EFFECT OF DIFFERENT SAWDUST SUBSTRATE ON THE GROWTH, YIELD AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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CERTIFICATE

This is to certify that the thesis entitled "EFFECT OF DIFFERENT SAWDUST SUBSTRATE ON THE GROWTH, YIELD AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (Pleurotus ostreatus)" submitted to the DEPARTMENT OF BIOCHEMISTRY, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (M.S.) IN BIOCHEMISTRY, embodies the results of a piece of bona fide research work carried out by PRINCE BISWAS, Registration. No. 09-03556, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in any other institution.

I further certify that any help or sources of information received during the course of this investigation has duly been acknowledged.

Dated: **Place: Dhaka, Bangladesh**	**Professor Dr. Kamal Uddin Ahmed** **Supervisor**

DEDICATED TO
MY
BELOVED PARENTS

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ABSTRACT

Five different sawdust viz. mango tree *(Mangifera indica)*, rain tree *(Albiia saman)*, teak tree *(Tectona grandis)*, mahogony tree (*Swietenia mahagoni*) and mixture of all four sawdust supplemented with 30 % wheat bran and 1 % $CaCO_3$ as basal substrate were selected for studies their performance on growth, yield and proximate composition of oyster mushroom (*Pleurotus ostreatus)*. The highest mycelium running rate in spawn packet (0.72 cm/day) was found in mixture of sawdust, the maximum time from stimulation to primordial initiation (8.04 days) and time from primordial initiation to harvest (4.24 days) were recorded in mango tree sawdust. The highest number of primordial/packet (217.5), fruiting body/packet (115.5) and effective fruiting body/packet (29.02) were observed in mango tree sawdust. The highest weight of individual fruiting body (5.60 g), dry yield (35.15 g), biological yield (368.18 g) and economic yield (360.68 g) were also found in mango tree sawdust. The mahogany tree sawdust gave the highest moisture (89.13 %) content of oyster mushroom. This study also investigate the effect of sawdust supplements on nutritional status where the maximum amount of dry matter (10.18%), amount of lipid (4.46 %) were found in mixture of sawdust. The highest amount of Fe (42.52 %) was observed in rain tree sawdust supplemented with 30% wheat bran. The highest crude fiber (19.53 %) was recorded in mango tree sawdust supplemented with 30% wheat bran. The highest Protein (26.04 %) and Ca (30.73 %) were observed in mixture of sawdust supplemented with 30% wheat bran. Therefore, it can be concluded that mango tree sawdust supplemented with 30 % wheat bran can be further used as a better substrate for oyster mushroom *(Pleurotus ostreatus)* production reducing cost and increasing the yield and nutritional quality.

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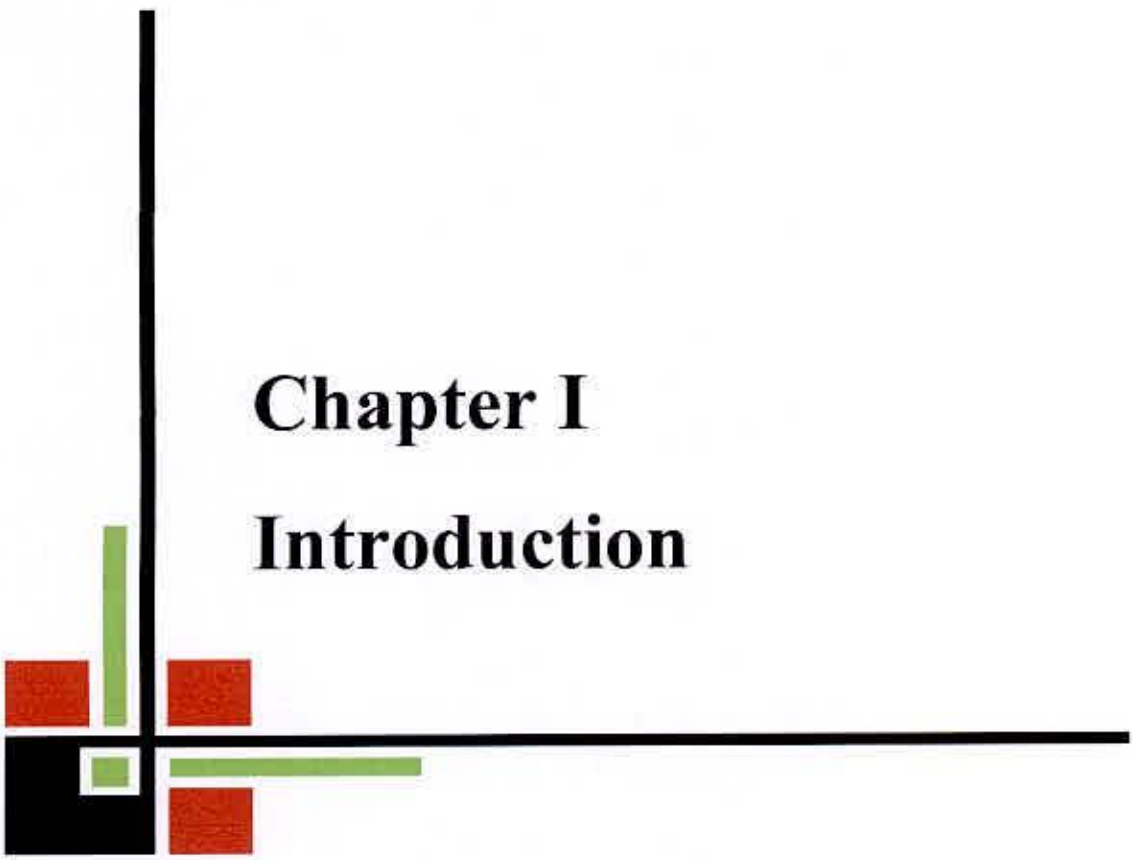
LIST OF ABBREVIATION AND ACRONYMS

BBS	=	Bangladesh Bureau of Statistics
FAO	=	Food and Agricultural Organization
N	=	Nitrogen
SA	=	Salicylic Acid
PGRs	=	Plant growth regulators
et. al.	=	And others
TSP	=	Triple Super Phosphate
MOP	=	Muirate of Potash
RCBD	=	Randomized Complete Block Design
DAS	=	Days after sowing
ha^{-1}	=	Per hectare
g	=	gram (s)
kg	=	Kilogram
SAU	=	Sher-e-Bangla Agricultural University
SRDI	=	Soil Resources and Development Institute
wt	=	Weight
LSD	=	Least Significant Difference
^0C	=	Degree Celsius
NS	=	Not significant
Max	=	Maximum
Min	=	Minimum
%	=	Percent
NPK	=	Nitrogen, Phosphorus and Potassium
CV%	=	Percentage of Coefficient of Variance



Chapter I

Introduction

INTRODUCTION

Mushroom is being widely used as food and food supplements from ancient times. They are increasingly being recognized as one of the important food items for their significant roles in human health, nutrition and diseases (Chang, 1996). There is a common saying that "medicines and foods have a common origin" (Kaul, 2001).

Bangladesh is a developing country. The land of our country is limited but the population is very high. Due to our huge population we have to produce more food in our limited land. Moreover due to the high population pressure the total cultivable lands have been decreasing day by day at a rate of one lac hectare per year for urbanization and other essentialities (BBS, 2010).

Substrate plays an important role in the yield and nutrient content of oyster mushroom. The substrates on which mushroom spawn (Merely vegetative seed materials) is grown, affects the mushroom production (Klingman, 1950).Oyster mushroom can grow on sawdust, rice and wheat straw and other agro-waste. Sarker *et al.* (2007) observed a remarkable variation in nutritional content of oyster mushroom in different substrates. A huge amount of rice straw is produced in Bangladesh annually. If we use a small part of this for oyster mushroom production, then we can produce notable amount of mushroom.

One of the world's biggest challenges is food insecurity. This problem is largely common in low and middle-income countries that mainly have poor food production systems and suffer from serious malnutrition. Such countries must find ways of improving food production to feed the vastly increasing human population (Diriba *et al.*, 2013).

Mashroom production is very good idea for supplying essential food nutrients using small area of land. In developing countries, governmental and non-governmental organizations have not given due attention to mushrooms as an important crop that can fetch farmers a substantial income to alleviate poverty (Olumide, 2007).

However, there is no enough mushroom cultivation practice in the country to fill the demands of people interested in the mushroom consumption. The trend of mushroom cultivation in Bangladesh is very recent. Mushroom cultivation in Bangladesh began in 1979 with assistance from Japanese organization JOCDV. In early 1980s commercial mushroom cultivation was initiated by Bangladesh Agricultural Research Council and Mushroom Culture Centre at Savar.

Apart from Savar, mushroom is being cultivated in Dinajpur, Jessore, Barisal, Chittagong, Sylhet, Comilla, Khulna, Mymensingh, Bandarban, Rangamati, Chapainawabganj and Rangpur (Asia Pulse News, 2008).

Mushroom contains 4.0% fat having good quantity of unsaturated fatty acids which are essential in our diet (Holman, 1976). Mushrooms with their flavor, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries (Chaube, 1995).

Mushroom cultivation offers benefit, when it is integrated into the existing production system by producing nutritious food at a profit, while using materials that would otherwise be considered "waste" (Beteez and Kustudia, 2004). Due to their nutritional, medicinal and ecological advantages, mushrooms have attracted the attention of many people in the world (Imtiaj and Rahman, 2008). Cultivation of edible mushrooms may be the only currently economical biotechnology for lignocellulosic organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution (Beteez and Kustudia, 2004).

The demand for mushroom has been increasing due to population growth, market expansions, changing of consumer behavior, and developments in the manufacturing 2 industries, storage, transportation, and retailing. Gradually, the world mushroom production reached 33.4 million tons in 2007 from the 26 million tons in 2000 (Chang, 1999).

China, United States of America and the Netherlands rank as the first three in mushroom production in the world (Atikpo *et al*., 2008).

Over 200 species have been collected from the wild vegetation and used for various traditional medicinal purposes, mostly in the Far East (James, 1995). The same author indicated that currently about 35 mushroom species have been cultivated commercially, and of these around 20 are cultivated on an industrial scale.

The mushroom cultivated worldwide are button mushroom *(Agaricus bisporus*), followed by Shiitake (*Lentinus edodes*), Oyster *(Pleurotus*), *Auriculaia* species, winter mushroom *(Flammulina velutipes*), straw mushroom *(Volveriella volvovacea*), *Grifola frondosa* and *Pholiota nameko* (Chang, 1999).

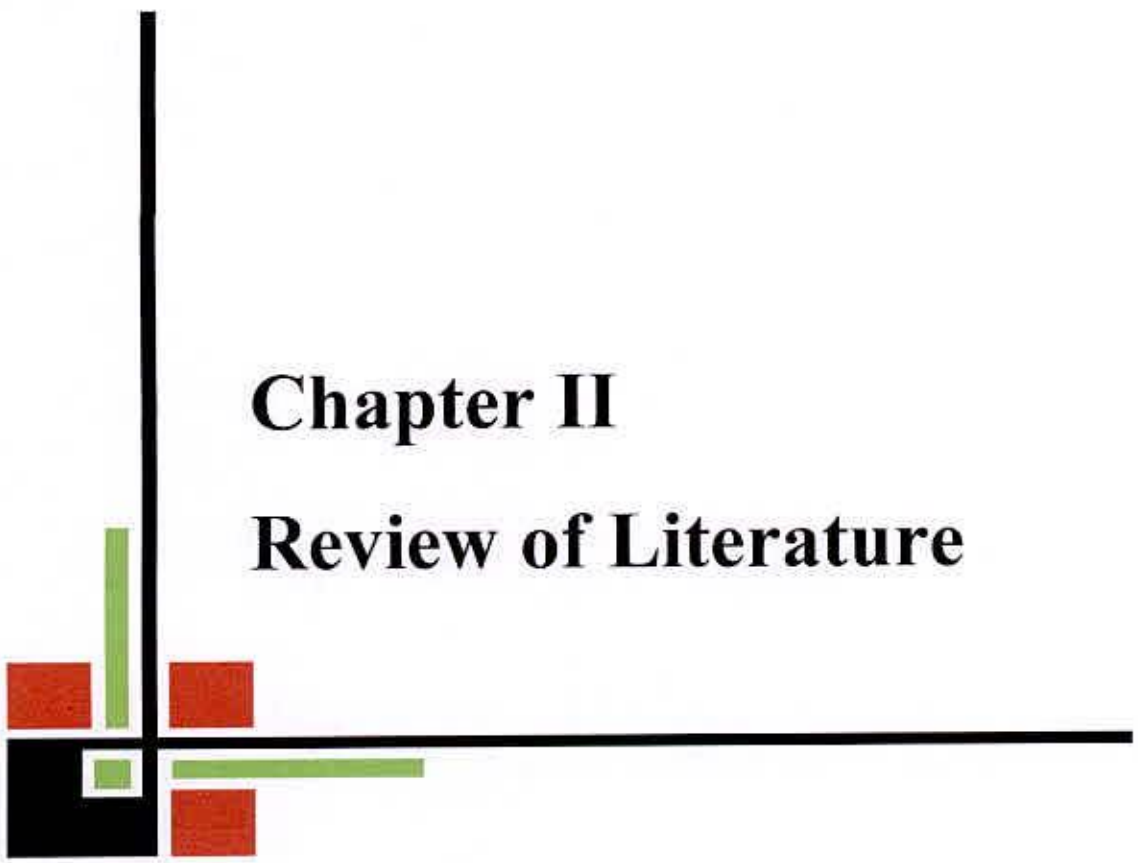
Oyster mushrooms are one of the most popular edible mushrooms and belong to the genus *Pleurotus* and the family *Pleurotaceae*. Many of the *Pleurotus* mushrooms are primary decomposers of hardwood trees and are found worldwide.

Two decades back, approximately about 70 species of *Pleurotus* had recorded and new species were subsequently discovered more or less frequently although some of them were considered identical with previously recognized species (Badshah *et al*., 1992). The cultivation of oyster mushroom requires the use of cellulosic materials or residues such as cereals straw, cotton stalks, various grasses, weeds, reed stems, maize and sorghum Stover, sugarcane bagasse, banana residues, coffee pulp and coffee husk, cottonseed and sunflower seed hulls, peanut shells, rice husks, waste paper, wood sawdust and chips (Dawit, 1998). In view of these facts, the present experiment was under taken five different sawdust Mango tree*(Mangifera indica*), Rain tree(*Albiia saman*), Teak tree (*Tectona grandis*), Mahogany tree (*swietenia mahagoni*) and all mixture of four sawdust supplemented with 30% wheat bran and 1 % lime as basal substrate were selected for studies their performance on growth,

yield and proximate composition of oyster mushroom (*Pleurotus ostreatus)* with the following objectives.

Objectives of the research work:

1. To improve the yield of oyster mushroom *(Pleurotus ostreatus).*
2. To know the suitable sawdust for oyster mushroom production.
3. To determine the physiochemical characteristics of oyster mushroom *(Pleurotus ostreatus).*



Chapter II

Review of Literature

REVIEW OF LITERATURE

Mushrooms are fruiting bodies of Fungi especially of Ascomycetes or Basidiomycetes, which are macro fungi with distinctive fruiting bodies large enough to be seen with the naked eye and to be picked up by hand (Chang and Miles, 1992). In history, human beings used to collect only wild mushrooms at the beginning (Imtiaj and Rahman, 2008). Mushrooms have been considered as a special kind of food since the earliest time. The Chinese were the first to grow mushrooms for the human consumption. In the last 25 years, worldwide mushroom production had increased over 300%, reaching approximately 2,961,491 tons in 2002 (USDA 2003). Presently, mushrooms have become popular throughout the world since they have wonderful food and medicinal values. The local demand for mushrooms is also progressively increasing (Nita, 2002). Tewari (1986) reported that the fresh mushroom contains about 85-90% moisture, 3% protein, 4% carbohydrates, 0.3-0.4% fats and 1% minerals and vitamins. The review of literature given below was based on the present information about the performance of oyster mushroom (*Pleurotus ostreatus*) and the effect of different kinds of substrate on mushroom cultivation.

