EFFECT OF DIFFERENT SAWDUST ON THE GROWTH, YIELD AND PROXIMATE COMPOSITION OF EAR MUSHROOM (*Auricularia auricula*)

MD. AMIR HAMJA

REGISTRATION NO.: 09-03538

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DEPARTMENT OF BIOCHEMISTRY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

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By

MD. AMIR HAMJA

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Approved By:

Supervisor
Prof. Md. Nuruddin Miah
Department of Biochemistry
Sher-e-Bangla Agricultural University

Co-Supervisor
Prof. Dr. Kamal Uddin Ahmed
Department of Biochemistry
Sher-e-Bangla Agricultural University

Prof. Md. Nuruddin Miah
Chairman of Examination
Committee

DEPARTMENT OF BIOCHEMISTRY
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207
Bangladesh

PABX: +88029144270-9
Ext. 309 (Off)
Fax: +88029112649
e-mail:bioc_sau@yamil.com

Ref. No:

Date:

CERTIFICATE

This is to certify that the thesis entitled "EFFECT OF DIFFERENT SAWDUST ON THE GROWTH, YIELD AND PROXIMATE COMPOSITION OF EAR MUSHROOM (Auricularia auricula)" submitted to the DEPARTMENT OF BIOCHEMISTRY, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (M.S.) IN BIOCHEMISTRY, embodies the results of a piece of bona fide research work carried out by MD. AMIR HAMJA, Registration. No. 09-03538, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in any other institution.

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

Dated: 10.08.16

Place: Dhaka, Bangladesh

Prof. Md. Nuruddin Miah

Supervisor

DEDICATED TO
MY
BELOVED PARENTS

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EFFECT OF DIFFERENT SAWDUST ON THE GROWTH, YIELD AND PROXIMATE COMPOSITION OF EAR MUSHROOM (*Auricularia auricula*)

ABSTRACT

Five different types of sawdust viz: Teak tree *(Tectona grandis)* sawdust, Mahogany tree (*Swietenia mahagoni*) sawdust, Mango tree (*Mangifera indica*) sawdust, Rain tree (*Albiia saman*) sawdust and mixture of all four supplemented sawdust (Teak, Mahogany, Mango and Rain tree) with 30% wheat bran and 1% lime as basal substrates were selected for studied their effect on growth, yield and nutritional composition of ear mushroom.

Data on growth, yield and nutrient composition and mineral content were recorded and significant variation was found for different studied parameter. The highest mycelium running rate (0.88 cm) and the highest average number of primordial per packet (74.34) were recorded in mixed sawdust. The highest average number (115.5) and weight (3.67 g) of individual fruiting body were obtained from mixed sawdust. The lowest average weight of individual fruiting body (2.76 g) was found in mango sawdust. The highest average length of stalk (2.70 cm) was found in mixed sawdust. The highest biological yield (276.21 g/packet) and the lowest biological yield (248.57 g/packet) were found in mixed and rain tree sawdust respectively. The highest benefit cost ratio (5.00) was found from mango sawdust and the lowest benefit-cost ratio (4.01) was obtained from rain tree sawdust. In case of proximate analysis the highest protein content (24.54%) was recorded from mahogany tree sawdust. The highest carbohydrate (38.52%) was observed from mango sawdust. The highest amount of phosphorus (1.53%) was obtained from mixed, again the lowest phosphorus content (1.29%) was found in rain tree. Among the treatments mixed sawdust with 30% wheat bran was found contributed significantly growth, yield, nutrient composition and mineral content of ear mushroom (*Auricularia auricula*).

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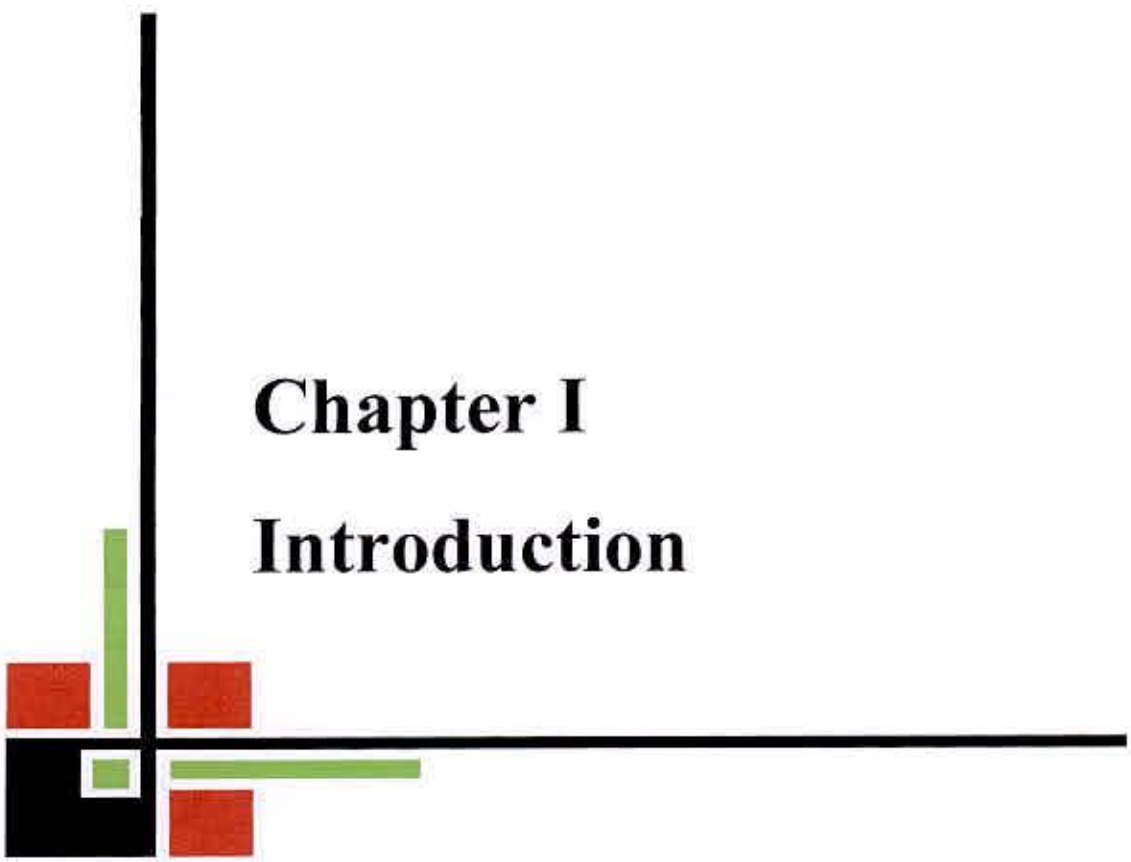


Chapter I

Introduction

CHAPTER I

INTRODUCTION

Mushrooms are the members of higher fungi, belonging to the class Ascomycetes (e,g, Morchella, Tuber etc) and basidiomycetes (e.g, Agaricus, Auricularia, Tremella, etc).They are characterized by having heterotropic mode of nutrition. According to Chang and Hayes (1978) edible mushroom refers to both epigeous and hypogeous fruiting bodies of macroscopic fungi that are already commercially cultivated or grown in half culture process or implemented under controlled conditions. Mushroom is being widely used as food and food supplements from ancient times. They are increasingly being recognized as one of the important food items for their significant roles in human health, nutrition and diseases (Chang, 1996).

There is a common saying that "medicines and foods have a common origin" (Kaul, 2001). Out of 2000 species of prime edible mushrooms about 80 have been grown experimentally, 20 cultivated commercially and 4-5 produced on industrial scale throughout the world (Chang and Miles, 1988). It contains 4.0% fat having good quantity of unsaturated fatty acids which are essential in our diet (Holman, 1976). Edible mushrooms are recommended by the FAO as food, to meet protein requirement of developing countries, the large proportion of which depends mainly on cereals (World Bank, 2004). Mushrooms are the fruiting bodies of macro fungi. Mushroom cultivation has become one of the most profitable agribusiness that could produce food products from different substrates and help to dispose them in an environmentally friendly manner (Bano *et al.*, 1993). Two species of *Auricularia*, a group of jelly fungi, are often used in Asian cuisine. Both are sold dried in Asian markets and are reasonably priced compared to many wild or cultivated mushrooms. For culinary purposes, they are identical. Ear Mushroom (*Auricularia auricular*) "cloud ear" "Judas' ear" is a smaller fungus with a brown to black cap surface and is dull brown underneath. *A. auricula* is not restricted to Asian countries. *Auricularia polytricha* is variously called "wood ear," "tree ear," "black fungus,"or"muk nge".

The dried ear-shaped cap is medium sized, dull in texture, and dark brown to black. The wavy lower surface has a contrasting powdery gray color. The stem is absent or rudimentary. It has no gills. It is a native of Asia and some Pacific Ocean islands in humid climates.

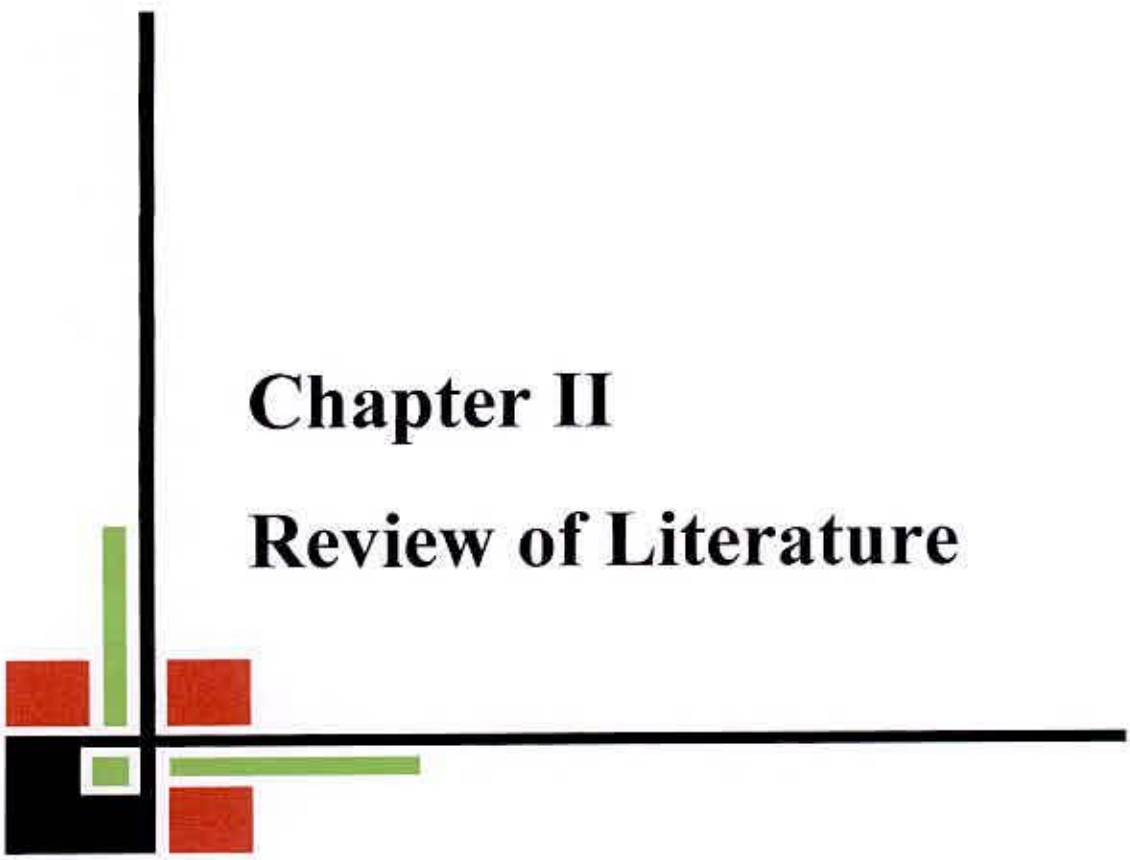
Bangladesh is a thickly populated country with an area of 147,570 km^2 and the country has limited cultivable land and per capita land area is decreasing at an alarming rate of 0.005 ha/capita/year since 1989 (Hossain and Bari, 1996). So, we have to increase intensive use of land for increasing crop production also 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. 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. For an over populated country supplying food to the person is a great challenge. So, cultivation of mushroom can remove the food shortage & nutritional problem. Beside there it keeps the environment clean.

