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Dr. Nirod Chandra Sarker
Editor-in-Chief
Bangladesh Journal of Mushroom
and
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Sobhanbag, Savar, Dhaka
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Nutritional Status of Different Strains of Maple Oyster and Ear Mushroom

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

Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

Pleurotus and *Auricularia* mushroom are considered as a good source of nutrition, because of the presence of high amount of proteins, vitamins and minerals. Apart from having high nutritional value, it also possesses medicinal properties because of low fat and cholesterol content. The nutritional composition of five strains of maple oyster and three strains of ear mushrooms such as Pcys-1, Pcys-2, Pcys-3, Pcys-4, Pcys-5, Au-3, Au-5 and Au-mix were determined. The protein content was found highest in Au-mix (9.0g/100g dry sample) followed by Au-3 (8.0g/100g) and Au-5 (6.0g/100g). The protein content was found lowest in Pcys-1 (3.6g/100g dry sample) followed by Pcys-3 (5.04g/100g), pcys-4 (5.10g/100g). The highest lipid content (4.10 g/100g) was estimated in Pcys-1 which was followed by Pcys-3(3.31g/100g) and Pcys-4(2.92g/100g). The lowest lipid content (1.20 g/100g) was estimated in Au-mix which was followed by Au-5 (1.60g/100g) and Au-3 (1.90g/100g). The carbohydrate content was found highest in Au-5 (63.23g/100g dry sample) followed by Au-mix (57.24g/100g) and Au-3(56.77g/100g). The carbohydrate content was found lowest in Pcys-5 (32.23g/100g dry sample) followed by pcys-2 (43.29g/100g), pcys-1 (44.79/100g). The fiber content was found highest in Pcys-5 (30.3 g/100g dry sample) followed by Pcys-2 (37.24g/100g), Pcys-1 (36.43g/100g). The fiber content was found lowest in Au-5 (19.14 g/100g dry sample) followed by Au-mix (21.80g/100g), Au-3 (22.80g/100g). The total ash content was found highest in *P. cystidiosus ie* Pcys-3 (11.50 g/100g dry sample) followed by *Pcys-5* (11.36g/100g), *Pcys-2* (11.32g/100g). The total ash content was found lowest in *A. auricula ie* Au-5 (10.03 g/100g dry sample). Obtained findings from the study might be helpful for deciding the choice/selection of suitable strain of maple oyster and ear mushroom to fulfill the specific nutritional requirement and hence it can take part to overcome nutritional problems.

Keywords: Lipid, Fibre, Moisture, Protein, Minerals, Carbohydrate, Ash, Metabolizable energy.

INTRODUCTION

Nutritional value of edible mushrooms is a complex task. Moreover, nutritional composition is affected by many factors; these include differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting, specific portion of the fruiting bodies used for analysis, time interval between harvest and measurement methods (Benjamin, 1995). Mushrooms are rich in protein, minerals and vitamins and they contain an abundance of essential amino acids (Sadler, 2003). The people of Bangladesh are still not very aware of nutritional and medicinal importance of mushrooms, but the popularity of mushroom as a food supplement is increasing day by day, so it is necessary to detect nutritional status of commercially cultivated mushroom. In Bangladesh, oyster mushrooms are cultivated and harvested all over the year (Amin *et al.*, 2007). These mushrooms are the most prospective mushrooms in Bangladesh. Abalone mushroom or maple oyster (*P. cystidiosus)*, is a choice edible with small dark greyish caps and fruiting bodies of this mushroom are valued as a source of nutrients and biologically active substances. Wood ear mushrooms are known for being low in fat and calories but rich in protein and other nutrients. There is shortage of protein around the world, especially in developing countries. To combat this problem, it is necessary to find some solution which is cheap and can be

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

easily available with high nutritional value. One such solution can be the cultivation of mushrooms, which are highly proteinaceous in nature. Therefore, the objectives of this study were to evaluate and select strain(s) which is/are biologically efficient and could produce higher yield with sound nutritional quality.

MATERIALS AND METHODS

Mushroom was cultivated and harvested in the culture house and the study was carried out in the 'Quality Control and Quality Assurance' laboratory of Mushroom Development Institute (MDI), Savar, Dhaka from June 2014 to March 2015.

Treatments: Fruiting body of five different strains of maple oyster (*Pleurotus cystidiosus*) and three different strains of ear mushroom (*Auricularia auricula*) such as Pcys-1, Pcys-2, Pcys-3, Pcys-4, Pcys-5, Au-3, Au-5 and Au-mix were selected in this study for investigation. Mushroom was cultivated on sawdust and fruiting bodies were harvested and taken for nutritional analysis. Fresh mushroom was taken and then they were dried for the estimation of protein, lipid, crude fibre and total ash.

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

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

Determination of crude fibre: Ten gram of moisture and fat-free sample was taken in a beaker and 200ml of boiling 0.255N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker, and 200ml of boiling 0.313N NaOH added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100^{0}C and weighed (We) in an electric balance. The crucible was heated in a muffle furnace at 600^{0}C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber.

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

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

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

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

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

RESULTS AND DISCUSSION

Several nutritional parameters were measured. Table 1. shows the nutritional parameters of dry mushrooms.

Total ash content: Considering total ash the findings were varied from 10.03 – 11.50% per 100g of dried mushroom. The total ash content was found highest in *P. cystidiosus ie* Pcys-3 (11.50 g/100g dry sample) followed by *Pcys-5* (11.36g/100g), *Pcys-2* (11.32g/100g). The total ash content was found lowest in *A. auricula ie* Au-5 (10.03 g/100g dry sample) followed by Au-3 (10.53g/100g), Au-mix (10.76g/100g) .The total ash content of *P. cystidiosus* (maple oyster) is comparatively more than ear mushroom in case of all strains.

Lipid content: The lipid contents varied in different strains from 1.20 – 4.10g per 100g of dried sample. The highest lipid content (4.10 g/100g) was estimated in Pcys-1 which was followed by Pcys-3(3.31g/100g) and Pcys-4(2.92g/100g). The lowest lipid content (1.20 g/100g) was estimated in Au-mix which was followed by Au-5 (1.60g/100g) and Au-3 (1.90g/100g) (Table 1). It was observed that the lipid content has low in ear mushroom.

Fibre content: The fibre contents in different strains of fruiting body were 19.14 – 47.76g per 100g of dried *mushroom.* The fiber content was found highest in Pcys-5 (30.3 g/100g dry sample) followed by Pcys-2 (37.24g/100g), Pcys-1 (36.43g/100g). The fiber content was found lowest in Au-5 (19.14 g/100g dry sample) followed by Au-mix (21.80g/100g), Au-3 (22.80g/100g). It was observed that the fibre content has low in ear mushroom whereas maple oyster is high fibre content mushroom. Oyster mushroom can serve as the least fattening food because it contains no starch, low sugar content and high amount of fibre (Samuel et al., 2012). In general, mushroom contains 90% water and 10% dry matter.

Protein content: The protein content was found highest in Au-mix (9.0g/100g dry sample) followed by Au-3 (8.0g/100g) and Au-5 (6.0g/100g). The protein content was found lowest in Pcys-1(3.6g/100g dry sample) followed by Pcys-3 (5.04g/100g), pcys-4 (5.10g/100g). It was observed that the protein content has low in maple oyster whereas ear mushroom is high protein content mushroom.

Table 1. Nutritional composition of different strains of maple oyster mushrooms and ear mushrooms (g/100g of dried sample)

Mushroom strains	Total ash (g/100g)	lipid	Fibre	Protein	Carbohydrate
Pcys-1	11.08	4.10	36.43	3.6	44.79
Pcys-2	11.32	2.55	37.24	5.6	43.29
Pcys-3	11.50	3.31	31.74	5.04	48.41
Pcys-4	11.09	2.92	33.96	5.10	46.96
Pcys-5	11.36	2.89	47.76	5.76	32.23
Au-3	10.53	1.90	22.80	8.0	56.77
Au-5	10.03	1.60	19.14	6.0	63.23
Au-mix	10.76	1.20	21.80	9.0	57.24

Carbohydrate content: The carbohydrate content was found highest in Au-5 (63.23g/100g dry sample) followed by Au-mix (57.24g/100g) and Au-3(56.77g/100g). The carbohydrate content was found lowest in Pcys-5 (32.23g/100g dry sample) followed by Pcys-2 (43.29g/100g), Pcys-1 (44.79/100g). It was observed that the carbohydrate content has low in maple oyster whereas ear mushroom is high carbohydrate content mushroom. This result is partially supported by Yang *et al.* (2001) he observed that the protein content of *P. cystidious* is 15.4g/100g, carbohydrate 63.1g/100g, lipid 3.10g/100g, fibre 8.74g/100g and total ash content 9.62g/100g dry sample.

The present study suggests that oyster mushrooms differ from each other in nutritional composition although they are of same genus, however each species are nutritious with high protein and fiber value with low fat. Hence fruiting bodies of oyster mushrooms can be taken regularly as a protein supplement or as an alternative to fish and meat. Vegetarians could also eat mushrooms because it might serve as alternative protein supplements in their diet. The low lipid and high fiber contents of the oyster mushrooms make it health beneficial food items especially against heart diseases and diabetes.