Nuruddin *et al.* (2010) carried out an experiment to investigate the effect of different levels of cowdung (0, 5, 10, 15 and 20%) on yield and proximate composition of *Pleurotus ostreatus*. The highest number of primordia (70.63) and fruiting body (51.92) were observed in rice straw supplemented with 5% level of cowdung. The highest weight of individual fruiting body (4.71g), biological yield (234.24g), economic yield (227.72g), dry yield (22.83g), biological efficiency (140.26%) and benefit cost ratio (5.69) were observed in rice straw supplemented with 10% level of cowdung.

Kulsum *et al.* (2009) conducted an experiment to determine the effect of five different levels of cow dung (0%, 5%, 10%, 15% and 20%) as supplement with sawdust on the performance of oyster mushroom.

All the treatments performed better over control. The mycelium running rate in spawn packet and the highest number of primordia/packet were found to be differed due to different levels of supplements used. The highest weight of individual fruiting body was observed in sawdust supplemented with cow dung @ 10% (3.69g).

The supplementation of sawdust with cow dung had remarkable effect on biological yield, economic yield, the dry yield, biological efficiency and cost benefit ratio. The highest biological yield (217.7 g), economic yield (213g), dry yield (21.27g) biological efficiency (75.06%) and cost benefit ratio (8.41) were observed due to sawdust supplemented with cow dung @ 10%. Among the chemical characteristics highest content of protein (31.30%), ash (8.41%), crude fiber (24.07%), the lowest lipid (3.44 %) and carbohydrate (32.85%) were observed due to sawdust supplemented with cow dung @ 10%. Among the minerals the highest amount of nitrogen (5.01%), potassium (1.39%), calcium (22.15%), magnesium (20.21%), sulfur (0.043%), iron (43.4%) and the lowest phosphorus (0.92) were observed due to sawdust supplemented with cow dung @ 10%.

Bhuyan (2008) conducted an experiment to study the effect of various supplements at different levels with sawdust showed significant effort on mycelium running rate and reduced the required days to complete mycelium running in the spawn packet. The supplementation of sawdust found to be significant in yield and yield contributing characters of oyster mushroom with some extent. The highest biological yield, economic yield, dry yield, biological efficiency (BE) and benefit cost ratio (BCR) of 270.5 g, 266.5 g, 26.34 g, 93.29, 9.57%, respectively was observed in sawdust supplemented with NPK mixed fertilizer (N=0.6%, P=0.3%, K=0.3%). Sawdust supplemented with different levels has a profound effect on chemical composition of oyster mushroom. Sawdust supplemented at different substrate found to be significant with mineral content of the fruiting body. Considering all the parameters in five experiments, NPK mixed fertilizer (N=0.6%,

P=0.3%, K=0.3%) supplemented with sawdust is found promising for lowering the cost of production as well as increasing the yield and quality of fruiting body. Cow dung (11.5%) and starch (5.5%) as supplement with substrate may be the fair choice.

Mane *et al.(* 2007) carried out an experiment to find out the performance of *Pleurotus* species are popular and widely cultivated throughout the world mostly in Asia and Europe owing to their simple and low cost production technology and higher biological efficiency.

Amin *et al.* (2007) carried out an experiment to find out the primordial and fruiting body formation and yield of oyster mushroom (*pleurotus ostreatus*) on paddy straw supplemented with wheat bran (WB) wheat flour (WF), maize powder (MP), rice bran (RB) and their three combination (WB+MP, 1:1), (WB+MP+RB, 1:1:1) and wheat broken (WBr) at six different levels namely 0,10,20,30,40 and 50% were studied. The minimum time (4.5 days) for primordial initiation was observed in the MP at 20% level and the highest number of effective fruiting bodies (60.75) was obtained in WF at 50% level. The highest biological yield (247.3 g/packet) was recorded at 10% level of (WBr).

Sarker *et al.* (2007 a) carried out an experiment to find out the performance of different cheap agricultural household by products, grasses and weeds as substrate available in Bangladesh. Mycelium growth rate and time required to complete mycelium running in spawn packet varied significantly in different substrates. The minimum duration to complete mycelium running was 17.75 days in waste paper, which differed significantly from that in all other substrates. Significant variation was found in duration from stimulation to primordial initiation, primordial initiation to first harvest and stimulation to first harvest in different substrates. The minimum duration required from stimulation to first harvest was observed in sugarcane bagasse (6.75 days), which was statistically identical to that in waste paper, wheat straw and sawdust (7.00 days).

The number of fruiting body grown on different substrates differed significantly and the highest number of fruiting body per packet (183.25) was recorded on waste paper, which was significantly higher as compared to all other substrates. The lowest number of fruiting body (19.25) was observed in water hyacinth. Significant variation in biological efficiency, biological yield and economic yield of oyster mushroom were observed in different substrates. The highest economic yield (225.43 g/packet) was estimated from the waste paper followed by wheat straw (215.72 g/packet).The economic yield on sugarcane bagasse was 191.98g/packet, which was statistically identical to that grown on rice straw (183.28 g/packet), kash (182.93 g/packet) and ulu (175.15g/packet).The economic yield on sawdust was 160.40g/packet, which was statistically identical to that on ulu. The lowest economic yield was observed in water hyacinth (33.59g/packet). Performances of the substrates were compared based on benefit cost ratio (BCR). Sawdust from *D. sisso* was the most suitable for spawn preparation and all types of sawdust amended with cotton waste were found to give optimum conditions for spawn running.

Ali *et al.* (2007) evaluate the influence of pasteurization methods on cotton waste substrate on yield of oyster mushroom (*Pleurotus* spp. Cotton waste subjected to different methods of pasteurization, namely pasteurization with steam, hot-water treatment and chemical sterilization with formalin, which were compared with control (without pasteurization). Three species of *Pleurotus* i.e. *Pleurotus florida, Pleurotus pulmonarius* and *Pleurotus ostreatus* were selected. Steam pasteurization produced the best results as for as the performances of individual species are concerned, *Pleurotus pulmonarius* completed the mycelial growth in the shortest time. Formalin treatment behaved poorly as the different *Pleurotus* spp, took maximum time to complete mycelial growth. Steam pasteurization technique produced more yield, whereas *Pleurotus florida* behaved better in all the treatments than other species. Substrate was analyzed chemically for N: P: K to determine their contents at different stages. N: P: K contents were increased after the

completion of mycelial growth in all the treatments, but were decreased after fructification as the fruiting bodies consumed nutrients for their growth.

Ali *et al.* (2010) conducted an experiment to investigate the performance of different levels of wheat bran (0, 10, 20, 30 and 40%) as supplement with sugarcane bagasse on the yield and proximate compositions of oyster mushroom. They reported that the highest mycelium growth rate (0.96 cm/day), the highest average number of primordia/packet (70.67), average number of fruiting body/packet (61.00) were observed in sugarcane bagasse supplemented with 40% wheat bran as supplement with sugarcane bagasse. The lowest time from primordia initiation to harvest (3.23 days) and the highest average weight of individual fruiting body (3.69 g) were observed in 30% level of wheat bran. The highest biological yield (254.7 g/500 g wet substrate), economic yield (243.3 g), dry matter (23.40 g), biological efficiency (87.82%) and benefit cost ratio (8.29) also observed in 30% level of wheat bran.

Sarker *et al.* (2007 b) found that remarkable difference in nutrient content of oyster mushroom was observed in respect of different substrates. Wide variation was recorded in the protein content of fruiting body. On dry weight basis, the highest protein content (11.63%) was observed in fruiting body grown on sugarcane bagasse. The 2nd highest protein (11.00%) was observed in that grown on wheat straw and water hyacinth. The lowest protein (7.81%) was observed in that grown on rice straw. Mushrooms are good source of minerals. Maximum of 18400 ppm Ca was found in mushroom which was grown on wheat straw. On other substrates its content varied from 1600 ppm to 18400 ppm. The content of Fe in the mushroom grown on different substrates varied from 92.09 ppm to 118.40 ppm. The highest Fe content was found in waste paper cultured oyster mushroom and lowest on water hyacinth.

Namdev *et al.* (2006) conducted a study to determine the effect of different straw substrates on spawn growth and yield of oyster mushroom. The number of days required for spawn run was significantly less (14 days) in case of gram straw, parthenium straw, sugarcane straw and wheat straw, compared with 20 days for sunflower stalk, mustard straw and paddy straw. Yield was very poor on parthenium straw (95 g/500 g dry substrates) and it was highest on paddy straw (666 g/500 g), followed by wheat straw and mustard straw (427 and 400 g/500 g respectively). Ramjan (2006) in his study found that high concentration of IAA is effective for mycelial growth and mustard straw performed best as a substrate for the production of fruiting bodies of oyster mushroom.

Sainos *et al.* (2006) conducted a study to determine the mycelial growth, intracellular activity of proteases, laccases and beta -1,3-glucanases, and cytoplasmic protein were evaluated in the vegetative phase of *Pleurotus ostreatus* grown on wheat straw and in wheat-grain-based media in Petri dishes and in bottles. The productivity of the wheat straw and wheat-grain-based spawn in cylindrical polyethylene bags containing 5 kg of chopped straw was also determined. We observed high activity of proteases and high content of intracellular protein in cultures grown on wheat straw. This suggests that the proteases are not secreted into the medium and that the protein is an important cellular reserve. On the contrary, cultures grown on wheat straw secreted laccases into the medium, which could be induced by this substrate. *Pleurotus ostreatus* grown on media prepared with a combination of wheat straw and wheat grain showed a high radial growth rate in Petridishes and a high level of mycelial growth in bottles. The productivities of wheat straw and wheat-grain-based spawn were similar. Our results show that cheaper and more productive mushroom spawn can be prepared by developing the mycelium on wheat straw and wheat-grain-based substrates.

Zape *et al.* (2006) conducted a study to determine the spawn run, days taken to pin head initiation, yield and biological efficiency of three oyster mushroom species viz. *Pleurotus florida, P. eous* and *P. flabellatus* were grown on wheat straw substrate. Time required for spawn run and pinning was significantly less in *Pleurotus eous* followed by *P. florida*. However, the yield and biological efficiency did not differ significantly but was higher in *P. florida* than *P. flabellatus* and *P. eous*. In analyzing the physico-chemical composition of dehydrated fruit bodies of *Pleurotus* species revealed that among different species *P. eous* was rich in protein (33.89%), moderate in fat (3.10%), carbohydrate (32.60%) and ash (8%) followed by *P. florida*. However, *P. flabellatus* was rich in crude fibre, carbohydrate and ash but low in protein and fat content as compare to *P. eous* and *P. florida*.

Thangamuthu (1990) in an investigation used sugarcane bagasse for growing *Pleurotus spp*. The two species gave similar yields at 400 g substrate, reaching maximum of 506-508 g on pretreated bagasse, 407-411 g on paddy straw and 379-391 g on wheat straw alone.

Ancona-Mendex *et al.* (2005) conducted an experiment to grow oyster mushroom (*Pleurotus ostreatus* (Jacq.: Fr.) Kummer in either maize or pumpkin straw. Samples were taken for each one of the three harvests and analyzed for total nitrogen (N) content and amino acids profile. The substrate had no effect ($P>0.05$) on N content and amino acid profile of the fruits. However, N (g/100 g DM) increased ($P<0.05$) from 4.13 g in the first harvest to 5.74 g in the third harvest. In general, the amino acids tended to be higher on the first harvest samples, but no changes were found ($P>0.05$) in the amino acid profile due to substrate or harvest, except for valine decreasing ($P<0.05$) from 3.96 to 3.15 g/16 g N. Changes in the N content of the fruit could be explained by changes in the stipe and pileus proportions as they had different N content (3.15 and 5.48 + or 0.031 g N/ 100 g DM respectively). The amino acid profile of the mushroom was adequate according to the FAO/WHO/UNU adult human amino acid requirements.

Habib (2005) tested different substrates such as sawdust, sugarcane bagasse, rice straw, wheat straw and waste paper for the production of oyster mushroom in polypropylene bag. Different substrates significantly affected the number of primordial, number of fruiting bodies and amount of fresh weight or yield. This experiment revealed that the highest number of primordial and fruiting bodies were found in waste paper 43.75 and 31.00 respectively. The highest amount of fresh weight was also found in waste paper 94.25 g.

Khlood and Ahmad (2005) conducted an experiment to study the ability of oyster mushroom (*Pleurotus ostreatus*) P015 strain to grow on live cake mixed with wheat straw. The treatments comprised : 90% straw + 5% wheat bran + 5% gypsum (control); 80% straw + 10% olive cake + 5% wheat bran + 5% gypsum (T_1); 70% straw + 20% olive cake 5% wheat bran + 5% gypsum (T_2); 60% straw + 30% olive cake + 5% wheat bran + 5% gypsum (T_3); 50% straw + 40% olive cake + 5% wheat bran +.5% gypsum (T_4); and 90% olive cake + wheat bran + 5% gypsum (T_5). After inoculation and incubation, transparent plastic bags were used for cultivation. The pinheads started to appear after 3 days and the basidiomata approached maturity 3-7 days after pinhead appearance. Several growth parameters including primordial induction and fructification period, earliness, average weight of individual basidiomata, average yield for each treatment, diameter of the pileus and biological efficiency percentage (BE%) were examined and proximate analyses for protein, crude fat, crude fiber, ash, carbohydrates, mineral and moisture contents were performed. The addition of 30% olive cake to the basal growing medium gave the highest yield (400 g/500 g dry substrate), average weight (21.5 g/cap) and average cap diameter (7.05 cm/cap) and BE% (80%).

Mousa (2005) conducted an experiment to study the edible oyster mushroom *Pleurotus ostreatus* produced the cellulose and hemicellulose degrading enzymes carboxymethylcellulase (CMCase), cellobiohydrolase, cellobiase and xylanase in liquid medium containing milled rice straw as a carbon source. Optimum CMCase production was obtained in a culture containing 2 % milled

rice straw, 0.2 % peptone, initial pH of 4 and incubated for 20 days at 35 ^{0}C. Maximum levels of cellobiohydrolase and cellobiase were achieved in a culture containing 2 % milled rice straw, 0.2 % peptone, initial pH of 5 and after incubation for 15 days at 40 degrees C. The optimum condition for xylanase production were obtained after 20 days with 2 % milled rice straw, 0.2 % peptone at pH 5 and 40 degrees C. The purified CMCase was a monomeric protein with a molecular weight 43 kDa. The optimum pH and temperature for the activity of CMCase were at 5 and 40 degrees C, respectively. The purified enzyme was stable at the pH range from 3 to 7 and temperature up to 45 degrees C.

Amin (2004) in his experiment revealed that the highest number of primordia of oyster mushroom was found in sterilized paddy straw at first flush; whereas the lowest was obtained with saw dust.

Maniruzzaman (2004) in his study used wheat, maize, rice and sawdust for the production of spawn in oyster mushroom and found that substrate rice was the best for spawn production of oyster mushroom.

