# EFFECT OF DIFFERENT SAWDUST SUBSTRATE ON THE GROWTH, YIELD, AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus high-king*)

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**MASTER OF SCIENCE**
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**DEPARTMENT OF BIOCHEMISTRY**
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By

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## CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF DIFFERENT SAWDUST SUBSTRATE ON THE GROWTH, YIELD, AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus high-king*)"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN BIOCHEMISTRY**, embodies the result of a piece of *bona fide* research work carried out by **Md.Reduan Rishad**, Registration No. **08-02999,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in any other institutes.

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

**Dated:** 10/08/16
**Place: Dhaka, Bangladesh**

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# DEDICATION

*DEDICATED TO*

**THIS THESIS IS LOVINGLY DEDICATED**

**TO**

**MY PARENTS**

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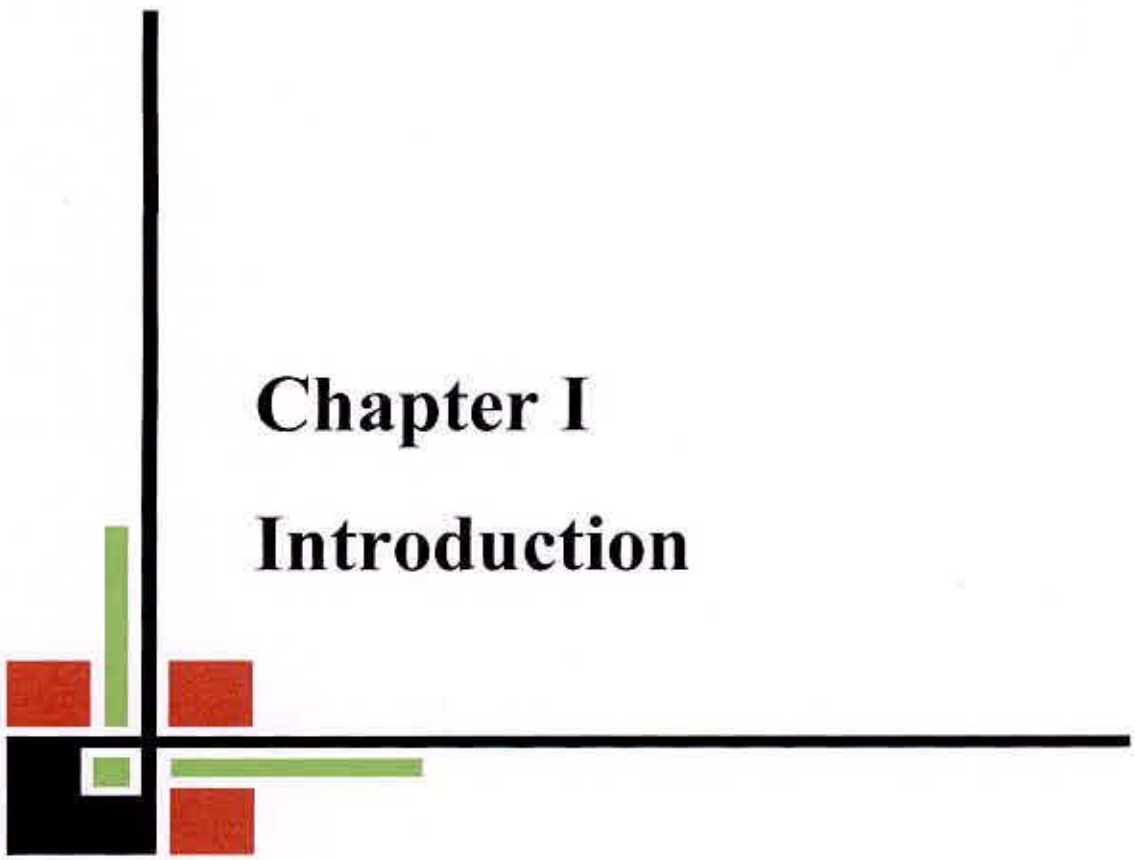
Dated: 10/08/16

The author: MD. REDUAN RISHAD

# EFFECT OF DIFFERENT SAWDUST ON THE GROWTH, YIELD AND PROXIMATE COMPOSITION OF PINK OYSTER MUSHROOM (*Pleurotus high-king*)