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Lentinus edodes has Ability for Improving Different Blood Lipids of Female Subjects

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

Abstract

As *Lentinus edodes* is one of the famous edible mushrooms in home and abroad, it is important to know its health benefit in our contest. The study was conducted at Mushroom Development Institute (MDI), savar, Dhaka, to find out its effect on different blood lipids of female subjects. A total 28 female subject aged from 22 to 73 years and free from any acute or chronic disease was included in the study. Fasting blood sample were collected for analysis. Capsule made with 500 mg dried *Lentinus edodes* powder was supplied to the subjects to take 2 capsules 3 times daily for three months. In the study a significant ($p = 0.019$) 7.17% reduction of plasma total cholesterol (TC) and ($p = 0.0412$) 6.53% reduction of plasma triglyceride (TG) were observed after 3 months supplementation of *Lentinus edodes* capsule. Considering plasma low density lipoprotein cholesterol (LDL-C) a significant ($p = 0.0019$) 11.27% reduction was also observed. Again a non significant ($p = 0.115$) 7.07 % elevation of plasma High Density Lipoprotein Cholesterol (HDL-C) was observed after three months. All the above findings suggest that *Lentinus edodus* may improve plasma lipid status of female subjects.

Keywords: *Lentinus edodes*, Total Cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein Cholesterol (LDL-C), High Density Lipoprotein Cholesterol (HDL-C).

INTRODUCTION

Natural products play a very important role in the process of discovery and development of drugs, including the treatment of chronic diseases (Newman and Cragg, 2007). Mushrooms have a great nutritional value and present medicinal molecules including polysaccharides, terpenoids, sterols and lipids that participate actively in several human disorders and modulate mechanisms involved in the immune system regulation.

For hundreds of years, medicinal mushrooms are used as decoctions and essences, and are applied as alternative medicine in different countries like Korea, China, Japan and eastern Russia (Lull, *et al.*, 2005). *Lentinus edodes* (Shiitake) is a traditionally renowned mushroom in Far East countries that has been used as a food and medicine for thousands of years. Shiitake is one of the five most cultivated edible mushrooms in the world. Its production is second only to button mushroom (Chang, 1999; Stamets, 2000).

Lentinus edodes is one of the members of macrofungus family with huge potential for therapeutic applications. The genus *Lentinula* sp. grows in gregarious on fallen wood of a wide variety of deciduous trees, in a warm, moist climate. Most of these are raised for artificial cultivation of shiitake mushroom and occurs naturally throughout Southeast Asia (Wasser, 2002). *Lentinus edodes* species is the most famous, and has been used as a model to investigate the functional properties and isolate pure compounds for pharmaceutical use. *Lentinus edodes* has shown to present medicinal compounds which are effective in treating

[1]Department of Biochemistry, Manikganj Medical College, Manikganj, Bangladesh; [2]Department of Agriculture Studies, Nabajug College, Dhamrai, Dhaka, Bangladesh; [3]Department of Oral & Maxillofacial Surgery, Dhaka Medical College Hospital, Dhaka, Bangladesh.

various tumors and infections, among other activities which are still being studied (Wang and Zhang, 2009).

Cardiovascular disease is one of the leading cause of cholesterol levels in the blood throughout the world and is an important risk factor for the high mortality, thus hypocholesteremic effects are of great importance. Evidence also shows that mushrooms may protect against chronic disease like CVD. Oxidative stress and inflammation are closely linked to atherogenesis (Lindequist, *et al.*, 2005). The mechanism of action is due to a significant reduction in binding of quiescent monocytes and also stimulated by cytokines (Martin, 2010_a; Martin, 2010_b). It was also observed that *Lentinus edodes* has the ability for reducing cholesterol and triglyceride (Yamac, *et al.*, 2008). The ability of shiitake in lowering sanguine cholesterol was first reported in the 1960s (Bisen, *et al.*, 2010). To date, some studies demonstrate the ability of *Lentinus edodes* in both decrease very low density lipoproteins (VLDL) as well as high density lipoproteins (HDL), preventing the increase of blood pressure (Oba, *et al.*, 2009; Shimada, et al., 2002).

The aims of this research were to find out the blood lipid status after consumption of shiitake mushroom on female volunteers.

MATERIALS AND METHODS

The study was conducted at Mushroom Development Institute Sobhanbag, Savar, Dhaka. A Total 28 female subjects aged (years) from 22 to 73 who were at the available location of the monitoring team were considered. The subjects were clarified about the study and after getting their written consent showing willingness to participate in the study they were included. During the study any acute or chronic disease or medication, malabsorption and any addiction were excluded.

At the beginning of study, health status of the subjects was being evaluated. The details history was taken from the subjects which included age, sex, occupation, educational status, marital status, family history and drug history. Fasting blood sample was collected for analysis of TC, TG, HDL-C and LDL-C.

Mushroom capsules were supplied to take two capsules three times daily. Each capsule contains 500 mg *Lentinus edodus* powder, so that each subject took 3 gms mushroom powder daily. After three months the subjects were evaluated and all the investigations were repeated. If any drug previously getting by the subjects, it was continued.

Ten ml fasting blood sample was collected from median cubital vein with all aseptic precaution. After collecting blood, immediately it was poured into test tube containing fluoride and EDTA. The test tube then gently shaken so that anti coagulant and fluoride mix with the blood properly. Then it was centrifuged by 3000 rpm for 5 minutes. Plasma was separated which were transferred into two eppendorf containing 1 ml in each. All the tests were carried out as early as possible.

Fresh fruiting body of *Lentinus edodes* was collected from culture house of Mushroom Development Institute. Collected mushrooms were sun dried at moisture level 4-5% then grinded and pour into capsule shell which contains 500 mg powder. Prepared capsules were preserved into moisture free glass containers which were ready to dispense.

All the biochemical parameters for the measurement of lipid profile were estimated by semi-auto analyzer (3000 evaluation) using the available reagent kit. The recorded characteristics of the subjects analyzed by standard statistical methods using computer software, SPSS package programme.

RESULTS AND DISCUSSION

In this study, mean age of the subjects was 47.35 years. Among the study population 68% subjects was house wife, 27% service holder and 6% others (Fig. 1).

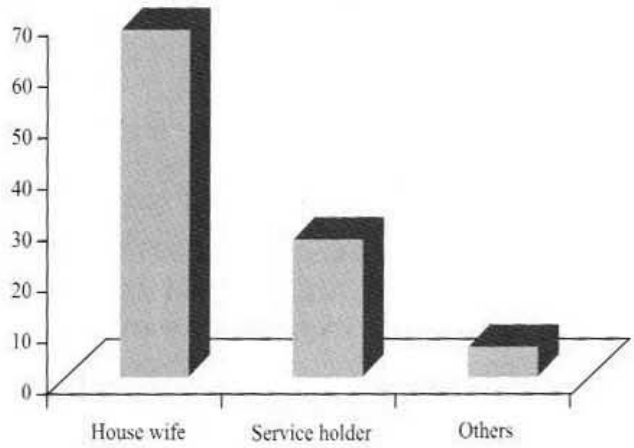

Fig. 1. Distribution of study population.

The mean (± SE) plasma total cholesterol (TC) (mg/dl) before- and after- mushroom capsule supplementation was 173.85 ± 7.02 and 161.39 ± 6.98 respectively. A statistically significant ($p = 0.019$) 7.17% reduction of TC was observed (Fig. 2).

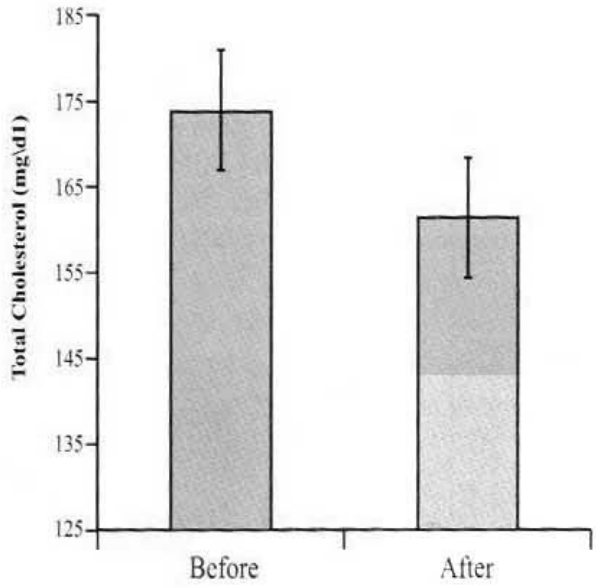

Fig. 2. Plasma concentration of Total Cholesterol (TC).

Considering triglyceride (TG), the mean (± SE) plasma TG (mg/dl) before and after mushroom supplementation was 149.93 ± 8.68 and 140.14 ± 7.72 respectively. Here also a significant (p = 0.0412) 6.53% reduction observed (Fig. 3).

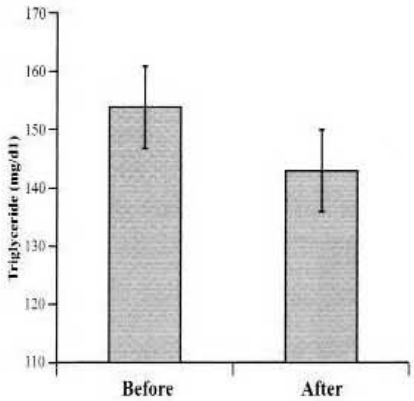

Fig. 3. Plasma concentration of Triglyceride (TG).