A healthy man should eat 200-250 gm vegetables daily where the persons of developed country eat about 400-500gm vegetables. Because vegetables contain vitamins, minerals and fibers. But we eat only 40-45gm vegetables except potato. So about 87% persons are suffering in malnutrition. The population incensement rate in our country is very high so the lands are decreased. Every year about 80000 hectare lands are decreased. So, increasing the mushroom production will play a important role for the fulfillment of nutrition. The substrates on which mushroom spawn (Merely vegetative seed materials) is grown, affects the mushroom production (Klingman, 1950). Ear mushroom can grow on sawdust, wheat and paddy straw, banana leaves, sugarcane bagasse and leaves, wheat barn, rice husk etc. and their culture can be concentrated within a relatively small space. In the present study five different sawdust viz: Teak tree *(Tectona grandis)* sawdust, Mahogany tree (*Swietenia mahagoni*) sawdust, Mango tree (*Mangifera indica*) sawdust, Rain tree (*Albiia saman*) sawdust and mixture of all four supplemented sawdust (Teak, Mahogany, Mango and Rain tree) with 30% wheat bran and 1%lime as basal substrates were selected for

studied their performance on growth, yield and nutritional composition of *Auricularia auricula.* If different sawdust can be used in mushroom production then low price and easily available sawdust could be select and which one is better and also best for mushroom production can be identified. So, the investigation is undertaken to fulfill the following aim and objectives:

Objectives of the research work

- To improve the yield of ear (*Auricularia auricula)* mushroom.
- To prepare suitable sawdust based spawn packet.
- To determined the physiochemical characteristics of ear (*Auricularia auricula)* mushroom.
- To find out benefit cost ratio of the sawdust based spawn packet.



Chapter II

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Mushrooms have been considered as a special kind of food since the earliest time. It grows well in waste materials. There are many scientific reports on the effect of different substrates on mushroom cultivation still there are major scope to investigate the effects of different sawdust on ear 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.

Adenipekun *et al.* (2015) conducted an experiment to determine determine the effect of additives on the performance of the fungus on the substrate; M. altissima sawdust. The treated and untreated substrates with additives at different percentages were analyzed for lignocelluloses composition, macro element, C-N ratio and proximate composition. The result of this study showed that A. auricular reduced the lignocelluloses composition of M. altissima sawdust. The lignin content reduced from 7.97% (control) to 1.59% in 20%SC treated substrate. The macro elements (Ca, Mg, K, Na) compositions were low in all the treated substrate - additives combination. The least was recorded in Na (25.8 - 84.5ppm), Ca (2.04% in control and 0.50% in 20%SC). The proximate composition showed that the substrate had an average moisture content of 50% - 61%, low protein (4.85-0.60%), high carbohydrate and high ash contents compared to the control.

Ahila *et al.* (2013) surveys were conducted in the hills of Nilgiris, Shervoys and Lower Pulneys during rainy season. A wood ear mushroom was collected from coffee plantations of Horticultural Research Station, Yercaud. The fungus was identified as Auricularia polytricha (Mont.) Sacc. based on cultural and morphological characters. The studies conducted at MR & TC, TNAU, Coimbatore revealed that the paddy straw +wheat bran (3:1) ratio recorded minimum days for spawn run (21.3 days), pin head formation(31.3) and first harvest (35.6 days).

The same combination also recorded the highest yield of 147.6 g/bed bioefficiency of 59.04 percent. The total cropping period was also the minimum in the same treatment.

In the trials conducted at Vijaya Mushrooms, Coimbatore (North), paddy straw+ wheat bran (3:1) ratio again recorded a significantly higher yield of 132.0 g/bed and bio efficiency of 58.20 percent with minimum cropping period of 47.3 days. The yield performance trials conducted at Maha Mushroom, Kovaipudur and Coimbatore (South) also revealed the same trend as paddy straw+wheat bran (3:1) again recorded significantly higher yield of 130g/bed and bioefficiency of 52.00 percent.

Onyango *et al.* (2011) stated that different organic substrates namely maize cobs, wheat straw, grass straw and sugarcane bagasse supplemented with either wheat or rice bran were evaluated for production of two Kenyan native strains of wood ear mushroom (*Auricularia auricula*). The objective was to evaluate the suitability of these substrates for cultivation of Kenyan native wood ear mushroom. Plastic bag technology was used with treatments arranged in a completely randomized design replicated three times. Samples of black and brown strains of the wood ear mushroom collected from woody stems of dead and dying trees within Kakamega forest were used in this study. Data was collected on days to pinning, fruit body quality, fruit body yields (number and fresh weight) and biological efficiency. The data collected was subjected to analysis of variance using SAS version 9.1. Mean separation was done using LSD and effects declared significant at 5% level. The two mushroom strains were not significantly ($p>0.05$) different in performance except for the number of fruit bodies where the black strain yielded significantly ($p<0.05$) higher than the brown one. The best performance was obtained from maize cobs and wheat straw substrates supplemented with wheat bran and these combinations were recommended to wood ear mushroom growers.

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 ear 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.

Pathan *et al.* (2009) was observed that the oyster mushroom, *Pleurotus sajor-caju* was cultivated by on fresh, dried and chopped sugarcane leaves in polythene bags at 400 g per bag. The spawning was done at 50 g/bag, followed by soaking and boiling of substrate. The pinheads were noted first (11.25 days after spawning) in case of soaking till it starts boiling followed by soaking and boiling for 15 and 75 minutes. Minimum period for maturation of fruiting bodies (5.25 days) was in case of soaking and boiling for 30 minutes and soaking and boiling for 75 minutes. The minimum period between flushes (4.25 days) was recorded in soaking and boiling for 30 minutes, followed by soaking and boiling for 75 and 90 minutes. The maximum number of flushes (2.75) was recorded in case of soaking and boiling for 75 minutes followed by 90 and 60 minutes.

The maximum fresh yield (61.75%) was obtained in case of soaking and boiling for 75 minutes, followed by soaking and boiling for 90 and 60 minutes. Data revealed that 75 minutes soaking was the best in relation to yield and other studied characters.

Kulsum *et al.* (2009) conducted an experiment to determine the effect of five different levels of cow dung (0%, 5%, 10%, 15% and 20%) as supplement with sawdust on the performance of oyster mushroom. All the treatments performed better over control. The mycelium running rate in spawn packet and the highest number of primordia/packet were found to be differed due to different levels of supplements used. The highest weight of individual fruiting body was observed in sawdust supplemented with cow dung @ 10% (3.69g). The supplementation of sawdust with cow dung had remarkable effect on biological yield, economic yield, the dry yield, biological efficiency and cost benefit ratio. The highest biological yield (217.7 g), economic yield (213g), dry yield (21.27g) biological efficiency (75.06%) and cost benefit ratio (8.41) were observed due to sawdust supplemented with cow dung @ 10%. Among the chemical characteristics highest content of protein (31.30%), ash (8.41%), crude fiber (24.07%), the lowest lipid (3.44 %) and carbohydrate (32.85%) were observed due to sawdust supplemented with cow dung @ 10%. Among the minerals the highest amount of nitrogen (5.01%), potassium (1.39%), calcium (22.15%), magnesium (20.21%), sulfur (0.043%), iron (43.4%) and the lowest phosphorus (0.92) were observed due to sawdust supplemented with cow dung @ 10%.

Bhuyan (2008) conducted an experiment to study the effect of various supplements at different levels with sawdust showed significant effort on mycelium running rate and reduced the required days to complete mycelium running in the spawn packet.

The supplementation of sawdust found to be significant in yield and yield contributing characters of oyster mushroom with some extent. The highest biological yield, economic yield, dry yield, biological efficiency (BE) and benefit cost ratio (BCR) of 270.5 g, 266.5 g, 26.34 g, 93.29, 9.57%, respectively was observed in sawdust supplemented with NPK mixed fertilizer (N=0.6%, P=0.3%, K=0.3%).

Sawdust supplemented with different levels has a profound effect on chemical composition of oyster mushroom. Sawdust supplemented at different substrate found to be significant with mineral content of the fruiting body. Considering all the parameters in five experiments, NPK mixed fertilizer (N=0.6%, P=0.3%, K=0.3%) supplemented with sawdust is found promising for lowering the cost of production as well as increasing the yield and quality of fruiting body.