Shah *et al.* (2004) carried out an experiment to investigate the performance of Oyster mushroom on the following substrates: 50 % sawdust + 50 % wheat straw, 75 % sawdust + 25 % leaves, 50 % wheat straw + 50 % leaves, 100 % sawdust, 100 % wheat straw and 100 % leaves. The temperature was kept at 25 degrees C for spawn running and 17-20 degrees C for fruiting body formation. The time for the completion of mycelial growth, appearance of pinheads and maturation of fruiting bodies on different substrates were recorded. The number of fruiting bodies and the biological efficiency of substrates were observed. The results show that spawn running took 2-3 weeks after inoculation, while small pinhead-like structures formed 6-7 days after spawn running. The fruiting bodies appeared 3-6 weeks after pinhead formation and took 27-34 days later after spawn inoculation.

Sawdust at 100 % produced the highest yield (646.9 g), biological efficiency (64.69 %) and the number of fruiting bodies (22.11). Therefore, sawdust is recommended as the best substrate for Oyster mushroom cultivation.

Baysal *et al.* (2003) conducted an experiment to spawn running, pin head and fruit body formation and mushroom yield of oyster mushroom (*Pleurotus ostreatus*) on waste paper supplemented with peat, chicken manure and rice husk (90+10; 80+20 W:W). The fastest spawn running (mycelia development) (15.8 days), pin head formation (21.4 days) and fruit body formation (25.6 days) and the highest yield (350.2 g) were realized with the substrate composed of 20% rice husk in weight. In general, increasing the ratio of rice husk within the substrate accelerated spawn running, pin head and fruit body formation and resulted increased mushroom yields, while more peat and chicken manure had a negative effect on growing.

Badshah *et al.* (1994) mentioned that *Pleurotus ostreatus* and *P. florida* were grown on wheat straw, sugarcane bagasse, corn cobs or sawdust and fruiting bodies were harvested at maturity. *P. ostreatus* and *P. florida* yields ranged from 49.8 and 277.7 g/2 kg substrate respectively on sawdust, to 432.8 and 420.5 g/ 2 kg substrate respectively, on wheat straw. Controls (grown in the field) yielded only 18.5 and 28.5 g/2 kg substrate for *P. ostreatus* and *P. florida*, respectively. In both species, wheat straw and sugarcane bagasse substrates resulted in the highest mushroom ascorbic acid contents and protein, fat and fiber contents were also affected by substrate. *Pleurotus florida* had higher fat but lower protein contents than *P. ostreatus*.

Obodai *et al.* (2003) evaluated eight lignocellulosic by-products as substrate, for cultivation of the oyster mushroom. *Pleurotus ostreatus* (Jacq. ex. fr.) Kummer. The yields of mushroom on different Substrates were 183.1, 151.8, 111.5, 87.5, 49.5, 23.3, 13.0 and 0.0 g for composted Sawdust of *Triplochiton scleroxylon*, Rice straw, Banana leaves, Maize stover, Corn husk, Rice husk, Fresh Sawdust and Elephant grass respectively. The biological efficiency (BE) followed the same pattern and ranged from 61.0%, for composted Sawdust to

50.0% for elephant grass. The Yield of mushroom was positively correlated to cellulose (r^2= 0.6). Lignin (r^2 =0.7) and fiber (r^2= 0.7) contents of the substrates. Based on the yield and BE of the substrates tested, Rice straw appeared to be the best alternate substrate for growing oyster mushroom.

Manzi *et al.* (2001) analyzed fresh and processed mushrooms (*Agaricus bisporus*, *Pleurotus ostreatus* and Boletus group). Results showed that botanical variety, processing and cooking are all effective determinants of mushroom proximate composition. Dried mushrooms (Boletus group) after cooking show the highest nutritional value, essentially due to insufficient dehydration. Dietary fiber, chitin and beta glucans, all functional constituents of mushrooms are present in variable amounts. Chitin level ranges from 0.3 to 3.9 g/100 g, while beta glucans which are negligible in *Agaricus*, range from 139 to 666 mg/100 g in *Pleurotus ostreatus* and Boletus group.

On an average, a serving (100 g) of mushroom will supply 9 to 40% of the recommended of dietary fiber. Ayyappan *et al.* (2000) used sugarcane trash and coir waste alone and in combination with paddy straw (3:1, 1:1 and 1:3 w/w) for sporophore production of two species of *Pleurotus*. The highest yields of *P. florida* (1395 g) and *P. citrinopileatus* (1365 g) were recorded in a mixture of sugarcane.

Upamanya and Rathaiah (2000) conducted an experiment to test the effect of fortification of rice straw with rice bran on the yield and quality of oyster mushroom (*Plelrrotus ostrentus*) in Jorhat, Assam, India. Treatments comprised: (i) addition of rice bran at 5% w/w (weight of rice bran/weight of dry substrate) at the time of spawning and (ii) control (without rice bran). Rice straw fortified with rice bran exhibited a higher yield compared to the control. Rice bran application had no effect on the crude protein content of mushroom but increased the yield by 44% over the control.

Wani and Sawant (1998) reported that among the various edible fungi, oyster mushroom (*Pleurotus spp.*) has a broad adaptability due to having a wide

range of suitable substrates, a simple cultivation technique and minimal cultural requirements. Various substrates on which oyster mushroom can be cultivated are mentioned.

Mathew *et al.* (1996) investigated that *Pleurotus ostreatus* were evaluated for their yield performance on various substrates, both for spawn production and cultivation, in the plains and in the high ranges of Kerala in studies conducted in the summer and rainy seasons. Sorghum, wheat and paddy grains were equally good for spawn production.

Badshah *et al.* (1994) mentioned that *Pleurotus ostreatus* and *P. florida* were grown on wheat straw, sugarcane bagasse, corn cobs or sawdust, by mixing 120-130 g of spawn with 2 kg of substrate and placing the mixture in sterilized polyethylene bags which were kept in the dark at 25°C for 2-3 weeks. Once the bags became full of mycelial growth, they were removed, leaving the substrate uncovered. Watering was carried out 2-3 times a day. Fruiting bodies were harvested at maturity. *P. ostreatus* and *P. florida* yields ranged from 49.8 and 277.7 g/2 kg substrate respectively on sawdust, to 432.8 and 420.5 g/ 2 kg substrate respectively, on wheat straw. Controls (grown in the field) yielded only 18.5 and 28.5 g/2 kg substrate for *P. ostreatus* and *P, florida*, respectively. In both species, wheat straw and sugarcane bagasse substrates resulted in the highest mushroom ascorbic acid contents and protein, fat and fiber contents were also affected by substrate. *Pleurotus florida* had higher fat but lower protein contents than *P. ostreatus*.

Dhanda *et al.* (1994) conducted an experiment on the use of fermented, semi-fermented and unfermented paddy straw as substrate for *Pleurotus spp.* (oyster mushroom). PAU-4 strain showed early primordial initiation, giving 60% biological efficiency whereas PAU-3 exhibited these effects much earlier with 70% biological efficiency.

Ijaz and Khan (1992) reported that mushroom has been recently introduced in Pakistan. Different species/strains i.e. *Pleurotus sajor-caju., P. ostreatus* strain

XI, *P. ostreatus* strain 467 and *P. ostreatus* were cultivated on cotton waste. *P. ostreatus* strain XI gave higher (260 g) basidiocarps out of 750 g of substrates per flush. It had 104 percent biological efficiency and 49 percent sustenance potential. In the same manner cotton waste scored maximum yield, biological efficiency and sustenance potential by defeating paddy straw + 25 percent synthetic compost, paddy straw and wheat straw in descending order.

Khan *et al.* (1991) used sawdust to prepare compost for spawn running amended with lime and different combinations of wheat chaff, wheat bran, paddy straw and cotton waste. Sawdust from *D. sisso* was the most suitable for spawn preparation and all types of sawdust amended with cotton waste were found to give optimum conditions for spawn running.

Thangamuthu (1990) in an investigation used sugarcane bagasse for growing *Pleurotus spp*. The two species gave similar yields at 500 g substrate, reaching maximum of 506-508 g on pretreated bagasse, 407-411 g on paddy straw and 379-391 g on wheat straw alone.

Ramesh and Ansari (1987) evaluated several locally available substrates such as rice straw, banana leaves, sawdust with more than 60% conversion efficiency on dry weight basis. The mean weight of the fruiting body was high (7.1 g) on banana leaves compared to other substrates (2.1-5.0 g). *Pleurotus ostreatus* was successfully grown under local conditions utilizing chopped wheat straw, cotton waste, maize cobs or rice straw as bedding material. Wheat straw and cotton waste gave the highest yields with the shortest incubation period; fruiting bodies were appeared after 15-18 days as compared to 4-5 weeks on the other substrates. The first flush gave the highest yield in all treatments and was a gradual decline in the yield of successive flushes.

Suprapti (1987) measured the mushroom yield and harvesting frequency after cultivation on Rubber wood (Hevea brasiliensis) sawdust mixed with 5, 10, 15 or 20 %, of leaves of either turi (Sesbonia grandiflora) or lamtoro gung (Leucaena leucocephala).

Average total yield per treatment was 643.00 g (532.29-744.69) per kg dry wt. of substrate. Addition of 40% lucerne hay (w/w) or 20% rapeseed meal (w/w) to the barley or wheat straw substrate gave the highest yields (275-300 kg/substrate) of *Pleurotus ostreatus*.

Bisht and Harsh (1985) mentioned the use of Lantana, straw and waste paper as substrate for button mushroom cultivation. The substrate was first biodegraded by Pleurotus ostreatus (Jacq.) Fr, then the sterilized substrate was used for button mushroom cultivation. Thus both mushrooms (Pleurotus ostreatus and Agaricus bisporus) can he grown economically in succession on the same substrate. Chang et al. (1981) reported that the fruiting bodies of mushrooms contained (82.5-92.2) % moisture, (4.30-50.7) % carbohydrate, (26.6-34.1) % crude protein and (1.1-8.0) % fat.

Baysal *et al.* (2003) conducted an experiment to spawn running, pin head and fruit body formation and mushroom yield of oyster mushroom (*Pleurotus ostreatus*) on waste paper supplemented with peat, chicken manure and rice husk (90+10; 80+20 W:W). The fastest spawn running (mycelia development) (15.8 days), pin head formation (21.4 days) and fruit body formation (25.6 days) and the highest yield (350.2 g) were realized with the substrate composed of 20% rice husk in weight. In general, increasing the ratio of rice husk within the substrate accelerated spawn running, pin head and fruit body formation and resulted increased mushroom yields, while more peat and chicken manure had a negative effect on growing.

Khlood and Ahmad (2005) conducted an experiment to study the ability of oyster mushroom (*Pleurotus ostreatus*) P015 strain to grow on live cake mixed with wheat straw. The treatments comprised: 90% straw + 5% wheat bran + 5% gypsum (control); 80% straw + 10% olive cake + 5% wheat bran + 5% gypsum (T_1); 70% straw + 20% olive cake 5% wheat bran + 5% gypsum (T_2); 60% straw + 30% olive cake + 5% wheat bran + 5% gypsum (T_3); 50% straw + 40% olive cake + 5% wheat bran +.5% gypsum (T_4); and 90% olive cake + wheat bran + 5% gypsum (T_5). After inoculation and incubation,

transparent plastic bags were used for cultivation. The pinheads started to appear after 3 days and the basidiomata approached maturity 3-7 days after pinhead appearance. The addition of 30% olive cake to the basal growing medium gave the highest yield (400 g/500 g dry substrate), average weight (21.5 g/cap) and average cap diameter (7.05 cm/cap) and BE% (80%).

Obodai *et al.* (2003) evaluated eight lignocellulosic by-products as substrate, for cultivation of the oyster mushroom. *Pleurotus ostreatus* (Jacq. ex. fr.) Kummer. The yields of mushroom on different Substrates were 183.1, 151.8, 111.5, 87.5, 49.5, 23.3, 13.0 and 0.0 g for composted Sawdust of *Triplochiton scleroxylon*, Rice straw, Banana leaves, Maize stover, Corn husk, Rice husk, Fresh Sawdust and Elephant grass respectively. The biological efficiency (BE) followed the same pattern and ranged from 61.0%, for composted Sawdust to 50.0% for elephant grass.

Amin *et al.* (2007) carried out an experiment to find out the primordia and fruiting body formation and yield of oyster mushroom (*Pleurotus ostreatus*) on paddy straw supplemented with wheat bran (WB) wheat flour (WF), maize powder (MP), rice bran (RB) and their three combination (WB+MP, 1:1), (WB+MP+RB, 1:1:1) and wheat broken (WBr) at six different levels namely 0,10,20,30,40 and 50% were studied. The minimum time (4.5 days) for primordial initiation was observed in the MP at 20% level and the highest number of effective fruiting bodies (60.75) was obtained in WF at 50% level. The highest biological yield (247.3 g/packet) was recorded at 10% level of (WBr).

Bhuyan (2008) conducted an experiment to study the effect of various supplements at different levels with sawdust showed significant effort on mycelium running rate and reduced the required days to complete mycelium running in the spawn packet. The supplementation of sawdust found to be significant in yield and yield contributing characters of oyster mushroom with some extent. The highest biological yield, economic yield, dry yield, biological efficiency (BE) and benefit cost ratio (BCR) of 270.5 g, 266.5 g,

26.34 g, 93.29, 9.57%, respectively was observed in sawdust supplemented with NPK mixed fertilizer (N=0.6%, P=0.3%, K=0.3%).

Nuruddin *et al.* (2010) carried out an experiment to investigate the effect of different levels of cowdung (0, 5, 10, 15 and 20%) on yield and proximate composition of *Pleurotus ostreatus*. The highest number of primordia (70.63) and fruiting body (51.92) were observed in rice straw supplemented with 5% level of cowdung.

The highest weight of individual fruiting body (4.71g), biological yield (234.24g), economic yield (227.72g), dry yield (22.83g), biological efficiency (140.26%) and benefit cost ratio (5.69) were observed in rice straw supplemented with 10% level of cowdung.

Upamanya and Rathaiah (2000) conducted an experiment to test the effect of fortification of rice straw with rice bran on the yield and quality of oyster mushroom (*Plelrrotus ostrentus*) in Jorhat, Assam, India. Treatments comprised: (i) addition of rice bran at 5% w/w (weight of rice bran/weight of dry substrate) at the time of spawning and (ii) control (without rice bran). They reported that rice bran application had no effect on the crude protein content of mushroom.

Ancona-Mendex *et al.* (2005) conducted an experiment to grow oyster mushroom (*Pleurotus ostreatus* in either maize or pumpkin straw. Samples were taken for each one of the three harvests and analyzed for total nitrogen (N) content and amino acids profile. The substrate had no effect on N content and amino acid profile of the fruits. However, N (g/100 g DM) increased from 4.13 g in the first harvest to 5.74 g in the third harvest. In general, the amino acids tended to be higher on the first harvest samples, but no changes were found in the amino acid profile due to substrate or harvest, except for valine decreasing from 3.96 to 3.15 g/16 g N. Changes in the N content of the fruit could be explained by changes in the stipe and pileus proportions as they had different N content (3.15 and 5.48 + or 0.031 g N/100 g DM respectively).

The amino acid profile of the mushroom was adequate according to the FAO/WHO/UNU adult human amino acid requirements.

