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Oyster Mushroom (*Pleurotus ostreatus*) Improves Glycemic Status of Normotensive Diabetic Male Volunteers of Bangladesh

Md. Bazlul Karim Choudhury[1], A. J. Kakon, Taufiqur Rahman, Ferdousi Rahman Mowsumi, Md. Burhan Uddin[2], M. Shahabuddin Kabir Choudhuri[3] and Md. Shahdat Hossain[4]

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Abstract

The study was undertaken to investigate the effect of *Pleurotus ostreatus* on glycemic status of normotensive diabetic male volunteers. The human fasting plasma level of creatinine and glucose associated with glycated hemoglobin (HbA1c) were estimated before and three months after administration of mushroom capsule. The feeding of three grams mushroom capsule daily showed no significant effect on plasma creatinine level ($p = 0.903$) but there was significant reduction of fasting plasma glucose ($p = 0.001$). There was also significant reduction of glycated hemoglobin ($p = 0.000$). All of these findings suggest that *Pleurotus ostreatus* may able to improve diabetic status significantly without interfering the renal function.

Key words: Diabetes, *Pleurotus ostreatus,* Plasma glucose, HbA1c.

INTRODUCTION

Pleurotus is a genus of gilled mushrooms which includes one of the most widely eaten mushrooms, *Pleurotus ostreatus*. Species of *Pleurotus* may be called oyster, abalone, or tree mushrooms, and are some of the most commonly cultivated edible mushrooms in the world (Chang and Miles, 2004). It is a delicious edible mushroom and is found throughout the world.

Oyster mushrooms have been revered for thousands of years as both a food and a medicine in the world. Oyster mushrooms are rich in protein, vitamin C, Niacin, Folic acid and potassium. For people with hypertension, obesity and diabetes, oyster mushroom can form part of a diet that is low in sodium, starch, fat and calories. Oyster mushrooms help in reducing the cholesterol level. In addition to their nutritional value oyster mushrooms are claimed to exhibit hypoglycemic and hypotensive properties (Choudhury *et al.*, 2008).

Oyster mushrooms are used in traditional medicine to treat infections, hyperlipidemia, diabetes, and cancer. Increasing evidence in both experimental and clinical studies suggest that there is a close link between hyperglycemia and diabetic complications

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(Rabbani *et al.*, 2009). Studies have shown that the Oyster mushroom has anti-tumor, anti-fungal, lipid lowering, hypoglycemic properties and is known to help regulate the immune system. Mushroom significantly reduced blood pressure, blood glucose, TG and cholesterol of diabetic subjects without any deleterious effect on liver and kidney (Khatun *et al.*, 2007). Oyster mushrooms are high potassium yet have a low sodium/potassium ratio, and are also low in starch, fat and caloric value to control hypertension, obesity and diabetes.

The diabetic animals showed a significant decrease in liver glycogen level but the liver glycogen level was significantly increased when treated with *Pleurotus ostreatus* (Krishna *et al.*, 2009). They have traditionally been used in the treatment and prevention of diabetes, obesity, heart disease, hyperacidity, constipation, cancer, blood pressure and hypertension (Suguna and Usha, 1995).

Almost all of us are familiar with mushrooms and their miraculous and magical powers. Mushrooms can be an ideal low energy diet for controlling blood glucose. The number of diabetic patients in Bangladesh is increasing at an alarming rate. The aim of the study was to evaluate efficacy of oyster mushroom (*Pleurotus ostreatus*) on glycemic control and improving quality of life in type 2 diabetic patients.

MATERIALS AND METHODS

The experiment was conducted between April 2010 and May 2011 in the laboratory of Strengthening Mushroom Development Project, National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka.

In the study 27 normotensive male subjects (systolic BP ranged from 90-130 mmHg and diastolic BP ranged from 60-85 mmHg) aged (years) from 28 to 77, suffering type 2 diabetes and resided at Savar were considered. After getting written consent, the subjects were included. The details history was taken from the subjects including age, sex, occupation, educational status, marital status, family history and drug history.

During the study any acute or chronic disease or medication, malabsorption and any addiction except cigarette smoking were excluded. Female subjects were not included in the study.

Study design: At the beginning of study, health status was being evaluated of the subjects. Fasting blood sample was collected for analysis. Mushroom capsules were supplied to take two capsules three times daily. Each capsule contains 500 mg *Pleurotus ostreatus* powder, so that each subject took 3 gms mushroom powder daily. After three months the subjects were evaluated and all the investigation procedures were repeated. If any drug previously getting by the subjects, it was continued.

Collection of blood sample: 10 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.

Preparation of Mushroom capsule: Fresh fruiting body of *Pleurotus ostreatus* was collected from culture house of National Mushroom Development and Extension Centre. 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.

Measurement of blood pressure: Blood pressure was measured using sphygmomanometer by trend physician. Cuff of the sphygmomanometer was binded on upper two third of the right arm of the subjects. Bell of the stethoscope was placed over bifurcation of right brachial artery, hand pump was used to block the brachial artery and reading of systolic and diastolic blood pressure was taken according to changes of corrotcoffs sound.

Biochemical analysis: Plasma creatinine was estimated to detect renal impairment by using 'alkaline picrate' method. Plasma glucose level was estimated using enzymatic 'Glucose oxidase method'. By using commercially available reagent kit, analysis was done by semi auto biochemical analyzer 3000 evaluation.

Estimation of Glycated hemoglobin (HbA1c): Glycated hemoglobin was estimated by a photometric method using 'Stanbio reagent kit'. In the method a preparation of hemolyzed whole blood was mixed with a weakly binding cation-exchange resin. The non-glycosylated hemoglobin (HbA_0) binded to the resin, leaving (HbA1) free which were removed by means of a resin separator in the supernate. The percent of HbA1 was determined by measuring the absorbance values at 415 nm of the HbA1 fraction and of the total Hb fraction, calculating the ratio of absorbances (R), and comparing this ratio to that of a glycohemoglobin standard carried through the same procedure. Results were expressed as HbA1 but it was converted as HbA1c by using a conversion factor.

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

RESULTS AND DISCUSSION

Oyster mushroom (*Pleurotus spp*) is known in the Indian traditional system of medicine for its antihyperglycemic and antihyperlipdemic potential. Mushrooms are edible fungi confirmed to have definite human health properties and nutrition. Oyster mushrooms have been demonstrated to have beneficial effects in animal and human studies individually as well as in combination. The present study was performed to observe the effect of oyster mushroom (*Pleurotus ostreatus*) on glycemic control and diabetes quality of life.

In this study the mean ± SE plasma creatinine (mg/dl) before and three months after mushroom treatment was 0.81 ± 0.11 and 0.82 ± 0.09 respectively. No significant mean difference of creatinine ($p = 0.903$) was observed (Fig. 1). This finding suggests that there was no significant effect of plasma creatinine in the mushroom-fed subjects.

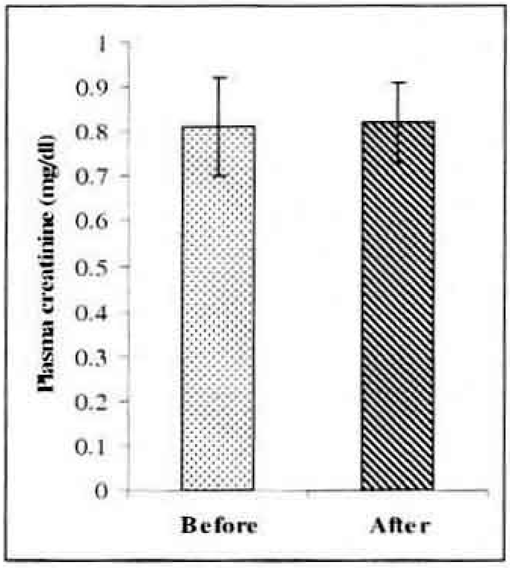

Fig. 1. Mean (± SE) plasma creatinine before and 3 months after mushroom supplementation.

It was observed that the mean ± SE fasting plasma glucose before and 3 months after mushroom supplement was 11.08 ± 1.08 and 8.54 ± 0.63 respectively (Fig. 2). A highly significant mean difference of plasma glucose was observed ($p = 0.001$) between the two periods.

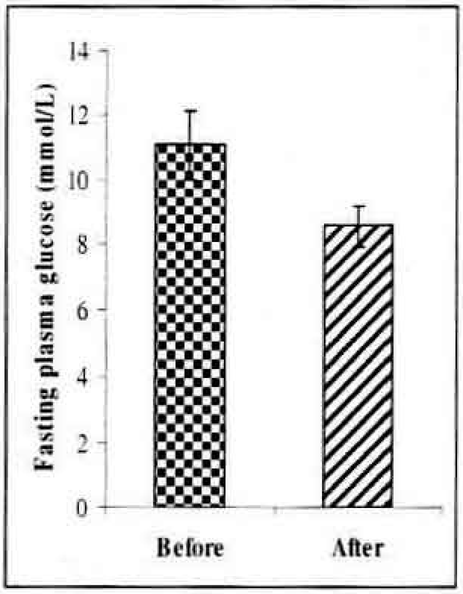

Fig. 2. Mean (± SE) fasting plasma glucose before and 3 months after mushroom supplementation.

The mean ± SE HbA1c (%) before and 3 months after mushroom treatment were 7.99 ± 0.28 and 7.1 ± 3.1 respectively. Here also, a significant mean difference of HbA1c ($p = 0.000$) was observed (Fig. 3).

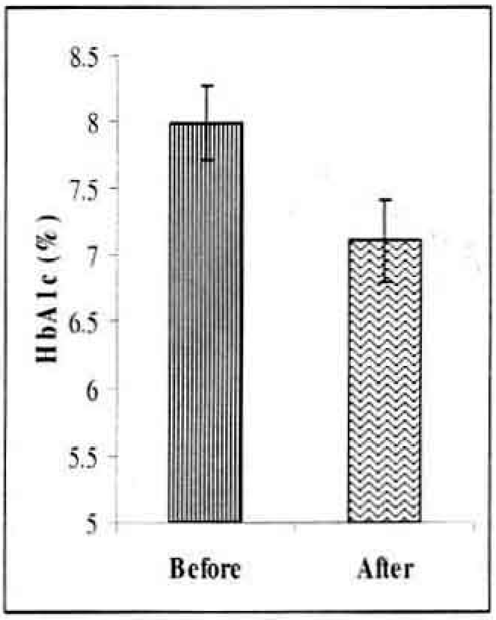

Fig. 3. Mean (± SE) HbA1c before and 3 months after mushroom treatment.

The present results showed that after a 3 month period, the fasting plasma glucose level was significantly reduced in diabetic male patients ($p = 0.001$). There was also a significant decreases of HbA1c ($p = 0.000$). The effect of *Pleurotus ostreatus* on reduction of blood glucose in diabetic patients has been evaluated in this clinical investigation of 27 subjects. In a study Oyster mushroom consumption significantly reduced systolic and diastolic blood pressure, lowered plasma glucose, total cholesterol and triglycerides significantly, whereas there was no significant change in body weight, there were no deleterious effects on liver or kidney function (Khatun *et al.*, 2007).

This study showed reduction of fasting plasma glucose and there was significant effect on glycemic control (HbA1c) by supplementation of *Pleurotus ostreatus*. The significant fall in fasting blood glucose and HbA1c may be attributed to the hypoglycemic potential of the oyster mushroom supplement. Reduction in glycated hemoglobin in streptozotocin-induced diabetic mice after mushroom supplement was observed by others (Swanston *et al.*, 1989).

The blood glucose and triglyceride (TG) lowering effects of water soluble extracts from *Lentinus edodes*, *Pleurotus ostreatus* and *Phellinus linteus* in the streptozotocin-induced diabetic model have been clearly demonstrated (Kim *et al.*, 1997 and Kim *et al.*, 2001). Such results strongly suggest that these mushrooms have potential preventive and therapeutic action in diabetes mellitus (type 1 and type 2). Our findings are also supportive with them. But further studies with large sample size are needed to verify these observations. In conclusion the results throw light on the potential use of oyster mushroom for better glycemic control and better quality of life.

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RAPD Fingerprinting in Four Different Species of Oyster Mushroom Available at NAMDEC

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Abstract

Genetic diversity in four different species of oyster mushrooms was studied based on their random amplification of polymorphic DNA. Four different species of oyster mushroom i.e. *Pleurotus djamor* (NO-3), *Pleurotus florida* (NO-4), *Pleurotus ostreatus* (NO-5, White snow) and *Pleurotus salmoneostramineus* (NO-6) were taken from National Mushroom Development and Extension Center (NAMDEC), Bangladesh. The dendrogram based on RAPD analysis generated 2 major clusters 'C_1' and 'C_2'. Cluster 'C_2' also formed sub cluster SC_1 and SC_2. Out of six random primers, the maximum polymorphism was observed by primers OPA 09 (18.18%). The two species, *Pleurotus djamor* (NO-3) and *Pleurotus ostreatus* (White snow) were observed to be least similar having value 43.0% and constituting a sub cluster SC_2. The present work revealed that RAPD primers showed different pattern of genetic diversity among the selected different oyster mushrooms.

Key words: Genetic diversity, Oyster mushroom, RAPD.

INTRODUCTION

Oyster mushroom belongs to the genus– *Pleurotus*, family- Pleurotaceae, order Agaricales, and class- Basidiomycota (Ainsworth, 1971). In nature oyster mushrooms appear in cluster on dead trees from late fall to spring, and are distributed almost all round the world. Oyster mushroom has been extensively used as food since ancient time, due to its nutritive and medicinal values (Manzi *et al.*, 2001). With the passage of time, there occurred an increase in awareness about mushrooms nutritive and medicinal value (Cheung, 1999).

Selected oyster mushroom has complicated morphological variations of basidiospores, resulting in taxonomic confusion and difficulties in delimiting species boundaries (Alam, 2011).Assessment of genetic and phenotypic diversity is nessesary to distinguish genotypes of *pleurotus djamor, pleurotus florida, pleurotus ostreatus and pleurotus salmoneostramineus* when seeking traits of interest and to identify strains with high yield potential. Various molecular genetics tools have been introduced for the verification of mushrooms such as restriction fragment length polymorphism (RELP), random amplication of polymorphic DNA (RAPD), small subunit ribosomal DNA (ssur DNA) and internal transcribed spacer (ITS) sequence analysis.

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Among the molecular approaches, the RAPD is convenient method for detecting genetic diversity (Alam *et al.*, 2009). Recent genetic analysis on the mushrooms species has shown that RAPD was superior to rDNA sequence based methods when distinguishing strains within species. RAPD was particularly successful when applied for verifying mushroom strains from various strains from various hosts with a wide range of geographical origins (Alam *et al.*, 2010). The present work was carried out to investigate the genetic relationship in four different species of oyster mushrooms.

MATERIALS AND METHODS

Four species; *Pleurotus djamor* (NAMDEC Oyster – NO 3), *Pleurotus florida* (NAMDEC Oyster – NO 4), *Pleurotus ostreatus* (NAMDEC Oyster – NO 5) and *Pleurotus salmoneostramineus* (NAMDEC Oyster – NO 6) were taken from National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka.

DNA extraction: Filamentous fungi have strong cell walls, which are often difficult to rupture. In the present investigation, Modified method of Aljanbi *et al.* (1999) has been used to isolate the total genomic DNA from mushroom. DNA of four species of oyster mushroom were extracted from 0.2-0.3 g fruiting body of each species. It was grinded in extraction buffer (200 mM Tris-HCl, pH-8.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) with a morter pestle. The lysates were incubated at 65°C for 40 min in water bath and centrifuge 30 mins at 10000 rpm. DNA was precipitated from the supernatant by adding equal volumes of isopropanol and resultant pallet was washed with 70% ethanol. The DNA pellet was air dried and dissolved in 50 µL TE buffer. DNA quantification was performed and a dilution of 50ng/µl was used in downstream application.

RAPD analysis: Genomic DNA was amplified by the RAPD technique (Williams *et al.*,1990) in which six sorts of arbitrary 10-base oligonucleotide primers (Operon technologies Inc.) such as OPA-02 (sequence 5' TGCCGAGCTG 3'); Tube E-1 (sequence 5' GTGACATGCC 3'); OPA-07 (sequence 5' GAAACGGGTG 3'); OPA-09 (sequence 5' GGGTAACGCC 3'); OPA-10 (sequence 5' GTGATCGCAG 3'); OPA-15 (sequence 5' TTCCGAACCC 3'). RAPD-PCR reaction was performed using a thermal cycler with an initial denaturation stage of 5 min at 94°C, followed by 40 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 36°C, extension for 2 min at 72°C and a final extension for 10 min at 72°C.

Gel electrophoresis and RAPD data scoring: RAPD products were electrophoresed on 1.4% agarose gels in 1X TBE buffer for 1.15 hr at 100v with 1kb DNA ladder as a size marker and stained while agitated in an EtBr solution (0.5% µg/ml). The stained gels were visualized under a UV transilluminator and photographed using Bio-Rad gel documentation system. The amplification products generated by each RAPD primer were scored as '1' or '0' for presence or absence of specific allele, respectively. To estimate the similarity and genetics distance among different species, cluster analysis based on Nei's unweighted pair-group with arithmetic average (UPGMA) was performed using the 'statistica' software and a dendrogram was constructed.

RESULTS AND DISCUSSION

DNA isolation, qualification and quantification are the prerequisite for DNA fingerprinting and genetic diversity study of varieties based on molecular markers. An easy and efficient method for DNA isolation of mushroom was adopted using available chemicals, simple equipments. The isolated DNA of five mushroom varieties (NO-3, NO-4, NO-5, NO-6) were successfully used for DNA fingerprinting using the six RAPD markers (OPA-02, Tube E-1, OPA-07, OPA-09, OPA-10, OPA-15). The DNA purity was measured by dividing the absorbance at 260nm by the absorbance at 280 nm. The A260/A280 ratio higher than 2.0 generally indicates RNA contamination. For A260/A280 ratios lower than 1.8 normally indicates protein contamination during extraction process. Good quality DNA should give the A260/A280 ratio in the range of 1.8 –2.0. However, Aljanabi *et al.*, (1999) reported the range of A260/A280 ratio from 1.25-2.2 for good quality DNA of mushroom. On the other hand, in this investigation, the ranges of A260/A280 ratios among five mushroom varieties were from 1.9–2.2 which indicates the good quality of DNA.

The amount of DNA recovered was enough for perform PCR amplification of Random Amplified Polymorphic DNA. The amount of recovered DNA was 958-1526 ng/µl. The highest amount of DNA was recovered from the variety NO-3 (1526 ng/µl) and the lowest amount was obtained from the variety NO-6 (958 ng/µl).

Table 1. RAPD primers with corresponding bands scored and their size ranges in selected mushrooms

Primer	Size ranges (bp)	Total number of bands scored	Number of polymorphic bands	Polymorpihism (%)
OPA-02	376-1955	6	0	00.00
Tube E-1	200-1530	10	0	00.00
OPA-07	150-1629	25	4	16.00
OPA-09	162-1370	22	4	18.18
OPA-10	131-1562	42	5	11.90
OPA-15	219-1620	24	4	16.66

The sizes of the amplified bands in the four mushroom varieties ranged from 131 to 1955bp (Table 1). Among the six RAPD primers, OPA-02 revealed band sizes that ranged from 376 bp to 1955 bp, primer Tube E-1 ranged from 200bp-1530bp, primers OPA-07 ranged from 150 bp to 1629 bp; primer OPA-09 ranged from 162 bp to 1370 bp, primer OPA-10 ranged from 131 bp to 1562 bp and primer OPA-15 ranged from 219 bp to 1620 bp. Six RAPD primers were used to amplify the segments of DNA in four different species of oyster mushrooms, which were found to be efficient for amplifying the genomic DNA (Table 2). These primers showed significant band profiles on the tested species of *Pleurotus* and high possibilities to screening of each genotype (Fig. 1, 2, 3, 4, 5 and 6). The primer OPA-10 amplified the highest number of bands (42), primer OPA-09 amplified a total of 22 bands, Tube E-1 amplified a total of 10 bands, OPA-15 amplified a total of 25 bands, OPA-07 amplified a total of 25 bands and primer OPA-02 amplified the lowest number of bands (6).

