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Improvement of Nutrition and Reduction of Poverty Through Mushroom Cultivation Project
Mushroom Development Institute
Department of Agricultural Extension
Ministry of Agriculture
Sobhanbag, Savar, Dhaka-1340
Bangladesh

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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.

Roberts, D. W. 1980. Toxins of entomopathogenic fungi. **In : Microbial control of Pests and Plant Diseases** (Ed) H. D. Burgess, New York Academic Press. pp. 441-463.

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Survey on Status of Mushroom Cultivation and Spoilage of Spawn in Bangladesh

Khadija Akhter[1], M. Salahuddin M. Chowdhury[1], Nirod Chandra Sarker and Akhter Jahan Kakon

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Abstract

The survey was carried out during the period of 2013 to study on production status and activities of mushroom farms in Bangladesh. Survey study on 110 mushroom growers of 59 upazillas in 21 districts revealed that oyster mushroom PO2 was the most cultivated popular species. Seed crisis and spawn contamination were identified as the main problem of farmers where 30% contamination occurred during incubation, 30% in summer and less than 20% in winter. 52.7% farmers conducted substrate sterilization through hot water for 1 hour. Rice straw (55.7%) and saw dust (21.6%) was mainly used as substrate.

Keywords: Survey, mushroom, respondents, spawn, substrates, sterilization.

INTRODUCTION

Mushroom is the fleshy large reproductive structure of edible fungi. belongs to either Ascomycotina or Basidiomycotina typically consisting of a cap (pileus) at the end of a stem arising from an underground mycelium. There are various types of edible mushrooms such as oyster mushroom, milky white mushroom, shitake mushroom, button mushroom, straw mushroom etc. which are cultivated in our country. The oyster mushroom (*Pleurotus ostreatus)*, first cultivated in Germany as a subsistence measure during World War I (Eger *et al*., 1976) is now grown commercially around the world for food. Mushrooms have been cultivated since ancient time for their nutritional value and flavor especially in the far eastern countries. Mushroom is now-a-days one of the promising concepts for crop diversification in Bangladesh. The climatic condition of Bangladesh is completely suitable for mushroom cultivation. Nwanze *et al*., (2005) stated that environmental factors such as temperature, O_2, CO_2, humidity, light, p^H affect the mycelial growth in the spawn. *Pleurotus* spp grows in wide range of temperature (15-30^0 C) which also varies from species to species (Sarker *et al.,* 2008). Uddin *et al*., (2011) reported that winter (temperature zone 14-27^0 C with relative humidity (70-80%) is suitable for better production and biological efficiency of *Pleurotus* spp in Bangladesh. It does not require any cultivable land. Mushroom cultivation can be a very interesting hobby with delicious results that could easily become a profitable small business, due to the low cost of inputs and high value of the crop. It requires short time, little capital and easy technique for cultivation. This is why all types of people like male and female, youth and old even children and disabled can easily participate in its cultivation. Its cultivation can transfer as a cottage industry and create a good opportunity for export. Therefore, it can generate huge scope of employment opportunities for unemployed people. Considering the above facts, the present investigation the survey was conducted to collect information about present status of mushroom cultivation and spoilage of spawn.

MATERIALS AND METHODS

Survey duration, area and selection of mushroom growers: The survey was conducted during 2014-2015. The locations of survey were mushroom centers of Department of Agricultural Extension (DAE) of the Peoples' Republic of Bangladesh and mushroom farms in 21 districts of the country. A total of 110 mushroom growers were selected from 110 farms including mushroom centers in different districts (Table 1).

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Table 1. List of surveyed area of mushroom farms/ centers in Bangladesh

Sl. No.	District(s)	Name of center/ farm(s)/proprietors	Number (s) of mushroom farm
01	Dhaka	i. Mushroom Culture House, SAU, Dhaka. ii. Dainty Mushroom centre, Mirpur, Dhaka. iii. Khadem mushroom centre, Khilgaon, Dhaka. iv. Marry mushroom, Dhakhin Khan. v. Jashim, mushroom, Dhaka. vi. Kanunj protein, Uttarkhan. vii. Rani mushroom, Dakhhin Khan.	07
2	Savar	i. NAMDEC, Sobhanbag, Savar, Dhaka. ii. DD mushroom, Savar, Dhaka. iii. Paradise Agrofarm, savar, Dhaka. iv. Organic Agrofarm, ganda, Savar, Dhaka. v.Tahsan, Dagarmora, Dhaka. vi. Kulsum mushroom, Dhaka. vii. Mollah, Joyapara, Dhaka. viii. Holy mushroom, Ashulia, Dhaka. ix. Golapi, Savar, Dhaka x. Munni mushroom, savar, Dhaka. xi. A. k. Mushroom, Savar, Dhaka. xii. Jahangir baul, Savar, Dhaka.xiii. Zaman, savar, Dhaka.	13
03	Gazipur	i. World mushroom. ii. Bhuyan mushroom. iii. Voawal mushroom. iv. M. R. C. L.Multifarm.	04
04	Mymensingh	i. Md. Edrisullah Hossain. ii. Winner Mushroom. iii. Usuf. iv. Hannan Mia. v. Sayed.	05
05	Noakhali	i. Lakhipur Mushroom Prakalpa. ii. Noakhali Mushroom. iii. Jahirpathan, Chhagalnaya. iv. Ujjibon Centre, sonaimuri. v. Sajedamushroomcentre, Chhagalnaya. vi. Asa mushroom Centre, Sunaimuri.	06
06	Bogra	i. Mansur Ali, Bagurasadar. ii. Aklima Mushroom Centre. iii. Khairul Islam, rajshahisadar. iv. Sajib, Bagurasadar. v. Fazlulhaque, atria. vi.Shafiqul Islam, BagiraSadar. vii. Hasan Mushroom, kahalo. viii. Raju, Bogura. ix. Mijanurrahman, Gaibandha. x. Surovi, Bagurasadar. xi. Rehana, BaguraSadar. xii. Kajal, Sonatala. xiii. Labu Mia, Sonatala. xiv. Alamin, sajahanpur. xv. Based, BaguraSadar. xvi. Aklima, BaguraSadar.	16
07	Dinajpur	i. Nasrin. ii. Khurshid Jahan, sadar. iii. Laki, Nayanpur. iv. NahinaAkhter, sadar. v. Arnaba Mushroom, Mirjapur, Sadar. vi. IsratJahan, Mirjapur. vii. Anjumanara, Trishbnadar. viii. Mamtaj Lavli.	08
08	Comilla	i. Mohoshin, Muradnagar. ii. Khidmah, Choddogram. iii. Chowdhory mushroom & Strawberry, Haziganj.	03
09	Bandorban	i. Mushroom Sub Centre. ii. Sabura, Gudarpara. iii. Sanuara, Gudarpara. iv. Samud Khatun. v. Tipu, sadar. vi. Juni, sadar. vii. Kallan Barua, Sadar.	07
10	Rangamati	i. Mushroom sub-centre. ii. Janani Mushroom Centre, Sada. iii. Sagar Mushroom, sadar. iv. Anika, rangamati, sadar. v. Jewel Mushroom, Sadar. vi. Sanchita Chakma. vii. Anjana Dey, sadar. viii.Yasin Centre, Sadar.	08
11	Sylhet	i. Mushroom Garden. ii. Yakob Ali. iii. Mostak.	03
12	Cox's Bazar	i. SahidulhaqbKajal. ii. Rupban, Sadar. iii. Laila begum, Sadar. iv. Dildar Begum. v. TanjilaAlam, Sadar. vi. Nurakter. vii. Nasima, Adarshagram. viii. Lutfunnesa, adarsha gram, Sadar. ix. Nurnahar, adarsha gram, Sadar. x. Nilofa Yasmin, adarsha gram, Sadar. xi. Morshida, Sadar.	11
13	Chittagong	i. Mahiuddin. ii. Jenin, Hathazari. iii. Usouf, Sitakundu. iv. Himelbarua, Patia.	04
14	Khulna	i. Rabiul mushroom farm, Magura. ii. Shadul Islam. iii. Nizamuddin. iv. Adarsa manna mushroomi. v. Makka-Madina.	05
15	Khagrachhari	i. Ataur Rahman. ii. Hmmam Rekhanjali Chakma. iii. Suman mushroom.	03
16	Nilphamari	i. Fatema Mushroom. ii. Hafijur Rahman.	02
17	Barisal	i. kamrul mushroom. ii. Joy.	02
18	Rajshahi	i. Md. Khairul Islam, Rangdhonu.	02
19	Naogaon	ii. Md. Fazlul Haque.	01
20	Gaibandha	iii. Md.Mijanur Rahman.	01
21	Patuakhali	iv. Mother mushroom.	01
Total			110

Data were recorded on the following parameters using a pre-tested questionnaire: Demographic information of the farmers, name of mushroom species and strains cultivated by growers, mushroom species and strains of oyster mushroom cultivated and seasons of cultivation, preparation and sources of mother culture and mother spawn, spoilage of mother spawns due to contamination, problems of mushroom cultivation, methods of substrate sterilization used by farmers, farmers response about contamination during incubation and cultivation during summer and winter, types of mushroom house and sources of water for growing mushroom, activities of mushroom farmers performed, various problems identified by mushroom famers. The data collected during survey based on questionnaire were analyzed following SPSS computer program.

RESULTS AND DISCUSSION

Mushroom species of mushroom cultivated and different seasons of cultivation in Bangladesh: In summer, only strain PO_2 of oyster mushroom was cultivated above 51.2% and in winter above 36.4%. Strains PCYS (*Pleurotus cystidiosus*), HK (High king) and WS (White snow) of oyster mushroom were reported to grow well in both winter and summer. Only POP (pink oyster mushroom) was cultivated 25% in winter and 6.7% in summer whereas, PO_2 and POP were cultivated together 18.5% in winter. PO2, POP, WS, PCYS, and HK were 11.4% in summer and 7.1% in winter. WS, PCYS, HK with PO2 were cultivated by 19.1% in summer, WS, PCYS, and HK with POP were cultivated by 6.0% in winter (Table 2).

So, it has been found that different species of mushrooms are cultivated in different seasons of Bangladesh. The findings indicate that different species and strains of mushroom are suitable to cultivate in the country. Among these PO2 of oyster mushroom is very popular to farmers and consumers. Other researchers also reported that cultivation of oyster mushroom is gaining importance in tropical as well as subtropical regions due to its simple way of cultivation and high bio efficiency (Singh *et al.*, 1990). Amin (2004) revealed that oyster mushrooms are ideally suitable for cultivation under both temperate and tropical climatic conditions and can be harvested all over the year (Some of oyster mushrooms are adaptable both in summer and winter season. In Bangladesh, summer is the most long lasting season and it is possible to cultivate many kinds of oyster varieties in this season. So, it is possible to mitigate the needs of mushroom all year round, which was corroborated with present study.

Table 2. Different strains of oyster mushroom cultivated by farmers of Bangladesh in summer and winter season

Strains of oyster mushroom and other species	% Famers cultivated oyster mushroom in two seasons	
	Summer	Winter
PO2	51.2	36.45
POP	6.71	25.06
PO2+WS+PCYS+HK	19.1	6.0
PO+POP+WS+HK	11.4	7.1
POP+WS+ PYCS	7.7	-
POP+PYCS	2.9	-
POP+HK+PCYS	1.0	-
POP+WS	-	6.9
PO2+POP	-	18.5

PO2 (*P. ostreatus 2*), HK- High King, WS-White snow, Pop-pink oyster, *PCYS-P. cystidiosus*, FLO- *P. florida*,- *Not cultivated.*

Sources of mother culture and preparation of mother spawn: The highest 51.3% farmers produced their own mother culture from tissue of fruiting bodies, while 25.6% farmers used either tissue or another mother culture. The lowest 23.1% farmers produced mother culture from previous mother

culture or mother spawn (Fig. 1). Pure culture is grown on PDA from tissues of fruiting body and then transferred onto grain or plant parts for media preparation of mother culture. As per Mbogoh (2011), spawn is pure culture of mycelium grown on a solid substrate such as cereal grain. It is the mushroom seed, comparable to the seed of crop plants. It serves as the planting material in mushroom cultivation. Different materials are used to multiply the mother spawn (seed) for large scale production. viz. cereal grains such as wheat (Elhami et. al., 2008; Chang 2009; Stanely, 2010), rye (Chang, 2009) rice (Oei, 1996), millet sorghum, white maize (Stanely, 2010) were reported to use as media for mother spawn production. Kumbhar (2012) reported that mycelium of P. eous had marked preference for cereal grains over pulses and crop residues and ragi grains took only six days for mycelial colonization followed by maize, pearl millet, sorghum, wheat and paddy grains while pulses did not allowed growth of the fungal mycelium. Sofi *et al.*, (2014) evaluated wheat grains, barley maize and millets for spawn production and reported maximum growth rate in the corn (38.60 mm) and minimum in millet (26.80 mm) after 12 days of inoculation.

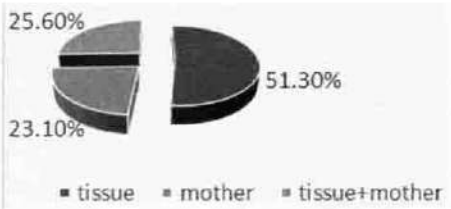

Fig. 1. Different sources for collection of mother cultures.

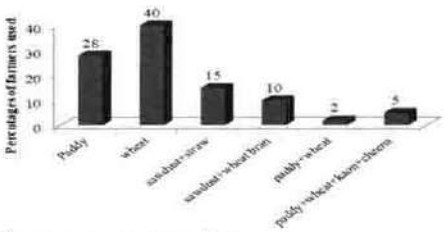

Fig. 2. Materials used for mother spawn preparation.

Spoilage of mother spawns due to contamination: The lowest 10.0% spoilage was reported by the maximum of 28.33% farmers, 20.0% by 27.0%, 30% spoilage reported by 18.9%, 40% by 40% farmers and 50% by 2.7% farmers and above 2.7% spoilage was reported by more than 50% farmers (Fig. 3).

Information about substrates for oyster mushroom cultivation: Among 110 mushroom growers selected for data collected, the maximum of 55.7% used rice straw, 21.6% used saw dust, 15.1% used rice straw mixed with saw dust, 3.4% used wheat straw, another 3.4% used wheat straw mixed with saw dust and the minimum 1.1% famers used sugarcane baggase as substrates (Fig. 4). Munsur *et al.*, (2012) reported that wheat substrate was the best substrate for spawn production of oyster mushroom followed by rice and wheat bran. From survey report, it was noticed that in Bangladesh, oyster mushrooms are most popular and different species of this mushroom like *Pleurotus ostreatus, P. florida, P. sajor-caju* and *P. high king* are commercially cultivated all over the year by using sawdust and/or rice straw as main substrate, which was also reported by Amin *et al.*, (2008). Maniruzzaman (2004) in his study found that substrate rice straw was the best for spawn production of oyster mushroom. Sarker *et al.*, (2007) tested waste paper, wheat straw, rice straw, sugarcane bagasse and *Saccharams pontaneum*as the substrates of *Pleurotus ostreatus* and indicated possibility of their commercial use. Substrate selection depends on the availability or costs or area for mushroom production in Bangladesh. Different substrates can, therefore, be recommended per region depending on local availability of agricultural wastes (Cohen *et al.* 2002).

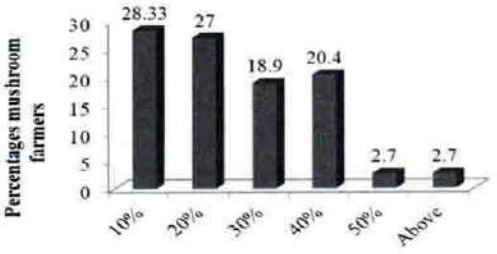

Fig. 3. Wastages of mother spawn of mushroom.

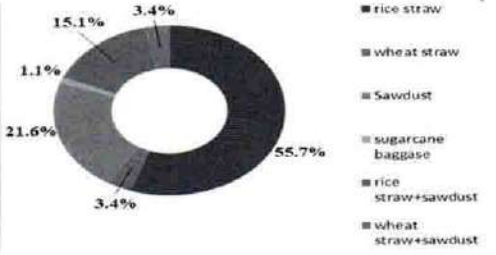

Fig. 4. Substrates for oyster mushroom cultivation.