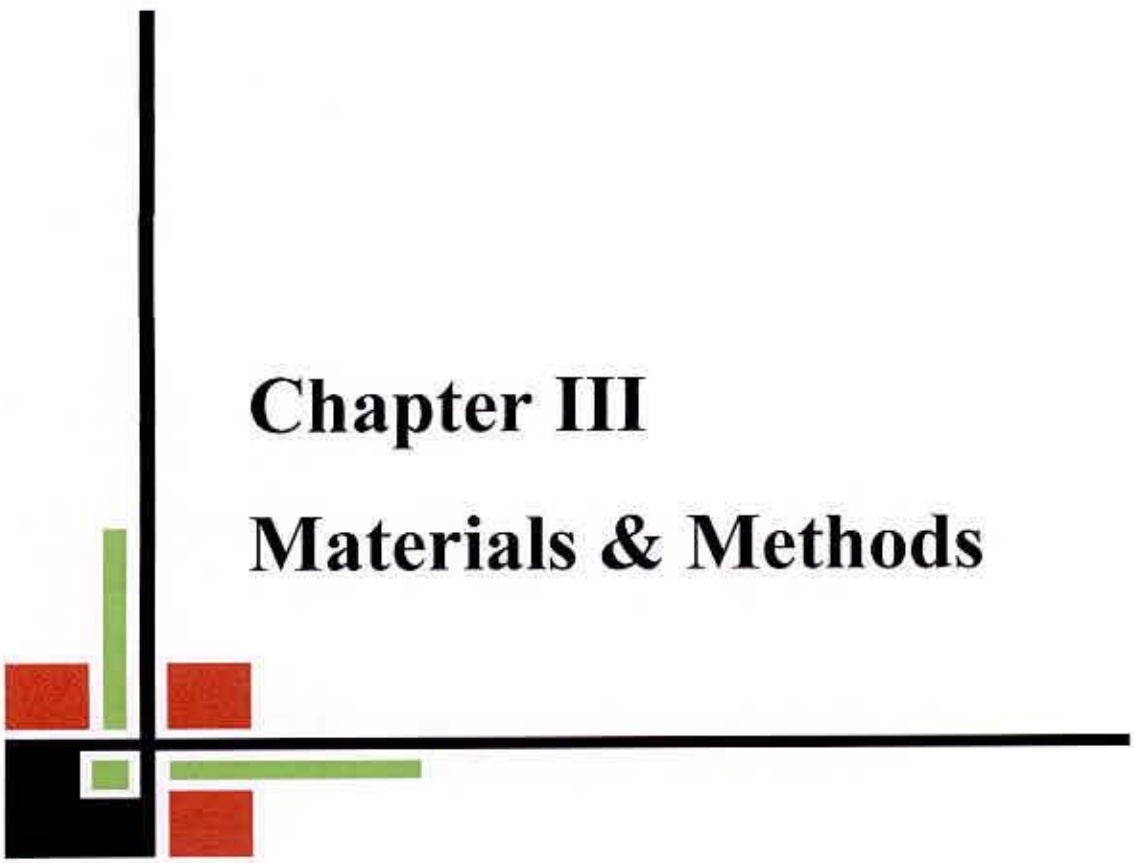
Khlood and Ahmad (2005) conducted an experiment to study the ability of oyster mushroom (*Pleurotus ostreatus*) P015 strain to grow on live cake mixed with wheat straw. The treatments comprised: 90% straw + 5% wheat bran + 5% gypsum (control); 80% straw + 10% olive cake + 5% wheat bran + 5% gypsum (T_1); 70% straw + 20% olive cake 5% wheat bran + 5% gypsum (T_2); 60% straw + 30% olive cake + 5% wheat bran + 5% gypsum (T_3); 50% straw + 40% olive cake + 5% wheat bran +.5% gypsum (T_4); and 90% olive cake + wheat bran + 5% gypsum (T_5). Carbohydrate, protein and fiber contents were high in the *P. ostreatus* basidiomete, ash contents were moderate, while fat content was low. For mineral contents in the mushrooms the trend was the same in all treatments. The K and P contents were high compared to the other minerals in all treatments, sodium was moderate while both Mg and Ca were found at low concentrations (Mg was relatively higher than Ca). Fe and Zn were relatively high compared to Cu and Mn which had very low concentrations.

Habib (2005) tested different substrates such as sawdust, sugarcane bagasse, rice straw, wheat straw and waste paper for the production of oyster mushroom in polypropylene bag. Different substrates significantly affected the number of primordia, number of fruiting bodies and amount of fresh weight or yield. This experiment revealed that the highest number of primordia and fruiting bodies were found in waste paper 43.75 and 31.00 respectively. The highest amount of fresh weight was also found in waste paper 94.25 g.

Kulsum *et al.* (2009) conducted an experiment to determine the effect of five different levels of cowdung (0%, 5%, 10%, 15% and 20%) as supplement with sawdust on the performance of oyster mushroom. Among the chemical characteristics highest content of protein (31.30%), ash (8.41%), crude fiber (24.07%), the lowest lipid (3.44%) and carbohydrate (32.85%) were observed due to sawdust supplemented with cowdung @ 10%.

Among the minerals the highest amount of nitrogen (5.01%), potassium (1.39%), calcium (22.15%), magnesium (20.21%), sulfur (0.043%), iron (43.4%) and the lowest phosphorus (0.92) were observed due to sawdust supplemented with cowdung @ 10%.

Manzi *et al.* (2001) analyzed fresh and processed mushrooms (*Agaricus bisporus*, *Pleurotus ostreatus* and Boletus group). Results showed that botanical variety, processing and cooking are all effective determinants of mushroom proximate composition. Dietary fiber, chitin and beta glucans, all functional constituents of mushrooms are present in variable amounts. Chitin level ranges from 0.3 to 3.9 g/100 g, while beta glucans which are negligible in *Agaricus*, range from 139 to 666 mg/100 g in *Pleurotus ostreatus* and Boletus group. On an average, a serving (100 g) of mushroom will supply 9 to 40% of the recommended of dietary fiber.



Chapter III

Materials & Methods

MATERIALS AND METHODS

The experiment was carried out to find out on the growth, yield and proximate composition of oyster mushroom (*Pleurotus ostreatus*) grown on supplemented sawdust. This chapter deals with a brief description on location and design of experiment, experiments and treatments, preparation of substrates, preparation of packets, cultivation of spawn packet, collection of produced mushrooms, proximate analysis of the mushrooms, data recording and their analysis under the following headings and sub-headings:

3.1 Location of experiment

The experiment was carried out at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, during the period from June' 2015 to Noverber' 2015.

3.2 Experimental materials

Mother culture of oyster mushroom was collected from Mushroom Development Institute (MDI), Savar, Dhaka, Bangladesh.

3.3 Varietal characteristics of oyster Mushroom

Oyster mushrooms (*Pleurotus ostreatus*) are characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. If the temperature increases above 32°C, its production markedly decreases. NAMDEC oyster-1, creamy white color first introduction oyster variety in Bangladesh released in 2012 as NAMDEC oyster-1 round the year.

3.4 Experiments and treatment

The experiment consists of five different types of sawdust with 30% wheat bran was taken as basal substrates. The experiment consists of the following treatments:

T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime

T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime

T_3: Teak tree (Tectona grandis) sawdust supplemented with 30% wheat bran and 1% lime

T_4: Mahogany tree (*swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime

T_5: Control (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

3.5. Medium Preparation

A medium was prepared using different sawdust. With spawn preparing substrate; different supplements (at the different rate on dry weight basis) and $CaCO_3$ (1%) was added. The measured materials were taken in a plastic bowl and mixed thoroughly by hand and moisture was increased by adding water.

Moisture was measured by using the moisture meter and adjusted the moisture content at 50%. The mixed substrates were filled into 9×12 inch polypropylene bag @ 200 g. The filled polypropylene bags were prepared by using plastic neck and plugged the neck with cotton and covered with brown paper placing rubber band to hold it tightly in place.

3.6. Sterilization, inoculation and mycelium running in spawn packets

Determination of spawn run

The prepared packets were sterilized about 2.5 hrs and then these were kept for cooling. After cooling, 5g mother spawn were inoculated into the packets in the laminar airflow cabinet and were kept at 20-22°C temperature until the packets become white with the mushroom mycelium. After completion of the mycelium running the rubber band, brown paper, cotton plug and plastic neck of the mouth of spawn packet were removed and the mouth was wrapped tightly with rubber band. Than this spawn packets were transferred to the culture house.

The growth of mycelium (linear length) in each bag was measured by a measuring tape at 6 days interval. Using this data, spawn run rate (cm/day) was determined for every spawn type. When the mycelium fully covered the substrate bag (spawn run completed), bags were kept open in the growing house. The days required for the completion of spawn running in the substrate bag were recorded. Days for pinhead formation, fruit body (flush) formation and for harvest was recorded.

3.7. Cultivation of spawn packet

Two ends, opposite to each other of the upper position of plastic bag were cut in "D" shape with a blade and opened by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a blade for removing the thin whitish mycelial layer. Then the spawn packets were soaked in water for 15 minutes and invested to remove excess water for another 15 minutes. The packets of each type were placed separately on the floor of culture room and covered with news paper. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3 times a day. The light around 300-500 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22°C to 25°C. The first primordia appeared 2-4 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

3.8. Collection of produced mushroom

Oyster mushrooms matured within 2-3 days after primordia initiation. The matured fruiting body was identified by curial margin of the cap, as described by Amin,(2002). Mushrooms were harvested by twisting to uproot from the base.

3.9 Data collection

3.9.1 Mycelial growth (%): Mycelial growth was counted by taking the full packet as a full unit and generally the data was taken at every six days intervals.

3.9.2 Mycelium running rate in spawn packet (cm): Mycelium running rate (MRR) for each type of substrate was measured after the mycelium colony cross the shoulder of the packet.

The linear length was measured at different places of packet using the following formula (Sarker, 2004) MRR= L/N cm/day.

Where,

L= Average length of mycelium running for different places (cm)

N= Number of days

3.9.3 Days required for completing mycelium running: Days required from inoculation to completion of mycelium running were recorded.

3.9.4 Time from stimulation to primordial initiation (days): Time from stimulation to primordial initiation (days) were recorded.

3.9.5 Time from primordial initiation to harvest (days): Time required form primordial initiations to harvest were recorded.

3.9.6 Number of primordial/packet: Number of primordial/packet was recorded.

3.9.7 Number of fruiting body/packet: Number of well developed fruiting body / packet was recorded. Dry and pinheaded fruiting bodies were discarded but tiny fruiting bodies were included in counting.

3.9.8 Number of effective fruiting body per packet: Number of well-developed fruiting body was recorded. Tiny fruiting bodies were discarded in counting.

3.9.9. Weight of individual fruiting body per packet: Average weight of individual fruiting body was calculated by dividing the total weight of fruiting body per packet by the total number of fruiting body per packet.

3.9.10. Biological yield (g): Biological yield per 500 g packet was measured by weighing the whole cluster of fruiting body without removing the lower hard and dirty portion.

3.9.11 Economic yield: Economic yield per 500 g packet was recorded by weighing all the fruiting bodies in a packet after removing the lower hard and dirty portion.

3.9.12 Drying of mushrooms: The collected fruiting bodies of the mushroom were transferred to the laboratory. Therefore data were collected on different parameter. After collection of the data the fruiting bodies were dried in the sun separately as per treatment. In the time of drying the stipe and the pileus were separated for better drying.

3.9.13 Dry yield:

About 50 g of randomly selected mushroom sample was taken in a paper envelop and was weighed correctly. The mushroom was oven dried at 72°C temperature for 24 hours and weighed again. The weight of blank envelop was subtracted from both the initial weight. The dry yield was calculated using the following formula (Sarker, 2004):

$$\text{Dry yield (g/500g packet)} = \text{Economic yield} \times \frac{\text{Oven Dry Weight of sample (g)}}{\text{Fresh weight of sample (g)}}$$

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

$$\text{Biological efficiency} = 100 \times \frac{\text{Total biological weight (g)}}{\text{Total weight substitute used (g)}}$$

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

3.9.16 Cultural operations for subsequent flushes: After completing the first harvest again the packets were scraped at the place where the 'D' shaped cut had been done and were soaked in a bucket for five minutes and then placed in the culture house and water was sprayed regularly.

The primordial appeared 9-10 days after first harvest and 7-8 days after second harvest. Water spraying was continued until the mushrooms were ready to be harvested.

3.10 Proximate analysis of the mushrooms

3.10.1 Collection of the samples: Mushrooms grown from the spawn were collected packet wise and all the wastes and dusts were removed from the fruiting body. Therefore they were ready to be analyzed.

3.10.2 Moisture:

About 10-20g of the material of each sample were weighed into separated and weighed petridishes and dried in an oven at 100°C to 105°C till the weight of the petridishes with their contents was constant. The moisture content was expressed as percent of the fresh fruiting bodies or mushrooms.

$$\text{Moisture \%} = 100 \times \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of the sample}}$$

3.10.3 Dry matter

The dry matter content of sample was calculated by subtracting of the percent moisture of each sample from 100. The process was repeat 3-4 times for achieving constant weight of the sample used, The constant weight of the dry sample was termed as dry matter % dry matter=100-% moisture content.

3.10.4 Grinding:

The dried plant materials were cut into small pieces with a knife or scissor. The sample was grinded in a plant grinder fitted with a suitable screen. If the

grinding takes a long time, the sample will absorb moisture and it is necessary to dry the sample again in the oven at 105°C overnight.

3.10.5 Determination of crude fiber:

Ten gram of moisture and fat-free sample was taken in a beaker and 200 ml of boiling 0.255 N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker and 200 ml of boiling 0.313 N NaOH added.

After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100°C and weighed (We) in an electric balance *(KEY: JY-2003; China)*. The crucible was heated in a muffle furnace *(Nebertherm: Mod-L9/11/c6; Germany)* at 600°C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber.

$$\text{Therefore, Crude fiber (g/100g sample)} = \frac{[100-(\text{moisture} + \text{fat})] \times (\text{We-Wa})}{\text{Weight of sample}}$$

(Raghuramulu et *al.*, 2003).

3.10.6 Determination of protein The Protein contents of the fruiting bodies of the mushrooms were determined by the standard Micro-kjeldhal procedure of AOAC (1975). According to this method total nitrogen contents of the samples were estimated and proteins contents were find out by multiplying by 6.25 to the total nitrogen values. The total nitrogen was determined by the Kjeldahl method, which depends upon the conversion of protein nitrogen into

ammonium sulfate, by digestion. Ammonia liberated from the ammonium sulfate by making the solution alkaline was distilled into known volume of a standard acid, which was then back titrated.

Reagents

a) Concentrated sulfuric acid (H_2SO_4)

b) Digestion Mixture: Potassium sulfate: Copper sulfate (98: 2 w/w)

c) 40% Sodium hydroxide in distilled water

d) N/10 Sulfuric acid and N/10 Sodium hydroxide

e) 0.1% methyl red indicator: 0.1 g of the indicator was dissolved in 60 ml of alcohol and the volume was made 100ml with the distilled water

Procedure

Weighed dried sample 2.0g, 5.0g of the digestion mixture, 25 pieces of the glass beads, and 25ml of concentrated sulfuric acid were taken in a Kjeldahl flask. The content of the flask was digested in a flame chamber until the total content became clear. The digested materials were quantitatively transferred into a one liter flat-bottomed flask and the volume was made up to about 400ml with distilled water. Then about 40% NaOH and some pumice stone were added to prevent bumping, and distilled immediately in the distillation chamber of the Kjeldahl apparatus. The distillation was continued till its volume diminished to one-half of the initial. The distillate was collected in a receiver containing 100ml of N/10 sulfuric acid containing 2/3 drops of methyl red indicator. The liberated ammonia absorbed in the sulfuric acid solution was titrated against standard (N/10) NaOH solution.

