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

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CERTIFICATE

This is to certify that the thesis entitled **'Effect of Different Sawdust on the Growth, Yield and Proximate Composition of White Oyster Mushroom (*Pleurotus ostreatus*)'** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE** in **BIOCHEMISTRY**, embodies the result of a piece of bonafide research work carried out by **Asma Begum**, Registration number: **08-03129** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Dated:
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DEDICATED
TO
MY BELOVED PARENTS

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

ABSTRACT

The study was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka during the period from August to December 2014 to study the effect of different sawdust on the growth, yield and proximate composition of white oyster mushroom (*Pleurotus ostreatus*). Mother culture of white oyster mushroom was collected in National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka. The experiment consists of six different type of sawdust with five replications. The experiment considered the following treatments: T_0: Mixture of all sawdust, T_1: Mango sawdust; T_2: Raintree sawdust; T_3: Segun sawdust; T_4: Gamari sawdust and T_5: Mahogony sawdust. The experiment was laid out in single factor Completely Randomized Design (CRD). The highest average number of fruiting body per packet (57.20) was observed in T_5, whereas the lowest (47.00) in T_2. The highest average weight of fruiting body (4.45 g) was observed in T_5 while the lowest (3.76 g) in T_4. The highest biological yield (227.68 g) was observed in T_5, while the lowest (204.78 g) in T_4. The highest economic yield (207.58 g) was observed in T_5, while the lowest (181.96 g) in T_4. The highest BCR (4.25) was observed in T_5, whereas the lowest (3.62) in T_4. The highest moisture content (87.77%) was observed in T_4, while the lowest (85.84%) in T_5. The highest dry matter content (14.16%) was observed in T_5, whereas the lowest (12.23%) in T_4. The highest protein content (24.97%) was observed in T_5, while the lowest (24.12%) in T_4. The highest lipid content (6.15%) was observed in T_5, while the lowest (5.72%) in T_4. The highest calcium content (17.33 mg/100 g) was observed in T_0, while the lowest (15.72 mg/100 g) in T_4. The highest magnesium content (14.50 mg/100 g) was observed in T_5, while the lowest (13.35 mg/100 g) in T_4. The highest iron content (47.46 mg/100 g) was observed in T_1, while the lowest (44.89 mg/100 g) in T_4.

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LIST OF ABBREVIATED TERMS

ABBREVIATION	FULL NAME
AEZ	Agro-Ecological Zone
et al.	and others
BBS	Bangladesh Bureau of Statistics
BCR	Benefit Cost Ratio
cm	Centimeter
^{0}C	Degree Celsius
etc	Etcetera
FAO	Food and Agriculture Organization
MoP	Muriate of Potash
m^2	Square meter
UNDP	United Nations Development Program
SAU	Sher-e-Bangla Agricultural University

CHAPTER I

INTRODUCTION

Mushroom usually umbrella like bodies belongs to the class Basidiomycetes and order Agaricales in fungal classification. It has been used as a food and medicine by different civilizations since immemorial time due to its delicious taste and dietetic qualities. Mushroom has qualities like lowering the blood cholesterol level, defense against cancer and invigorating hair growth. Kovfeen (2004) reported that the fresh mushroom contains about 85-90% moisture, 3% protein, 4% carbohydrates, 0.3-0.4% fats and 1% minerals and vitamins. *Pleurotus* species are very much effective in reducing harmful plasma lipids (Alam *et al.*, 2007) and thus reduce the chance of atherosclerosis and other cardiovascular and artery-related disorders. As a vegetable, mushroom can play an important role to meet up the nutritional requirements of the population of our country. It is also a highly nutritious, delicious, medicinal and economically potential vegetable.

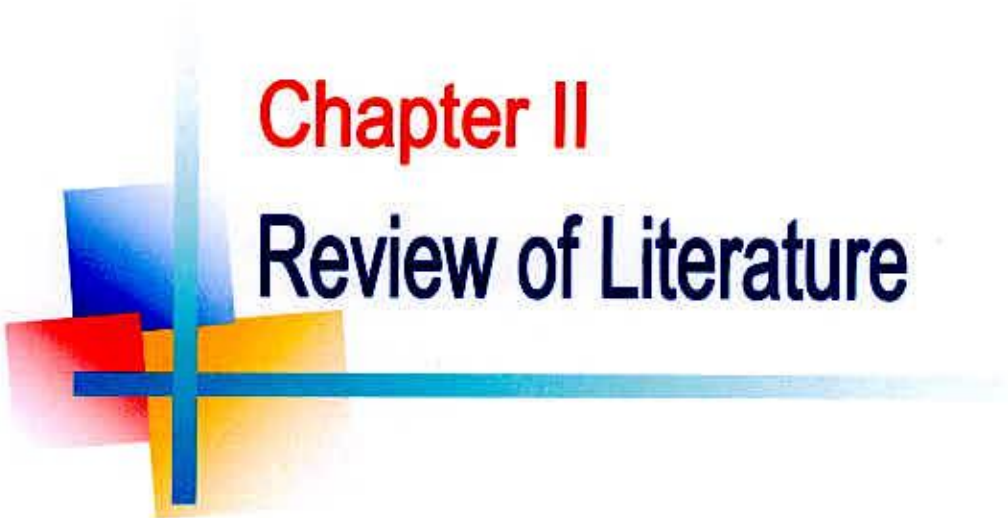
Bangladesh is a thickly populated country and we have to increase intensive use of land for increasing crop production considering natural resources. In this case mushroom cultivation can be a huge opportunity for increasing crop production per unit area with the vertical use of land. There are 2000 species of prime edible mushrooms of which about 80 have been grown experimentally and among them 20 species cultivated commercially and 4-5 species cultivated in industrial scale throughout the world (Chang and Miles, 1988). Only some species of mushrooms are now cultivated in Bangladesh and among this *Pleurotus florida* are popular and widely accepted after *Pleurotus ostreatus* (Amin *et al.*, 2007). The Greeks believed that mushrooms provided strength for warriors in battle. The Pharoaphs prized mushrooms as a delicacy and the Romans regarded mushrooms as the "Food of the Gods," which was served only on festive occasions. The Chinese treasured mushrooms as a health food, the "Elixir of life." The Mexican Indians used mushrooms as hallucinogens in religious ceremonies and in witchcraft as well as for therapeutic purposes (Chang and Miles, 1988).

Edible mushrooms have been treated as important tool in modern medicine for their medicinal values (Kovfeen, 2004). The low calorie and cholesterol free mushroom diets also display certain medicinal properties. Mushroom reduces the diabetic on regular feeding. It also reduces the serum cholesterol in human bodies which reduces hypertension (Suzuki and Oshima, 1979). It inhibits the growth of tumor and cancer (Mori, 1986). Oyster mushroom contains 19-35% protein on dry weight basis as compared to 7.3% in rice, 13.2% in wheat and 25.2% in milk (Chang and Miles, 1988). It contains 4.0% fat having good quantity of unsaturated fatty acids which are essential in our diet (Holman, 1976). Mushroom is rich in essential minerals and trace elements (Chandha and Sharma, 1995). It is the source of Niacin (0.3 g) and Riboflavin (0.4 mg). Mushroom is also a good source of trypsin enzyme. It is rich of low in calories, riches in vegetable proteins, iron, copper, calcium, potassium, zinc, chitin fibre and also contain vitamin D and folic acid. Mushrooms are valuable health food which is (Alam and Saboohi, 2001). Mushroom reduces serum cholesterol and high blood pressure (Mori, 1986).

There are various types of mushrooms such as oyster mushroom, milky white mushroom, button mushroom etc. Among them, several species of oyster mushroom are widely cultivated in our country. Oyster (*Pleurotus ostreatus*) mushrooms are the easiest and least expensive commercial mushrooms to grow because they are well known for conversion of crop residues to food protein (Banik and Nandi, 2004). Oyster mushroom is an edible mushroom having excellent fragrant and taste and its cultivation on crop residues is considered as potential source of income, an alternative food production, provision of employment, and for recycling of agricultural wastes. *P. ostreatus* possesses antitumor activity (Yoshioka *et al.*, 1985) and hypoglycaemic effects in experimentally induced diabetic rats (Chorvathova *et al.*, 1993). *Pleurotus sajor-caju* and *Pleurotus florida* are popular and widely accepted after *Pleurotus ostreatus* among the different species of mushrooms are now cultivated in our country (Amin *et al.*, 2007).

Different factors influence the growth, yield and proximate composition of mushroom. Among these factors substrate plays an important role in growth, yield and proximate composition 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.* (2007a) observed a remarkable variation in nutritional content of oyster mushroom in different substrates. The National Mushroom Development and Extension Centre (NAMDEC), Savar, grows oyster mushroom using sawdust. Bhuyan (2008) in his study observed that the proximate composition of oyster mushroom is greatly changed due to different supplement used in sawdust based substrates. If we use sawdust as substrate then expected yield performance may be achieved. But there are different type of sawdust which influences differently in respect to growth, yield and proximate composition of mushroom that is why it is necessary to find out the suitable sawdust for attaining maximum economic return in mushroom cultivation. Considering the above all circumstances the investigation was undertaken to fulfill the following objectives:

- To increase the yield of white oyster mushroom (*Pleurotus ostreatus*) by using suitable sawdust.
- To prepare suitable substrate based spawn packets.
- To know the physio-chemical characteristics of white oyster mushroom grown on different sawdust.
- To find out cost benefit ratio of suitable sawdust based spawn packets in cultivation of white oyster mushroom.



Chapter II

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Mushrooms grow well in waste materials have been considered as a special kind of food since the earliest time. There are many scientific reports on different aspects of mushroom cultivation especially different variety, pasteurization method, use of substrate etc. but still there are major scopes to investigate the effects of different sawdust as substrate to cultivate white oyster mushroom. The review includes reports of several investigators which appear pertinent in understanding the problem and which may lead to the explanation and interpretation of results of the present investigation. Several research works have been conducted in different parts of the world to improve the growth, yield and proximate composition of mushroom but the research work so far done in Bangladesh is not adequate and conclusive. Nevertheless, some of the important informative works and research findings in this respect at home and abroad have been reviewed below-

A study was carried out by Ashrafi *et al.* (2014) with aimed at utilizing this spent mushroom substrate (SMS) in a productive and sustainable way. Experiment was carried out to reuse of SMS of oyster mushroom for the production of oyster mushroom at Bangladesh Agricultural University (BAU), Mymensingh. Two mushroom species (*Pleurotus ostreatus* and *P. florida*) were grown on SMS supplemented with sawdust and wheat bran at different proportions. The results showed that SMS supplement with 60% sawdust +20% wheat bran demonstrated the highest biological yield, economic yield and biological efficiency for both species. Yield parameters were increased with increasing C/N ratio where as 36:1 C/N ratio exhibited the highest yield and the C/N ratio below or above 36:1 decreased yield of both species of oyster mushroom. The optimum C/N ratio for economic yield varied between the two oyster mushroom species and found to be 35.2 for *P. ostreatus* against C/N ratio of 40.1 for *P. florida*.