The mean (± SE) plasma high density lipoprotein cholesterol (HDL-C) (mg/dl) before- and after- mushroom supplementation was 31.12 ± 1.28 and 33.32 ± 1.21 respectively. A non significant (p = 0.115) 7.07 % elevation of HDL-C was observed after three months (Fig. 4).

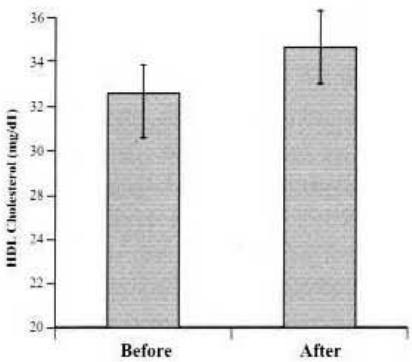

Fig. 4. Plasma concentration of High Density Lipoprotein Cholesterol (HDL-C).

Again the mean (± SE) plasma low density lipoprotein cholesterol (LDL-C) (mg/dl) before- and after- supplementation was 112.56 ± 6.34 and 99.87 ± 4.88 respectively. A significant (p = 0.0019), 11.27% reduction was observed (Fig. 5).

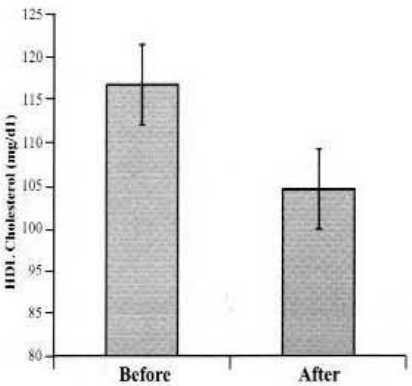

Fig. 5. Plasma concentration of Low Density Lipoprotein Cholesterol (LDL-C).

Obtained findings of this study shows that *Lentinus edodes* significantly reduces plasma total cholesterol and LDL- cholesterol. It also reduces plasma TG, although it is statistically non significant. Also it is observable that *Lentinus edodes* has ability to elevate plasma HDL-cholesterol which was termed as "good cholesterol" significantly. Although few studies were conducted in different part of the World with relationship between *Lentinus edodes* consumption with blood lipid profile but most of them were limited in animal subjects. In a study it was observes that dietary *Lentinus edidus* decreased serum concentrations of a number of polar lipids in rats (Yu, *et al.* 2010).

To date, some studies demonstrate the ability of *Lentinus edodes* in both decrease very low density lipoproteins (VLDL) as well as high density lipoproteins (HDL) (Oba, *et al.*, 2009; Shimada, *et al.*, 2002). The reported cholesterol-lowering effects of dietary *Lentinus edodes* have been attributed to the mycochemical eritadenine (Fukushima, *et al.*, 2001; Kaneda and Tokuda, 1966; Chibata, *et al.*, 1969; Rokujo, *et al.*, 1970). Dietary eritadenine regulates lipid metabolism with differing effects on lipid molecular types. For example, eritadenine increased the proportion of the 16:0–18:2 molecular species and decreased that of the 18:0–20:4 species in the plasma lipoprotein phosphatidylcholines of male rats respectively fed cholesterol-free and cholesterol-enriched diets (Shimada, *et. al.*, 2003). Again Free radicals and reactive oxygen and reactive nitrogen species play important roles in the pathogenesis of cardiovascular and cerebrovascular diseases and various cancers (Niki, 2011). Antioxidants, which can reduce oxidative stress, are thought to be of central importance in the prevention of such diseases. *Lentinus edodes* also contain another active compound Chitin. Chitin is insoluble in water and grouped as functional fibre to lower blood lipids (Van Der Kamp, *et al.*, 2003; Nwe and W. Stevens, 2002).

Findings of the study suggests that regular consumption of *Lentinus edodes* can improve different blood lipid status of female subjects and thus able to improve various life threatening diseases like, ischemic heart disease, brain stroke etc.

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Performance of Golden Oyster Mushroom (*Pleurotus citrinopileatus*) Strains Available at MDI

Afsana Mimi[1], Md. Anwarul Haque[1], Md. Ruhul Amin, Nirod Chandra Sarker and Akhter Jahan Kakon

Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

The present study was to evaluate the performance of different strains of golden oyster (*Pleurotus citrinopileatus*) mushroom. All the strains are commonly shown similar qualitative characters. Yield and growth related attributes such as days required for opening to pinhead initiation, first harvest, total harvest, number of fruiting body, number of effective fruiting body, length and diameter of stipes and biological efficiency (%) were showed significant difference among the strains. The diameter and thickness of pileus were not differed significantly. The highest (40.00) numbers of fruiting body was found in PO96-2 and the lowest (20.25) number of fruiting body was found in PY-1. The highest NEFB (22.56) was found in PO96-2 which was statistically simillar to PO96-1 (20.06) and the lowest NEFB was found in PY-1 (12.81) which was followed by PY-2 (12.88). The highest biological yield (126.90g/packet) was found in PO96-2 and the lowest biological yield was found in PY-1 (72.88g/packet). Present study also revealed that the strains were mostly similar with each other except some variation.

Keywords: Golden oyster, Biological efficiency, Strains.

INTRODUCTION

Golden oyster mushroom is definitely an impressing edible mushroom species especially due to its distinctive taste and delightful color. It is native to eastern Russia, northern China and Japan and like other species of oyster mushroom; it is a wood-decay fungus. The fruiting bodies of *P. citrinopileatus* grow in clusters of bright yellow to golden brown caps with a velvety, dry surface texture. It contains useful antitumor polysaccharides (Zhang *et al*., 1994 and Wang *et al*., 2005) and it has antioxidant activities (Hu *et al*., 2006). This mushroom enhances immunity and delay aging (Wang *et al*., 2001). It is delicious in taste and rich in nutrients (Ghosh *et al*., 1991). Commercial production of edible mushrooms represents unique exploitation of the microbial technology for the bioconversion of the agricultural, industrial, forestry and house-hold wastes into nutritious food (mushrooms). Mushroom plays important roles in nutritional, medicinal, environmental and socio-economical fields. Edible mushrooms provide a good supplement to the diet including proteins, carbohydrates, valuable salts and vitamins, in addition to meat and vegetables.

Though different types of oyster mushrooms (*Pleurotus spp.*) are cultivated in our country for their growing suitability and have nutritional as well as medicinal importance but among these, *Pleurotus citrinopileatus* is the special one. The present study was to evaluate the growth and yield performance of different strains of golden oyster mushroom.

MATERIAL AND METHODS

The experiment was carried out in the laboratory, workshop and culture house of Mushroom Development Institute (MDI), Sobhanbag, Savar, Dhaka from January to June 2015. Strains

[1] Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh.

of golden oyster mushroom (*Pleurotus citrinopileatus)* used in the experiment which was collected from germplasm centre of MDI. Four strains of *Pleurotus citrinopileatus* (PO96-1, PO96-2, PY-1 and PY-2) were available at MDI.

Preparation of pure culture: Pure culture of four strains were prepared on potato dextrose agar (PDA) medium containing 200g peeled and sliced potato, 20g dextrose and 20g agar per liter. The medium was poured into test tube at 10 ml/tube. The medium in test tube was sterilized in an autoclave for 20 minutes at 121^0 C under 1.5 kg/cm^2 pressure. After sterilization and solidification, the tubes were inoculated separately with the inoculants of above mentioned 4 strains. After inoculation, the tubes were covered with cotton plug. All operations were done under sterile condition in a clean bench. The inoculated tubes were incubated in a growth chamber at 22 ± 2^0 C. After completion of the whitish mycelium, this culture was used for inoculation of mother culture.

Preparation of mother culture: To prepare mother culture of test mushroom sawdust and wheat bran mixed together at the ratio of 2:1 (v/v). Water was added to adjust moisture content at 65% and $CaCO_3$ was mixed at the rate of 0.2% of the mixture. Polypropylene bags of 18 cm × 25 cm size were filled with 300 g of the above prepared mixture and packed tightly. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the volume of the bag was made for space to put the inoculums. The neck was plugged with cotton and covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for one (1) hour at 121^0 C under 1.5 kg/cm^2 pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. Individually, a piece of stock PDA culture of each strain containing mycelium was placed aseptically in the hole of mother culture packet and the packet was again plugged as mentioned above. The inoculated packets were placed on a rack in the laboratory at 22 ± 2^0 C temperatures for incubation. The substrate of the mother culture was colonized by the growth of whitish mycelium within 15 - 20 days after inoculation. The fully colonized packets were used for spawning.

Preparation of spawn packet: The substrate of spawn packets were prepared using sawdust and wheat bran mixture at the ratio of 2:1. Water was added to make the moisture level about 65% and $CaCo_3$ was added at 0.2% (w/w) of the mixture. The substrate mixture was poured into 18 cm × 25 cm polypropylene bags at 500 g/bag. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the bag was made for space to introduce the inocula. The neck of each poly bags was plugged with cotton, covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for 2 h at 121^{0}C under 1.1 kg/cm^2 pressures. After sterilization, the packets were cooled and transferred to an inoculation chamber. The packets were inoculated separately with the mother culture of the four strains at the rate of two tea spoonful per packet. The inoculated packets were incubated at 22 ± 2^0 C.