Calculation

$$\% \text{ N in the supplied fiber sample} = \frac{(a \times MHCl\text{-}b \times MNaOH) \times 1.401}{c}$$

Where,

a = ml HCI taken into the conical flask (usually 20.00 ml)

b = ml NaOH used for titration of the content in the conical flask

M_{HCl} = Molarity of the HCl

M_{NaOH} = Molarity of the NaOH

c = g of mushroom powder used for the analysis

3.10.7 Total fat estimation: Fat was estimated as crude ether extraction of the dry materials. The dried sample (about 5.0 g) was weighted into a conical flask and plugged with fat free cotton. The flask was then placed in an electric shaker and extracted with anhydrous ether for about 16 hours. The ether extract was filtered into another weighed conical flask. The flask containing the original ether extract was washed 4 to 5 times with small quantities of ether and the washings were also transferred to the filter paper.

The ether in the conical flask was then removed by evaporation, and the flask with the residual was dried in an oven at 80°C to 100°C, cooled in desiccators and weighted. The result was expressed as follows:

$$\text{Fat contents (g) per 100g of dried sample} = \frac{\text{Weight of either x Percentage of dried sample}}{\text{Weight of the dried sample}}$$

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

$$\text{Ash content (g/100 g sample)} = \frac{\text{Weight of the ash} \times \text{Percentage of dried sample}}{\text{Weight of sample taken}}$$

(Raghuramulu *et al.*, 2003)

3.10.9 Total carbohydrate estimation: The content of the available carbohydrate was determined by the following equation:

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

(Raghuramulu *et al.*, 2003)

3.11 Elementary composition analysis

3.11.1 Equipments: Electric balance, Grinding machine, Desiccators, Atomic Absorption Spectrometer (AAS), Spectrophotometer, Porcelain crucible, Muffle furnace, Oven, Beaker and Flame Photometer.

3.11.2 Determination of total Nitrogen Total nitrogen was determined by a micro kjaldhal apparatus in the traditional method and calculated using the following formula.

$$\% \text{ N in the supplied fiber sample} = \frac{(a \times MHCl - b \times MNaOH) \times 1.401}{C}$$

Where,

a = ml HCI measured into the conical flask

b = ml NaOH used for titration

M_{HCl} = molarity of the HCI

M_{NaOH} = molarity of the NaOH

c = g powder of mushroom used for the analysis

3.11.3 Determination of Ca, Mg, Fe, Zn and P

The sample was digested with nitric acid to release of Ca, Mg, Fe, Zn and P. Ca, Mg, Fe and Zn were determined by atomic absorption spectrophotometer and P was determined by spectrophotometer.

3.11.3.1 Digestion

1. 0.500 g of dried sample was taken into each of 18 nitrogen digestion tubes. The two remaining tubes were kept blanks. 5 ml nitric acids were added to each of all 20 tubes. The tubes were left overnight mixing the contents in the tubes. Covering with the exhaust manifold, the tubes were placed in the digester and the temperature was set to 125°C, turning on the digester, the digestion was continued for 4 hours after boiling started. Every tube was observed to avoid drying.

2. After cooling, the digestion mixture was transferred with distilled water to a 100 ml volumetric flask. Water was added to the flask to make the volume up to the mark.

3. Filtration was performed on a dry filter into a dry bottle, which could be closed with a screw cap. Keeping the filtrate in the closed bottle Ca, Mg, K, Fe, Mn, Zn, Cu and P were determined in the filtrate.

3.11.3.2 Estimation of Ca

20 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette. 5 ml $LaCl_3$-solution was added and the volume was made with water and mixed. Then the content of Ca was measured by atomic absorption spectrometer (AAS).

3.11.3.3 Estimation of Mg

20 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette. 5 ml $LaCl_3$-solution was added and the volume was made with water

and mixed. Then the content of Mg was measured by atomic absorption spectrometer (AAS).

3.11.3.4 Estimation of P

5 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette. 30 ml water was added, mixed and then 10 ml ammonium molybdate-ascorbic acid solution was added to volume with water and mixed. After 15 minutes, the absorbance was measured on a spectrophotometer at 890 nm.

3.11.3.5 Estimation of Fe, Zn and Co

The content of Fe elements were measure by atomic absorption spectrometer (AAS) directly in the undiluted filtrate.

3.11.3.6 Calculations

For Ca, Mg, K. P

$$\text{mg per kg sample} = \frac{a \times 25000}{b \times c}$$

Where,

a= mg/L Ca, Mg, P measured on atomic absorption spectrometer, flame photometer or spectrophotometer

b= ml diluted filtrate transferred into the 50 ml volumetric flask for determination of Ca, Mg, K or P

c = g sample weighed into the digestion tube

If an additional dilution is made before the transfer to the 50 ml volumetric flask, the result is multiplied with the dilution factor. But the above elements were in trace. So addition of dilution was not to be performed.

For Fe, Zn and Co

mg per kg sample = d x 100/c

Where,

d = mg/L Zn and Fe measured on atomic absorption spectrophotometer

c = g sample weighed into the digestion tube

3.12 Statistical analysis of data

Data on various parameters were analyzed by following standard statistical method by Gomez and Gomez, 1984. Computer package program MSTAT-C is also used. The experiment was laid out in single factor RCBD (Randomized Complete Block Design).The experiment consider 6 treatment with 3 replication and 1 spawn packet in each packet replication. The analysis of variance was conducted and means were separated and compared by least significant difference (LSD) and DMRT test respectively.

Chapter IV

Results and Discussion

RESULTS AND DISCUSSION

The study was conducted to find out the effect of different sawdust substrate on the yield and yield contributing character of oyster mushroom (*Pleurotus ostreatus*). The results have been presented and discussed with the help of tables, graphs and possible interpretations given under the following headings:

4.1 Experiment 1: Effect of different sawdust substrate on the yield and yield contributing characters of oyster mushroom (*Pleurotus ostreatus)*

4.1.1 Effect on mycelial growth

4.1.1.1 Effect of different sawdust substrate on mycelium running rate in spawn (cm)

Statistically significant variation of mycelium running rate (MRR) of oyster mushroom (*Pleurotus ostreatus*) showed due to different sawdust under the present trial (Table 1). The highest mycelium running rate was recorded from T_5 (0.72 cm) that is mixture of sawdust: mango, rain tree, teak tree and mahogany + 30% wheat bran, while the lowest mycelium running rate was observed from T_4 (0.56 cm) that mahogany sawdust + 30% wheat bran which is statistically similar with T_1 (mango sawdust + 30% wheat bran) (0.57 cm) and T_2 (rain tree sawdust + 30% wheat bran) (0.57 cm).

Different sawdust showed different mycelium running because of different carbohydrate based on availability and the environment of the spawn. The present findings found more or less similar with the previous workers. Khan *et al.* (1991) reported that sawdust amended with different organic supplement like wheat chaff, wheat bran, paddy straw, cotton waste etc. provided suitable condition for spawn running. Sarker (2004) found that the mycelium running rate of oyster mushroom greatly influenced with the supplement of wheat barns in different levels. Bhuyan (2008) also found similar result as found in the present experiment.

4.1.1.2 Effect of different sawdust substrate on time from stimulation to primordial initiation (days)

The duration between stimulation to primordial initiation ranged was recorded from 6.0 days to 8.0 days. The highest time from stimulation to primordial initiation was recorded in T_1 (8.04 days). Statistically similar lowest stimulation primordial initiation was recorded in the treatment T_2 (6.04 days) which is near to T_4 (6.70 days). The other treatments were statistically near to T_3 (7.37 days) and T_5 (7.70 days) of time from stimulation to primordial initiation (Table 1). The result of the present findings keeps in with the findings of previous scientists (Sarker, 2004, Ruhul Amin *et al.*, 28 2007; Bhuyan, 2008). Ruhul Amin *et al.* (2007) found significant differences among the level of supplements used for preparing the substrates. Sarker (2004) observed that duration from primordial initiation to first harvest of oyster mushroom was significantly lower as compared to control where no supplement was used and the duration required for total harvest of oyster mushroom increased with the level of supplement used. In the present study, the time required for total harvest also decreased with the levels of supplements increased compared to sugarcane rice straw alone.

4.1.1.3 Effect of different sawdust substrate on time from primordial initiation to harvest (days)

Statistically significant variation was recorded in terms of time from primordial initiation to harvest of oyster mushroom due to different sawdust substrate (Table 1). The highest time from primordial initiation to harvest was attained in T_1 (4.24 days) which was statistically similar with T_3 (4.12 days), T_2 (3.69 days). On the other hand, the lowest time from primordial initiation to harvest was found in T_5 (3.02 days) which was statistically identical with T_4 (3.33 days). Similar results were also reported by Khan *et al.* (2001); Dhoke *et al.* (2001). Khan *et al.* (2001) reported that time from primordial initiation to harvest vary from 3-5 days.

Dhoke *et al.* (2001) found significant effect of different agro-wastes on that time from primordial initiation to harvest of oyster mushroom and the days required for the final picking complete from 2.25 to 3.50 days depending on different substrates.

Table 1. Effect of sawdust substrate on mycelia growth of oyster mushroom (*Pleurotus ostreatus*)

Treatment	Mycelium running rate in spawn packet (cm)	Time from stimulation to primordial initiation (days)	Time from primordial initiation to harvest (days)
T_1	0.57 c	8.04 a	4.24 a
T_2	0.57 c	6.04 c	3.69 ab
T_3	0.63 b	7.37 ab	4.12 a
T_4	0.56 c	6.70 bc	3.33 bc
T_5	0.72 a	7.70 a	3.02 c
$LSD_{(0.05)}$	0.06	0.88	0.59
CV (%)	6.30	6.90	8.96

T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

4.1.2 Effect on yield contributing character and yield

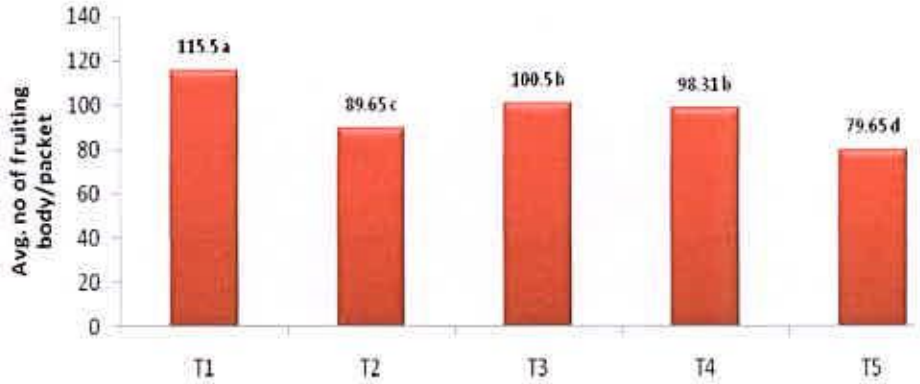
4.1.2.1 Average number of primordial/packet

T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

Figure 1. Performance of sawdust substrate on the average number of primordial/packet of oyster mushroom (*Pleurotus ostreatus*)

Statistically significant variation of average number of primordial/packet of oyster mushroom (*Pleurotus ostreatus*) showed due to different sawdust under the present trial (Figure 1). The highest average number of primordial/packet was observed in the treatment T_1 (217.5) and the lowest average number of primordial/packet was in the treatment T_5 (192.5). The result of the present findings keeps in with the findings of previous scientists (Ahmed, 1998; Dey, 2006; Bhuyan, 2008). Ahmed (1998) reported significantly different number of primordial on different substrates. Dey (2006) found that the number of primordial and the average yield significantly varied with the substrates used in production of oyster mushroom. Bhuyan (2008) found similar findings growing oyster mushroom on saw dust supplemented with different levels of cow dung.

4.1.2.2 Average number of fruiting body/packet



T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

Figure 2. Performance of sawdust substrate on the average number of fruiting body/packet of oyster mushroom

Statistically significant variation of fruiting body/packet of oyster mushroom *(Pleurotus ostreatus)* showed due to different sawdust under the present trial (Figure 2). The highest average number of fruiting body per packet was recorded from T_1 (115.5) while the lowest average number of fruiting body per packet was observed from T_5 (79.65) where T_3 (100.5), T_4 (98.31) are statistically similar. The result of the present findings keeps in with the findings of previous scientists (Yoshida *et al.,* 1993; Al Amin, 2004; Sarker, 2004, Bhuyan, 2008). Yoshida *et al.* (1993) reported that the number of fruiting bodies was lower, but increased when the substrates was mixed with different supplements. Al Amin (2004) reported that the number of primordial grown on different substrates differed significantly. Sarker, (2004) found that the number of primordial increased with the levels of supplement and continued up to a certain range and decline thereafter. In the present study the average number of fruiting body in creased up to 10 % of cow dung used as supplement and decreased thereafter. Bhuyan (2008) in a same type of experiment found similar results.

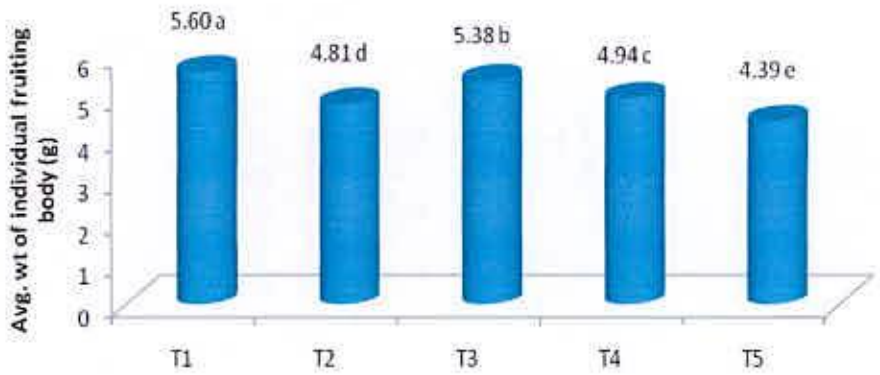
4.1.2.3 Average number of effective fruiting body/packet

T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

Figure 3. Performance of sawdust substrate on the average number of effective fruiting body/packet of oyster mushroom (*Pleurotus ostreatus*)

The highest average number of effective fruiting body/packet was recorded from treatment T_1 (29.02), the lowest average number of effective fruiting body/packet was recorded from treatment T_5 (17.02). Statistically similar to T_4 (20.35) followed by T_2 (19.69) in terms of average number of primordial/packet (Figure 3). The result of the present findings keeps in with the findings of previous scientists (Yoshida *et al.*, 1993 ;) who reported that the no of fruiting body was low but increase when the substrate was mixed with different substrate.

4.1.2.4 Average weight of individual fruiting body (g)



T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

Figure 4. Performance of sawdust substrate on the average weight of individual fruiting body of oyster mushroom (*Pleurotus ostreatus*)

Statistically significant variation was recorded from average weight of individual fruiting body of oyster mushroom due to different sawdust substrate (Figure 4). The average weight of individual fruiting body in different treatment ranged from 5.60 g to 4.39 g. The highest average weight of individual fruiting body was recorded from treatment T_1 (5.60 g), the lowest average weight of individual fruiting body was recorded treatment T_5 (4.39 g). The other treatments varied significantly over control in terms of average weight of individual fruiting body (Figure 4). The present study matches with the study of the previous scientist (Bhuyan, 2008). Bhuyan (2008) found significant effect of supplementation on the weight of fruiting body but he found comparatively higher weigh of individual fruiting body ranged from (5.02g to 7.01g), which may be due to environmental conditions or growing season. Erkel E. I. 2009 found that 30 minutes soaking was the best in relation to studied characters.