Sofi *et al.* (2014) carried out an investigation by using various grains for spawn production, and waste paper, wood chips were used in comparison with wheat husk for mushroom production at Department of Botany, Singhania University, Rajasthan. The results of the analysis of variance showed that diameter of colony extension in various grains are different and were significantly affected by substrate type. The maximum and minimum growth rates were seen in the corn and millet substrates, respectively. It is concluded that wheat straw in combination with wood chips are best substrate for oyster mushroom cultivation.

Romero *et al.* (2013) conducted an experiment to evaluate the production of the CP-50 of *Pleurotus ostreatus* in coffee bagasse dehydrated (Coffeaarabica) in relation to other agricultural waste of the Municipality of Tetela of Ocampo-Puebla, the coffee bagasse was collected in the zone of Cuahutempan, Puebla-Mexico and the other agricultural waste as they are: wheat straw (*Triticum aestivum*), straw of barley (*Hordeum vulgare*), bean straw (*Phaseolus vulgaris*) and Corn stubble (*Zea mays*). The CP-50 showed adequate growth of aerial mycelium in coffee bagasse dehydrated, reaching a production rate of 1.5+or-0.2%, the highest biological efficiency (EB) was obtained in the wheat straw substrate with 119.24+or-7.1%, in remainders of coffee bagasse dehydrated with 109.03+or-0.4% and corn stover obtained the lowest EB 77.47+or-0.2%.

Studies were undertaken by El-Sawy (2011) to find out the effect of water hyacinth (WH) and rice straw (RS) substrates and their mixtures (25% WH+75% RS, 50% WH+50% RS and 75% WH+25% RS) on yield, quality and chemical components of oyster mushroom (*Pleurotus ostreatus*). Results indicate that the three substrates mixtures of water hyacinth and rice straw significantly stimulated the fungal growth (decreased number of days from spawning till first flush) and increased early, late and total yields, total yield to weight of wet substrate (%), biological efficiency (%), number of fruit bodies, crude protein (%), total carbohydrates (%) and nitrogen content (%) of oyster mushroom fruiting bodies, when compared with the rice straw of water hyacinth alone, and that the lowest values were recorded with the WH alone. The highest values of these

measurements were obtained using the mixture of 75% WH and 25% RS, followed by the mixture of 50% WH and 50% RS and the mixture of 25% WH and 75% RS. The highest fruit body weight, cap weight, cap/fruit body (%), step length and cap diameter of early, late and total yields was also obtained using the mixture of 75% WH and 25% RS. Using WH alone significantly increased P and K contents of fruit bodies especially RS alone.

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. The highest protein content (30.90%), crude fiber (24.03%) and the lowest lipid (3.34%) were found in 10% cowdung.

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 were studied. 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. 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) were also observed in 30% level of wheat bran. The highest content of protein (30.31 %), ash (9.15 %) and crude fiber (24.07 %) and the lowest content of lipid (3.90 %) and carbohydrate (32.57 %) were recorded in 30% wheat bran.

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. 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 cowdung @ 10% (3.69g). The supplementation of sawdust with cowdung 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 cowdung @ 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 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%.

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.

Sangeetha (2007) carried out an experiment to study the effect of organic amendments on yield performance of pink mushroom. The organic amendments viz., groundnut cake powder, neem cake powder, rice bran and black gram powder were added at 3 and 5% levels to mushroom beds as amendments during cultivation. Neem cake at 5% level significantly increased the sporophore production (690.1 g) followed by 3% level (675.3 g). These treatments produce fruiting bodies earlier (10.8 to 11 days) than other amendments tried (11.1 to 12 days). Except neem cake powder and rice bran, all the other amendments had little effect on increasing the yield.

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).

Sarker *et al.* (2007a) carried out an experiment to find out the performance of different cheap agricultural household byproducts, grasses and weeds as substrate available in Bangladesh. 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 was positively correlated with biological efficiency, biological yield and economic yield of oyster mushroom. 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). No fruiting body and economic yield were obtained from para and nepier grasses. Performances of the substrates were compared based on benefit cost ratio (BCR). The highest BCR (6.50) was estimated when wheat straw was used as substrate followed by sugarcane bagasse (5.90), waste paper (5.65), rice straw (5.58) and kash (5.25) The lowest BCR was obtained from water hyacinth (1.05) followed by ulu (4.74) and sawdust (4.90).

Sarker *et al.* (2007b) 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.

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.*

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).

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%). 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.

Ancona-Mendex *et al.* (2005) conducted an experiment to grow oyster mushroom (*Pleurotus ostreatus* (Jacq.: Fr.) 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.

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.

Moni *et al.* (2004) cultivated the oyster mushroom (*Pleurotus sajor-caju*) on paddy straw, banana leaves, sugarcane baggase, water hyacinth and beetle nut husk. The fruit bodies were sun-dried and analyzed for various nutritional parameters. Considerable variation in the composition of fruit bodies grown on different substrates was observed. Moisture content varied from 88.15 to 91.64%. On dry matter basis, the percentage of nitrogen and crude protein varied from 4.22 to 5.59 and 18.46 to 27.78%, respectively and carbohydrate from 40.54 to 47.68%. The variation in content of crude fat and crude fiber ranged from 1.49 to

1.90 and 11.72 to 14.49% respectively whereas, energy value of fruit bodies was between 310.00 and KCal/100 g of fruit body weight.

Banik and Nandi (2004) carried out an experiment on oyster mushroom for its ease of cultivation, high yield potential as well as its high nutritional value. Laboratory experimentation followed by farm trial with a typical oyster mushroom *Pleurotus sajor-caju* revealed that the yield potential of these mushrooms can be increased significantly when grown on a lignocellulosic crop residue - rice straw supplemented with biogas residual slurry manure in 1:1 ratio as substrate. Residual slurry manures obtained from biogas plants utilising either cattle dung or poultry litter, jute caddis or municipal solid waste as substrates for biogas production were all effective in increasing the yield of *Pleurotus sajor-caju* significantly although to different extents. Disinfection of straw and manure by means of 0.1 % $KMnO_4$ plus 2 % formalin solution in hot water caused 42.6 % increase in yield of *Pleurotus sajor-caju* over control, i.e., when disinfection done with hot water. In addition to increased yield, the above treatments caused significant increase in protein content, reduction in carbohydrate and increase in essential mineral nutrients in mushroom sporophores. Thus, it is concluded from the study that supplementation of rice straw with biogas residual slurry manure has strong impact in improving the yield potential, protein and mineral nutrient contents of *Pleurotus sajor-caju* mushroom in Indian subcontinent or similar climatic conditions.

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.

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. Based on the yield and BE of the substrates tested, Rice straw appeared to be the best alternate substrate for growing oyster mushroom.

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.

Shen and Royse (2001) evaluated the effects of various, combinations of wheat bran, rye and millet (At 20% and 30% of total dry substrate Wt) on crop cycle time, biological efficiency (BE) and mushroom quality for a commercially used isolate of *Grifola frondoso* (maitake). Supplements were combined with a basal ingredient of mixed oak (primarily red oak) sawdust and the resulting mixture was pasteurized, cooled, inoculated and bagged with an autoclaving mixer. Times to mushroom primordial formation and mushroom harvest were recorded, and mushroom quality was rated on a scale of 1-4, where 1 was the highest quality and 4 was the lowest quality. The combinations of 10%, wheat bran, 10% millet and 10% rye (BE 47.1%, quality 1.5 and crop cycle 12 weeks) and 10% wheat bran plus 20% rye (BE 44%, quality 1.7 and crop cycle 10 weeks) gave the most consistent yields and best basidiome quality over time.

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.

Dhoke *et al.* (2001) studied the effect of different agro-wastes on cropping period and yield of *Pleurotus sajor-caju* the experiments carried out in Prabhani and Maharashtra in India, during 1998-99. Various plant materials, i.e. soybean, paddy, cotton, wheat and jowar (*Sorghum bicolor*) were used. Cropping period on different substrates was recorded for first, second and third picking. The cropping period for third picking varied from 42.25 to 43.50 days in different substrates. The days required for first picking indicated that soybean straw took 22.00 days to produce first crop of harvestable mushroom while a minimum of 21.25 days were required for paddy and wheat straw. For second picking, jowar and cotton waste took the maximum days of 32.75 days while soybean took the minimum of 31.50days. The final and third picking was completed in 43.50 days in case of soybean straw which was statistically higher compared to paddy and wheat straw (42.25) and cotton and jowar straw (42.75). The highest yield of 993.00 g/kg was obtained from cotton, followed by soybean straw (935.25 g/kg) and paddy straw (816.0 g/kg). The lowest yield of 445.50 g/kg was recorded in jowar straw.

Khan *et al.* (2001) investigated the different aspects of the cultivation of Oyster mushroom on industrial wastes to push it as a new biotechnology and as a commercial crop in Pakistan. They found that after spawn running, pinhead formation took 7-8 days and sporocarps formed after 10-12 days. Cotton waste recorded the highest yield of 198.67 g. Wheat straw yielded 129.253 g, paper waste + wheat straw yielded 58.95 g and paper waste alone recorded no yield. The best mycelium growth was observed in cotton waste substrate. The average time taken for complete spawn running was 17 days. The second best mycelium

growth was on wheat straw, where the average time for spawn running was 19 days. In paper waste, the average time for spawn running was 22 days. However, the average time taken for completion of spawn running on paper waste + wheat straw was 20 days. The differences among the phase of mycelium growth and their interaction with substrate were statistically significant.

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.

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.

Rathaiah and Shill (1999) in their experiment found that parboiled paddy was as good as wheat for spawn production of oyster mushroom. The spawn prepared from parboiled paddy was also compared with conventionally prepared paddy spawn. The suitability of parboiled paddy for spawn of paddy straw mushroom (*Volvariella volvacea*) was also confirmed.

Patil and Jadhav (1999) reported that *Pleurotus sajor-caju* was cultivated on cotton, wheat, paddy, sorghum and soyabean straws in Marathwada, India. Cotton stalks + leaves was the best substrate for production (yield of 1039 g/kg dry straw), followed by soyabean straw (1019 g/kg). Paddy and wheat straw yielded 650 and 701g/kg. The lowest yield (475 g/kg) was obtained on sorghum straw. Pileus size and stipe length of *P. sajor-caju* were greatest on sorghum straw.

Zhang-Ruihong *et al.* (1998) cultivated oyster mushroom (*P. sajor-caju*) on rice and wheat straw without nutrient supplementation. The effects of straw size reduction methods and particle sizes spawn inoculation level and types of substrate (rice straw vs. wheat straw) on mushroom yield, biological efficiency and substrate degradation were determined. The protein content of mushrooms produced was 27.2% on an average. The dry matter loss of the substrate after mushroom growth varied from 30.1 to 44.3%. Yields were higher from substrates which had been ground-up to 2.5 cm lengths; further size reductions lowered yields. Mushroom cultivation is a highly efficient method for disposing of agricultural residues as well as producing nutritious human food.

