covered with polypropylene bag place on floor (T_3), Total open and covered with polypropylene bag place on rack (T_4), Only cotton plug open and place on floor (T_5), No open and place on floor (T_6), Only cotton plug open and place on rack (T_7), No open and place on rack (control) (T_8), Total open and place on rack (T_9), Total open and place on floor (T_{10}), No open and place on culture house floor (T_{11}). These opening were done in incubation period for early bump initiation. Two strains of shiitake mushroom (*Lentinus edodes*), namely Le 8 and Le 16 were used as test materials (Plate 1).

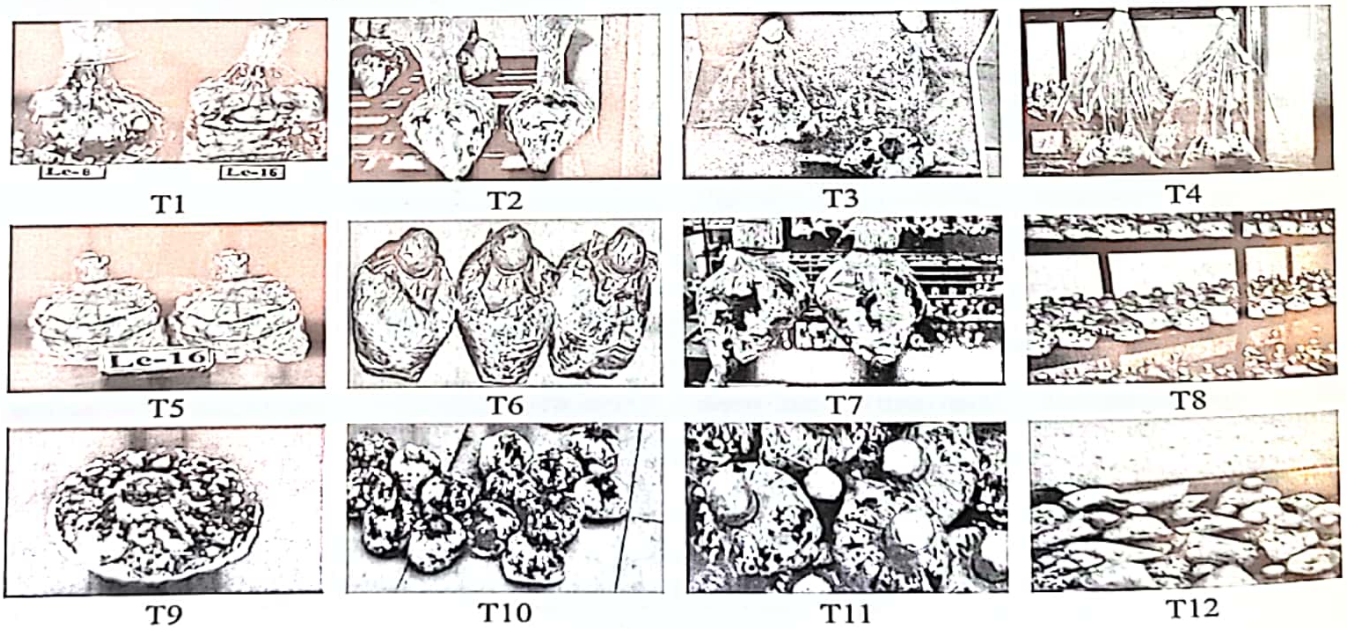

Plate 1. Different types of opening pattern (T_1-T_{11}) during incubation for early bump formation.

Preparation of pure culture: Pure culture of selected mushroom strain was prepared on potato dextrose agar (PDA) medium containing 200g peeled and sliced potato, 20g dextrose and 20g agar per liter. The mixture was boiled on gas burner until the agar dissolved. The medium was poured into test tube at 10 ml/tube. The medium in test tube was sterilized in an autoclave for 20 minutes at 121°C under 1.5 kg/cm^2 pressure. After sterilization and solidification, the tubes were inoculated separately with the inoculants of selected shiitake mushroom strains. Pieces of inner tissues of the joint of stalk and pileus were used as inocula. A fresh and full grown sporophore of shiitake mushroom was surface sterilized with 70% ethanol by rubbing cotton soaked in the alcohol. The stalk was peeled from out site. Tissues were collected from inner region of the joint of stalk and pileus. The tissues were cut into small pieces and placed on the solidified tubes containing PDA. After inoculation, the tubes were covered with cotton plug. All operations were done under sterile condition in a clean bench. The inoculated tubes were incubated in a growth chamber at 22 ± 2°C for 10-12 days. After completion of the whitish mycelium, this 10 days' culture was used for inoculation of mother culture.

Preparation of mother culture: To prepare mother culture of test mushroom sawdust and wheat bran mixed together at the ratio of 2:1 (v/v). Water was added to adjust moisture content at 65% and $CaCO_3$ was mixed at the rate of 0.2% of the mixture. Polypropylene bags of 18 cm × 25 cm size were filled with 300g of the above prepared mixture and packed tightly. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the volume of the bag was made for space to put the inoculums. The neck was plugged with cotton and covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for one (1) hour at 121°C under 1.5 kg/cm^2 pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. A piece of pure PDA culture medium containing mycelium of shiitake mushroom was placed aseptically in the hole of mother culture packet and again plugged the packet as mentioned above. The inoculated packets were placed on a rack in the laboratory at 22 ± 2°C temperatures for incubation. The substrate of the mother culture was colonized by the growth of whitish mycelium within 15-20 days after inoculation. The fully colonized packets were used for spawning.

Preparation of spawn packets: The substrate of spawn packets was prepared using sawdust and wheat bran mixture at the ratio of 2:1. Water was added to make the moisture level about 65% and $CaCO_3$ was added at 0.2% (w/w) of the mixture. The substrate mixture was poured into 18 cm × 25 cm polypropylene bags at 500g/bag. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the bag was made for space to introduce the inocula. The neck of each poly bags was plugged with cotton, covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for 2 h at 121°C under 1.1 kg/cm^2 pressures. After sterilization, the packets were cooled and transferred to an inoculation chamber. The packets were inoculated separately with the mother culture of the twenty-three strains at the rate of two tea spoonful per packet. The inoculated packets were incubated at 22 ± 2°C.

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

Opening of spawn packets: After completion of mycelium running spawn packets were opened and placed according to the treatments to determine the right opening pattern for early bump initiation.

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

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

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

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

RESULTS AND DISCUSSION

Time required for bump formation (days): The time required for bump formation was non-significant influenced by different opening pattern with strain. The highest time (120.80 days) required for bump formation was recorded in the treatment combination of Le 8 with the treatment T_8 where spawn packets were no open and place on rack i.e. control which was statistically similar (120.00 days) to the treatment combination of Le 8 with the treatment of $T_9$where spawn packets were total opened and place on rack. The lowest time (105.00 days) required for bump formation was found in the treatment combination of Le 16 with T_5 where spawn packets were only cotton plug open and place on floor, which was statistically similar to the same strain with T_7 where the spawn packets were only cotton plug open and place on rack (Table 1).

Time required for bump formation after treatment (days): The time required for bump formation after treatment was also non-significant influenced by different opening patterns with strain. The highest time (12.75 days) required for bump formation after treatment was found from the treatment combination of T_8 where spawn packets were no open and place on rack i.e. control with the strain Le 8. The lowest time (3.00 days) required for bump formation after treatment was found from the treatment combination of T_5 where spawn packets were only cotton plug open and place on floor with Le 16 (Table 1).