## ABSTRACT

The study was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka - 1207 during the period from June to December 2015 to study effect of different sawdust on the growth, yield and proximate composition of oyster mushroom (*Pleurotus high-king*). Mother culture of oyster mushroom (*Pleurotus high-king*) was used as experimental materials. The experiment consists of six different type of sawdust. The experiment was laid out in single factor Completely Randomized Design (CRD) with three replications. Data on different growth, yield and nutrient composition and mineral content were recorded and significant variation was found for different studied parameter. The highest mycelium running rate (0.64 cm) was recorded from $T_3$, while the lowest mycelium running rate (0.47cm) was observed in $T_2$. The maximum average number of fruiting body per packet (83.33) was observed from $T_4$, again the minimum average number of fruiting body per packet (50.00) was found in $T_2$. The highest average weight of individual fruiting body (4.0 g) was attained from $T_1$ and the lowest average weight of individual fruiting body (2.99 g) was found in $T_2$. The highest biological yield observed in $T_4$ and $T_1$ was (297.00g) and the lowest attaind from $T_5$ was (280.4). The highest economic yield (296.7g) was found from $T_4$ and $T_1$.the lowest economic yield was found in $T_5$ (280.0g).The highest benefit cost ratio (4.13) was found from $T_4$, and the lowest benefit-cost ratio (3.88) was attained in $T_2$ and $T_3$. In case of proximate analysis, the highest moisture content (90.38%) was observed from $T_5$, while the lowest moisture content (90.02%) was found in $T_2$. The highest protein content (26.24%) was recorded from $T_4$, while the lowest protein content (21.43%) was observed in $T_2$. The highest carbohydrate (42.23%) was observed from $T_2$, whereas the lowest carbohydrate content (36.85%) was observed in $T_5$.The highest amount of calcium content (2.13%) was attaind from $T_4$, while the lowest (1.53%) from $T_3$. The highest amount of lipid content (4.45%) was attained from $T_4$, whereas the lowest lipid content (3.51%) was found in $T_6$. Among the treatments $T_4$: Mahagony sawdust + 30% wheat bran was found contributed significantly different growth, yield and nutrient composition and mineral content of oyster mushroom (*Pleurotus high-king*).



# Chapter I

# Introduction

# Chapter I

## Introduction

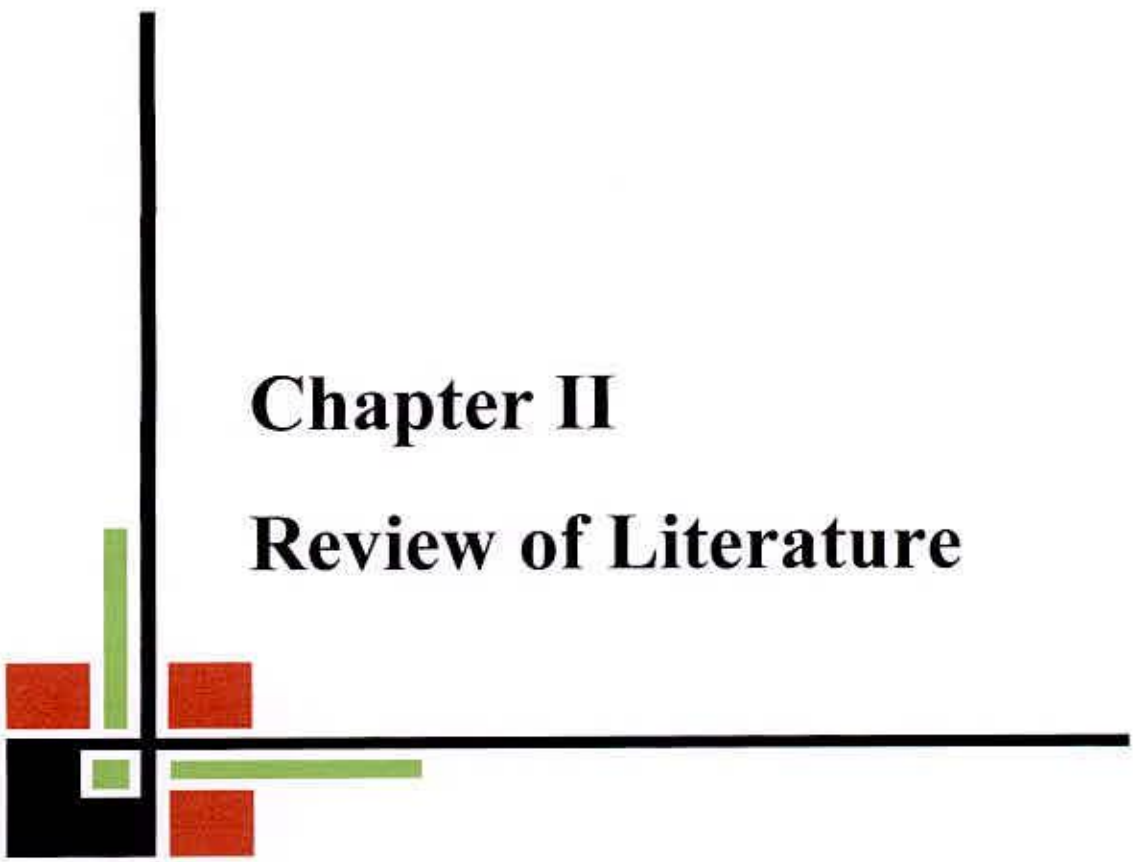
Mushrooms are being recognized as important food items from ancient times. Their usage is being increased day by day for their significant role in human health, nutrition and disease. Mushrooms of *Pleurotus* spp. are commonly known as oyster mushrooms which occupy the second position among cultivated edible mushrooms worldwide due to their nutritional and medicinal values. The environmental factor is very important for the production of oyster mushrooms. Various mushrooms are known to be sensitive to the climatic conditions. The major environmental factors like temperature, humidity, fresh air and compact materials affect in mushroom production.