Pani and Mohanty (1998) used water hyacinth alone and in combination with paddy straw (3:1, 1:1 and 1:3 ratios) for cultivation of *Pleurotus sajor-caju* and *P. Florida*. Paddy straw alone sustained highest mushroom yield (83.3-84.6% BE). Water hyacinth in combination with paddy straw produced higher yields than when used alone.

Chowdhury *et al.* (1998) examined the effects of adding rice husks, soybean meal, pea meal, wheat bran, poultry manure or neem cake (each at 2 or 5%) to rice straw for growing oyster mushrooms (*P. sajor-caju*). Adding 5% soybean or pea meal gave the highest yield of 630 g/kg dry straw.

Patrabansh and Madan (1997) used three different kinds of biomass, namely *Pofulus deltoides*, *Isuhatoriium adenophorum* and sericulture waste individually for the cultivation of *Pleurotus sajor-caju*, alone and mixed with paddy straw. *P. sajor-caju*, when used alone, exhibited a very good colonizing ability on these substrates except in sericulture waste.

Kalita *et al.* (1997) studied the growth of *Pleurotus sajor-caju* in polyethylene bag on different combinations of substrates viz. only rice straw, rice straw plus rice husk mixture (1:1 v/v), water hyacinth, chopped banana leaves, areca nut husk and sugarcane bagasse. They found that only rice straw, rice straw plus rice husk

mixture and areca nut husk substrates completed spawn running comparatively within short time (12-14 days) but other substrates took longer time.

Biswas *et al.* (1997) reported that methods including spawning percentage, combinations of paddy straw, wheat straw and supplements, to improve the biological efficiency (BE) of *P. florida* were investigated in Madhya Pradesh, India. Increasing spawning rates reduced the time required for spawn runs. The highest BEs (66.8-101.25%) was observed after the use of the highest spawning percentages. A 1:1 mixture of paddy straw wheat straw promoted a high BE (106.5%); supplementation of this substrate with 5% rice flour also promoted BE (125.75%).

Ragunathan *et al.* (1996) investigated that the fruiting bodies of oyster mushroom were rich in nutrients such as carbohydrate, protein, amino nitrogen and minerals and low fat content. The moisture content of the fruiting bodies ranged from 84.70 to 91.90 % and the carbohydrate content ranged from 40.6 to 46.3 %, the crude protein content ranged from 31.9 to 42.5 %, 26.92 to 38.8%, and 30.0 to 42.5% in *Pleurotus sajor-caju*, *Pleurotus platypus* and *Pleurotus citrinopileatus*, respectively.

Mathew *et al.* (1996) investigated that *Pleurotus sajor-caju*, *Pleurotus* citrinopileatus, *Pleurotus florida*, *Pleurotus platypus* and *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. *Pleurotus sajor-caju*, *Pleurotus citrinopileatus* and *Pleurotus florida* were the most suitable species for cultivation in both the plains and the high ranges. These 3 species were successfully cultivated on paddy straw, *Eliocharis plantogena* [*Eleocharis plantaginea*] and rubber wood [Hevea] sawdust, although for commercial cultivation of *Pleurotus sajor-caju*, rubber wood sawdust was not rated as an ideal medium.

Jadhav *et al.* (1996) reported that oyster mushroom (*Pleurotus sajor-caju*) was cultivated on wheat straw, paddy straw, talks and leaves of maize or cotton, jowar, soyabean straw, groundnut creepers plus wheat straw (1:1), soyabean straw plus groundnut creepers (1:1), or groundnut creepers alone. Cotton stalks and leaves gave the best results with respect to sporophore number, weight of sporophore (5.12 g) and total yield (914 g/kg of dry straw). Yields obtained on other substrates were: 796 g on paddy straw; 557 g on soyabean straw; and 508 g on soyabean + wheat straw. The lowest yield was recorded on groundnut creeper (258 g).

Singh *et al.* (1995) reported that the *Pleurotus florida* was cultivated on wheat straw, paddy straw and sugarcane trash (dried leaves) used either separately or in 1:1 ratio, yield and biological efficiency were the highest in paddy straw. The effects of different forest wastes on the radial growth of *Lentinus edodes* Berk were studied. Three types of sawdust from Shishum (*Dalbergia sisso*) 'Kikar' (*Acacia arabica*) and Poplar (*Populus alba*) amended with wheat bran and lime were used for spawn preparation.

Patra and Pani (1995) mentioned that five species of *Pleurotus* were cultivated in polythene [polyethylene] bags containing chopped paddy straw (2 kg) + spawn (200 g) + boiled wheat (200 g). Highest yield was observed in *P. Florida*, followed by *P. sajor-caju*, *P. citrinopileatus*, *P. sapidus* and *P. flabellatus*. The fungi took 13-I6 days for complete mycelial run in the bags and 20-24 days for initiation of fruiting bodies. *P. sajor-caju* produced the heaviest fruiting bodies (12.2 g) and *P. citrinopileatus* the lightest (6.9 g).

Murugesan *et al.* (1995) cultivated mushroom P. *sajor-caju* (Fr.) Sing, on water hyacinth (*Elchhorni crassipe*). They compared water hyacinth with other conventional substrates paddy straw. Total yields for 20 bags of the two substrates were 15.0 and 10.5 kg respectively, although the time taken to reach the pin-head stage was longer on the water hyacinth substrate (17 days in water hyacinth and 10 days in paddy straw). The high yield on water hyacinth was attributed to the C:

N ratio (24.3 compared with 53.5) and low lignin content (9% compared with 17%) of this substrate. Use of water hyacinth would provide a cheap substrate and a means of eradicating a troublesome aquatic weed.

Isik *et al.* (1995) conducted an experiment to find out the best preparation formulas of horse manure and synthetic compost. Horse manure, wheat straw, gypsum as basic materials and wheat bran, cotton seed meal, sunflower meal, malt sprout, chicken food, molasses, ammonium sulphate, urea as activators were used. The nitrogen content of the starting mixture was brought up 2 in all applications. According to the results, the highest yields with horse manure compost were obtained from the combinations of 1000 kg of horse manure, 50 kg of wheat bran, 3.1 kg of ammonium sulphate, 1.5 kg of urea, 35 kg of gypsum 40 kg of chicken food or malt sprout.

Marimuthu *et al.* (1994) investigate *Pleurotus sajor-caju*, *P. citrinopileatus* and *P. platypus* on paddy straw were tested for their response to substrate amendment with neem cake, rice bran, wheat bran and tapioca thippi (Factory waste). Neem cake at 5% level increased the yield of *P. citrinopileatus, P. sajor-caju* and *P. pathypus* by 26-49, 24-79 and 16% respectively and reduced the number of days required for completion of spawn run by 2-6, 5 and 6 days, respectively compared with control.

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.

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.

Royse *et al.* (1991) found that yields of *Pleurotus sajor-caju* strain 537 from the substrate supplemented with the commercial nutrient were 1.7-fold higher than yields from non-supplemented substrate. As the supplement level increased from 6 to 12%, the mushroom yields increased.

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.

From the review of literature illustrated above, it may be concluded that the growth, yield and proximate composition of mushroom were greatly influenced due to different sawdust. Therefore, the present study will be carried out to find out the comparative study of different sawdust on the growth, yield and proximate composition of mushroom.

Chapter III

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The study was conducted during the period from August to December 2014 to study effect of different sawdust on the growth, yield and proximate composition of white oyster mushroom (*Pleurotus ostreatus*). The chapter includes a brief description of the location of experimental site and climate condition, materials used for the experiment, design of the experiment, preparation of substrates, preparation of packets, cultivation of spawn packet, collection of produced mushrooms, proximate analysis of the mushrooms, data collection and data analysis procedure. The details materials and methods are presented below under the following headings-

3.1 Experimental site

The experiment was conducted at the laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka. Details of the meteorological data during the period of the experiment was collected from the Bangladesh Meteorological Department, Agargoan, Dhaka and presented in Appendix I.

3.2 Planting materials

Mother culture of white oyster mushroom was collected from National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka.

3.3 Varietal characteristics of White Oyster Mushroom

White oyster mushroom is *Pleurotus ostreatus* is a whitish to light cream colored cap depending upon the strain and growing conditions. Primordia and young mushrooms are bright white but become less intensely colored as the mushroom matures. White oyster mushroom is characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. Their fruiting bodies are shell or spatula shaped with white color. If the temperature increases above 32^0C, its production markedly decreases.

3.4 Treatment of the experiment

The experiment considered the following treatments:

T_0: Mixture of all sawdust

T_1: Mango sawdust

T_2: Raintree sawdust

T_3: Segun sawdust

T_4: Gamari sawdust

T_5: Mahogony sawdust

3.5 Design and layout of the experiment

The experiment was laid out in single factor Completely Randomized Design (CRD). The experiment considered to six treatments with five replications and three spawn packets in each replication

3.6 Preparation of substrates

At first weight of dry sawdust of mango, raintree, segun, gamari, mahogany and mixture of all sawdust was taken. Then the sawdust, 30% wheat bran and 1% $CaCO_3$ were mixed. 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 65%.

3.6.1 Preparation of spawn packets

The mixed substrates were filled into 7×10 inch polypropylene bag @ 500 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.

3.6.2 Sterilization, inoculation and mycelium running in spawn packets

The spawn packets were sterilized about 1 hour and then these were kept for cooling. After cooling, 5 g mother spawn was inoculated into the spawn packets

in the laminar airflow cabinet and the packets were kept at 20-22^{0}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. Then these spawn packets were transferred to the culture house.

3.6.3 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 tea spoon 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 newspaper. 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^{0}C to 25^{0}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.

3.6.4 Harvesting of mushrooms

White 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.

3.7 Data collection

3.7.1 Mycelial growth

Mycelial growth was counted by taking the full packet as a full unit and generally the data was taken at every two days intervals.

3.7.2 Time required to complete mycelium running

Days required from inoculation to completion of mycelium running were recorded.

3.7.3 Mycelium running rate in spawn packet

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):

$$\text{MRR} = \frac{L}{N} \text{ cm/day}$$

Where,

L= Average length of mycelium running (cm)

N= Number of days

3.7.4 Time from stimulation to primordia initiation

Days required from stimulation to primodia initiation were recorded.

3.7.5 Average number of fruiting body per packet

Number of well-developed fruiting body of specific species of oyster mushroom was recorded. Dry and pinheaded fruiting bodies were discarded but tiny fruiting bodies were included in counting.

3.7.6 Average 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 of specific species of oyster mushroom.

3.7.7 Dimension of fruiting body (stipe and pileus)

Length of the pileus of three randomly selected fruiting bodies was measured using a slide calipers. Diameter of stipe, diameter and thickness of pileus were also measured.

a. Length of stipe (cm)

b. Diameter of stipe (cm)

c. Diameter of pileus (cm)

d. Thickness of pileus (cm)

3.7.8 Biological yield

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

3.7.9 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.