Methods of substrate sterilization used by farmers: Methods of sterilization or pasteurization, duration of sterilization or pasteurization and percentage of farmers used different methods are shown in Fig. 5. During survey it was found that 52.7% farmers pasteurized substrates of mushroom using hot water treatments for one hour, 26.7% using autoclave for one hour, 8.6% using steam pasteurization in drum for one hour, 1.2% for 5 hours, 1.5% using sundry and 7.0% growers pasteurized the substrates in NAMDEC steam oven for 2 hours while 2.3% for 6 hours. NAMDEC steam oven is special type oven for pasteurization of substrate developed by NAMDEC (Fig. 3). Siqueira *et al.,* (2012) proposed a number of different methods for substrate pasteurization or sterilization viz. autoclaving (axenic), axenic and inoculation with thermophilic microorganisms, rapid substrate steam treatment between 80^0 C and 100^{0}C for several hours, pasteurization at 72^{0}C for four or five days and pasteurization by substrate steam treatment for several days (60^{0}C) in a tunnel.

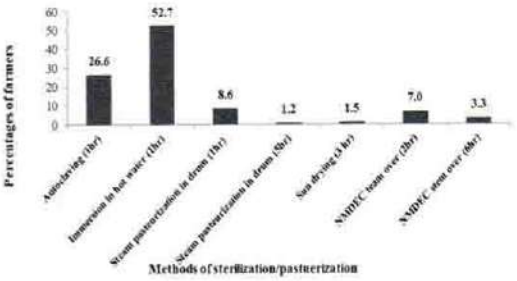

Fig. 5. Methods of sterilization/pasteurization of mushroom substrates in survey areas of Bangladesh (NAMDEC= National Mushroom Development and Extension Center).

Sources of water for growing mushroom: The sources of water used for irrigation were tap, tube, pond and well. The water sources were found to be used by 48.6, 44.8, 4.8 and 1.9% mushroom farmers, respectively (Fig. 6). Tewari (1986) reported that the mushroom fungus requires a considerable amount of water for fruiting bodies formation, due to the high content of water in mushrooms. Sarker *et al.,* (2007) reported that time required from stimulation to primordia initiation, stimulation to first harvest, total harvest, the number of fruiting bodies, biological efficiency and economic yield of *Pleurotus ostreatus* were influenced significantly by frequency of watering. López *et al.*, (1995) determined the quality of water for pasteurizing substrate (coffee wastes) of *P. ostreatus* cultivation. They found that residual water could become highly polluted. As a consequence, this factor may have affected mushroom yield.

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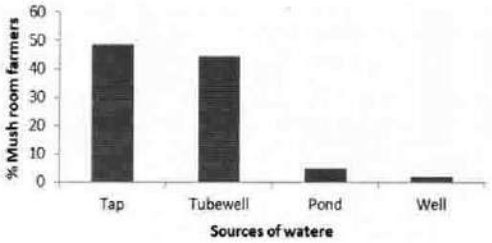

Fig. 6. Different sources of water used by different percentage of mushroom famers.

Farmers response about contamination during cultivation in summer and winter: The maximum of 46.3% followed by 27.4, 9.5, 8.4, 5.3 and 3.2% farmers reported >10, 10, 20, 30 and 40% spoilage of spawn packets during incubation in summer (Fig. 7). Less than 10, 10, 20, 30 and 40% spoilage of spawn packet during cultivation was recorded by 47.1, 27.6, 14.9, 8.0 and 2.3% mushroom farmers during cultivation in winter (Fig. 8). Mazumder *et al.*, (2005) observed month wise variation in spawn contamination and found that the combination was highest during the monsoon season (28.57%) followed by pre-monsoon (21.9%). They also reported that paddy grain based spawn recorded significantly lowest (15.00%) contamination as compared to wheat grain based (30.0%) spawn.

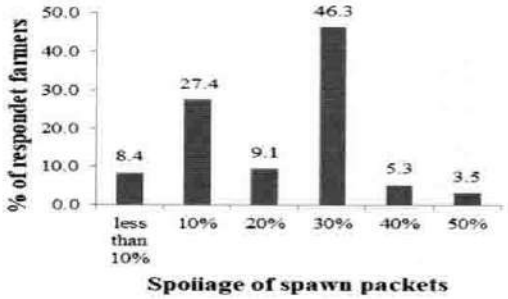

Fig. 7. Percentage of farmers reported spoilage of spawn packets during cultivation in summer.

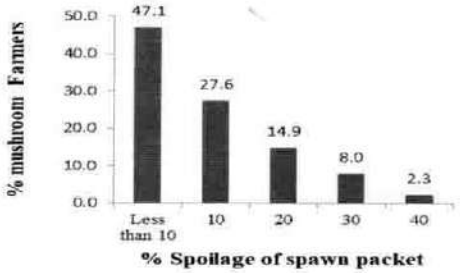

Fig. 8. Percentage of farmers reported spoilage of spawn packets during cultivation in winter.

Various problems identified by mushroom famers: In surveyed area, seed crisis and spawn packet contamination were identified as important problems of mushroom cultivation by 58.0% famers, seed crisis was identified by 25.0% famers, only seed contamination was identified by 16.0% farmers, marketing problem was identified by 4.0%, and varietal problem reported by 3.0% mushroom famers (Fig. 9).

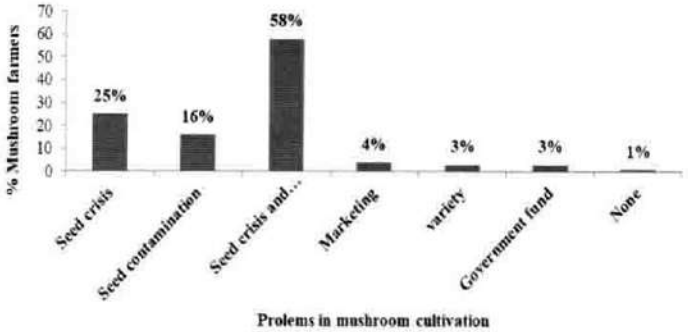

Fig. 9. Miscellaneous problems faced by mushroom farmers during cultivation.

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Effect of Casing Materials on Growth and Yield of Milky White Mushroom (*Calocybe indica*)

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Abstract

Casing the surface of composted substrate fully colonized by mycelium of mushroom is an essential function for stimulation and promoting the development of fruit bodies. Casing is an important cultural practice of milky white mushroom cultivation. Therefore the present experiment was carried out to select the suitable casing materials for successful cultivation of milky white mushroom. Eleven different combination of casing materials were used in this experiment, such as T_1= coconut coir dust; T_2 = coconut coir dust+ decomposed cow dung (1:1); T_3 = coconut coir dust+ loamy soil (1:1); T_4 = ash; T_5 = ash + loamy soil (1:1); T_6 = loamy soil + sand (3:1); T_7 = decomposed spent mushroom substrate; T_8 = decomposed spent mushroom substrate + loamy soil (1:1); T_9 = decomposed spent mushroom substrate + decomposed cow dung (1:1); T_{10} = decomposed spent mushroom substrate + ash (1:1) and T_{11} = loamy soil (control). Spawn packet covered with coconut coirdust in combination with decomposed cow dung (T_2) produced highest number of effective fruiting body, fruiting body flushes, economic yield and biological efficiency (90.6%) but loamy soil in combination with sand at 3:1 ratio promoted earlier fruiting body primordial initiation. The result revealed that coconut coir dust in combination with decomposed cow dung was the best casing material for successful cultivation of milky white mushroom.

Keywords: Casing materials, effects, milky white mushroom (*Calocybe indica*).

INTRODUCTION

Casing is an important cultural practice of milky white mushroom cultivation. Casing means covering the mycelial enriched substrate with a layer of soil or soil like material after spawn run which enhances the transformation of vegetative stage to reproductive stage (Pani, 2012; Suess and Curtis, 2009). Casing the surface of composted substrate fully colonized by mycelium of mushroom is an essential function in stimulation and promoting the development of fruit bodies (Farsi *et al.*, 2011). Recent studies on the constraints in the cultivation of milky mushroom indicated casing is the most important factor affecting the yield. The production of *Calocybe indica* depends on top dressing after the substrate has been fully colonized with mycelium. After complete mycelial formation casing is done to provide a reservoir of water for the developing fruiting body. The composition of casing mixture determines its quality (texture, structure, pH, water holding capacity, C: N ratio etc.), which directly affect the mycelial growth in casing layer and initiation of fruiting bodies (Tewari, 2005). Before the early 1950s, sterilized soil or subsoil was used for casing, and this is still used by some farms. The characteristics of a good casing medium are that it should have an open texture, good water-holding capacity, freedom from pests and diseases, and a pH between 6.5 and 8.0. A peat moss mixture with the pH adjusted by lime, chalk, or ground limestone fulfills the requirements of a good casing soil and is now widely used in many advanced mushroom industries. With the help of modern technology, understanding the specific relationship between water tension in the casing layer and mushroom yield could add a great deal to the future success of mushroom crop management.

Although different materials may adequately function as a casing layer, peat is commonly used and recommended as a good casing in mushroom cultivation (Gulser and Peksen, 2003). Peat is not so available in many mushroom growing areas. It is a costly and nonrenewable input. Not only the import cost of peat but also the depletion of its available resources worldwide discourages the investors to use peat as casing layer (Sassine et al., 2005). After that many materials, alone or in combination, have been used as casing both commercially and experimentally (Gimenez and Gonzalez, 2008).

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Different materials are used in the shape of casing throughout the world, but a few casing substances have been developed and suggested for use. Mantel (1973) recommended the use of compost with slaked lime and sand (4:1:1). It was stated that farmyard manure and loamy soil in 1:1 ratio in the production of *Agaricus bisporus* (Hayes and Shandilya, 1977) and the mixture of soil and sand 1:1 ratio in the production of *Calocybe indica* (Purkayastha and Chandra, 1985) had supported better fructification compared to other substrates. Sharma *et al.* (1997) evaluated 16 combinations of casing material and reported that 2 years old cow dung patties were excellent casing material, using 1-inch-thick in milky mushroom cultivation.

Beside physical, chemical and biological factors of the suitable casing material, cost and availability are more important factors in successful application and acceptance by the mushroom growers. Therefore, the present study was conducted to evaluate different casing materials for successful production of milky white mushroom.

MATERIALS AND METHODS

The experiment was conducted at Mushroom Development Institute (MDI), Department of Agricultural Extension, Savar, Dhaka, Bangladesh from May 2019 to July 2019. Eleven different combination of casing materials were used in this experiment. The treatments were: T_1 = coconut coir dust (CC); T_2 = coconut coir dust(CC) + decomposed cow dung (CD) (1:1); T_3 = coconut coir dust(CC) + loamy soil (LS) (1:1); T_4 = ash (AS); T_5= ash (AS) + loamy soil (LS) (1:1); T_6 = loamy soil (LS) + sand (S) (3:1); T_7 = decomposed spent mushroom substrate (SMS); T_8 = decomposed spent mushroom substrate (SMS) + loamy soil (LS) (1:1); T_9 = decomposed spent mushroom substrate (SMS) + decomposed cow dung (CD) (1:1); T_{10} = decomposed spent mushroom substrate (SMS) + ash (AS) (1:1) and T_{11} = loamy soil (LS) (control).

Spawn preparation: Rice straw was used for the cultivation of milky white mushroom. The straw was chopped to convenient length of 2.5 to 5 cm. The substrate was mixed with appropriate amount of water and then filled in net bag. The net bag filled with substrate were placed in the sterilization cum inoculation chamber. Door of the chamber was closed and tightened with the help of screws. Burner was turned on to produce steam that flows in to the chamber. When the temperature of the chamber rises to 60°C, the steam flow was adjusted to maintain a constant temperature of 70°C – 80°C up to 90 minutes. After 90 minutes, burner was turned off and kept it for about 20 hours. After 20 hours' substrate was taken out and used for preparation of spawn packet. Pasteurized substrate was filled into the polythene bags (12"x18") and inoculated with 10% sawdust based mother culture by thorough mixing. Then the spawn packets were transferred to the culture house for mycelium run. After 16-25 days the substrate was completely colonized by the mycelium and polythene cover was opened.

Preparation of casing materials: Different casing materials such as loamy soil, coconut coirdust, ash, sand, well decomposed cow dung and well decomposed spent mushroom substrates were collected locally. Collected materials were sterilized at 65°C for 4 hours separately. Casing materials were mixed as per treatments. In case of treatment T_{11} (control) sterilized loamy soil was made small clods and used for covering the completely colonized spawn packets.

Casing and after care: After completion of mycelial colonization, cotton, brown paper and neck were removed from the packets and the mouth of the plastic bags was folded 4-5 cm above the spawn. Previously sterilized casing materials were used according to treatments to cover over the mycelium on the substrate up to 4 cm thickness. Watering was done at regular interval to maintain moisture at 60 to 70%. Fruiting body primordial initiated within 12-24 days and developed in to fruiting bodies. The fruiting bodies were harvested at 7-8 days of primordial initiation. Data were collected on days to primordial initiation, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, number of effective fruiting body (NEFB), number of flushes, yield and biological efficiency (BE). The BE was measured by the formula;

$$\text{BE (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate.}} \times 100$$

Data analysis: The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. The data were statistically analyzed following SPSS (version 20.0) computer program. Difference among the treatment means were determined by Tukey's Test (Tukey, 1977) at $P \leq 0.05$.

RESULTS AND DISCUSSION

Days to primordial initiation, Number of flushes and Number of effective fruiting body- Casing materials has significant influence on primordial initiation, number of flushes and number of effective fruiting body of milky white mushroom (Table-1). Spawn packet covered with loamy soil and sand at 3:1 ratio (T_6) produces earlier (12 days) fruiting body primordia than all other casing materials which was statistically similar to T_3, T_5 and T_4. Whereas Spawn packet covered with decomposed spent mushroom substrate (SMS) and ash (AS) at 1:1 ratio (T_{10}) required highest time (24 days) for fruiting body primordial initiation which was significantly higher than all other casing materials. Time required for fruiting body primordial initiation in T_2, T_{11}, T_7, T_1, T_9 and T_8 was between 15.0-15.9 days which were statistically similar. T_3, T_5 and T_4 required similar time (13.0-13.1days) for fruiting body primordial initiation. This result supported the findings of Ashrafi et al. (2017) who also observed earlier primordial initiation after casing with soil + sand (3:1). Amin *et al.* (2010) also reported that primordia initiation with the different substrates and casing materials was between the 13th and 19th day.

Table 1. Effects of casing materials on primordial initiation of fruiting body, number of flushes and number of effective fruiting body of milky white mushroom

Treatments	Days to primordial initiation	Number of flushes	Number of effective fruiting body
T_1- Coconut coir dust(CC)	15.8c	2.6ab	6.1ab
T_2- Coconut coir dust(CC) + Cow dung (CD) (1:1)	15.0bc	3.1b	7.6b
T_3- Coconut coir dust(CC) + Loamy soil (LS) (1:1)	13.0ab	2.8ab	6.8ab
T_4- Ash (AS)	13.1ab	2.4ab	6.8ab
T_5- Ash (AS) + Loamy soil (LS) (1:1)	13.0ab	2.5ab	7.1ab
T_6- Loamy soil (LS) + Sand (S) (3:1)	12.0a	2.4ab	6.5ab
T_7- Decomposed spent mushroom substrate (SMS)	15.6c	2.6ab	6.5ab
T_8- Decomposed spent mushroom substrate (SMS) + Loamy soil (LS) (1:1)	15.9c	2.3ab	6.5ab
T_9- Decomposed spent mushroom substrate (SMS) + Cow dung (CD) (1:1)	15.8c	1.9a	5.9a
T_{10}- Decomposed spent mushroom substrate (SMS) + Ash (AS) (1:1)	24.0d	2.5ab	6.3ab
T_{11}- Loamy soil (LS) (control)	15.2c	2.3ab	6.8ab
P	<0.001	= 0.070	= 0.048

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. T_1- Coconut coir dust (CC), T_2- CC + Cow dung (CD) (1:1), T_3- CC + Loamy soil (LS) (1:1), T_4- Ash (AS), T_5- AS + LS (1:1), T_6- LS + Sand (S) (3:1), T_7- Decomposed spent mushroom substrate (SMS), T_8- SMS + LS (1:1), T_9- SMS + CD (1:1), T_{10}- SMS + AS (1:1) and T_{11}- LS (control).