Pleurotus spp. Grows in wide range of temperature (15-30 0C) which also varies from species to species. Bano and Rajarathnam observed maximum yield of oyster mushroom (Pleurotus sajor-caju) during rainy seasons, when the temperature was nearly 20-26 0C and relative humidity 70-90%. A fairly good yield can be obtained up to 30 0C. Production of P. Fossulatus prefers 20±1 0C but P. Eous prefers 21-35 0C and humidity of 65 to 100%. Maximum growth of P. Ostreatus was recorded at 25 0C by Rangad and Jandaik, whereas P. Florida gave the highest yield at 30 0C. P. Flabellatus also have a similar temperature requirement. Kong reported that P. Ostreatus, P. Florida, P. Sajor-caju reach their optimum growth at 25 0C, while P. Cornucopiae and P. Cystidiosus reach their optimum growth at 25-35 0C temperature.

In Bangladesh, oyster mushrooms are most popular and four different species of this mushroom like *Pleurotus ostreatus, P. florida, P. sajor-caju* and *P. high-king* are commercially cultivated all over the year by using sawdust and/or rice straw as main substrate. But the productions of these mushrooms are not economically beneficial in every season. The environmental variation is supposed to be the main cause behind this problem. But the performances of these species of oyster mushroom have not yet properly been investigated in the climatic conditions of different seasons. Therefore the present study was undertaken to identify the specific season or cultivation time for the specific species of oyster mushroom.

Bangladesh Council of Scientific and Industrial Research (BCSIR) investigated the efficacy of sawdust supplemented with rice or wheat bran as substrate, and found the 9:1 ratio of sawdust and rice bran/wheat bran to be effective for the cultivation of *Pleurotus high-king* with elevated production, even in the large scale. Although extensively used in several Asian and tropical countries, unfortunately its application as medicine in Bangladesh is still in scarce. Commercial-based cultivation of such mushroom in this country is thus critical to confer its extended medicinal use as it could be a suitable alternative to synthetic drugs with less adverse effects. Along these lines, the present study assessed the best cultivation media for achieving high yield, biological efficiency, and growth (mycelial, primordial and fruiting body) rate of *Pleurotus high-king*. The substrates on which mushroom spawn (Merely vegetative seed materials) is grown, affects the mushroom production (Klingman, 1950). *Pleurotus high-king* 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: Jackfruit (*Artocarpus heterophyllus*) sawdust, Mango (*Mangifera indica*) sawdust, Rain tree(*Albizia saman*) sawdust, Shegun (*Tectona grandis*) sawdust ,Mahagony (*Swietenia mahagony*) sawdust and Mixture of all five supplemented sawdust (Jackfruit, Mango, Rain tree, Shegun, Mahagony) with 30% wheat bran and 1%lime as basal substrates were selected for studied their performance on growth yield and nutritional composition of *Pleurotus high-king*. 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:

1. To prepare suitable sawdust based spawn packet.
2. To find out physio-chemical characteristics (*Pleurotus high-king)* mushroom.
3. 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 sawdusts on oyster species. 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.

Mushrooms, a highly priced delicacy for more than two thousand years, are now consumed by many people. Mushroom cultivation is profitable agribusiness. Many agricultural and industrial wastes can be utilized as substrates for production of *Pleurotus* species (Zadrazil & Brunnert, 1981).