Table 2. DNA bands of four different species of oyster mushroom by random amplification poly morphic DNA assay on selected primers

Primers	DNA bands (kb)	Four different species of oyster mushroom			
		1	2	3	4
OPA-02	2.0	-	-	+	-
	1.5	-	+	-	-
	1.0	-	-	-	-
	0.8	-	-	-	+
	0.6	-	-	-	+
	0.5	-	-	-	+
	0.3	-	-	-	+
	0.2	-	-	-	-
Tube E-1	2.0	-	-	-	-
	1.5	-	+	+	-
	1.0	-	-	+	-
	0.8	-	-	-	+
	0.5	-	-	+	+
	0.3	-	-	-	-
	0.2	-	-	-	+
OPA-07	2.2	-	-	-	-
	1.5	+	+	+	-
	1.0	+	+	+	-
	0.8	+	+	+	-
	0.5	-	+	+	-
	0.3	+	-	+	-
	0.2	+	+	+	-
OPA-09	2.2	-	-	-	-
	1.5	+	+	-	-
	1.0	+	+	-	-
	0.8	+	+	-	+
	0.5	+	+	-	+
	0.3	+	+	-	+
	0.2	-	+	-	-
OPA-10	2.2	-	-	-	-
	1.5	-	-	+	+
	1.0	+	+	+	+
	0.8	+	+	+	+
	0.5	+	+	+	+
	0.3	+	+	+	+
	0.2	-	-	-	+
OPA-15	2.2	-	-	-	-
	1.5	+	+	+	-
	1.0	+	+	+	-
	0.8	+	+	-	-
	0.5	+	+	+	-
	0.3	-	-	-	-
	0.2	+		+	-

Lane 1. NO-3 (NAMDEC oyster-3); Lane 2. NO-4 (NAMDEC oyster-4); Lane 3. NO-5 (NAMDE-C oyster-5); Lane 4. NO-6 (NAMDEC oyster-6).

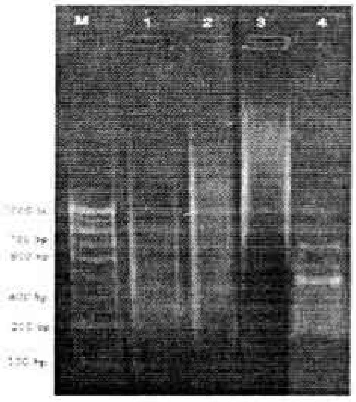

Fig. 1. RAPD Profile of Four Different Oyster Mushroom Varieties. Primer OPA-02 Showing Polymorphism among the varieties. Lane M – Marker, 1000 bp DNA ladder; Lane 1 = NO-3; Lane 2 = NO-4; Lane 3 = NO-5; Lane 4 = NO-6.

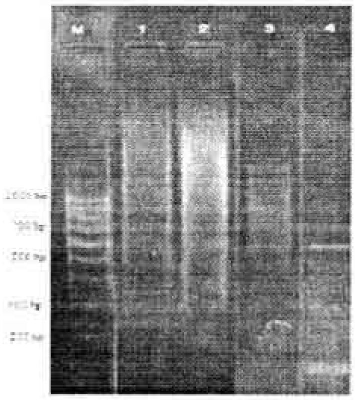

Fig. 2. RAPD Profile of Four Different Oyster Mushroom Varieties. Primer Tube E-1 Showing Polymorphism among the varieties. Lane M – Marker, 1000 bp DNA ladder; Lane 1 = NO-3; Lane 2 = NO-4; Lane 3 = NO-5; Lane 4 = NO-6.

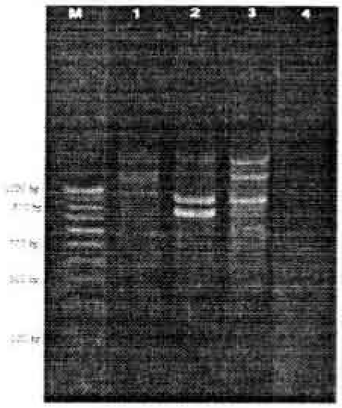

Fig. 3. RAPD Profile of Four Different Oyster Mushroom Varieties. Primer OPA-07 Showing Polymorphism among the varieties. Lane M – Marker, 1000 bp DNA ladder; Lane 1 = NO-3; Lane 2 NO-4; Lane 3 = NO-5; Lane 4 = NO-6.

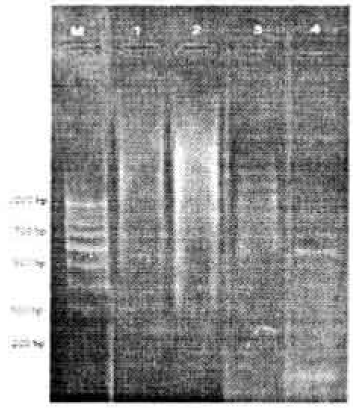

Fig. 4. RAPD Profile of Four Different Oyster Mushroom Varieties. Primer OPA-09 Showing Polymorphism among the varieties. Lane M – Marker, 1000 bp DNA ladder; Lane 1 = NO-3; Lane 2 = NO-4; Lane 3 = NO-5; Lane 4 = NO-6.

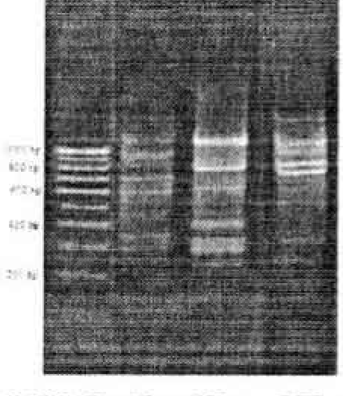

Fig. 5. RAPD Profile of Four Different Oyster Mushroom Varieties. Primer OPA-10 Showing Polymorphism among the varieties. Lane M – Marker, 1000 bp DNA ladder; Lane 1 = NO-3; Lane 2 = NO-4; Lane 3 = NO-5; Lane 4 = NO-6.

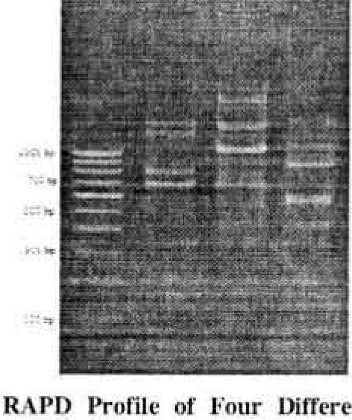

Fig. 6. RAPD Profile of Four Different Oyster Mushroom Varieties. Primer OPA-15 Showing Polymorphism among the varieties. Lane M – Marker, 1000 bp DNA ladder; Lane 1 = NO-3; Lane 2 = NO-4; Lane 3 = NO-5; Lane 4 = NO-6.

The values of pair-wise comparisons of genetic distances analyzed by using computer software "Statistica" between varieties were computed from combined data for the six primers, ranged from 43.0 to 56.0 (Table 3). The highest linkage distance (56.0) was recorded between variety pairs NO-6 and NO-4. The linkage distance (53.0) was recorded between variety NO-3 and NO-6. The linkage distance (52.0) was recorded between variety NO-5 and NO-6. The linkage distance (50.0) was recorded between variety NO-4 and NO-5; The another linkage distance (45.0) was recorded between variety pairs NO-4 and NO-3; The lowest linkage distance (43.0) was recorded between variety NO-3 and NO-5.

Table 3. Summary of linkage distances for different pairs of selected species of oyster mushroom by RAPD

Variety	NO-3	NO-4	NO-5	NO-6
NO-3	0.0	45.0	43.0	53.0
NO-4	45.0	0.0	50.0	56.0
NO-5	43.0	50.0	0.0	52.0
NO-6	53.0	56.0	52.0	00.0

Genetic relationships among the varieties at the average distance of 53.66 showed two major clusters (C_1 and C_2) presented in the Fig.7. NO-6 only in major cluster C_1 while the major cluster C_2 included all three varieties (Pop 2, NO-4, and NO-5). At the linkage distance of 47.33 the cluster C_2 produced sub-cluster SC_1 and SC_2. Sub-cluster SC_1 separated the variety NO-4 from others varieties of sub-cluster SC_2. Again, the genotypes of sub cluster SC_2 comprises NO-3 and NO-5 Variety.

The basic DNA sequence of an organism can be presumed to be insensitive to short term environmental change and thus should provide a more stable alternative for strain/ species identification. Therefore, the random and genomic wide nature of the RAPD technique is best to indicate over all genetic relatedness/dissimilarity than the morphological analysis (Ravash *et al.*, 2009). The different primers produced different number of bands in PCR. This variation in the number of bands may be due to the sequence of primer, availability of annealing sites in the genome and template quality (Kernodle *et al.*, 1993). Despite this, all the species were grown on similar culture but the variations in the banding pattern were reflected. Chandra *et al.*, (2010) used the RAPD markers to discriminate the eight *Pleurotus* species of mushrooms and also found variations in banding pattern. All the primers except OPA 02 and Tube E-1 were polymorphic. The maximum polymorphism was produced by the primer OPA 09 having polymorphism percentage 18.18. The polymorphism produced by RAPD primers may be due to the base substitution, insertion and deletion or collection of genetic material from different sources (Chopra, 2005; Jusuf, 2010). The maximum similarity (56%) was observed between NO-4 and NO-6 belonging to major cluster 'C1' and 'C2'. Cluster C2 divided into sub cluster SC1 and SC2. SC1 comprises NO-4 and SC2 comprises NO-3 and NO-5. This is due to their same culture medium. The minimum (43%) similarity was observed between NO-3 and NO-5 which belonged to same sub- cluster S C_2. It is proved that they were grown well in winter.

These findings revealed that the genetic make up is correlated with environmental heterogeneity (Nevo, 1998).

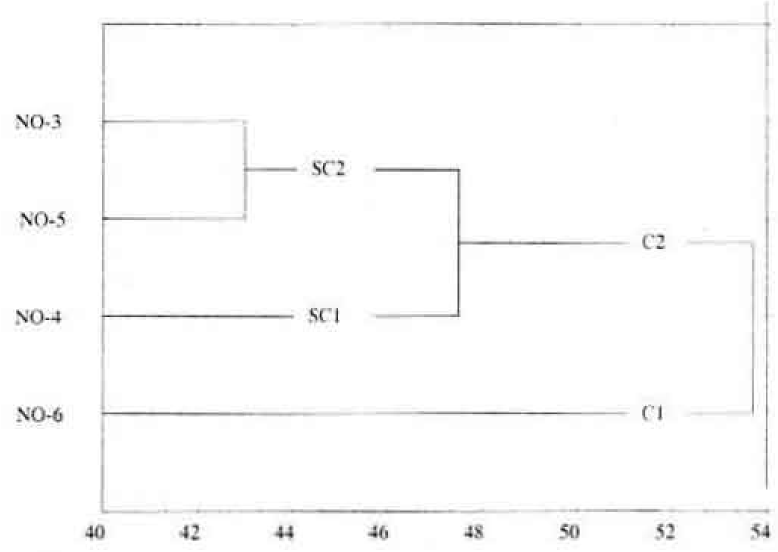

Fig. 7. Cluster analysis by unweighted pair group method of arithmetic means (UPGMA) of four species of oyster mushroom. NO-3 (NAMDEC oyster-3), NO-4 (NAMDEC oyster-4), NO-5 (NAMDEC oyster-5), NO-6 (NAMDEC oyster-6).

The mushroom varieties belonging to ecological proximity or different geographical origins can be classified through morphological and molecular markers. The current study demonstrated that, the RAPD analysis is useful for characterization, genetic diversity and identifying relationships among the oyster mushrooms. Study also revealed that, RAPD analysis can be very useful tool for mushroom grower for classification and maintenance of good quality spawns.

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Determination of Minimum Nutrient for *in vitro* Mycelial Growth and preservation in Pure Culture

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Abstract

The experiment was conducted to evaluate the effect of nutrient on the mycelial growth rate of some commercially important oyster mushroom. The fastest mycelium growth rate and the minimum days required for completion of mycelium running in Petri plate were observed when *Pleurotus djmour* (POP_2) inoculated in PDA media at the ratio of 15 : 150 (Dextrose: Potato). Minimal mycelial growth rate and maximum days required for the completion of mycelium running were observed when *Pleurotus djmour* (POP_2) inoculated in PDA media at the ratio of 25 : 250 (Dextrose : Potato). No mycelial growth was observed when all variety inoculated in PDA media at the ratio of 0:0.

Key words: Dextrose, Potato, PDA, *Pleurotus ostreatus*, *Pleurotus djmour*.

INTRODUCTION

Cultures of edible mushroom can be preserved either as spores or as vegetative mycelia. The main objectives of culture preservation are maintenance of viability for long periods, thus permitting survival of the culture, maintenance of genetic, morphological and physiological stability, maintenance of culture purity from microorganisms. The traditional method for maintaining mushroom cultures is by periodic transfer into a nutrient media such as potato dextrose agar, malt agar, oatmeal agar and complete agar. As a general rule, a nutritionally weak medium is preferred, because it lowers the metabolic rate of the organism and thus prolongs the period between transfers into a subculture (Chang and Miles, 1993). In National mushroom development and extension centre, we have 157 strains, for the maintenance and preservation of those strains and to save time, manpower and money it is necessary to determine the minimum amount of nutrient for preservation. So, the aim of this study was to determine the suitable concentration of potato & dextrose are used to prepare PDA media for the production of vigorous mycelium within short time.

MATERIALS AND METHODS

This experiment was conducted in the tissue culture laboratory of National Mushroom Development and Extension Centre (NAMDEC) during August to September 2010. Two oyster mushroom species such as *Pleurotus ostreatus* (Po 2), *Pleurotus djmour* (Pop 1; Pop 2.) and different concentration of dextrose and Potato in Potato Dextrose Agar media (PDA), were used in this study. The inocula were collected from the germplasm centre of NAMDEC.

Preparation of synthetic (PDA) media: Different amount of potato and dextrose were used for PDA media. The PDA media was prepared by 0g, 5g, 10g, 15g, 20g, 25g of dextrose and 0g, 50g, 100g, 150g, 200g, 250g, potato (According to treatment combination) mixed with 20g agar at pH 6.5. The mixture was boiled on gas burner until the agar dissolved. The media was poured into Petri plate at 20 ml/plates and sterilized in an autoclave for 20 minutes at 120°C under 1.5 kg/cm^2 pressure. After sterilization and solidification, the plates were inoculated with the inocula of testing mushroom species. Then plates were transferred in incubation room for mycelium running at 20-25° C temperature.

Statistical Analysis: The experiment was laid out in Completely Randomized Designs (CRD) with 4 replications. Data on mycelial growth rate and days to complete mycelium running, were collected and analyzed following standard methods (Gomez and Gomez, 1984) using MSTAT-C computer Program. Means were computed following DMRT using the same computer program.

RESULTS AND DISCUSSION

Mycelial growth rate (cm/day): The result of mycelial growth rate in different ratio of potato and dextrose used in culture media as well as variety is shown in Table 1. Highest mycelial growth rate (0.33cm/day) was observed from the treatment combination V_3T_4 when *Pleurotus djmour* (Pop 2) was inoculated in PDA at ratio of dextrose (g/ liter): Potato (g/liter) (15:150) which was statistically similar to V_1T_2 and V_2T_2. Similar findings was reported by Khandaker *et al.* (2009), who reported that PDA media gave highest (0.33cm/day) mycelial growth of *Grifola frondosa.* The lowest mycelial growth rate (0.26 cm/day) was observed from the treatment combination V_3T_6 when *Pleurotus djmour* (Pop 2) was inoculated in PDA at ratio of dextrose (g/ liter): Potato (g/ liter) (25:250). Incase of T_1 treatment no mycelial growth was observed in above variety.

Table 1. Mycelial growth rate of different mushroom species on different ratio of PDA media

Treatment	Mycelial growth rate (cm/day)		
	Pleurotus ostreatus (V_1)	*Pleurotus djmour* (V_2)	*Pleurotus djmour* (V_3)
T_1	-	-	-
T_2	0.32 A	0.32 A	0.28 AB
T_3	0.29 AB	0.30 AB	0.30 AB
T_4	0.29 AB	0.29 AB	0.33 A
T_5 (control)	0.29 AB	0.31 AB	0.31 AB
T_6	0.28 AB	0.30 AB	0.26 B
CV (%)	**9.57**	**9.57**	**9.57**

Means followed by a common letter are not significantly different at 5% level by DMRT. [T_1 = Dextrose (g/liter): Potato (g/liter) (0:0). T_2 = Dextrose (g/liter): Potato (g/liter) (5:50)T_3= Dextrose (g/liter): Potato (g/liter) (10:100). T_4 = Dextrose (g/liter): Potato (g/liter) (15:150). T_5 = Dextrose (g/liter): Potato (g/liter) (20:200) (control). T_6 = Dextrose (g/liter): Potato (g/liter) (25:250)].

Duration of mycelial completion (days): Duration of mycelia completion in different ratio of potato and dextrose used in culture media as well as variety is shown in Table 2. Maximum days (14.00 days) required for the completion of mycelium running was observed from the treatment combination V_3T_6 when *Pleurotus djmour* (Pop 2) was inoculated in PDA at ratio of dextrose (g/ liter): Potato (g/ liter) (25:250) because excess nutrient delay the mycelial growth. Minimum days required for completion of mycelium running was observed from the treatment combination V_3T_4 when *Pleurotus djmour* (Pop 2) was inoculated in PDA at ratio of dextrose (g/ liter): Potato (g/ liter) (15:150) which was statistically similar to V_1T_2.

Table 2. Days required to mycelium completion of different mushroom species on different ratio in PDA media

Treatment	Days required to mycelium complete (days)		
	Pleurotus ostreatus (V_1)	*Pleurotus djmour* (V_2)	*Pleurotus djmour* (V_3)
T_1	-	-	-
T_2	11.50 B	12.00 AB	13.75 A
T_3	13.00 AB	12.00 AB	13.00 AB
T_4	13.00 AB	12.75 AB	11.50 B
T_5 (control)	12.50 AB	12.50 AB	12.25 AB
T_6	13.50 AB	12.00 AB	14.00 A
CV (%)	**9.83**	**9.83**	**9.83**

Means followed by a common letter are not significantly different at 5% level by DMRT. [T_1 = Dextrose (g/liter): Potato (g/liter) (0:0), T_2 = Dextrose (g/liter): Potato (g/liter) (5:50), T_3 = Dextrose (g/liter): Potato (g/liter) (10:100), T_4 = Dextrose (g/liter): Potato (g/liter) (15:150), T_5 = Dextrose (g/liter): Potato (g/liter) (20:200) (control), T_6 = Dextrose (g/liter): Potato (g/liter) (25:250)].