Bhatti *et al.* (2007) conducted that the ear mushroom (*Auricularia auricula*) was cultivated on wheat straw in polythene bags (containing 500 g wheat straw on dry weight basis per bag) using sorghum grain spawn at different rates. The spawning was done followed by boiling of substrate and sterilization of bags. The bags were kept in mushroom growing room at 25 to 35°C with 80 to 100% humidity under regular white fluorescent light arranged by the tube lights in mushroom growing room (10'x14'x14'). The pinheads first appeared 32.33 days after spawning by using 70 g spawn rate per kg on substrate dry weight basis. The minimum period of 4.66 days after pinhead formation for maturation of fruiting bodies was recorded by using 60, 70, 80, 90 and 100 g spawn rate. The minimum period between flushes (6.33 days) was taken by using 20 g spawn rate. The maximum flushes (4.00) were harvested by using 70 g spawn rate. The maximum number of bunches per bag (7.66) were obtained by using 100 g spawn rate. The maximum number of fruiting bodies per bunch (7.30) was observed by using 70 g spawn rate. The maximum yield on fresh weight basis (45.4%) as well as on dry weight basis (4.63%) was also obtained by using 70 g of spawn rate per bag. The results were highly significant from each other. It is concluded that spawning at 70 g per kg on substrate dry weight basis found to be the best dose for obtaining early and high yielding crop of oyster mushroom, with minimum period for maturation of fruiting bodies, maximum number of flushes and fruiting bodies per bag.

Namdev *et al.* (2006) conducted a study to determine the effect of different straw substrates on spawn growth and yield of oyster mushroom. The number of days required for spawn run was significantly less (14 days) in case of gram straw, parthenium straw, sugarcane straw and wheat straw, compared with 20 days for

sunflower stalk, mustard straw and paddy straw. Yield was very poor on parthenium straw (95 g/500 g dry substrates) and it was highest on paddy straw (666 g/500 g), followed by wheat straw and mustard straw (427 and 400 g/500 g respectively). Ramjan (2006) in his study found that high concentration of IAA is effective for mycelial growth and mustard straw performed best as a substrate for the production of fruiting bodies of oyster mushroom. Sainos *et al.* (2006) conducted a study to determine the mycelial growth, intracellular activity of proteases, laccases and beta - 1,3-glucanases, and cytoplasmic protein were evaluated in the vegetative phase of *Pleurotus ostreatus* grown on wheat straw and in wheat-grain-based media in Petri dishes and in bottles. The productivity of the wheat straw and wheat-grain-based spawn in cylindrical polyethylene bags containing 5 kg of chopped straw was also determined. We observed high activity of proteases and high content of intracellular protein in cultures grown on wheat straw. This suggests that the proteases are not secreted into the medium and that the protein is an important cellular reserve. On the contrary, cultures grown on wheat straw secreted laccases into the medium, which could be induced by this substrate. *Pleurotus ostreatus* grown on media prepared with a combination of wheat straw and wheat grain showed a high radial growth rate in Petridishes and a high level of mycelial growth in bottles. The productivities of wheat straw and wheat-grain-based spawn were similar. Our results show that cheaper and more productive mushroom spawn can be prepared by developing the mycelium on wheat straw and wheat-grain-based substrates.

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

However, *P. flabellatus* was rich in crude fibre, carbohydrate and ash but low in protein and fat content as compare to *P. eous* and *P. florida.*

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.

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 utilizing 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 < sub>4</ sub> 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.

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.

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

Ayyappan *et al.* (2000) used sugarcane trash and coir waste alone and in combination with paddy straw (3:1, 1:1 and 1:3 w/w) for sporophore production of two species of *Pleurotus*. The highest yields of *P. florida* (1395 g) and *P. citrinopileatus* (1365 g) were recorded in a mixture of sugarcane.

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.

Kalita *et al.* (1997) studied the growth of *Auricularia auricula* 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%).

Jadhav *et al.* (1996) reported that oyster mushroom *Auricularia auricula* 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.

Marimuthu *et al.* (1994) investigate *Pleurotus sajor-caju*, *P. citrinopileatus* and *P. platypus* on paddy straw 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% and reduced the number of days required for completion of spawn run by 2-6, 5 and 6 days, respectively compared with control.

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. The yields were observed 3.56 kg/m^2 for non-supplemented substrate to 7.36 kg/m^2 for substrate supplemented (12% DW) with formaldehyde soybean meal.

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 400 g substrate, reaching maximum of 506-508 g on pretreated bagasse, 407-411 g on paddy straw and 379-391 g on wheat straw alone.

Suprapti (1987) measured the mushroom yield and harvesting frequency after cultivation on Rubber wood (*Hevea brasiliensis*) sawdust mixed with 5, 10, 15 or 20%, of leaves of either turi (*Sesbonia grandiflora*) or lamtoro gung (*Leucaena leucocephala*). Average total yield per treatment was 643.00 g (532.29-744.69) per kg dry wt. of substrate.

Chapter III

Materials & Methods

CHAPTER III

MATERIALS AND METHODS

The study was conducted during the period from June to November' 2015 to study effect of different sawdust on the growth, yield and proximate composition of ear mushroom (*Auricularia auricula*). 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 Biochemistry 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 ear mushroom was collected from National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka.

3.3 Varietal characteristics of ear Mushroom

The fruiting body is distinguished by its noticeably ear-like shape and brown color. It grows upon wood, especially elder. The fruit body of *A. auricula* is normally 3 to 8 centimeters (1.2 to 3.1 in) across, but can be as much as 12 centimeters (4.7 in). It is distinctively shaped, typically being reminiscent of a floppy ear, though the fruit bodies can also be cup-shaped. It is normally attached to the substrate laterally and sometimes by a very short stalk.

The species has a tough, gelatinous, elastic texture when fresh, but it dries hard and brittle. The outer surface is a bright reddish-tan-brown with a purplish hint, often covered in tiny, downy hairs of a grey color.

The color becomes darker with age. The inner surface is a lighter grey-brown in color and smooth. The spores of *A. auricula* are long and sausage shaped, ranging in size from 16 to 18 micrometers (μm) long by 6 to 8 μm thick. The spores themselves are white, cream or yellowish and are hyaline. The spores can sometimes be seen in a whitish mass on the underside of the fruit body. The species is found all over the world.

3.4 Treatment of the experiment

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

T_1:Teak tree *(Tectona grandis)* sawdust supplemented with 30% wheat bran and 1% lime

T_2: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime

T_3: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime

T_4: Rain tree (*Albiia saman*) sawdust supplemented with 30% wheat bran and 1% lime

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

3.5 Design and layout of the experiment

The experiment was laid out in single factor Completely Randomized Design (CRD). The experiment included five treatments.

3.6 Preparation of substrates

At first weight of dry sawdust of teak tree, mahogany, mango and rain tree was taken. Then the sawdust was soaked in water over night. Thereafter the sawdust was taken off from water and left on a perforated sieve for removing the excess water for few hours. Then wheat bran @ 30% and $CaCO_3$ @ 1% on dry weight basis were added with spawn preparing substrate. 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 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 primordial 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 mushroom

Ear mushrooms matured within 5-6 days after primordial 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 Mycelia growth

Mycelia 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 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):

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

Where, L= Average length of mycelium running (cm)

N= Number of days

3.7.3 Days required for completing mycelium running

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

3.7.4 Average number of fruiting body per packet

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

3.7.5 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.

3.7.6 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)

3.7.7 Biological yield

Biological yield per 600 g packet was measured by weighing the whole cluster of fruiting body without removing the lower hard and dirty portion.

3.7.8 Economic yield

Economic yield per 600 g packet was recorded by weighing all the fruiting bodies in a packet after removing the lower hard and dirty portion.

3.7.9 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.

3.7.10 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):

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

3.7.11 Biological efficiency

Biological efficiency was determined by the following formula:

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

3.7.12 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 were soaked in a bucket for five minutes and then were placed in the culture house 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.

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 analyzed.

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. 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 weighted. Again, the container was placed in the oven at 105^0C for 2 hours. It was cooled in a desiccator and weighed again. Repeat drying, cooling and weighing were continued until the weight became constant. The dried sample was stored in an airtight container. The moisture content of the sample was calculated.

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:

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

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 washed with hot water till free from acid. The material was then transferred to the same beaker and 200 ml of boiling 0.313 N NaOH 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 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 were finding out 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

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

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°C to 100°C, 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 elementary composition analysis the equipment were used as electric balance, desiccators, atomic absorption apectrophotometer (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 of 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 was added to each of all 20 tubes. The tubes were left overnight mixing the contents in the tubes. Covering with the exhaust manifold, the tubes were placed in the digester and the temperature was set to 125°C, turning on the digester, the digestion was continued for 4 hours after boiling started. Every tube was observed to avoid drying.
2. After cooling, the digestion mixture was transferred with distilled water to a 100 ml volumetric flask. Water was added to the flask to make the volume up to the mark.
3. Filtration was performed on a dry filter into a dry bottle, which could be closed with a screw cap. Keeping the filtrate in the closed bottle Ca, Mg, K, Fe, Mn, Zn, S, Cu and P were determined in 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 $LaCl_3$-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 $LaCl_3$-solution was added and the volume was made with water and mixed. Then the content of Mg was measured by atomic absorption spectrophotometer (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 to volume with water and mixed. 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,

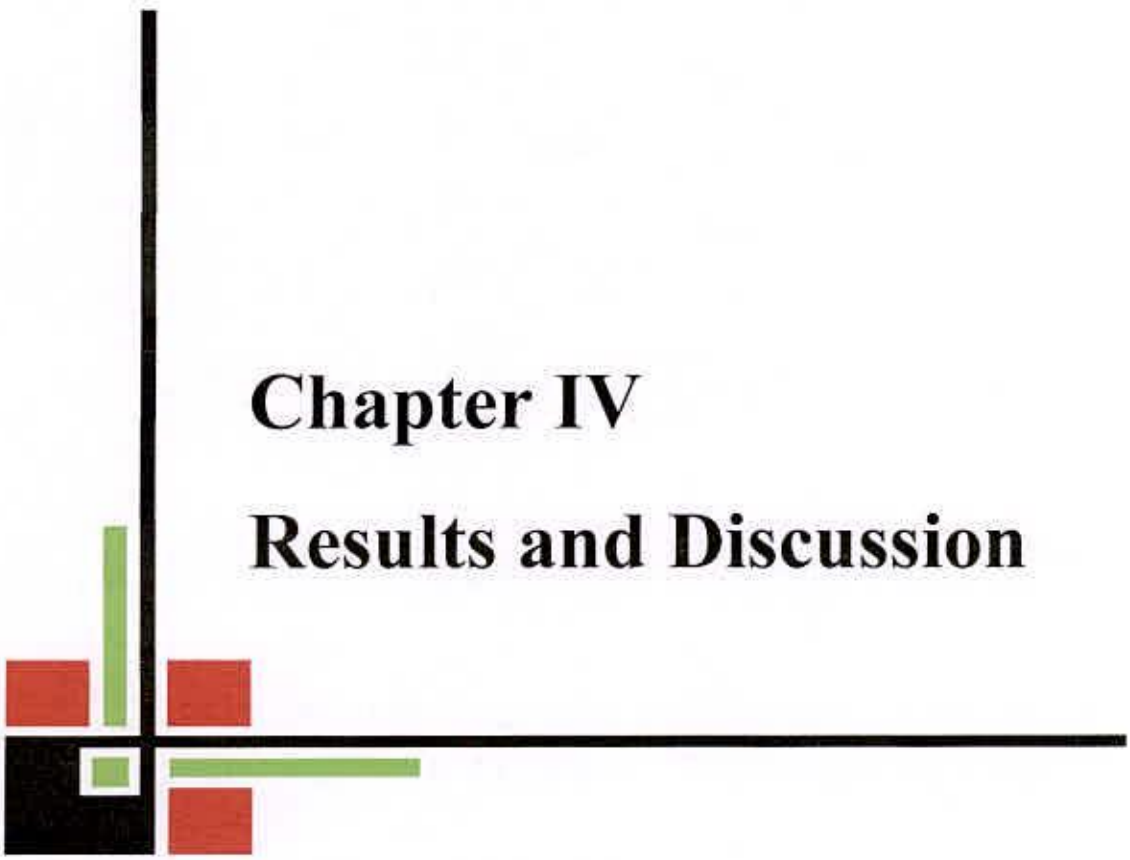
A = weight of $BaSO_4$ g

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. All the data collected on different parameters were statistically analyzed by Duncan's Multiple Range Test (DMRT). The mean values of all the characters were evaluated and analysis of variance was performing by the 'F' test. 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 DISCUSSIONS