Studies conducted by Tan (1981) revealed that cotton waste was the best substrate for the cultivation of Pleurotus ostreatus. Cereal bran rich in protein is usually added to the substrate in *P. ostreatus* cultivation to stimulate mycelia growth and increase the yield of mushroom (Kinugawa et al., 19940)

Sawdust and sugarcane bagasse were the best substrates for growing of Oyster Mushroom than other agro-based substrates (Ahmed, 1998).

Obodai et al. (2002) reported that sawdust substrate for mushroom production should undergo a period of composting to breakdown the cellulose and lignin components of the wood in order to release the essential materials for the establishment of mushroom mycelium. The ligno-cellulosic materials in sawdust are generally low in protein content and thus insufficient for the cultivation of mushrooms, and therefore require additional nitrogen, phosphate and potassium.

Baysal (2003) investigated paper waste supplemented with rice husk, chicken manure and peat for *Pleurotus ostreatus* cultivation. Highest yield for fresh weight was recorded as 350.2 grams in the substrate containing 20% rice husk.

The values of commercial cultivation of mushrooms, especially in a developing economy like Nigeria, is the availability of large quantities of several agro–industrial wastes which can serve as substrates for the cultivation of mushrooms (Banjo et al.,

2004) has been reported that mushrooms can grow on chopped cocoa pods, cotton waste, dried chopped maize straw, oil palm (fibre and bunch) wastes, tobacco straw, used tea leaves, rice straw, sugarcane bagasse, newsprint, old rags and sawdust.

Silva et al. (2005) reported that mycelium extension is related to bio availability of nitrogen when they found that eucalyptus residues supplemented with cereal bran supported fast growth. However, the low amount of available nitrogen (N) in the ligno-cellulosic substrate of wood components is often considered as a limitation to its use as mushroom substrate.

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

Moonmoon *et al.*, (2010) studied king Oyster mushroom *Pleurotus eryngii* on saw dust and rice straw in Bangladesh and found that saw dust showed the highest biological efficiency (73.5%) than other strains. He has also reported on saw dust, the yield and efficiency were better than those cultivated on rice straw, however, on straw; the mushroom fruiting bodies were larger in size. This study shows the prospects of *P. eryngii* cultivation in Bangladesh and suggests further study in controlled environment for higher yield and production.

Stanley et al., 2011 has evaluated the effect of supplementing corn cob substrate with rice bran on yield of *Pleurotus pulmonarius* (Fr) Quel. Un-supplemented corn cob (0% supplementation) gave the best yield in terms of the mean diameter of pileus 5.50cm, mean fresh weight of fruiting bodies 53.2g, mean height of stipe 3.64cm and number of healthy fruiting bodies as 12. The least yield was recorded with 30% supplementation as follows: mean diameter 3.20cm, mean fresh weight of fruiting bodies 30.0g, mean height of stipe 1.65cm and number of healthy fruiting bodies as 5.in terms of quantity and quality, the un-supplemented substrate produced better edible mushrooms

Nasir Ahmad Khan (2012) has observed that *Pleurotus ostreatus* gave the maximum yield in the first flush followed by second and third flush.The maximum yield was obtained on Kikar sawdust 282.2gm followed by Mango sawdust 257.7gm, mixed sawdust 233gm, Simbal sawdust 216.5gm and Kail 200.5gm.Oyster mushroom

showed relatively more yield on control treatment of cotton waste as compared to other substrates. The maximum biological efficiency was obtained in kikar sawdust which was 70.56 %. The lowest biological efficiency was obtained in kikar sawdust which was 50.12 %. Among all substrates, sawdust of Kikar proved the best substrates for the effective cultivation of Oyster mushroom.

Oyster mushrooms are a diverse group of saprotrophic fungi belonging to the genus *Pleurotus* (Kong, 2004). According to Croan (2004), these mushrooms are a good source of non-starchy carbohydrates, with high content of dietary fiber and moderate quantity of proteins, including most amino acids, minerals, and vitamins. The protein content varies from 1.6 to 2.5%, and the niacin content is about ten times higher than that of any other vegetable. Moreover, Randive (2012) reported that oyster mushrooms are rich in Vitamin C, B complex, and mineral salts required by the human body.

Mushroom production in rural communities can alleviate poverty and improve the diversification of agricultural production (Godfrey et al., 2010). For successful cultivation, it is important to select high-yielding strains. However, the production and yield performance of commercial strains of mushrooms tend to decrease after consecutive subculturing (Naraian et al., 2011). Therefore, more information about this genus and its species is necessary to identify good strains to ensure continuous yield improvement (Uhart et al., 2008) and to screen the efficient varieties for Bangladesh.