Time required from opening to first harvest (TRFOH): The time required from opening to first harvest was highly significant influenced by different opening patterns with two strains of shiitake mushroom. Significantly the highest time (11.00 days) required from opening to first harvest was recorded in the treatment combination of T_9 where spawn packets were total open and place on rack with strain Le 16. On the other hand, the lowest time (6.00 days) required from opening to harvest was found in the treatment combination of T_5 where spawn packets were only cotton plug open and place on floor with strain Le 16 which was statistically similar to T_8 where spawn packets were no open and place on rack i.e. control with Le 16 and the in the treatment from T_7, where spawn packets were only cotton plug open and place on rack with the same strain and also T_{11} where no open and place on culture house floor with the strain Le 16 (Table 1).

Time required for harvest (TRH): The effect of two strains and different opening patterns on time required for harvest was non-significant. The highest time (129.50 days) required for harvest was found in the treatment combination of T_8, where the spawn packets were no open and place on rack i.e. control with the strain Le 8 which was statistically similar (128.50 days) to the treatment combination of T_9, where the spawn packets were total open and place on rack with the strain Le 8. The lowest time (111.00 days) required for harvest was found in the treatment combination of T_5 where the spawn packets were only cotton plug open and place on floor with the strain Le 16 which was statistically similar (111.80 days) to the treatment combination of T_7 where the spawn packets were only cotton plug open and place on rack with the same strain (Table 1).

Table 1. Effect of strain and different opening patterns on growth of shiitake mushroom

Opening pattern	Time required for bump formation (days)	Time required for bump formation after treatment (days)	Time required from opening to first harvest (days)	Time required for harvest (days)
Strains of shiitake mushroom (Le 8)				
T_1	116.00cd	8.00cde	8.00cde	124.00bcd
T_2	117.00bc	9.00bcd	8.00cde	125.50bc
T_3	115.00de	7.00ef	10.00b	125.0bc
T_4	115.00de	7.00ef	8.00cde	123.00cd
T_5	114.00e	6.00fg	8.00cde	122.00de
T_6	117.00bc	9.00bcd	8.00cde	125.00bc
T_7	115.00de	7.00ef	8.00cde	123.00cd
T_8	120.80a	12.75a	8.75cd	129.50a
T_9	120.00a	12.00a	8.50cd	128.50a
T_{10}	117.50bc	9.50bc	8.00cde	125.50bc
T_{11}	118.00b	10.00b	8.00cde	126.00b
Strains of shiitake mushroom (Le 16)				
T_1	106.00ij	4.00hi	7.00ef	113.00hij
T_2	107.00hi	5.00gh	6.50f	113.50ghij
T_3	106.00ij	4.00hi	8.00cde	114.00ghi
T_4	106.00ij	4.00hi	9.00c	115.00fgh
T_5	105.00j	3.00i	6.00f	111.00j
T_6	107.00hi	5.00gh	7.00ef	114.00ghi
T_7	105.00j	3.50hi	6.25f	111.80ij
T_8	110.00f	8.00cde	6.00f	116.00fg
T_9	109.00fg	7.00ef	11.00a	120.00e
T_{10}	109.30fg	7.25def	8.00cde	116.80f
T_{11}	108.00gh	6.00fg	6.00f	114.00ghi
CV (%)	**1.06**	**17.00**	**8.62**	**1.41**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.T_1= Top open and place on floor, T_2= Top open and place on rack, T_3= Total open and covered with polypropylene bag place on floor, T_4= Total open and covered with polypropylene bag place on rack, T_5= Only cotton plug open and place on floor, T_6= No open and place on floor, T_7= Only cotton plug open and place on rack, T_8= No open and place on rack (control), T_9= Total open and place on rack, T_{10}= Total open and place on floor, T_{11}= No open and place on culture house floor.

Number of fruiting body (NFB): The number of fruiting body was significantly influenced by different opening pattern with two strains of shiitake mushroom. The highest number (62.00) of fruiting body was recorded in the treatment combination of T_5, where spawn packets were only cotton plug open and place on floor with Le 16. The lowest number (2.00) of fruiting body was recorded in the treatment combination of T_{10}, where spawn packets were total open and place on floor with both the strain (Table 2).

Number of effective fruiting body (NEFB): The number of effective fruiting body was significantly influenced by different opening pattern with two strains of shitake mushroom. The highest number (37.25) of effective fruiting body was recorded in the treatment combination of T_5, where spawn packets were only cotton plug open and place on floor with Le 16. The lowest number (1.25) of effective fruiting body was recorded in the treatment combination of T_{10}, where the spawn packets were total open and place on floor with Le 8 (Table 2).

Table 2. Effect of strain and different opening patterns on yield attributes and yield of shiitake mushroom

Opening pattern	Number of fruiting body	Number of effective fruiting body	Yield (g)	Biological efficiency (%)
Strains of shiitake mushroom (Le 8)				
T_1	23.00f	18.50def	114.00g	65.14h
T_2	18.00h	14.00gh	126.00f	72.00g
T_3	17.00hi	15.00fg	98.00hi	56.00j
T_4	19.00gh	12.25gh	89.00j	50.86kl
T_5	9.00k	8.00i	153.00d	87.93e
T_6	14.00ij	13.00gh	146.00e	83.18f
T_7	12.00jk	11.00hi	87.00j	50.22kl
T_8	13.00j	11.00hi	92.00ij	52.57k
T_9	4.00l	3.00j	50.00l	28.82n
T_{10}	2.00l	1.25j	29.00n	16.57p
T_{11}	22.00fg	18.00ef	104.00h	59.43i
Strains of shiitake mushroom (Le 16)				
T_1	53.00b	31.50b	158.50d	90.57de
T_2	33.00e	20.25de	156.00d	89.14e
T_3	30.00e	18.00ef	85.00jk	48.57l
T_4	25.00f	15.00fg	80.00k	45.72m
T_5	62.00a	37.25a	193.00a	110.30a
T_6	40.00d	28.00c	179.00c	102.30c
T_7	44.00c	21.75d	187.50ab	107.10b
T_8	56.00b	35.00a	160.00d	92.79d
T_9	5.00l	3.00j	98.00hi	56.00j
T_{10}	2.00l	1.50j	39.00m	22.28o
T_{11}	56.50b	34.75	181.50bc	103.50c
CV (%)	**9.45**	**13.61**	**3.89**	2.79

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.T_1= Top open and place on floor, T_2= Top open and place on rack, T_3= Total open and covered with polypropylene bag place on floor, T_4= Total open and covered with polypropylene bag place on rack, T_5= Only cotton plug open and place on floor, T_6= No open and place on floor, T_7= Only cotton plug open and place on rack, T_8= No open and place on rack (control), T_9= Total open and place on rack, T_{10}= Total open and place on floor, T_{11}= No open and place on culture house floor.

Yield (g): The yield was highly significant influenced the effect of two strain of shiitake mushroom with different opening patterns. The highest yield (193.00g) was obtained from the treatment combination of T_5 where the spawn packets were only cotton plug open and place on floor with Le 16 and the lowest yield (29.00g) was obtained from the treatment combination of T_{10} where the spawn packets were total open and place on floor with Le 8 (Table 2).

Biological efficiency (Be%): The biological efficiency was also highly significant influenced by the combined effect of two strains of shiitake mushroom with different opening patterns. The highest biological efficiency (110.30%) was obtained from the treatment combination of T_5 where the spawn packets were only cotton plug open and place on floor with the strain Le 16 and the lowest yield (16.57%) was obtained from the treatment combination of T_{10} where the spawn packets were total open and place on floor with the strain Le 8 (Table 2).

Length of stalk (LS): The effect of strain and different types of opening pattern on length of stalk was highly significant. The highest length (1.88 cm) of stalk was found from the treatment combination of T_2 where the spawn packets were top open and place on rack with Le 16. The lowest length (1.10 cm) of stalk was recorded from the treatment combination of T_4 where the spawn packets were total open and covered with polypropylene bag place on rack with the strain Le 16(Table 3).