The study was conducted to find out the effect of different sawdust on the growth, yield and proximate analysis of ear mushroom (*Auricularia auricula).* Data on growth, yield contributing characters, proximate composition of mushroom were recorded. The results have been presented and discussed with the help of table, graphs and possible interpretations given under the following headings:

4.1 Growth and yield contributing characters

4.1.1 Mycelium running rate in spawn packets (cm)

Mycelium running rate (MRR) of ear mushroom (*Auricularia auricula)* showed statistically significant variation due to different sawdust under the present trial (Table 1). The highest mycelium running rate was observed from T_5 (0.88 cm) (Mixed sawdust: teak tree, mahogany, mango tree and rain tree + 30% wheat bran), whereas the lowest mycelium running rate was observed from T_4 (0.73 cm) (rain tree sawdust + 30% wheat bran). On the other hand T_2 (0.79 cm) and T_3 (0.78 cm) treatment were statistically similar. Different sawdust showed different mycelium running because of different carbohydrate based on availability and the environment of the spawn. The present findings found more or less similar with the previous workers. Khan *et al.* (1991) reported that sawdust amended with different organic supplement like wheat chaff, wheat bran, paddy straw, cotton waste etc. provided suitable condition for spawn running. Sarker (2004) found that the mycelium running rate of oyster mushroom greatly influenced with the supplement of wheat barns in different levels. Bhuyan (2008) also found similar result as found in the present experiment.

Table 1. Effect of different sawdust on the growth and yield contributing characters of ear mushroom (*Auricularia auricula)*

Treatments	Mycelium running rate in spawn packets (cm)	Time from stimulation to primordial initiation (days)	Average number of primordial per packet	Time from primordial initiation to harvest (days)
T_1	0.85 b	2.35 c	72.68 b	6.40 ab
T_2	0.79 c	3.13 a	67.34 d	6.80 a
T_3	0.78 c	2.54 b	69.01 c	6.60 a
T_4	0.73 d	2.67 b	64.68 e	6.00 bc
T_5	0.88 a	2.09 d	74.34 a	5.80 c
$LSD_{(0.05)}$	0.029	0.143	2. 343	0.559
CV(%)	4.54	3.88	6.10	13.00

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

4.1.2 Time from stimulation to primordial initiation

There was significant variation in terms of time from stimulation to primordial initiation of ear mushroom due to different sawdust (Table 1). The highest time from stimulation to primordia initiation was found from T_2 (3.13 days), whereas the lowest time from stimulation to primordia initiation was recorded in T_5 (2.09 days). On the other hand T_3 (2.54 days) was statistically similar with T_4 (2.67 days). The result of the present finding was found similar with Gupta (1989); Khan *et al.* (2001); Royse (2002); Sarker (2004) and Amin *et al.* (2007). Sarker (2004) observed that duration from primordia initiation of oyster mushroom was significantly lower as compared to control i.e. no supplement was used. Ruhul Amin *et al.* (2007) found significant differences on time from stimulation to primordia initiation among the level of supplements used for preparing the substrates. Bhuyan (2008) also found similar effect as found in the present study.

4.1.3 Average number of primordial per packet

Average number of primordial per packet of ear mushroom was varied significantly due to different sawdust under the present trial (Table 1). The maximum average number of primordial per packet was observed from T_5 (74.34) and the minimum average number of primordial per packet was found in T_4 (64.68). The result of the present study supported with the previous findings (Amin, 2004; Sarker, 2004 and Dey, 2006). Amin (2004) in his experiment found that the highest number of primordial of oyster mushroom was found in sterilized paddy straw but lowest was found in saw dust. Dey (2006) found that the number of primordia and the average yield of Oyster mushroom give the lowest value with sawdust. Ahmed (1998) reported significantly different number of primordial on different substrates. Bhuyan (2008) found similar findings when he growing oyster mushroom on saw dust supplemented with different levels of cow dung.

4.1.4 Time from primordial initiation to harvest (days)

Numerically the highest time required from primordial initiation to harvest was in the treatment T_2 (6.80 days) which is statistically similar with T_1 (6.40 days) and T_3 (6.60 days). On the other hand the lowest time required from primordial initiation to harvest was observed in the treatment T_5 (5.50 days) which is statistically similar with T_4 (6.00 days). The result of present findings keeps in with the findings of previous scientists [(Basunia *et al.*2007, Rahman *et al.*2007 and Gomez (2002)]. Basunia *et al.* (2007) reported that after spawn running pinhead formation took 12-15 days and fruiting body formed after 7-8 days, sporocarps may be harvested after 10-12 days. Rahman *et al.* (2007) found significant effect of different agro-waste on the yield of mushroom. The days required for first picking varied from 11.25-12.00 days and the final picking varied from 42-43.50 days depending on different substrates. Gomez (2002) found as the spawn rate increased the number of days to production decreased.

Table 2. Effect of different sawdust on the growth and yield contributing characters of ear mushroom (*Auricularia auricula)*

Treatments	Avg. no of fruiting body/packet	Avg. no of effective fruiting body/packet	Average weight of individual fruiting body (g)
T_1	79.65 d	17.02 d	3.55 b
T_2	89.65 c	19.69 c	3.20 c
T_3	100.5 b	24.02 b	2.76 e
T_4	98.31 b	20.35 c	2.89 d
T_5	115.5 a	29.02 a	3.67 a
$LSD_{(0.05)}$	3.038	1.956	0.196
CV (%)	1.79	5.17	6.55

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

4.1.5 Average number of fruiting body per packet

Different sawdust substance showed statistically significant differences in terms of fruiting body per packet of ear mushroom (Table 2). The highest average number of fruiting body per packet was observed in T_5 (115.5), whereas the lowest average number of fruiting body per packet was found in T_1 (79.65) treatment. Sarker *et al.* (2011) reported that the number of effective fruiting bodies was the highest (21.00) in autoclaved sawdust with pasteurized straw (1:2) and it was the lowest (7.50) in autoclaved sawdust with pasteurized straw (1:1).

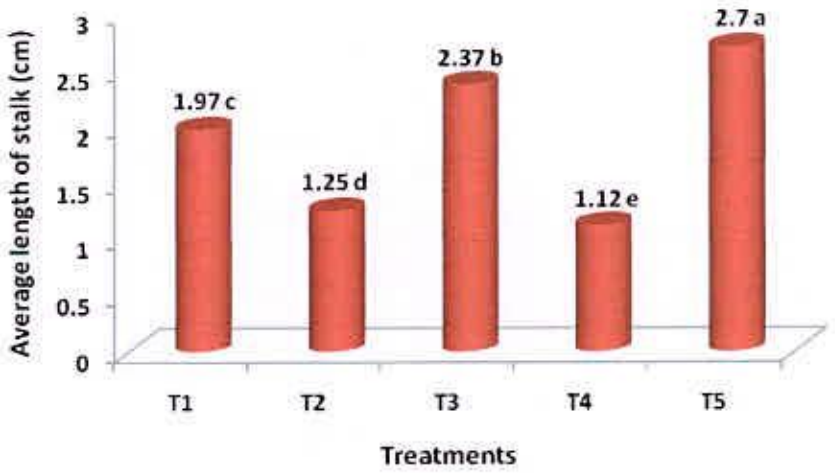
4.1.6 Average number of effective fruiting body per packet

Different sawdust substance showed statistically significant differences of effective fruiting body per packet of ear mushroom (Table 2). The highest average number of effective fruiting body per packet was observed in T_5 (29.02), whereas the lowest average number of effective fruiting body per packet was found in T_1 (17.02) treatment. Wasser (2005) reported that the number of effective fruiting bodies was the highest (30.00) in autoclaved sawdust with Eucalyptus sawdust with 30%rice bran.

4.1.7 Average weight of individual fruiting body (g)

Statistically variation was observed in case of average weight of individual fruiting body of ear mushroom for different sawdust under the present trial (Table 1). The highest average weight of individual fruiting body was found from T_5 (3.67 g), where as the lowest average weight of individual fruiting body was found in T_3 (2.76 g). The findings of this experiment were also supported by the findings of 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 from (5.02g to 7.01g).