Oyster mushroom can grow at moderate temperatures, ranging from 20 to 30°C, and at a humidity of 55–70%, on various agricultural waste materials used as substrate. Because of its flexible nature, the *Pleurotus* genus is more cultivated than any other mushroom species (Rosado et al., 2002). The climatic conditions and seasonal diversity of Bangladesh is ideal for the cultivation of the oyster mushroom (Amin et al., 2007b). The collection of the National Mushroom Development and Extension Centre (Namdec) in Bangladesh includes different strains of oyster mushrooms. In recent years, four new strains have been introduced: *Pleurotus high-king* (PHK), *P. ostreatus* (PO3), and *P. geesteranus* (PG1 and PG3). However, the performances of these strains have not yet been properly investigated in the climatic conditions of

Bangladesh. Moreover, studies concerning the nutritive analysis of oyster mushrooms are not available in the country.

Ramesh and Ansari (1987) evaluated several locally available substrates such as rice straw, banana leaves, saw dust, oil palm refuse, oil palm bunch refuse or grass straw in Andamans to study conversion efficiency of *Pleurotus sajor-caju*.

Suprapti (1987) measured the mushroom yield and harvesting frequency after cultivation on Rubber wood (*Hevea brasiliensis*) sawdust mixed with 5, 10, 15 or 20 %, of leaves of either turi (*Sesbonia grandiflora*) or lamtoro gung (*Leucaena leucocephala*). Average total yield per treatment was 643.00 g (532.29-744.69) per kg dry wt. of substrate. Addition of 40% lucerne hay (w/w) or 20% rapeseed meal (w/w) to the barley or wheat straw substrate gave the highest yields (275-300 kg/substrate) of *Pleurotus ostreatus*.

Patil (1989) cultivated *P. sajor-caju* on six different substrates, i.e. wheat straw, bajra (*Pennisetunz americana*), maize straw, paddy straw, jower and cotton stick. The results indicated that all the substrates could be used for commercial cultivation of the oyster mushroom.

Qin (1989) conducted an experiment to evaluate the performance of five species of *Pleurotus* grown on cotton seed hulls, wheat, rice and maize straw. The crude protein content of the fruiting bodies was varied with different substrates. *Pleurotus sajor-caju* contained 41.26 % crude protein when cultivated on rice straw and 29 % when cultivated on wheat straw.

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

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.

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 ranged from (3.56kg/$m^2$ for non-supplemented substrates) to (7.36 kg/$m^2$ for substrate) supplemented (12% DW) with formaldehyde soybean meal.

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.

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

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

Isik *et al.* (1995) conducted an experiment to find out the best preparation formulas of horse manure and synthetic compost. Horse manure, wheat straw, gypsum as basic materials and wheat bran, cotton seed meal, sunflower meal, malt sprout, chicken food, molasses, ammonium sulphate, urea as activators were used. The nitrogen content of the starting mixture was brought up 2 in all applications. According to the results, the highest yields with horse manure compost were obtained from the combinations of 1000 kg of horse manure, 50 kg of wheat bran, 3.1 kg of ammonium sulphate, and 1.5 kg of urea, 35 kg of gypsum 40 kg of chicken food or malt sprout. The highest yields with synthetic compost were obtained from the combinations of 1000 kg of wheat straw, 282 kg of wheat bran, 13 kg of urea, 23.5 kg of ammonium nitrate, 40 kg of molasses, 60 kg of gypsum, 65 kg of cotton seed meal or 100 kg of chicken food.

Murugesan *et al.* (1995) cultivated mushroom P. *sajor-caju* (Fr.) Sing, on water hyacinth (*Elchhorni crassipe*). They compared water hyacinth with other conventional substrates paddy straw. Total yields for 20 bags of the two substrates were 15.0 and 10.5 kg respectively, although the time taken to reach the pin-head stage was longer on the water hyacinth substrate (17 days in water hyacinth and 10 days in paddy straw). The high yield on water hyacinth was attributed to the C: N ratio (24.3 compared with 53.5) and low lignin content (9% compared with 17%) of this substrate. Use of water hyacinth would provide a cheap substrate and a means of eradicating a troublesome aquatic weed.

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

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.