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Influence of Different Covering Materials on Yield Attributes and Yield of Milky White Mushroom

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Abstract

An experiment was conducted at National Mushroom Development and Extension Centre, Savar, Dhaka to observe the effect of light intensity on yield and yield related attributes of milky white mushroom (*Calocybe indica*). The days required from casing to primordia initiation was the lowest (9.75 days) at 7 lux and the highest (13.25 days) at 570 lux. The lowest (17.00 days) required for first harvest at 7 lux and it was the highest (22.00 days) at 570 lux. Days required for final harvest ranged from 42.00 to 46.25 and the highest (46.25 days) was found at 12 lux. The length of stipe ranged from 6.20 to 14.13 cm and the highest (14.13 cm) was found at 5 lux and the lowest (6.20 cm) was found at 300 lux. The highest (3.47 cm) diameter of stipe was found at 570 lux and the lowest (2.2 cm) was found at 5 and 12 lux. The highest (7.67 cm) and the lowest (3.65 cm) diameter of pileus were found at 450 lux and at 40 lux respectively. The highest (2.00 cm) and the lowest (1.00 cm) thickness of pileus were found at 570 lux and at 12 lux respectively. Effective fruiting body was obtained maximum (6.00/ packet) at 7 lux and it was minimum (3.50/ packet) at 20 lux. Significant variation was observed in yield and it ranged from 130.00 to 187.30 g/packet. The highest yield (187.30 /packet) was recorded at 570 lux and the lowest (130.00 g/packet) was recorded at 15 lux. Similar trend was observed in dry yield and biological efficiency of milky white mushroom.

Key words: *Calocybe indica,* Light intensity, Covering materials.

INTRODUCTION

Different species of mushrooms are cultivated in Bangladesh such as oyster, milky, button, straw, shitake, reishi etc. Though oyster mushrooms (*Pleurotus spp.*) are mainly cultivated for their nutritional and medicinal importance (Khan *et al*., 2008 and Alam *et al*., 2008) and suitability under Bangladeshi condition (Amin *et al*., 2007), but *Calocybe indica* is the special one. The Bengali and English name of this mushroom is dudh and milky white mushroom, respectively. Its robust size, sustainable yield, attractive color, delicacy, long shelf-life, and lucrative market value have attracted the attention of both mushroom consumers and prospective growers (Chakraborty and Sikdar, 2010). Milky white mushroom is rich in protein, lipids, fiber, carbohydrates and vitamins and contains essential amino acids (Alam *et al.,* 2008). The key factors to increase mushroom production, is to manage the proper environmental conditions for their optimum growth and yield. The major environmental factors affecting mushroom crops are light, temperature, relative humidity and carbon dioxaide etc. Among them, light intensity largely influences the yield of this mushroom. The light is often required for one or more phases in fruiting body production in the Basidiomycetes. Marsh *et al.* (1959) reviewed on the effects of light on reproduction of fungi, including many species of

Basidiomycetes. Many Basidiomycetes require light for normal pileus development, such as *Collbia velutipes* (Plunkett, 1956 and Aschan, 1960) and *Coprinus myceliocephalus* (Lange, 1948). Stipe elongation often occurs in the absence of light (Brefeld, 1877 and Chakrabarty, 1941). The illumination time and the intensity of light are both significant variables in the effect of light on fruiting (Vorderberg, 1950 and Medelin, 1956). Moreover, low light intensity delayed fruiting in *Polyprus brumalis* (Plunkett, 1956). Some Basidiomycetes are able to initiate and complete fruiting body development in the absence of light (Campbell, 1938).

The production and marketing potential of the milky white mushroom in Bangladesh is promising because this mushroom requires a temperature of 30 -35° C and a relative humidity of 70~80% for cultivation, which is conducive to the environmental conditions of Bangladesh (Krishnamoorthy *et al.*, 2000). Moreover, because of suitable condition, high local demand and export potential of this mushroom, many private entrepreneurs are interested in its commercial cultivation. But the suitable range of light requirement has not yet been determined in Bangladesh condition for the cultivation of milky white mushroom.

Therefore, the present experiment was undertaken to determine the appropriate light intensity and environmental factors for higher yield and better quality of milky white mushroom.

MATERIALS AND METHODS

The study was carried out in the laboratory, workshop and culture house of National Mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka from February to July 2010. The experiment conducted with fifteen (15) different covering materials. these were considered as treatments ie. T_1= Covered by a single layer of black cloth and a poly propylene (pp) sheet, T_2= Covered by a single layer of black cloth and white cloth and a pp sheet , T_3= Covered by a single layer of hessian (Jute mat) and a pp sheet, T_4= Covered by only a pp sheet, T_5= Covered by a double layer of black cloth and a pp sheet, T_6= Covered by a double layer of white cloth and a pp sheet, T_7 = Covered by a double layer of hessian (Jute mat) and a pp sheet, T_8 = Covered by a single layer of black cloth, T_9 = Covered by a single layer of white cloth, T_{10} = Covered by a single layer of black cloth and white cloth, T_{11} = Covered by a single layer of hessian (Jute mat), T_{12}= Open, i.e. no covering, T_{13}= Covered by a double layer of black cloth, T_{14}= Covered by a double layer of white cloth, T_{15}= Covered by a double layer of hessian (jute mat).

The spawn packets with complete mycelium 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 casing was performed with casing soil. Top layer of spawn packet covered with mixture of soil, Farm Yard Manure, cow dung, sand etc is called casing. The loamy soil and cow dung (3:1) were used as casing soil in this experiment. All casing materials were sterilized at 65°C for 4 hours. The packets were placed separately side by side on the rack in the culture house. The relative humidity was 80-90% and the temperature was 30-35°c. The relative humidity (%) and the temperature were maintained by watering thrice daily. The light intensity was maintained according to the treatments and measured by Lux meter and proper ventilation in culture house was maintained for fruiting body development. Data on days required from casing to primordia initiation was determined by counting the days which were required to primordia initiation from the casing date of spawn packet. Number of primordia was determined by counting the number of primordia after primordia initiation. Days required to first harvest was determined by counting the days from the casing date of spawn packet. Days required for final harvest was determined by counting the days which were required to final harvest from the casing date of spawn packet. Number of well developed fruiting body was recorded. Dry and pinheaded fruiting body was discarded. Thickness of the pileus, diameter of pileus, diameter of stipe (cm) and length of stipe (cm) of four randomly selected fruiting bodies were recorded. Yield (g/500g packet) was recorded by weighing the whole cluster of fruiting body without removing the lower hard and dirty portion. Biological efficiency was determined according to Ahmed, 1998.

The experiment was laid out following completely randomized design (CRD) with 16 treatments and 4 replications. Data were analyzed following using MSTAT-C computer program. Means separation were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Days required from casing to primordial initiation: The days required from casing to primordial initiation differed significantly and ranged from 9.75 to 13.25 days (Table 1). The lowest DRPI (9.75 days) was found at 7 lux in the treatment T_7. It was designed by covering a double layer of hessian (Jute mat) and a pp sheet, where the temperature 33.0^{0}C and relative humidity 87% were measured. The highest 13.25 days required for primordial initiation (DRPI) was found at 570 lux in the treatment T_{12} which was statistically similar to T_{11}, T_5, T_{10}, T_9, T_{15}, T_{14}, T_{13}, T_2 and T_8. The treatment T_{12} was designed by Open, i.e. no covering, where the temperature 31.4^{0}C and relative humidity 80% were measured. Panna *et al.* (2010) showed minimum 14 days and maximum 17 days required for primordia initiation when *C. indica* was cultured on 500g substrate, 500-800 lux light, 80% relative humidity and 30-35^{0}C temperature which corroborates the result of Tandon *et al.* (2006) where they observed that the days required for primordia initiation was 15.6 days when casing with vermicompost.

Days required from casing to first harvest: The days required to first harvest (DRFH) differed significantly and ranged from 17.00 to 22.00 days (Table 1). The lowest DRFH (17.00 days) was found at 7 lux in the treatment T_7. The treatment T_8 was designed by covering with poly propylene sheet and Jute mat double layer, where the temperature 33.0^{0}C and relative humidity 87% were measured. The highest DRFH (22.00 days) was found at 570 lux in the treatment T_{12} which was significantly similar to the treatment T_{15} T_{11}, T_{10}, T_{13}, T_5, T_9, and T_{14}. It was designed by Open, i.e. no covering, where the temperature 31.4^{0}C and relative humidity 80%were measured. Panna *et al.* (2010) reported that 18-21 days required for first harvest which is more or less similar with the present finding.

Days required from casing to final harvest: This parameter showed significant difference and ranged from 42.00 to 46.25 days. The lowest (42.00 days) required for total harvest (DRTH) was found at 7 lux in the treatment T_7 (Table 1). It was designed by covering with poly propylene sheet and Jute mat double layer, where the temperature 33.0^{0}C and relative humidity 87% were measured. The highest DRTH (46.25 days) was found at 12 lux in the treatment T_{10}, which was statistically similar to other treatments except T_1, T_2, T_3, T_4 and T_7. It was designed by covering with single layer of black and white cloths, where the temperature 32.0^{0}C and relative humidity 85% were measured.

The length of stipe (cm): The length of stipe differed significantly and ranged from 6.20 to 14.13 cm (Table 1). The highest length (14.13 cm) was found at 5 lux in the treatment T_{15} which was statistically similar to T_5, T_2, T_7 and T_3. It was designed by covering with Jute mat double layer, where the temperature 32.5^{0}C and relative humidity 86 % were measured. The lowest length (6.20 cm) was found at 300 lux in the treatment T_6, which was statistically similar to T_4, T_9, T_{12}, T_{14} and designed by rapping with poly propylene (pp) sheet and white cloth double layer, where the temperature 32.0^{0}C and relative humidity 86% were measured. It showed that the lower light intensity may cause the stipe elongation. A negative linear relationship was observed between light intensity and length of stipe (Fig. 2) which showed that the length of stipe increased as the light intensity decreased. Chakrabarty (1941) and Brefeld (1877) reported that stipe elongation often occurs in the absence of light, which is similar with the present findings.

Diameter of stipe (cm): The diameter of stipe differed significantly and ranged from 2.20 cm to 3.47 cm (Table 1). The highest diameter (3.47 cm) was found in the treatment T_{12} which was statistically similar to the treatment T_9, T_8, T_1, T_4, T_{13} and T_{14}. It was designed by no covering, where the temperature 31.4^{0}C and relative humidity 80% were measured. The lowest diameter of stipe (2.2cm) was found at 0 lux and 12 lux light in the treatment T_5 and T_{10} respectively. The treatment T_6 was designed by rapping with poly propylene sheet and black cloth double layer, where the temperature 32.8^{0}C and relative humidity 85% were measured. The treatment T_{11} was designed by covering with black-white cloth single layer, where the temperature 32.0^{0}C and relative humidity 85% were measured. It indicated that diameter of stipe increased in the presence of high light intensity and decreased in lower light intensity. Panna *et al.* (2010) observed that the highest diameter of stipe (3.7 cm) was found at 500-800 lux which is similar with the present investigation.

Diameter and thickness of pileus (cm): The diameter of pileus differed significantly and ranged from 3.65 to 7.67 cm (Table 1). The highest diameter (7.67 cm) was found at 450 lux in the treatment T_4, which was statistically similar to the treatment T_{12}. It was designed by rapping with only polypropylene sheet and where the temperature 32.0^{0}C and relative humidity 80% were measured. The lowest (3.65 cm) diameter was found in the treatment T_3 which was statistically similar to T_5 and T_{10}. It was designed by rapping with poly propylene sheet and jute single layer, where the temperature 32^0C and relative humidity 86% were measured. The thickness of pileus differed significantly and ranged from 1.00 to 2.00 cm (Table 4). The highest thickness (2.00 cm) was found at 570 lux in the treatment $T_{12,}$ which was statistically similar to the treatment T_4 and designed by no covering, where the temperature 31.5^0C and relative humidity 80% were measured. The lowest thickness (1.00 cm) was found at 12 lux in the treatment $T_{10,}$ which was statistically similar to $T_{5,}$ T_7, T_3, $T_{1,}$ $T_{15,}$ and designed by covering black-white cloth single layer, where the temperature 32.0^0C and relative humidity 85% were measured. The result showed that light influenced pileus development. The highest diameter (11.38 cm) and thickness (2.78 cm) of pileus were observed at 500-800 lux in an experiment conducted by Panna *et al.* (2010). Plunkett (1956), Aschan (1960) and Lange (1948) also reported that many Bacidiomycetes require light for normal pileus development, such as *Collbia velutipes* and *Coprinus myceliocephalus* respectively, which also corroborates with the present findings.

Number of primordia: The number of primordia (NP) differed significantly and ranged from 8.75 to 15.50 (Table 1). The highest NP (15.50) was found at 15 lux in treatment T_1 and T_7 which is statistically similar to the treatment T_4, T_{15}, T_3 and T_{10}. It was designed by covering a single layer of black cloth and a poly propylene sheet. The temperature 32.0^0C and relative humidity 85% were measured. The lowest NP (8.75) was recorded at 570 lux in the treatment T_{12} and was designed by Open, i.e. no covering, where the temperature 31.4^0C and relative humidity 80 %were measured. According to Okwujiako (2001), light enhanced primordia formation and necessary for the production of fruiting bodies which corroborates with the present study.

Number of effective fruiting body: The number of effective fruiting body (NEFB) obtained from 3 flushes differed significantly and ranged from 3.50 to 6.00 per packet (Table 1). The highest NEFB (6.00/ packet) was observed at 7 lux in treatment T_7, which was statistically similar to the treatment T_5, T_{14}, $T_{6,}$ $T_{3,}$ T_2, T_9 and $T_{10.}$ It was designed by rapping poly propylene sheet and jute mat double layer, where the temperature 33.0^0C and relative humidity 87 % were measured. The lowest NEFB (3.50/ packet) was observed at 20 lux in treatment T_8, which was designed by rapping black cloth single layer, where the temperature 31.5^0C and relative humidity 83 % were measured. Panna *et al.* (2010) observed that the average number of effective fruiting body (5.25) was found at 500-800 lux, which was similar with the present study.

Yield /packet (g): Significant variation was observed in case of yield in different treatments and it ranged from 130.00 to 187.30 g/packet (Table 1). The highest (187.30 g/packet) yield was recorded at 570 lux in the treatment T_{12} followed by T_2 and designed by no covering i.e. open, where the temperature 31.4^0C and relative humidity 80 % were measured. The lowest yield 130.00 g/packet was recorded at 15 lux in treatment T_1 which was designed by rapping poly propylene sheet and black cloth single layer, where the temperature 32.2^0C and relative humidity 85% were measured.

Dry yield /packet (g): Significant variation was observed in dry yield in different treatments (Table 1). The DY was counted only from the first flash. It ranged from 16.10 g to 21.38 g/packet. The highest 21.38 g/packet was recorded at 570 lux in the treatment T_{12}, followed by the treatment T_2. It was designed by no covering and the temperature 32.5^{0}C and relative humidity 80 % were measured. The lowest dry yield of 16.10 g/packet was recorded in treatment T_1, which was statistically similar to T_8, T_9, T_{10} and T_{13}. It was designed by covering with polypropylene sheet and black cloth single layer, where the temperature 32.5^{0}C and relative humidity 86 %were measured.

Biological Efficiency (%): Biological efficiency (%) in the different treatments was shown in (Fig. 1). It was ranged from 65.00 to 93.63 %. The highest BE (93.63%) was recorded at 570 lux in the treatment T_{12} followed by T_2. It was designed by no covering and the temperature 31.5^{0}C and relative humidity 80 % were measured. The lowest BE (65.00 %) was recorded at 15 lux in the treatment T_1, which was statistically similar to the treatment T_{10} and T_{13}. It was designed by rapping with poly propylene (pp) sheet and black cloth single layer, where temperature 32.2^{0}C and relative humidity 85% were measured. Panna *et al.* (2010) recorded that biological yield, economic yield, dry yield and biological efficiency was the highest at 500-800 lux which was approximately similar with the present study.

Table 1. Influence of covering materials on light intensity and their effect on yield

Treatment	Light intensity (lux)	Days to primordia initiation	Days to first harvest	Days to final harvest	Length of stipe (cm)	Diameter of stipe (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	No. of primordia/ packet	No. of fruiting body/ packet	Yield /packet (g)	Dry yield /packet (g)
T1	15	10.50 b-d	19.00 b-d	42.75 cd	10.73c-e	3.15 a-c	4.67 d	1.22 fg	15.50 a	4.00 c-e	130.0 e	16.10 f
T2	10	11.00 a-d	20.00 a-c	42.75 cd	12.70 a-c	2.67 c-f	4.67 d	1.30 ef	09.25 de	5.00 a-d	175.5 ab	20.42 ab
T3	40	10.00 cd	18.75 cd	43.25 b-d	12.13 a-d	2.27 ef	3.65 g	1.17 fg	12.50 a-d	5.00 a-d	165.3 b-d	18.00 c-e
T4	450	10.50 b-d	19.00 b-d	43.25 b-d	6.90 fg	2.97 a-d	7.67 a	1.90 ab	14.25 ab	4.50 b-e	166.5 b-d	19.15 bc
T5	0	12.50 a-c	21.50 a-c	44.50 a-d	13.43 ab	2.20 f	3.97 e-g	1.12 fg	10.50 c-e	5.75 ab	166.8 b-d	18.52 c-e
T6	300	10.50 b-d	19.75 a-c	43.75 a-d	6.20 g	2.42 d-f	4.30 d-f	1.55 c-e	9.00 e	5.25 a-c	167.3 bc	19.35 bc
T7	7	9.75 d	17.00 d	42.00 d	12.48 a-c	2.52 d-f	4.75 d	1.15 fg	15. 50 a	6.00 a	163.0 b-d	19.23 bc
T8	20	10.75 a-d	19.00 b-d	44.50 a-d	8.75 ef	3.40 ab	6.92 b	1.72 b-c	11.50 b-e	3.50 e	157.5 c-e	17.88 c-f
T9	350	11.75 a-d	20.00 a-c	45.00 a-c	7.07 fg	3.27 a-c	6.32 c	1.62 c-d	9.00 e	5.00 a-d	150.8 c-e	17.76 c-f
T10	12	13.25 a	21.50 a-c	46.25 a	10.15 de	2.20 f	3.80 fg	1.00 g	12.50 a-d	4.75 a-e	139.0 ef	16.95 d-f
T11	80	12.75 ab	21.75 ab	45.25 a-c	8.80 ef	2.80 b-f	6.27 c	1.75 a-c	11.50 b-e	3.75 de	158.8 c-e	17.92 c-e
T12	570	13.25 a	22.00 a	45.50 a-c	6.35 g	3.47 a	7.20 ab	2.00 a	8.75 e	4.50 b-e	187.3 a	21.38 a
T13	0	11.25 a-d	21.50 a-c	45.00 a-c	11.75 b-d	2.97 a-d	4.52 de	1.40 d-f	10.25 c-e	4.50 b-e	146.0 d-f	16.90 ef
T14	330	11.25 a-d	21.50 a-c	44.25 a-d	6.62 fg	2.95 a-d	6.20 c	1.62 c-d	10.75 c-e	5.25 a-c	162.5 b-d	18.80 b-d
T15	5	11.25 a-d	21.75 ab	45.00 a-c	14.13 a	2.82 b-e	4.92 d	1.22 fg	13.00 a-c	4.50 b-e	153.8 c-e	18.48 c-e
CV (%)		13.82	8.22	3.42	14.37	13.14	7.60	11.90	17.21	17.96	7.87	6.11

In a column, means followed by a common letter are not significantly different at 5% level by DMRT test. [T_1= Covered by a single layer of black cloth and a poly propylene (pp) sheet, T_2= Covered by a single layer of black cloth and white cloth and a pp sheet , T_3= Covered by a single layer of hessian (Jute mat) and a pp sheet, T_4= Covered by only a pp sheet, T_5= Covered by a double layer of black cloth and a pp sheet, T_6= Covered by a double layer of white cloth and a pp sheet, T_7 = Covered by a double layer of hessian (Jute mat) and a pp sheet, T_8 = Covered by a single layer of black cloth, T_9 = Covered by a single layer of white cloth, T_{10} = Covered by a single layer of black cloth and white cloth, T_{11} = Covered by a single layer of hessian (Jute mat), T_{12}= Open, i.e. no covering, T_{13}= Covered by a double layer of black cloth, T_{14}= Covered by a double layer of white cloth, T_{15}= Covered by a double layer of hessian (jute mat)].