4.2 Effect of different sawdust substrate on the development and size of fruiting body



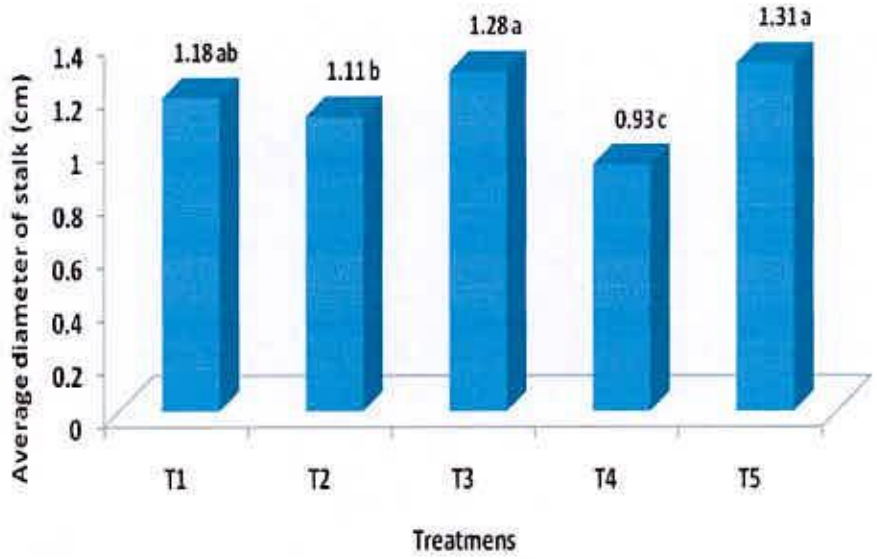
T_1:Teak tree *(Tectona grandis)* sawdust supplemented with 30% wheat bran and 1% lime
T_2: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Rain tree (*Albiia saman*)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

Figure 1: Effect of different sawdust on the average length of stalk of *Auricularia auricula*

4.2.1 Length of stalk (cm)

Length of stalk of ear mushroom varied significantly due to different sawdust (Table 3). The highest length of stalk was observed in T_5 (2.70 cm) treatment, whereas the lowest length of stalk was found in T_4 (1.12 cm).

4.2.2 Diameter of stalk

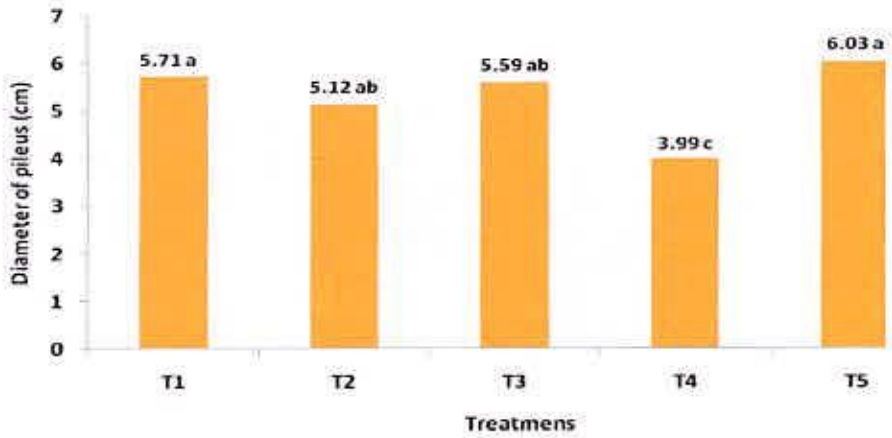


T_1:Teak tree *(Tectona grandis)* sawdust supplemented with 30% wheat bran and 1% lime
T_2: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Rain tree (*Albiia saman*)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

Figure 2. Performance of sawdust substrate on the average diameter of stalk of ear mushroom (*Auricularia auricula*)

Statistically significant variation was recorded in terms of diameter of stalk of ear mushroom due to different sawdust treatment (Figure 2). The highest diameter of stalk was found in T_5 (1.31 cm) treatment was statistically similar with T_3 (1.28 cm) and T_1 (1.18 cm) treatment, while the lowest diameter of stalk was attained in T_4 (0.93 cm) treatment. Chen (1998) reported significant effects of various substrates on diameter of stalk. Powell (2006) found that stalk of ear mushroom on different sawdust varied from 0.99 cm to 2cm.

4.2.3 Diameter of pileus (cm)

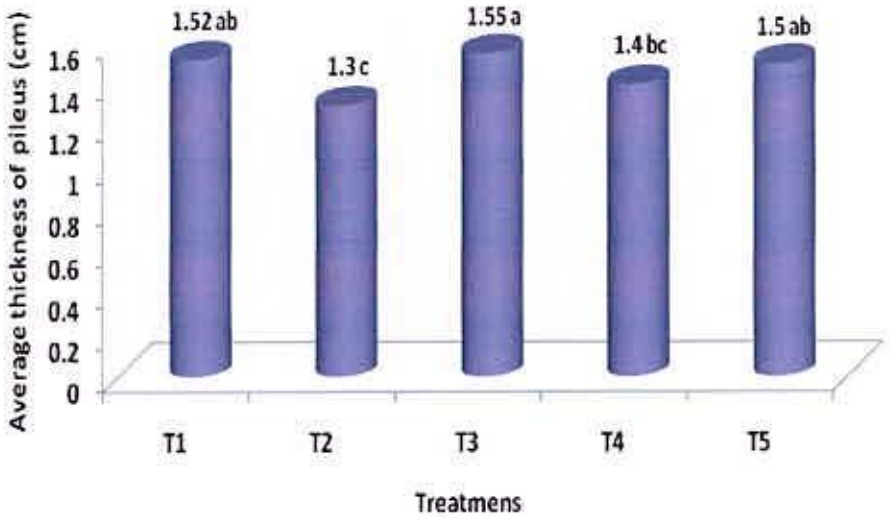


T_1:Teak tree *(Tectona grandis)* sawdust supplemented with 30% wheat bran and 1% lime
T_2: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Rain tree (*Albiia saman*)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

Figure 3. Performance of sawdust substrate on the average diameter of pileus of ear mushroom (*Auricularia auricula*)

Different sawdust method showed statistically significant variation in terms of diameter of pileus of ear mushroom (Figure 2). The highest diameter of pileus was recorded in T_5 (6.03 cm) treatment which was statistically similar with T_1 (5.71 cm) and T_2 (5.12 cm) and T_3 (5.59 cm) whereas the lowest diameter of pileus was observed in T_4 (3.99 cm) treatment. Paterson (2006) reported the diameter pileus ranged from 5.66 to 7.44 cm. The highest diameter of pileus (7.44 cm) was found in autoclaved sawdust mixed with wheat bran.

4.2.4 Thickness of pileus



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

Figure 4. Performance of sawdust substrate on the average thickness of pileus of ear mushroom (*Auricularia auricula*)

Significant difference was recorded in terms of thickness of pileus of ear mushroom due to different sawdust packet (Figure 3). The highest thickness of pileus was observed in T_3 (1.55 cm) treatment which was closely followed with T_1(1.52 cm) and T_5 (1.50 cm) treatment. On the other hand, the lowest thickness of pileus was found in T_2 (1.30 cm) treatment which was closely followed by T_4 (1.40 cm). Powell (2006) reported the thickness of pileus ranged from 0.47 to 1.55 cm respectively.

4.3 Effect of different sawdust substrates on biological yield

4.3.1 Biological yield

Different sawdust substrates had the significant variation on biological yield of ear mushroom which show in Table 4. The highest biological yield was recorded from T_5 (276.21 g/packet), while the lowest biological yield was recorded in T_4 (248.57 g/packet). The result of the present study found similar with the previous studies (Gottlieb *et al.*, 1998; Wasser *et al.*, 2005 and Yang *et al.*, 2003). Erkel *et al.* (2009) found the highest biological yield 287.3 g/packet. Gottlieb *et al.* (1998) examined the effects of adding various lime percentage and temperature fluctuation gave the highest yield of ear mushroom.

4.3.2 Economic yield

Economic yield of ear mushroom grown on different sawdust showed statistically significant variation (Table 4). The highest economic yield was recorded from T_5 (261.11 g/packet), whereas the lowest economic yield was observed in T_4 (237.41 g/packet). The finding of experiment also supported by the earlier findings of Paterson, *et al.* (2006) and Amin *et al.* (2007). Amin *et al.* (2007) found that the trend of economic yield corresponded with different supplements at different level.

4.3.3 Dry yield

The highest dry yield was observed from T_5 (26.46 g), which was statistically similar with T_5 (25.35 g/packet), On the other hand the lowest dry yield was attained in T_1 (18.33 g/packet). The result of the present study was supported by the study of previous researcher Gomez *et al.* (2007) who found the range of dry yield ranged from 14.28 to 29.98 g/packet of *Auriculari* grown on different sawdust. Kulsum *et al.* (2009) found that the highest dry yield was 31.27 g due to mixture of different sawdust. Chen & Fang (1998) observed that the diameter of pileus increased the quality and yield mushroom and highest dry yield from mango sawdust.

4.3.4 Biological efficiency

The highest biological efficiency was recorded from T_2 (81.41%) and the lowest biological efficiency was observed in T_4 (55.18%). Muhammad (2011); Shen and Royse (2001); and many other researchers reported earlier similar findings from their experiment. Shen and Royse (2001) found supplements combined with basal ingredient results better mushroom quality as well as Biological efficiency.

Table 3. Effect of sawdust on the yield, biological efficiency and benefit cost ratio of *Auricularia auricula*

Treatments	Biological yield (g/packet)	Economic yield (g/packet)	Dry yield (g/packet)	Biological efficiency (%)	Benefit cost ratio
T_1	255.64 d	244.44 d	18.33 e	73.33 d	4.65 c
T_2	268.41 c	251.57 c	20.01 d	81.41 a	4.45 d
T_3	271.71 b	258.23 b	22.64 b	74.72 c	5.00 a
T_4	248.57 e	237.41 e	21.14 c	55.18 e	4.01 e
T_5	276.21 a	261.11 a	25.35 a	79.14 b	4.78 b
$LSD_{(0.05)}$	0.177	0.176	0.025	0.111	0.112
CV%	0.04%	0.04%	0.05%	0.07%	0.86%

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

4.3.5 Benefit cost ratio

Different sawdust substrate showed statistically significant variation in terms of benefit cost ratio of ear mushroom (Table 4). The highest benefit cost ratio was found from T_3 (5.00), on the other hand the lowest benefit cost ratio was recorded in T_4 (4.01). The present findings found similar with the findings of previous research. Lim *et al.* (1997) analyzed the cost and return of different species of *Auricularia* mushroom production and found the BCR of 8.9 and 5.1. Sarker *et al*, (2007) mentioned the performances of substrates were significantly differed based on benefit cost ratio. They reported the highest cost benefit ratio of 6.50 of wheat bran.

4.4 Effect of different sawdust substrates on proximate analysis of *Auricularia auricula*

4.4.1 Moisture content

Statistically significant variation was recorded in terms of moisture content of ear mushroom due to different sawdust treatment (Table 5). The highest moisture content was found in T_3 (86.44%) treatment which was statistically identical with T_2 (86.34%) treatment, while the lowest moisture content was recorded in T_1 (84.13%) treatment which was statistically similar with T_5 (84.64%). The findings of the present experiment corroborate with the Mohammad *et al.* (1996) cultivated the Reishi mushroom on paddy straw, banana leaves, sugarcane baggase, water hyacinth, betel nut husk and he found moisture content varied from 88.15 to 91.64%. Yung (2008) found no significant differences among the mushrooms produced in sawdust.