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

Mathew *et al.* (1996) investigated that *Pleurotus sajor-caju*, *Pleurotus* citrinopileatus, *Pleurotus florida*, *Pleurotus platypus* and *Pleurotus ostreatus* were evaluated for their yield performance on various substrates, both for spawn production and cultivation, in the plains and in the high ranges of Kerala in studies conducted in the summer and rainy seasons. Sorghum, wheat and paddy grains were equally good for spawn production. *Pleurotus sajor-caju*, *Pleurotus citrinopileatus* and *Pleurotus florida* were the most suitable species for cultivation in both the plains and the high ranges. These 3 species were successfully cultivated on paddy straw, *Eliocharis plantogena Eleocharis plantaginea* and rubber wood Hevea sawdust, although for commercial cultivation of *Pleurotus sajor-caju*, rubber wood sawdust was not rated as an ideal medium.

Ragunathan *et al.* (1996) investigated that the fruiting bodies of oyster mushroom were rich in nutrients such as carbohydrate, protein, amino nitrogen and minerals and low fat content. The moisture content of the fruiting bodies ranged from 84.70 to 91.90 % and the carbohydrate content ranged from 40.6 to 46.3 %, the crude protein

content ranged from 31.9 to 42.5 %, 26.92 to 38.8%, and 30.0 to 42.5% in *Pleurotus sajor-caju*, *Pleurotus platypus* and *Pleurotus citrinopileatus* respectively.

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%).

Kalita *et al.* (1997) studied the growth of *Pleurotus sajor-caju* in polyethylene bag on different combinations of substrates viz. only rice straw, rice straw plus rice husk mixture (1:1 v/v), water hyacinth, chopped banana leaves, areca nut husk and sugarcane bagasse. They found that only rice straw, rice straw plus rice husk mixture and areca nut husk substrates completed spawn running comparatively within short time (12-14 days) but other substrates took longer time.

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

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

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

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.

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

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

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

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

on the crude protein content of mushroom but increased the yield by 44% over the control.

Khan *et al.* (2001) investigated the different aspects of the cultivation of Oyster mushroom on industrial wastes to push it as a new biotechnology and as a commercial crop in Pakistan. They found that after spawn running, pinhead formation took 7-8 days and sporocarps formed after 10-12 days. Cotton waste recorded the highest yield of 198.67 g. Wheat straw yielded 129.253 g, paper waste + wheat straw yielded 58.95 g and paper waste alone recorded no yield. The best mycelium growth was observed in cotton waste substrate. The average time taken for complete spawn running was 17 days. The second best mycelium growth was on wheat straw, where the average time for spawn running was 19 days. In paper waste, the average time for spawn running was 22 days. However, the average time taken for completion of spawn running on paper waste + wheat straw was 20 days. The differences among the phase of mycelium growth and their interaction with substrate were statistically significant.

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

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.

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

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.

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

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

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

Moni *et al.* (2004) cultivated the oyster mushroom (*Pleurotus sajor-caju*) on paddy straw, banana leaves, sugarcane baggase, water hyacinth and beetle nut husk. The fruit bodies were sun-dried and analyzed for various nutritional parameters. Considerable variation in the composition of fruit bodies grown on different substrates was observed. Moisture content varied from 88.15 to 91.64%. On dry matter basis, the percentage of nitrogen and crude protein varied from 4.22 to 5.59 and 18.46 to 27.78%, respectively and carbohydrate from 40.54 to 47.68%. The variation in content of crude fat and crude fiber ranged from 1.49 to 1.90 and 11.72 to 14.49% respectively whereas, energy value of fruit bodies was between 310.00 and KCal/100 g of fruit body weight.

Shah *et al.* (2004) carried out an experiment to investigate the performance of Oyster mushroom on the following substrates: 50 % sawdust + 50 % wheat straw, 75 % sawdust + 25 % leaves, 50 % wheat straw + 50 % leaves, 100 % sawdust, 100 % wheat straw and 100 % leaves. The temperature was kept at 25 degrees C for spawn running and 17-20 degrees C for fruiting body formation. The time for the completion of mycelial growth, appearance of pinheads and maturation of fruiting bodies on different substrates were recorded. The number of fruiting bodies and the biological efficiency of substrates were observed. The results show that spawn running took 2-3 weeks after inoculation, while small pinhead-like structures formed 6-7 days after spawn running. The fruiting bodies appeared 3-6 weeks after pinhead formation and took 27-34 days later after spawn inoculation. Sawdust at 100 % produced the highest yield (646.9 g), biological efficiency (64.69 %) and the number of fruiting bodies (22.11). Therefore, sawdust is recommended as the best substrate for Oyster mushroom cultivation.