Number of fruiting body flushes and number of effective fruiting body were also affected by casing materials (Table-1). Highest number of flushes (3.1) and effective fruiting body (7.6) per packet were recorded in spawn packet covered with coconut coir dust and well decomposed cow dung at 1:1 ratio (T_2). Number of flushes and effective fruiting body were lowest (1.9) in spawn packet covered with decomposed spent mushroom substrate and cow dung at 1:1 ratio (T_9). This result was similar to that of Ashrafi *et al.* (2017) who also reported that number of effective fruiting body significantly affected by different casing materials. But was different from that of Amin *et al.* (2010) who reported that number of effective fruiting body were statistically similar in all of the casing materials used.

Length and diameter of stalk, diameter and thickness of pileus- Length of stalk and diameter & thickness of pileus were significantly affected by casing materials (Table- 2). Highest stalk length (11.0 cm) was recorded in spawn packet covered with decomposed spent mushroom substrate and decomposed cow dung at1:1 ratio (T_9) which was statistically similar to spawn packet covered with only decomposed spent mushroom substrate (T_7). Stalk length was lowest (7.1 cm) when spawn packets were covered with loamy soil and sand at 3:1 ratio (T_6). This result was similar to that of Amin *et al.* (2010) and Ashrafi *et al.* (2017) who also observed greatest stalk length with spent mushroom substrate as casing material. Stalk diameter was not significantly affected by casing materials except coconut coir dust (T_1). Stalk diameter was significantly smaller (1.8 cm) in T_1 than all other treatments. Diameter of pileus was highest (7.6 cm) when spawn packets were covered with coconut coir dustand well decomposed cow dung at 1:1 ratio (T_2) which was statistically similar to spawn packet covered with decomposed spent mushroom substrate and decomposed cow dung at1:1 ratio (T_9). Pileus thickness was highest (2.5 cm) when spawn packets were covered with decomposed spent mushroom substrate and ash at 1:1 ratio (T_{10}) which was statistically similar to all other treatments except T_1. Pileus thickness was lowest (1.8 cm) in T_1.

Table 2. Effects of casing materials on length & diameter of stalk and diameter & thickness of pileus of milky white mushroom

Treatments	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1- Coconut coir dust(CC)	7.4a	1.8a	4.5a	1.8a
T_2- Coconut coir dust (CC) + Cow dung (CD) (1:1)	7.7a	2.5b	7.6d	2.3ab
T_3- Coconut coir dust(CC) + Loamy soil (LS) (1:1)	7.2a	2.4b	6.2abc	2.2b
T_4- Ash (AS)	7.8a	2.8b	6.0ab	2.1ab
T_5- Ash (AS) + Loamy soil (LS) (1:1)	7.6a	2.4b	6.5abc	2.1ab
T_6- Loamy soil (LS) + Sand (S) (3:1)	7.1a	2.9b	6.0ab	2.2ab
T_7- Decomposed spent mushroom substrate (SMS)	10.2bc	2.7b	6.4abc	2.4b
T_8- Decomposed spent mushroom substrate (SMS) + Loamy soil (LS) (1:1)	9.8b	2.6b	6.3abc	2.1ab
T_9- Decomposed spent mushroom substrate (SMS) + Cow dung (CD) (1:1)	11.0c	2.6b	7.2cd	2.3b
T_{10}- Decomposed spent mushroom substrate (SMS) + Ash (AS) (1:1)	9.4b	2.7b	6.6bc	2.5b
T_{11}- Loamy soil (LS) (control)	8.3a	2.5b	6.6bc	2.3b
P =	<0.001	<0.001	<0.001	=0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. T_1- Coconut coir dust (CC), T_2- CC + Cow dung (CD) (1:1), T_3- CC + Loamy soil (LS) (1:1), T_4- Ash (AS), T_5- AS + LS (1:1), T_6- LS + Sand (S) (3:1), T_7- Decomposed spent mushroom substrate (SMS), T_8- SMS + LS (1:1), T_9- SMS + CD (1:1), T_{10}- SMS + AS (1:1) and T_{11}- LS (control).

Economic Yield (g): Economic yield of milky white mushroom was significantly affected by different casing materials used (Table-3). Yield was highest (374.1g) when spawn packets were covered with coconut coir dust and well decomposed cow dung at 1:1 ratio (T2) which was significantly different from T1, T6 and T3 but was similar to other treatments. Lowest yield (126.4g) was recorded in spawn packet covered with only coconut coir dust (T1) which was significantly lower than all other treatments. This result supported the findings of Ashrafi et al. who also reported that biological yield of milky mushroom was significantly affected by different casing materials. But Amin et al. reported that the biological and economic yields and biological efficiency were statistically similar in all of the casing materials tested. Among different fruiting body flashes maximum mushroom were harvested from 1st flash (73.88%) and minimum from 4th flash (0.61%) (Fig. 1).

Considering all the yield contributing parameters only coconut coir dust as a casing material (T_1) performed worse than all other treatments. This might be due to reduced aeration and access water retention on the top layer of spawn packet. Cocopeat has a very high water holding capacity which causes poor aeration in the root zone. Evans *et al.* (1996) obtained water holding capacities of coir dust samples ranged from 750% to 1100% of dry weight. This will later affect the oxygen diffusion to the roots. Depending on the handling and processing technique, the physical properties of cocopeat can easily affect the air capacity and water retention (Abad *et al.* 2002). Incorporation of coarser material into cocopeat media will solve this problem and improve aeration (Yahya *et al.* 2009).For this reason coconut coir dust mixed with decomposed cow dung (T_2) performed better than only coconut coir dust (T_1).

Table 3. Effects of casing materials on economic yield of milky white mushroom

Treatments	Yield of 1st flush (g)	Yield of 2nd flush (g)	Yield of 3rd flush (g)	Yield of 4th flush (g)	Total yield (g)
T_1- Coconut coir dust (CC)	85.6a	29.3a	8.8a	0.0a	126.4a
T_2- Coconut coir dust (CC) + Cow dung (CD) (1:1)	239.0bcd	77.9b	41.4a	10.3b	374.1c
T_3- Coconut coir dust(CC) + Loamy soil (LS) (1:1)	187.8b	71.6b	31.5a	2.4ab	289.0b
T_4- Ash (AS)	229.0bcd	70.5b	12.3a	0.0a	315.5bc
T_5- Ash (AS) + Loamy soil (LS) (1:1)	226.0bcd	69.6b	19.1a	3.0ab	316.5bc
T_6- Loamy soil (LS) + Sand (S) (3:1)	208.6bc	59.1b	11.6a	0.0a	279.4b
T_7- Decomposed spent mushroom substrate (SMS)	242.0bcd	57.6b	17.5a	3.4ab	316.0bc
T_8- Decomposed spent mushroom substrate (SMS) + Loamy soil (LS) (1:1)	235.4bcd	52.6ab	7.0a	0.0a	297.5bc
T_9- Decomposed spent mushroom substrate (SMS) + Cow dung (CD) (1:1)	272.8d	51.5ab	4.6a	0.0a	328.8bc
T_{10}- Decomposed spent mushroom substrate (SMS) + Ash (AS) (1:1)	235.8bcd	59.0b	20.5a	0.0a	309.3bc
T_{11}- Loamy soil (LS) (control)	262.4cd	49.4ab	14.5a	1.2a	324.2bc
P	< 0.001	< 0.001	= 0.058	= 0.022	< 0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. T_1- Coconut coir dust(CC), T_2- CC + Cow dung (CD) (1:1), T_3- CC + Loamy soil (LS) (1:1), T_4- Ash (AS), T_5- AS + LS (1:1), T_6- LS + Sand (S) (3:1), T_7- Decomposed spent mushroom substrate (SMS), T_8- SMS + LS (1:1), T_9- SMS + CD (1:1), T_{10}- SMS + AS (1:1) and T_{11}- LS (control).

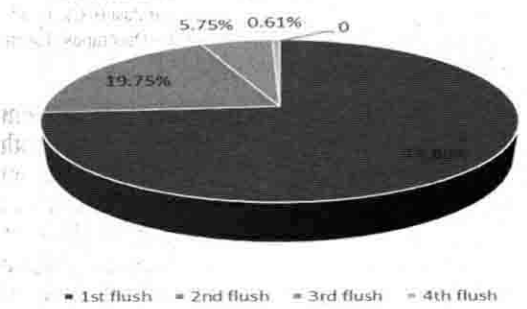

Fig. 1. Flush wise yield of milky white mushroom.

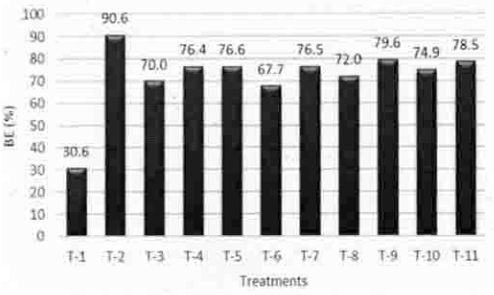

Fig. 2. Effects of casing materials on biological efficiency of milky white mushroom.

Biological efficiency (BE): Different casing materialshas significant effect on biological efficiency of milky white mushroom (Fig. 2.). It was highest (90.6%) when spawn packets were covered with coconut coir dustand well decomposed cow dung at 1:1 ratio (T_2) which was significantly higher than T_1, T_6 and T_3 but was similar to other treatments. Biological efficiency was lowest (30.6%) when spawn packets were covered with only coconut coir dust (T_1). Chakraborty *et al.* (2016) also reported that casing materials significantly influenced the biological efficiency of milky mushroom.

From the above study it can be suggested that casing materials has significant influence on fruiting body initiation, growth, yield and biological efficiency of milky white mushroom. Coconut coir dust in combination with decomposed cow dung at 1:1 ratio was better than other casing materials as it helped to produce highest number of fruiting body, number of flushes, yield and biological efficiency of milky white mushroom. Therefore, coconut coir dust in combination with decomposed cow dung at 1:1 ratio can be used to produce milky white mushroom.

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Arsenic Levels of Arsenicosis Patients are Reduced Tremendously by Oral Application of Fruiting Body of *Pleurotus ostreatus*

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Abstract

The study is focused on the analysis of As levels in human hair and nail samples before and after administering mushroom *Pleurotus ostreatus* (PO), collected from 184 arsenic affected people of Eruine village in the Laksham upazilla of Comilla district of Bangladesh. The arsenic in toxic level for cancer risk was found in ground water of Eruine. The highest level of two tube wells are 50 in Hazard Quotient (HQ) test and maximum tube-wells (33) are 23.33 times greater than safe level, which is 1. All the patients are divided into two groups initially, medicinal and control groups; and in these two are again divided in two sub-groups, drinking arsenic water (DAW) and drinking fresh water (DFW). In medicinal group, the mean arsenic concentrations (mg/kg) of hair and nail for before and after treatment were taken. In the result of the study, the increasing values were found in all arsenic in taking sections DAW of both medicinal (Hair- 27.5%, Nail- 97.7%) and control (Nail- 503.9%) groups; but only decreasing values were observed in medicinal group of fresh water taking DFW section (Hair- 45.6% and Nail- 70.3%). On the other hand, in control groups of both DAW & DFW are increased arsenic concentration rates in Nails (503.9% and 122.8%). After oral application of the fruiting body of PO for one year, remarkable cutaneous changes were observed in the patients. The keratosis (n=87), melanosis (n=138) and leuco-melanosis (n=105) are mostly dissolved from the patients cutaneous layer of the skin. In a differential study, the recovery rate of keratosis of half dose was more efficient than the full dose, these were 72% and 50%; in melanosis, the symptoms of full dose patients were increase rate than half dose, 46% and 43%, in leuco-melanosis, the result of half dose was better than full dose in the patients, and were respectively 60% and 54%. After that study no one has had affected by arsenic induced cancer. In all respect, the half dose of fruiting body of *Pleurotus ostreatus* was more effective for preventing arsenic induced cancers in arsenicosis patients.

Keywords: Arsenicosis, DAW, DFW, CSTA, Hair and Nail samples, As levels, Keratosis, Melanosis, Leuco-melanosis.

INTRODUCTION

An emerging disease named arsenicosis is the witness of 20th century. Till now arsenicosis and arsenic induce cancers are threat to mankind, as they are not curable disease. (Das and Sengupta, 2008). Minimum dose and prolonged consumption of metallic arsenic with drinking water leads to different internal as well as external disorders with cutaneous necrosis and cancer, with myriads of internal organ involvement, as well as cancers (Kapaj, *et al.*, 2006).

A study conducted at 2007 found that over 137 million people in more than 70 countries are probably affected by arsenic poisoning from drinking water (Ravenscroft, 2007). More affected people of the countries are China, Bangladesh, Chili, Argentina, Thailand and Taiwan. World Health Organization (WHO) considered the arsenic poisoning in drinking water by the ground of Bangladesh is the worst incident of the world and called it as the "largest mass poisoning of a population in history" (Smith *et al.*, 2000 and Wikipedia, 2016). About 60 districts out of 65 of Bangladesh are affected by arsenic contamination in ground water (Jiang J-Q, *et al.*, 2013).

It is known that long-term intake of small doses of inorganic arsenic compounds with drinking water is responsible for the disorders and cancer of lungs, liver, bladder, skin, kidney and reproductive organs cancer, and many other diseases and disorders of vital organs (Chatterjee *et al.*, 1995). There is

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an evidence of skin ulcer and cancer on the chest found in Sagarkandi of Pabna district and the patients are dying with this skin cancer (Jahangir, 2009). So it is confirming that death from cancer is the last destination of arsenic affection in human beings.

Flanagan, *et al.* (2012) were afraid on consequences of delaying action of arsenic in human body that the long-term arsenic exposure will last for several decades and cause death and sufferings by arsenic induced cancers and other diseases. They had mentioned the diseases as an increased risk of fetal loss, infant death, reduced birth weight and impaired cognitive function in child, and also high risk of lung function, renal cancer and death from lung cancer, lung disease and acute myocardial infarction later in life.

Generally arsenic found in blood serum, and urine after few hours of consumption of arsenic with drinking water. But the long duration consumption of arsenic found in higher concentration in keratin-rich tissues, such as hair and nails than in the other parts of the body (Yanez, *et al*, 2005). The SH-groups of keratin may bind trivalent arsenic. Arsenic can bind with hair in a high concentration rather than other biological tissues for the high affinity of arsenic with keratin tissues (Hindmarsh, 2000). The World Health Organization asserts that a level of 0.01 mg/L (10ppb) poses a risk of 6 in 10,000 chance of lifetime skin cancer risk and contends that this level of risk is acceptable (WHO, 2012).

Pleurotus ostreatus is commonly known as oyster mushroom and zhenuk mushroom in Bangla. It is recommended for reducing cholesterol level in human body for containing alkaloids, statins and lovastatin (Gunde-Cimerman and Cimerman, 1995). *Pleurotus ostreatus* is a good source of dietary fiber and other valuable nutrients. It has some medicinal important, such as – influencing and regulating immune system, decrease blood lipid level, lowered high blood pressure and prevent atherosclerosis, as well as hypoglycemic and antithromboic activities (Alam, *et al.*, 2010). On the other hand, it has a strongest chelating capacity (85.66%) of the acetone extract toward ferrous ions was investigated (Alam, *et al.*, 2010). and it is said that it has health promoting and environmental restoring capacity rather than other medicinal mushrooms resulting in an upsurge in their R and D activities during past two decades (Patel, *et al.*, 2012).

To the best of our knowledge, there is no remedy to prevent arsenic induced cancers. In these cases, medicinal research is very much important for arsenicosis treatment. According to Das and Sengupta (2008), "No effective therapy is known till date" (Das and Sengupta, 2008). Considering no active medicine is identified till now, we are proceeding to give a new medicine to prevent cancer, as well as treatment of arsenicosis and to save the life of arsenic affected people from the consequences (lungs affection, liver diseases, respiratory diseases, skin ulcers and cancers *etc.*) of arsenicosis.