3.7.10 Drying of mushrooms

The collected fruiting bodies of the mushroom were transferred to the laboratory. Then 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.

3.7.11 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 72^0C 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):

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

3.7.12 Biological efficiency

Biological efficiency was determined by the following formula:

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

3.7.13 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).

3.7.13 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 water was sprayed regularly. The primordia 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. Similarly three harvest were taken per 1 spawn packet.

3.8 Proximate analysis of the mushrooms

3.8.1 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. Then the samples were ready to be found approximate composition.

3.8.2 Determination of Moisture

About 10-20 g of each sample were weighed into separated and weighed petridishes and dried in an oven at 100^0C to 105^0C till the weight of the petridishes with their contents was constant. Then the constant weight was subtracted from the fresh weight to determine moisture content. The moisture content was expressed as percent of the fresh fruiting bodies.

3.8.3 Determination of dry matter

A clean container (dish or beaker) was place in an oven at 105^0C overnight. The container was allowed to cool in a desiccator and was weighed. The sample was kept into the container and weighed with the sample. The container was placed in the oven at 105^0C for 24 hours. The container was allowed to cool in a desiccator and was weighed. Again, the container was placed in the oven at 105^0C for 2 hours. It was cooled in a desiccator and weighed again. Drying, cooling and

weighing were repeated until the weight became constant. The dried sample was stored in an airtight container. The constant weight of dry sample is known as dry matter.

3.8.4. 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 105^0C overnight.

3.8.5 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 desiccator 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 (Raghuramulu *et al.*, 2003):

$$\text{Ash content (g/100 g sample)} = \frac{\text{Weight of ash}}{\text{Weight of sample taken (g)}} x100$$

3.8.6 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 Moslin cloth and the residue was washed with hot water till it was free from acid. The material was then transferred to the same beaker and 200 ml of boiling 0.313 N NaOH was added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a Moslin cloth and the residue was washed with hot water till it was 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 (Raghuramulu *et al.*, 2003).

Therefore,

Crude fiber (g/100 g sample) = [100-(moisture + fat)] x (We-Wa)/Wt. of sample.

3.8.7 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).

3.8.8 Determination of protein

The Protein contents of the fruiting bodies of the mushrooms were determined by the standard Micro-kjeldhal procedure. According to this method total nitrogen contents of the samples were estimated and protein contents calculated by multiplying by 6.25 to the total nitrogen values. The total nitrogen was determined by the Kjeldahl methods, which depends upon the conversion of protein nitrogen into ammonium sulfate, by digestion ammonia liberated from the ammonium sulfate by making the solution alkaline were distilled into known volume of a standard acid, which was then back titrated.

Reagents

a) Concentrated sulfuric acid

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 2.0 g, 5.0 g 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

$$\text{Percentage of nitrogen} = \frac{(A-B)\times 14\times 100}{W\times 1000}$$

Where A = ml of NaOH required in the titration of blank

B = ml of NaOH required in the titration of sample

N = Normality of the NaOH

W = Weight of the sample

The protein content in gram per 100 g of the dried sample

$$= \frac{\text{Percentage of nitrogen} \times 6.25 \times D}{100}$$

Where, D = Percentage of dried sample from the fresh sample

3.8.9 Total fat estimation

Fat was estimated as crude ether extraction of the dry materials. The dried sample (about 5.0 g) was weighed 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 80^0C to 100^0C, cooled in a dessicator and weighed. The result was expressed as follows:

Fat contents (g) per 100 g of dried sample =

$$\frac{\text{Weight of ether extract} \times \text{Percentage of dried sample}}{\text{Weight of the dried sample taken}}$$

3.9 Estimation of minerals

3.9.1 Equipments

For analysis of elementary composition analysis the equipments used were electric balance, desiccators, atomic absorption spectrophotometer (AAS), spectrophotometer, porcelain crucible, beaker and flame photometer etc.

3.9.2 Determination of Ca, Mg, K, Fe, S, Zn and P

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

3.9.2.1 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 acid 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.
2. After cooling, the digestion mixture was transferred with distilled water to a 100 ml volumetric flask water added 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, S, Cu and P were determined from the filtrate.

3.9.2.2 Estimation of Ca

20 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette. 5 ml $LaC1_3$-solution was added and the volume was made with water and mixed. Then the content of Ca was measured by atomic absorption spectrophotometer (AAS).

3.9.2.3 Estimation of Mg

20 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette. 5 ml $LaC1_3$-solution was added and the volume was made with water and mixed. Then the content of Mg was measured by AAS.

3.9.2.4 Estimation of K

10 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette to volume with water and mixed. The content of K was measure by flame photometer.

3.9.2.5 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 mixed and volume was made with water. After 15 minutes, the absorbance was measured on a spectrophotometer at 890 nm.

3.9.2.6 Estimation of Fe and Zn

The content of Fe and Zn elements were measured by atomic absorption spectrophotometer (AAS) directly in the undiluted filtrate.

3.9.2.7 Calculations: For Ca, Mg, K, P

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

Where, a= mg/L Ca, Mg, K or P measured on atomic absorption spectrophotometer, 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 Zn and Fe

$$\text{mg per kg sample} = \frac{d \times 100}{c}$$

Zn and Fe measured on atomic absorption spectrophotometer

c = g sample weighed into the digestion tube

3.9.2.8 Determination of total sulphur

Organic matter is destructed and sulphur is oxidized to sulphate by digestion with a mixture of nitric and perchloric acid. The sulphate is determined by precipitation as barium sulphate using the following formula.

$$\% S = \frac{A \times 1374}{M \times W} \qquad \% SO_3 = \% S \times 2.50$$

Where,

A = weight of $BaSO_4$ g

M = amount of soln. transferred to beaker for precipitation of $BaSO_4$ (ml)

W = weight of sample in g

3.10 Statistical analysis

The data obtained for different parameters were statistically analyzed to find out the significance of the difference among the treatment. The mean values of all the characters were evaluated and analysis of variance was performing by the 'F' test. All the data collected on different parameters were statistically analyzed by Duncan's Multiple Range Test (DMRT). The significance of the difference among the treatments means was estimated by the least significant difference (LSD) test at 5% level of probability (Gomez and Gomez, 1984).

Chapter IV

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

The study was conducted to study effect of different sawdust on the growth, yield and proximate composition of white oyster mushroom (*Pleurotus ostreatus*). The results have been presented and discussed with the help of table and graphs and possible interpretations given under the following headings:

4.1 Growth and yield contributing characters

4.1.1 Mycelium running rate

Mycelium running rate of white oyster mushroom showed statistically significant variation due to different sawdust use (Table 1). The highest mycelium running rate (0.76 cm) was observed in T_5 (Mahogony sawdust) which was statistically similar (0.75 cm and 0.70 cm) to T_1 (Mango sawdust) and T_0 (Mixture of all sawdust), respectively and closely followed (0.68 cm) by T_3 (Segun sawdust), while the lowest mycelium running rate (0.63 cm) was found in T_4 (Gamari sawdust) which was statistically similar (0.64 cm) to T_2 (Raintree sawdust). Different sawdust showed different mycelium running because of different carbohydrate based on availability and the environment of the spawn. Mycelium running rate varied due to use of different sawdust. Statistically highest mycelium running rate was observe in Mahogony sawdust followed by Mango sawdust and mango sawdust and mixture of all sawdust which was statistically similar to the olive segun and followed by gamari sawdust. 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.

Table 1. Effect of different sawdust on growth and yield contributing characters of white oyster mushroom (*Pleurotus ostreatus*)

Treatments	Mycelium running rate in spawn packets (cm)	Time required to complete mycelium running	Average number of fruiting body per packet	Average weight of individual fruiting body (g)
T_0	0.70 ab	18.60 abc	50.60 bcd	4.21 b
T_1	0.75 a	18.20 c	54.00 ab	4.42 a
T_2	0.64 c	19.20 a	47.00d	3.87 cd
T_3	0.68 bc	18.40 bc	51.60 bc	4.03 c
T_4	0.63 c	19.00 ab	48.00 cd	3.75 d
T_5	0.76 a	18.40 bc	57.20 a	4.45 a
LSD (0.05)	0.058	0.608	4.016	0.165
CV(%)	6.55	7.69	5.99	3.04

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability

T_0: Mixture of all sawdust

T_1: Mango sawdust

T_2: Raintree sawdust

T_3: Segun sawdust

T_4: Gamari sawdust

T_5: Mahogony sawdust

4.1.2 Time required to complete mycelium running

Statistically significant variation was recorded in terms of time required to complete mycelium running for different sawdust (Figure 1). The maximum time required to complete mycelium running (19.20 days) was observed in T_2 which was statistically similar (19.00 days and 18.60 days) with T_4 and T_0, respectively, whereas the minimum time required to complete mycelium running (18.20 days) was found in T_1 which was statistically similar (18.40 days) to T_3 and T_5. The result of the present finding was found similar with Gupta (1989); Khan *et al.* (2001); Amin *et al.* (2007). Sarker (2004) observed that duration in primordia initiation of oyster mushroom was significantly lower as compared to control i.e. no supplement was used.

4.1.3 Time required to stimulation of primordia initiation

Time required to stimulation of primordia initiation varied significantly due to different sawdust (Figure 2). The maximum time required to stimulation of primordia initiation (4.00 days) was observed in T_4 which was statistically similar (3.60 days and 3.40 days) to T_2, T_3 and T_0, respectively, whereas the minimum time required to stimulation of primordia initiation (2.60 days) was found in T_5 which was statistically similar (2.80 days) to T_1. The result of the present finding was found similar with Gupta (1989); Khan *et al.* (2001) and Amin *et al.* (2007). Sarker (2004) observed that duration in primordia initiation of oyster mushroom was significantly lower as compared to control i.e. no supplement was used.

4.1.4 Average number of primordia per packet

Average number of primordia per packet of white oyster mushroom showed statistically significant variation due to different sawdust (Table 1). The highest average number of primordia per packet (75.20) was observed in T_5 which was statistically similar (73.60 and 71.20) to T_1 and T_3, respectively, while the lowest average number of primodia per packet (65.80) was found in T_2 and T_4 which was statistically similar (69.60) to T_0. Dey (2006) found that the number of primordia and the average yield of oyster mushroom give the lowest value with sawdust.