4.4.2 Dry matter content

Different sawdust treatment varied significantly in terms of dry matter content of ear mushroom (Table 5). The highest dry matter content was found from T_1 (15.82%) treatment which was statistically identical with T_5 (15.36%), whereas the lowest dry matter content was recorded in T_3 (13.56%) treatment which was statistically similar with T_2 (13.67%).

The result of the present study matches with the findings of previous one that reported by Bhuyan (2008), they revealed that the dry matter percentage of the fruiting body was ranged from 9.40 to 9.98 due to sawdust supplemented with different levels of cow dung.

4.4.3 Protein content

All the treatment contains a considerable amount of protein (table 5). The highest protein content was recorded in T_5 (24.74%) treatment which was statistically identical with T_2 (24.54%). On the other hand, the lowest protein content was observed in T_3 (23.18%) treatment which was statistically similar with T_4 (23.45%) treatment. Zhang-Ruihong *et al.* (1998) reported the protein content of mushrooms produced was 27.2% on an average. Sarkar *et al.* (2007) cultivated the ear mushroom and found 26.6-34.1% crude protein.

4.4.4 Lipid content content

Statistically significant variation was recorded in terms of lipid content of *Auricularia* mushroom due to different sawdust treatment (Table 5). The highest lipid content was observed from T_5 (6.83%) treatment, whereas the lowest lipid content was obtained in T_1 (5.91%) which was statistically similar with T_4 (5.94%) treatment. The result 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 ear mushroom.

4.4.5 Ash content

Different sawdust treatment varied significantly in terms of ash content of ear mushroom under the present trial (Table 5). The highest ash content was found in T_2 (8.36%) treatment which was statistically identical with T_1 (8.33%), T_3 (8.15%), again the lowest ash content was recorded in T_5 (7.95%) treatment which was statistically similar with T_4 (8.04 %) treatment. The findings of the present study was supported by the study of Fang, Q. H. & Zhong, J. J. (2002) who found that ash content was ranged from 6.58 to 8.41%.

Table 4. Effect of different sawdust on proximate nutrient composition of *Auricularia auricula*

Treatment	Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	Carbo hydrate (%)	Crude fiber (%)
T_1	84.13 d	15.82 a	23.94 b	5.91 c	8.33 a	37.68 b	24.13 b
T_2	86.34 ab	13.67 c	24.54 a	6.23 b	8.36 a	37.55 b	23.40 c
T_3	86.44 a	13.56 c	23.18 c	5.77 d	8.15 ab	38.52 a	23.75 c
T_4	85.13 c	14.87 b	23.45 c	5.94 bc	8.04 b	37.39 bc	24.79 a
T_5	84.64 d	15.36 a	24.74 a	6.83 a	7.95 bc	36.86 d	23.28cd
$LSD_{(0.01)}$	2.117	0.696	0.601	0.079	0.025	0.008	0.273
CV(%)	0.99	2.13	1.02	0.52	0.16	0.04	0.46

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

4.4.6 Carbohydrate content

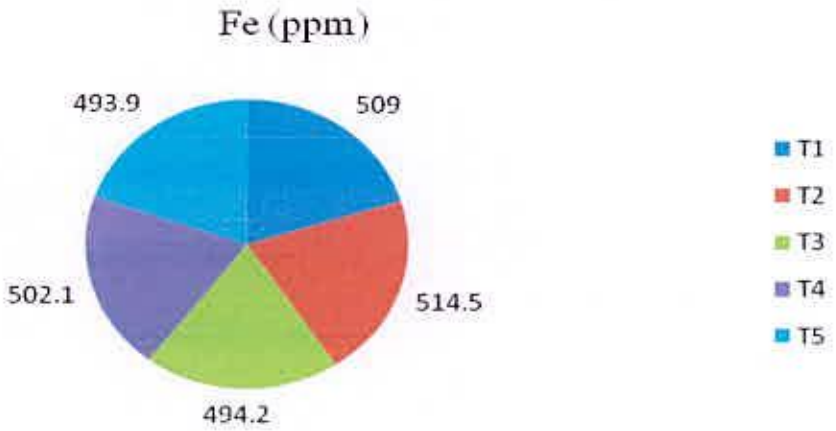
Statistically significant variation was recorded in terms of carbohydrate content of ear mushroom due to different sawdust treatment (Table 5). The highest carbohydrate content was recorded in T_3 (38.52 %) treatment, whereas the lowest was observed in T_5 (36.86 %) treatment. The findings of the present study were not supported by the study of Chang *et al.* (2009) who found that carbohydrate content was ranged from 32.85 to 56.38% which showed a high rate of variation.

4.4.7 Crude fiber content

Statistically significant variation was recorded in term of crude fiber content showed due to different sawdust (Table 5). The highest crude fiber content was found in T_4 (24.79%), while the lowest crude fiber content was obtained in T_5 (23.28%) treatment which was statistically similar with T_2 (23.40%) and T_3 (23.75%) treatment. The findings of the present study corroborate with the study Alam *et al.* (2007) reported 22.87g/100g to 23.29g/100g of fiber in *Ganoderma spp.* Manzi *et al.* (2001) reported that on an average, a serving (100 g) of mushroom will supply 9 to 40% of the recommended of dietary fiber which was also differ from the present study.

4.5 Effect of different sawdust on the mineral content of *Auricularia auricula*

4.5.1 Iron (Fe) content

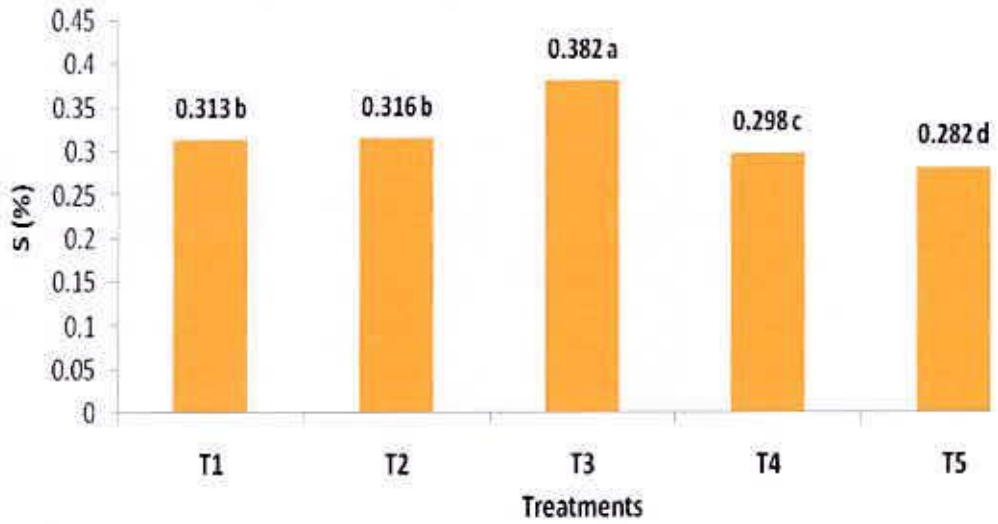


T_1:Teak tree *(Tectona grandis)* sawdust supplemented with 30% wheat bran and 1% lime
T_2: Mahogany tree (*Swietenia mahagoni*) sawdust supplemented with 30% wheat bran and 1% lime
T_3: Mango tree (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime
T_4: Rain tree (*Albiia saman*)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

Figure 5. Performance of sawdust substrate on the Fe(ppm) content of ear mushroom (*Auricularia auricula*)

Fe content of ear mushroom showed statistically significant variation (Figure 4). The highest Fe content was recorded in T_2 (514.5 ppm). On the other hand, the lowest Fe content was observed in T_5 (493.9 ppm) treatment which was statistically similar with T_3 (494.2 ppm) treatment. Sharma *et al.* (1995) reported that content of Fe in the mushroom grown on different substrates varied from 92.09 ppm to 118.40 ppm.

4.5.2 Sulphur (S) content



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

Figure 6. Performance of sawdust substrate on the Sulphur (S) content of ear mushroom (*Auricularia auricula*)

Statistically significant variation was recorded in terms of S content of ear mushroom due to different sawdust treatment (Figure 5). The highest S content was found in T_3 (0.382%), whereas the lowest S content was recorded in T_5 (0.282%). The findings of the present study were supported with the findings of Mizuno *et al.* (2007) who recorded 0.238 to 0.321% of sulphur from their earlier study in oyster mushroom varieties.

4.5.3 Phosphorus (P) content

Different sawdust treatment showed significant differences in terms of P content of ear mushroom (Table 6). The highest P content was observed in T_5 (1.53%) treatment which was statistically identical with T_3 (1.48%), while the lowest P content was found in T_4 (1.29%) treatment.

4.5.4 Potassium (K) content

Significant variation was recorded in terms of K content of ear mushroom due to different treatment (Table 6). The highest K content was recorded in T_5 (2.67%) treatment which was statistically identical with T_1 (2.59 %) and T_3 (2.64%) treatment, whereas the lowest K content was observed in T_2 (2.40%) treatment which was statistically similar with T_4 (2.51%) treatment. The findings of the present study similar with the study of Chang *et al.* (1981) who reported that the fruiting bodies of *Auricularia* contained 1.43 to 1.88 g of K on dry weight basis. Sarker *et al.* (2007) also found 1.3% potassium in ear mushroom which was smaller than the findings of present study.

4.5.5 Calcium (Ca) content

Calcium content of ear mushroom showed statistically significant variation due to different sawdust used under the present trial (Table 6). The highest amount of calcium was observed from T_3 (1.97%) which was followed by T_1 (1.91%), T_2 (1.93%) and T_5 (1.92%), whereas the lowest calcium content was observed in T_4 (1.74 %) which was statistically similar with T_4 (1.57%).

The findings of the present study were lower than the previous reports. 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.

4.5.6 Magnesium (Mg) content

Effect of different sawdust, statistically significant variation was recorded in terms of Mg content of ear mushroom (Table 6). The highest Mg content was found in T_5 (0.73%) treatment which was statistically identical with T_2 (0.73 %) and T_3 (0.72%). On the other hand the lowest Mg content was recorded in T_4 (0.68%) treatment. Sharma *et al.* (2004) also found 0.21% magnesium in ear mushroom which was smaller than the findings of this experiment.