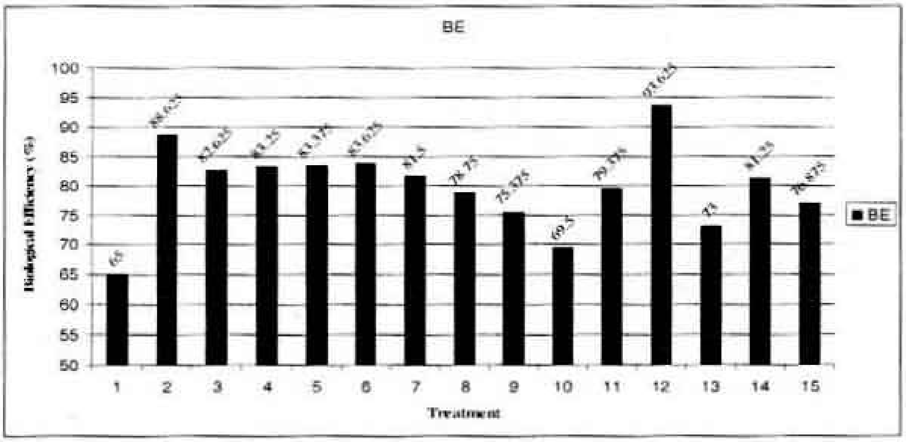

Fig. 1. Effect of light intensity on biological efficiency (%) of milky white mushroom.

Relationship between light intensity and length of stipe of milky white mushroom: A negative linear relationship was observed between light intensity and length of stipe (Fig. 2). Here the equation y = -0077x + 11.221 gave a good fit to the data and the value of co-efficient of determination (R^2 = 0.5214**) showed that the fitted regression line had a significant regression co-efficient at 1% level of probability which showed that the length of stipe increased as the light intensity decreased.

Relationship between light intensity and biological yield: A positive linear relationship was observed between light intensity and biological yield per packet (Fig. 3). It was observed that the equation y = 0.0314x + 155.1 gave a good fit to the data and the value of co-efficient of determination (R^2 = 0.284*) showed that the fitted regression line had a significant regression co-efficient at 5% level of probability. It indicated that biological yield per packet increased as the intensity of light increased.

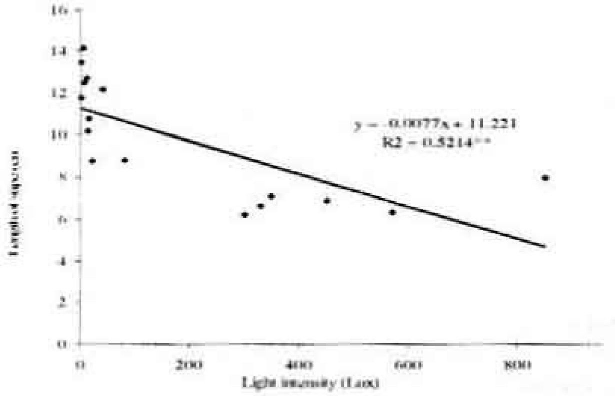

Fig. 2. Relationship between light intensity and stipe length of milky white mushroom.

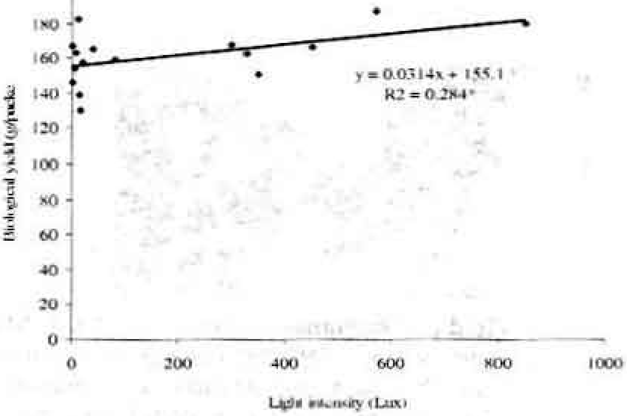

Fig. 3. Relationship between light intensity and biological yield of milky white mushroom.

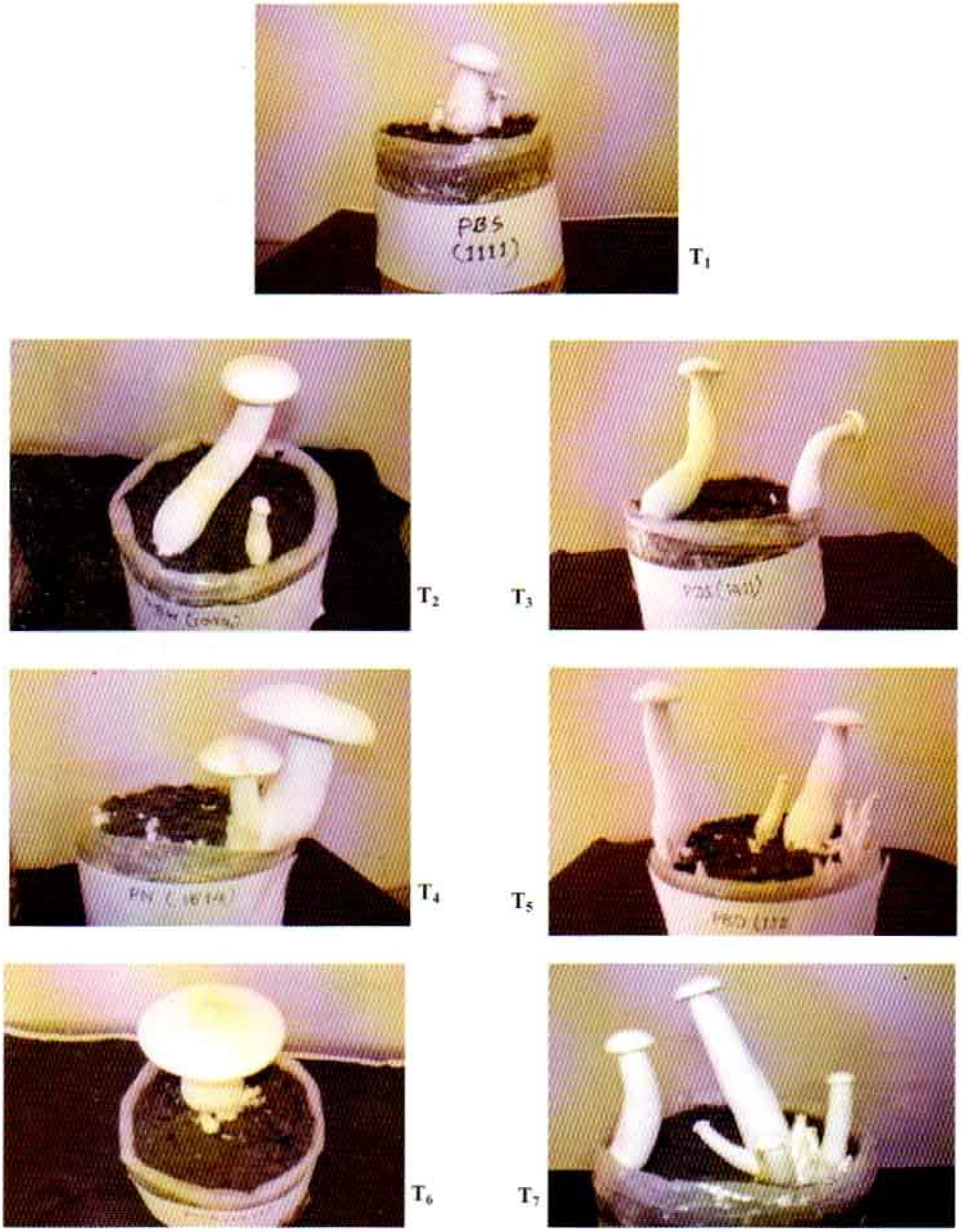

Fig. 4. Yield performance of milky white mushroom in treatment T_1-T_7. T_1= Covered by a single layer of black cloth and a poly propylene (pp) sheet, T_2= Covered by a single layer of black cloth and white cloth and a pp sheet , T_3= Covered by a single layer of hessian (Jute chot) and a pp sheet, T_4= Covered by only a pp sheet, T_5= Covered by a double layer of black cloth and a pp sheet, T_6= Covered by a double layer of white cloth and a pp sheet, T_7 = Covered by a double layer of hessian (Jute chot) and a pp sheet.

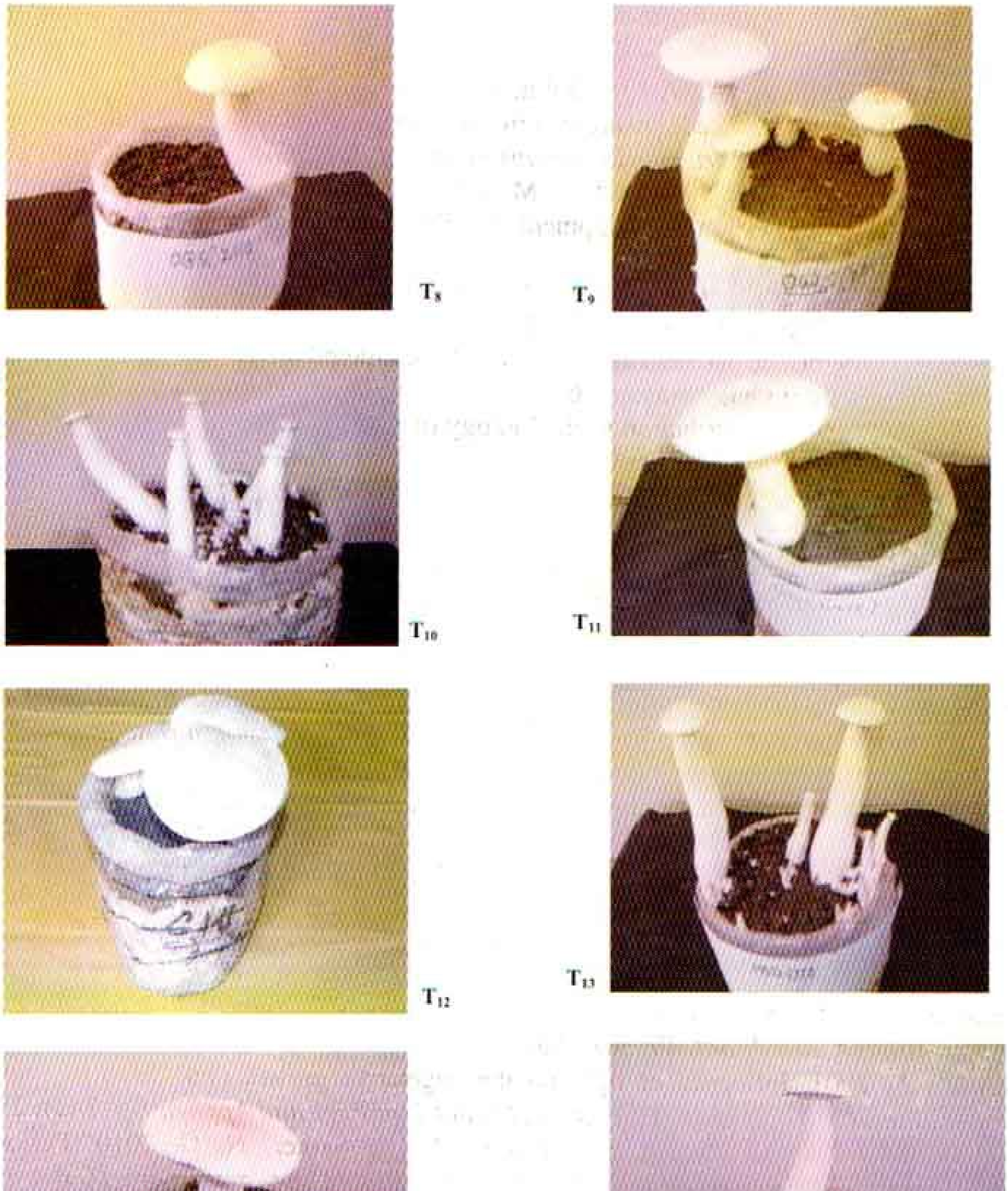

Fig: 5. Yield performance of milky white mushroom in treatment T_8-T_{15}. T_8 = Covered by a single layer of black cloth, T_9 = Covered by a single layer of white cloth, T_{10} = Covered by a single layer of black cloth and white cloth, T_{11} = Covered by a single layer of hessian (Jute chot), T_{12}= Open, i.e. no covering, T_{13}= Covered by a double layer of black cloth, T_{14}= Covered by a double layer of white cloth, T_{15}= Covered by a double layer of hessian (jute chot).

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Effect of Different Media, p^H and Temperature on Mycelial Growth and Substrates on Yield of *Pleurotus djamor*

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Abstract

A study was undertaken to determine the optimum culture media, P^H, temperature and substrate on yield of pink oyster mushroom. The highest mycelial growth rate (0.24 cm) was observed in potato dextrose agar media and the lowest mycelium growth (0.11 cm) was observed in yeast media. The highest mycelial growth rate (0.41 cm) was observed in 25^{0}C, whereas the lowest mycelial growth rate (0.20 cm) was observed in 30^{0}C. Among the p^H ranges the highest mycelial growth rate (0.45 cm) was observed in the p^H 6.5 and the lowest (0.11 cm) mycelial growth rate was observed in the p^H 4.5 which is statistically similar with p^H 9.0. Minimum days (13.25) required for completion of mycelial growth was observed in pasteurized rice straw substrate whereas the highest days (31.75) required for mycelial growth was observed in sugarcane baggase. The highest yield (253g) was obtained in paper substrate and the lowest yield (152.3 g) was shown by lentil + chickpea substrate. The highest biological efficiency (144.57%) was found in paper substrate and the lowest (87.03%) biological efficiency was found in lentil + chickpea substrate.

Key words: *Pleurotus djamor*, Substrate, Yield, Biological efficiency.

INTRODUCTION

The mycelial growth depends on several factors such as growing media, p^H, temperature, nutrient element and some environmental factors (Clam, 1981). A substrate is the surface that an organism , plant on animal live on. It can also be described as the medium it exists on. Vigorous mycelium and suitable substrate is important for the production of *Pleurotus djmou*r because growth & yield depend on those factors. The mycelial growth of different mushroom species greatly influence on media (Fasidi and Olorunmaiye, 1994; Eswaran and Rambadran, 2000).

Fasidi and kadiri (1993) reported that the successful growth of mushrooms on lignocellulose waste such as bagasse, banana leaves, plantain leaves, cereal straw, casava peals, coconut core, cotton waste (Kapola) and paper wastes, which provided the essential nutrients required for its growth. Others factors that affect mushroom growth include moisture content, temperature p^H and light intensity (Stamet, 1993; Kadiri and Kehinde, 1999). Chang and Miles (1989) also reported that Mushroom production was influenced by p^H of substrate medium, type of substrate medium, light availability, temperature and degree of aerations. This study examines the factors that enhance the optimum mycelial growth and yield of *Pleurotus djmour*.

MATERIALS AND METHODS

The present study was carried out in National Mushroom Development and Extension Centre, Savar, Dhaka from September to November 2010.

Preparation of culture media: Pure culture was prepared on different media such as potato dextrose agar (PDA), malt extract agar (MEA), potato yeast dextrose agar (PDYA) and Yeast extract agar (YEA).The culture media was prepared according to Moonmoon *et al.* (2008). Average mycelium growth and duration of complete mycelial growth was calculated.

Temperature: The selected medium was employed to evaluate the suitable temperature for mycelium growth of *P. djamor*. Same composition was adjusted at 6.5 p^H value before autoclave. After cooling the Petri plates were inoculated with the inoculums of the same mushroom. Then the inoculated plates were incubated at five different temperatures such as 10^0C, 15^0C, 20^0C, 25^0C, 30^0C. PDA media was used as a base medium.

p^H: Ten p^H levels such as 4.5, 5.0, 5.5, 6.0, 6.5, 7.0,7.5, 8.0, 8.5 and 9.0. The media were evaluated for getting the best p^H level for the *pleurotus djamor* mushroom cultivation. The p^H levels were adjusted by adding 1N HCL or NaOH before autoclaving. The PDA medium was used as the base medium.

Preparation of substrates and spawn packets: Ten different substrates as, saw dust, sugarcane baggage, rice straw (hot water treated), news paper, coir, lentil husk, chickpea, lentil + chickpea, rice straw (autoclaved) and wheat straw. Spawn packets of all the substrates except the rice straw (hot water treated) were prepared, inoculated, and incubated following the procedure mentioned by Sarker *et al.* (2007). The hot water treated rice straw was prepared following Shelly *et al.* (2010). All the packets were 500 g. After completion of mycelium running, spawn packets were opened by "D" shaped cut on the shoulder and removed the polypropylene sheet then transferred to the culture house for fruiting body initiation. The relative humidity and temperature of the culture house were maintained at 80-90% and 20-25 ^{0}C respectively by spraying water.

Data collection and Analysis: The experiment was laid out in a completely randomized design (CRD) with four replications. Data was recorded and analyzed following standard methods using MSTATC computer program and means were computed following DMRT using the same computer program. Biological efficiency was measured by the following formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

RESULTS AND DISCUSSION

Effect of culture media: The highest mycelial growth rate (0.24) was observed in the potato dextrose agar media which was similar to Khan *et al.* (1991) who reported that malt extract agar, wheat extract agar and potato extract agar were the best media for cultivation of *Auricularia politrica*, the lowest mycelial growth rate (0.11) was observed in yeast media. Minimum days required (5.25) to complete mycelial growth was observed in potato dextrose agar media and the maximum (10.25) days to complete mycelial growth was observed in yeast media.

Table 1. Effect of culture media on mycelial growth of pink oyster mushroom.

Media	Mycelial growth rate (cm/day)	Days to complete mycelial growth
Potato dextrose agar	0.24 a	5.25 d
Malt extract agar medium	0.20 a	6.75 c
Potato yeast dextrose agar	0.16 ab	8.50 b
Yeast agar medium	0.11 b	10.25 a
C.V (%)	**9.16**	**6.77**

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

Effect of Temperature: The temperature range was observed from 10^{0}C to 35^{0}C. Here mycelium growth was not observed in 10^{0}C and 35^{0}C. The highest (0.41) mycelial growth rate was observed in 25^{0}C that was similar with Amin *et. al.* (2008) who reported that 25-27^{0}C is suitable temperature for the mycelium growth of *Coriolus versicolor* . The lowest (0.20) mycelial growth rate was observed in 30^{0}C. Lowest days required for completion of mycelial growth was observed in 20^{0}C and the highest days observed were 30^{0}C.

Table 2. Effect of temperature on mycelial growth of pink oyster mushroom

Temperature	Mycelial growth rate (cm/day)	Days to complete mycelial growth
10^{0}C	-	-
15^{0}C	0.28 b	6.50 a
20^{0}C	0.26 bc	5.20 a
25^{0}C	0.41 a	7.00 a
30^{0}C	0.20 c	7.75 a
35^{0}C	–	–
C.V %	**4.01**	**9.24**

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

Effect of pH: Among the pH ranges, the highest mycelial growth rate (0.45) was observed in the pH 6.5 which is supported by the result with Ahmed *et al.* (2009) who reported that *Morchella asculenta* requires p^H 6.0-7.5 for its best mycelium running and the lowest mycelial growth rate (0.11) was observed in the pH 4.5 which is statistically similar with pH 5.0,5.5 8.5 and 9.0 decrease in mycelial growth at lower p^H could be due to the toxicity of very acidic p^H to the hyphae. This report is in line with the findings of Ibekwe *et al.* (2008) who reported that optimum mycelia production was recorded at P^H 6.4 while P^H range less than 5.0 showed no significant growth. Minimum days (5.50)required for completion of mycelial growth was observed in p^H 6.5 which is statistically similar to 6.0 and maximum days required for completion of mycelial growth rate was observed in the pH 4.5 which is statistically similar with 9.0. This study proved that mushroom mycelium prefers alkaline condition.