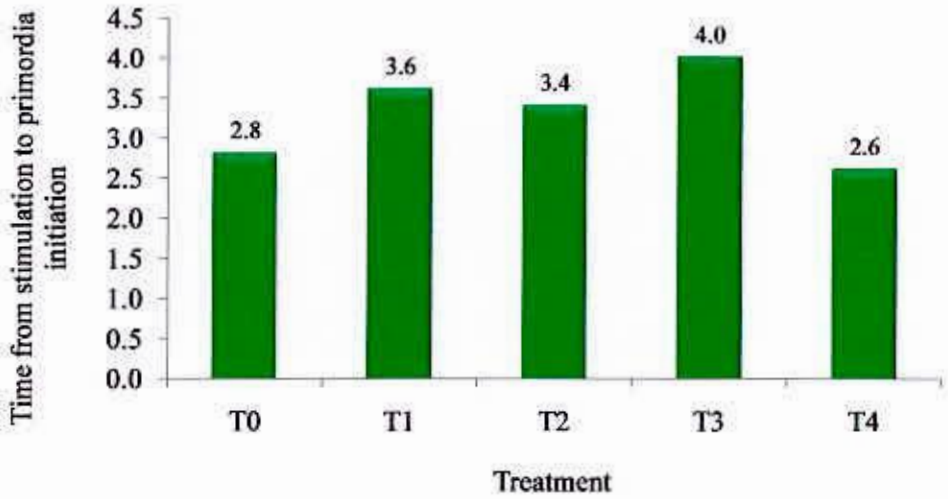


Figure 1. Effect of different sawdust on time from stimulation to primordia initiation of white oyster mushroom

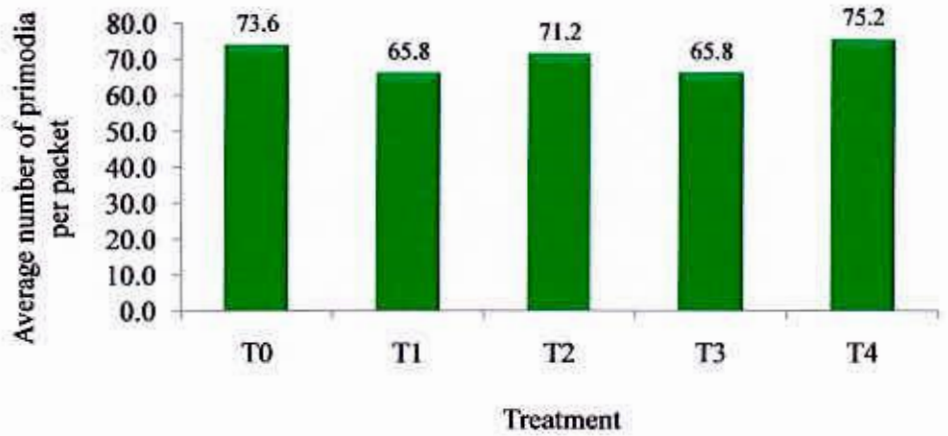


Figure 2. Effect of different sawdust on average number of primodia per packet of white oyster mushroom

T_0: Mixture of all sawdust
T_1: Mango sawdust
T_2: Raintree sawdust
T_3: Segun sawdust
T_4: Gamari sawdust
T_5: Mahogony sawdust

4.1.5 Average number of fruiting body per packet

Statistically significant variation was recorded in terms of average number of fruiting body per packet for different sawdust (Table 1). The highest average number of fruiting body per packet (57.20) was observed in T_5 which was statistically similar (54.00) to T_1 and closely followed (51.60) by T_3, whereas the lowest average number of fruiting body per packet (47.00) was found in T_2 which was statistically similar (48.00 and 50.60) to T_4 and T_0. This variation might be due to variation among the sawdust. The result of the present study found similar with the previous findings of Yoshida *et al.* (1993); 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. Sarker (2004) found that the number of fruiting body increased with the levels of supplement and continued up to a certain range and decline there after. Bhuyan (2008) found similar results in his experiment.

4.1.6 Average weight of individual fruiting body

Average weight of individual fruiting body of white oyster mushroom showed statistically significant variation due to different sawdust (Table 1). The highest average weight of fruiting body (4.45 g) was observed in T_5 which was statistically similar to T_1 (4.42 g) and closely followed by T_0 (4.21 g), while the lowest average weight of individual fruiting body was found in T_4 (3.76 g) which was statistically similar (3.87 g) to T_2. The findings of this experiment were also supported by Sarker *et al.* (2007) and Bhuyan (2008). Sarker (2004) found significant increase in weigh of fruiting body in gram per sporocarps over control in spawn packet containing different supplement in compared with sawdust alone. Bhuyan (2008) found comparatively higher weigh of individual fruiting body ranged in (5.02g to 7.01g).

4.1.7 Length of stipe

Statistically significant variation was recorded in terms of length of stripe for different sawdust (Table 2). The highest length of stripe (2.69 cm) was observed in T_1 which was statistically similar to T_5 (2.63 cm) and closely followed by T_0 (2.46 cm) and T_3 (2.40 cm), respectively, while the lowest length of stripe (2.13 cm) was found in T_4 which was statistically similar (2.20 cm) to T_2. Ahmed (1998) reported significant effects of various substrates on length of stalk. Habib (2005) found that the length of stipe of oyster mushroom on different substrates varied in 1.93cm to 2.97cm.

4.1.8 Diameter of stipe

Diameter of stripe showed statistically significant variation for different sawdust (Table 2). The highest diameter of stripe (1.15 cm) was observed in T_5 which was statistically similar (1.14 cm) to T_1 and closely followed (1.08 cm) by T_3, whereas the lowest diameter of stripe (1.01 cm) was found in T_4 which was statistically similar (1.02 cm) to T_2. Ahmed (1998) reported significant effects of various substrates on diameter of stalk. Habib (2005) found that stipe of oyster mushroom on different substrates varied in 0.74 cm to 1.05 cm.

4.1.9 Diameter of pileus

Statistically significant variation was recorded in terms of diameter of pileus for different sawdust (Table 2). The highest diameter of pileus (6.88 cm) was observed in T_5 which was statistically similar (6.83 cm) to T_1 and closely followed (6.70 cm) by T_0, while the lowest diameter of pileus (6.37 cm) was found in T_4 which was statistically similar (6.42 cm) to T_2. Ahmed (1998) reported significant effects of various substrates on diameter 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 in mango sawdust. Habib (2005) found that the diameter of pileus ranged in 4.85 cm to 8.95 cm.

Table 2. Effect of different sawdust on the dimension of fruiting body of white oyster mushroom (*Pleurotus ostreatus*)

Treatments	Length of stipe (cm)	Diameter of stipe (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_0	2.46 b	1.09 b	6.70 b	0.75 b
T_1	2.69 a	1.14 a	6.83 a	0.80 a
T_2	2.20 c	1.02 c	6.42 cd	0.69 cd
T_3	2.40 b	1.08 b	6.53 c	0.73 bc
T_4	2.13 c	1.01 c	6.37 d	0.68 d
T_5	2.63 a	1.15 a	6.88 a	0.82 a
LSD (0.05)	0.154	0.041	0.124	0.413
CV(%)	4.96	3.55	1.41	5.18

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability

T_0: Mixture of all sawdust

T_1: Mango sawdust

T_2: Raintree sawdust

T_3: Segun sawdust

T_4: Gamari sawdust

T_5: Mahogony sawdust

4.1.10 Thickness of pileus

Thickness of pileus showed statistically significant variation due to different sawdust (Table 2). The highest thickness of pileus (0.80 cm) was observed in T_1 which was statistically similar to T_5 (0.82 cm) and closely followed (0.75 cm and 0.73 cm) by T_0 and T_3, while the lowest thickness of pileus (0.68 cm) was found in T_4 which was statistically similar (0.69 cm) to T_2. Ahmed (1998) reported significant effects of various substrates on thickness of pileus. He found that lower thickness of pileus produced the lowest yield and concluded that the thickness of pileus increased with the quality and yield of mushroom. Habib (2005) found that thickness of the pileus ranged in 0.45cm to 0.70 cm due to different substrates.

4.1.11 Biological yield

Statistically significant variation was recorded in terms of biological yield of mushroom for different sawdust (Table 3). The highest biological yield (227.68 g) was observed in T_5 which was statistically similar to T_1 (227.03 g), T_0 (226.97 g) and T_3 (219.51 g) and closely followed (212.24 g) by T_2, while the lowest biological yield (204.78 g) was found in T_4 which was statistically similar (212.24 g) to T_2. Chowdhury *et al.* (1998) examined the effects of adding different supplements to substrates for growing oyster mushrooms (*Pleurotus sajor-caju*) and found adding 5% supplements gave the highest yield of oyster mushroom. Dhoke *et al.* (2001) found significant effect of different agro-wastes on yield of oyster mushroom. Baysal *et al.* (2003) found the highest yield of Oyster mushroom (*Pleurotus ostreatus*) with the substrate composed of 20% rice husk in weigh.

Table 3. Effect of different sawdust on the yield, biological efficiency and benefit cost ratio of white oyster mushroom (*Pleurotus ostreatus*)

Treatments	Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)	Benefit cost Ratio
T_0	226.97 a	199.55 abc	18.07 ab	129.69 a	3.93 bc
T_1	227.03 a	206.42 ab	18.85 a	129.73 a	4.18 ab
T_2	212.24 bc	189.40 cd	17.51 b	121.28 c	3.71 cd
T_3	219.51 ab	194.98 bc	17.93 ab	125.43 b	3.76 cd
T_4	204.78 c	181.96 d	17.26 b	117.01 d	3.62 d
T_5	227.68 a	207.58 a	18.88 a	130.10 a	4.25 a
LSD (0.05)	11.84	10.91	1.276	2.232	0.286
CV(%)	4.13	4.25	5.22	3.89	5.61

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability

T_0: Mixture of all sawdust

T_1: Mango sawdust

T_2: Raintree sawdust

T_3: Segun sawdust

T_4: Gamari sawdust

T_5: Mahogony sawdust

4.1.12 Economic yield

Economic yield of mushroom showed statistically significant variation due to use of different sawdust (Table 3). The highest economic yield (207.58 g) was observed in T_5 which was statistically similar (206.42 g and 199.55 g) to T_1 and T_0 and closely followed (194.98 g) by T_3, while the lowest economic yield (181.96 g) was found in T_4 which was statistically similar (189.40 g) to T_2. 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 there after.

4.1.13 Dry yield

Dry yield of mushroom showed statistically significant variation due to different sawdust (Table 3). The highest fry yield (29.39 g) was observed in T_5 which was closely followed (27.58 g) by T_1, while the lowest dry yield (22.25 g) was found in T_4 which was closely followed (24.60 g) by T_2. Sarker *et al.* (2007) who found that the range of dry yield ranged in 4.28 to 29.98 g/packet of *Pleurotus ostreatus* grown on different substrate. Kulsum *et al.* (2009) found that the highest dry yield was 21.27 g due to sawdust. Ahmed (1998) observed similar result in case of dry yield.

4.1.14 Biological efficiency

Biological efficiency of mushroom showed statistically significant variation was recorded due to different sawdust (Table 3). The highest biological efficiency (130.10%) was observed in T_5 which was statistically similar to T_1 (129.73%) and T_0 (129.69%), respectively and closely followed (125.43%) by T_3, while the lowest biological yield (117.01%) was found in T_4 which was closely followed (121.28%) by T_2. 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 in 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.