Diameter of stalk (DS): The effect of strain and different types of opening pattern on diameter of stalk was statistically significant. The highest diameter (5.10 cm) of stalk was observed in the treatment combination of T_5 where the spawn packets were only cotton plug open and place on floor with Le 8 and the lowest diameter (3.10 cm) of stalk was observed in the treatment combination of T_9 where the spawn packets were total open and place on rack with Le 16 (Table 3).

Diameter of pileus (DP): The effect of strain and different types of opening pattern was statistically significant on diameter of pileus. The highest diameter (11.50 cm) of pileus was recorded in the treatment combination of T_9 where the spawn packets were total open and place on rack with the strain Le 16 and the lowest diameter (3.90 cm) of pileus was observed in the treatment combination of T_4 where the spawn packets were total open and covered with polypropylene bag place on rack which was statistically similar to the treatment of T_3 where the spawn packets were total open and covered with polypropylene bag place on floor with the strain Le 16 (Table 3).

Thickness of pileus (TP): The thickness of pileus was also highly significant influenced by the effect of strain and different types of opening pattern. The highest thickness (2.50 cm) of pileus was recorded from the treatment combination of T_9 where the spawn packets were total open and place on rack with Le 16 while the lowest thickness (1.10 cm) of pileus was recorded in the treatment combination of T_1 where the spawn packets weretop open place on floor with Le 8 (Table 3).

Table 3. Effect of strain and different opening patterns on size of fruit body of shiitake mushroom

Opening pattern	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Strains of shiitake mushroom (Le 8)				
T_1	1.40c-f	4.10cde	6.60g	1.10f
T_2	1.20fg	3.50fgh	7.45e	1.30def
T_3	1.25efg	3.90def	7.48e	1.10f
T_4	1.40c-f	4.30bcd	6.55g	1.50cde
T_5	1.60bc	5.10a	9.00c	1.55cd
T_6	1.60bc	4.05cde	10.40b	1.70c
T_7	1.33d-g	3.30gh	7.20ef	1.15f
T_8	1.20fg	4.17b-e	7.15ef	1.22ef
T_9	1.70ab	4.28bcd	8.10d	1.32def
T_{10}	1.40c-f	4.02cde	7.50e	1.20f
T_{11}	1.55bcd	4.20b-e	7.10ef	1.60cd
Strains of shiitake mushroom (Le 16)				
T_1	1.20fg	3.73efg	6.53g	1.55cd
T_2	1.88a	4.45bc	7.53e	2.05b
T_3	1.20fg	3.83def	4.05i	1.21ef
T_4	1.10g	3.50fgh	3.90i	1.12f
T_5	1.30d-g	4.60b	6.58g	1.40def
T_6	1.20fg	4.10cde	6.50g	1.60cd
T_7	1.35c-g	4.08cde	6.75fg	1.35def
T_8	1.23fg	3.95def	4.00i	1.20f
T_9	1.53bcd	3.10h	11.50a	2.50a
T_{10}	1.50b-e	5.05a	8.90c	1.40def
T_{11}	1.33d-g	4.25bcd	5.88h	1.55cd
CV (%)	**11.42**	**7.41**	**5.31**	**12.37**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.T_1= Top open and place on floor, T_2= Top open and place on rack, T_3= Total open and covered with polypropylene bag place on floor, T_4= Total open and covered with polypropylene bag place on rack, T_5= Only cotton plug open and place on floor, T_6= No open and place on floor, T_7= Only cotton plug open and place on rack, T_8= No open and place on rack (control), T_9= Total open and place on rack, T_{10}= Total open and place on floor, T_{11}= No open and place on culture house floor.

REFERENCES

Chadha, K. L. & Sharma, S. R. 1998. Mushroom Research in India.history, infrastructure and achievements. **In advances in Horticulture**, Vol. 13. Malhotra Publishing House, New Dilhi, India. p.113.

Chang, S. T. & Miles, P. G. 2004. Mushrooms: Cultivation, nutritional value medicinal effect, and environmental impact, 2nd ed. Boca Raton, Fl: CRC Press. pp. 2-3.

Chang, S. T. 1999. World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinula edodes*inChina.*Int. J. Med. Mushroom.* **1**: 291-300.

Chiu, S. W., Wand, Z. M., Chiu, W. T., Lin, F. C. & Moore, D. 1999. An integrated study of individualism in *Lentinula edodes*in nature and its implication for cultivation strategy. *Mycol. Res.* **103**: 651-660.

Fan, L., Pan, H., Wu, Y. & Choi, K. W. 2005. Shiitake bag cultivation in China. Mushroom Grower's Handbook, Mushworld, Korea. pp. 121-131.

Iizuka, H. 1997. Production of *Lentinus edodes*mycelia extracts (LEM). *Food Rev. Intern.* **13**: 343-348.

Kawai, G., Kobayashi, H., Fukushima, Y., Yamada, S., Fuse, H. K. &Ohsaki, K. 1997. Continuous manufacturing system of solid culture media packet in film bags for cultivation of shiitake. *Food Rev. Intern.* **13**: 349-356.

Lin, L. Y., Tzeng, Y. H. & Mau, J. L. 2008.Quality of Shiitake stipe bread. *J.Food Proc.* Press. **32**: 1002-1015.

Martínez-Carrera, D. 2002. Current development of mushroom biotechnology in Latin America. *Micol.Apl. Int.* **14**: 61-74.

Miles, P. G. & Chang, S. T. 1989. Edible Mushrooms and Their Cultivation, Boca Raton, Fl: CRC Press. pp. 189-223.

Poppe, J. A. & Hofte, M. 1995. Twenty wastes for twenty cultivated mushrooms. **In: Science and cultivation of edible fungi.** Ed. T. J. Ellioit, Balkema, Rotterdam. pp. 171-179.

Ramkumar, L., Thirunavukkarasu, P. & Ramanathan, T. 2010. Development of improved technology for commercial production and preservation of shiitake mushroom (*Lentinus edodes*). *American-Eurasian J. Agric. & Environ. Sci.* 7(4): 433-439.

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Effect of Shiitake Mushroom Consumption on Blood Pressure Status of Randomly Selected Adult Male Population

Md. Bazlul Karim Choudhury[1], Akhter Jahan Kakon, Mohammad Golam Mohsin[2], Md. Erfan Reza[3] and Nirod Chandra Sarker
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

The study was conducted in the National Mushroom Development and Extension Center (NAMDEC) Sobhanbag, Savar, Dhaka during the period of 5th March 2014 to 29th December 2014. A total 32 adult male subjects were included in the study. Fasting plasma glucose and Plasma creatinine were estimated for diagnosis of diabetes mellitus and chronic kidney disease (CKD). A significant reduction of fasting plasma glucose was observed ($p = 0.002$) after 3 months consumption of mushroom capsule (3 gm shiitake mushroom powder/day), indicating shiitake mushroom has the ability of reducing blood glucose level. Also feeding of mushroom capsule showed non-significant mean difference of plasma creatinine between the two periods of observation ($p = 0.186$), which indicates limited amount of shiitake mushroom consumption has no harmful effect on kidney functions. Considering systolic and diastolic blood pressure, 3 months consumption of shiitake mushroom capsule causes significant reduction of both systolic and diastolic blood pressure (133.57 ± 3.39 & 124.41 ± 2.45, $p < 0.001$ and 82.19 ± 1.77 & 73.33 ± 1.44, $p < 0.001$ respectively). Findings of the study suggest that, shiitake mushroom causes reduction of both systolic and diastolic blood pressure of randomly selected male volunteers.

Keywords: Shiitake mushroom, Systolic blood pressure, Diastolic blood pressure.

INTRODUCTION

Hypertension is a major risk factor for various life threatening conditions. Even moderate elevation of blood pressure is associated with a shortened life expectancy. Dietary and life style changes can improve blood pressure control.