MATERIALS AND METHODS

Description of the Study Area and Populations: The study is adopted for the sample design in a rural area of Bangladesh. We have selected Eruine village of Laksham upazila of comilla district as the study area. Laksham upazila is bounded by Comilla sadar and Barura upazila on the north, Chatkhil, Begumganj and shenbagh upazilas on the south, Nangolcot and Choddogram upazilas on the east, Barura and Shasrasti upazilas on the west. Main rivers are Dakatia and Shoto Feni. The village Eruine of Kandirpar union is bounded by Boikali union on the north, Gobindopur and Moisatua union on the south, mozaffargonj union on the west and Laksham pouroshava on the east. In the village, the drinking water source is only shallow tube-well, few ponds are dried in winter season. Rivers are in a distance and nearer to Laksham pouroshava, dirty with pouroshava waste drainage.

Arsenic unit of Community Care Hospital, Dhaka was established a tank in the middle of the village before two years, recently it was out of order due to sand in the bottom of the well. And all the tube-wells are arsenic contaminated. Only rain water harvesting is the main source of arsenic free water.

The present study is a descriptive cross-sectional population based study conducted in the village Eruine. Each and every personal of selected 113 houses of 15 Bari's (residential area) were the

principal samples. Then the samples were divided into two parts: one- arsenic affected personals and another- arsenic free people. Primarily they were separated by observing the presence of cutaneous symptoms of arsenicosis. Among them we have selected 200 fresh human samples (FHS) for the study. The FHS were not taking any drugs and nutritional supplements from hospitals or any other NGO's. All the samples were divided into two parts: a) Experimental group, and b) Control group. Again both the groups were divided into two groups: 1) drinking arsenic water (DAW), and 2) drinking fresh water (DFW).

Statistical Analysis for Population Based Survey: Statistical analysis was done using Epi-info (ver.3.5.1, 2008) and SPSS 15.0 (SPSS, Inc., chikago, IL. USA). Univariate and bivariate analysis of socio-demographic and other variables were performed. The pearson's chi-square test, the adjusted Mantel Haenzel's test were used to analyze the differences in the categorical data. A p-value was considered significant.

Sampling and Sample Preparation

Water: Water samples were collected from tube wells used by the participants visiting the household of each family. Maximum tube wells were found shallow. Approximately 5 (five) liters of water were pulled out, then water samples was collected from each tube well. Water samples collected in polyethylene bottles which was prewashed with aqueous nitric acid (1:1). Water samples were analyzed by proposed and reported method immediately after the collection at the field.

Water Sample Preparation and Analysis: Prepared the bung and taken 50 ml water sample in the reaction vessel of the Wagtech Digital Arsenator. Added 1sachet of reagent 1 to the sample in the reaction vessel and dissolved the acid sufficiently by swirling the reaction vessel. Added 1 tablet from reagent 2 to the contents of the reaction vessel and inserted the bung onto the mouth of the reaction vessel immediately. Removed the bung after waiting for 20 minutes, then detached the black slide from the bung. Matched the color of black slide with the color chart, otherwise inserted the black slide onto the arsenator (for below 100 µgm/l) and noted the reading of all samples.

Cancer Risk Assessment: Cancer risk assessment of arsenic water was performed by the formula –

$$I (CDI) = C \times (CR \times EF \times ED) / (BW \times AT)$$

Where, I is intake rate, CDI is Chronic Daily Intake, C= average concentration of arsenic in drinking water, CR= contact rate is 2L/day, EF= Exposure frequency is 365 days/year, ED= Exposure duration is 10 years, BW= Body weight is 60 kg average adult, AT= Period over which exposure is averaged (365 days).

To assess the Risk, the Hazard Quotient (HQ) = I / RfD

Where, RfD (Reference Concentration) = NOAEL / UF;

(NOAEL = No Observable Average Effect Level, UF = Uncertainty Factor)

Biological Samples: Hair samples were collected from different part of the scalp of the head using a stainless still scissors and nail samples were collected from the cleaned fingers of the arsenicosis patients. All biological samples were placed in polyethylene bags for transport and preserved in 4°C in the laboratory of the Institute until the laboratory works would be done.

Sample Preparation: For each hair and nail samples, 0.05 to 0.1 gm of sample was taken in a 50 ml Borosil glass flask. 5 ml concentrate nitric acid (HNO_3) was added in it and dissolved the sample. The color of the liquid samples were changed to brown for hair and yellow for nail, then heated them on a hot plate with a watch glass at the top of the beaker at 90-100°C temperature for a few minutes. Stopped the heat and kept them for overnight. Next morning, samples were evaporated at about 100°C

in an exhaust chamber. Nitric acid was added if necessary till the color of the solution turned into pale yellow for hair and colorless for nail samples. On reaching a final volume of about 1 ml heating was discontinued. Samples were diluted with de-ionized water and filtered through a Millipore membrane (0.45μm) filtering apparatus, then adjusted to a 5-6 ml volume for total arsenic concentration analysis by AAS.

Instrumentation for Arsenic Speciation: Arsenic speciation was performed using graphite furnace atomic absorption spectrophotometer (GF-AAS). According to Shimadzu Corporation, the atomization method using a flame is still popularly used as the standard atomization method due to good reproducibility of measured volumes and easy use. In the electro-thermal atomic absorption method, the samples were injected in the formed graphite tube and an electric current of 300 ampere is applied to the tube. The graphite was heated to a high temperature (100^{0}C for evaporating water and 1000^{0}C for organic matter and other coexistent matter to dissolve and evaporate) and the elements in the samples were atomized. Then the light was sent through the tube, light was absorbed when they were atomized. Lastly, in the atomizing stage, it was heated to 1400^{0}C to 3000^{0}C and metallic salts left in the tube were atomized. Arsenic analysis results were shown in AAS monitor and preserved in computer; result data were – Sample ID, True value (ppb), Concentration (ppb), absorbance, Position and %RSD.

Medicinal Source Collection and Capsule Preparation: Samples of mushrooms (*Pleurotus ostreatus*) were collected from The National Mushroom Development and Extension Centre (NMDEC), Sobhanbagh, Savar, Dhaka- 1340 and Center for Mass Education in Science (CMES), Dhanmondi, Dhaka- 1209. Specialty mushrooms were harvested using standard mycology protocols and harvested on peak production days. Harvested mushroom crops were randomly sampled, cleaned, sliced and stored at 0^{0}C for 24h. Samples were later freeze-fried, ground to a fine powder and sieved through a 16 mesh screen. Mushroom powders were collected in sterile sample bags (Fisher Scientific, Pittsburgh, PA) and stored in the dark at room temperature.

Empty hard gelatin capsules (Global Capsules Ltd. Barisal, Bangladesh), size # 0, quantity- 1.00 Lac, color- maroon opaque (cap and body) were filled with powered mushroom and powered fried wheat (Placebo) at Helal & Company Ltd., Khilgaon, Dhaka- 1219.

Study Design: This treatment criterion was named as "Curative Short-course Treatment for Arsenicosis" (CSTA) patients. Total field program was designed in 3 phases, which took a total period of 18 months. First 6 months was for physical examination of the patients, drinking water analysis and initial biological samples analysis for total arsenic by AAS. Second 3 months were a provision period for a sensitivity test program (STP) and it continued to third phase of 9 months, they were for medicinal application of the patients. The STP was a provision for Identification of Intermediate Improvement (III) for development assessment notification (DAN) by physical signs analysis to take necessary actions. After that lab works were full-fledged by digestion of biological samples and total arsenic analysis by AAS.

RESULTS AND DISCUSSION

Arsenic Concentration in Drinking Water: The population of Eruine village mainly consumes aquifer water by shallow tube wells. We had 45 tube wells in danger level out of 48. The highest arsenic concentration found in this drinking water source was 500 μgm/L, more than 50 times the accepted international level (10 μgm/L) and 10 times greater than the Bangladesh standard (50 μgm/L). Maximum tube wells (33) were found up to 300 μgm/L, one (1) for under danger level (50 μgm/L) according to Bangladesh standard, one (1) for WHO standard and one (1) with nil arsenic concentration (Table 1).

Table 1. Arsenic Concentration Level in Drinking Water

Sl. No.	Arsenic Concentration (µgm/L) or ppb	Average As mgm/L	Number of Tube wells	I (CDI) (mg/ kg.day)	HQ (HQ<= 1 is Safe)
01	>500	-	0	-	
02	401-500	0.45	2	$1.5 X10^{-2}$	50
03	301-400	0.35	2	$1.0 X10^{-2}$	33.33
04	201-300	0.25	33	$0.7 X10^{-2}$	23.33
05	101-200	0.15	4	$0.4 X10^{-2}$	13.33
06	51-100	0.07	4	$0.2 X10^{-2}$	6.66
07	11-50	0.03	1	$0.1 X10^{-2}$	3.33
08	1-10	0.006	1	$0.02 X10^{-2}$	0.66
09	0	0.00	1	$0.0X10^{-2}$	0.00
Total			48		

Cancer Risk Assessment of Arsenic in Water: On every arsenicosis patients daily water intake was 2 litres, average intake of arsenic per day with 50% absorption for a life time, in 10 years of consumption time of a 60 kg weight body the result of Hazard Quotient (HQ) is belongs to average arsenic concentration of individuals. Only two tube wells were found safe in HQ estimation (0.66 and 0), rest of all were unsafe in different scale of arsenic toxicity. Highest toxicity for cancer risk levels were found in two tube wells, which were 50 times greater in HQ test. Maximum tube wells (33) were 23.33 times greater than safe level. It is found that arsenic-related mortality in Taiwan started to decline gradually about 20 or 25 years after measures to reduce exposure were initiated and coronary heart diseases mortality declined ever faster (Chang, *et al.*2004).

Arsenic Concentration for Biological Sample Speciation: At the beginning we have selected 200 fresh human samples for the study. In between 1st phase of analysis (physical examination of the patients, and drinking water and initial biological samples analysis) 16 patients were discontinued and again in 2nd and 3rd phase more 25 patients were discontinued, remaining 159 patients were continued up to the end of the study.

We had randomly selected 20 human samples out of 184 for biological study, remain 16 were shifted to other district, absent and one died at the beginning of the study. There were 8 hair and 12 nail samples within these 20 human samples, and 9 were drinking arsenic water (DAW) and 11in fresh water (DFW) group. We had divided them again in two groups, one- medicinal group and another-control group. Medicinal group contains 16 samples and control group contains 4 samples. Two human samples were found extremely arsenic concentration in their hair and nails, respectively 101.3 and 105.0 mg/kg. These are separated for special care in DFW category with 2 capsules daily and taken a keen eye observation in the research.

Maximum arsenicosis patients have had complained of about drowsiness, headache, anxiety, confusion, diarrhea, thirst, muscles and stomach cramp, vertigo, weakness, hair falls as physical general symptoms. Few cancer affected patients were deducted from the research and shifted them to cancer hospital to take special care of cancer. All the drinking water sources are tested for arsenic and asked villagers to drink only fresh water, except few for the time being who were involved in this research drinking arsenic water (DAW).

Randomly selected 20 biological samples were studied under atomic absorption spectrophotometer (AAS) for arsenic analysis. The initial arsenic concentrations of the human samples were mentioned in Table. 2.

Two other human samples of hair and nail respectively 101.3 and 105.0 mg/kg were placed in DFW group to estimate separately for their extreme values. We had unequal human sample values found in

Table-02. Maximum arsenic level had found in hair (39.3 mg/kg), which was 39 times greater than the normal value (1.0 mg/kg) and the lowest one was 5.2 mg/kg in hair, it was 5 times greater than normal value (1.0 mg/kg) and the highest nail value was obtained 14.3 mg/kg, whereas the normal value of arsenic is 1.5 mg/kg in nail. The average initial value of all samples was 9.5 mg/kg.

Comparing with two villages (Esquin˜a and Illapata) of the Atacama Desert, Chile were found that on average, the total arsenic concentrations in hair from individuals of Esquin˜a and Illapata were 0.7 and 6.1 $\mu g\ g^{-1}$ (mg/kg) respectively (Yanez, *et al.*, 2005). But there was symmetry with the patients of Illapata village with Eruaine village rather than Esquin˜a, that was the opposite of the rate of drinking water arsenic concentration. We had a concentration from 5 to 105 mg/kg of the hair and nail of the patients of Eruaine village. But the average was 9.5 mg/kg (Table 2).

Table 2. Initial arsenic concentrations in different groups of human samples

Groups	Drinking water Category	Sub-groups	Arsenic Concentrations (mg/kg)			
			Individual	Total	Sub-group Average	Total Average
Medicinal	DFW	Hair	39.3			
			6.3			
			5.2	80.6	13.4	
			9.1			
			10.2			
			10.5			
	DAW	,,	6.9	6.9	6.9	
	DFW	Nail	14.3	21.9	10.9	
			7.6			
	DAW	,,	8.9			9.5
			7.0			
			11.0	44.7	8.9	
			9.0			
			8.8			
Control	DFW	Hair	-	-	-	
	DAW	,,	-	-	-	
	DFW	Nail	9.2	9.2	9.2	
	DAW	,,	6.3			
			8.0	21.9	7.6	
			7.6			

Out of 159 human samples, 114 were treated for a year with *Pleurotus ostreatus* under experimental group, rest 45 patients were in control group. The selected 20 biological samples were again collected and analyzed under AAS after one year. Medicinal group had two types of deviations; we had selected two capsules daily for a person. But some patients had some complains like- headache, nausea, diarrhea *etc.*, we discarded one capsule from their requisite. And every person of control group also contains a capsule daily. The obtained result and the initial result were compared in Table 3.

In differential study of medicinal group, Patients with DAW had found a slight to moderate increasing rate (27.5 for hair and 97.7% for nails) of arsenic in their samples. But an impressive result found in DFW. Only this group had a decreasing value of arsenic in their samples, respectively hair was 45.6% and nail was 70.3%.

Table 3. Comparison between Medicinal and Control Groups, Before and After Treatment

Groups	Drinking Water	Sub-groups	Average arsenic concentration (mg/kg)		Increased or Decreased Rate (%)
			Before treatment	After treatment	
Medicinal	DAW	Hair	6.9	8.8	27.5 (I)
		Nail	8.9	17.6	97.7 (I)
	DFW	Hair	13.4	9.2	45.6 (D)
		Nail	10.9	6.4	70.3 (D)
Control	DAW	Hair	-	-	-
		Nail	7.6	45.9	503.9 (I)
	DFW	Hair	-	-	-
		Nail	9.2	20.5	122.8 (I)

*WHO recommended Maximum arsenic level in Hair is 1.0 mg/kg and in Nail is 1.5 mg/kg.

Comparing with other treatments found that the manifestations of chronic arsenicosis, melanosis and keratosis showed clinical improvement on treatment with vitamin A, E and C. Amongst the patients who had used both arsenic safe drinking water and regular medications 90.5% showed improvement of melanosis, 86.4% showed improvement of keratosis but none showed deterioration (Ahmed *et al.*1998) and Ahmed recommended that only vitamin supplement was not sufficient without withdrawing arsenic contaminated water, and according to the observation of Bangladesh Arsenic Control Society (2003) a combination of arsenic-safe water and anti-oxidant was the most effective for the management of arsenicosis patients.

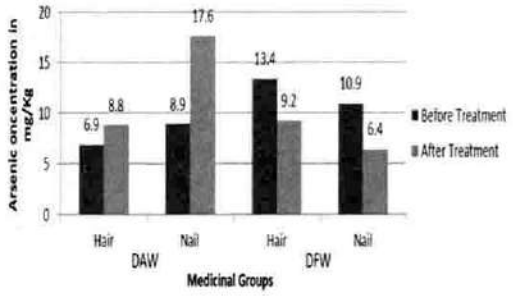

Fig. 1. Mean Arsenic Concentration in Medicinal Group. DAW = Drinking Arsenic Water, DFW = Drinking Fresh Water.

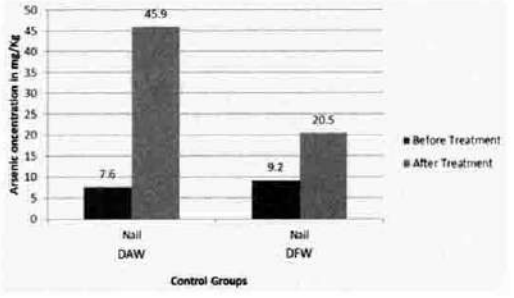

Fig. 2. Mean Arsenic Concentration in Control Group. DAW = Drinking Arsenic Water, DFW = Drinking Fresh Water.