4.1.15 Benefit cost ratio

Statistically significant variation was recorded in terms of benefit cost ratio (BCR) for different sawdust (Table 3). The highest BCR (4.25) was observed in T_5 which was statistically similar (4.18) to T_1 and closely followed (3.93) by T_0, whereas the lowest BCR (3.62) was found in T_4 which was statistically similar (3.71) to T_2. The present findings found similar with the findings of previous research. Lim *et al.* (1997) analyzed the cost and return of *Volvariella* and *Pleurotus* mushroom production and found the BCR of 8.9 and 5.1, respectively. Ahmed (1998) also observed the benefit cost ratio of 7.32, 23.78 and 16.23 in case of *Pleurotus sajor-caju*. These variation is due to different genotype.

4.2 Proximate composition

4.2.1 Determination of Moisture content

Statistically significant variation was recorded in terms of moisture content of mushroom for different sawdust (Table 4). The highest moisture content (87.77%) was observed in T_4 which was statistically similar (87.01%) to T_2 and closely followed (86.49% and 86.64%) by T_0 and T_3, respectively, while the lowest moisture content (85.84%) was found in T_5. The result of the present study found more or less similar with the study of previous researchers (Moni *et al.*, 2004; Alam *et al.*, 2007 and Rahman, 1994). Moni *et al.* (2004) cultivated the oyster mushroom (*Pleurotus sajor-caju*) on paddy straw, banana leaves, sugarcane baggase, water hyacinth, betel nut husk and he found moisture content varied in 88.15 to 91.64%. Bhuyan (2008) found no significant differences in moisture content of the mushrooms produced in sawdust supplemented with wheat bran.

Table 4. Effect of different sawdust on proximate nutrient composition of white oyster mushroom (*Pleurotus ostreatus*)

Treatments	Moisture (%)	Dry matter (%)	Ash (%)	Carbohydrate (%)	Crude fiber (%)
T_0	86.49 bc	13.51 ab	13.24 ab	42.20 c	14.03 a
T_1	86.64 bc	13.36 ab	13.28 a	41.89 c	13.91 ab
T_2	87.01 ab	12.99 bc	13.03 bc	43.32 b	13.59 ab
T_3	86.64 bc	13.36 ab	13.11 ab	43.02 b	13.57 ab
T_4	87.77 a	12.23 c	12.81 c	44.02 a	13.33 b
T_5	85.84 c	14.16 a	13.35 a	41.52 c	14.01 a
LSD (0.05)	0.772	0.772	0.226	0.697	0.569
CV(%)	2.68	4.46	2.13	1.42	1.84

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability

T_0: Mixture of all sawdust

T_1: Mango sawdust

T_2: Raintree sawdust

T_3: Segun sawdust

T_4: Gamari sawdust

T_5: Mahogony sawdust

4.2.2 Determination of Dry matter

Dry matter content of mushroom showed statistically significant variation for different sawdust (Table 4). The highest dry matter content (14.16%) was observed in T_5 which was statistically similar (13.51% and 13.36%) to T_0, T_1 and T_3, respectively, whereas the lowest dry matter content (12.23%) was found in T_4 which was statistically similar (12.99%) to T_2. The result of the present study matches with the findings of previous one that reported by Kulsum *et al.* (2009), they revealed that the dry matter percentage of the fruiting body was ranged in 9.40 to 9.98 due to sawdust supplemented with different levels of cow dung. These variation might be due to use of different genotype.

4.2.3 Determination of Protein content

Statistically significant variation was recorded in terms of protein content of mushroom for different sawdust (Figure 3). The highest protein content (24.97%) was observed in T_5 which was statistically similar (24.83% and 24.50%) to T_1 and T_0, respectively and closely followed (24.37%) by T_3, while the lowest protein content (24.12%) was found in T_4 which was statistically similar (24.17%) to T_2. Chang *et al.* (1981) reported that the fruiting bodies of mushrooms contained 26.6-34.1% crude protein.

4.2.4 Determination of Lipid content

Statistically significant variation was recorded in terms of lipid content of mushroom for different sawdust (Figure 4). The highest lipid content (6.15%) was observed in T_5 which was statistically similar (6.10% and 6.03%) to T_1 and T_0, respectively and closely followed (5.94%) by T_3, while the lowest lipid content (5.72%) was found in T_4 which was closely followed (5.89%) to T_2. The results of the present study was found more or less similar with the findings of Alam *et al.* (2007) who reported 4.30 to 4.41% lipids in oyster mushroom grown on different substrates. Kulsum *et al.* (2009) also found that lipid content was ranged in 3.44 to 5.43% where sawdust supplemented with different levels of cowdung which is more or less similar to the present study.

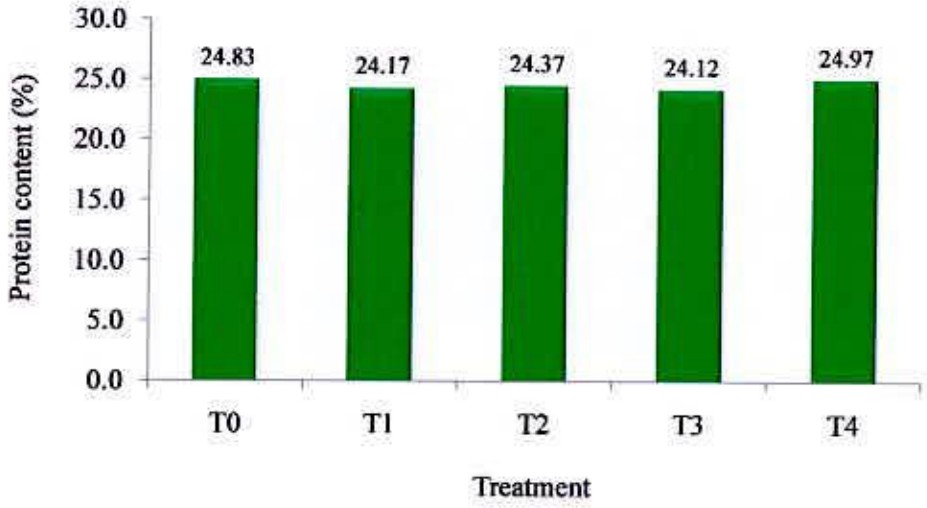


Figure 3. Effect of different sawdust on protein content of white oyster mushroom

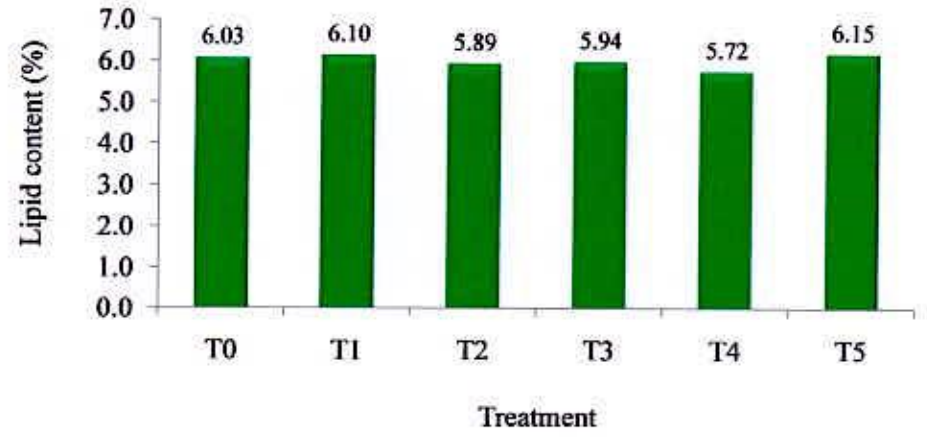


Figure 4. Effect of different sawdust on lipid content of white oyster mushroom

T_0: Mixture of all sawdust
T_1: Mango sawdust
T_2: Raintree sawdust
T_3: Segun sawdust
T_4: Gamari sawdust
T_5: Mahogony sawdust

4.2.5 Determination of Ash

Ash content of mushroom showed statistically significant variation due to different sawdust (Table 4). The highest ash content was observed in T_5 (13.35%) which was statistically similar (13.28%, 13.24% and 13.11%) to T_1, T_0 and T_3, respectively, whereas the lowest ash content (12.81%) was found in T_4 which was statistically similar (13.03%) to T_2. The findings of the present study was didder with the findings of Kulsum *et al.* (2009) who found that ash content was ranged from 6.58 to 8.41% due to sawdust supplemented with different levels of cow dung.

4.2.6 Determination of Carbohydrate content

Statistically significant variation was recorded in terms of carbohydrate content of mushroom for different sawdust (Table 4). The highest carbohydrate content (44.02%) was observed in T_4 which was closely followed (43.32%) by T_2, while the lowest carbohydrate content (41.52%) was found in T_5 which was statistically similar (41.89% and 42.20%) to T_1 and T_0, respectively. The findings of the present study are supported by the study of Kulsum *et al.* (2009) who found that carbohydrate content was ranged in 32.85 to 56.38% due to sawdust supplemented with different levels of cow dung.

4.2.7 Determination of Crude fiber content

Crude fiber content of mushroom showed statistically significant variation due to different sawdust (Table 4). The highest crude fiber content (14.03%) was observed in T_0 which was statistically similar (14.01%, 13.91%, 13.59% and 13.57%) to T_5, T_1, T_2 and T_3, respectively, whereas the lowest crude fiber content (13.33%) was found in T_4. The findings of the present study differ with the study Alam *et al.* (2007) and they reported 22.87 g/100g to 23.29 g/100g of fiber in *Pleurotus spp.*

4.3 Determination of Mineral content

4.3.1 Nitrogen (N)

Statistically significant variation was recorded in terms of nitrogen content of mushroom for different sawdust (Table 5). The highest nitrogen content (4.00%) was observed in T_5 which was statistically similar (3.97% and 3.92%) by T_1 and T_0, respectively, while the lowest nitrogen content (3.86%) was found in T_4 which was statistically similar (3.87%) to T_2.

4.3.2 Phosphorus (P)

Phosphorus content of mushroom showed statistically significant variation for different sawdust (Table 5). The highest phosphorus content (0.89%) was observed in T_5 which was statistically similar (0.87% and 0.86%) by T_1 and T_0, respectively, while the lowest phosphorus content (0.74%) was found in T_4 which was statistically similar (0.78%) to T_2. The findings of the present study agree with the study of Sarker *et al.* (2007a) who found 0.97% phosphorus in oyster mushroom grown on sawdust based substrates. Kulsum *et al.* (2009) also found that phosphorus content was ranged in 0.84 to 0.92% due to sawdust supplemented with different levels of cow dung.

4.3.3 Potassium (K)

Statistically significant variation was recorded in terms of potassium content of mushroom for different sawdust (Table 5). The highest potassium content (1.35%) was observed in T_5 which was statistically similar (1.33% and 1.32%) by T_1 and T_0, respectively, while the lowest potassium content (1.24%) was found in T_2 which was statistically similar (1.25%) to T_4. The findings of the present study similar with the study of Chang *et al.* (1981) who reported that the fruiting bodies of *Pleurotus* contained 1.432 to 1.88 mg/g of K on dry weight basis. Sarker *et al.* (2007) also found 1.3% potassium in oyster mushroom grown on sawdust based substrates.