Proper dietary substances can protect people from chronic diseases such as coronary heart disease, hypertension, cancer, obesity and diabetes. The consensus is that regular consumption of fruits and vegetables reduce the risk of cardiovascular disease (CVD). This is due to the antioxidant activity and immunomodulation exerted by this class of food (Martin, 2010). Evidence also shows that mushrooms may protect against chronic disease like CVD. Oxidative stress and inflammation are closely linked to atherogenesis (Lindequist *et al.*, 2005). Now a days it is established that atherogenesis is the important cause of hypertension. Traditional medicines such as mushrooms are increasingly being used for treatment of certain health problems.

Mushrooms are nutritive and are richer in protein than cereals, pulses, fruits and vegetables on dry weight basis (Ghosh, 1990). Due to their low caloric value, mushrooms can be consumed by patients with hyperlipidemia (Bano, 1982) which is one of the important

[1] Department of Biochemistry, Manikganj Medical College, Manikganj, Bangladesh; [2] Department of Agriculture Studies, Nabajug College, Dhamrai, Dhaka, Bangladesh; [3] Department of Biochemistry, Rajshahi Medical College, Rajshahi, Bangladesh.

causes of hypertension. Edible fungi produce secondary metabolites which possess various therapeutic properties. Mushrooms also contain ample minerals such as calcium, phosphorous, potassium, iron and copper. They have traditionally been used in the treatment and prevention of various diseases including hypertension (Suguna and Usha, 1995).

Shiitake Mushroom is one of the most popular mushrooms Worldwide. It has a great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fiber. The dietary fiber present in shiitake mushroom consists of soluble and insoluble structures. Shiitake mushroom is the most famous, and has been used as a model to investigate the functional properties and isolate pure compounds for pharmaceutical use. Shiitake Mushroom has shown to present medicinal compounds, including polysaccharides, terpenoids, sterols and lipids, which are effective in treating various tumors and infections, among other activities which are still being studied (Wang and Zhang, 2009).

Our body has antioxidant defense systems that are often insufficient to completely prevent the damage caused by oxidative stress (Da-Silva and Jorge, 2011). Thus, natural products such as mushrooms containing bioactive compounds can be used to help reduce such damage in the body (Mohsin *et al.*, 2011). Numerous bio-components present in shiitake mushroom aid in its pharmacological potency against hypertension, hyperlipidemia and cardiovascular complications, depressed immunity, hepatic disorders and cancer. In addition, its antioxidative, anti-fungal and anti-microbial aspects have been duly attributed to its bio-functional components (Bisen *et al.*, 2010). The present study was conducted to observe the effect of shiitake mushroom on blood pressure status of randomly selected adult male population.

MATERIALS AND METHODS

The study was conducted in the laboratory of Mushroom Development Institute, Sobhanbag, Savar, Dhaka, during the period of 5th July 2014 to 29th June 2015. A total 32 randomly selected non-diabetic adult male volunteers, who were free from chronic kidney diseases (CKD), aged (years) from 25 to 69 were included. Informed written consent was taken from the subjects. Then the details history was taken from the subjects including age, sex, occupation, educational status, marital status, family history and drug history. Any acute or chronic diseases were excluded from the study. Fasting plasma glucose < 7 mmol/L were considered as non-diabetes. Plasma creatinine > 1.4 mg/dl were considered as CKD.

After collecting 8-10 hours fasting blood sample from the subjects, capsules made from shiitake mushroom were supplied to take two capsules three times daily. Each capsule contains 500 mg shiitake mushroom powder. Thus each subject took 3 gms mushroom powder daily. After three months the subjects were evaluated and all the investigation procedures were repeated. If any drug previously getting by the subjects, it was continued. No additional antihypertensive drugs were allowed for the subjects during the study period.

With all aseptic precaution 10 ml of blood sample was collected from median cubital vein of each subject. Then it was immediately poured into test tube containing fluoride and EDTA. The test tube then gently shaken so that anti coagulant and fluoride mix with the blood properly. Then it was centrifuged by 3000 rpm for 5 minutes. Plasma was separated which were transferred into two eppen dorf containing 1 ml in each.

Fresh fruiting body of shiitake mushroom was collected from the culture house of NAMDEC. They were dried using an electric drier at moisture level 4-5%. Then the mushrooms were grinded and poured into capsule shell which contains 500 mg powder. Prepared capsules were preserved into moisture free glass containers.

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

Results were expressed as mean ± SE. Paired Student's 't' test was used to see the level of significance. 95% confidence limit was taken as level of significance.

RESULTS AND DISCUSSION

Mean age (years) of the subjects was 38.7, ranged from 25 -69. The mean ± SE of fasting plasma glucose (mmol/L) before mushroom treatment was 5.83 ± 0.26, ranged from 3.9 - 6.8. After 3 months' mushroom treatment the value was 5.13 ± 0.34 ranged from 3.7 - 6.5. A significant reduction of plasma glucose was observed ($p = 0.002$) between the two periods (Table 1), indicating shiitake mushroom has the ability of reducing blood glucose level.

The mean ± SE plasma creatinine (mg/dl) before mushroom treatment was 0.82 ± 0.04, ranged from 0.51 -1.22. After 3 months' mushroom treatment, the value was 0.86 ± 0.05, ranged from 0.49 – 1.31. There was no statistically significant mean difference ($p = 0.186$) between the two periods of observation (Table 1). This finding suggests that limited amount of shiitake mushroom consumption has no harmful effect on kidney functions.

Table 1. Evaluation of plaasma glucose and creatinine level of the subjects

Parameter	Number of subjects (n)	Values				p
		Pre Treatment (mean ± SE)	Range	Post treatment (mean ± SE)	Range	
Glucose (mmol/L)	32	5.83 ± 0.26	3.9 - 6.8	165.32 ± 6.06	3.7 - 6.5	0.002
Creatinine (mg/dl)	32	0.82 ± 0.04	0.51 -1.22	0.86 ± 0.05	0.49 – 1.31	0.186

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

The Mean (± SE) systolic blood pressure (mmHg) before mushroom treatment was 133.57 ± 3.39, ranged from 115 – 165. After 3 months of mushroom treatment, the value was 124.41 ± 2.45, ranged from 110 – 155 (Fig. 1.). A highly significant mean difference of systolic blood pressure between the two periods was observed ($p < 0.001$).

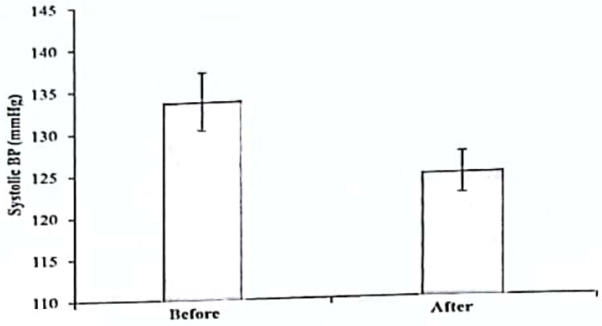

Fig. 1. Systolic blood pressure (Results show mean ± SE. Data were analyzed by Pair t test. Means were significantly different at $p < 0.05$ at 95% confidence limit).

Considering diastolic blood pressure (mmHg), the mean (± SE) before mushroom supplementation was 82.19 ± 1.77, ranged from 65 - 110 and after mushroom supplementation it was 73.33 ± 1.44, ranged from 65 – 105 (Fig. 2). Here also a highly significant mean difference was observed ($p < 0.001$).