But the *Pleurotus ostreatus* gives a better result than vitamin a, E and C, or anti-oxdents. The treatment revealed in DAW types, nail sub-groups of control and medicinal groups had an increased rate respectively were 503.9 and 97.7. The differences are more than 5 times due to PO administration. On the other hand, in the DFW types of Control and medicinal groups variation of results are 122.8% increased and 70.3% decreased. So the difference due to medicinal impact is (122.8 + 70.3) 193.1%.

In differential study of control group, only nail samples were available there. The average arsenic concentrations (mg/kg) in two groups were from 7.6 mg/kg to 45.9 mg/kg in DAW with an increasing rate of 503.9% and from 9.2 mg/kg to 20.5 mg/kg in DFW respectively with an increasing rate of 122.8% (Table. 2). Both the groups were too much increased in values. Bar charts are showing the differences between medicinal and control groups in Fig. 1 and Fig. 2.

Comprising both medicinal and control groups together, only decreasing rate found in DFW of medicinal group (figure-3.10). So it is important that the medicine alone can't reduce the arsenic in the body, if the person is drinking arsenic water. DFW and medicine is the solution to treat the patients. On the other hand,

medicinal dose can be increase to tolerable position. As we know, most of the tube-wells were arsenic contaminated, among them 33 tube-wells out of 45 had the arsenic range (201-300) ppb. Some were more and bellow than that level, and only 3 were below 50 ppb level (table-3.23).

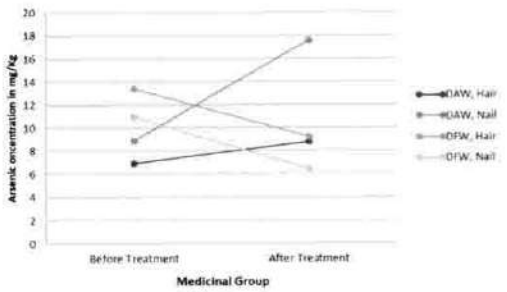

Fig. 3. Trend of arsenic concentration in medicinal group.

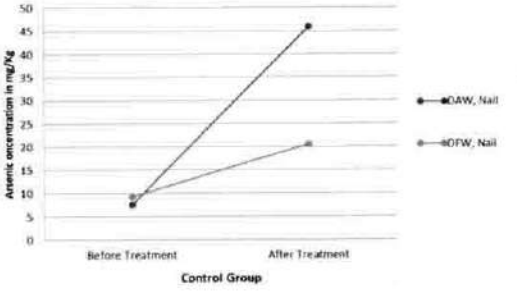

Fig. 4. Trend of arsenic concentration in control group.

As per sensitivity test (STP) study result, we had 159 patients for the study of experimental and control groups; rest 25 patients were absent or/and discontinued (Table 4).

Table 4. Total Results summary of Full, Half and BLK Doses of the Patients after Treatment of 12 Months

Doses	No. of Patients	Keratosis Recovered		Melanosis Recovered		Leucomelanosis Recovered		Total Recovered (%)	
Full Dose	57	38	19 (50%)	48	22 (46%)	39	21 (54%)	125	62 (50%)
Half Dose	57	29	21 (72%)	47	20 (43%)	37	22 (60%)	113	63 (56%)
BLK (Blank/ Null)	45	20	0 (0%)	43	6 (14%)	29	1 (3%)	92	92 (7%)
Total (Disease Wise)	159	87	40 (46%)	138	102 (74%)	105	44 (42%)	330	132 (40%)

In the result of the study, the curative rate of full dose, half dose and BLK were respectively 50%, 56% and 8%. In a differential study, the recovery rate of keratosis of half dose was more efficient than full dose, these were 72% and 50%; in melanosis, full dose was somehow increase rate than half dose, 46% and 43%, in leuco-melanosis, the result of half dose was maximum than full dose, and was respectively 60% and 54% (Table- 04). In all respect, the half dose was too much efficient for Bangladeshi patients. Bangladesh Arsenic Control Society (2003) revealed improvement of skin lesions of arsenicosis on comparing photographs by the treatment with anti-oxidents and arsenic free drinking water, where the treatment duration is 12 months Bangladesh Arsenic Control Society (2003). A study of forty-one patients of chronic arsenic poisoning was conducted with spirulina extract (250 mg) plus zinc (2 mg) for 24 patients and placebo for 17 patients treated twice daily for 16 weeks. The result revealed with an improvement of skin manifestations with drinking arsenic free drinking water (Misbahuddin, *et al.*, 2006).

CONCLUSION

The study envisaged that the result is more than 62% improvement among the patients, who were taking medicines and fresh water. So it is seemed that it will take more one or two years treatment for full placed recovery. Because of that it is named "Curative Short-course Treatment for Arsenicosis" (CSTA) patients. And it is found that the mushroom *Pleurotus ostreatus* is a good source for removing metallic arsenic from the body, and treating arsenicosis and arsenic induce cancer.

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Effects of Substrate Moisture Content on the Growth and Yield of Milky White Mushroom (*Calocybe indica*)

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Abstract

The effects of moisture content of rice straw substrate on the growth and yield of milky white mushroom (*Calocybe indica*) were studied. Eight different moisture levels of rice straw substrate were used in this experiment such as 35, 40, 45, 50, 55, 60, 65 and 70 percent respectively. Mycelium colonization, substrate contamination, harvesting time, yield and biological efficiency were significantly affected by the substrate moisture level. Faster mycelium colonization, no substrate contamination and highest yield and biological efficiency were recorded in substrate containing 70% moisture level. Biochemical analysis of whole fruiting body showed that protein, dietary fiber, Mo, Co and Se content were varied significantly due to variation in substrate moisture level though some other nutrient content were more or less similar.

Keywords: Substrate, moisture content, nutrient, milky white mushroom (*Calocybe indica*).

INTRODUCTION

Milky white mushroom (*Calocybe indica*) normally grows on humus and at high temperature on summer season. It can be cultivated on varieties of cellulosic substrates (Patel and Trivedi, 2016). A good substrate for mushroom growth must be suitable both chemically and physically, as well as having the proper condition for microbial activities (Chang and Miles, 2004).Rice straw is the most common lignocellulosic substrate whose major component is cellulose and it is also the best substrate for cultivating milky white mushrooms (Mangat *et al.*,2008 and Amin, *et. al.*, 2010).

Moisture content in substrate is very important factor for the growth, development and yield of oyster mushroom (Sarker, *et. al.*, 2007). Optimum mycelial growth and mushroom production are dependent on adequate moisture and gas exchange within the substrate (Shen *et al.,* 2008). It is generally recognized that most mushrooms grow well at substrate moisture levels of 50 to 75% (Bratkovich and Stephen, 2004). Yoshida *et. al.* (1993) adjusted the moisture content between 65-70% to either chopped straw or sawdust for *P. ostreatus* production. The moisture content of 80 percent influenced negatively the yielding because of the high share of misshaped carpophores (Siwulski *et al.,* 2007). But suitable moisture level for the production of milky white mushroom on rice straw substrate is yet to be standardized. Therefore, the present experiment was undertaken to determine the moisture level suitable for production of milky white mushrooms.

MATERIALS AND METHODS

The experiment was conducted at Mushroom Development Institute (MDI), Department of Agricultural Extension, Savar, Dhaka, Bangladesh from May to July 2019. Eight different moisture levels, 35, 40, 45, 50, 55, 60, 65, and 70 percent, of the substrate were tested in this experiment. Rice straw and Cid-1 were used as substrate and milky white mushroom strain respectively in this experiment.

Preparation of substrate and spawn packet: Rice straw substrate was chopped to convenient length of 2.5 to 5 cm. Existing moisture content of the substrate was measured by using moisture analyzer 'weighed moisture box (A&D company Ltd. N-92; P1011656, Japan)'. Then the required amount of water was added to the substrate according to the treatment and mixed thoroughly and filled in net bags. The net bags filled with substrate were placed in the sterilization cum inoculation chamber.

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Door of the chamber was closed and tightened with the help of screws. Water heater was turned on to produce steam that flows in to the chamber. When the temperature of the chamber rises to 60^{0}C, the steam flow was adjusted to maintain a constant temperature of 60^{0}C – 80^{0}C up to 90 minutes. After 90 minutes water heater was turned off and kept it for about 20 hours. After 20 hours substrate was taken out and moisture content was checked. Moisture content of the pasteurized substrate was adjusted to 35, 40, 45, 50, 55, 60, 65 and 70 percent by spreading the substrate in the air if necessary. Substrate was filled into the polypropylene bags (12"x16") and inoculated with 10% sawdust mother culture by thorough mixing. A plastic neck was fitted to the filled bags with rubber band and sealed with cotton plug and covered with brown paper. Then the spawn packets were transferred to the culture house for mycelium run. After 14-19 days the substrate was completely colonized by the mycelium and polypropylene covered was opened.

Casing and after care: Loamy soil was used as casing material and was pasteurized at 65^{0}C for 4 hours. Casing material was covered over the mycelium on the substrate up to 4 cm thickness. Watering was done at regular interval to maintain moisture at 60 to 70%. Primordia initiated at 12-19 days and developed in to fruiting bodies.

The fruiting bodies were harvested at 7-8 days of primordia initiation. Data were collected on days to complete spawn run, rate of spawn packet contamination, days to primordia initiation, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, number of effective fruiting body (NEFB), days to final harvest, yield and biological efficiency (BE). The BE was measured by the formula;

$$\text{Biological Efficiency (BE)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

Biochemical analysis: After harvest, fruiting bodies were divided into small pieces, dried and pulverized. Protein, lipid, total ash, and minerals of whole fruiting body were analyzed from the pulverized mushroom powder in the laboratory.

Determination of total protein: Five grams of dried sample was taken and mixed with 50 ml 1N NaOH and boiled for 30 minutes. The solution was cooled at room temperature and centrifuged at 1000 rpm by a table centrifuge machine (DIGISYSTEM: DSC- 200T; Taiwan). The supernatant was collected and total protein was measured according to the Biuret method (Burtis and Ashwood, 2006) with a diagnostics kit (Total protein: Colorimetric test- Biuret method/ Crescent Diagnostics, Saudi Arabia).

Determination of total lipid: Total lipid content of mushroom was determined following the methods of Folch *et al.* (1957) with slight modification. Five grams of grinding mushroom powder was suspended in 50 ml of chloroform: methanol (2:1 v/v) mixture. The content was mixed thoroughly and let stand for 3 days. The solution was filtered through Whitman filter paper No. 2 and centrifuged at 1000 rpm by a table centrifuge machine. The upper layer of menthol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid.

Determination of crude fiber: Ten grams of moisture and fat free sample was taken in a beaker and 200 ml of boiling 0.255N H2SO4 was added. The mixture was boiled for 30 minutes keeping the volume constant by frequent adding of water at regular intervals. The mixture was then filtered through a muslin cloth and the residue was washed with hot water till free from acid. The material was transferred to the same beaker and 200 ml of boiling 0.313N NaOH was added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue was washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was transferred to a crucible, dried overnight at 80-1000C and weighted (We) in an electric balance (KEYI: JY-2003; China). The crucible was heated in a muffle furnace (Nebertherm: Mod-L9/11/c6; Germany) at 600^{0}C for 5-6 hours, cooled and weighted again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber.

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

Determination of total ash: One gram of sample was weighted accurately in a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5-6 hours at 600^{0}C. It was then cooled in a desiccator and weighted. This was repeated till two consecutive weights were the same and the ash was almost white or grayish white in color. Total ash was calculated following the equation shown below:

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

Mineral content: Content of Calcium (Ca), Copper (Cu), Iron (Fe), Zinc (Zn), Selenium (Se) and Cobalt (Co) was determined following flame method of atomic absorption spectrophotometer (AAS 240, Varian) and the Molybdenum (Mo) was determined following graphite furnace method (GTA 120, Varian).

Total ash as determined earlier was taken in a beaker. Two milliliter of concentrated HNO_3 was added to the ash and heated for 2 minutes. One drop of hydrogen peroxide was added in to the solution to remove turbidity. The solution was transferred in to a volumetric flask and total volume was made 100 ml by adding de-ionized water.

For each mineral, one milliliter of the primary standard solution was taken in a 100 ml volumetric flask and the volume was adjusted to 100 ml with de-ionized water and mixed properly. The solution was considered as the secondary stock solution of the respective mineral. Standard solution of the mineral was prepared as per the instruction of the AAS for the particular mineral (Fluka Analytical SIGMA-ALDRICH product of Switzerland).

Data analysis: The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. The data were statistically analyzed following SPSS (version 20.0) computer program. Difference among the treatment means were determined by Tukey's Test (Tukey, 1977) at $P \leq 0.05$.

RESULT AND DISCUSSION

Days to complete mycelium run in the spawn packet, fruiting body primordia initiationand harvesting time: Mycelium run in the spawn packet and fruiting body primordia initiation was significantly influenced by substrate moisture content (Table 1). Mycelium colonization was faster (14.5 days) in the substrate containing 70% moisture which was statistically similar to substrate containing 60% (16.0 days) and 65% (15.7days) moisture. Suganthi and Krishnakumari (2018) also reported that maximum mycelium growth of *Pleurotus cornucopiae* was observed in sugarcane bagasse at 70 ± 2% moisture. Mycelium colonization was slowest (19.0 days) in the substrate containing 45% moisture. No mycelium colonization was observed in the substrate containing 35% moisture and only 12.5% mycelium colonization was observed in the substrate containing 40% moisture. Sarker *et al.* (2007) also reported that duration of mycelium running in spawn packet of *Pleurotus ostreatus* decreased with the increase in substrate moisture level up to 70%. Fruiting body primordial initiation was also significantly influenced by substrate moisture level. Lowest time (12.2 days) was required for fruiting body primordia initiation in substrate containing 65% moisture which was statistically similar to substrate containing 60% (12.3days) and 70% (12.6 days) moisture.

Substrate moisture content had a significant influence on harvesting time of milky white mushroom (Table 1). Lowest time was required from spawning to first harvest (44.0 days) and spawning to last harvest (50.0 days) in substrate containing 70% moisture which was statistically similar to substrate containing moisture level 65% and 60% respectively. Highest time was required both for spawning to first (62.2 days) and last (77.0 days) harvest of milky white mushroom in substrate containing 45% moisture. This results are an agreement with the findings of Sarker*et al.* (2007). They reported that time required to first harvest and total harvest were significantly influenced by the moisture levels in substrate of *Pleurotus ostreatus*.

Table 1. Effect of substrate moisture content on spawn run, fruiting body primordial initiation, and harvesting time of milky white mushroom (*Calocybe indica*)

Substrate moisture level	Days to spawn run	Days to primordial initiation	Days to first harvest	Days to last harvest
35%	-	-	-	-
40%	-	-	-	-
45%	19.0a	19.0a	62.2a	77.0a
50%	17,0b	14.9b	47.7b	53.8bc
55%	17.0b	16.5b	51.6bc	54.5b
60%	16.0bc	12.3c	44.4bc	46.6bc
65%	15.7bc	12.2c	44.4bc	45.0c
70%	14.5c	12.6c	44.0c	50.0bc
P	< 0.001	<0.001	<0.001	<0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. '–' indicate no data were recorded due to maximum spawn packet were contaminated.

Substrate contamination: Rate of substrate contamination was significantly influenced by substrate moisture level (Fig. 1). The rate of substrate contamination was highest (100%) in substrate with 35% moisture level which was statistically similar (87.5%) to substrate with 40% moisture level and the contamination rate was gradually decreased with increasing the moisture level of the substrate. No substrate contamination was recorded in 65% and 70% moisture level.

The rate of contamination in substrate containing 35% and 40% moisture was very high, this might be due to uneven pasteurization of the substrate. Dry rice straw is a good thermal insulating material and have low conductivity. Ashour *et al.* (2011) and Goodhew *et al.*(2004) reported that rice straw has been used as a building insulation material due to their low density and high heat insulation. Therefore, when adequate water added to the substrate, its thermal conductivity become high and temperature can easily reach inside the substrate filled net bag. In case of 35 and 40% moisture content substrate remain relatively drier and temperature cannot reach inside the substrate properly, resulting uneven pasteurization of the substrate.