Table 5. Effect of different sawdust on mineral contents of white oyster mushroom (*Pleurotus ostreatus*)

Treatments	N (%)	P (%)	K (%)	Ca (mg/100 g)	S (mg/100 g)	Zn (mg/100 g)
T_0	3.92 abc	0.86 ab	1.32 a	17.33 a	0.263 abc	15.36 ab
T_1	3.97 ab	0.87 ab	1.33 a	17.25 a	0.273 a	15.69 ab
T_2	3.87 c	0.78 c	1.24 c	16.16 ab	0.256 bc	15.19 ab
T_3	3.90 bc	0.83 b	1.30 ab	16.15 ab	0.266 ab	15.34 ab
T_4	3.86 c	0.74 c	1.25 bc	15.72 b	0.251 c	15.00 b
T_5	4.00 a	0.89 a	1.35 a	16.91 ab	0.274 a	15.81 a
LSD (0.05)	0.075	0.413	0.058	1.222	0.013	0.719
CV(%)	4.15	4.42	3.84	5.64	4.37	3.58

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability

T_0: Mixture of all sawdust

T_1: Mango sawdust

T_2: Raintree sawdust

T_3: Segun sawdust

T_4: Gamari sawdust

T_5: Mahogony sawdust

4.3.4 Calcium (Ca)

Calcium content of mushroom showed statistically significant variation for different sawdust (Table 5). The highest calcium content (17.33 mg/100 g) was observed in T_0 which was statistically similar (17.25, 16.91, 16.16 and 16.15 mg/100 g) by T_1, T_5, T_2 and T_3, respectively, while the lowest calcium content (15.72 mg/100 g) was found in T_4. Alam *et al.* (2007) who found 22.15 to 33.7 mg/100 g calcium in different oyster mushroom varieties. Sarker *et al.* (2007b) also found 2400 ppm calcium in oyster mushroom grown on sawdust based substrates.

4.3.5 Magnesium (Mg)

Statistically significant variation was recorded in terms of magnesium content of mushroom for different sawdust (Figure 5). The highest magnesium content (14.50 mg/100 g) was observed in T_5 which was statistically similar (14.39, 14.28 and 14.04 mg/100 g) by T_1, T_0 and T_3, respectively, while the lowest magnesium content (13.35 mg/10) was found in T_4. %). Sarker *et al.* (2004) also found 0.21% magnesium in oyster mushroom grown on sawdust based substrates.

4.3.6 Iron (Fe)

Iron content of mushroom showed statistically significant variation for different sawdust (Figure 6). The highest iron content (47.46 mg/100 g) was observed in T_1 which was statistically similar (47.14 and 46.90 mg/100 g) by T_5 and T_0, respectively, while the lowest iron content (44.89 mg/100 g) was found in T_4. The result of the present study found iron higher than the value found by Alam *et al.* (2007) who found that iron content of different oyster mushroom varieties ranged in 33.45 to 43.2 ppm.

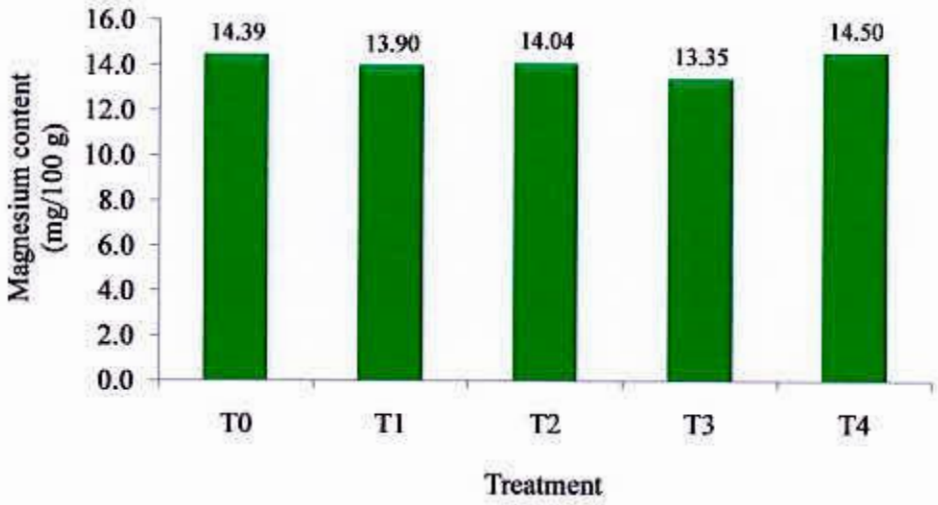


Figure 5. Effect of different sawdust on magnesium content of white oyster mushroom

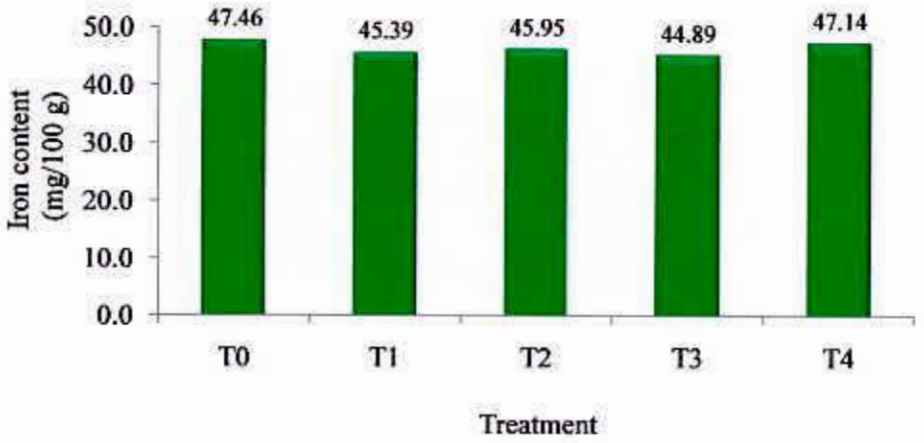


Figure 6. Effect of different sawdust on iron content of white oyster mushroom

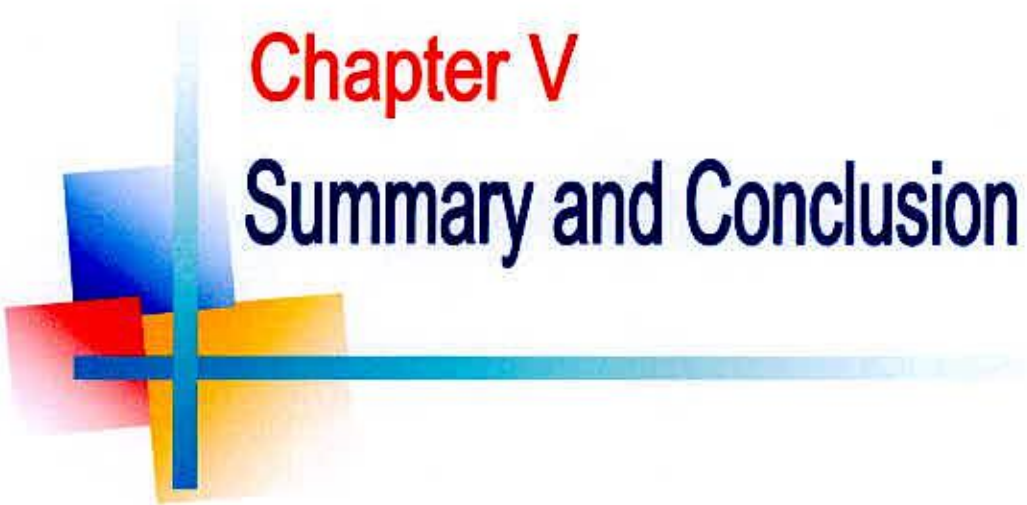
T_0: Mixture of all sawdust
T_1: Mango sawdust
T_2: Raintree sawdust
T_3: Segun sawdust
T_4: Gamari sawdust
T_5: Mahogony sawdust

4.3.7 Sulphur (S)

Statistically significant variation was recorded in terms of sulphur content of mushroom for different sawdust (Table 5). The highest magnesium content (0.274 mg/100 g) was observed in T_5 which was statistically similar (0.273, 0.266 and 0.263 mg/100 g) by T_1, T_3 and T_0, respectively, while the lowest sulphur content (0.251 mg/10) was found in T_4 which was statistically similar (0.256 mg/100 g) to T_2. The findings of the present study were supported with the findings of Alam *et al.* (2007) who recorded 0.238 to 0.321% of sulphur in their earlier study in oyster mushroom varieties.

4.3.8 Zinc (Zn)

Zinc content of mushroom showed statistically significant variation for different sawdust (Table 5). The highest zinc content (15.81 mg/100 g) was observed in T_5 which was statistically similar (15.69, 15.36, 15.34 and 15.19 mg/100 g) by T_1, T_0, T_3 and T_2, respectively, while the lowest zinc content (15.00 mg/100 g) was found in T_4. The results of the present study was similar with the study of Alam *et al.* (2007) found in their earlier experiment that zinc content of different oyster mushroom ranged in 16 to 20.9%. Sarker *et al.* (2007a) found 30.92 ppm zinc in oyster mushroom grown on sawdust based substrates.



Chapter V

Summary and Conclusion

CHAPTER V

SUMMARY AND CONCLUSION

The study was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka during the period in August to December 2014 to study effect of different sawdust on the growth, yield and proximate composition of white oyster mushroom (*Pleurotus ostreatus*). Mother culture of white oyster mushroom was collected in National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka. The experiment consists of six different type of sawdust with five replications. The experiment considered the following treatments: T_0: Mixture of all sawdust and T_1: Mango sawdust; T_2: Raintree sawdust; T_3: Segun sawdust; T_4: Gamari sawdust and T_5: Mahogony sawdust. The experiment was laid out in single factor Completely Randomized Design (CRD).