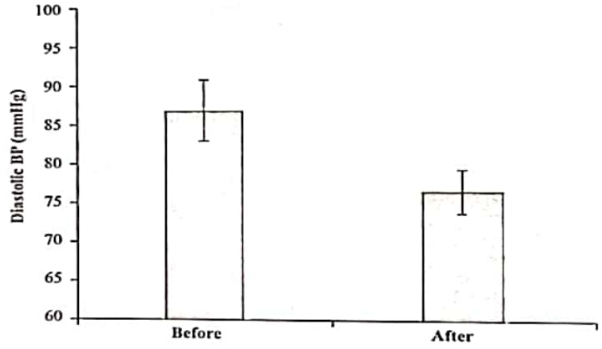

Fig. 2. Diastolic blood pressure (Results show mean ± SE. Data were analyzed by Pair t test. Means were significantly different at $p < 0.05$ at 95% confidence limit).

Mushrooms are edible fungi confirmed to have definite human health properties and nutrition. Shiitake mushrooms have been demonstrated to have beneficial effects in animal and human studies individually as well as in combination. However, the effect of shiitake mushroom on hypertension was not clear in previous studies. Hence, the present study was performed.

In the study it was observed that there was no significant variation of serum creatinine level of subjects before and after three months supplementation of mushroom capsules (3 gms/ day), indicating shiitake mushroom has no harmful effect on kidney.

Obtained findings of this study indicate that shiitake mushrooms reduced both systolic and diastolic blood pressure significantly after three months mushroom treatment. Findings of previous animal trial support this observation. In a study Kabir *et al.* (1987) observed that,

the systolic blood pressure of rats fed either Shiitake or Maitake was significantly lower ($p<0.001$) than that of the control (Kabir *et al.*, 1987).

Although there is no sufficient human data of antihypertensive effect of shiitake mushroom, it was reported that shiitake mushroom reduces cholesterol and teiglyceride level (Yamac *et al.*, 2008). Cardiovascular disease is the leading cause of cholesterol levels in the blood throughout the world and is an important risk factor for the high mortality, therefore hypocholesteremic effects are of great importance. The ability of shiitake in lowering sanguine cholesterol was first reported in the 1960s (Bisen *et al.*, 2010). To date, some studies demonstrate the ability of shiitake mushroom in both decrease very low density lipoproteins (VLDL) as well as high density lipoproteins (HDL), preventing the increase of blood pressure (Oba *et al.*, 2009; Shimada *et al.*, 2002).

Cholesterol lowering is an alternative therapy that may potentially target arterial stiffness, and thus blood pressure, through effects on endothelial function and arterial wall composition. A number of studies in hypercholesterolemic patients have shown improvement, particularly in peripheral artery properties, with cholesterol-lowering therapy (Smilde *et al.*, 2000; Matthews *et al.*, 1993; Yasuaki *et al.*, 1996). It is unknown whether a cholesterol reduction within the normal clinical range in patients with isolated systolic hypertension (ISH) might also reduce stiffness of the large arteries, and thereby systolic blood pressure (SBP). Our current study is in agreement with these findings.

Again, regular consumption of fruits and vegetables reduce the risk of cardiovascular disease (CVD). This is due to the antioxidant activity and immunomodulation exerted by these class of food (Martin, 2010). Reactive oxygen free radicals have been reported to be important in ischemia reperfusion injury cascades which are an important factor for hypertension. In a study Mowsumi *et al.* (2011) demonstrated that *Calocybe indica* and *Pleurotus djamor* mushroom extracts are capable of scavenging free radicals. Considering theses observations it is maintainable that shiitake mushroom is able to improve both systolic and diastolic blood pressure by their free radical scavenging activity.

REFERENCES

Bano, Z. 1982. Pleurotus mushroom as nutritional food. Tropical mushroom - biological nature and cultivation method. Hong Kong: *The Chinese University Press*. pp. 362 – 363.

Bisen, P. S., Baghel, R. K., Sanodiya, B. S., Thakur, G. S. & Prasad, G. B. K. S. 2010. *Lentinus edodes:* a macrofungus with pharmacological activities. *Curr. Med. Chem.* **17**: 2419 – 2430.

Da-Silva, A. C. & Jorge, N. 2011. Antioxidant Properties of *Lentinus edodes* and Agaricus Blazei Extracts. *Journal of Food Quality*. **34**: 386 - 394.

Ghosh, C. 1990. Nutritional value of edible mushroom. In the biology and cultivation of edible mushroom. *New York: Academic press.*

Kabir, Y., Yamaguchi, M. Y. & Kimura, S. 1987. Effect of shiitake (*Lentinus edodes*) and maitake (*Grifola frondosa*) mushrooms on blood pressure and plasma lipids of spontaneously hypertensive rats. *J Nutr Sci Vitaminol.* **33**(5): 341-346.

Lindequist, U., Niedermeyer, T. H. & Julich, W. D. 2005. The Pharmacological Potential of Mushrooms. *Evidence- Based Complementary and Alternative Medicine*. **2**: 285-299.

Martin, K. R. 2010. Both Common and Specialty Mushrooms Inhibit Adhesion Molecule Expression and *in Vitro* Binding of Monocytes to Human Aortic Endothelial Cells in a Pro-Inflammatory Environment. *Nutrition Journal*. **9**(29): 9 - 29.

Matthews, P. G., Wahlqvist, M. L., Marks, S. J., Myers, K. A. & Hodgson, J. M. 1993. Improvement in arterial stiffness during hypolipidaemic therapy is offset by weight gain. *Int J Obes Relat Metab Disord*. **17**: 579 – 583.

Mohsin, M., Negi, P. & Ahmed, Z. 2011. Determination of the Antioxidant Activity and Polyphenol Contents of Wild Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (W.Curt. Fr.) P. Karst. (Higher Basidiomycetes) from Central Himalayan Hills of India. *International Journal of Medicinal Mushrooms*. **13**: 535 - 544.

Mowsumi, F. R., Rahaman, A., Choudhury, M. B. K., Sarker, N. C. & Hossain, S. 2021. Comperative *in vitro* Free Redical Scavenging Effects of *Calocybe indica* and *Pleurotus djamor*. *Bangladesh J Mushroom*. **5**(1): 9 - 15.

Oba, K., Kobayashi, M., Matsui, T., Kodera, Y. & Sakamoto, J. 2009. Individual Patient Based Meta-Analysis of Lentinan for Unresectable/Recurrent Gastric Cancer. *Anticancer Research*. **29**: 2739 - 2745.

Shimada, Y., Morita, T. & Sugiyama, K. 2002. Effects of *Lentinus edodes* on Fatty Acid and Molecular Species Profiles of Phosphatidylcholine in Rats Fed Different Levels of Corn Oil. *Bioscience, Biotechnology, and Biochemistry*. **66**: 1759 - 1763.

Smilde, T. J., van-den-Berkmortel, F. W., Wollersheim, H., van-Langen, H., Kastelein, J. J. & Stalenhoef, A. F. 2000. The effect of cholesterol lowering on carotid and femoral artery wall stiffness and thickness in patients with familial hypercholesterolaemia. *Eur J Clin Invest*. **30**: 473–480.

Suguna, S. & Usha, M. 1995. Cultivation of oyster mushroom. *J food sci technol*. **32**: 351-352.

Wang, X. & Zhang, L. 2009. Physicochemical Properties and Antitumor Activities for Sulfated Derivatives of Lentinan. *Carbohydrate Research*. **344**: 2209 - 2216.

Yamac, M., Kanbak, G., Zeytinoglu, M., Bayramoglu, G., Senturk, H. & Uyanoglu, M. 2008. Hypoglycemic Effect of *Lentinus Strigosus* (Schwein.) Fr. Crude Exopolysaccharide in Streptozotocin-Induced Diabetic Rats. *Journal of Medicinal Food*. **11**: 513 -517.

Yasuaki, T., Fumio, O., Nobuaki, T., Yuichiro, W., Ikuo, T., Shumpei, A., Chieko, M., Shiro, O., Kazuyoshi, O. & Masunori, M. 1996. Improvement of atherosclerosis and stiffness of the thoracic descending aorta with cholesterollowering therapies in familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol*. **16**: 955 – 962.