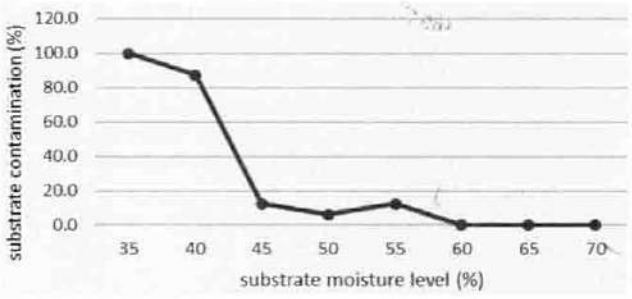

Fig. 1. Effect of substrate moisture content on the rate of substrate contamination.

Number and size of fruiting body: Number of effective fruiting body was not affected by substrate moisture level but the size of fruiting body was significantly affected by substrate moisture level (Table 2). Stalk length of fruiting body was highest (9.5cm) but stalk diameter was lowest (2.3 cm) in substrate having 45% moisture. That means substrate containing 45% moisture produces thinner fruiting body. Big size fruiting body was produced in substrate containing 70% moisture. Stalk diameter was highest (2.6 cm) in substrate containing 70% moisture which was significantly higher than other moisture level. Both diameter (7.2 cm) and thickness (2.8 cm) of pileus was highest in substrate containing 70% moisture and lowest (6.1 cm and 2.1 cm) in substrate containing 55% moisture.

Table 2. Effect of substrate moisture content on number and size of fruiting body of milky white mushroom

Substrate moisture level	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
35%	-	-	-	-	-
40%	-	-	-	-	-
45%	5.8a	9.5a	2.3c	6.7ab	2.3bc
50%	5.6a	8.3b	2.3c	6.2b	2.2bc
55%	5.4a	8.4b	2.4bc	6.1b	2.1c
60%	6.1a	8.3b	2.4bc	6.6ab	2.4bc
65%	6.0a	8.0b	2.5b	6.5ab	2.4bc
70%	6.4a	8.2b	2.6a	7.2a	2.8a
P	<0.001	<0.001	<0.001	<0.001	<0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. '–' indicate no data were recorded due to maximum spawn packet were contaminated.

Yield and biological efficiency: Yield and biological efficiency (BE) of milky white mushroom was significantly influenced by substrate moisture level (Fig. 2). Highest yield (361.1g) and biological efficiency (87.4%) was recorded in substrate containing 70% moisture which was statistically similar to substrate containing 60% (315.2g & 76.3%) and 65% (303.8g & 73.6%) moisture. Yield and biological efficiency was lowest (271.3g & 65.7%) in substrate containing 45% moisture. No yield was recorded in substrate containing 35% and 40% moisture because no mycelium colonization was observed in substrate containing 35% moisture and only12.5% mycelium colonization was observed in substrate containing 40% moisture. This result was similar to that of Sarker *et al.* (2007) who also reported wide variation was found in the biological and economic yield among the moisture levels in substrate of oyster mushroom.

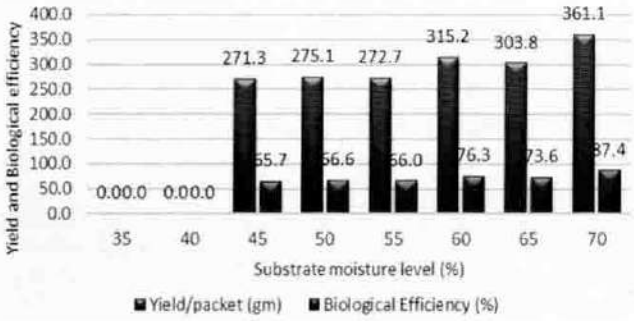

Fig. 2. Effect of substrate moisture content on yield and biological efficiency of milky white mushroom.

Nutrient content: Protein and dietary fiber content of whole fruiting body of milky white mushroom was significantly affected by moisture level of the substrate but ash and lipid content was not affected (Table 3). Protein and dietary fiber are important compounds of mushroom. Amount of protein was ranges from 19.6g to 33.3g and fiber from 10.85 to 11.48g per 100g dried mushroom. Highest amount of protein (33.3g) was observed at 65% moisture level of the substrate and lowest (19.6g) at 50% moisture level. Fiber content was highest (11.48g) at 45% moisture level and lowest (10.85g) at 65% moisture level. Ash and lipid content of milky mushroom was observed 10.17 to 11.95g and 4.52 to 5.58g respectively in this study. This result supports the findings of Alam *et al.* (2008), Breene (1990), Sumathy *et al.* (2015) and Zahid *et al.* (2010).

Calocybe indica have a mix of minerals and their fruiting bodies are characterized by high level of assimilable mineral constituents. In the present study it was observed that among the minerals Ca, Cu, Fe and Zn content was not affected by moisture level of the substrate but amount of Co, Mo and Se was affected by substrate moisture level (Table 3). Ca content was varied from 6.71 to 8.02 mg, Cu from 7.43 to 8.19 mg, Fe from 23.17 to 29.83 mg and Zn from 6.82 to 7.48mg per 100g dry weight of milky mushroom. Co content was maximum (0.74mg) at 60% moisture level of the substrate and minimum at 50% moisture level (0.65mg). Mo content was ranges from 232 to 275µg. Maximum Mo content was observed at 70% moisture level and minimum at 45% moisture level of the substrate. Se content was highest (25.0µg) at 65% moisture level and lowest (18.5µg) at 45% moisture level. It was reported that the individual chemical composition of the mushroom largely varies with species and also depends on the age of the fruiting body, composition of the compost and substrate. It was also reported that the nutritional property changes by flush to flush (Sumathy *et al.*, 2015). In some cases resultof the present study differed with the findings of Alam *et al.* (2008) may be due to variations of environmental condition, water, soil, substrate etc. which influences the quality of mushrooms.

Table 3. Nutrient content of milky white mushroomgrownon rice straw substrate at different moisture level (per 100g dry weight)

Substrate moisture level	Protein g	Fibre g	Ash g	Lipid g	Ca mg	Cu mg	Fe mg	Co mg	Mo µg	Zn mg	Se µg
35%	-	-	-	-	-	-	-	-	-	-	-
40%	-	-	-	-	-	-	-	-	-	-	-
45%	21.2c	11.48a	11.36a	4.98a	7.22a	8.19a	24.10a	0.69ab	232c	7.48a	18.5d
50%	19.6c	11.32a	10.77a	5.58a	7.51a	7.61a	23.17a	0.65b	245bc	7.42a	20.5cd
55%	26.1b	10.96b	11.95a	4.92a	8.02a	7.43a	24.17a	0.67ab	260ab	6.82a	21.6bc
60%	31.2a	10.92b	10.41a	4.52a	7.82a	7.79a	26.17a	0.74a	241bc	7.21a	23.1ab
65%	33.3a	10.85b	10.43a	4.91a	6.93a	7.99a	29.83a	0.66ab	272a	7.28a	25.0a
70%	27.1b	10.90b	10.17a	4.67a	6.71a	7.52a	28.23a	0.67ab	275a	7.38a	19.5cd
P	<0.001	0.002	0.061	0.108	0.041	0.365	0.107	0.038	<0.001	0.278	<0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. '–' indicate no data were recorded due to maximum spawn packet were contaminated.

Relationship between substrate moisture content and rate of substrate contamination: Significant negative correlation (r = - 0.803) was observed between substrate moisture content and the rate of substrate contamination. The relationship between the two variables was linear and could be expressed by the equation y = 173.44 + -2.78x (R^2 = 0.645*) where y = rate of substrate contamination, x = substrate moisture content. The R^2 value indicated that 64.5% substrate contamination was attributed to the substrate moisture content and the rate of substrate contamination was gradually decreased with the increase of substrate moisture content (Fig. 3).

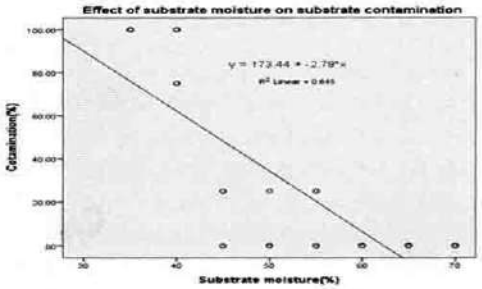

Fig. 3. Relationship between substrate moisture content and the rate of substrate contamination.

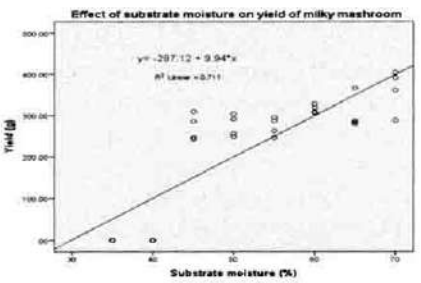

Fig. 4. Relationship between substrate moisture content and yield of milky white mushroom.

Relationship between substrate moisture content and yield: Significant positive correlation (r= 0.845) was observed between substrate moisture content and yield per packet of milky white mushroom. The relationship between the two variable was linear and could be expressed by the equation y = -297.12 + 9.94x (R^2 = 0.711*) where y = yield per packet, x = substrate moisture content. The R^2 value indicated that 71.1% yield per packet was attributed to the substrate moisture content and the yield was increased with the increase in substrate moisture content (Fig. 4).

Moisture content in substrate is very important factor for the growth, development and yield of milky white mushroom. A good substrate for mushroom growth must be suitable both chemically and physically, as well as having the proper condition for microbial activities. Appropriate moisture can provide suitable physical and chemical condition of substrate. From the above experiment it can be concluded that there is a positive relationship between yield and moisture content and negative relationship between substrate contamination and moisture content of rice straw substrate up to 70% moisture level. Protein, dietary fiber, Mo, Co and Se content of the whole fruiting body varied with substrate moisture content though some other mineral content does not varies. Therefore 70% moisture

of rice straw substrate can be used for milky white mushroom cultivation as it provide faster mycelium colonization, no substrate contamination and highest yield whereas below 60% moisture level mycelium colonization become slower, yield decreases and rate of substrate contamination increases gradually. Hence highest substrate moisture level of this experiment was 70% and at this moisture level yield was highestand substrate contamination was zero so further investigation is needed to identify the maximum moisture level of rice straw substrate above which yield may decreases and rate of contamination increases again.

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Effect of Different Maize Straw Substrates Ratio on Growth and Yield performance of Oyster Mushroom

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Abstract

An experiment was carried out with two different varieties, viz. V_1 (*Pleurotus ostreatus*) and V_2 (*Pleurotus djamor*), and five different amount of substrates combination T_1 = 500g (450g maize straw + 50g mother culture) per packet, T_2 = 750 g (675g maize straw + 75g mother culture) per packet, T_3 = 1000 g (900g maize straw + 100g mother culture) per packet, T_4 = 1500 g (1350g maize straw + 150g mother culture) per packet and T_5 = 2000 g (1800g maize straw + 200g mother culture) per bed in Mushroom Development Institute, Savar, Dhaka, during the period from July to December 2019 to find out the effect of different amount of maize straw substrates on the growth and yield of oyster mushroom. The experiment was laid out in Completely Randomized Design (CRD) with four replications. Significant variation was found in all parameter due to the effect of substrates amount. The highest number of fruiting body (16.00), number of effective fruiting body (10.25), length of stalk (5.93 cm), length of pileus (8.21 cm), diameter of pileus (8.80 cm) and thickness of pileus (0.90 cm) were recorded in T_3 treatment with V_1 variety. On the other hand, V_2 variety of mushroom showed influence on all parameter. The maximum number of fruiting body (13.50), number of effective fruiting body (8.25), length of stalk (3.39 cm), diameter of stalk (0.98 cm), length of pileus (6.47 cm), diameter of pileus (7.60 cm) and thickness of pileus (0.78 cm) were recorded in T_3 treatment. Biological efficiency and benefit cost ratio also found highest in T_3 treatment. All the combination between different two variety and different substrates amount was found significant variation on the yield.

Keywords: Oyster mushroom, Maize straw, Mother culture.

INTRODUCTION

Oyster mushrooms belong to the genus *Pleurotus* under the class Basidiomycetes are one kind of edible saprophytic fungi which are growing dead organic matters (Mondal *et al.*, 2010). Having outstanding flavor and good taste oyster mushroom is rich source of both macro and micro nutrients where dried protein (25-50%), fat (2-5%), sugars (17-47%), mycocellulose (7-38%) and minerals (potassium, phosphorus, calcium, sodium) of about 8- 12% (Sher *et al.*, 2011). The pileus of mushroom is fan liked shaped. The vegetative part of mushroom consists of thread like long thin mycelium which under suitable condition forms fruiting body or sporocarps. This fruiting body is used as edible mushroom. Mushroom is a highly nutritious, delicious, medicinal and economically potential vegetable (Alam and Saboohi, 2001).

Mushrooms are recognized as a food from ancient times and now it is cultivated all over the world. Mushroom as a food are now very popular and having significant role in human health and nutrition (khan *et al.*, 2008). The easiest and least expensive commercial mushrooms to grow from oyster mushrooms because they are well known for conversion of crop residues to food protein. Mushroom cultivation is one of the most commercially important steps towards diversification of agriculture.

Oyster mushroom can grow on a wide variety of substrate. However, the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates (Badu *et al*, 2011 and Patil *et al*, 2010). Again agricultural substrates such as paddy straw, vegetable residues, maize stalks and cotton waste are utilized for cultivation of oyster mushroom (Hassan *et al*., 2011). Substrates having high levels of nitrogen and carbohydrate contents are categorized as ideal for mushroom growth (Khare *et al*., 2010).

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Majority of farmer use rice straw for oyster mushroom production as and they are not familiar with efficiency and methods of Pleurotus mushroom production using other agro bi-products like maize straw, maize cob, lentil straw, wheat straw etc. Maize straw is very common and suitable substrate for oyster mushroom cultivation. The utilization of maize residues as substrate for oyster mushroom cultivation under controlled conditions has been reported by Atikpo *et al.,* (2008), Obodai *et al.,* (2003), and Onyango *et al.*, (2010).

In our country oyster mushroom is now widely cultivated because the weather and climate of Bangladesh is suitable for its cultivation and the necessary materials required for oyster mushroom cultivation such as maize straws are available and cheap. During the main harvesting period, maize straw is in abundance and farmers dispose of them by burning. If grounded maize straw can support the growth of oyster mushroom, then it would serve as a cheap source of substrate for mushroom growers. The main objective was to find out appropriate combination of substrate ratio and varietal performance.

MATERIALS AND METHODS

The experiment was conducted at mushroom Development Institute, Savar, Dhaka with five different size of spawn packets during the period from July to December 2019.

Experimental materials: Two oyster Mushroom varieties such as-V_1 (*Pleurotus ostreatus*) & V_2 (*Pleurotus djamor)* were tested on different amount of maize straw as substrates with supplement. Four spawn packets of 500 g, 750 g, 1000 g, 1500 g sizes and one bed of 2000 g was prepared by through spawning method and was maintain the definite substrates amount.

Treatments: The experiment was consisted with the following two treatment factors-

Factor-A: Mushroom variety

V_1 = *Pleurotus ostreatus (PO2).*

V_2 = *Pleurotus djamor (Pop).*

Factor-B: Substrates amount-

T_1 = 500g (450g maize straw + 50g mother culture) per packet.
T_2 = 750 g (675g maize straw + 75g mother culture) per packet.
T_3 = 1000 g (900g maize straw + 100g mother culture) per packet.
T_4 = 1500 g (1350g maize straw + 150g mother culture) per packet.
T_5 = 2000 g (1800g maize straw + 200g mother culture) per bed.

Experimental design: The study was laid out in Completely Randomized Design (CRD) with 4 replications and five treatment combinations.

Preparation of pure culture and mother culture: Pure culture and paddy grain mother culture were prepared, inoculated and incubated following the procedure that developed and explained by Luckey *et al.,* 2018. After completion of mycelium running the paddy grain containing mother culture were used for spawn packet preparation.

Preparation of substrates: Maize straw were used as substrates for this study. 30 kg maize straw mixed with 28 liter of water after chopped 4-5 cm were kept in pasteurization chamber at 60-70^{0}C temp. for one hour. Maize straw were placed for 18-20 hours to reduce moisture at 63%.

Preparation of spawn packets: Different size of polypropylene bags were used for preparing 500g, 750g, 1000g, 1500g/bag and 2000g/bed.