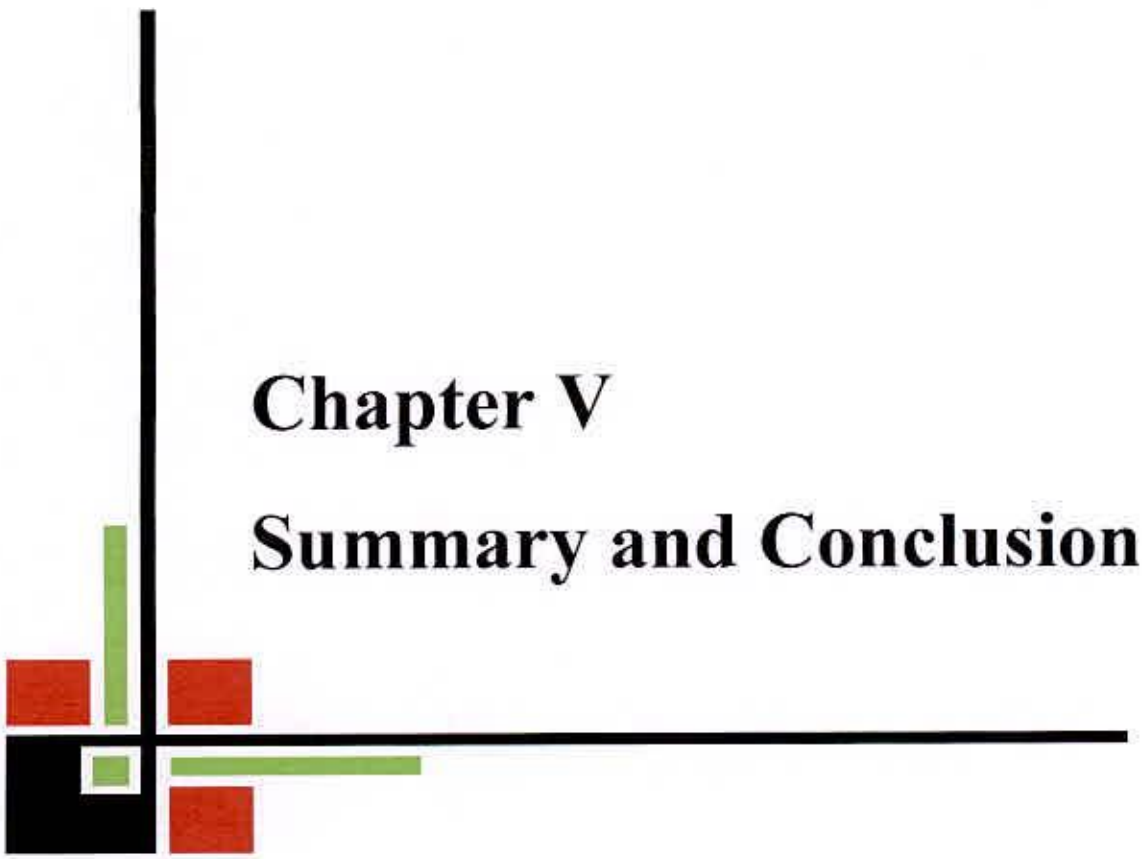
4.5.7 Zinc (Zn) content

Statistically significant variation was recorded in terms of Zn content of ear mushroom due to different sawdust treatment (Table 6). The highest Zn content was observed in T_5 (15.55 %) treatment and the lowest Zn content in T_4 (15.16%) treatment. The results of the present study have the similarity with the study of Alam *et al.* (2007) found from their earlier experiment that zinc content of different ear mushroom ranged from 16 to 20 .09.

Table 5. Effect of different sawdust on major mineral contents of *Auricularia auricula*

Treatments	P (%)	K (%)	Ca (%)	Mg (%)	Zn (%)
T_1	1.46 b	2.59 ab	1.91 a	0.71 b	15.33 d
T_2	1.44 b	2.40 c	1.93 a	0.73 a	15.50 b
T_3	1.48 a	2.64 ab	1.97 a	0.72 a	15.37 c
T_4	1.29 c	2.51 bc	1.74 b	0.68 c	15.16 e
T_5	1.53 a	2.67 a	1.92 a	0.73 a	15.55 a
$LSD_{(0.01)}$	0.008	0.137	0.447	0.008	0.009
CV(%)	0.70	2.29	9.68	0.13	0.06

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



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, and Dhaka during the period from June to November, 2015 to evaluate the performance of different sawdust on the growth, yield and proximate composition of ear mushroom (*Auricularia auricula*). The experiment consists of five different type of sawdust as T_1:Teak tree sawdust +30% wheat bran, T_2: Mahogany sawdust + 30% wheat bran, T_3: Mango sawdust + 30% wheat bran, T_4: Rain tree sawdust + 30% wheat bran and T_5: mixture (Teak tree, Mahogany, Mango and Rain tree) sawdust + 30% wheat bran.

The experiment was laid out in single factor Completely Randomized Design. Data on different growth, yield and nutrient composition and mineral content were recorded and significant variation was recorded for different studied parameter. The highest mycelium running rate (0.88 cm) was recorded from T_5, while the lowest mycelium running rate (0.73 cm) was observed in T_4. The highest time from stimulation to primordial initiation (3.13 days) was found from T_2, whereas the lowest time from stimulation to primordial initiation (2.09 days) was recorded in T_5. The highest time from primordial initiation to harvest (6.80 days) was attained from T_2 and the lowest time from primordial initiation to harvest (5.80 days) was found in T_5. The maximum average number of primordial/packet (74.34) was observed from T_5, again the minimum average number of primordial/packet (64.68) was found in T_4. The highest average number of individual fruiting body (115.5) was attained from T_5 and the lowest average number of individual fruiting body (79.65) was found in T_1. The highest average weight of individual fruiting body (3.67 g) was found in T_5 and the lowest average weight of individual fruiting body (2.76 g) was found in T_3.

The longest length of stalk (2.70 cm) was recorded from T_5, while the shortest length of stalk (1.12 cm) was found in T_4. The highest diameter of stalk (1.31 cm) was found from T_5, whereas the lowest diameter of stripe (0.93 cm) was recorded in T_4. The highest diameter of pileus (6.03 cm) was recorded from T_5, again the lowest diameter of pileus (3.99 cm) was found in T_4. The highest thickness of pileus (1.55 cm) was observed from T_3, and the lowest thickness of pileus (1.30 cm) was found in T_2. The highest biological yield (276.21 g) was attained from T_5, while the lowest biological yield (248.57 g) was recorded in T_4. The highest economic yield (261.11 g) was recorded from T_5, whereas the lowest economic yield (237.41 g) was observed in T_4. The highest dry yield (25.35 g) was observed from T_5, while the lowest dry yield (18.33 g) was attained in T_1. The maximum biological efficiency (81.41%) was recorded from T_2, again the lowest biological efficiency (55.18%) was observed in T_4. The highest benefit cost ratio (5.00) was found from T_3, and the lowest benefit cost ratio (4.01) was attained in T_4.

The highest moisture content (86.44%) was observed from T_3, while the lowest moisture content (84.13%) was found in T_1. The lowest dry matter content (13.56%) was found from T_3, whereas the highest dry matter content (15.82%) was recorded in T_1. The highest protein content (24.74%) was recorded from T_5, while the lowest protein content (23.18%) was observed in T_3. The highest lipid content (6.83%) was found from T_5, again the lowest lipid content (5.91%) was recorded in T_1. The highest ash content (8.36%) was recorded from T_2.

The highest carbohydrate (38.52 %) was observed from T_3, whereas the lowest carbohydrate content (36.86 %) was observed in T_5. The highest crude fiber (24.79%) was recorded from T_4, and the lowest crude fiber content (23.28%) was found in T_5.The highest amount of iron content (514.5 ppm) was attained from T_2, whereas the lowest iron content (493.9 ppm) was found in T_5. The highest amount of sulphur (0.382%) was attained from T_3, again the lowest sulphur content (0.282%) was found in T_5.

The highest amount of phosphorus (1.53%) was observed from T_5, whereas the lowest phosphorus content (1.29%) was observed in T_4. The highest amount of potassium (2.67%) was attained from T_5 and the lowest potassium content (2.40%) was found in T_2. The highest amount of calcium (1.97%) was observed from T_3, whereas the lowest calcium content (1.74%) was observed in T_4. The highest amount of magnesium (0.73%) was found from T_5, while the lowest magnesium content (0.68%) was attained in T_4. The highest amount of zinc (15.55 %) was observed from T_5, whereas the lowest zinc content (15.16%) was recorded in T_4.

Conclusion

From the above discussion, it was observed that treatment T_5 [Mixed sawdust (Teak tree, Mahogany, Mango and Rain tree) + 30% wheat bran], among the treatments performed significantly better on growth, yield, nutrient and mineral content of ear mushroom (*Auricularia auricula)*.

Recommendations

In this experiment, mixed sawdust (Teak tree, Mahogany, Mango and Rain tree) + 30% wheat bran performed better in respect of different growth, yield and nutrient composition and mineral content of ear mushroom. Therefore, mixed sawdust (Teak tree, Mahogany, Mango and Rain tree) + 30% wheat bran substrate can be recommended for wide range cultivation of ear mushroom.

Reference

REFERENCES

Adenipekun, C. O., Lawal, R. and Isikhuemhen, O. S. (2015). Effect of growth supporting additives on the performance of *Auricularia auricula* on *Mansonia altissima* A. chev sawdust. *Int. Food R. J.* **22**(5): 2167-2173.

Ahila, D. P., Veeralakshmi, S., Prakasam, V. and Vinothini, M. (2013). Saw dust and wheat bran substrates for the cultivation of new wood ear mushroom (Auricularia polytricha). *American-Eurasian J. Agric. & Environ. Sci.* **13**(12): 1647-1649.

Ahmed, S. (1998). Performance of differerent substrates on the growth and yield of Oyster mushroom (*Pleurotus sajor-caju* (Fr.) Sing). M.S. thesis, Institute of Postgraduate Studies in Agriculture, Salna. Gazipur. p. 78

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

Amin, M.A. (2004). Studies on mycelium, spawn and production of certain edible mushrooms. M.S. Thesis, Department of Biotechnology, BAU, Mymensingh. 81 P.

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

Ayyappan, S., Chandrasehar, G., Gnanasambandan, S. and Kumaran, K. (2000). Utilization of sugarcane trash and coir waste for the cultivation of Oyster mushroom (*Pleurotus sp.*). *J. Ecobiol.* **12**(4): 317-319.

Banik, S. and Nandi, R. (2004). Effect of supplementation of rice straw with biogas residual slurry manure on the yield, protein and mineral contents of oyster mushroom. *Industrial Crops and Products.* **20**(3): 311-319.