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Effect of Substrate Ratio on Growth and Yield of Maple Oyster Mushroom

Nirod Chandra Sarker, Shamima Khatun, Rakib Al Hasan, Mustafizur Rahman[1] and Tasnim Farzana[2]
Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

Different amount of pasteurized rice straw and sawdust filled with 10 x 14 size of polypropylene bag were used for cultivation of maple oyster mushroom and the yield as well as yield related attributes was compared. Maximum days (16.50) required from opening to harvest when substrates were used as 2:2 ratio (T_2) and minimum days (8.50) required from opening to harvest when substrates were used as 1:3 ratio (T_1). The highest number of fruiting bodies (60.00), effective fruiting bodies (46.00), the maximum AVY (81.75g/packet) and the highest yield (327.00g) were recorded in T_1. The lowest number of fruiting bodies (24.50), effective fruiting bodies (23.00), the minimum AVY (52.50g/packet) and yield (210.0g) were found in T_3 respectively. The maximum AIW (9.14g/packet) was recorded in T_3 and it was minimum (7.12g/packet) in T_1. The length of stalk ranged from 3.43 to 4.90 cm with significant difference. The diameter of stalk was significant and ranged from 0.76 to 0.95 cm. The diameter of pileus was non- significant and ranged from 5.98 cm to 6.53 cm. The thickness of pileus in different treatments were non- significant and ranged from 0.56cm to 0.60cm. The highest biological efficiency (107.90%) was found in T_2 followed by T_1 (103.80%) and the lowest biological efficiency was found in T_3 (85.71%).

Keywords: Pasteurization, Ratio, Sawdust, Spawning, 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 yield of the domestically grown oyster mushroom were greatly affected by the substrate media. Some researchers have already observed that the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates (Badu *et al.*, 2011; Tesfaw *et al.*, 2015). Hence the objective of this study is to determine the effect of different mixture of substrate media on growth characteristics and yield of domestically grown oyster mushroom.

MATERIALS AND METHODS

The experiment was conducted in the culture house of Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh, from March 2014 to October 2015. In this experiment pasteurized sawdust and rice straw were used as substrate according to treatment for the cultivation of maple oyster mushroom. Different ratio of sawdust and rice straw (substrate) filled with 10×14 size

[1] Ministry of Agriculture, Bangladesh Secretariat, Dhaka, Bangladesh; [2] Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh.

of polypropylene bag was used as treatments. The treatments were T_1= Rice straw: sawdust (1:3), T_2= Rice straw: sawdust (2:2), T_3= Rice straw: sawdust (3:1). One strain of *Pleurotus cystidious*, namely Pcys-2 was used as test materials which is also known as maple 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 separately. Then the mixture of sawdust and rice straw was poured separately (3-4 kg/ bag) in net's bag. The bags were kept in a rack of MDI developed sterilization cum chamber at 60-70°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 at 65%.

Preparation of spawn packets: The polypropylene bags were filled with pasteurized sawdust and rice straw according to treatments. Pasteurized substrate and sawdust based spawn packet were mixed thoroughly without supplementation. Twelve pieces of spawn packet (30%spawning rate) were mixed with twenty kg of prepared substrates. Then their mouths were plugged by inserting absorbent cotton with the help of plastic neck. The neck of the bag was prepared by using heat resistant plastic pipe. Substrate mixture was poured into polypropylene bags according to substrate ratio at 1:3, 2:2, 3:1/ bag. The prepared packets were incubated in culture house at 25-30°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°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°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 time required from opening to harvest, 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

Time required from opening to harvest (days): Time required from opening to harvest under different treatments differed significantly (Table 1). Maximum days (16.50) required from opening to harvest when substrates were used as 2:2 ratio and minimum days (8.50) required from opening to harvest when substrates were used as 1:3 ratio.

Number of fruiting body: Number of fruiting bodies under different treatments differed significantly (Table 1). The highest number of fruiting bodies (60.00) was found in T_1 followed by T_2 (46.00) which were statistically different to other treatments. The lowest number (24.50) of fruiting bodies was found in T_3. 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.

Number of effective fruiting body: Number of effective fruiting bodies under different treatments differed significantly (Table 1). The highest number of effective fruiting bodies (46.00) was found in T_1 which was followed by T_2 (38.00). The lowest number (23.00) of effective fruiting bodies was found in T_3 which was statistically differing to other treatments.

Individual weight of fruiting body (g): Significant difference was observed in individual weight of fruiting body among the different ratio of rice straw and sawdust (Table 1). The maximum AIW (9.14g/packet) was recorded in T_3 which was followed by T_2 (7.96g/packet) and it was minimum (7.12g/packet) in T_1. It was observed that individual weight of fruiting body increased when amount of rice straw increased/packet.

Table 1. Effect of substrate ratio on growth and yield of maple oyster mushroom

Treatments(Rice straw:sawdust)	Time required from opening to harvest (days)	Total number of fruiting bodies	Number of effective fruiting bodies	Individual weight of fruiting body (g)	Average yield (g)	Total yield (g)
T_1= 1:3	8.25c	60.00a	46.00a	7.12c	81.75a	327.00a
T_2= 2:2	16.50a	46.00b	38.00b	7.96b	75.50b	302.00b
T_3= 3:1	13.50b	24.50c	23.00c	9.14a	52.50c	210.00c
CV(%)	**9.34**	**4.66**	**5.12**	**5.36**	**0.97**	**0.97**

Average yield (g): Significant difference was observed in average yield among the different substrate ratio (Table 1).The maximum AVY (81.75g/packet) was recorded in T_1 which was followed by T_2 (75.50g/packet) and it was minimum (52.50g/packet) in T_3.

Yield/ Packet (g): Significant variation was observed in yield under different treatments (Table 1). The highest yield (327.00g) was found in T_1 followed by T_2 (302.00g).The lowest yield was found in T_3(210.0g). Yield was counted in the harvest period 4th flush. This result is partially supported by Khan *et al.* (2012) who reported that yield of oyster mushroom in rice straw substrate ranged from 106g–534.50g and also reported yield increased with increasing the amount of rice straw. Hence yield increased with decreasing the amount of rice straw.

Size of fruiting body: The length of stalk ranged from 3.43 to 4.90 cm with significant difference (Table 2).The highest length of stalk was found in T_1 (4.90cm) which was statistically identical to other treatments. The lowest length of stalk was found in T_3 (3.43cm) which was statistically similar to T_2. The diameter of stalk was significant and ranged from 0.76 to 0.95cm (Table 2). The highest diameter of stalk was found in T_1 (0.95cm) while the lowest diameter of stalk (0.76cm) was found in T_3.

The diameter of pileus was non- significant and ranged from 5.98 cm to 6.53 cm (Table 2).The highest diameter of pileus (6.53cm) was found in T_1 followed by T_3(6.43cm) and the lowest diameter of pileus (5.98cm) was found in T_2. The thickness of pileus in different treatments was non-significant and ranged from 0.56cm to 0.60cm (Table 2). The highest thickness was found in T_2 (0.60cm) and the lowest thickness of pileus (0.56cm) was found in T_1.

Table 2. Effect of substrate ratio on size of fruiting body of maple oyster mushroom

Treatments(Rice straw: sawdust)	Length of stipe(cm)	Diameter of stipe (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1=1:3	4.90a	0.95a	6.53a	0.56a
T_2=2:2	3.73b	0.83ab	5.98a	0.60a
T_3=3:1	3.43b	0.76b	6.43a	0.58a
CV(%)	6.29	9.75	6.65	8.09

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

Biological efficiency (%): The highest biological efficiency (107.90%) was found in T_2 followed by T_1 (103.80%) and the lowest biological efficiency was found in T_3 (85.71%).As substrate rice straw quantities increased, substrate utilization decreased (Fig 1).