4.1.3.1 Biological yield (g)

Statistically significant variation was recorded from biological yield of oyster mushroom due to different sawdust substrate (Table 2). The highest biological yield was recorded from treatment T_1 (368.18 g), the lowest biological yield was recorded from treatment T_5 (316.28 g), which was statistically similar with T_3 (348.58 g) and T_4 (344.68 g) treatment. Baysal *et al.* (2003) found the highest yield of oyster mushroom (*Pleurotus ostreatus*) with the substrate composed of 20% rice husk in weigh. Ruhul Amin *et al.* (2007) found the highest biological yield 247.3g/packet. He also found that the trend of economic yield corresponded with different supplements at different level.

4.1.3.2 Economic yield (g)

The highest economic yield was recorded under treatment T_1 (360.68 g) and the lowest economic yield was counted under T_2 (309.98 g). The other treatments varied significantly over control (Table 2), which was statistically similar with T_3 (342.28 g) and T_4 (338.28 g) treatment. Payapanon *et al.* (1994) mentioned that suitable amount of supplements added to sawdust medium maximized economic yield of oyster mushroom at optimum production cost. Sarker (2004) found appreciable variations in economic yield also observed at different levels of supplements under different substrate-supplement combinations. Bhuyan (2008) observed that the yield of *Pleurotus ostreatus* responded with the levels of supplements used with sawdust and increased with the level of supplementation and declined thereafter.

4.1.3.3 Dry yield

The dry yield of mushroom was maximum under the treatment T_1 (35.15 g) and the lowest dry yield was counted under T_2 (30.08 g). The other treatments were varied significantly over control (Table 2), which was statistically similar with T_3 (33.31 g) and T_4 (32.91 g). The result of the present study corroborates with Ahmed (1998) who observed significant effects of various substrates on

diameter and length of stalk also diameter and thickness of pileus. He also found that lower diameter of pileus produced the lowest yield and concluded that the diameter of pileus increased the quality and yield of mushroom and highest dry yield from mango sawdust. Sarker *et al.* (2007) found the range of dry yield from 4.28 g to 29.98 g, which was more or less similar to this study.

4.1.3.4 Biological efficiency

Different sawdust showed statistically significant variation for biological efficiency of oyster mushroom under the present trial (Table 2). The maximum biological efficiency was recorded from T_1 (200.48%) again the lowest biological efficiency was observed in T_2 (174.38%), which was statistically similar with T_3 (195.88%) and T_4 (194.08 %). Kalita *et al.* (1997); Shen and Royse (2001); Obodai *et al.* (2003) and many other researchers reported earlier similar findings from their experiment. Kalita *et al.*, (1997) observed biological efficiency for different substrates ranged from 35.2 to 60.9%. Obodai *et al.* (2003) found biological efficiency (BE) followed a pattern and ranged from 61.0% to 80.0%. But Biswas *et al.* (1997) found supplementation of substrate promoted biological efficiency (125.75%). Shen and Royse (2001) found supplements combined with basal ingredient results better mushroom quality as well as Biological efficiency.

4.1.3.5 Cost ratio

The highest cost benefit ratio was calculated in treatment T_5 (5.28) which is statistically similar with T_4 (5.24) and T_1 (5.09). The lowest cost benefit ratio was calculated in T_2 (4.68) which were statistically similar with T_3 (4.87). The other treatments differed significantly in terms of cost benefit ratio (Table 2). The present findings keep in with the findings of previous workers (Lim *et al.*, 1997; Ahmed, 1998; Sarker *et al.*, 2007). Lim *et al.* (1997) analyzed the cost and return of *Volvariella* and *Pleurotus* mushroom production and found the ROI of 8.9 and 5.1, respectively. Ahmed (1998) also observed the benefit cost ratio of 73.2, 23.78 and 16.23 in case of *Pleurotus sajor-caju.*

The cause of these variations between the results of this study might be due to consideration of other costs involved in the production of oyster mushroom or might be due to measuring system. Sarker *et al.* (2007) mentioned the performances of substrates were significantly differed based on benefit cost ratio. They reported the highest cost benefit ratio of 6.50 with wheat straw.

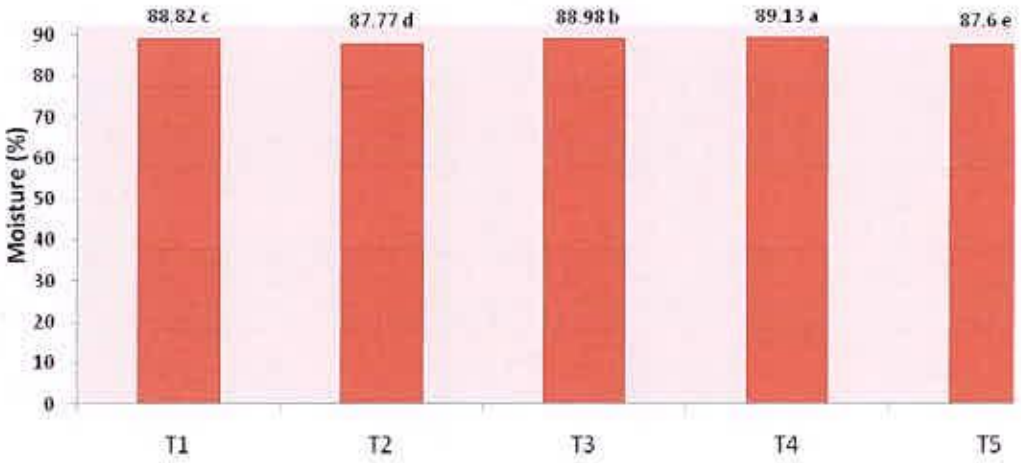
Table 2. Effect of different sawdust substrate on the yield of oyster mushroom (*Pleurotus ostreatus*)

Treatment	Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)	Cost benefit ratio
T_1	368.18 a	360.68 a	35.15 a	200.48 a	5.09 ab
T_2	329.08 d	309.98 e	30.08 e	174.38 d	4.68 c
T_3	348.58 b	342.28 b	33.31 b	195.88 b	4.87 bc
T_4	344.68 bc	338.28 bc	32.9 bc	194.08 b	5.24 a
T_5	316.28 e	322.98 d	31.38 d	185.18 c	5.28 a
$LSD_{(0.05)}$	7.009	7.193	0.718	2.554	0.237
CV (%)	1.13	1.18	1.18	0.73	2.59

T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

4.2.1 Effect on proximate content

4.2.1.1 Moisture



T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

Figure 5. Performance of sawdust substrate on the moisture content of individual fruiting body of oyster mushroom (*Pleurotus ostreatus*)

Statistically significant variation was recorded in terms of moisture content of oyster mushroom due to different sawdust treatment (Figure 5). The moisture percent of fruiting body ranged from 89.13 % to 87.6 %. The highest moisture content was found in T_4 (89.13 %) treatment while the lowest moisture content was recorded in T_5 (87.6%) treatment. The result of the present study keep in with the findings of previous workers (Rahman, 1994; Moni, 2004; Alam *et al.*, 2007, Bhuyan, 2008). Rahman (1994) observed more or less 90% moisture in the mushroom *Pleurotus ostreatus*. Moni *et al.* (2004) found 88.15 to 91.64 % moisture. Alam *et al.* (2007) reported 87 % to 87.5 % moisture in oyster mushrooms grown on different substrates. Bhuyan (2008) found no significant differences among the mushrooms produced in sawdust supplemented with wheat bran.

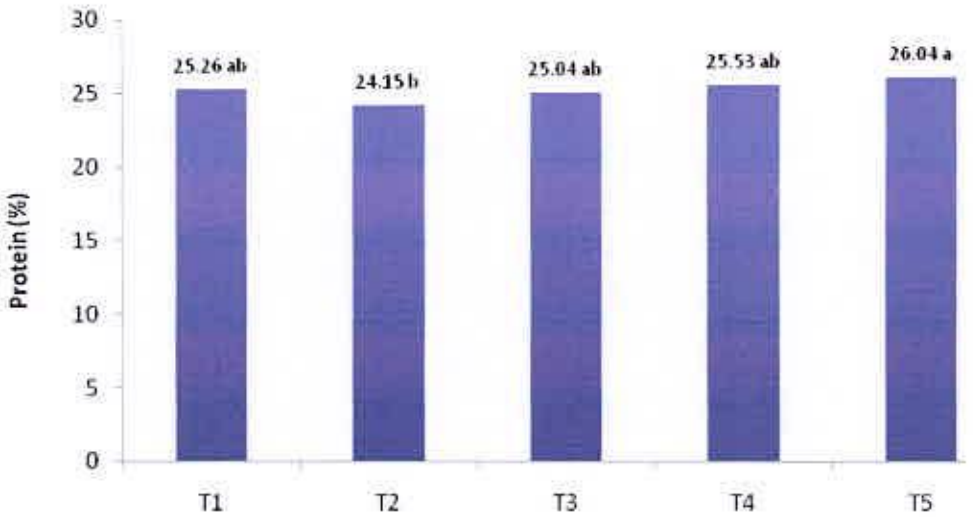
4.2.1.2 Dry matter

T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

Figure 6. Performance of sawdust substrate on the dry matter content of individual fruiting body of oyster mushroom (*Pleurotus ostreatus*)

The dry matter percentage of the fruiting body shows significant difference. The dry matter percent of fruiting body ranged from 10.18% to 8.64 %. The highest dry matter percentage was observed in treatment T_5 (10.18%) and the lowest dry matter percentage was observed in T_4 (8.64%) (Figure 6). The result of the present study matches with the findings of previous scientists. Bhuyan (2008) found no significant differences among the treatments when cow dung used as supplement. But in this study there was significant differences found among the treatments. This may be due to different levels of cultural practices.

4.2.1.3 Protein content

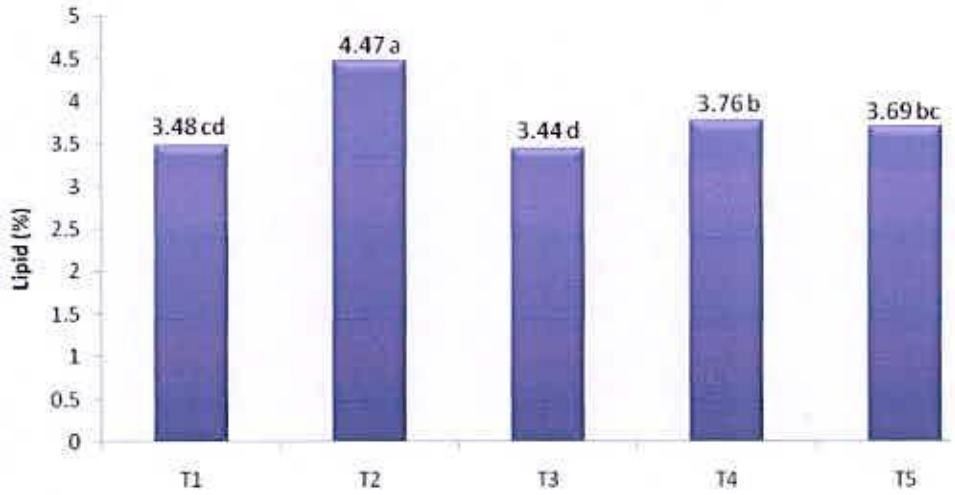


T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

Figure 7. Performance of sawdust substrate on the protein content of individual fruiting body of oyster mushroom (*Pleurotus ostreatus*)

All the treatments contain a considerable amount of protein. The content of protein varied from 26.04%-24.15% (w/w) in the mushroom grown on different sawdust substrate. The highest content of protein was found in treatment T_5 (26.04%) and the lowest protein was found in T_2 (24.15%). The other treatments varied significantly over control in respect to protein content (Figure 7). The result of the present study corroborates with the study of Chang *et al.* (1981) who reported that the fruit bodies of oyster mushrooms contained 26.6-34.1% protein. Zhang-RuiHong *et al.* (1998) found the protein content of oyster mushroom was 27.2% on an average.

4.2.1.4 Lipid content

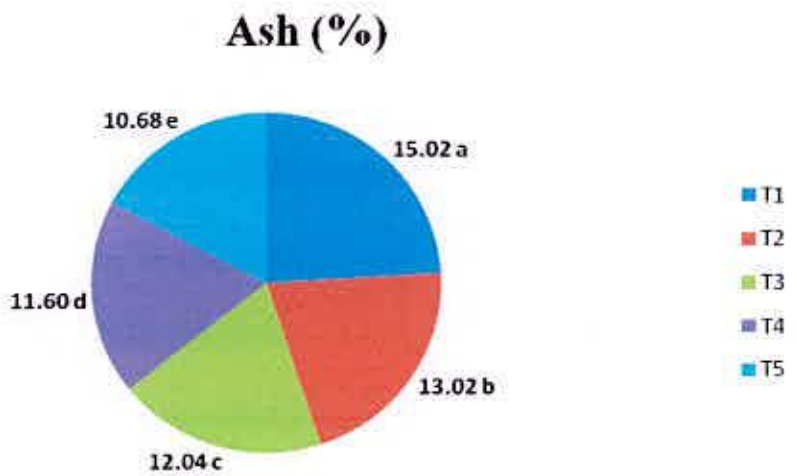


T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

Figure 8. Performance of sawdust substrate on the lipid content of oyster mushroom (*Pleurotus ostreatus*)

The lowest lipid percentage was calculated in treatment T_3 (3.44 %) followed by T_1 (3.48 %). The highest lipid percentage was counted under T_2 (4.47 %). The rest of the treatments were statistically similar (Figure 8). The result of the present study showed that the lipid content of the mushroom decreased as the supplements added with the substrates. The result of the present study keep in with the findings of Chang *et al.* (1981) who found 1.1-8.0 lipid in oyster mushroom varieties. Moni *et al.*, (2004) found 1.49 to 1.90 % crude fats in oyster mushroom; Alam *et al.* (2007) reported 4.30 to 4.41 % lipids in oyster mushroom grown on different substrates.

4.2.1.5 Ash content

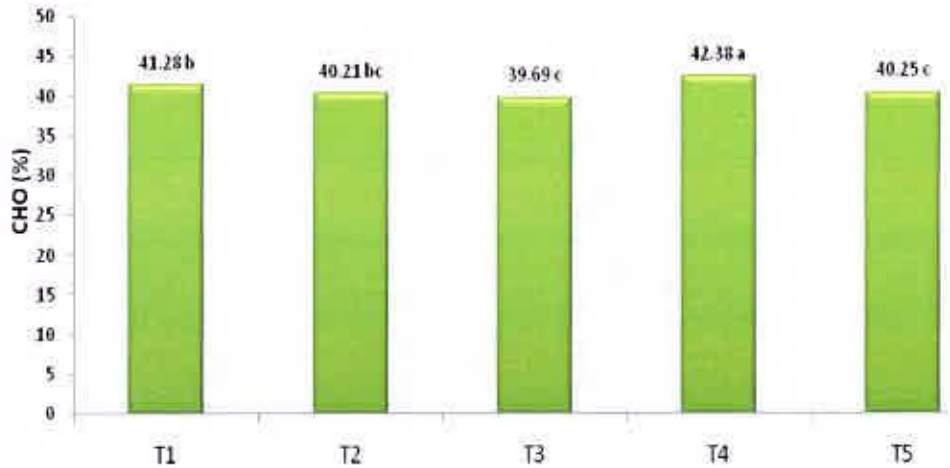


T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

Figure 9. Performance of sawdust substrate on the ash content of oyster mushroom (*Pleurotus ostreatus*)

The highest percentage of ash was calculated in treatment T_1 (15.02 %) and the lowest percentage of ash was calculated in treatment T_5 (10.68 %). The other treatments were statistically different but differed significantly in terms of percentage ash content (Figure 9). The findings of the present study are supported by the study of Khlood-Ananbeh *et al.* (2005) who reported ash contents were moderate in the fruiting bodies. Alam *et al.* (2007) reported 8.28 to 9.02 % of ash in *Pleurotus spp.* In the present study the ash content is as high as 12.80 may be due to the newly introduced varieties.