Ancona-Mendex *et al.* (2005) conducted an experiment to grow oyster mushroom (*Pleurotus ostreatus* (Jacq.: Fr.) in either maize or pumpkin straw. Samples were taken for each one of the three harvests and analyzed for total nitrogen (N) content and amino acids profile. The substrate had no effect ($P>0.05$) on N content and amino acid profile of the fruits. However, N (g/100 g DM) increased ($P<0.05$) from 4.13 g in the first harvest to 5.74 g in the third harvest. In general, the amino acids tended to be higher on the first harvest samples, but no changes were found ($P>0.05$) in the amino

acid profile due to substrate or harvest, except for valine decreasing ($P<0.05$) from 3.96 to 3.15 g/16 g N. Changes in the N content of the fruit could be explained by changes in the stipe and pileus proportions as they had different N content (3.15 and 5.48 + or 0.031 g N/ 100 g DM respectively). The amino acid profile of the mushroom was adequate according to the FAO/WHO/UNU adult human amino acid requirements.

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.

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

content was low. For mineral contents in the mushrooms the trend was the same in all treatments. The K and P contents were high compared to the other minerals in all treatments, sodium was moderate while both Mg and Ca were found at low concentrations (Mg was relatively higher than Ca). Fe and Zn were relatively high compared to Cu and Mn which had very low concentrations.

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).

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.*

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

wheat straw and sawdust (7.00 days). The number of fruiting body was positively correlated with biological efficiency, biological yield and economic yield of oyster mushroom. The number of fruiting body grown on different substrates differed significantly and the highest number of fruiting body per packet (183.25) was recorded on waste paper, which was significantly higher as compared to all other substrates. The lowest number of fruiting body (19.25) was observed in water hyacinth. Significant variation in biological efficiency, biological yield and economic yield of oyster mushroom were observed in different substrates. The highest economic yield (225.43 g/packet) was estimated from the waste paper followed by wheat straw (215.72 g/packet).The economic yield on sugarcane bagasse was 191.98g/packet, which was statistically identical to that grown on rice straw (183.28 g/packet), kash (182.93 g/packet) and ulu (175.15g/packet).The economic yield on sawdust was 160.40g/packet, which was statistically identical to that on ulu. The lowest economic yield was observed in water hyacinth (33.59g/packet). No fruiting body and economic yield were obtained from para and nepier grasses. Performances of the substrates were compared based on benefit cost ratio (BCR). The highest BCR (6.50) was estimated when wheat straw was used as substrate followed by sugarcane bagasse (5.90), waste paper (5.65), rice straw (5.58) and kash (5.25) The lowest BCR was obtained from water hyacinth (1.05) followed by ulu (4.74) and sawdust (4.90).

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

Sangeetha (2007) carried out an experiment to study the effect of organic amendments on yield performance of pink mushroom. The organic amendments viz., groundnut cake powder, neem cake powder, rice bran and black gram powder were

added at 3 and 5% levels to mushroom beds as amendments during cultivation. Neem cake at 5% level significantly increased the sporophore production (690.1 g) followed by 3% level (675.3 g). These treatments produce fruiting bodies earlier (10.8 to 11 days) than other amendments tried (11.1 to 12 days). Except neem cake powder and rice bran, all the other amendments had little effect on increasing the yield.