Table 3. Effect of pH on mycelial growth of pink oyster mushroom

pH	Mycelial growth rate (cm/day)	Days to complete mycelial growth
4.5	0.11 c	10.50 a
5.0	0.13 c	9.75 a
5.5	0.15 c	8.50 b
6.0	0.31 b	5.50 d
6.5	0.45 a	5.50 d
7.0	0.31 b	6.50 c
7.5	0.30 b	6.50 c
8.0	0.26 b	8.75 b
8.5	0.15 c	10.0 a
9.0	0.12 c	10.0 a
C.V%	**4.93**	**7.15**

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

Effect of substrates: effect of different substrates on yield and yield attributes were determined by using selected substrate.

Days required to complete mycelial growth: Days required from inoculation to complete mycelial growth rate ranged from 13.25 to 31.75 days in different substrates differed significantly at 5% level (Table 4). Minimum days (13.25) required for completion of mycelial growth was observed in hot water treated rice straw substrate which was supported by Sarker *et al.*(2007) who reported that 15 days required for mycelium running from pasteurized rice straw substrate of oyster mushroom. Whereas the highest days (31.75) required for mycelial growth was observed in sugarcane baggasse.

Days required from stimulation to first harvest: Days required from stimulation to first harvest ranged from 4.50 to 7.25 days in different substrates differed significantly at 5% level. The minimum days (4.50) required from stimulation to first harvest was found in paper substrate which is statistically similar to sawdust. The maximum days required from stimulation to first harvest was found (7.25 days) in lentil and chickpea mixed substrate.

Days required from stimulation to primordia initiation: Days required from stimulation to primordia initiation ranged from 3.50 to 7.50 days in different substrates differed significantly at 5% level (Table 4).The findings were agreed with the Patra and Pani (1995) who reported that oyster mushroom took 4-8 days for initiation of fruiting bodies. The minimum days required from stimulation to primordia initiation was found (3.50 days) was observed in wheat straw which was statistically similar to paper wastes (3.75) substrate. The maximum days required from stimulation to primordia initiation were found in Lentil + chickpea (7.50 days) which is statistically similar to lentil substrate.

Table 4. Effect of different substrates on growth of pink oyster mushroom

Substrates	Days to complete mycelium growth	Stimulation to pinhead initiation (Days)	Days to first harvest (Days)
Paper	22.50d	3.75 d	4.50 d
Sawdust	24.50c	4.75 c	4.50 d
Sugarcane bagasse	31.75a	4.50 c	6.50 a-c
Rice straw (hot water treated)	13.25e	6.00 b	6.50 a-c
Coir	30.25ab	5.75 b	6.70 ab
Lentil	28.25b	7.25 a	6.00 bc
chickpea	28.00b	6.25 b	5.00 c
Lentil + chickpea	31.25a	7.50 a	7.25 a
Rice straw	30.25ab	6.25 b	6.25 bc
Wheat straw	21.50d	3.50 d	6.50 a-c
C.V (%)	**5.51**	**8.83**	**8.33**

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

Diameter of pileus: The diameter of pileus ranged from 5.62 cm to 7.02 cm .The highest diameter (7.02) of pileus was observed in sugarcane baggase and the lowest diameter of pileus was observed in coir substrate.

Thickness of pileus (cm): The thickness of pileus ranged from 0.47 cm to 0.72 cm. The highest t thickness (0.72 cm) was found in sugarcane bagasse and lowest thickness (0.47) was found in rice straw (hot water treated) and lentil + chickpea substrate. This result is more or less similar with Sarker *et al.* (2004) reported that the thickness of oyster mushroom from that substrate was ranged from 0.53cm to 0.62 cm.

Table 5. Effect of different substrates on yield attributes of pink oyster mushroom

Substrates	Diameter of Pileus (cm)	Thickness of Pileus (cm)
Paper	6.27 bc	0.55 cd
Sawdust	6.25 bc	0.65 ab
Sugarcane bagasse	7.02 a	0.72 a
Rice straw (hot water treated)	5.95 cd	0.47 d
Coir	5.62 d	0.62 bc
Lentil	6.00 cd	0.62 bc
Chickpea	5.92 cd	0.65 ab
Lentil + Chickpea	6.27 bc	0.47 d
Rice straw	6.30 bc	0.62 bc
Wheat straw	6.60 b	0.55 cd
C.V (%)	**4.18**	**8.71**

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

Number of fruiting body: The number of fruiting bodies obtained from different substrates varied significantly at 5% level of significance and ranged from 22.50 to 39.00. The highest numbers of fruiting bodies was found in paper (39.00). The findings of this experiment was agreed with Sarker *et al.* (2007) who observed the highest fruiting body on waste paper and the lowest number of fruiting bodies was found in rice straw (25.50) which was significantly lower as compared to all the treatments.

Yield (g/packet): Significant variation was observed on yield of *P.djamor*. The yield was obtained from four flashes in the harvest period. The highest biological yield (253 00 g) was observed in paper substrate. Similar result was obtained by Sarker *et al.*, 2007 and Baysal *et al.* (2003) they found the highest yield on waste paper substrate supplemented with 20% rice husk and the lowest (152.3e) yield was obtained from lentil + chickpea substrate.

Table 6. Effect of substrate on yield of pink oyster mushroom

Substrate	Number of fruiting body	Yield g/packet
Paper	39.00 a	253.00a
Sawdust	31.75 b	248.30ab
Sugarcane bagasse	25.50 de	228.50c
Rice straw(hot water treated)	28.25 c	196.00d
Coir	27.50 cd	185.00d
Lentil	24.00 ef	181.00d
Chickpea	26.75 cd	189.50d
Lentil + Chickpea	22.50 f	152.30e
Rice straw autoclaved	26.75 cd	180.80d
Wheat straw	38.25 a	236.00bc
C. V.%	**5.29**	**5.08**

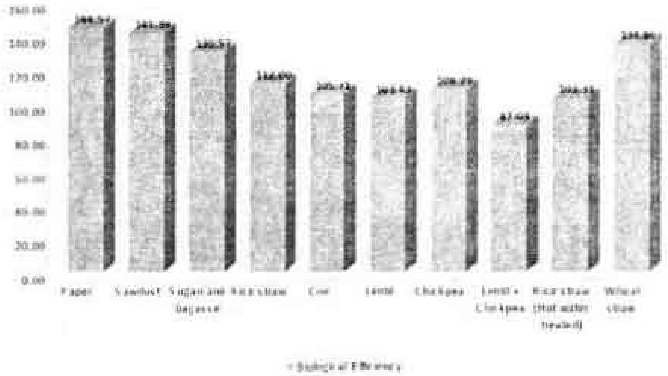

Fig. 1. Biological efficiency of pink oyster mushroom in different substrate.

Biological efficiency: The highest (144.57%) biological efficiency was found in paper substrate which is followed by sawdust (141.89) substrate, this result is supports by Howlader *et al.* (2010) she showed that wastepaper and sawdust mixed substrate gives the highest biological efficiency and the lowest (87.03%) biological efficiency was found in lentil + chickpea substrate.

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Effect of Pretreated Sawdust and Pasteurized Straw with Various Combinations on Yield of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract

Different amount of pasteurized rice straw and pretreated sawdust used for cultivation of oyster mushroom and the yield as well as yield related attributes were compared. The minimum days (6.50) required to pinhead initiation (DRPI) of oyster mushroom was recorded in autoclaved sawdust with pasteurized straw (1:1) while the maximum DRPI (13.00) was recorded in without autoclaved mixture of spawn packet with pasteurized straw. The minimum days (14.00) required to final harvesting was found in without autoclaved sawdust with pasteurized straw at the ratio of 1:2 and the maximum (35.00) was found in autoclaved sawdust with pasteurized straw. The number of effective fruiting bodies was the highest (21.00) in autoclaved sawdust with pasteurized straw (1:2) and it was the lowest (7.50) in autoclaved sawdust with pasteurized straw (1:1). The length of stalk ranged from 1.80 to 2.57cm. The highest length of stalk (2.57 cm) was found in without autoclaved mixture of spawn packet with pasteurized straw (1:2) and the lowest length (1.80 cm) of stalk was found in sundry sawdust with pasteurized straw (1:1) The diameter of stalk, pileus and thickness of pileus ranged from 0.70 to 1.11 cm; 5.66 to 7.44 cm and 0.47 to 0.55 cm respectively. The highest diameter of stalk (1.11 cm) and pileus (7.44 cm) were found in sundry sawdust with pasteurized straw (1:2). The highest yield and biological efficiency was 179.80g and 89.90% respectively, in autoclaved sawdust with pasteurized straw at 1:2 ratio respectively.

Key words: Pasteurization, Sun dry, Autoclave, Ratio, Sawdust, Straw.

INTRODUCTION

The oyster mushroom is one of the useful mushrooms and can easily be grown, if proper requirements of food and humidity are maintained for its growth (Amin *et al.*, 2007). Mushrooms are cultivated on agricultural and industrial wastes after boiling, pasteurizing and fermenting (Jiskani, 1999). Mushroom spawn is the mushroom mycelium growing on a given substrate. It serves as the planting material in mushroom cultivation. To produce spawn, inoculate a pasteurized medium, usually grain, with the sterile culture of a particular mushroom species. After the culture has grown throughout the medium, it is called spawn. It is actually the first stage of mushroom production. Spawn quality is counted the most important part in mushroom production (Mohammadi Goltapeh and Purjam, 2003). Spawn production is a very technical process and requires a lot of expertise and specialized knowledge and care on the part of people producing it (Chinda and Chinda, 2007).

Various agricultural wastes are being used as substrates for cultivation of oyster mushrooms. Some of these wastes include banana leaves, mango fruits, wheat straw and rice straw (Thomas *et al.*, 1998). The widely used substrate for cultivation of the oyster mushroom in Asia is rice straw (Thomas *et al.*, 1998). It is also considered the best substrate in terms of yield and high protein content. Oyster mushroom can be cultivated on any type of ligno and cellulosic materials like (saw dust, wheat straw and rice husk). The Oyster mushrooms can be cultivated on a wide range of cellulosic materials (Ficior *et al.*, 2006). Diffenernt sterlization methods can be used for cultivation of oyster mushroom production and its yield improvement . Using such appropriate methods, spawning will assure better resistance against any disturbance of competitive micro-organisms. Oei (1996) determined that sterilization of substrates is much more appropriate method for effective and smooth cultivation of mushrooms to remove the existence of a number of microorganisms. Bioconversion of lignocellulosic residues through cultivation of *Pleurotus* species offers the opportunity to utilize renewable resources in the production of edible, protein-rich food that will sustain food security for people in developing countries (Sanchez *et al.*, 2002). Cultivation of edible mushrooms is one of the most economically viable processes for the bioconversion of lignocellulosic wastes (Bano *et al.*, 1993 and Cohen *et al.*, 2002). The technology can also limit air pollution associated with burning agriculture wastes as well as to decrease rodents,pests and deleterious fungal inoculum populations.

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

MATERIALS AND METHODS

The experiment was conducted in the culture house of National Mushroom Development and Extention Centre, Sobhanbag, Savar, Dhaka, Bangladesh from January to March 2011. In this experiment rice straw and sawdust were used as substrate for the cultivation of *Pleurotus ostreatus* mushroom. Different amount of pasteurized straw and treated sawdust were used as treatments. The treatments were T_1= Sun dry sawdust + pasteurized straw (1:1) T_2= Sun dry sawdust + pasteurized straw (1:2) T_3= Autoclaved sawdust + pasteurized straw(1:1) T_4= Autoclaved sawdust + pasteurized straw (1:2) T_5 = Without autoclaved sawdust + pasteurized straw (1:1), T_6= Without autoclaved sawdust + pasteurized straw (1:2), T_7 = Mixture for commercial spawn packet (without autoclaved) +pasteurized straw (1:1) T_8 = Mixture for commercial spawn packet (without autoclaved)+ pasteurized straw (1:2).

Preparation of substrate: The straw was chopped to 4-5 cm length and then poured in water at 60°C for one hour and then drained out the water and kept it to get cool slowly. After about 22 hours the straw was spread over polythene sheet in the open place to reduce the moisture level at 65%. Then the sawdust was prepared by autoclaving, sundry, without autoclave (normal), mixture for commercial spawn packet preparation The sawdust was spread over polythene sheet and sun dry for 12 hours.

Preparation of spawn packets: The polypropylene bags of 9" x 12" 0r 23×30 cm size was filled with pasteurized straw and treated sawdust according to treatments. Then the packets were spawning (three layers) with the spawn packets of *Pleurotus ostreatus*, and their mouths were plugged by inserting absorbent cotton with the help of plastic necks.

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

Biological efficiency was calculated according to the formula:

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

Data collection and statistical analysis: The experiment was laid out following completely randomized design (CRD) with 4 replications. Data on days required to pinhead initiation, , number of fruiting bodies, number of effective fruiting bodies,length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, days required to harvesting, yield, number of flush and biological efficiency were recorded and analyzed following Gomez and Gomez (1984) using MSTAT-C computer program. Means separation were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

The analysis of variance for days to pinhead initiation, days to final harvest, number of fruit body, number of effective fruit body, stalk length, stalk diameter, pileus diameter, and thickness of pileus of oyster mushroom indicated significant differences among the pretreated sawdust and pasteurized straw with various combinations (Table 1).

Days required to pinhead initiation (DRPI): Appreciable variation was found in days required to pinhead initiation in different treatment of oyster mushroom and ranged from 6.50 to 13.00 (Table1). The DRPI was maximum (13.03) inT_8 followed by T_6 and T_7. The lowest DRPI (6.50) was found in T_3. Similar results were reported by Pathan *et al.* (2009), with different methods of boiling, pasteurization and fermentation. The results were varied with the findings of Amin *et al.* (2007) who reported that DRPI for oyster mushroom ranged 3- 4 days. This might be attributed to different environmental factors and culture substrates.

Days required to final harvest: The minimum days (14.00) required from opening to final harvest were observed in T_6 which was statistically similar to T_7 because single flush counted. After first harvest, the packets were contaminated due to several causes. Kurtzman (2010) reported several causes of mushroom substrate contamination. The maximum days (35.00) required from opening to final harvest were observed in T_4 which was statistically similar to T_8. The maximum days (35.00) required from opening to final harvest were observed in T_4 because three flushes counted and it was possible the packets were not contaminated.

Number of fruiting body: The number of fruiting body under different treatments varied significantly (Table 1). The highest number of fruiting body was observed in T_4 (34.75) followed by T_8 (25.25) and T_3 (20.25). The lowest number of primordia was observed in T2 (11.00).

Number of effective fruiting body: The number of effective fruiting bodies under different treatments differed significantly (Table 1). The highest number of effective fruiting bodies (21.00) was found in T_4 followed by T_8 (16.00) which were statistically different to other treatments. The lowest numbers of effective fruiting bodies were found in T_3 (7.50) which were statistically similar to T_2. The finding of the present study was supported by Sarker *et al.* (2008) who observed that the number of fruiting body of oyster mushroom ranged from 20 to 98.25/packet on wheat straw supplemented with different levels of wheat and rice bran.

Size of fruting body: The length of stalk ranged from 1.80 to 2.57 cm with significant difference (Table 1). The highest length of stalk was found in T_8 (2.57cm) which was statistically identical to all treatments except T_1. The lowest length of stalk was found in T_1 (1.80).The diameter of stalk differed significantly and ranged from 0.702 to 1.11cm (Table 1). The highest diameter was found in T_2 (1.11cm) followed by T_5 (.82 cm) while it was lowest (0.702cm) in T_7.

The diameter of pileus ranged from 5.66 cm to 7.44 cm with significant difference among the treatments (Table 1). The highest diameter of pileus was found in T_2 (7.44cm) followed by T_6 (6.54cm) and the lowest diameter of pileus was found in T_7 (5.66 cm).

The thickness of pileus in different species differed significantly and ranged from 0.47cm to 0.55 cm (Table 1). The highest thickness was found in T_2 (0.55 cm) which was statistically similar to T_1 (0.54). The lowest thickness was found in T_7 which was statistically similar to T_3, T_4 and T_8.

Yield /packet (g): Significant variation was observed in yield under different treatments (Fig. 1) because single to third flushes was counted. The highest yield (179.80g) was found in T_4 followed by T_3 (147.80g) and the lowest yield was found inT_5 (54.44g). This result was almost similar to the findings of Amin *et al.* (2007) who reported that in some species of oyster mushroom yield ranged from 43.00 g to 58.00 g/packet from one flush.

Biological efficiency (%): Significant variation was observed on biological efficiency (BE) (Fig 2). The highest biological efficiency (89.90%) was found in T_4 followed by T_3 (73.90%) and the lowest biological efficiency was found in T_5 (27.22%).

Contamination rate: There was a significant difference in percent contamination rate, which ranged from 25 to100% by green mould (*Trichoderma harzianum*) and other bacteria during cultivation (Table 2). The highest contamination rate (100%) was found in T_1 followed by T_5, T_6, T_7, T_8 (100%) and the lowest contamination rate was found inT_4 (25%) during third harvest. The ability of sterilization method to eliminate substrate contaminants is shown by the presence or absence of contaminants in the substrate after sterilization, spawning and incubation. According to Balasubramanya and Kathe (1996), the microorganism species that competed with *Pleurotus* sp. after pasteurisation with hot water (80°C for 2h) were the fungi *Penicillium* sp. and *Trichoderma* sp. probably due to the partial breakdown of cellulose and hemicelluloses, thus making them available to competitors.

Table 1. Effect of pretreated sawdust and pasteurized straw with various combinations on yield attributes of oyster mushroom

Treatment	Days to pin head initiation	Days to final harvest	Number of fruit body	Number of effective fruit body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1	8.50 c	28.75c	16.00d	9.50e	1.80b	0.87c	6.45b	0.54a
T_2	8.25c	20.75e	11.00e	8.50ef	2.49a	1.11a	7.44a	0.55a
T_3	6.50d	27.00d	20.25c	7.50f	2.48a	0.81cd	6.22bc	0.49b
T_4	7.25cd	35.00a	34.75a	21.00a	2.30a	0.77d	6.02cd	0.47b
T_5	8.50 c	32.00b	12.00e	12.00d	2.45a	0.82 cd	5.94cd	0.51ab
T_6	11.25b	14.00f	17.00d	14.00c	2.28a	0.94b	6.54b	0.51ab
T_7	10.75b	15.00f	16.00d	9.50e	2.60a	0.70e	5.66d	0.47b
T_8	13.00a	33.75a	25.25b	16.00b	2.57a	0.77d	5.81d	0.49b
CV (%)	**8.83**	**3.38**	**4.32**	**6.67**	**8.24**	**5.40**	**3.93**	**6.2**

In a column do not differ significantly at 5 % level according to DMRT. [T_1=sun dry sawdust + pasteurized straw (1:1) T_2= sun dry sawdust + pasteurized straw (1:2) T_3=autoclaved sawdust + pasteurized straw(1:1) T_4=autoclaved sawdust +pasteurized straw (1:2) T_5 = without autoclaved sawdust + pasteurized straw (1:1). T_6= without autoclaved sawdust+ pasteurized straw (1:2). T_7 = mixture for commercial spawn packet (without autoclaved)+ pasteurized straw (1:1) T_8 = mixture for commercial spawn packet (without autoclaved)+ pasteurized straw (1:2)].