Cultivation conditions for fruiting: After mycelium maturation all the packets were transferred to the culture house and the brown paper, rubber bands, cotton plug and plastic neck of the spawn packets were removed and the mouths of the polypropylene bags were wrapped and tied with rubber bands. The plastic bags were opened by "D" shaped cut on the shoulder side and removed the sheet. The opened surface of substrate was scraped slightly with a blade for removing the thin whitish mycelial layer. Then the packets were placed separately on the rack of culture house under natural condition. Water was applied per day as required and proper aeration was maintained in culture house for the release of excess CO_2 and supply of sufficient O_2 as required for the development of primordia and fruiting body.

Experiment design, data collection and analysis: The experiment was laid out following completely randomized design (CRD) with 4 replications and each replication contain sixteen populations. Data on days required for pin head initiation, days required for first harvesting, days required for total harvest, number of fruiting bodies, number of effective fruiting bodies, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, biological yield and biological efficiency were recorded. Biological yield gm/500g packet was recorded by weighing the whole cluster of fruiting body without removing the lower hard and dirty portion. The Biological Efficiency was determined by using the following formula:

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

Data were analyzed according to Gomez and Gomez method (1984) using MSTAT-c computer program and Excel software. Means separation were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

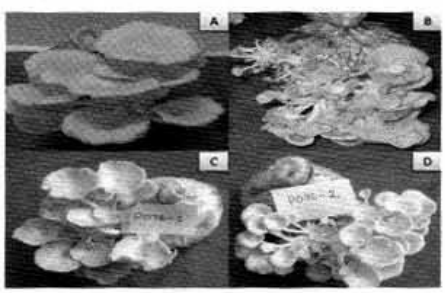

Fig. 1. Fruiting bodies of different strains of Golden oyster mushroom. Here, A=PY-1; B=PY-2; C=PO96-1; D=PO96-2.

RESULTS AND DISCUSSION

The four strains of golden oyster mushroom were commonly shown similar qualitative characters (Table 1). Pileus convex at first depressed when old, tapering to downwards, pileus color yellow to golden rod yellow, pileus glabrous, pileus texture smooth, pileus margin laciniate, firm, smooth, margin often splitting. Stipe usually lateral, short, sometimes elongated, hollow, stipe color navajo white. Gill spacing was always crowded of all these four strains of *Pleurotus citrinopileatus.*

Table 1. Qualitative characters of different strains of *Pleurotus citrinopileatus*

Characters	Golden oyster mushrooms			
	PY-1	PY-2	PO96-1	PO96-2
Pileus shape	Convex at first tapering to downward	Convex at first tapering to downward	Convex at first tapering to downward	Convex at first tapering to downward
Pileus color	Yellow to golden rod yellow	Yellow to golden rod yellow	Yellow to golden rod yellow	Yellow to golden rod yellow
Pileus texture	Smooth	Smooth	Smooth	Smooth
Pileus margin	Laciniate	Laciniate	Laciniate	Laciniate
Stipe color	Navajo white	Navajo white	Navajo white	Navajo white
Stipe texture	Smooth	Smooth	Smooth	Smooth
Gill attachment to stipe	Descending	Descending	Descending	Descending
Gill spacing	Crowded	Crowded	Crowded	Crowded

Days Required for Opening to Pinhead Initiation (DROPI): The DROPI varied significantly among the strains of *Pleurotus citrinopileatus* ranged from 3.25 to 4.87 days (Table 2). The highest days required in PY-1 (4.87 days) for DROPI which was significantly different from others. The lowest (3.25) days required from opening to PI in PO96-2, which was similar to other strain.

Days Required to First Harvest after Pinhead Initiation (DFH): Days required to first harvest after pinhead initiation was significantly influenced by the strains and ranged from 2.87 to 4.00 days (Table 2). The highest days required for the first harvest was observed in PY-1(4.00 days), which was statistically similar to PY-2 (3.62days). On the other hand, it was the lowest (2.87 days) in PO96-2 which was followed by PO96-1 (3.06 days).

Days Required for Total Harvest from Pinhead Initiation (DTH): The DTH (three flush) after pinhead initiation was shown significantly difference among the strains of *Pleurotus citrinopileatus* (Table 2). The highest DTH was found in PO96-2 (34.31days) which is statistically similar to PO96-1 (33.44 days). The lowest DTH was found in PY-1 (29.81 days) which is significantly different from other strains. The PY-2 required 31.06 days for total harvest after pinhead initiation.

Number of Fruiting Body (NFB): The number of fruiting body significantly differed among the strains (Table 2). The highest (40.00) numbers of fruiting body was found in PO96-2. In case of PO96-1 and PY-2 the number of fruiting body were 34.50 and 23.25 respectively. The lowest (20.25) number of fruiting body was found in PY-1.

Table 2. Significance of different strains of Golden oyster mushroom on yield and yield related characters

Name of strains	DROPI	DFH	DTH	NFB	NEFB	BY
PY-1	4.87 a	4.00 a	29.81 b	20.25 d	12.81 b	72.88 d
PY-2	3.62 b	3.62 a	31.06 ab	23.25 c	12.88 b	91.56 c
PO96-1	3.50 b	3.06 b	33.44 a	34.50 b	20.06 a	110.00 b
PO96-2	3.25 b	2.87 b	34.31 a	40.00 a	22.56 a	126.90 a
CV(%)	8.88	10.59	5.06	6.53	14.00	2.04

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

Here, DROPI=Days Required from Opening to Pinhead Initiation, DFH=Days Required to First Harvest after Pinhead Initiation, DTH=Days Required for Total Harvest from Pinhead Initiation, NFB=Number of Fruiting Body, NEFB=Number of Effective Fruiting Body, BY=Biological yield (g/packet).

Number of Effective Fruiting Body (NEFB): NEFB was shown significantly difference among the strains of *Pleurotus citrinopileatus* (Table 2). The highest NEFB (22.56) was found in PO96-2 which is statistically simillar to PO96-1 (20.06). The lowest NEFB was found in PY-1(12.81) which is followed by PY-2 (12.88).

Biological yield (g/packet): Significant variation was observed in biological yield (BY) of different strains of golden oyster mushroom (Table 2). The BY was counted from 3 flushes.

The highest BY (126.90g/packet) was found in PO96-2 followed by PO96-1 (110.00g/packet) and the lowest biological yield was found in PY-1 (72.88g/packet). The recorded biological yield of PY-2 was 91.56g/packet.

Biological efficiency (%): Significant variation was observed in biological efficiency (BE). The highest biological efficiency (63.43%) was found in PO96-2 followed by PO96-1 (55.00%) and the lowest biological efficiency was found in PY-1 (36.43%) which was preceded by PY-2 (45.78%) (Fig. 2).

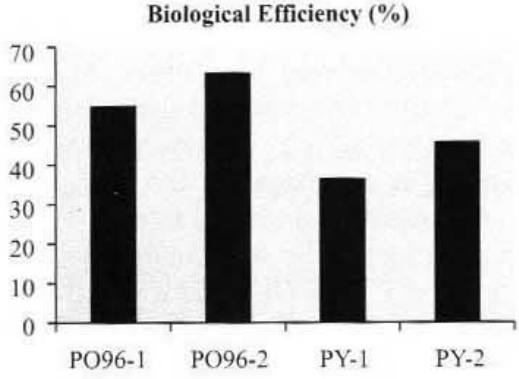

Fig. 2. Biological efficiency of different strains of *P. citrinopileatus* mushrooms.

Diameter of Pileus (DP): The diameter of pileus ranged from 3.46 to 3.81 cm with no significant difference among the strains (Table 3). The highest diameter of pileus was found in PY-1(3.81cm) followed by PO96-1 (3.51cm). The lowest diameter of pileus was found in PO96-2 (3.46 cm) which was followed by PY-2 (3.48cm).

Thickness of Pileus (TP): The thicknesses of pileus in different strains were not significantly different (Table 3). The highest thickness was found in PY-1 and PO96-2 (0.31 cm) which was statistically similar to PO96-1 (0.29 cm) and it was the lowest in PY-2 (0.27 cm).

Length of stipe (cm): The length of stipe ranged from 2.58 to 3.03 cm with significant difference (Table 3). The highest length of stipe was found in PO96-1 (3.03cm) which was statistically similar PO96-2 (2.96cm). It was significantly different from PY-1 (2.83cm). The lowest length of stipe was found in PY-2 (2.58cm).

Diameter of stipe (cm): The diameter of stipe was differed significantly among the different strains (Table 3). The highest diameter was found in PY-2 (0.47 cm) followed by PO96-2 (0.45 cm) and the lowest diameter of stipe was found in PO96-1 (0.38cm). On the other hand, the diameter of stipe was 0.41cm in PY-1.