Mycelium running in spawn packets/incubation: The packets were kept at room temp. in culture house in Mushroom Development Institute until the packets were full of mycelium.

Opening the packet: After completion mushroom mycelium running, "D" shaped cut were done two ends of the packets.

Cultivation of spawn packet: The packets and bed were placed at 20-25^{0}C room temp. with 80-85% relative humidity and 150-200 lux light.

Harvesting and data analysis: The packets were arranged in culture house following completely randomized design each treatment with 4 replications. Data on days required pin head initiation to first harvest, number of fruiting body and effective fruiting body, length and diameter of stalk, diameter and thickness of pileus, Weight of fruiting body, yield (g/packet), were recorded. Weight of fruiting body was recorded after removing the lower hard and dirty portion of stipe.

Biological efficiency: Biological efficiency was determined by the following formula:

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

Benefit cost ratio: The benefit cost ratio for different low cost substrates were computed based on present market price of mushroom and cost of different inputs in the markets (Sarker, 2004).

$$\text{Benefit cost ratio} = \frac{\text{Price per packet}}{\text{Production cost per packet}}$$

Statistical analysis: Recorded data were analyzed by CRD design to find out the statistical significance of experimental results by suing Statistic 10X and Microsoft Excel soft. The mean difference was adjudged by LSD test.

RESULTS AND DISCUSSION

Effect of maize straw as a substrate for oyster mushroom yield performance which are presented several figures and tables for better understanding. The findings of the study represented significant variation between varieties and significant differences among treatments which influenced yield performance and other attribute characters.

Days required from pinhead initiation to 1st harvest: Significant variation was observed in days required from pinhead initiation to 1st harvest due to the effect of substrates amount. The lowest time (3.00 days) from pinhead initiation to 1st harvest was in the treatment T_1 & T_5 that was cultivated in 500g and bed and the highest time (4.38 days) from pinhead initiation of 1st harvest was observed in the treatment T_3 (Table 1). Royse (2003) found that there was a significant opposite relation between spawn rate and days to production. If spawn rate increased, then days to production decreased.

Interaction effect of varieties and substrates amount was found significant on days required from pinhead initiation to 1st harvest. The highest time for V_1 (*Pleurotus ostretus*) (3.56 days) in treatment T_3 and the V_2 (*Pleurotus djamor*) (4.38 days) in treatment T_3 (Table 1)

Number of fruiting body/packet: The number of fruiting body was influenced by the amount of maize straw. The highest amount number of fruiting body was observed in treatment T_3 (900g maize straw + 100g mother culture/per packet) in both variety V_1 (16.00) and V_2 (13.50) respectively. The lowest number of fruiting body was found in treatment T_5 (1800g maize straw + 200g mother culture/ per bed) (Table 1). Amin (2004) reported that the number of primordia grown on different substrates differed significantly.

Result showed that there were significant differences in number of fruiting body per packet among varieties (Table 1). V1T3 (Pleurotus ostretus) found the highest (16.00) number of fruiting body per packet where V_2T_5 (*Pleurotus djamor*) found the lowest (5.25) number of fruiting body per packet (Table 1).

Number of effective fruiting body/packet: Significant amount of variation was found in the number of fruiting effective body which was influenced by the amount of maize straw. The highest amount number of effective fruiting body was observed in treatment T_3 (900g maize straw + 100g mother culture/per packet) in both variety V_1 (10.25) and V_2 (8.25) respectively (Table 1).Sarker (2004) found that the number of primordia increased with the levels of supplement and continued up to a certain range and decline thereafter.The treatment may be ranked in order of $T_3>T_4>T_2>T_1>T_5$.

Result showed that there were significant differences in number of fruiting body per packet among varieties (Table 1). The maximum number of effective fruiting body per packet (10.25) was found from Variety V_1 (*Pleurotus ostretus*) in Treatment T_3 where Variety V_2 (*Pleurotus djamor*) and Treatment T_5 found the lowest (2.25) number of effective fruiting body per packet (Table 1).

Length of stalk: Significant variation was found in the length of stalk influenced by the amount of maize straw substrate. The maximum length of stalk was found Variety V_1 (*Pleurotus ostretus*) in Treatment T_3 (5.93 cm) where the minimum length of stalk was found same variety in Treatment T_5 (2.55 cm) (Table 1). And from Variety V_2 (*Pleurotus djamor*), Treatment T_3 (3.39 cm) and Treatment T_5 (1.83 cm) was found the maximum and minimum length of stalk respectively. V_2T_5 found the lowest (1.83 cm) length of stalk in that study (Table 1).

Table 1. Combined effect of varieties and different substrates amount on yield contributing of oyster mushroom

Treatments	Pinhead initiation to 1st harvest	Number of fruiting body	Number of effective fruiting body	Length of stalk	Diameter of stalk	Length of pileus	Diameter of pileus	Thickness of pileus
V_1T_1	3.00 F	9.25 E	4.75 D	3.32 D	1.40 A	7.21 C	7.23 D	0.60 D
V_1T_2	3.06 EF	12.25 C	7.00 C	4.22 C	0.85 E	7.60 BC	8.03 BC	0.70 C
V_1T_3	3.56 C	16.00 A	10.25 A	5.93 A	1.09 C	8.21 A	8.80 A	0.90 A
V_1T_4	3.25 DE	13.00 B	8.25 B	4.59 B	1.17 B	7.78 AB	8.28 B	0.80 B
V_1T_5	3.00 F	6.75 F	3.00 E	2.55 F	0.59 G	6.72 D	6.57 E	0.53 E
V_2T_1	3.00 F	7.00 F	3.25 E	2.24 G	0.58 G	5.86 F	5.64 F	0.53 E
V_2T_2	3.31 D	9.00 E	5.00 D	2.67 F	0.63 G	5.95 F	6.68 E	0.63 D
V_2T_3	4.38 A	13.50 B	8.25 B	3.39 D	0.98 D	6.47 DE	7.60 CD	0.78 B
V_2T_4	3.88 B	10.50 D	6.75 C	2.93 E	0.73 F	6.13 EF	7.25 D	0.70 C
V_2T_5	3.00 F	5.25 G	2.25 F	1.83 H	0.50 H	5.31 G	5.14 G	0.48 F
$LSD_{(0.05)}$	0.2298	0.7326	0.4351	0.2429	0.0612	0.4622	0.4908	0.0461
CV (%)	4.04	4.20	4.35	4.24	4.22	4.04	4.05	4.07

Here, V_1T_1 = PO_2 × 500g substrate, V_1T_2 = PO_2 × 750 g substrate, V_1T_3 = PO_2 × 1000 g substrate, V_1T_4 = PO_2 × 1500 g substrate, V_1T_5 = PO_2 × 2000 g substrate and V_2T_1 = POP × 500g substrate, V_2T_2 = POP × 750 g substrate, V_2T_3 = POP × 1000 g substrate, V_2T_4 = POP × 1500 g substrate, V_2T_5 = POP × 2000 g substrate.

Diameter of stalk: Among the varieties a significant variation in the diameter of stalk was clearly observed. The largest stalk diameter (1.40 cm) was obtained from V_1 treatment that was T_1, and the shortest stalk diameter (0.50 cm) was obtained from V_2 where T_5 treatment was used (Table 1).

Length of pileus: Length of pileus is important yield contributing parameters of oyster mushroom cultivation. Length of pileus statistically significant to the different substrate composition. The highest pileus length (8.21 cm) was found in V_1T_3 (*Pleurotus ostreatus*) (900g maize straw + 100g mother culture/per packet) and the lowest pileus length (5.31 cm) was found in V_2T_5 (*Pleurotus djamor)* (1800g maize straw + 200g mother culture/per bed) (Table 1).

Diameter of pileus: Significant variation was found in diameter of pileus influenced by the amount of maize straw substrate. The highest diameter of pileus (8.80 cm) was found in V1T3 (Pleurotus

ostreatus) (900g maize straw + 100g mother culture/per packet) followed by V2T3 (Pleurotus ostreatus) (900g maize straw + 100g mother culture/per packet) (7.60 cm) (Table 1). Where the lowest diameter of pileus (5.14 cm) was found in V_2T_5 (*Pleurotus djamor)* (1800g maize straw + 200g mother culture/per bed) (Table 1).

Thickness of pileus: Thickness of pileus is one of the yields contributing character of mushroom. Thickness of pileus significantly varied due to the substrates amount. The highest (0.90 cm) thickness of pileus was recorded from V_1T_3, While V_2T_5 gave the minimum (0.48 cm) thickness of pileus (Table 1).

Yield (g/packet and bed): Yield per packet was varied significantly due to the effect of different amount of substrates (Figure 1). The highest yield (128.67 g) was recorded under 900g maize straw + 100g mother culture per packet (T_3) application, whereas the lowest yield (55.50 g) was recorded under 1800g maize straw + 200g mother culture per bed (T_5). Several authors have recommended supplementing the cereals such as maize straw with bean straw in order to achieve high yields. For example, Tikdari and Bolandnazar (2012) stated that small quantities of protein rich additives are recommended to boost yield. The supplementation of cereals with beans becomes very crucial under the smallholder sector.

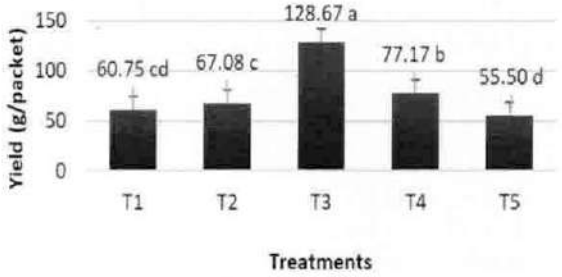

Fig. 1. Effect of different substrates amount on yield of oyster mushroom. Here, T_1 = 500g (450g maize straw + 50g mother culture) per packet, T_2 = 750 g (675g maize straw + 75g mother culture) per packet, T_3 = 1000 g (900g maize straw + 100g mother culture) per packet, T_4 = 1500 g (1350g maize straw + 150g mother culture) per packet & T_5 = 2000 g (1800g maize straw + 200g mother culture) per bed.

Significant variation was found on yield due to the varietal effect of oyster mushroom (Figure 2). However, maximum yield (75.6 g) was recorded from *Pleurotus ostreatus* (V_1), whereas the minimum yield (65.30 g) was recorded from *Pleurotus djamor* (V_2) (Fig. 2).

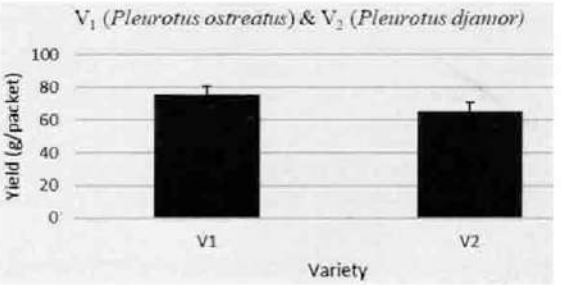

Fig. 2. Effect of variety on yield of oyster mushroom.

The interaction between different variety and different substrates amount was found significant variation on the yield. The maximum yield (117.25 g) was produced by V_1T_3 and the minimum yield (49.75g) was produced by V_2T_1which followed by V_2T_5 treatment (Figure 3). *Pleurotus ostreatus* V_1 was more productive than *Pleurotus djmor* V_2 and treatment T_3 was found out the best combination for both of this variety.

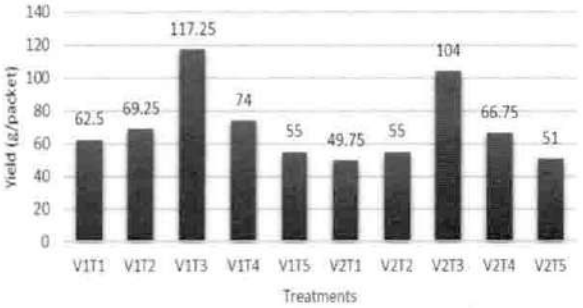

Fig. 3. Combined effect of varieties and different substrates amount on yield of oyster mushroom. Here, V_1=*Pleurotus ostreatus*, V_2= *Pleurotus djamor*, T_1 = 500g (450g maize straw + 50g mother culture) per packet, T_2= 750 g (675g maize straw + 75g mother culture) per packet, T_3= 1000 g (900g maize straw + 100g mother culture) per packet, T_4= 1500 g (1350g maize straw + 150g mother culture) per packet & T_5= 2000 g (1800g maize straw + 200g mother culture) per bed.

Biological efficiency: The highest biological efficiency 37.54% was found in V_1T_1 (*Pleurotus ostreatus*) (450g maize straw + 50g mother culture/per packet) where the lowest biological efficiency 7.66% was found in V_2T_5(*Pleurotus djamor)* (1800g maize straw + 200g mother culture/per bed) (Table 2). Treatment V_1T_3 produced 35.21 % higher biological efficiency from V_2T_3 (31.23 %) (Table 2).

Benefit cost ratio: The highest benefit cost ratio 2.67 was calculated from V_1T_3 followed by V_2T_3 (2.36) and the lowest benefit cost ratio 0.71 was calculated from V_2T_5 followed by V_1T_5 (0.76) (Table 2).

Table 2. Combined effect of varieties and different substrates amount on biological efficiency and benefit cost ratio of oyster mushroom

Treatments	Biological efficiency %	Benefit cost ratio
V_1T_1	37.54 A	1.95 C
V_1T_2	27.73 D	1.82 D
V_1T_3	35.21 B	2.67 A
V_1T_4	14.81 F	1.28 F
V_1T_5	8.26 G	0.76 H
V_2T_1	29.88 C	1.56 E
V_2T_2	22.02 E	1.45 E
V_2T_3	31.23 C	2.36 B
V_2T_4	13.36 F	1.15 G
V_2T_5	7.66 G	0.71 H
LSD	1.7094	0.1148
CV	4.41	4.29

Here, V_1T_1 = PO_2 × 500g substrate, V_1T_2 = PO_2 × 750 g substrate, V_1T_3 = PO_2 × 1000 g substrate, V_1T_4 = PO_2 × 1500 g substrate, V_1T_5 = PO_2 × 2000 g substrate, V_2T_1 = POP × 500g substrate, V_2T_2 = POP × 750 g substrate, V_2T_3 = POP × 1000 g substrate, V_2T_4 = POP × 1500 g substrate and V_2T_5 = POP × 2000 g substrate.

Considering the stated findings, it may be concluded that yield and yield contributing characters were significant influenced by the amount of substrates. However, *Pleurotus ostreatus* in combination of 900g maize straw + 100g mother culture/per packet would be beneficial for farmers.

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Effects of Spawn Density on Mycelium Running, Yield and Yield Attributes of Milky White Mushroom

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Abstract

The present study has been carried out to evaluate the effect of spawn quantities on the growth and yield of milky white mushroom. Five different spawn densities viz. 10%, 20%, 30%, 40% and 50% (dry weight basis of substrate) were used to inoculate the rice straw substrate. Partial colonization of mycelium in spawn packets was completely disappeared with the increase of spawn density. The relationship between spawn density and economic yield and biological efficiency was significant and quadratic. Maximum growth and yield contributing parameters of milky white mushroom were increased with the increase of spawn density. The spawn density of 50% gave the highest yield and biological efficiency though the benefit cost ratio was highest at 40% spawn density.

Keyword: spawn quantities, spawn densities, economic yield.

INTRODUCTION

Spawn serves as the planting material in mushroom cultivation (Romaine *et al.*, 2007) and it provides the backbone to any mushroom growing operation. It is as the equivalent of seeds for a mushroom farm. The spawn is used to transfer mycelium onto any material from which mushrooms will grow, called a substrate. The amount spawn, moving to bulk substrate, has a great effect over any other contaminants due to its full colonization of the highly nutritious substrate and its many points of inoculation throughout the bulk substrate. The quantity of spawn used does not directly affect the yield of mushrooms (Quimio *et al.*, 1990). However, the use of more spawn has been found to influence mushroom growth, development and yield. Growers have sought, in the past, to optimize the amount of spawn used to inoculate their substrate. Increasing the amount of spawn used has led to higher yields (Royse, 2002). Specifically, raising spawn rates from 1.25% to 5% resulted in a yield increase of nearly 50%. Dahmardeh *et al.* (2010) found out that the maximum average yield (1810 g/2 kg wet barely substrate) of mushrooms was estimated from the barley substrate at 150 g spawn level. Fan *et al.* (2000) carried out the studies with 2.5-25% spawn rates, 25% spawn rate appeared superior, but recommended 10% spawn rate in view of the process economics. In our country rice straw substrates are generally inoculated with 10% saw dust spawn for milky white mushroom cultivation which creates partial coverage of substrate with mycelium but fully mycelium colonized substrate is prerequisite for better yield and biological efficiency. Therefore, the present study has been carried out to evaluate the effect of spawn quantities on the growth and yield of milky white mushroom.