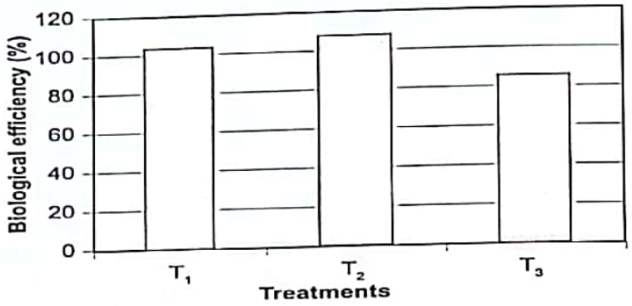

Fig.1. Effect of substrate ratio on biological efficiency of maple oyster.

REFERENCES

Badu, M., Twumasi, S. K. &Boadi, N. O. 2011. Effect of lignocellulosic in wood used as substrate on the quality and yield of mushrooms. *Food Nutr. Sci.* **2**: 780-784.

Chang, S. T. & Miles, P. G. 1988. Edible Mushroom and their cultivation.CRC Press, Inc. Boca Raton, Florida U.S.A. pp. 27-28.

Gomez, A. C. & Gomez, A. A. 1984. Statistical procedures for agricultural research. John Wiley & Sons, Inc.New York.p.680.

Hadwan, H. A., Al-Jaboury, M. H. & Hassan, A.O. 1997.Suitability of different substrates and amendments on the cultivation of oyster mushroom Collection of Thesis Materials, S & T, Development, Environment and Resources.Proc. 96 FUZHOU international, Symposium on the development of juncau industry. pp. 215–221.

Klingman, A. M. 1950. Hand book of mushroom culture (2nd edition). CRC Publishing Co. J. B. Kenneth Square, Pennsylvania, USA.pp. 663 - 674.

Mata, G. & Savoie, J. M. 2005. Wheat straw (Chapter 4). Shiitake Cultivation, Mushroom Growers' Handbook 2, Mush World: Seoul. pp. 125 – 130.

Sarker, N. C., Hossain, M. M., Sultana, N., Mian, I. H., Karim, A. J. M. S. & Amin, S. M. R. 2007. Performance of different substrates on the growth and yield of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer. *Bangladesh J. Mushroom.* **1**(2): 44 - 49.

Tesfaw, A., Tadesse, A. & Kiros, G. 2015.Optimization of oyster (*Pleurotus ostreatus*) mushroom cultivation using locally available substrates and materials in Debre Berhan, Ethiopia. *J. Appl. Biol. Biotechnol.* 3(1): 15 - 20.

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Effect of Different Packet Size on Yield of Pearl Oyster Mushroom

Akhter Jahan Kakon, Shamima Khatun, Md. Masud Rana and Md. Bazlul Karim Choudhury[1]

Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

Different amount of pasteurized rice straw and sawdust filled with different size of polypropylene bag were used for cultivation of pearl oyster mushroom and the yields as well as yield related attributes were compared. The highest yield (316.30g) and number of fruiting body (34.25) was obtained in 9×12 size polypropylene bag with 700g substrate (T_2) respectively whereas the lowest yield (190.00g) and fruiting body (11.0) was obtained in 7×10 size polypropylene bag with 400g substrate (T_1).The biological efficiency was the highest (142.10%) in 7×10 size polypropylene bag (T_1) and (54.55%) in 8×16 size polypropylene bag with 1100g substrate(T_5). The number of effective fruiting bodies was the highest (27.00) in 8×14 size polypropylene bag T_3 and lowest (9.00) inT_1. The length of stalk ranged from 3.90 to 5.65cm. The highest length of stalk (5.65 cm) was found in (T_3) and the lowest length (3.90 cm) of stalk was found in 8×16 size polypropylene bag (T_5). The diameter of stalk, pileus and thickness of pileus ranged from 0.63 to 1.10 cm; 5.43 to 6.63 cm and 0.55 to 0.70 cm respectively. The highest diameter of stalk (1.10cm) and pileus (5.65 cm) were found in using 8×14 size polypropylene bag with 900g substrate (T_3).

Keywords: Pasteurization, Ratio, Sawdust, Spawning, Mother culture.

INTRODUCTION

Agricultural wastes are the good source for the cultivation of mushrooms. *Pleurotus spp.* can consume a vast variety of crop residues because it has a great ability to grow on residues (Mamiro, *et al.,* 2011). For the cultivation of *Pleurotus* rice straw, wheat straw, sugarcane bagasse, waste paper and sawdust are the substrates that are commonly used. Sawdust when used in combination with paddy straw resulted in high yield of oyster mushroom. The large amount of agricultural wastes and appropriate climatic conditions provide massive scope for oyster mushroom cultivation in Bangladesh. There are several species of *Pleurotus* identified in the world. Most of them are suitable for cultivation. However, the most important cultivated species is *Pleurotus ostreatus*, being easier to cultivate, favorable to eat, and grow economically on different kinds of organic waste raw material (Kong, 2004). The objectives of this study were to determine the quantity of substrate that would maximize mushroom production and to determine the effect of bag size on yield of oyster mushroom.

MATERIALS AND METHODS

The experiment was conducted in the culture house of Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh, from December 2014 to August 2015. In this experiment pasteurized sawdust and rice straw were used as substrate for the cultivation of pearl oyster mushroom. Different amount of substrate filled with different size of polypropylene bag was used as treatments. The treatments were T_1=7×10(400g),T_2=9×12(700g), T_3=8×14(900g),

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

T_4=10×14(1000g), T_5=8×16(1100g) and T_6=12×18 (1400g). 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. Fifteen kg sawdust and five kg rice straw with 17liter 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 inoculation chamber at 60-70°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 at 65%.

Preparation of spawn packets: The polypropylene bags were filled with pasteurized sawdust and rice straw according to treatments. Pasteurized substrate and sawdust based spawn packet were mixed thoroughly without supplementation. Twelve pieces of spawn packet (30% spawning rate) were mixed with twenty kg of prepared substrates. Then their mouths were plugged by inserting absorbent cotton with the help of plastic neck. The neck of the bag was prepared by using heat resistant plastic pipe. Substrate mixture was poured into polypropylene bags according to bag size at 400, 700, 900, 1000, 1100, 1400g/ bag. The prepared packets were incubated in culture house at 25-30°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°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°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) except treatment T_4 and T_5. The highest number of fruiting bodies (34.25) was found in T_2 followed by T_3 (32.00) which were statistically different to other treatments. The lowest number (11.00) of fruiting bodies was found in T_1 which was statistically differing 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.

Number of effective fruiting body: Number of effective fruiting bodies under different treatments differed significantly (Table 1). The highest number of effective fruiting bodies (27.00) was found in T_3 which was statistically similar toT_2 (26.50). The lowest number (9.00) of effective fruiting bodies was found in T_1 which was statistically differing to other treatments.