Table 3. Quantitative characters of different strains of *Pleurotus citrinopileatus*

Name of strains	Pileus diameter (cm)	Pileus thickness (cm)	Stipe length (cm)	Stipe diameter (cm)
PY-1	3.81 a	0.31 a	2.83 ab	0.41 bc
PY-2	3.48 a	0.27 a	2.58 b	0.47 a
PO96-1	3.51 a	0.29 a	3.03 a	0.38 c
PO96-2	3.46 a	0.31 a	2.96 a	0.45 ab
CV(%)	11.37	8.55	7.07	6.71

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

The four strains of golden oyster mushroom were commonly shown similar qualitative characters (Table 1). Pileus convex at first depressed when old, tapering to downwards, pileus color yellow to golden rod yellow, pileus glabrous, pileus texture smooth, pileus margin laciniate, firm, smooth, margin often splitting. Stipe usually lateral, short, sometimes elongated, hollow, stipe color navajo white. Gill spacing was always crowded of all these four strains of *Pleurotus citrinopileatus*. Afsary *et al.* (2013) reported that pileus color yellow to golden red yellow, pileus fragile, basidiospores oblong, sporeprint ochrosporae of *Pleurotus citrinopileatus*.

The highest days required from opening to pin head initiation (DROPI) was in PY-1 (4.87 days) which is significantly different from others (Table 2). This result is partially similar with Howlader *et al.* (2010) who reported that oyster mushroom needs 3 to 17.75 days for pinhead initiation. The lowest 3.25 days were required from opening to pinhead initiation in PO96-2. This result is supported by the findings of Amin *et al.* (2007) who reported that DROPI for oyster mushroom ranged from 3-4 days. These may be due to use of different substrates or supplements. The maximum days required for first harvest after pinhead initiation was observed in PY-1 (4.00) and it was the lowest days in PO96-2 (2.87 days). Moonmoon *et al.* (2012) reported that PY-1and PY-2 required for first harvest were 3.50 and 3.25 days respectively which corroborate with the present study. The highest days required for total harvest (three flush) was found in PO96-2 (34.31days) and the lowest DTH required for PY-1 (29.81 days) which is significantly different from other strains.

In case of yield and yield related attributes, the number of fruiting body, effective fruiting body, biological yield and biological efficiency were significantly differed among the different strains. The highest (40.00) and the lowest (20.25) numbers of fruiting body was found in PO96-2 and PY-1 respectively. This result is partially similar with Moonmoon *et al.* (2012) who observed that the minimum number of fruiting body was found in PY-1 (27.25) and PY-2 (28.75). The highest number of effective fruiting body (22.56) was found in PO96-2 which is statistically simillar to PO96-1 (20.06) and it was the lowest in PY-1(12.81). This result differed with Shelly *et al.* (2010) who found that the NEFB was 47.00 in *P. citrinipileatus* when rice straw substrate was supplied. It may be due to use of different substrate, because sawdust was used as substrate in the present investigation.

Significant variation was observed in biological yield (BY) of different strains of golden oyster mushroom. The highest BY (126.90g/packet) was found in PO96-2 followed by PO96-1 (110.00g/ packet) and the lowest biological yield was found in PY-1 (72.88g/packet). This result differed with Shelly *et al.* (2010) who found that the BY of *P. citrinopileatus* was 155.50 g/packet on rice straw substrate. The highest biological efficiency (63.43%) was found in PO96-2 and the lowest biological efficiency was found in PY-1 (36.43%). This result is supported by the findings of Moonmoon *et al.* (2010) who observed that the biological efficiency of *P. citrinopileatus* was ranged from 12% to 83% due to different supplement supplied in saw dust.

On the other hand, the diameter and thickness of pileus were not differed significantly among the strains. The highest diameter of pileus was found in PY-1(3.81cm) and the lowest diameter of pileus was found in PO96-2 (3.46 cm) and PY-2 (3.48cm) respectively. Moonmoon *et al.* (2012) observed diameter of pileus ranged from 3.15 cm to 3.22 cm in PY-1 and PY-2. The highest thickness was found in PY-1 and PO96-2 (0.31 cm) and it

was lowest in PY-2 (0.27 cm). Shelly *et al.*, 2010 observed thickness of pileus ranged from 0.35cm on *Pleurotus citrinopileatus* which is similar with the present study.

The length and diameter of stipe differed among the different strains significantly. The highest length of stipe was found in PO96-1 (3.03cm). The lowest length of stipe was found in PY-2 (2.58cm). This result is supported by the findings of Afsary *et al.* (2013) who observed that the length of stipe was 3.4 cm in *P. citrinopileatus* species. The highest diameter was found in PY-2 (0.47 cm) and the lowest diameter of stipe was found in PO96-1 (0.38cm). Afsary *et al.* (2013) also reported that the diameter of stipe was 0.4 cm in *P. citrinopileatus*.

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Effect of Age of Spawn Packet on the Growth and Yield of Shiitake Mushroom (*Lentinus edodes*)

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

Abstract

The present study was conducted at Mushroom Development and Extension Centre, Sobhanbagh, Savar, Dhaka during the period from October 2012 to March 2013 to determine the right age of spawn packet and to select the best strain for shiitake mushroom cultivation. In this experiment ten different age (40, 50, 60, 70 80, 90, 100, 110, 120 and 130 days) of spawn packets and two strains (Le 8 and Le 16) of shiitake mushroom were cultured. A wide variation was observed in yield and biological efficiency in different ages. The highest yield (179.50g) and highest biological efficiency (102.60%) were recorded from the treatment combination of 90 days old spawn packet with the strain Le 16, followed by 100 and 110 days old spawn packets with same strain. The lowest yield (36.75g) and lowest biological efficiency (21.00%) were found in 130 days old spawn packet with Le 16 strain. No yield was obtained from the 40 and 50 days old spawn packets. Findings of this study give idea to take decision about the right age and strain of shiitake mushroom which might be helpful for mushroom growers.

Keywords: Age, Growth, Strain, Yield.

INTRODUCTION

Shiitake mushroom (*Lentinus edodes*) is commonly used in China and Japan as high valued food and medicine. It is produced on different kinds of lignocelluloses substrates. The production system of shiitake mushroom is quite different from other edible mushrooms. There are two period of mushroom cultivation, the incubation period and the harvesting period. During the incubation period, the mycelium colonizes on the substrates with some distinct stages, such as, mycelium running, thickening, pigmentation, hardening and bumping for growth improvement (Stamets, 1993). It is gaining popularity among the potential mushroom growers as well as perspective consumers owing to attractive shape and size, simple growing technique, low capital investment, wide substrate range, sustainable yield, long shelf-life and ability to thrive in a wide range of climatic conditions. The incubation period of shiitake mushroom is very crucial which can affect the yield. Quality and quantity of spawn play an important role in the successful production of any mushroom species. Spawn age is an important factor for growth and development of shiitake mushroom. Short days/ age of spawn packet and so many older days of spawn packet give lower yield. But an appropriate day the spawn packet gives the better performance. Mushroom yield decreased with increase in spawn age after a certain age. Considering the facts, the present work was undertaken to determine the right age of spawn packet for shiitake mushroom cultivation to get higher yield and better quality.

MATERIALS AND METHODS

The experiment was conducted at National Mushroom Development and Extension centre, Savar, Dhaka during October 2012 to March 2013 to determine the right age of spawn packet and to select the best strain for shiitake mushroom cultivation. Ten

different age (40, 50, 60, 70 80, 90, 100, 110,120 and 130 days) of spawn packets and two strain (Le 8 and Le 16) of shiitake mushroom were used in the experiment (Plate 1).

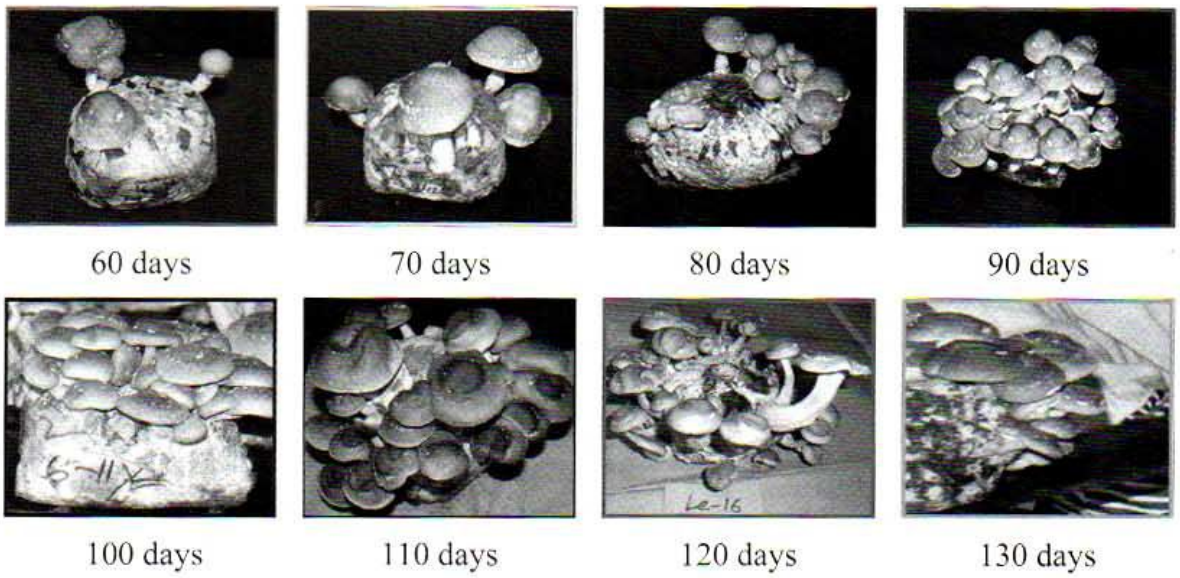

Plate: Different age of spawn packet

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

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

placed aseptically in the hole of mother culture packet and again plugged the packet as mentioned above. The inoculated packets were placed on a rack in the laboratory at 22 ± 2°C temperatures for incubation. The substrate of the mother culture was colonized by the growth of whitish mycelium within 15-20 days after inoculation. The fully colonized packets were used for spawning.