MATERIALS AND METHODS

The experiment was conducted at Mushroom Development Institute, Department of Agricultural Extension, Savar, Dhaka, Bangladesh from July 2020 to September 2020. Five different spawn density, viz. T_1 = 10%, T_2 = 20%, T_3 = 30%, T_4 = 40% and T_5 = 50%, considered as the treatments in this experiment were used to inoculate the substrate, rice straw (dry weight basis).

Preparation of rice grain mother culture: Rice grain spawn was used as mother culture. Fresh dry rice grain was soaked in water for overnight and boiled for about 30 minutes so that the grain hull began to crake. After 30 minutes boiling was stopped and excess water was drained out from the grain

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and the grains were air dried. The grain was treated with calcium carbonate @ 5g/kg grain. Calcium carbonate treated grain, 250g, was filled in 7″x10″ sized polypropylene bag. A plastic neck was fitted with rubber band and sealed with cotton plug and covered with brown paper. Then the grain filled bags were sterilized in an autoclave machine for an hour at 121°C and 15 PSI pressure. After cooling the grain filled bags were inoculated with fully colonized pure culture of milky white mushroom in a clean bench. The inoculated grain bags were kept in an inoculation room at 25°C temperature for two weeks. After completion of mycelium running the rice grain mother culture was used as spawn for bulk substrate.

Preparation of spawn packets: Rice straw was used for the cultivation of milky white mushroom. The straw was chopped to convenient length of 2.5 to 5 cm. The substrate was mixed with appropriate amount of water and then filled in net bag. The net bags filled with substrate were placed in the Sterilization cum Inoculation Chamber. Door of the chamber was closed and tightened with the help of screws. Water heater was turned on to produce steam that flows in to the chamber. When the temperature of the chamber rises to 60°C, the steam flow was adjusted to maintain a constant temperature of 60°C – 80°C up to 90 minutes. After 90 minutes water heater was turned off and kept it for about 20 hours. After 20 hrs. substrate was taken out and used for preparation of spawn packet. Pasteurized substrate was mixed thoroughly with rice grain based mother culture of different densities according to the treatments and filled into the polythene bags (12″x16″). A plastic neck was fitted to the filled bags with rubber band and sealed with cotton plug and covered with brown paper. Then the spawn packets were transferred to the culture house for mycelium run. After 16-25 days the substrate was completely colonized by the mycelium and polythene cover was opened.

Casing and after care: After completion of mycelium colonization, cotton, brown paper and neck were removed from the packets and the mouth of the polypropylene bags were folded 4-5 cm above the spawn. Previously sterilized loamy soil casing material was used to cover over the mycelium on the substrate up to 4 cm thickness. Watering was done at regular interval to maintain moisture at 70 to 80%. Fruiting body primordia initiated within 9-15 days and developed in to fruiting bodies.

Harvesting and data collection: The fruiting bodies were harvested at 7-8 days of primordial initiation and data were collected on days to primordial initiation, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, number of effective fruiting body (NEFB), number of flushes, yield and biological efficiency (BE). The BE was measured by the formula:

$$\text{Biological Efficiency (BE)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

Experimental design and statistical analysis: The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. The data were statistically analyzed following SPSS (version 26.0) computer program. Difference among the treatment means were determined by Tukey's Test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Days to spawn run, pinhead formation and time to complete total harvest: Days to complete spawn run and pinhead formation was significantly influenced by the spawn density used for commercial spawn production of milky white mushroom but time to complete total harvest was insignificant (Table 1). Maximum time (23.83 days) was required to complete spawn run in the rice straw substrate when 10% rice grain mother culture was used for inoculation and minimum time (12.90 days) was required to complete spawn run when 50% mother culture was used. This might be due to higher spawn density provides the higher number of points of spreading mycelia. Earlier pinhead of fruiting body formation (9.58 days) was observed in case of 50% spawn density and longest time (14.53 days) was required in 10% spawn density. From the study it was observed that spawn run time and fruiting body pinhead

formation was gradually decreased with the increase of amount of spawn used. To complete total harvest, spawn packets inoculated with 40% mother culture takes longest time (65.23 days) and spawn packets inoculated with 10% mother culture takes shortest time (59.98 days).

In our country rice straw substrates are generally inoculated with 10% saw dust or rice grain spawn for milky white mushroom cultivation which creates partial coverage of substrate with mycelium but fully mycelium colonized substrate is prerequisite for better yield and biological efficiency. These results revealed that partial mycelium colonization problem was gradually disappeared with the increase of spawn density. These results are in accordance with the findings of Pani (2011) who also observed that there was quicker substrate colonization, earlier pinhead appearance and higher number of sporophores as the amount of spawn increased in the cultivation substrate. Kuforiji and Fasidi (2009) similarly observed that high spawning rate led to more rapid colonization of substrate.

Table 1. Effects of spawn density on spawn run, pinhead formation and harvesting time of milky white mushroom

Spawn density (%)	Days to spawn run	Days to pinhead formation	Number of effective fruiting body	Number of flush	Time complete to total harvest (days)
10	23.83a	14.53a	6.15c	1.65c	59.98a
20	20.40b	13.25b	7.08bc	1.90c	62.65a
30	16.78c	12.88b	8.05ab	2.35b	62.08a
40	15.45d	10.43c	8.53ab	2.85a	65.23a
50	12.90e	9.58d	9.10a	3.10a	65.13a
P	<0.001	<0.001	0.001	<0.001	0.079

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Number of effective fruiting body and number of flush: Density of spawn used to commercial spawn packet production of milky white mushroom had significant influence on number of effective fruiting body and number of flush (Table 1). Highest number of effective fruiting bodies (9.10) and flushes (3.10) were recorded from the spawn packets which were inoculated with 50% rice grain spawn. Number of effective fruiting bodies and flushes were gradually decreased with the decrease of amount of spawn used. Lowest number of effective fruiting bodies and flushes were recorded from the spawn packet which were inoculated with 10% rice grain spawn. Highest number of fruiting body and number of flushes produced at 50% spawn density might be due to evenly and densely colonized mycelium and more amount of grain spawn acted as a supplement for mycelium. This result was similar to the findings of Pani (2011) who also reported that number of sporophore was increased with the increase of spawn density in paddy straw substrate and the highest number of sporophore (8.0) was recorded at 500 g spawn/kg substrate. Idowu *et al.* (2016) also reported that as the psawn density increased number of fruiting body increased.

Relationship between spawn density, time to complete spawn run and fruiting body pinhead formation: There was a strong negative correlation ($r = - 0.986^{**}$) between the spawn density used and time to complete spawn run. It was observed that the equation $y = -2.680x + 25.910$ gave a good fit to the data and the value of the co-efficient of determination ($R^2 = 0.971^{**}$) showed that the fitted regressing line had a significant regression co-efficient (Fig. 1). The R^2 value indicated that 97.10% time to complete spawn run was attributed to the amount of spawn used to prepare commercial spawn packet and the spawn run time was gradually decreased with the increase of amount of spawn used.

There was also a strong negative correlation ($r = - 0.960^{**}$) between the amount of spawn used and time to fruiting body pinhead formation of milky white mushroom. A significant linear relationship was observed between the two variables and the relationship could be described by the equation $y = -1.273x + 15.948$ (Fig. 2). The value of co-efficient of determination ($R2 = 0.922^{**}$) indicated that

92.20% time to pinhead formation was attributed to the spawn used to prepare spawn packet. Higher grain spawn supplies more energy to the mushroom mycelium, so it grows faster and reduce the time to complete spawn run and fruiting body pinhead formation. Idowu *et al.* (2016) also reported that time to complete spawn run and fruiting body pinhead formation was decreased with the increase of spawn density.

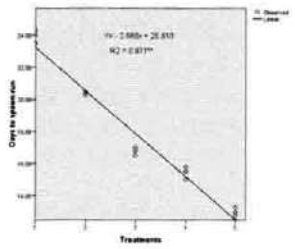

Fig. 1. Relationship between spawn density and time to complete spawn run.

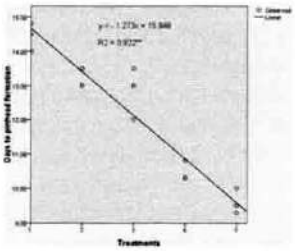

Fig. 2. Relationship between the spawn density and days to pinhead formation.

Relationship between the spawn density, number of effective fruiting body and number of flush: There was a significant positive correlation ($r = 0.824^{**}$) between the spawn density and number of effective fruiting body. It was observed that the equation $y = 0.735x + 5.575$ gave a good fit to the data and the value of co-efficient of determination ($R^2 = 0.679^{**}$) showed that the fitted regression line had a significant regression co-efficient (Fig. 3). The R^2 value indicated that 67.90% number of effective fruiting body was attributed to the spawn density and the number of effective fruiting bodies were increased with the increase of amount of spawn used.

As like number of effective fruiting body, there was also a significant positive correlation ($r = 0.964^{**}$) between the amount of spawn and number of flushes. The relationship between the two variable was linear and could be expressed by the equation $y = 0.385x + 1.215$ ($R^2 = 0.929^{**}$) (Fig. 4). The R^2 value indicated that 92.90% number of flushes were attributed to the amount of spawn used for commercial spawn production of milky white mushroom and it was also indicated that the number of flushes were increased with the increase of amount of spawn used. These results are an agreement with the findings of Idowu *et al.* (2016) who also reported that number of effective fruiting body and number of flushes increased with the increase of spawn density

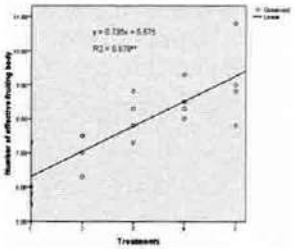

Fig. 3. Relationship between the spawn density and number of effective fruiting body.

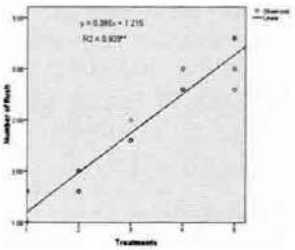

Fig. 4. Relationship between the spawn density and number of flushes.

Length & diameter of stalk and diameter & thickness of pileus: Stalk length and stalk diameter was significantly influenced by the amount of spawn used for preparation of spawn packets but the effect of spawn density on diameter and thickness of pileus was insignificant (Table 2). Highest stalk length (9.55 cm) was recorded from the spawn packet inoculated with 50% rice grain spawn which was significantly higher than other treatments. Stalk length was lowest (8.38 cm) in spawn packet inoculated with 10% mother culture. Stalk diameter was also highest (2.83 cm) when spawn packets were inoculated with 50% rice grain mother culture which was significantly higher than other treatments but stalk diameter was lowest (2.38cm) when spawn packets were inoculated with 30% mother culture.

Diameter of pileus was highest (6.90 cm) when spawn packets were inoculated with 40% mother culture which was similar to all other treatments. Pileus diameter was lowest (5.85 cm) when spawn packets were inoculated with 10% mother culture. Thickness of pileus was highest (2.35 cm) when spawn packets were inoculated with 50% mother culture which was similar to all other treatments. Pileus thickness was lowest (2.23 cm) when spawn packets were inoculated with 10% mother culture. These results were partially supported by the findings of Idowu *et al.* (2016) who also reported that longest stipe and widest pileus of *Pleurotus ostreatus* was observed at highest spawn density they used. This might be the difference of mushroom species studied.

Table 2. Effects of spawn density on length & diameter of stalk and diameter & thickness of pileus

Spawn density (%)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
10	8.38b	2.40b	5.85a	2.23a
20	8.63b	2.40b	5.93a	2.28a
30	8.48b	2.38b	6.70a	2.28a
40	8.75b	2.45b	6.90a	2.33a
50	9.55a	2.83a	6.65a	2.35a
P	<0.001	<0.001	0.114	0.792

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Weight of fruiting body, economic yield, biological efficiency and benefit cost ration: Economic yield and biological efficiency of milky white mushroom was significantly influenced by the spawn density used for commercial spawn production but effect on average weight of fruiting body was insignificant (Table 3). Highest average weight (51.55 g) of fruiting body was recorded from the spawn packets which were inoculated with 40% rice grain mother culture and lowest fruiting body weight (45.28 g) was recorded from spawn packets inoculated with 10% mother culture. Economic yield (454.88 g) and biological efficiency (109.61%) was highest when the spawn packets were inoculated with 50% rice grain spawn which was similar to 40% spawn density (436.40 g & 105.23%). Economic yield and biological efficiency were lowest (273.08 g & 65.80%) when the spawn packets were inoculated with 10% rice grain mother culture. Benefit Cost Ratio (BCR) was highest when 40% spawn density was used. This finding implied that sufficient amount of spawn added to the fruiting substrate, the mycelium grows faster and has more energy available for fruiting body formation, hence the increased yield and better biological efficiency. This result supports the findings of Pani (2011), Idowu *et al.* (2016) who also reported that yield and biological efficiency were increased with the increase in spawn doses of *Clocybe indica* and *Pleurotus pulmonarius* respectively.

Table 3. Effects of spawn density on weight of fruiting body, economic yield, biological efficiency and benefit cost ratio

Spawn density (%)	Weight of fruiting body (g)	Economic yield per packet (g)	Biological efficiency (%)	Benefit Cost Ratio (BCR)
10	45.28a	273.08d	65.80d	2.84
20	47.29a	332.28c	80.07c	3.26
30	49.88a	397.28b	95.73b	3.68
40	51.55a	436.40a	105.23a	3.83
50	50.86a	454.88a	109.61a	3.79
P	0.555	<0.001	<0.001	

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Relationship between spawn density, economic yield and biological efficiency: There was a strong positive correlation ($r = 0.960^{**}$) between the spawn density and economic yield of milky white mushroom. Relationship between the two variables were quadratic and could be expressed by the equation $y = -7.688x^2 + 92.928x +$

184.620 (Fig. 5). The value of co-efficient of determination (R^2=0.956**) showed that the fitted regression line had a significant regression co-efficient. The graph also indicated that spawn density had a diminishing increase effect on economic yield of milky white mushroom. These results are in accordance with the findings of Idowu *et al.* (2016) who also observed that as the spawn level increased, the yield of *Pleurotus ostreatus* increased.

There was also a significant correlation was observed between spawn density and biological efficiency of milky white mushroom. The relationship between the two variable was also quadratic and could be described by the equation $y = -1.853x^2 + 22.394x + 44.487$ (Fig. 6). The value of co-efficient of determination (R^2= 0.956**) indicated that 95.60% biological efficiency was attributed to the spawn density. The equation also indicated that spawn density had a diminishing increase effect on the biological efficiency of milky white mushroom. This result supports the findings of Pani (2011), Idowu *et al.* (2016) who also reported that biological efficiency was increased with the increase in spawn doses of *Clocybe indica* and *Pleurotus pulmonarius* respectively.

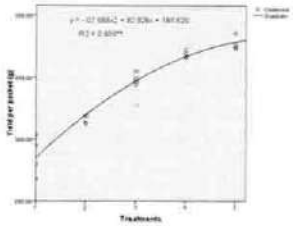

Fig. 5. Relationship between the spawn density and economic yield per packet.

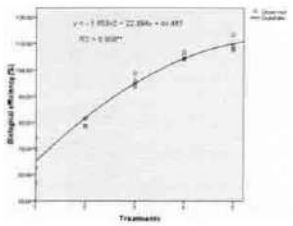

Fig. 6. Relationship between the spawn density and biological efficiency of milky white mushroom

Fully mycelium colonization in the substrate is very important for a good harvest. Partial colonization of mycelium in spawn packets was completely disappeared with the increase of spawn density. The relationship between spawn density and economic yield and biological efficiency was significant and quadratic. Maximum growth and yield contributing parameters of milky white mushroom were increased with the increase of spawn density and 50% spawn density gave the highest yield but benefit cost ratio was highest at 40% spawn density.

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