Table 1. Effect of different packet size on yield and yield attributes of pearl oyster mushroom

Treatments	No of fruiting body	No of effective fruiting body	Yield (g)	Biological efficiency (%)	Contamination rate (%)
T1=7×10 (400g)	11.00e	9.00d	190.00d	142.10a	0.0d
T2= 9×12(700g)	34.25a	26.50a	316.30a	129.10b	0.0d
T3=8×14 (900g)	32.00b	27.00a	292.00b	92.70c	33.33c
T4=10×14 (1000g)	26.00d	21.50b	300.30b	85.78d	0.0d
T5=8×16 (1100g)	25.00d	18.00c	210.00d	54.55e	50.0b
T6=12×18 (1400g)	30.00c	23.00b	280.00c	57.14e	66.67a
CV(%)	**4.96**	**5.77**	**2.87**	**2.68**	**8.07**

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

Yield/ Packet (g): Significant variation was observed in yield under different treatments (Table 1). The highest yield (316.30g) was found in T_2 followed by T_3 (300.30g) and T_4 (292. 0g). The lowest yield was found in T_1 (190.0g). 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 106g–534.50g and also

reported yield increased with increasing the amount of rice straw. Amin *et al.* (2008) also reported yield was increased with increasing the amount of rice straw for the cultivation of oyster mushroom. Shelly *et al.* (2010) observed that the total yield of *Pleurotus ostreatus* 176.30g/packet on paddy straw substrate. It is also apparent from the results that increasing substrate quantities above 1kg per bag did not result in increases in mushroom production and will be wasteful of time and labour. These results were in contrast with the results obtained by Shah *et al.* (2004) and Singh and Prasad, (2012) who obtained higher yields (647- 645 g) from 1000 g dry substrates. Actually, the total yields should be associated with the amounts of substrates placed in the bags in grams or kilograms. Pathmashini *et al.* (2008) obtained highest and lowest total fresh yields of 276.78 and 107.87 g, respectively from 800 g dry substrates.

Biological efficiency (Be) (%): The highest biological efficiency (142.10%) was found in T_1 followed by T_2 (129.10%) and the lowest biological efficiency was found in T_5 (54.55%) where Shelly *et al.* (2010) was observed that the biological efficiency (121.30%) of *Pleurotus ostreatus* packet on paddy straw substrate (Table 1). As substrate quantities increased, substrate utilization decreased.

Contamination rate (%): There was a significant difference in percent contamination rate, which ranged from 0.0% to 66.67% (Table 1) by green mould and other bacteria. The highest contamination rate (66.67%) was found in T_6 which was followed by T_5 and the lowest contamination rate (0.0%) was found in T_1 which was statistically similar to T_2 and T_4. 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. The ability of sterilization methods to eliminate substrate contaminants is shown by the presence or absence of contaminants in the substrate after sterilization, spawning and incubation. Kurtzman (2010) reported several causes of mushroom substrate contamination. It is concluded that contamination was increased with increasing the amount of substrates and bag size for the cultivation of pearl oyster mushroom. According to Balasubramanya and Kathe (1996), the microorganism species that competed with *Pleurotus sp.* after pasteurization with hot water (80°C for 2h) were the fungi *Penicillium sp.* and *Trichoderma sp.* probably due to the partial breakdown of cellulose and hemicelluloses, thus making them available to competitors. Contamination by faster growing organisms is consequently a major problem since it causes decreased yields and production rates (Royse, 1989).

Size of fruiting body: The length of stalk ranged from 3.90 to 5.65 cm with significant difference (Table 2). The highest length of stalk was found in T_5 (5.65cm) which was statistically similar to treatment T_6 (5.20cm). The lowest length of stalk was found in T_5 (3.90cm). The diameter of stalk was non- significant and ranged from 0.63 to 1.10 cm (Table 2). The highest diameter of stalk was found in T_3 (1.10cm) while the lowest diameter of stalk (0.63cm) was found in T_5.

The diameter of pileus ranged from 5.43 cm to 6.63 cm with significant difference among the treatments (Table 2). The highest diameter of pileus (6.63cm) was found in T_3 followed by T_4 (6.40cm) and the lowest diameter of pileus (5.43 cm) was found in T_5. The thickness of pileus in different treatments differed significantly and ranged from 0.55cm to 0.70cm (Table 2).

Table 2. Effect of different packet on size of fruiting body of pearl oyster mushroom

Treatments	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T1=7×10 (400g)	4.83b	0.85a	5.80b	0.70a
T2= 9×12(700g)	4.68b	0.93a	5.54b	0.68a
T3=8×14 (900g)	5.65a	1.10a	6.63a	0.60b
T4=10×14 (1000g)	4.63b	1.05a	6.40a	0.58bc
T5=8×16 (1100g)	3.90c	0.63a	5.43b	0.55c
T6=12×18 (1400g)	5.20ab	0.70a	5.70b	0.70a
CV(%)	**9.66**	**5.52**	**6.84**	**3.79**

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_6 (0.70cm) which was statistically similar to T_1 (0.70). The lowest thickness of pileus (0.55cm) was found in T_5 which was statistically similar to T_4.

REFERANCES

Amin, S. M. R., Sarker, N. C., Alam, N., Hossain, K. & Uddin, M. N. 2008. Influence of different amount of rice straw per packet and rate of inocula on the growth and yield of oyster mushroom (*pleurotus ostreatus*). *Bangladesh J. Mushroom.* **2**(1): 15 - 20.

Balasubramanya, R. H. & Kathe, A. A. 1996. An inexpensive pre-treatment of cellulosic materials for growing edible oyster mushrooms. *Biol. Resour. Technol.* **57**: 303 – 305.

Gomez, K. A. & Gomez, A. A. 1984. Statistical Procedures of Agricultural Research. John Wiley and Sons. Inc. New York. pp. 304 - 307.

Khan, A. S., Sarker, N. C., Howlader, R. K. & Kakon, A. J. 2012. Effect of different Amount of Rice Straw on Growth and Yield of pleurotus salmoneostramineus. *Bangladesh J. Mushroom.* **6**(1): 37 - 43.

Kong, W. 2004. Oyster Mushroom Cultivation (Descriptions of Commercially Important Pleurotus species), Mushroom Growing Handbook1, Mushworld all rights reserved, ISSN 1739-1377. 54 - 61.

Kurtzman, J. R. 2010. Pasteurisation of mushroom substrate and other solids. *African J. Environ. Sci. Technol.* **4**: 936 – 941.

Mamiro, D.P. & Mamiro, P.S. 2011. Yield and Mushroom Size of *Pleurotus ostreatus* Grown on Rice Straw Basal Substrate Mixed and Supplemented with Various Crop Residues, *Journal of Animal & Plant Sciences*. **10**(1): 1211 - 1218.

Moonmoon, M., Mahjabin, T., Sarker, N. C., Khan, A. S., Rahman,T. & Kakon, A. J. 2012. Performance of oyster mushroom variety on rice straw and sawdust in summer season. *Bangladesh J. Mushroom.* **6**(2): 35 - 40.

Pathmashini, L., Arulnandhy, V. and Wijeratnam, R. S. 2008. Cultivation of Oyster Mushroom (*pleurotus ostreatus*) on Sawdust, Cey. *J. Sci. Bio. Sci.* **37**(2): 177 - 182.

Royse, D. J. 1989. Factors influencing the production rate of shiitake. Mushroom Journal of the Tropics. **9**: 127 - 138.

Sarker, N. C., Hossain, M. M., Sultana, N., Mian, I. H., Karim, A. J. M. S. & Amin, S. M. R. 2007. Performance of different Substrates on the Growth and Yield of *Pleurotus ostreatus* (jacquin ex Fr.) Kummer. *Bangladesh J. Mushroom.* **1**(2): 9 - 20.

Shah, Z. A., Ashraf, M. & Ishtiaq, M. C. 2004. Comparative Study on Cultivation and Yield Performance of Oyster Mushroom (*Pleurotus ostreatus*) on Different Substrates (Wheat Straw, Leaves, and Sawdust), *Pakistan Journal of Nutrition.* **3**(3): 158 - 160.

Shelly, N. J., Rahman, M. M., Moonmoon, M. & Sarker, N. C. 2010. Performence of Different Speices of Oyster Mushroom on Rice straw. *Bangladesh J. Mushroom.* **4**(1): 51 - 57.

Singh, S. D. & Prasad, G. 2012. Effect of Different Substrate Supplements on the Growth and Yield of two Species of Mushroom *Pleurotus florida* and, *P. Sajor-Caju. International Multidisciplinary Research.* **2**(3): 61 - 64.

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