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

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

Opening of spawn packet: The inoculated packets were placed on a steel rack at 22 ± 2°C temperature for incubation. Whitish mycelia began to grow on the substrate and after full colonization a thick mycelial coat forms on the outer surface of colonized substrate. To determine the right age of spawn, the packets were fully opened at 40, 50, 60, 70, 80, 90, 100, 110, 120 and 130 days after inoculation.

Cultivation conditions for fruiting: Treatment wise, the packets were moved to culture house and placed on racks. The temperature, relative humidity and light were maintained at 18-22 °C, 70-80% and 10-20 lux respectively. Watering was done 3 to 4 times per day to maintain temperature and relative humidity. Excess CO_2 was removed by exhaust fan.

Data collection and analysis: The experiment was laid out in a completely randomized design (CRD) with 4 replications. Data on time required from opening to first harvest (days), time required for harvest (days), number of fruiting body, number of effective fruiting body, length of stalk (cm), diameter of stalk (cm), diameter of pileus (cm), thickness of pileus (cm), yield (g) and biological efficiency (%) were recorded. Yield in g/packet was recorded by removing the dirty portion of fruiting bodies. Biological efficiency was determined by following formula:

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

The data were analyzed following MSTAT-C computer program and means were computed and separated following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSIONS

Time required from opening to first harvest (TROFH): The effect of strain on different age of spawn packet on time required from opening to first harvest was found to be significant. The highest time (30.00 days) required from opening to first harvest was found from the strain Le 8 with 60 days old spawn packet treatment combination and the lowest time (4.00 days) required from opening to first harvest was found from the strain Le 16 with 90 days old spawn packet treatment combination (Table 1).

Table 1. Effect of strain and different age of spawn on growth and yield attributes of shiitake mushroom

Age of spawn(days)	Time required from opening to first harvest (days)	Time required for harvest	Number of fruiting body	Number of effective fruiting body
Strain of shiitake mushroom (Le 8)				
40	-----	-----	-----	-----
50	-----	-----	-----	-----
60	30.00a	90.00i	2.50i	1.50g
70	25.00b	95.00h	5.75hi	3.50fg
80	17.25e	97.25g	15.25f	12.50e
90	11.25f	101.30f	21.00de	17.00cd
100	7.75g	107.80d	29.00b	22.50b
110	5.75ghi	115.80c	29.75b	25.00b
120	6.00ghi	126.00b	17.75ef	14.25de
130	7.00gh	137.00a	21.50cd	15.75cde
Strain of shiitake mushroom (Le 16)				
40	-----	-----	-----	-----
50	-----	-----	------	-----
60	22.75c	82.75j	4.75hi	3.00fg
70	19.75d	89.75i	7.25gh	5.00f
80	11.75f	91.75i	4.75hi	3.00fg
90	4.00i	94.00h	44.50a	32.25a
100	5.25hi	105.30e	45.25a	32.75a
110	6.75gh	116.80c	23.00cd	15.25de
120	4.75hi	125.50b	25.00c	19.00c
130	5.00hi	135.00a	9.50g	5.75f
CV (%)	**12.83**	**1.36**	**12.29**	**15.51**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT. ---- = Not produce fruit body.

Time required for harvest (TRH): Time required for harvest was found being significant in different strains of shiitake mushroom under different age of spawn packet. The highest time (137.00 days) required for harvest was found from the strain Le 8 with 130 days old of spawn packet treatment combination which was statistically similar to the treatment combination of Le 16 with 130 days old of spawn packet. The lowest time (82.75 days) required for harvest was found from the strain Le 16 with 60 days old spawn packet treatment combination (Table 1).

Number of fruiting body (NFB): Significant variations were observed in number of fruiting body by the combined effect of strain of shiitake mushroom with different age of spawn packet. The number of fruiting bodies ranged from 2.50 to 45.25. The highest

number (45.25) of fruiting body was recorded from the strain Le 16 with 100 days old spawn packet which was statistically similar to the same strain with 90 days old spawn packet. The lowest number (2.50) of fruiting body was recorded in 60 days age of spawn packet with the strain Le 8 (Table 1).

Number of effective fruiting body (NEFB): The number of effective fruiting body was found to be significant by the combined effect of strain and different age of spawn packet. The highest number (32.75) of effective fruiting body was recorded from the strain Le 16 with 100 days old spawn packet which was statistically similar to the same strain with 90 days old spawn packet. The lowest number (1.50) of effective fruiting body was recorded in 60 days age of spawn packet with the strain Le 8 (Table 1).

Length of stalk (LS): The length of stalk was significantly different in two strains of *Lentinus edodes* when cultured on different age of spawn packet ranged from 3.15 to 5.75 cm. The highest length (5.75cm) of stalk was found from the treatment combination of strain Le 8 with 80 days old of spawn packet which was statistically similar to the treatment combination of Le 8 with 60 days old of spawn packet and Le 16 with 60 days old of spawn packet. The lowest length (3.15cm) of stalk was recorded from the treatment combination of strain Le 16 with 130 days old of spawn packet (Table 2).

Table 2. Effect of strain and different age of spawn packet on size of fruiting body of shiitake mushroom

Age of spawn (Days)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Strain of shiitake mushroom (Le 8)				
40	-----	-----	-----	-----
50	-----	-----	-----	-----
60	5.58a	1.35c-f	6.68efg	1.70b
70	4.95b	1.25def	7.00def	1.38cd
80	5.75a	1.75ab	6.00h	1.10e
90	3.83d	1.18ef	6.53fgh	1.38cd
100	3.60d	1.55bc	6.58fg	1.10e
110	3.75d	1.48cd	6.30gh	1.18cde
120	3.85d	1.15ef	7.35d	1.10e
130	4.63bc	1.30c-f	7.00def	1.43c
Strain of shiitake mushroom (Le 16)				
40	-----	-----	-----	-----
50	-----	-----	-----	-----
60	5.55a	1.38cde	9.28c	1.88b
70	5.02b	1.48cd	10.00b	1.43c
80	4.68bc	1.95a	10.95a	2.15a
90	3.83d	1.10f	6.25gh	1.28cde
100	3.63d	1.43cde	5.38i	1.13de
110	4.88bc	1.43cde	7.18de	1.70b
120	4.48c	1.28c-f	6.43gh	1.23cde
130	3.15e	1.30c-f	4.40j	1.02e
CV (%)	**6.30**	**11.85**	**5.02**	**11.91**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.---- = Not produce fruit body.

Diameter of stalk (DS): The diameter of stalk was highly significant by the combined effect of strain and different age of spawn packet. The highest diameter (1.95 cm) of stalk was observed from the treatment combination of strain Le 16 with 80 days old of spawn packet and the lowest diameter (1.10 cm) of stalk was observed from the treatment combination of strain Le 16 with 90 days old of spawn packet (Table 2).

Diameter of pileus (DP): The combined effect of two strains and different age of spawn packet on diameter of pileus were found significant. The highest diameter (10.95 cm) of pileus was recorded from the treatment combination of strain Le 16 with 80 days old of spawn packet. The lowest diameter (4.40 cm) of pileus was observed from the treatment combination of strain Le 16 with 130 days old of spawn packet (Table 2).

Thickness of pileus (TP): The thickness of pileus was found significant by the combined effect of two strains and different age of spawn packet. The highest thickness (2.15cm) of pileus was recorded from the treatment combination of strain Le 16 with 80 days old of spawn packet. The second highest thickness (1.88cm) of pileus was recorded from the treatment combination of strain Le 16 with 60 days old of spawn packet which was statistically similar to the treatment combination of Le 8 with 60 days old of spawn packet. The lowest thickness (1.02 cm) of pileus was observed from the treatment combination of strain Le 16 with 130 days old of spawn packet (Table 2).

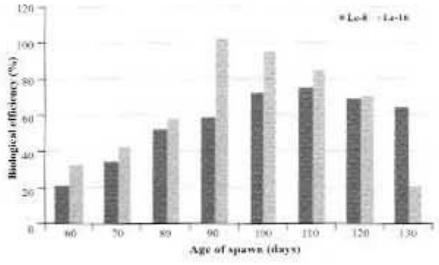

Fig. 1. Effect of strain and age of spawn packets on yield of shiitake mushroom.