Table 2. Effect of pretreated sawdust and pasteurized straw with various combinations on contamination rate of oyster mushroom in different stage

Treatment	Incubation	1 Flash	2 Flash	3 Flash
T1	-	--	43.75 c	100.0 a
T2	-	--	0.00 e	50.00 c
T3	-	--	0.00 e	62.50 b
T4	-	--	0.00 e	25.00 d
T5	-	--	87.50 a	100.0 a
T6	-	--	25.00 d	100.0 a
T7	-	--	82.25 b	100.0 a
T8	-	--	25.00 d	100.0 a
CV (%)	**-**	**--**	**4.40**	**2.11**

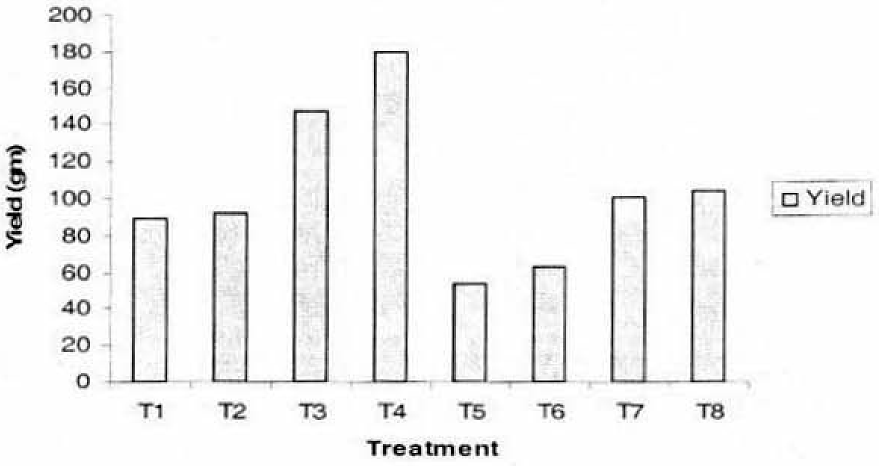

Fig. 1. Yield of *Pleurotus ostreatus* on pretreated sawdust and pasteurized straw with various combinations.

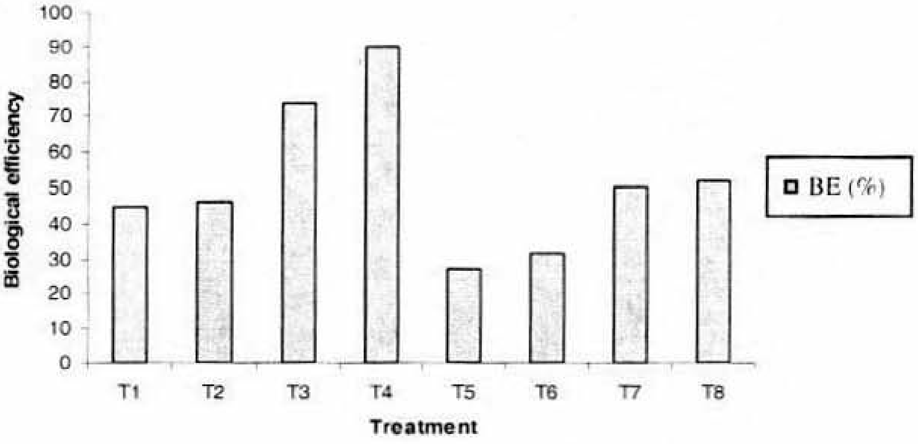

Fig. 2. Biological efficiency of *Pleurotus ostreatus* on pretreated sawdust and pasteurized straw with various combinations.

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Influence of Different Supplements on the Growth and Yield of Straw Mushroom

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Abstract

Effect of different supplements on the growth, yield and quality of straw mushroom (*Volvariella volvacea*) was investigated. Rice straw substrate was used with eight different supplements such as wheat bran, rice bran, maize powder, saw dust, rice husk, sugarcane baggage, mastered cake, cotton waste with a control. Straw mushroom cultivation on maize powder (4.25 Days) and control (4.50 days) had significantly faster mycelial growth as compared to other supplements. Cultivation of straw mushroom on wheat barn gave the highest production (1010 g/bed) and biological efficiency (33.75 %). The lowest biological yield (285.00 g/bed) and biological efficiency (9.50 %) was recorded when it was culture on sugarcane baggage. The present results suggest that commercial production of straw mushroom wheat bran is most effective and for mycelium growth maize powder is the best.

Key words: Yield, *Volvariella volvacea,* Supplements, Mycelium growth.

INTRODUCTION

Volvariella volvacea, the fourth most important cultivated edible mushroom in the world (Chang, 1983) known as 'Ogiri-agbe' among the Yoruba's of Nigeria, is a popular edible fungus of the tropics and subtropics which grows well on cellulosic agricultural residues and industrial wastes (Chang, 1978 and Chang and Yau, 1971). Large quantities of agricultural wastes (agro wastes) are generated all over the world. Mushrooms have been reported to be capable of transforming nutritionally worthless wastes into protein rich food and have been confirmed to be sources of single cell protein (Kurtzmam, 1981 and Alofe *et al.,* 1998). The cultivation of edible mushroom like *V. volvacea* on these wastes may thus be a value added process capable of converting these materials, which are otherwise considered to be wastes, into foods and feeds (Bisaria *et al.,* 1997). The cultivation of *V. volvacea* on agro wastes may also offer economic incentives for agribusinesses to examine these residues as valuable resources and develop new enterprises by using them to produce nutritious mushroom products (Bisaria *et al.,* 1987). In the humid tropics, pieces of land are cultivated continuously, leading to loss of soil fertility, nutrient deficiency symptoms on the crop and loss of yield. High cost and scarcity does not encourage the use of fertilizers, hence the need to the use of locally and readily available plant residues which are usually discarded.

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Paddy straw mushroom is also known as "warm mushroom" as it grows at relatively high temperature. It is a fast growing mushroom and under favorable growing conditions total crop cycle is completed with in 4-5 weeks time. This mushroom can use wide range of cellulosic materials and the C: N ratio needed is 40 to 60, quite high in comparison to other cultivated mushrooms. It can be grown quite quickly and easily on uncomposted substrates such as paddy straw and cotton waste or other cellulosic organic waste materials (Ahlawat and Kumar, 2005).

According to Zoberi (1972), the species grows naturally on dead leaves, dead wood, animal dropping, on trees and waste stumps. The major problem associated with the transfer of technology for mushroom cultivation is the lack of technical know how for its cultivation and the lower biological efficiency as compared to other tropical mushrooms. A variety of substrate including fruit and vegetable wastes and straw of different cereals and grasses has been used for cultivation of *Volvariella species* (Chang, 1979). The cultivation of *Volvariella spp* has been tested in various agricultural by-products as substrates for the cultivation of the paddy straw mushroom. Some of this wastes/ agro-waste includes banana leaves, saw dust, rice barn, wheat barn, sugarcane baggage (Tripathy, 1999), wheat and rice straw (Cangy and Peerally, 1995). The majority of these substrates can be used as animal feed.

Yield of straw mushroom may fluctuate due to certain factors such as nutritional property of raw material, pest and disease outbreak in the case of indoor farming, geographic region and cultivating season. To increase the yield of straw mushroom under indoor–farmed conditions using different supplements are necessary to be investigated. Therefore, the present investigation was carried out to find out the suitable supplement for mycelium growth and yield potential of straw mushroom.

MATERIALS AND METHODS

This experiment was carried out in a CRD with 5 replications at National Mushroom Development and Extension Centre, Savar, Dhaka from September to November 2010.

Preparation of culture media: There are several media on which the mushroom culture can grow, such as potato dextrose agar (PDA), malt extract agar (MEA), potato yeast dextrose agar (PDYA) and Yeast extract agar (YEA). The compositions of potato dextrose agar (PDA) are given below: Washing, peeling and slicing of 200g potatoes. Boiling in 1000 ml distilled water until potatoes become soft enough to be eaten but not over cooked. Straining through cheese cloth and collecting of liquid in graduated cylinder followed by restoring of volume to 1000 ml by adding fresh distilled water. The basal component of the media was mixed 20 g of dextrose and 20 g of agar at pH 6.5. The media was boiled on gas burner until the agar dissolved. The media was poured into Petri dishes (90 mm diameter) at 20 ml/plates and sterilized in an autoclave for 20 minutes at 120^0C under 1.5 kg/cm^2 pressure. After sterilization and solidification, the plates were transferred in to incubation room for mycelium running at 25^0C temperature.

Preparation of mother culture: A number of materials alone or in different combinations are popular as spawn substrates. The most common substrates are rice straw cuttings, wheat and maize grains, cotton waste, used tea leaves etc. The protocols of rice straw substrates are mentioned below: At first the rice straw cut into pieces of 4 to 5 cm long. Then rice straw was soaked in water for 2 to 4 hours. Wheat barn was mixed @ 30% wet basis and the polypropylene bags of 7" × 10" size were filled with 300 g of substrate and their mouths were plugged by inserting water absorbing cotton with the help of plastic neck. The bags were autoclaved at 121^0C and 1.5 kg/cm^2 pressure for 2 hours. After autoclaving and cooling, the bags were inoculated with the tissue culture of *Volvariella volvacea*. Then, the inoculated packets were kept for mycelium running at 25^0-30^0C.

Substrate Preparation: The substrate used for this experiment was rice straw. Rice straw substrate was chopped to convenient length of about 4 to 5 cm. and the chopped rice straw was poured into a net bag and treated with hot water at 60°C in a drum for one hour and allowed to drain out the excess water by hanging the bag for 20 hours.

Supplementation: For the experiment, nine supplements were selected. They were, rice straw (Control), Wheat bran (WB), Rice bran (RB), Maize powder (MP), Saw dust (SD), Rice husk (RH), Sugarcane baggage (SB), Mastered cake (MC), Cotton waste (CW).

Each supplement was applied 15% of dry substrate. Before spawning, these were added and mixed thoroughly with the substrate. A wooden rack (30cm × 30cm × 1m) = 0.09 m^3 is used for the bed preparation. At first mixed substrate sprayed in the wooden rack with 10 cm thickness and mother culture mixed thoroughly on the surface. After complete first layer another layer done by same technique. Same process repeated for five to six times but in opposite direction in each layer. The inoculated beds were covered with polythene sheet and incubated at 38 to 40^0 C temperature for ramifications of the mushroom mycelia. After full ramification, polythene sheet were removed and watering on bed was done. Thus, the supplements constituted 9 treatments including control.

Observation: Rice straw substrate was used with eight different supplements such as wheat bran, rice bran, maize powder, saw dust, rice husk, sugarcane baggage, mastered cake, cotton waste and control were used to make nine treatments. A total of eight yield and yield contributing data were collected viz. mycelium run period, pin head appearance, days to first harvest, days to total harvest, number of fruit bodies, yield, average fresh weight of single fruiting body and Biological efficiency.

Biological efficiency was determined by the following formula:

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

All the calculated data were statistically analyzed and the means were separated by DMRT.

RESULTS AND DISCUSSION

Mycelium running Period: Mycelium running period was found significantly different among the using supplements and ranged from 4.25 to 8.25 days. The highest mycelium run period was found the supplement maize powder (4.25 Days) and control (4.50 days) as compared to rice husk (8.25 days) and Mastered cake (7.75days). Chen *et al.* (1973) and Chua *et al.* (1973) reported that *Volvariella volvacea* mycelia grows very well on wide range of lignocelluloses waste such as banana leaves, sawdust, wheat barn, sugarcane baggage, waste tea dust, cotton waste, oil palm bunch wastes but their mean mycelia yields are comparably low in some these wastes. Substrates and supplements structure is an important factor for the growth of the mycelium as it should be suitable for penetration of the mycelium.

Days required to primordial initiation (DRPI): The days required to primordial initiation differed significantly and ranged from 6.75 to 11.00 days (Table 1). The highest 11.00 days required for primordial initiation (DRPI) was found at rice husk in the treatment T_6 which was statistically similar to the treatment T_8. The lowest DRPI (6.75 days) was found at maize powder in the treatment T_4. Tripaty *et al.* (2011) reported that where minimum 5 days and maximum 9 days required for primordia initiation when *Volvariella volvacea* was cultured on rice bran, wheat bran, rice straw, sawdust, banana leaf and sugarcane baggage supplements

Days required to first harvest: The days required for first harvest (DRFH) differed significantly and ranged from 10.00 to 14.50 days (Table 1). The lowest days required for first harvest (10.00 days) was found at control in the treatment T_1. The treatment (T_1) was designed by only rice straw used for the preparation of *Volvariella volvacea* bed. The highest DRFH (14.50 days) was found in the treatment T_6 which was statistically similar to the treatments T_8 (14.25 days).

Days required to total harvest: The lowest days (25.25 days) required for total harvest (DRTH) was found at sugarcane baggage in the treatment T_7 (Table 1). The highest DRTH (36.25 days) was found at saw dust in the treatment T_5, which was statistically similar to the treatments T_2 and T_4.

Number of effective fruiting body: The number of effective fruiting body (NEFB) differed significantly and ranged from (31.75 to 92.00)/bed (Table 1). The highest NEFB (92.00/ bed) was observed at saw dust, which was statistically differ to other treatments. The lowest NEFB (31.75/ bed) was observed at sugarcane baggage. Tripaty *et al.* (2011) observed that the average number of effective fruiting body ranged from (40 to 91)/bed, when *Volvariella volvacea* was cultured on rice bran, wheat bran, rice straw, sawdust, banana leaf and sugarcane baggage supplements which was similar with the present study where the highest number of fruiting bodies (91) was recorded with wheat barn and rice barn supplements.

Fresh weight of each fruiting body: In case of *Volvariella volvacea* significantly variation was observed in fresh weight of each fruiting body. Maximum fresh weight of each fruiting body was recorded with the supplement wheat bran (16.75 g) and cotton waste (13.25 g) in comparison to other supplement. The minimum average fresh weight of single fruiting body was produced by sugarcane baggage supplement (8.75 g).

Table 1. Effect of different supplements to the substrate on yield of *Volvariella volvacea*

Treatment	Mycelium run Period	Days required to primordial initiation	Days required to first harvest	Days required to total harvest	Number of effective fruiting body/ bed	Yield/ bed (g)	Fresh Weight of each Fruiting Body
T1	4.50 c	7.00 c	10.00 c	32.50 c	70.75 c	778.00 d	11.00 bc
T2	5.50 b	7.50 bc	12.00 b	35.75 a	61.25 d	1010.00 a	16.75 a
T3	6.00 b	8.50 b	12.25 b	31.25 d	78.75 b	720.00 e	9.25 e-g
T4	4.25 c	6.75 c	10.75 bc	34.25 b	79.00 b	935.50 b	11.75 c
T5	5.50 b	8.00 bc	11.25 bc	36.25 a	92.00 a	927.00 b	10.25 de
T6	8.25 a	11.00 a	14.50 a	28.50 e	59.75 de	542.50 f	9.00 fg
T7	5.50 b	8.00 bc	11.75 b	25.25 g	31.75 f	285.00 g	8.75 g
T8	7.75 a	10.00 a	14.25 a	26.00 fg	55.50 e	563.80 f	10.00 d-f
T9	5.50 b	8.00 bc	12.25 b	26.75 f	61.75 d	824.00 c	13.25 b
CV (%)	**10.17**	**9.48**	**8.02**	**2.69**	**4.67**	**3.87**	**7.04**

Means followed by a common letter in a column are not significantly different at 5% level according to DMRT. T1 = Control (Rice straw), T2 = Wheat bran (WB), T3 = Rice bran (RB), T4 = Maize powder (MP), T5 = Saw dust (SD), T6 = Rice husk (RH), T7 = Sugarcane Baggage (SB), T8 = Mastered cake (MC), T9 = Cotton waste (CW).

Yield/bed (g): Significant variation was observed in yield in different treatments and it was ranged from 285.00 to 1010.00 g/bed (Table 1). The highest yield (1010.00 g/bed) was recorded at wheat bran in the treatment T_2 followed by T_4 (935.50 g/bed), T_5 (927.00 g/bed). The lowest yield 285.00 g/bed was recorded at sugarcane baggage in the treatment T_7. Tripaty *et al.* (2011) observed that the yield ranged from (500 to 1360 g/bed), when *Volvariella volvacea* was cultured on rice bran, wheat bran, rice straw, sawdust, banana leaf and sugarcane baggage supplements which was similar with the present study. Where the highest biological yield (1360 g/bed) was recorded with wheat barn and rice barn supplements.

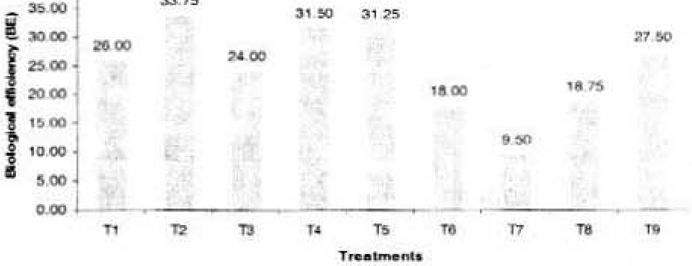

Fig. 1. Biological efficiency of different supplements to the substrate on yield of *Volvariella volvacea*.

Biological Efficiency: The biological efficiency was influnced by different supplements. Wheat bran supplement resulted with the highest biological efficiency in *Volvariella* *volvacea* (33.75 %)(Fig.1). Sugarcane baggage showed the lowest biological efficiency (9.50 %) in *Volvariella volvacea.* Bolton and Blair (1982) and Fasidi (1996) reported that rice husk is good for the production of *V. esculenta* because of its richness in oils and vitamins which are good stimulants for high mushrooms yield.

The highest yield of *Volvariella volvacea* (1010 g/bed) was obtained from wheat barn supplement. So the use of wheat barn supplemented is the most suitable due to improved growth and sporophore production. Rice straw as substrate for mushroom bed is recommended for its cheapest quality and readily available in tropical countries.

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Effect of Oyster Mushroom (*Pleurotus ostreatus*) on Renal Function of Hypertensive Diabetic Male Volunteers

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Abstract

The present study was carried out to evaluate the effect of oyster mushroom (*Pleurotus ostreatus*) on renal function of hypertensive diabetic male volunteers. The study was conducted in the National Mushroom Development and Extension Centre, Sobhanbag, Savar Dhaka in association with the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. Three grams of dried oyster mushroom (*Pleurotus ostreatus*) powder as capsule form was taken by the subjects in three divided doses for three months. The finding of the study showed no significant change of plasma urea (27.63 ± 1.80 and 29.84 ± 1.80, $p = 0.342$), creatinine (0.92 ± 0.05 and 1.01 ± 0.04, $p = 0.176$) and uric acid (5.14 ± 0.26 and 5.12 ± 0.23, $p = 0.914$). These findings suggest that *Pleurotus ostreatus* has no detrimental effect on kidney function of hypertensive diabetic male human subjects.

Key words: Urea, Creatinine, Uric acid, *Pleurotus ostreatus*.

INTRODUCTION

Mushrooms have been known for their nutritional and culinary values and used as medicines and tonics by humans for ages. Edible mushrooms have been widely utilized as human foods for centuries and have been appreciated for texture and flavor as well as some medicinal and tonic attributes (Manzi *et al.*, 2001). However, the awareness of mushrooms as a healthy food and as an important source of biological active substances with medicinal value has only recently emerged (Cheung *et al.*, 2003). Mushrooms are considered as healthy food because they are low in calories and fat but rich in proteins and dietary fibers (Manzi *et al.*, 1999). The mushroom protein contains all the essential amino acids required by humans. In addition to their good protein content, mushrooms are a relatively good source of the nutrients like phosphorus, iron and vitamins, including thiamine, riboflavin, ascorbic acid, ergo sterol, and niacin (Barros *et al.*, 2008).