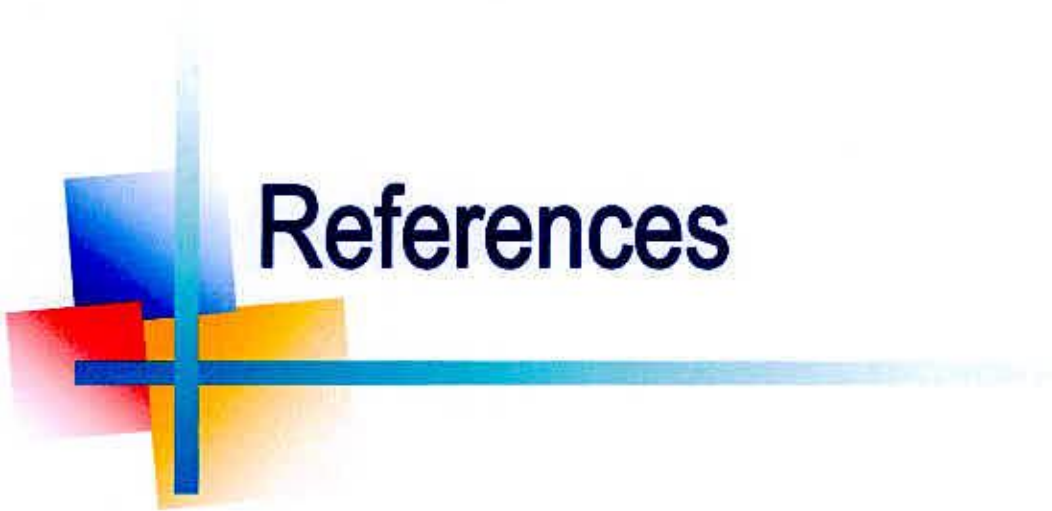
The highest mycelium running rate (0.76 cm) was observed in T_5, while the lowest mycelium running rate (0.63 cm) was found in T_4. The maximum time required to complete mycelium running (19.20 days) was observed in T_2, whereas the minimum time required to complete mycelium running (18.20 days) was found in T_1. The maximum time in stimulation to primordia initiation (4.00 days) was observed in T_4, whereas the minimum time in stimulation to primordia initiation (2.60 days) was found in T_5. The highest average number of primordia per packet (75.20) was observed in T_5, while the lowest average number of primordia per packet (65.80) was found in T_2 and T_4. The highest average number of fruiting body per packet (57.20) was observed in T_5, whereas the lowest average number of fruiting body per packet (47.00) was found in T_2. The highest average weight of fruiting body (4.45 g) was observed in T_5 while the lowest average weight of individual fruiting body (3.76 g) was found in T_4. The highest length of stripe (2.69 cm) was observed in T_1, while the lowest length of stripe (2.13 cm) was found in T_4. The highest diameter of stripe (1.15 cm) was observed

in T_5, whereas the lowest diameter of stripe (1.01 cm) was found in T_4. The highest diameter of pileus (6.88 cm) was observed in T_5 while the lowest diameter of pileus (6.37 cm) was found in T_4. The highest thickness of pileus (0.80 cm) was observed in T_1, while the lowest thickness of pileus (0.68 cm) was found in T_4. The highest biological yield (227.68 g) was observed in T_5, while the lowest biological yield (204.78 g) was found in T_4. The highest economic yield (207.58 g) was observed in T_5, while the lowest economic yield (181.96 g) was found in T_4. The highest dry yield (29.39 g) was observed in T_5, while the lowest dry yield (22.25 g) was found in T_4. The highest biological efficiency (130.10%) was observed in T_5, while the lowest biological yield (117.01%) was found in T_4. The highest BCR (4.25) was observed in T_5, whereas the lowest BCR (3.62) was found in T_4.

The highest moisture content (87.77%) was observed in T_4, while the lowest moisture content (85.84%) was found in T_5. The highest dry matter content (14.16%) was observed in T_5, whereas the lowest dry matter content (12.23%) was found in T_4. The highest protein content (24.97%) was observed in T_5, while the lowest protein content (24.12%) was found in T_4. The highest lipid content (6.15%) was observed in T_5, while the lowest lipid content (5.72%) was found in T_4. The highest ash content (13.35%) was observed in T_5, whereas the lowest ash content (12.81%) was found in T_4. The highest carbohydrate content (44.02%) was observed in T_4, while the lowest carbohydrate content (41.52%) was found in T_5. The highest crude fiber content (14.03%) was observed in T_0, whereas the lowest crude fiber content (13.33%) was found in T_4. The highest nitrogen content (4.00%) was observed in T_5, while the lowest nitrogen content (3.86%) was found in T_4. The highest phosphorus content (0.89%) was observed in T_5, while the lowest phosphorus content (0.74%) was found in T_4. The highest potassium content (1.35%) was observed in T_5, while the lowest potassium content (1.24%) was found in T_2. The highest calcium content (17.33 mg/100 g) was observed in T_0, while the lowest calcium content (15.72 mg/100 g) was found in T_4. The highest magnesium content (14.50 mg/100 g) was observed in T_5, while the

lowest magnesium content (13.35 mg/10) was found in T_4. The highest iron content (47.46 mg/100 g) was observed in T_1, while the lowest iron content (44.89 mg/100 g) was found in T_4. The highest magnesium content (0.274 mg/100 g) was observed in T_5, while the lowest sulphur content (0.251 mg/10) was found in T_4. The highest zinc content (15.81 mg/100 g) was observed in T_5, while the lowest zinc content (15.00 mg/100 g) in T_4.

From the above findings, it was revealed that among the sawdust T_5 (Mahogony sawdust) performed significantly better on growth, yield, nutrient and mineral content of white oyster mushroom (*Pleurotus ostreatus*) compare to the other sawdust under the study.



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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 2014

Month (2014)	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. Analysis of variance of the data on growth and yield contributing characters of white oyster mushroom (*Pleurotus ostreatus*) due to different sawdust

Source of variation	Degrees of freedom	Mean square					
		Mycelium running rate in spawn packets (cm)	Time required to complete mycelium running	Time from stimulation to primordia initiation (days)	Average number of primordia per packet	Average number of fruiting body per packet	Average weight of individual fruiting body (g)
Between	5	0.014**	0.753**	1.340**	76.640**	72.000**	0.404**
Within	24	0.002	0.217	0.233	8.900	9.467	0.016

** Significant at 0.01 level of probability;

Appendix III. Analysis of variance of the data on the dimension of fruiting body of white oyster mushroom (*Pleurotus ostreatus*) due to different sawdust

Source of variation	Degrees of freedom	Mean square			
		Length of stipe (cm)	Diameter of stipe (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Between	5	0.250**	0.018**	0.228**	0.016**
Within	24	0.014	0.001	0.009	0.001

** Significant at 0.01 level of probability;

Appendix IV. Analysis of variance of the data on the on the yield, biological efficiency and benefit cost ratio of white oyster mushroom (*Pleurotus ostreatus*) due to different sawdust

Source of variation	Degrees of freedom	Mean square				
		Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)	Benefit cost ratio
Between	5	448.585**	494.564**	2.178*	23.341**	0.335**
Within	24	82.344	69.795	0.645	3423	0.048

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Appendix V. Analysis of variance of the data on proximate nutrient composition of white oyster mushroom (*Pleurotus ostreatus*) due to different sawdust

Source of variation	Degrees of freedom	Mean square						
		Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)	Crude fiber (%)
Between	5	2.019**	2.019**	0.604**	0.123**	0.192**	4.540**	0.409*
Within	24	0.350	0.350	0.129	0.015	0.030	0.285	0.190

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Appendix VI. Analysis of variance of the data on the mineral contents of white oyster mushroom (*Pleurotus ostreatus*) due to different sawdust

Source of variation	Degrees of freedom	Mean square							
		N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	S (%)	Zn (%)
Between	5	0.197**	0.017**	0.010**	2.218*	0.873**	5.356**	0.0001**	0.463*
Within	24	0.039	0.001	0.002	0.877	0.157	0.381	0.0001	0.303

* Significant at 0.05 level of probability

* Significant at 0.05 level of probability

Appendix VII. List of Plates

Plate 1. Preparation of straw dust

Plate 2. Young fruiting body in the spawn packet

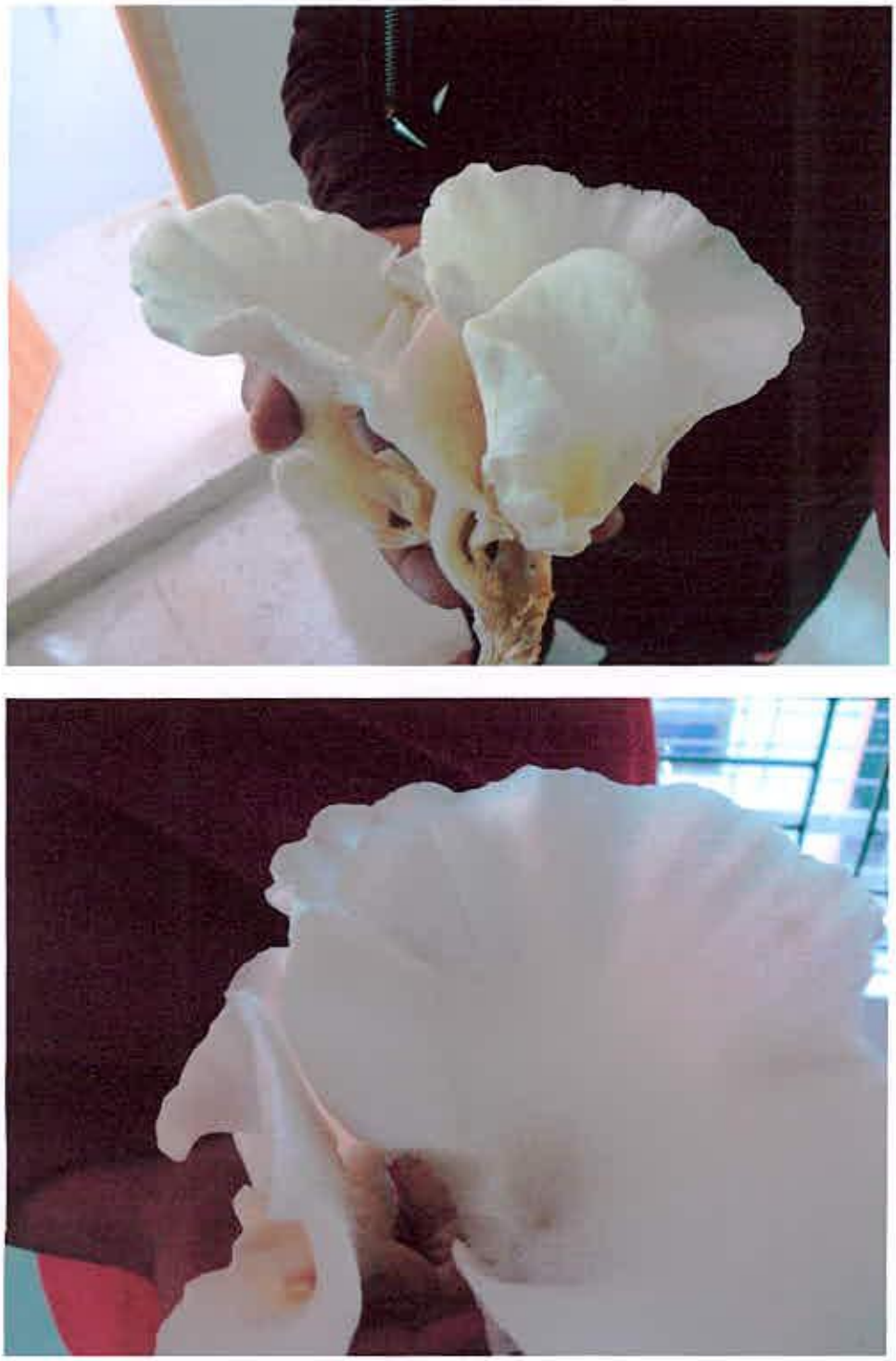

Plate 3. Mature fruiting body after harvest from spawn packet