Yield: Appreciable variation was found in the yield from the treatment combination of two strains of shiitake mushroom with different age of spawn packet. The highest yield (179.50g) was recorded from the treatment combination of strain Le 16 with 90 days old of spawn packet followed by 100 and 110 days old of spawn packet with the same strain. The lowest yield (36.75g) was recorded from the treatment combination of strain Le 16 with 130 days old of spawn packet which was statistically similar to 60 days old of spawn packet with Le 8. The yield decreased with the increase of age of spawn packet because more age of spawn packet becomes dry and aborted. 40 and 50 days old spawn packet did not produce any fruit body (Fig. 1.).

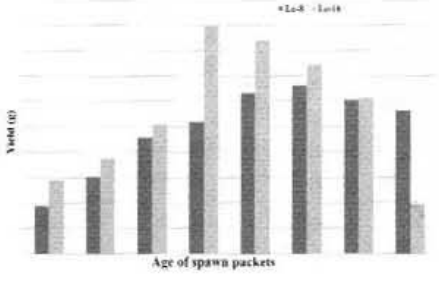

Fig. 2. Effect of strain and age of spawn packets on biological efficiency of shiitake mushroom.

Biological efficiency (BE %): Remarkable variation was observed in biological efficiency from the treatment combination of different strain of shiitake mushroom with different age of spawn packet. The highest biological efficiency (102.60%) was obtained from the treatment combination of Le 16 with 90 days old of spawn packet which was statistically similar to the same strain with 100 days old of spawn packet. The lowest biological efficiency (21.00%) was recorded from the treatment combination of Le 16 with 130 days old of spawn packet which was statistically similar to the strain Le 8 with 60 days old of spawn packet (Fig. 2.).

Results of the present experiment reveal that there are appreciable variations in yield and yield contributing attributes with the variation of age of spawn packets and strain of shiitake mushroom. In terms of yield and yield attributes performance of the strain Le 16 was better than Le 8. In case of different age of spawn packets 100 days old spawn packet gave the highest yield while 40 and 50 days old spawn packet did not produce any fruit body. The yield decreased with the increase of age of spawn packets. On the other hands the spawn opens too early the crop may be failed. Many other investigators also found variations in effect of age of spawn packets on growth, yield and yield contributing characters of shiitake mushroom.

Ahmed *et al.*, 2010 reported that the lowest days required from opening to first harvest was observed in spawn packets opened after 130 days of spawning which was statistically similar to all the spawn age except 80 and 90 days. The highest days required from opening to first harvest was recorded in 80 days spawn age.

Leatham (1985) reported that shorter period of spawn run gave limited fruiting bodies and longer period 105 to 150 days gave more fruiting bodies. Ahmed *et al.* (2010) also reported that highest number of fruiting body was recorded in 110 days old spawn packet which was statistically similar to 120 days old spawn packet where as the lowest number of fruiting body was recorded in 80 days old spawn.

Ahmed *et al.* (2010) described that yields were increased with the increase of age of spawn packets. Similar result was reported by Royse (1985). Ahmed *et al.* (2010) also reported the highest yield was obtained from 110 days old spawn packet which was statistically similar to 120 and 130 days old spawn and the lowest yield was recorded in 80 days old spawn.

Ahmed *et al.* (2010) reported that the highest biological efficiency was recorded in 110 days old spawn which was statistically similar to 120 days and 130 days old of spawn packet. The lowest biological efficiency was observed from 80 days old of spawn packet. Almost similar result was reported by Royse and Bahler (1986) stating that biological efficiency increased with the increase of incubation time.

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Effect of Media of Mother Culture on Yield of Milky White Mushroom

Akhter Jahan Kakon, Nirod Chandra Sarker, Rakib Al Hasan and Md. Bazlul Karim Choudhury[1]

Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

In this experiment pasteurized sawdust and rice straw were used as substrate for the cultivation of *Calocybe indica* mushroom. Different types of media for the preparation of mother culture were used as treatments. The highest number of fruiting body (7.98), the maximum total yield (463.0g) and biological efficiency (147.00%) were recorded from the treatment T_1 where maize grain was used as mother culture. The lowest number (4.00) of fruiting body, minimum total yield (208.0g) and biological efficiency (65.94%) were recorded from the treatment T_4 where sawdust was used as mother culture. The maximum average weight of fruiting body (61.85g) was recorded from the treatment T_3 where wheat grain was used as mother culture and the minimum average weight of fruiting body (50.50g) was recorded from the treatment T_2 where rice grain was used as mother culture. The highest length of stalk (11.27cm) and diameter of stalk (2.60 cm) were found from the treatment T_4 whereas the lowest length of stalk (7.75cm) and diameter of stalk (2.08 cm) was recorded from the treatment T_2. The highest diameter of pileus (8.75cm) and thickness of pileus (2.05 cm) were found from the treatment T_3.

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

INTRODUCTION

Calocybe indica is one of the best edible mushrooms which can be grown at high temperature. The cultivation process resembles that of oyster mushrooms but for the additional process of casing. The advantages of this mushroom over other mushrooms are easy method of cultivation, less investment, very attractive fruiting body, pleasing milk white color, long shelf life, more nutritious and less time to grow (Bokaria *et al.*, 2014). Spawn is the seed or vegetative mycelium of mushroom required for mushroom cultivation. Spawn quality is counted the most important part in mushroom production (Mohammadi Goltapeh and Purjam, 2003). A media of mother culture is an important substance for making spawn and growing mushrooms. Various kinds of grains including wheat, paddy, maize, bajra, millet and sawdust substrate can be utilized for cultivation of *Calocybe indica*. Various kinds of grains were successfully used by different workers to prepare the spawn (Amle *et al.*, 2007 and Senthilnambi *et al.*, 2011). Sorghum or wheat grains were found to be the best substrates for *C. indica* spawn production (Purkayastha & Chandra, 1976 and Krishnamoorthy & Muthuswamy, 1997). Spawn grains of advantages was mycelium the same growth in all grain surface and it was cause of the same distribution (Mottaghi, 2004). Now a days, grains are very suitable of carrier, because it had hardly shell which aleurone layer was containing of protein and starch. It too will attract very much water by swelling. Therefore, the present experiment was conducted to determine a suitable media of mother culture under Bangladesh condition on yield of milky white mushroom and asses the biological efficiency.

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

MATERIALS AND METHODS

The experiment was conducted in the culture house of Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh from February 2015 to June 2015. In this experiment pasteurized sawdust and rice straw were used as substrate for the cultivation of *Calocybe indica* mushroom. Different media were used as treatments. The treatments were T_1 = Maize grain, T_2 = Rice grain, T_3 = Wheat grain, T_4 = Sawdust. One strain of milky white mushroom (*Calocybe indica*), namely Cid-1 was used as test materials.

Preparation of master mother culture: To prepare mother culture of the test mushroom strain sawdust and wheat bran were mixed together at 2:1 (v/v) and supplemented with $CaCO_3$ at 0.2% (w/w) of the mixture. The moisture level of the mixed substrate was maintained at 65% with tap water. The substrate was poured into polypropylene bags (7" × 10") at 300g/bag. The substrate in bags was sterilized in an autoclave for 1 h at 121°C under 1.1 kg/cm^2 pressures and allowed to cool for 24h. Pure cultures of Cid-1 strains were grown on potato dextrose agar (PDA) following hyphal tip method. A piece of the PDA culture of test strain containing mycelium was placed aseptically in the opening of the mother culture packets. The inoculated packets were placed on a rack in the laboratory at 22 ± 2 °C for incubation. The substrate of the mother culture was covered by whitish mycelium within 15-20 days after inoculation. The fully colonized packets were used as master mother for inoculation of mother culture.

Preparation of mother culture (paddy): To prepare mother culture of the test mushroom (Cid-1) paddy grains were used as media of mother culture. At first grains collected which was free from diseases and not broken, old, and insect damaged. The grains were thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses, weeds, etc. Washed grains were then soaked in sufficient water for 2-3 hours and boiled in a container for 25-45 minutes till the skin started to crack. Excess water from the boiled grains was removed by stirring and heating. Then the grains were thoroughly mixed with calcium carbonate at 0.2% so that the pH of the grains was around 7.0 to 7.8. This mixing was done on the same container after wearing gloves. The substrate was poured into polypropylene bags (9" × 12") at 400-450 g/bag. The necks of the bags were heat resistant plastic and the neck was plugged with cotton wool, covered with paper piece and then tied together by a rubber band. The substrate in bags was sterilized in an autoclave for 2 h at 121°C under 1.1 kg / cm^2 pressures and allowed to cool for 24 h. Then the master mother was poured aseptically at 10% in the opening of paddy grain containing mother culture packets and substrate grain was covered by whitish mycelium within 10-15 days after inoculation. The fully colonized packets were used as mother culture for spawning.

Preparation of mother culture (wheat): Mother culture was prepared by mixing boiled (soft) wheat and calcium carbonate. For 400-450g mother, calcium carbonate was used at the rate of 0.5g per packet. Polypropylene bags of 9" × 12" size were filled with specific amount of the above mentioned mixture and packed tightly. The packets were sterilization, inoculation and incubation in the same process.

Preparation of mother culture (maize): Mother culture was prepared by mixing boiled maize and calcium carbonate. For 400-450g mother, calcium carbonate was used at the rate of 0.5g per packet. Polypropylene bags of 9" × 12" size were filled with specific amount of the above mentioned mixture and packed tightly. The packets were sterilization, inoculation and incubation in the same process.