In modern terms, they can be considered as functional foods which can provide health benefits beyond the traditional nutrients. There has been a recent upsurge of interest in

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mushrooms not only as a health food which is rich in protein but also as a source of biologically active compounds of medicinal value which include complementary medicine / dietary supplements for anticancer, antiviral, hepatoprotective, immunopotentiating and hypocholesterolemic agents. The major attribute of mushrooms is their medicinal properties which have been the main focus of researchers around the world. The various activities of mushrooms have been studied which includes hypotensive and renal effects (Ribeiroa *et al.*, 2009). Oyster mushrooms are best known medically for their cardiovascular and cholesterol controlling benefits. In addition, they have been shown to have the following qualities: anti-tumor, stimulate immune response, anti-inflammatory, antiviral, antibiotic. anecdotal reports suggest, oyster mushrooms improve liver and kidney function and help gastrointestinal disorders (Ying and Weil, 1987).

Jayakumar *et al.* (2008) studied the protective effect of the *Pleurotus ostreatus* on carbon tetrachloride (CCl_4)-induced toxicity in male Wistar rats. Histopathological studies confirmed the toxic effects of CCl4 on other organs such as kidneys, heart and brain and also tissue protective effect of the extract of *Pleurotus ostreatus*. These results suggest that an extract of *Pleurotus ostreatus* is able to alleviate the oxidative damage caused by CCl_4 in the kidneys, heart and brain of Wistar rats. Nuhu Alam *et al.* (2009) revealed that feeding of 5% oyster mushroom powder do not have detrimental effects on the liver and kidneys rather may provide health benefits for the cardiovascular-related complication by decreasing the atherogenic lipid profiles. Besides these mushroom consumption significantly reduced systolic and diastolic blood pressure, lowered plasma glucose, total cholesterol and triglycerides significantly, whereas there was no significant change in body weight. In a study it was observed that there were no deleterious effects on liver or kidney function (Khatun *et al.*, 2007). Sirag (2009) was studied to investigate the putative protective effect with antioxidant potential of the *Pleurotus ostreatus* in glycerol-induced Acute Renal Failure (ARF) in rats. He suggested that *Pleurotus ostreatus* may have ability to protect the renal damage involved in acute renal failure in rats.

The kidneys are vital organs that filter impurities out of the bloodstream, which body then flushes out though the excretory system. Protein is an essential nutrient to the body that helps repair muscle tissue and fight disease. Eating protein (especially complete proteins) is so important to staying healthy. The average person needs between 40 to 65 grams of protein each day. Kidneys help separate protein from waste and filter the protein throughout the body. But excessive amounts of protein can strain kidneys. Protein can be tricky for chronic kidney disease (CKD) patients. Although protein is a necessary nutrient, CKD patients are often faced with the dilemma of having to limit protein intake. In addition, high blood pressure makes the heart work harder and, over time, can damage blood vessels throughout the body. If the blood vessels in the kidneys are damaged, they may stop removing wastes and extra fluid from the body. The extra fluid in the blood vessels may then raise blood pressure even more. So, in these cases, our study was to investigate whether or not the oyster mushroom shows deleterious effect on renal function of hypertensive male volunteers.

MATERIALS AND METHODS

The study was conducted in the laboratory of Strengthening Mushroom Development project, National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka. A total of 19 hypertensive diabetic male volunteers (systolic BP 140-180 mmHg and/or diastolic BP 90-110 mmHg and fasting plasma glucose 7.2-15.6 mmol/l) age range 29 to 67 years, who were free from renal impairment and other known acute or chronic diseases were included in the study after taking their informed written consent. Subjects with a history of addiction other than smoking were also excluded from the study. They were allowed to continue the medication they were taking. Age, sex, occupation, educational status, marital status, family history and drug history were recorded in a preformed data collection sheet.

At the beginning of the study, subjects were evaluated for health status. Both systolic and diastolic blood pressure was measured following standard procedure using sphygmomanometer by a trained physician. Mean of duplicate measurements was taken. With all aseptic precautions, 10 ml of fasting blood was collected from median cubital vein. Immediately after collecting, blood was poured into fluoride and EDTA containing test tube. The test tube was then gently shaken for proper mixing with the anticoagulants. This anticoagulant-mixed blood was centrifuged at 3000 rpm for 5 minutes. Separated plasma was transferred into two eppendorf containing 1 ml in each. Plasma urea was estimated by enzymatic, colourimetric, endpoint – Berthelot method. Plasma creatinine was estimated by alkaline picrate method and plasma uric acid was estimated by uricase colorimetric method. Analysis was done by semi auto biochemical analyzer 3000 evaluation using commercially available reagent kit. All the tests were carried out as early as possible.

Fresh fruiting bodies of *Pleurotus ostreatus* were collected from culture house of National Mushroom Development and Extension Centre (NAMDEC). Collected mushrooms were then sun dried at moisture level 4-5%, then grinded and poured into capsule shells which contain 500 mg powder. Prepared capsules were ready to dispense and preserved into moisture free glass containers.

Mushroom capsules containing 500 mg *Pleurotus ostreatus* powder in each were supplied to take two capsules three times daily, so that each subject took 3 gms mushroom powder daily.

After three months the subjects were re-evaluated and all the laboratory investigations were repeated.

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

RESULTS AND DISCUSSION

Not only in Bangladesh, *Pleurotus* species is one of the most famous edible mushroom which have lot of ameliorative effect on health. But it is questionable, whether it has any detrimental effect on kidney. The present study was conducted to observe the effect of oyster mushroom (*Pleurotus ostreatus*) on the kidney function of hypertensive diabetic male subjects.

In the study it was observed that the mean ± SE plasma urea before and 3 months after mushroom supplement was 27.63 ± 1.80 and 29.84 ± 1.80 respectively (Fig. 1). There was no statistically significant mean difference ($p = 0.342$) between the two periods.

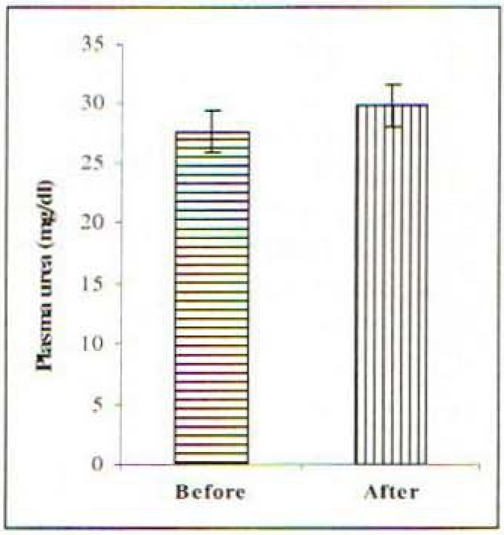

Fig. 1. Mean (± SE) plasma urea before and 3 months after mushroom supplementation.

The mean ± SE plasma creatinine (mg/dl) before and three months after mushroom treatment was 0.92 ± 0.05 and 1.01 ± 0.04 respectively. No significant mean difference of creatinine ($p = 0.176$) was observed (Fig. 2).

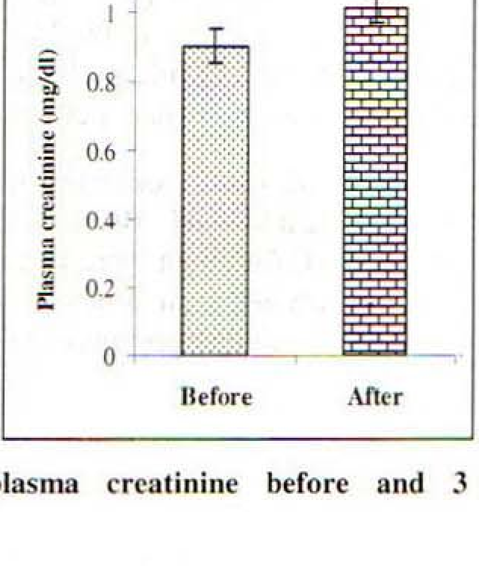

Fig. 2. Mean (± SE) plasma creatinine before and 3 months after mushroom supplementation.

The mean ± SE plasma uric acid (mg/dl) before and 3 months after mushroom treatment were 5.14 ± 0.26 and 5.12 ± 0.23 respectively. Here is also no significant mean difference of uric acid ($p = 0.914$) observed (Fig. 3).

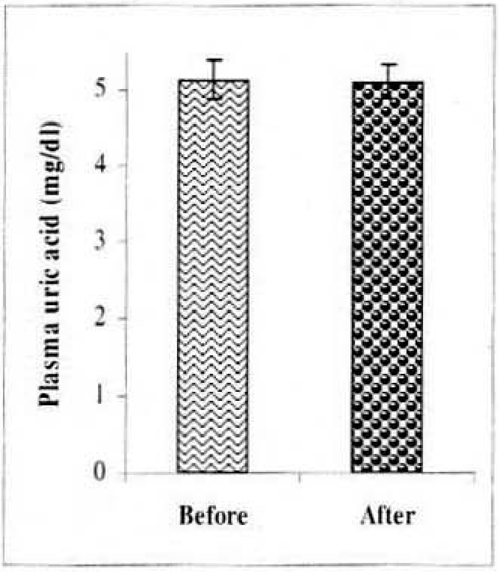

Fig. 3. Mean (± SE) plasma uric acid before and 3 months after mushroom supplementation.

In this study, although there is no significant change of plasma urea and creatinine, we observed a small rising of these two parameters. Previous studies shown that oyster mushroom is rich in high quality protein and this value is 19 to 39 gm in 100 gm dried matter (Breene, 1990). On the other hand urea and creatinine are protein metabolites. So, these two parameters (urea and creatinine) normally can rise in increasing protein intake. Again, this small rising of urea and creatinine is within normal safe limit (in case of urea 15-55 mg/dl and in case of creatinine 0.6-1.2 mg/dl). On the other hand, although statistically non significant, the change of uric acid shows declining tendency after three months treatment of mushroom capsule. Uric acid is the final oxidation (breakdown) product of purine metabolism and it accumulates in the blood due to renal impairment. Though this negligible declining tendency of plasma uric acid gives no statistically significant status but this trend indicates a stable renal performance.

In a study of animal model Alam *et al.* (2009) observed that there was no significant difference in plasma bilirubin, creatinin and BUN levels in 5% mushroom-fed hypercholesterolemic rats after 40 days. Although there is no sufficient human data, in a study, Khatun *et al.* (2007) observed no detrimental effect of oyster mushroom on renal function. This trend was also supported by other previous study of this author (Choudhury *et al.*, 2008).

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Influence of Multilayer Rack Cultivation on Growth and Yield of Milky and Straw Mushrooms

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Abstract

A study was undertaken to find out the effect of multilayered rack cultivation on growth and yield of straw mushroom (*Volvariella volvacea*) and milky mushroom (*Calocybe indica*). Straw mushroom was grown in beds which are arranged in rack and milky mushroom in polypropylene bags. Pasteurized rice straw was used as substrate. The racks and polypropylene bags were placed on shelves at the height of 0, 15, 60, 105, 150, 195 and 240 cm from the floor. In case of straw mushroom, the maximum yield of mushroom was obtained from 0 cm shelf height i.e. when the bed was placed on the floor followed by the shelf height 15 and 60 cm from the floor. The treatments gave 983.30, 448.80 and 437.50 g/bed, respectively. The lowest yield was observed at the height of 195 cm and no growth was observed at 240 cm from the floor. In case of milky mushroom, the growth and yield also reduced gradually with the increase of shelf height from the floor. In case of milky mushroom, the yield increased gradually up to 60 cm and decreased gradually thereafter. Its yield was 225.80, 245.80, 268.00, 161.30, 140.30, 135.30 and 82.75 g/packet at the height of 0, 15, 60, 105, 150, 195 and 240 cm from the floor, respectively. Based on findings of the study it may be concluded that the straw mushroom may be cultivated on floor and milky mushroom at the maximum height of 60 cm from the floor to get higher yield.

Key words: Rack cultivation, Multilayer, *Volvariella volvacea*, *Calocybe indica*, Cultivation.

INTRODUCTION

Straw mushroom (*Volvariella volvacea*) and milky mushroom (*Calocybe indica*) are two high yielding mushroom species (Chang, 1974 and Purkayastha and Chandra, 1974). They can be grown in door as well as outdoor (Quimio *et al.*, 1990). They require a temperature range of 30-35^{0}C and a relative humidity range of 70-80% for cultivation (Amin *et al.*, 2007 and Krishnamoorthy *et al.*, 2000), which is prevailed in Bangladesh. In indoor system, both the mushrooms are cultivated mostly on floor. Vertical cultivation of two mushroom species on multilayered shelves may give maximum yield per unit area. The present piece of research was undertaken to find out the effect of multilayered rack cultivation on shelves on growth and yield of straw and milky mushrooms.

MATERIALS AND METHODS

Experimental site and duration: Two experiments were conducted in a mushroom culture house of National Mushroom Development and Extension Centre, Savar, Dhaka during September to November 2011 to determine the effect of multilayered rack cultivation on shelves at different height from the floor on growth and yield of straw and milky mushrooms. In the first experiment, straw mushroom (*Volvariella volvacea*) was grown and in the second experiment, milky mushroom (*Calocybe indica*) was grown. The shelf height was 0, 15, 60, 105, 150, 190 and 240 cm from the floor.

Preparation of culture media: Potato dextrose agar (PDA) was used as a tissue culture medium of two mushroom species. The ingredients of PDA was 200 g peeled potato slices 20 g dextrose and 20 g agar. The potato slices were boiled in 1 L distilled water until potatoes become soft and the content was passed through cheese cloth. The extract was poured into a measuring cylinder. The agar and dextrose was added and required quatity of distilled water was also added to make the volume 1 L. The pH of the mixture was adjusted 6.5. The medium was heated until the agar completely melted. The media was poured into Petri dishes (90 mm diameter) at 20 ml/plates and sterilized in an autoclave for 20 minutes at 120^0C under $1kg/cm^2$ pressure. After sterilization and solidification, the plates were inoculated with the tissue of individual species. The plates were incubated in an incubation room at 25^0C temperature for mycelium running. After 07 days when the media is fully covered with mycelia then it is used for mother culture.

Preparation of mother culture of straw mushroom: Rice straw was cut into pieces of 2.5 to 5 cm long and soaked in water for 2 to 4 hours. Wheat bran was mixed @ 30% (w/w) Polypropylene bags of 7" × 10" size were filled with 300 g of the substrate and their mouth was plugged by inserting absorbing cotton with the help of plastic neck. The bags were autoclaved at 121^0C under 1.5 kg/cm^2 pressure for 2 hours. After autoclaving and cooling, the bags were inoculated with the tissue culture of *V. volvacea*. The inoculated packets were incubated at $25\text{-}30^0$C for mycelium running for 10 days.

Preparation of mother culture for milky mushroom: Mother culture of milky mushroom was prepared on wheat grain. The grain was soaked in water for 24 hours, washed in flowing water and boiled for 30-40 minutes. The excess water of the grain was drained off and the grain was being mixed with 0.2% $CaCo_3$. The moisture content was 60-65%. Exactly 300 g of substrate was poured into polypropylene bags (7"×10") and their mouth was plugged by inserting absorbing cotton with the help of plastic neck. The bags were autoclaved at 121^0C under 1.5 kg/cm^2 pressure for 2 hours. After autoclaving and cooling, the bags were inoculated with the tissue culture of milky mushroom. The inoculated packets were incubated at $25^0\text{-}30^0$C for mycelium running.

Substrate Preparation: For growing both the mushroom species, rice straw was used as the substrate. To prepare the substrate, fresh rice straw was chopped in to 4 to 5 cm pieces. The straw pieces were pasteurized at 60^0 C for 60 minutes using steam. The pasteurized straw was then incubated for 16-22 hours.

Growing straw mushroom: Wooden frame measuring 30 cm width × 30cm deep × 1m long (0.9 m^3) were used for bed preparation. At first, the substrate was spread in the wooden rack at 10 cm thickness then mother culture is spreaded over the outer portion of the substrate then another 10 cm layer of substrate and mother culture. The final layer was made at 6 cm after second layer and mother was thoroughly sprayed over the upper surface. After inoculation the wooden frame is removed and bed is covered with polythene sheet. The inoculated beds in the racks were placed on multilayered shelves in a mushroom house at the height of 0 (Floor cultivation), 15, 60, 105, 150, 195 and 240 cm from the floor. The racks were covered with polythene sheets and incubated at 38 to 40^0 C temperature for ramifications of mycelia. After full ramification, polythene sheet was removed. The mushroom was allowed to grow maintaining proper moisture level by regular watering the beds.

Growing milky mushroom: Polyethylene bags (9″×12″) were filled with the substrate prepared from rice straw as mentioned earlier. The substrate in the polyethylene bags was inoculated with 5% grain spawn of milky mushroom (*Calocybe indica*). The neck of poly bags was plugged with cotton and covered with brown paper and tide with a rubber band. The bags were incubated in a cropping room. Within 18-22 days substrate was completely colonized by mycelium and polythene bags was opened. Cowdung and loamy soil (3: 1, v/v) was used as the casing material and was sterilized at 65^0C for 4 hr. The mycelium grown on the substrate was covered with the casing materials up to 4 cm thickness. The prepared packets were placed on the multilayered shelves at the height of 0 (on floor), 15, 60, 105, 150, 195 and 240 cm from the floor of the mushroom house. Watering was done at regular interval to maintain proper moisture level in the substrate.

Data collection: Data on days to appearance of pin head or primordia, first harvest and total harvest, and number of effective fruiting bodies were recorded. Matured fruiting bodies were harvested and data on length and thickness of stipe pileus, and yield were determined. The BE was computed using the formula.

Design of the experiments and data analysis: The experiments were laid out in completely randomized design with 4 replications. The data were statistically analyzed following MSTAT-C computer programme. Means were compared following Duncan's Multiple Range Test (DMRT) using the same computer programme.

RESULTS AND DISCUSSION

Effect on *Volvariella volvacea*

Days required to primordial initiation (DRPI), first harvest and total harvest: In case of straw mushroom (*V. volvacea*) when the bed was placed at the height of 240 cm no primordia was appeared. 9 -14 days required for primordial initiation in different beds placed in different height. Significantly the highest DRPI was found at the height of 195 cm from the floor. On the contrary, significantly the lowest (DRPI) was recorded when the bed rack was placed on the floor (0 cm) followed by 15, 60, 105, and 150 cm. The effect of 15-195 cm height on this parameter was statistically similar. The results show that when *V. volvacea* was cultivated on the floor primordial initiation appeared earlier compared to height other bed placed in different height (Table 1).

Days required for first harvest ranged 12.00 to 17.50 on the beds placed at the height of 0-195 cm. significantly the lowest days required for first harvest was observed when the bed was placed on the floor. The parameter recorded at 15, 60 and 105 cm height was statistically similar and significantly lower compared to only the height of 195 cm (Table 1).

Days required to total harvest at the height of 0 cm (28.50) and 15 cm (28.0) was statistically similar but significantly higher compared to other height. The lowest days (19.00) required for total harvest was recorded from the bed at the height of 60 cm followed by 150 cm and 105 cm. Their differences were significant (Table 1).

Table 1. Effect of height of bed from the floor on growth and yield of straw mushroom

Placement of bed at different height (cm)	Days to primordial initiation	Days to first harvest	Days to total harvest	Number of effective fruiting body / bed	Yield (g/bed)
0	9.00 c	12.00 c	28.50 a	125.80 a	983.30 a
15	11.25 b	14.75 b	28.00 a	71.25 b	448.80 b
60	11.50 b	15.00 b	19.00 d	75.50 b	437.50 b
105	11.50 b	15.00 b	24.00 b	36.75 c	312.80 c
150	11.50 b	15.00 b	21.75 c	28.50 d	261.80 d
195	14.00 a	17.50 a	25.50 b	26.75 d	252.30 d
240	-	-	-	-	-
CV (%)	**7.62**	**7.21**	**5.77**	**8.52**	**6.50**

Means within the same column with a common letter(s) are not significantly different at 5% level according to DMRT.