4.2.1.6 Carbohydrate content

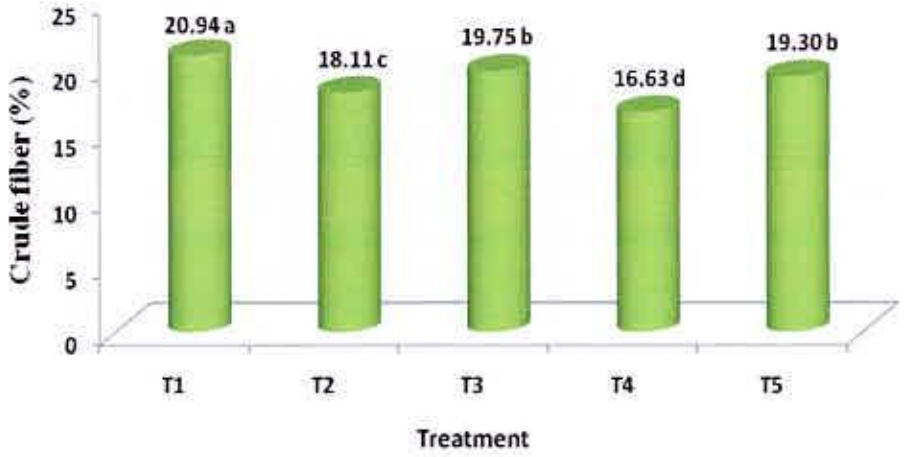


T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

Figure 10. Performance of sawdust substrate on the carbohydrate content of oyster mushroom (*Pleurotus ostreatus*)

The lowest percentage of carbohydrate was counted under treatment T_3 (39.69 %) and the highest carbohydrate percentage was counted under T_4 (42.38 %). The rest of the treatments were statistically different but differed significantly over control in respect to percent carbohydrate content (Figure 10). The findings of the present study does not match with the study of Chang *et al.* (1981) reported that the fruit bodies mushrooms contained 40.30-50.7 % of carbohydrates. But it was supported by Alam *et al.* (2007) who found 39.82 to 42.83 % of carbohydrates in *Pleurotus spp.*

4.2.1.7 Crude fiber content



T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

Figure 11. Performance of sawdust substrate on the crude fiber content of oyster mushroom (*Pleurotus ostreatus*)

The highest percentage of crude fiber was counted under treatment T_1 (20.94 %) and the lowest crude fiber percentage was counted under T_4 (16.63 %). The rest of the treatments were statistically different but varied significantly over control in respect to percent crud fiber content (Figure 11). The findings of the present study corroborate with the study Alam *et al.* (2007) reported 22.87g/100g to 23.29g/100g of fiber in *Pleurotus spp.*

4.2.2 Effect of different sawdust substrate on elemental content

4.2.2.1 Phosphorus (P) content

The highest percentage of phosphorus content counted under treatment T_1 (0.92), whereas the lowest phosphorus percentage was counted under treatment T_5 (0.60). The rest of the treatments were statistically similar (Table 3). The findings of the present study does not match with the study of Chang *et al.* (1981) who reported that the fruiting bodies of *Pleurotus* contained 5.87 to 8.40 mg/g of P on dry weigh of fruiting bodies. This may be due to the system of measurement. But Sarker *et al.* (2007) found 0.97% phosphorus, in oyster mushroom grown on sugarcane biogases based substrates.

4.2.2.2 Calcium (Ca) content

The highest percentage of calcium content (mg/100g) was counted under treatment T_5 (30.73) and the lowest calcium percentage was counted under T_1 (24.16). The rest of the treatments were statistically similar (Table 3). The findings of the present study matches with the study of Alam *et al.* (2007) who found 22.15 to 33.7 mg/100g of calcium in different oyster mushroom varieties. Sarker *et al.* (2007) found 2400ppm calcium, in oyster mushroom grown on sawdust based substrates.

4.2.2.3 Magnesium (Mg) content

The highest percentage of magnesium was observed under treatment T_4 (21.06) followed by T_1 (17.44) and T_3 (18.77). The lowest percentage of magnesium observed under T_5 (13.18) followed by T_2 (14.82). The rest of the treatments were statistically similar in respect to magnesium content (Table 3). The findings of the present study corroborates with the study of Alam *et al.* (2007) found 13.4 to 20.22 mg/100g of magnesium in different oyster mushroom varieties.

4.2.2.4 Zinc (Zn) content

The highest percentage of zinc content (mg/100g) was counted under treatment T_2 (27.61) which were followed by T_3 (26.89). The lowest zinc percentage was counted under T_5 (20.8). Where T_4 (22.87) and T_1 (24.27) are statistically similar in (Table 3). The result of the present study matches with the study of Alam *et al.* (2007) who found that zinc content of different oyster mushroom varieties ranged from 16 to 20.9 mg/100g. Sarker *et al.* (2007) found 30.92ppm zinc in oyster mushroom grown on sawdust based substrates.

4.2.2.5 Cobalt (Co) content

The highest percentage of cobalt content (g/100g) was counted under treatment T_3 (21.43) and the lowest cobalt percentage was counted under T_1 (12.23). The rest of the treatments were statistically different in respect to percent cobalt content (Table 3).

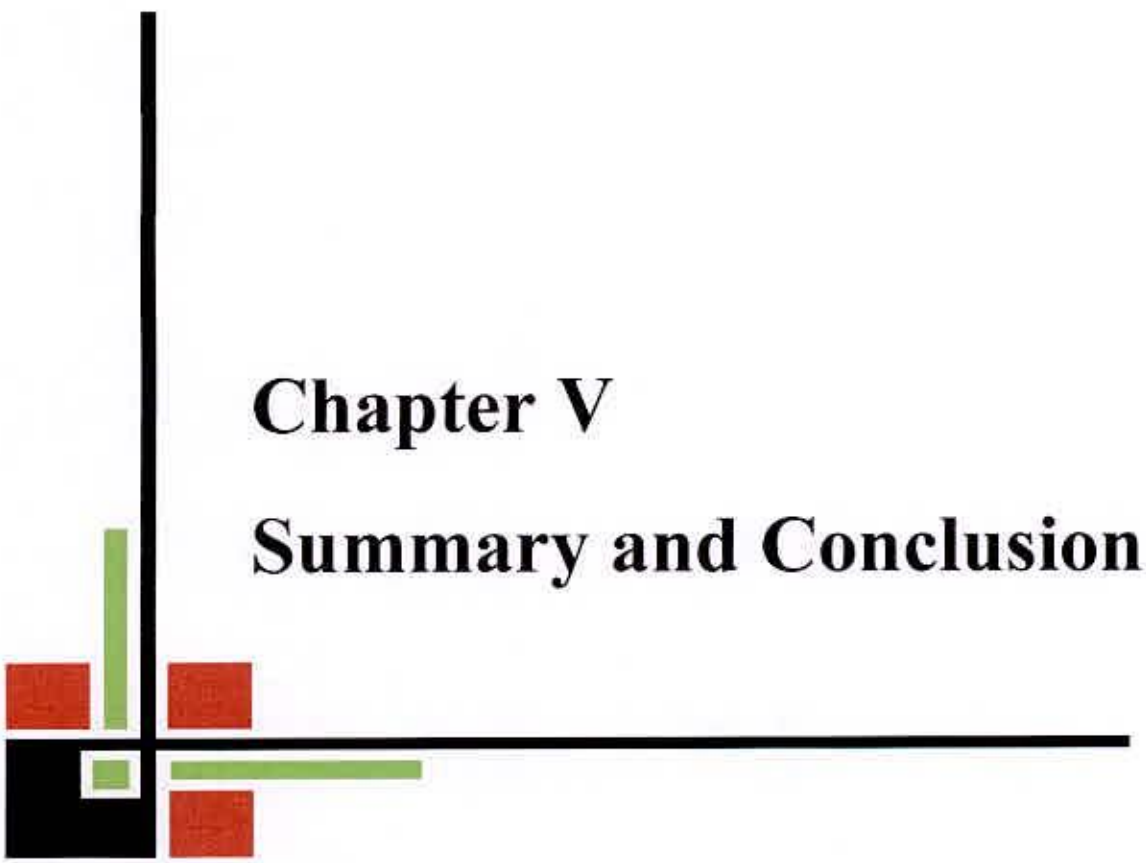
4.2.2.6 Iron (Fe) content

The highest percentage of iron content (g/100g) was counted under treatment T_2 (42.52) and the lowest iron percentage was counted under T_1 (39.75). The rest of the treatments were statistically similar in respect to percent iron content (Table 3). On the other side T_3 (40.45) is statistically similar with T_5 (40.53). The findings of the present study matches with the findings of Alam *et al.* (2007) found 33.45 to 43.2 mg/100g of iron in different oyster mushroom varieties. Sarker *et al.* (2007) found 92.09 ppm to 118.40 ppm iron, in oyster mushroom grown on sawdust based substrates.

Tabla 3. Effect of sawdust substrate on elemental contents of oyster mushroom (*Pleurotus ostreatus*)

Treatment	P (%)	Ca (%)	Mg (%)	Zn (mg %)	Co (mg %)	Fe (mg %)
T_1	0.92 a	24.16 b	17.44 ab	24.27 b	12.23 d	39.75 d
T_2	0.79 b	28.94 ab	14.82 bc	27.61 a	13.80 c	42.52 a
T_3	0.77 b	25.30 ab	18.77 ab	26.89 a	21.43 a	40.45 c
T_4	0.81ab	25.93 ab	21.06 a	22.87 b	16.48 b	41.50 b
T_5	0.60 c	30.73 a	13.18 c	20.8 c	12.57cb	40.53ca
LSD $_{(0.05)}$	0.11	6.20	4.34	1.54	1.48	0.58
CV (%)	7.32	12.24	13.80	3.46	5.09	0.81

T_1: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_2: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_5: Controled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime



Chapter V

Summary and Conclusion

SUMMARY AND CONCLUSION

The present study was carried out at the Biochemistry laboratory and Mushroom Culture House (MCH) of Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, during the month of June to November' 2015 to investigate the performance of different sawdust on the growth, yield and proximate composition of Oyster mushroom *(Pleurotus ostreatus)*.

The experiment consists of five different types of sawdust as- T_1: mango tree *(Mangifera indica)* sawdust + 30% wheat bran, T_2: rain tree (*Albiia saman*) sawdust + 30% wheat bran; T_3: teak tree *(Tectona grandis)* sawdust + 30% wheat bran; T_4: mahogany tree *(Swietenia mahagoni)* + 30% wheat bran; T_5: controlled (mixture of sawdust; mango, rain tree, teak tree, mahogany) sawdust + 30% wheat bran.

Mycelium running rate per day (MRR) for each type of substrates was measured after the mycelium colony crossed the shoulder of the packet. The highest running rate was observed in T_5 (0.72 cm) and the lowest running rate of mycelium was observed in T_4 (0.56 cm). The other treatment was statistically similar.

The highest time from stimulation to primordial initiation was observed in T_1 (8.04 days) and the lowest time from stimulation to primordial initiation was in the treatment T_2 (6.04 days). The other treatments varied significantly in terms of time from stimulation to primordial initiation. The highest time from primordial initiation to harvest was in the treatment T_1 (4.24 days) and the lowest time from primordial initiation to harvest was observed in the treatment T_5 (3.02 days). The other treatments were statistically varies in terms of time from primordial initiation to harvest (days).

The highest average number of primordial/packet was observed in the T_1 (217.5) whereas the lowest number of primordial/packet was in the T_5 (192.5). The other treatments were statistically and significantly varied in terms of average number of primordial/packet. The highest number of fruiting

body/packet was observed in T_1 (115.5) and the lowest number of fruiting body/packet was recorded in T_5 (79.65). Other treatments were statistically differed in terms of number of primordial/packet.

The highest number of effective fruiting body/packet was observed in T_1 (29.02) and the lowest number of effective fruiting body/packet was recorded in T_5 (17.02). The other treatments were statistically similar in terms of number of effective fruiting body/packet. The highest weight of individual fruiting body was observed in T_1 (5.60 g) the lowest weight of individual fruiting body was in T_5 (4.39 g).

The other treatments were statistically and significantly varied in terms of average weight of individual fruiting body. The highest biological yield was recorded in the T_1 (368.18 g/packet) which were significantly higher as compared to all the treatments. The lowest biological yield was recorded in T_5 (316.28 g/packet). The rest of the treatments varied significantly as compared with control in terms of biological yield.

The highest economic yield was recorded in T_1 (360.68 g/packet) and the lowest economic yield was under T_2 (309.98 g/packet). The rest of the treatments varied significantly over control. The maximum dry yield of mushroom was found in T_1 (35.15 g/packet). The minimum dry yield was recorded under T_2 (30.08 g/packet). The rest of the treatments were statistically different. The highest biological efficiency of 200.48% was calculated in T_1 and the lowest biological efficiency of 174.38% was calculated from T_2. The rest of the treatments significantly varied over control.

The highest cost benefit ratio was observed in T_5 (5.28) whereas the lowest observed in T_2 (4.68).The rest of observation were statistically different over control. Moisture percentage of the fruiting body shows significant difference. The moisture percent ranged from 89.13 to 87.60. The highest moisture percent was observed in T_4 (89.13) but the lowest was in T_5 (87.60). The rest of the treatments were statistically varied over control.

The maximum dry matter percent was observed in T_5 (10.18) whereas the minimum in T_4 (8.64) and the other treatments varied statistically over control. Protein is the most important constituent of food material. All the treatments contain a great amount of protein. The highest content of protein was found in T_5 (26.04%) and the lowest content of protein was in T_2 (24.15 %). The rest of the treatments were statistically different.

The highest lipid percentage was counted under T_2 (4.47) and lowest in T_1 (3.48). The other treatments were varied statistically in respect to percent lipid content. The highest percentage of ash was observed in T_1 (13.02) whereas the lowest percentage of ash was in T_5 (8.55). The rest of the treatments are statistically different.

The highest percentage of carbohydrate was recorded in T_4 (42.38) and the lowest carbohydrate percentage was recorded in T_3 (39.69). The rest of the treatments were statistically similar in respect to carbohydrate content.

The highest percentage of crude fiber (19.53) was recorded in T_1 and the lowest crude fiber percentage (16.13) was counted under T_4. The rest of the treatments were statistically different. The highest percentage of nitrogen (4.42) was recorded in the T_2 and the lowest nitrogen percentage (3.93) was counted under T_3 treatment.