Preparation of mother culture (sawdust): Mother culture was prepared by mixing sawdust and wheat bran at the ratio of 2:1. Calcium carbonate was used at the rate of 0.2% of the mixture. The moisture level of the mixture was maintained at 65% by adding tap water. Polypropylene bags of 7" × 10" size were filled with 300g of the above mentioned mixture and packed tightly. Then the packets were sterilization, inoculation and incubation in the same process.

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

Preparation of spawn packets: The polypropylene bags of 10" × 14" size were filled with pasteurized substrates and mother culture according to treatments. Pasteurized sawdust, rice straw and different mother culture mixed thoroughly separately without supplementation ie through spawning method was done. Then their mouths were plugged by inserting absorbent cotton with the help of plastic neck. The neck of the bag was prepared by using heat resistant plastic pipe. Substrate mixture was poured into polypropylene bags at 900g / bag. The prepared packets were incubated in culture house at 20-25^{0}C. Thorough spawning of the substrate was also followed in which the spawn was thoroughly mixed with the wet substrate before bagging.

Cultivation for fruiting body: After mycelium maturation, all the packets were fully opened and placed separately on the floor in the culture house. Then casing was performed with sterilized casing soil. Top layer of spawn packet covered with mixture of sand, soil and cow dung etc. is called casing. The casing soil is prepared by steaming garden soil (clay loam, pH around 8.0) for one hour. Apply casing soil on top of the spawn to a height of 1-2 cm. Temperature, relative humidity and light intensity of the culture house were maintained at 30-35^{0}C, 80-90% and 500-600 lux, respectively. Sufficient water was sprayed every day and proper aeration was maintained in culture house for the release excess CO_2 and supply of sufficient O_2 as required for the development of primordia and fruiting bodies. Pinheads appear in 8-10 days after casing and the first harvest can be made in 6-8 days after pinhead formation. After obtaining the first harvest the casing medium is gently ruffled, slightly compacted back and sprayed regularly with water. Second and third harvest may be obtained within 55-60 days of spawn preparation.

Re-casing: The yield obtained from the bags. After three harvesting, on the bags re-casing was done and observation was recorded for yield of *C. indica.*

Collection and analysis of data: The packets were arranged in culture house following completely randomized design with 4 replications. Data on number of fruiting body, length and diameter of stalk, diameter and thickness of pileus, yield (g/packet), average weight of fruiting body and biological efficiency were recorded. Weight of fruiting body was recorded after removing the lower hard and dirty portion of stipe. The biological efficiency was determined using the following formula:

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

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

RESULTS AND DISCUSSION

Total number of fruiting body/packet: The total number of fruiting body was significantly difference. The highest TNFB (7.98) was observed when maize grain containing mother culture was used for cultivation of mushroom which was statistically different to other treatments (Table 1).The lowest TNFB (4.00) was observed when sawdust based mother culture was used for cultivation of mushroom which was statistically similar to wheat grain containing mother culture.

Total Yield (g): The fresh yield of *C. indica* differed significantly with type of grains used, and it was ranged from 208.50 to 463.00g/packet (Table 1). The highest yield (463.0g/ packet) was obtained from maize grain containing mother culture which was followed by wheat grain containing mother culture (256.50 g/packet) while the lowest yield (208.50 g/packet) was observed in sawdust based mother culture. Ragupathi et al. 2016 reported that mean yield is 356 g/bed (contains 250g of paddy straw on dry weight basis). The findings of Amle *et al.* (2007) and Senthilnambi *et al.* (2011) are in close agreements with the present results. They found maize grains and sorghum grains as good substrates for spawn production of *C. indica*.

Yield of first flush/harvest (g): Significant difference was observed in YFH among the different media of mother culture (Table 1). The maximum YFH (260.00g/packet) was recorded in maize grain containing mother culture which was followed by rice grain containing mother culture and it was minimum (137.30g/packet) in wheat grain containing mother culture which was statistically similar to sawdust based mother culture.

Table 1. Effect of different media of mother culture on yield and yield attributes of milky mushroom

Treatments	Total no. of fruiting bodies	Yield (g)			
		1st harvest	2nd harvest	3rd harvest	Total yield
T_1=Maize	7.98a	260.00a	153.00a	50.00a	463.00a
T2=Rice grain	5.25b	158.00b	55.00c	18.50c	231.50c
T3=Wheat grain	4.25c	137.30c	77.25b	42.00b	256.50b
T4=Sawdust	4.00c	139.50c	57.50c	11.50d	208.50d
CV(%)	**3.75**	**1.92**	**2.60**	**6.96**	**1.95**

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

Yield of second flush (g): Significant difference was observed in YSH among the different media of mother culture (Table 1). The maximum YSF (153.00g/packet) was recorded in maize grain containing mother culture which was followed by wheat grain containing mother culture (77.25g/packet). The minimum yield was (55.00g/packet) obtained from rice grain containing mother culture which was statistically similar to sawdust based mother culture. It is very important that the second flush play an important role to give yield in all treatments.

Yield of third flush (g): Significant difference was observed in YTF among the different media of mother culture(Table 1).The maximum YTF (50.00g/packet) was recorded in maize grain containing mother culture which was followed by wheat grain containing mother culture(42.00g/packet). The minimum yield was (11.50g/packet) obtained from sawdust based mother culture.

Individual weight of fruit body (g): Significant difference was observed in average weight of fruiting body among the different media of mother culture (Fig.1). The maximum AVW (60.35g/packet) was recorded in wheat grain containing mother culture which was followed by maize grain containing mother culture (58.02g/packet) and it was minimum (44.09g/ packet) in rice grain containing mother culture. Ragupathi et al. 2016 reported that on an average single mushroom weighs 55-60 g. Anurag *et al.* 2017 also reported the average weight of sporophores varied from 54-85g, The maximum AVW (85.00g/packet) was recorded in wheat grain containing mother culture and it was minimum (54.00g/packet) in maize grain containing mother culture.

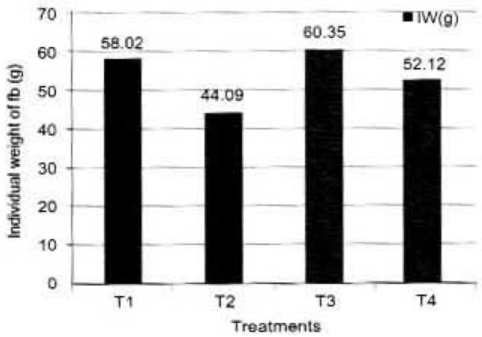

Fig. 1. Effect of media of mother culture on average individual weight of fruiting body.

Biological efficiency: The biological efficiency differed significantly with respect to different grains. The highest biological efficiency (147.00%) was found in T_1 followed by T_3 (81.43%) and the lowest biological efficiency was found in T_4 (65.94%) (Fig.1) whereas Ragupathi *et al.* 2016 reported that biological efficiency of milky mushroom is 143%.

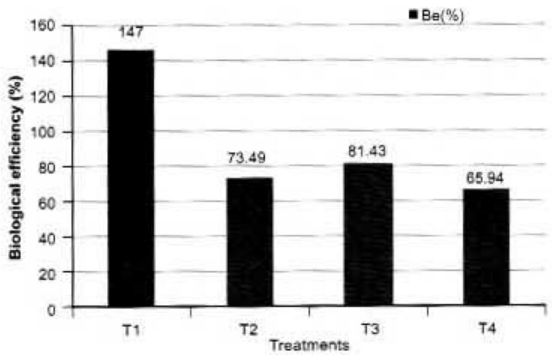

Fig. 2. Effect of media of mother culture on biological efficiency.

Length and diameter of stalk: The other parameters like stalk length differed significantly. The stipe length of *C. indica* was highest in the fruit body produced from spawn packet prepared with sawdust based mother culture (11.27 cm) followed by wheat grain (10.63 cm), and maize (9.38 cm). However, the minimum length was showed by rice (7.75cm)

grain. Stalk diameter did not show any significant difference with the type of grains used. The maximum stalk diameter (2.60 cm) was found from spawn packet prepared with sawdust based mother culture and the minimum length was showed by rice (2.08cm) grain.

Table 2. Effect of different media of mother culture on size of fruiting body of milky mushroom

Treatments	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
$T_{1=}$Maize	9.38c	2.38a	8.88a	1.70b
T2=Rice grain	7.75d	2.08a	8.50a	1.60b
T3=Wheat grain	10.63b	2.50a	8.75a	2.05a
T4=Sawdust	11.27a	2.60a	8.66a	1.65b
CV(%)	**3.49**	**7.79**	**3.91**	**9.62**

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

Diameter and thickness of pileus: Pileus diameter did not show any significant difference but thickness of pileus differed significantly with the type of grains used. The highest diameter of pileus (8.88 cm) was found from spawn packet prepared with maize grain based mother culture and it was minimum when spawn packet prepared with rice (8.50 cm) grain based mother culture. The highest thickness of pileus (2.05cm) was found from spawn packet prepared with wheat grain based mother culture and it was minimum when spawn packet prepared with rice (1.60 cm) grain based mother culture.

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