Bano, Z., Shasirekhaandm, M. N. and Rajarathnam, S. (1993). Improvement of the bioconversion of biotransformation efficiencies of the Oyster mushroom. *Enzyme and Microbial Technology.* **15**: 985-989.

Basunia, M. A., Amin, S. M., Sarker, N. C., Jamal, S., & Rahman, A. (2007). Study on the effect of opening patterns of spawn bag on the production of Ganoderma lucidum (Fr.) *Karst. Bangladesh J. Mushroom.* **1**(2): 57-62.

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

Bhatti, M. I., Jiskani, M. M., Wagan, K. H., Pathan, M. A. and Magsi, M. R. (2007). Growth, development and yield of Oyster mushroom, *Pleurotus ostreatus* as affected by different spawn rates. *Pak. J. Bot.* **39**(7): 2685-2692.

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

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

Chang, k. and Fang, H. (1998). The cultivation and nutritional value of *Pleurotus sajor cuju. Eur. J. Appl. Microbiol. Biotechnol.* **14**(1): 38-42.

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

Chang, S. T. and Miles, P. G. (1988). Edible mushroom and their cultivation. CRC Press, Inc. Boca Raton, Florida U.S.A. pp. 27-88.

Chang, S. T. and Hayes, W. S. (1978). The biology and cultivation of edible mushrooms. *Academic press, inc. A subsidiary or Harcourt Brace Jovanovich, Publishers.* pp. 31-42.

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

Chang, S.T., Lau, O.W. and Chowdhury, K.Y. (2009). The cultivation and nutritional value of *Pleurotus sajor cuju. Microbiol. Biotech.* **8**(1): 38-45.

Chen, A. W. (1998). Cultivation of the medicinal mushroom Ganoderma lucidum (Curt.:Fr.) P. Karst. (ear) in North America. *Int. J. Med. Mushroom*. **1**: 263-282.

Chen, H. M. (1998). Reutilization of waste materials from a rice distillery for the cultivation of Ganoderma lucidum. Taiwan: M. S. thesis, Tunghai University.

Chen, H. M., Yang, F. C., and Hsieh, C. (2003). "Use of stillage grain from a rice-spirit distillery in the solid state fermentation of Ganoderma lucidum," Process Biochem. **39**: 21–26.

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

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

Fang, Q. H. & Zhong, J. J. (2002). Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of Ganoderma lucidum. Process Biochemistry. **37**(7): 769-774.

Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for agricultural research.

Gomez, K.A. and Gomez, A.A. (2002). Statistical procedures for agricultural research. John Wiley & Sons, Inc. New York.

Gottlieb, A. M., Saidman, B. O. and Wright, J. E. (1998). Isoenzymes of Ganoderma species from South America. *Mycological Research*. **102**: 415-426.

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

Holman, R. I. (1976). Significance of essential fatty acids in human nutrition, in Lipids, *Raven press*, New York. **1**: 215.

Hossain, M. and Bari, M. A. (1996). Situation of cultivable land in Bangladesh. *Bangladesh J. Soil Sci*. **12**(1): 24-29.

Ijaz, M. and Khan, S.M. (1992). Biological efficiency of different species/strains of lignicolus fungus *Pleurotus* cultivated on different agro-wastes. *Agril. Res.*, **30**(8): 423-427.

Jadhav, A. B., Agal, P. K. and Jadhav, S. W. (1996). Effects of different substrates on yield of oyster mushroom. *J. Maharashtra Agril. Univ.* **21**(3): 424-426.

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

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

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

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

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

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

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

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

Marimuthu, T., Krishnamoorthy, A.S. and Nallathambi, P. (1994). Nam cake amendment for better yield of Oyster mushroom. *Indian J. Myco. Plant Path.*, **24**(2): 103-106.

Mizuno, T. (2011). Studies on bioactive substance and medicinal effect of Reishi - Ganoderma lucidum in Japan. Cannited.com 1-2. Beelman, R.B., D.J. Royse, and N. Chikthimmah. 2004. Bioactive components in Agaricus bisporus of nutritional, medicinal or biological importance. *Mushroom Sci.* **16**:1-16.

Mizuno, T. and Musa, S. (2007). Studies on bioactive substance and medicinal effect of Reishi - Ganoderma lucidum in Japan. Cannited.com 1-2. Beelman, R.B., D.J. Royse, and N. Chikthimmah. 2004. Bioactive components in Agaricus bisporus of nutritional, medicinal or biological importance. Mushroom Science16:1-16.

Mohammad, H. and Karim, H. A. (1996). Study on the effect of opening patterns of spawn bag on the production of Ganoderma lucidum (Fr.) *Karst. Bangladesh J. Mushroom.* **1**(2): 37-52.

Muhammad, I. and Khan, S. M. (1993). Yield performance of different species strains for oyster mushroom (*Pleurotus* spp.) on cotton waste. *Pakistan J. Phytopathol.* **5**(12): 53-57.

Namdev, J. K., Thakur, M. P. and Tripathi, P. N. (2006). Effect of different straw substrates on spawn growth and yield of oyster mushroom (*Pleurotus flabellatus*). *Flora-and-Fauna-Jhansi.* **12**(2): 210-212.

Nuruddin, M.M., Rahman, M.H., Ahmed, K.U., Hossain, A. and Sultana, N. (2010). Effect of Cowdung Supplements with Rice Straw on the Yield and Proximate Composition of Oyster Mushroom (*Pleurotus ostreatus*). *Bangladesh J. Mushroom.* **4**(2): 45-52.

Onyango, B. O., Palapala, V. A., Arama, P. F., Wagai, S. O. and Gichimu, B. M. (2011). Suitability of selected supplemented substrates for cultivation of kenyan native wood ear mushrooms (*Auricularia auricula*). *American J. of Food Tech.* **6:** 395-403.

Paterson, R. R. (2006). "Ganoderma": a therapeutic fungal biofactory, Phytochemistry. **68**(15): 1985-2001.

Pathan, A. A., Jiskani, M. M., Pathan, M. A., Wagan, K. H. and Nizamani, Z. A. (2009). Effect of soaking and boiling of substrate on the growth and productivity of oyster mushroom. *Pak. J. Phytopathol.* **21**(1): 01-05.

Powell, M. (2006). The use of Ganoderma lucidum in the management of histamine mediated.

Raghuramulu, N., Madhavan, N. K. and Kalyanasundaram, S. (2003). A Manual of LaboratoryTechniques. National Institute of Nutrition. Indian Council of Medical Reasearch, Hyderabad-500007, India. pp:56-58.

Rahman, S. M. (2007). Nutritional and Biochemical Analysis of edible mushrooms in three developmental stages. M. Sc. Thesis. Department of Biochemistry. University of Dhaka.

Royse, D.J., Fales, S.L. and Karunanandaa, K. (1991). Influence of formaldehyde treated soybean and commercial nutrient supplementation on mushroom (*Pleurotus sajor-caju*) yield and *in-vitro* dry matter digestibility of spent substrate. *Applied Microbiol. Biotechnol.*, **36**(3): 425-429.

Royse, D.J., Fales, S.L. and Karunanandaa, K. (2002). Influence of formaldehyde treated soybean and commercial nutrient supplementation on mushroom (*Pleurotus sajor-caju*) yield and *in-vitro* dry matter digestibility of spent substrate. *Applied Microbiol. Biotechnol.*, **36**(3): 425-429.

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

Sarker, N. C., Hossain, M. M., Sultana, N., Mian, I. H., Karim, A. J. M. S. and Amin, S. M. R. (2007). Performance of different substrates on the growth and yield of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer. *Bangladesh J. Mushroom.* **1**(2): 44-49.

Sarker, N. C., Kakon, A. J., Moonmoon, M., Khan, A. S., Mujib, T. B., Haque, M. M. and Rahman, T. (2011). Effect of pretreated sawdust and pasteurized straw with various combinations on yield of oyster mushroom (*Pleurotus ostreatus*). *Bangladesh J. Mushroom.*, **5**(2): 39-45.

Sharma, S. R., Chandha, K. L. and (1995). Advances in Horticulture. Mushroom, Malhotra Publication house, New Delhi.**13**: 649.

Sharma, S. R., Chandha, K. L. and (2004). Advances in Horticulture. Mushroom, Malhotra Publication house, New Delhi.**23**: 49.

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

Singh, A. K., Awasthi, S.K., Bharat and Rai, B. (1995). Utilization of sugarcane trash (dried leaves) for production of Oyster mushroom, *Pleurotus florida*. *Mushroom Res.* **4**(1): 35-38.

Suprapti, S. (1987). Utilization of wood waste for substrate of Oyster mushroom *Pleurotus ostreatus* cultivation. *J. Penelitian.* **4**(3): 50-53.

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

Wasser, S. P. (2005). Reishi or Lingzhi (Ganoderma lucidum), Encyclopedia of dietary Supplements, P. 603-622. Toronto. pp. 201-430. BioMed Research International. p. 1-13.

World Bank. (2004). World Development Reports. *Oxford University Press, Inc*, New York.

Yung, H. K. (2008). Edible mushroom and their cultivation. CRC Press, Inc. Boca Raton, Florida U.S.A. pp. 78-88.

Zape, A. S., Thakur, M. P., Bainade, P. S. and Nichal, S. S. (2006). Analysis of major chemical constituents and yield of three different species of *Pleurotus* on wheat straw. *J. of Plant Disease Sci.* **1**(2): 171-172.

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

Appendices

APPENDICES

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

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

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

Appendix II. Effect of different sawdust substrate on yield contributing character and yield of ear mushroom (*Auricularia auricula*)

Treatments	Average diameter of stalk (cm)	Average thickness of pileus (cm)	Diameter of pileus (cm)
T_1	1.18 ab	1.52 ab	5.71 a
T_2	1.11 b	1.30 c	5.12 ab
T_3	1.28 a	1.55 a	5.59 ab
T_4	0.93 c	1.40 bc	3.99 c
T_5	1.31 a	1.50 ab	6.03 a
$LSD_{(0.05)}$	0.137	0.137	1.023
CV (%)	4.85	4.00	7.88

Appendix III. Effect of different sawdust substrate on proximate composition of ear mushroom (*Auricularia auricula*)

Treatments	Fe (ppm)	S (%)	Zn (%)
T_1	509.0 b	0.313 b	15.33 c
T_2	514.5 a	0.316 b	15.50 b
T_3	494.2 d	0.382 a	15.37 c
T_4	502.1 c	0.298 c	15.16 d
T_5	493.9 d	0.282 d	15.55 a
$LSD_{(0.01)}$	0.009	0.008	0.009
CV (%)	0.04	0.32	0.06