Number of effective fruiting body and yield: The number of effective fruiting body and yield ranged 26.75-125.80 and 252.30-982.30 g per bed at the height of 0-195 cm. Significantly the highest number of effective fruiting body and yield were found when the bed was placed on the floor (0 cm height). Number of effective fruiting body as well as yield harvested from the bed place at height of 150 (28.5) and 195 cm (26.75) was statistically similar but significantly lower compared to the height of other racks. The second highest yield was obtained when the bed was placed at the height of 60 cm, which was statistically similar to 15 cm (Table 1).

Effect on *Calocybe indica*

Days required to primordial initiation, first harvest and total harvest: The days required to primordial initiation (DRPI) ranged 13.25 to 39.75 under different bed height from the floor. Significantly the highest DRPI was found at the maximum bed height of 240 cm. The second highest DRPI was recorded from the bed height of 195 cm, which was statistically similar to 0, 105 and 150 cm. The lowest DRPI (13.25 days) was found at the height of 60 cm which was statistically similar to 15 cm (Table 2).

The days required from opening to first harvest (DROFH) varied from 18.50 to 46.00 under different treatments. The lowest days (18.50) to first harvest was recorded from the bed placed at the height of 60 cm followed by 150 cm. Significantly the highest days to first harvest was recorded when the bed was placed at the height of 240 cm (Table 2).

Table 2. Effect of placement of milky spawn bags at different height on yield and yield attributing characters of milky mushroom

Placement of spawn at different height (cm)	Days to primordia initiation	Days required for first harvest	Days required for total harvest	Number of effective fruiting body	Yield (g)
0	15.50 bc	21.75 bc	58.50 b	6.25 ab	225.80 c
15	14.00 cd	20.25 cd	54.75 c	5.25 ab	245.80 b
60	13.25 d	18.50 e	63.50 a	6.50 a	268.00 a
105	14.75 bcd	21.25 bc	61.50 a	5.00 b	161.30 b
150	14.25 bcd	19.50 de	54.50 c	5.75 ab	140.30 e
195	15.75 b	22.25 b	61.50 a	5.25 ab	135.30 e
240	39.75 a	46.00 a	46.00 d	2.50 c	82.75 f
CV (%)	**5.73**	**4.68**	**3.30**	**17.26**	**3.42**

Means within the same column with a common letter(s) are not significantly different at 5% level according to DMRT.

Appreciable variation was found in days required for total harvest in different height within the range of 46.00 to 63.50 days. Significantly the lowest days to total harvest was recorded when the bed was placed on the shelf at the height of 240 cm. The second lowest days required for total harvest was found at the height of 150 cm, which was statistically simillar to 15 cm. The highest days required for total harvest was found at the height of 60 cm followed by 105 and 195. Their differences were also not significant (Table 2).

Number of effective fruiting body: The highest number of effective fruiting body (6.50) was found in bed rack placed at the height of 60 cm, which was statistically similar to the height of 0, 15, 150 and 195 cm. Significantly the lowest number of effective fruiting bodies was recorded from the bed placed at 240 cm height (Table 2).

Length of stalk: The length of stalk ranged from 5.70 to 9.87 cm under different height of bed from the floor. The highest length of stalk was found at the height of 0 cm (on floor) followed by 240 and 105 cm. The effect of three treatments on stalk length was statistically similar but significantly higher compared to other treatments. It was significantly different from other treatments. The length of stalk at the height of 150 and 195 was also statistically similar but significantly lower compared to other treatments. Effect of 15 and 60 cm height on this parameter was also significantly different (Table 3).

Diameter of stalk: The diameter of stalk ranged from 1.45 to 3.17 cm. Significantly the highest diameter of stalk was recorded when the mushroom was grown on the floor (0 cm height). The stalk diameter recorded under other treatments was statistically similar except 150 cm height (Table 3).

Diameter and thickness of pileus: Diameter and thickness of pileus under different treatments ranged 5.62-6.85 and 1.15-2.07 cm. The diameter of pileus recorded from beds placed on the floor and 15 cm above the froor was statistically similar and significantly higher compared to other height. Its diameter obtained from the bed placed at the height of 60, 105, 195 and 240 cm was not significantly different. The smallest pileus was recorded from the bed rack placed at the height of 150 cm. The maximum thickness of pileus was obtained from the bed placed at the height of 195 cm, which was statistically similar to 0 and 105 cm but significantly higher compared to other treatments. The lowest thickness was found in bed placed at the height of 15 cm followed by 60 and 195 cm (Table 3).

Table 3. Effect of placement of milky spawn bags at different height on size of fruiting body of milky mushroom

Placement of spawn at different height (cm)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pilus (cm)	Thickness of pilus (cm)
0	9.87 a	3.17 a	8.57 a	1.67 ab
15	8.10 b	2.10 b	8.65 a	1.15 c
60	7.35 c	2.07 b	6.67 b	1.60 b
105	9.57 a	2.17 b	6.42 b	1.90 ab
150	5.75 d	1.45 c	5.62 c	1.67 b
195	5.70 d	2.17 b	6.42 b	2.07 a
240	9.80 a	2.32 b	6.85 b	1.55 b
CV (%)	**5.57**	**13.42**	**6.22**	**15.45**

Means within the same column with a common letter(s) are not significantly different at 5% level according to DMRT.

Yield: Significant variation was observed in yield (BY) of different height (Table 2). The yield of mushroom under different treatments ranged 82.75-268.00 g/packet. Significantly the highest yield was recorded from the packet placed at the height of 60 cm followed by the packets placed at the height of 15 cm and the packets placed on the floor (0 cm height). The lowest yield was recorded from packets placed on rack at 240 cm height (Table 2).

Findings of two experiments reveal that higher yield of straw mushroom can be obtained through floor cultivation but to obtained higher yield milky mushroom may be grown on shelf at the bmaxium height of 60 cm.

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Effect of Different Casing Materials on the Yield Attributes and Yield of White Button Mushroom

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Abstract

An experiment was conducted to find out the effect of casing materials on yield and yield attributes of white button mushroom. Farm yard manure: burnt rice husk (2:1), peat soil, poultry manure and soil: sand (3:1) were evaluated as casing materials. The highest number of fruiting body, biological yield, economic yield and biological efficiency was recorded in farm yard manure and burnt rice husk (2:1). The highest economic yield (332.07g/ 3 kg bag) was estimated from farm yard manure and rice husk (2:1) followed by peat soil (313.62 g/ 3kg bag) and soil: sand (3:1) (301.25 g/ 3 kg bag). The lowest economic yield was observed on poultry manure (292.01kg/ 3 kg bag).

Key words: Casing, Button mushroom, Yield.

INTRODUCTION

Agaricus bisporus (Lange) Singer is the popular cultivar among the artificially grown edible fungi of the world. It contributes about 31.8% to the global mushroom production (Angrish *et al.*, 2003). *Agaricus bisporus* (Lange) Singer, the most popular and widely grown button mushroom requires two different substrates to form its fruiting bodies, *i.e.*, the compost in which it grows vegetatively and the nutritionally poor casing materials which provide suitable physical, chemical and biological conditions that stimulate the initiation of fruiting body formation (Coskuner and Ozdemir, 1997 and Segula *et al.*, 1987). Casing is a mixture designed to cover the nutritional composted substrate colonized with mycelium and has an essential function in stimulating and promoting the developments of sporophores (Pardo *et al.*, 2003 and Noble *et al.*, 2005). It is normally believed that fruiting bodies of mushrooms are produced when some stress is provided. Application of casing layer, which is not nutritionally as rich as compost, creates condition of stress, necessary for induction of fruiting bodies. Besides, the casing layer fulfils several functions (Bazerque and Laborde, 1976; Stames and Chilton, 1983 and Wuest and Beyer, 1996) it constitutes the physical support of the emerging carpophores and contributes to the maintenance of a moist microclimate to help feed the mycelium and support the formation of primordia; it acts as a suitable medium for the development of

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bacteria which stimulate fructification; it provides water for the growth and development of mushrooms, supplementing the water provided by the compost; it provides the mycelium with a suitably aerated environment, permitting gas interchange; and finally, it provides an environment of low osmotic value unlike compost, whose osmotic value is too high for mushrooms. For these ends, the casing material must fulfill certain conditions (Wuest and Beyer, 1996; Hayes, 1981 and and Flegg and Wood, 1985). As peat moss, which is universally accepted as best casing material in mushroom cultivation due to high water holding capacity and other favorable traits (Vijay and Gupta, 1995), is not available in Bangladesh, different casing mixtures based on locally available materials have to be tested. So the aim of the present study was to find out the best casing materials on yield attribute and yield of white button mushroom.

MATERIALS AND METHODS

Four different casing materials were selected and collected from local villages of Savar, Dhaka area and National Mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka. The materials were composed in the following manner T_1 = Farm Yard Manure (FYM): Burnt Rice Husk (BRH) (2:1), T_2 = Peat, T_3 = Poultry Compost and T_4 = Soil: Sand (3:1).

Preparation of compost: Paddy straw was used as the main substrate and the compost was prepared by long method of composting (LMC) using rice straw (300 Kg), wheat bran (30 Kg), gypsum (15 Kg), calcium carbonate (10 Kg), urea (9 Kg), triple super phosphate (6 Kg), muriate of potash (3 Kg), furadon (250 g) and bavistin (150 g). The ready compost was deep brown in colour, free from bad smell of ammonia and had 65-67% moisture.

Spawning and incubation: Three kilograms (3 kg) of compost were mixed with 75 g of mother culture of *Agaricus bisporus* and poured in a polypropylene bag. The open top of the bags were covered with wetted clean news papers and incubated in the incubation room at $24^0 \pm 2^0$C temperature 90% relative humidity for 20 days.

Casing: After completion the spawning of those bags, casing materials were applied to the top of spawn bags maintaining 3-4 cm thickness according to treatment combination. Before using the casing materials, it was sterilized by autoclaving for 1 hour with 121^0C temperature at 1.1 kg/cm^2 pressure. The packets were incubated in the same incubation room at $24^0 \pm 2^0$C temperature for 10 days.

Cropping and harvesting: Case run was considered complete when mycelia come in the valleys of casing layer. After case run, the environmental conditions changed by bringing down the temperature to 15-17°C (air), RH to 85% by opening of the fresh air ventilation and exhaust CO_2. This change in environmental parameters induced pinhead formation in 3-4 days. The pinheads developed into solid button sized mushrooms in another 3-4 days.

Mushroom was harvested before the fruiting body showed any detachment of the cap from the stipe. The yield of mushrooms and their parameters were recorded regularly. The number of fruiting body, biological and economic yield was estimated. Biological efficiency (BE) was determined by the formula:

BE (%) = Total biological yield (g) X 100/Total compost used (g).

Data analysis: Data were statistically analyzed MSTAT-C computer program. Means were computed following Duncan's Multiple Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Days required to primordia initiation (DRPI): All the four casing mixture were evaluated for their yield potential. Days to primordia initiation in compost bag ranged from 14.80-15.60 days on different casing materials and no significant difference was observed among the treatments. The highest DRPI was observed on soil and sand (3:1) (Table 1). Amin *et al.* (2007) supported the result.

Number of primordia in first flush (NPFF): Significant variation was observed in the number of primordia in first flush (NPFF) on different casing materials tested in the present experiment (Table 1). The highest NPFF was found on peat soil which was statistically similar to FYM: BRH (2:1) and soil: sand (3:1). The lowest NPFF was recorded on poultry manure.

Number of total fruiting body (NTFB): Significant variation was found in number of total fruiting body (NTFB) on different treatment tested in this experiment. The treatment FYM and BRH (2:1) showed the highest (95.60) NTFB which was statistically similar to the peat soil casing. The lowest (50.0) NTFB was recorded in soil and sand (3:1) which did not differ with poultry manure. These results partially supported the results of Amin *et al.* (2007) who reported that soil: sand: CD: FYM casing treatment produced the highest number of fruiting body. The results also satisfied the findings of Angrish *et al.* (2003). They found that FYM: SC (spent compost) casing mixture gave the highest number of fruiting body followed casing mixture, FYM+BRH (2:1).

Biological yield (BY) and economic yield (EY): Significant variation was observed in respect of biological yield (BY) and economic yield (EY) ranging from 350.80 to 399.60 g and 292.01 to 332.07 g/bag respectively (Table 1). The highest biological yield (399.60 g) was recoded in FYM+BRH (2:1) which was significantly higher as compare to all the treatments except peat soil. The lowest by (350.80 g) was observed in poultry manure. Almost similar trend was observed in economic yield.

Angrish *et al.* (2003) evaluated five casing materials of *Agaricus bisporus*: biogas plant slurry, burnt rice husk (BRH), farm yard manure (FYM), sandy soil (SS), spent compost(SC) and observed that FYM+SS (1:1) was the best which was followed by

FYM+SC (1:1) and FYM + SS (2:1). Gupta and Dhar (1993) also found the mixture to be good but equally good yield obtained with farm yard manure alone as well as with mixture of FYM + spent compost + loam Soil (1:1:1) which support the present result.

Table 1. Effect of different casing materials on the yield attributes and yield of white button mushroom

Treatment	Days required to primordia initiation	Number of primordia in first flush	Number of total fruiting body	Biological yield (g)	Economical yield (g)
FYM:BRH (2:1)	14.80 a	24.20 a	95.6 a	399.60 a	332.07 a
Peat soil	14.80 a	24.60 a	85.20 a	382.00 ab	313.62 ab
Poultry manure	14.80 a	16.20 b	59.60 b	350.80 c	292.01 b
Soil and sand (3:1)	15.60 a	22.40 a	50.0 b	371.80 bc	301.25 b
CV (%)	**5.16 a**	**9.97**	**9.82**	**3.54**	**4.05**

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

Biological efficiency (BE): The biological efficiency (BE) of *Agaricus bisporus* influenced by different casing materials were ranged from 11.69 to 13.32% (Fig. 1). The highest BE (13.32%) was observed in FYM+BRH (2:1) which was followed by peat soil (12.73%) and sand : soil (3:1) (12.39%). The lowest BE was recorded in poultry manure (11.69%).

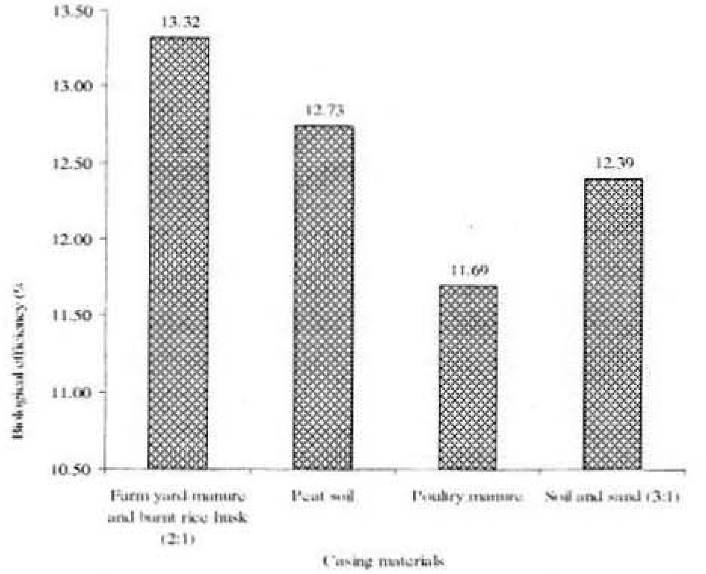

Fig. 1. Biological efficiency of white button mushroom in different casing materials.

Relation between number of fruiting body (NFB) and economic yield (EY): A positive linear relationship was observed between number of fruiting body and economic yield per packet (3 kg of compost) (Fig. 2). The equation $y = 0.7202x + 257.45$ gave a good fit to the data and the value of co-efficient of determination ($R^2 = 0.788^*$) showed that the fitted regression line had a significant regression co-efficient. So, it indicated that economic yield per packet increased as the number of fruiting body increased.

Relationship between biological yield (BY) and economic yield (EY): A positive linear relationship was observed between biological and economic yield per bag (Fig. 3). It was observed that the equation y=0.8291x-2.0367 gave a good fit to the data and the co-efficient of determination (R^2 = 0.952**) showed that the best fitted regression line had a significant regression co-efficient. It indicated that the economic yield per bag increased with the increase of biological yield. More over its value also indicate that 95.2% economic yield was attributed by the biological yield.

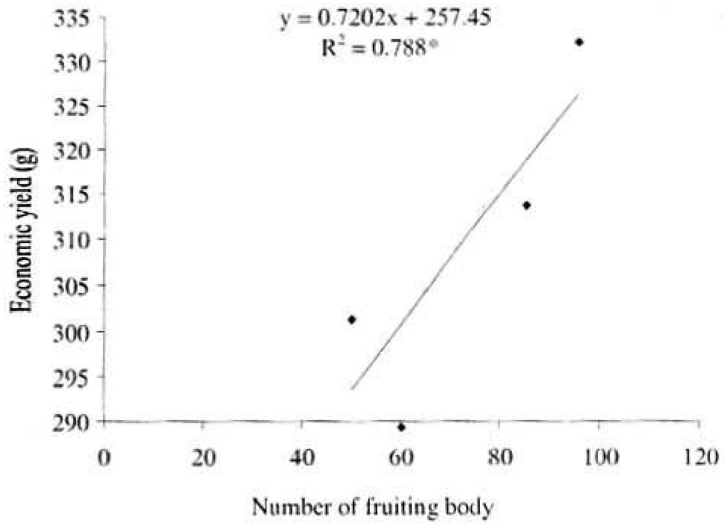

Fig. 2. Functional relationship between number of fruiting body and economic yield of white button mushroom.

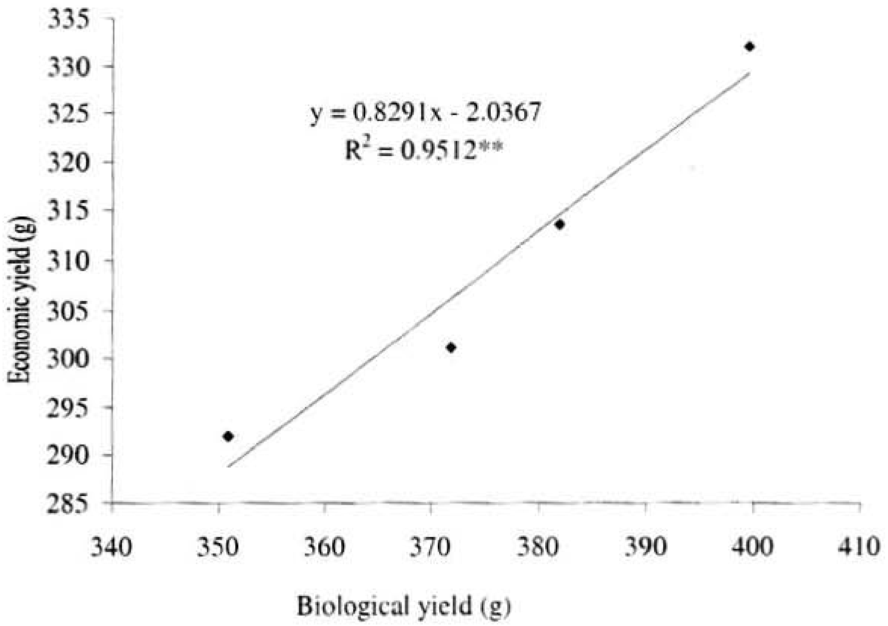

Fig. 3. Funcitional relationship between biological yield and economic yield of white button mushroom.

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