The rest of the treatments were statistically similar. The highest percentage of phosphorus was recorded in T_1 (0.92) and the lowest percentage of phosphorus was recorded in T_5 (0.60). The highest percentage of calcium recorded in T_5 (30.73) and lowest in T_1 (24.16). Rest of the treatments is statistically similar. The highest percentage of magnesium was observed in T_4 (21.06) while the lowest percentage was counted under T_5 (13.18). The rest of the treatments were statistically different over control in respect to percent magnesium content. The highest amount (mg) of Zinc was counted under T_2 (27.61) whereas the lowest

amount was counted under T_5 (20.8). The rest of the treatments were statistically different.

The highest amount (mg) of cobalt was counted from T_3 (21.43) whereas the lowest amount was counted under T_1 (12.23). The rest of the treatments were statistically different. The highest amount (mg) of iron was counted under T_2 (42.52) and the lowest amount was counted under T_1 (39.75). The rest of the treatments were statistically similar.

Conclusion

From the above discussion, it was observed that treatment T_1 [mango tree sawdust + 30% wheat bran] among the treatments performed significantly better on growth, yield, nutrient and mineral content of oyster mushroom *(Pleurotus ostreatus)*.

Recommendations

In this experiment, mango tree sawdust + 30% wheat bran performed better in respect of different growth, yield and nutrient composition and mineral content of oyster mushroom. Therefore, mango tree sawdust + 30% wheat bran substrate can be recommended for wide range cultivation of oyster mushroom. On the other hand, controlled (mixture of mango, rain tree, teak tree and mahogany tree sawdust) + supplemented with 30% wheat bran treatment may be fair option.

Reference

REFERENCES

Ahmed, S. (1998). Performance of differerent substrates on the growth and yield of Oyster mushroom (*Pleurotus Sajor-Caju* (Fr.) Sing). M.S. Thesis, Institute Of Postgraduate Studies In Agriculture, Salna, Gazipur.

Alam, N., Khan, A., Hossain, M. S., Amin, S. M. R. and Khan, L. A. (2007). Nutritional analysis of dietary mushroom *Pleurotus florida* Eger *and Pleurotus sajorcaj (*Fr.) Singer. *Bangladesh J. Mushroom.* **1**(2): 1-7.

Ali, M. A., Mehmood, M. I., Nawaz, R., Hanif, M. A. and Wasim, R. (2007). Influence of substrate pasteurization methods on the yield of Oyster mushroom (*Pleurotus species*). *Pakistan J. Agri. Sci.*, **44**(2): 300-303.

Ali, M. R., Hoque, M. S., Ahmed, K. U. and Rahman, M. H. (2010). Effect of wheat bran supplements with sugarcane bagasse on the yield and proximate composition of Oyster mushroom (*Pleurotus ostreatus*). *Bangladesh J. Mushroom.* **4**(2): 21-26.

Amin, M. A. (2004). Studies on mycelium, spawn and production of certain edible mushrooms. M.S. Thesis, Department of Biotechnology, BAU, Mymensingh.

Amin, S. M. R., Sarker, N. C., Khair, A. and Alam, N. (2007). Detection of novel supplements on paddy straw substrates on Oyster mushroom cultivation. *Bangladesh J. Mushroom.* **1**(2): 18-22.

Ancona-Mendex, M. L., Sandoval, C., Belmar-Casso, R. and Capetilo-Leal, C. M. (2005). Effect of substrate and harvest on the amino acid profile of Oyster mushroom (*Pleurotus ostreatus*). *J. Food Comp. Analysis.* **18**(5): 447-450.

Atikpo, M., Onokpise, O., Abazinge, M., Louime, C., Dzomeku, M., Boateng, L. (2008). Sustainable mushroom production in Africa: A case study in Ghana. *Afr. J. Biotec.* **7**: 24-25.

Badshah, N. N and Wahid, M. (1992). Yield and quality of mushrooms grown on different substrates. *Sarhad J. Agriculture*. **8**(6): 631-635.

Badshah, N., Wahid, M. and Ur-Rehman, N. (1994). Yield and quality of mushrooms grown on different substrates. *Sarhad J. Agril.* **8**(6): 631-635.

Baysal, E., Peker, H., Yalinkilic, M. K. and Temiz, A. (2003). Cultivation of Oyster mushroom on waste paper with some added supplementary materials. *Bio. Tech.* **89**(1): 95-97.

Baysal, E., Peker, H., Yalinkilic, M. K. and Temiz, A. (2003). Cultivation of Oyster mushroom on waste paper with some added supplementary materials. *Bio. Tech.* **89**(1): 95-97.

BBS (Bangladesh Bureau of Statistics). (2010). Statistical Yearbook of Bangladesh. Bangladesh Bureau of Statistics, Ministry of Planning. Dhaka, Bangladesh. pp. 76.

Beteez, A. and Kustudia, M. (2004). Mushroom cultivation and marketing Horticulture production. *ATTRA publication*. pp: 1-25.

Bhuyan, M. H. M. B. U. (2008). Study on preparation of low cost spawn packets for the production of Oyster mushroom *(Pleurotus ostreatus)* and its proximate analysis. M.S. Thesis, Department of Biochemistry, SAU, Dhaka-1207.

Bhuyan, M. H. M. B. U. (2008). Study on preparation of low cost spawn packets for the production of Oyster mushroom *(Pleurotus* ostreatus) and its proximate analysis. M.S. Thesis, Department of Biochemistry, SAU, Dhaka-1207.

Biswas, M. K., Shukla, C. S. and Kumar, S. M. (1997). Method for increasing biological efficiency of Oyster mushroom *(Pleurotus florida)* in Madhya Pradesh. *Adv. Plant Sci., Indira Gandhi Argil. Univ.* **10**(l): 69-74.

Chang, R. (1996). Functional properties of mushrooms. *Nutrition Reviews*. **54**: 91-93.

Chang, S. T., Lau, O. W. and Chowdhury, K. Y. (1981). The cultivation and nutritional value of *Pleurotus sajor cuju. Eur. J. Appl. Microbiol. Biotec.* **12**(1): 58-62.

Chaube, H. S., (1995). Nutritional and medicinal value of mushrooms, mushroom production technologies. *Pantngear.* **2:** 63-145.

Dawit, A. (1998). Mushroom cultivation: A practical approach. Berhanena Selam printing press. Addis Ababa. pp. 17-72.

Bhattacharjya, D. K., Paul, R. K., Miah, M. N. and Ahmed, K. U. (2014). Effect of different saw dust substrates on the growth and yield of Oyster mushroom *(Pleurotus ostreatus). IOSR J. Agril. Veterinary Sci.* 7(2): 38-46.

Dey, R. C. (2006). Mycelial growth and Oyster mushroom production with different hormone and media composition. M.S. Thesis, Department of Biotechnology, BAU, Mymensingh.

Dhoke, P. K., Chavan, R. A. and Jadhay, V. T. (2001). Cropping period and yield of Oyster mushroom *(Pleurotus sajor-caju)* on different agro-substrate. *Madras Agril. J.* **88**(4-6): 327-329.

Muleta, D., Gume, B. and Dawit, A. (2013). Evaluation of locally available substrates for cultivation of Oyster mushroom *(Pleurotus ostreatus). Afri. J. Microbiol. Res.* Jimma, Ethiopia. pp. 2228-2237.

Erkel, E. I. (2009). The effect of different substrate mediums on yield of Ganoderma lucidum (Fr.) Karst. *J. Food, Agril. & Environ.* 7(3&4): 841-844.

Habib, M. A. (2005). Comperative study on cultivation and yield performance of Oyster mushroom (*Pleurotus ostreatus*) on different substrates. M. S. Thesis, Department of Biotechnology, BAU, Mymensingh.

James, C. S. (1995). Analytical chemistry of foods 1st edn, chapman and hall, newyork cultivation of edible and medicinal fungi, Penn State University, Pennsylvania.

Kalita, M. K., Rathaiah, Y. and Bhagabati, K. N. (1997). Effects of some agro-wastes as substrate for Oyster mushroom (*Pleurotus sajor-caju*) cultivation in Assam. *Indian J. Hill Farming*. **10**(1-2): 109-110.

Kaul, T. N. (2001). Biology and conservation of mushrooms. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi. India. pp: 117-145.

Khan, A. M., Khan, S. M. and Khan, S. M. (2001). Studies on the cultivation of Oyster mushroom *Pleurotus ostreatus* on different substrates. *Pakistan J. Phytopath.* **13**(2): 140-143.

Khan, S. M., Mirza, J. H. and Khan, M. A. (1991). Studies on Shiitake mushroom (*Lentinula edodes*). *Proc. 13th Int. Con. Sci. Culti. Edible Fungi. Dublin, Irish Republic*. pp 503-508.

Khlood, A. and Ahmad, A. (2005). Production of Oyster mushroom *(Pleurotus ostreatus)* on olive cake agro-waste. *Dirasat Agril. Sci.* **32**(1):64-70.

Klingman, A. M. 1950. Hand book of mushroom culture. CRC Publishing co. J. B. Kenneth Square, Pennsylvania, USA.

Kulsum, U., Hoque, S. and Ahmed, K. U. (2009). Effect of different levels of cow dung with sawdust on yield and proximate composition of Oyster mushroom *(Pleurotus ostreatus)*. *Bangladesh J. Mushroom.* **3**(2): 25-31.

Lim, J., Mangaoang, Y. and Ranchey, C. (1997). Mushroom cultivation under the closed canopy high-diversity forest farming system. PCARRD highlights 1996. Philippine Council for Agriculture, forestry and Natural Resources, Research and Development. Los Banos, Laguna (Philippines). p. 91.

Manzi, P., Aguzzi, A. and Pizzoferrato, L. (2001). Nutritional value of mushroom widely consumed in Italy. *Food Chem.* **73** (3): 321-325.

Moni, K. H., Ramabardan, R. and Eswaran, A. (2004). Studies on some physiological, cultural and post harvest aspects of Oyster mushroom *(Pleurotus ostreatus)* (Berk). *Trop. Agril. Res.* **12**: 360-374.

Mori, K. (1986). Cultivated mushrooms in Japan. Proc. *Int. Sym. Sci. Tech.* Aspects of Culti. Edible Fungi. Penna. State Univ. USA. pp 21-24.

Nuruddin, M. M., Rahman, M. H., Ahmed, K. U., Hossain, A. and Sultana, N. (2010). Effect of cowdung supplements with rice straw on the yield and proximate composition of Oyster mushroom *(Pleurotus ostreatus)*. *Bangladesh J. Mushroom.* **4**(2): 45-52.

Obodai, M., Okine, C. and Vowotor, K. A. (2003). Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products. Food Res. Inst. Accra, Ghana. *J. Industrial Microbiol. Biotech.* **30**(3): 146-149.

Payapanon A., Butranu, P. and Ayuthaya, P. S. N. (1994). Optimum amount of the rice bran for Oyster mushroom (*Pleurotus florida*) cultivation. Kasetsart University, Bangkok (Thailand). Proceedings of the 24th National Conference: Poster Session. Bangkok. pp. 259-264.

Rahman, S. M. (1994). Nutritional and biochemical analysis of edible mushrooms in three developmental stages. M. Sc. Thesis. Department of Biochemistry. University of Dhaka.

Ruhul, S. M., Nirod, C. S., Mahbuba, M., Jebunnahar, K. and Rahman, M. (2007). Officer's Training Manual. National mushroom development and extension centre, Savar, Dhaka, Bangladesh. p: 13-17.

Sarker, N. C. (2004). Oyster mushroom (*Pleurotus ostreatus*) production technology suitable for Bangladesh and its nutritional and postharvest behavior. Ph.D. Thesis, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur.

Sarker, N. C., Hossain M. M., Sultana, N., Mian, I. H., Karim, A. J. M. S. and Amin, S. M. R. (2007). Impect of different substrates on nutrient content of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer. *Bangladesh J. Mushroom.* **1**(2): 35-38.

Shen, Q. and Royse, D. J. (2001). Effects of nutrient supplements on biological efficiency, quality and crop cycle time of Maitake (*Grifola frondosa*). *Appl. Microbial. Biotec.* **57**(1&2): 74-78.

Singh, Reeti and Singh, U. C. (2005). Modern mushroom cultivation. Jodhpur,Updesh Purohit for Agrobios.

Thangamuthu, P. (1990). Food from sugarcane waste. *SISSTA-sugar. J.* **16**(2): 45-50.

Upamanya, G. K. and Rathaiah, Y. (2000). Effect of fortification of rice straw with rice bran on yield and protein content of Oyster mushroom *(Pleurotus cornucopiae). Indian J. Hill Farm.* **13**(1-2): 104-105.

Yoshida, N., Takahashi, T., Nagao, T. and Chen, J. (1993). Effect of edible mushroom *(Pleurotus ostreatus)* cultivation on *in vitro* digestibility of wheat straw and sawdust substrate. *J. Japanese Soc. Grassland Sci.* **39**(2): 177-182.

Zhang-Ruihong, H., Li-Xiu, J., Fadel, J. G. and Li-XJ. (1998). Oyster mushroom cultivation with rice and wheat straw. *Biores. Tech.* **82**(3): 277-284.

Appendices

APPENDICES

Appendix I. Monthly record of air temperature, relative humidity, rainfall, and sunshine (average) of the experimental site during the period from August to December 2015

Month (2015)	Air temperature (^{0}c)		Relative humidity (%)	Rainfall (mm)	Sunshine (hr)
	Maximum	Minimum			
August	36.0	23.6	81	319	4.0
September	34.8	24.4	81	279	4.4
October	26.5	19.4	81	22	6.9
November	25.8	16.0	78	00	6.8
December	22.4	13.5	74	00	6.3

Source: Bangladesh Meteorological Department (Climate & weather division) Agargoan, Dhaka–1212*

Appendix II. Effect of sawdust substrate on mushroom on the yield attributes of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Number of primordial/packet	Number of fruiting body/packet	Number of effective fruiting body/packet	Weight of individual fruiting body (g)
T_1	217.5 a	115.5 a	29.02 a	5.60 a
T_2	202.2 d	89.65 c	19.69 c	4.81 d
T_3	205.5 c	100.5 b	24.02 b	5.38 b
T_4	207.9 b	98.31 b	20.35 c	4.94 c
T_5	192.5 e	79.65 d	17.02 d	4.39 e
$LSD_{(0.05)}$	2.195	3.038	1.956	0.115
CV (%)	0.60	1.79	5.17	1.60

Appendix III. Effect of sawdust substrate on proximate composition of oyster mushroom (*Pleurotus ostreatus*)

Treat--ment	Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	CHO (%)	Crude fiber (%)
T_1	88.82 c	8.96 c	25.26 ab	3.48cd	15.02 a	41.28b	20.94 a
T_2	87.77 d	10.01 b	24.15 b	4.47 a	13.02 b	40.21bc	18.11 c
T_3	88.98 b	8.82 d	25.04 ab	3.44 d	12.04 c	39.69 c	19.75 b
T_4	89.13a	8.64 e	25.53 ab	3.76 b	11.60 d	42.38 a	16.63 d
T_5	87.6e	10.18 a	26.04 a	3.69 bc	10.68 e	40.25bc	19.30 b
$LSD_{(0.05)}$	0.09	0.09	4.91	0.23	1.012	1.32	0.42
CV (%)	0 .026	0.515	10.012	3.319	5.412	1.709	1.185

Appendix IV. Plates

Plate 1: Preparing mycelium growth media packets

Plate 2: Mycelium growth packets on racks

Plate 3: Mycelium running complete in spawn packet

Plate 4: Matured fruiting body in the spawn packet

Plate 5: Taking biological yield in laboratory plate