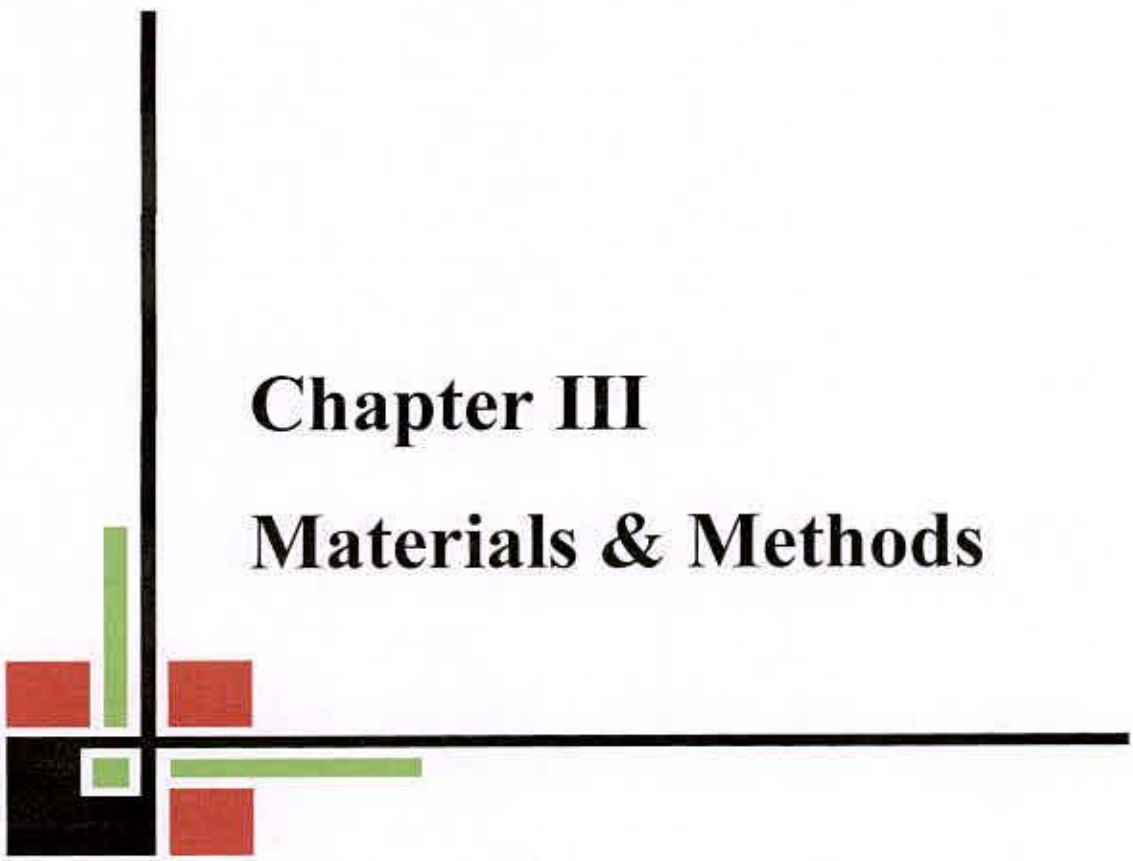
Bhuyan (2008) conducted an experiment to study the effect of various supplements at different levels with sawdust showed significant effort on mycelium running rate and reduced the required days to complete mycelium running in the spawn packet. The supplementation of sawdust found to be significant in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

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%.

Ali *et al.* (2010) conducted an experiment to investigate the performance of different levels of wheat bran (0, 10, 20, 30 and 40 %) as supplement with sugarcane bagasse on the yield and proximate compositions of oyster mushroom were studied. The highest mycelium growth rate (0.96 cm/day), the highest average number of primordia/packet (70.67), average number of fruiting body/packet (61.00) were observed in sugarcane bagasse supplemented with 40% wheat bran. The lowest time from primordia initiation to harvest (3.23 days) and the highest average weight of individual fruiting body (3.69 g) were observed in 30% level of wheat bran. The highest biological yield (254.7 g / 500 g wet substrate), economic yield (243.3 g), dry matter (23.40 g), biological efficiency (87.82%) and benefit cost ratio (8.29) were also observed in 30% level of wheat bran. The highest content of protein (30.31 %), ash (9.15 %) and crude fiber (24.07 %) and the lowest content of lipid (3.90 %) and carbohydrate (32.57 %) were recorded in 30% wheat bran.

Nuruddin *et al.* (2010) carried out an experiment to investigate the effect of different levels of cow dung (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 cow dung. 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 cow dung. The highest protein content (30.90%), crude fiber (24.03%) and the lowest lipid (3.34%) were found in 10% cow dung.



# Chapter III

# Materials & Methods

# CHAPTER III

# MATERIALS AND METHODS

The study was conducted during the period from June to December 2015 to study effect of different sawdust on the growth, yield and proximate composition of oyster mushroom (*Pleurotus high-king*). The chapter includes a brief description of the location of experimental site, soil 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-1207. Details of the meteorological data during the period of the experiment was collected from the Bangladesh Meteorological Department, Agargoan, Dhaka-1207 and presented in Appendix I.

## 3.2 Planting materials

Mother culture of *Pleurotus high-king* mushroom was collected from National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka.

## 3.3 Varietal characteristics of *Pleurotus high-king* mushroom

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

### 3.4 Treatment of the experiment

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

$T_1$: Jackfruit sawdust + 30% wheat bran

$T_2$: Mango sawdust + 30% wheat bran

$T_3$: Shegun sawdust + 30% wheat bran

$T_4$: Mahagony sawdust + 30% wheat bran

$T_5$: Rain tree sawdust + 30% wheat bran

$T_6$: Mixed sawdust (Jackfruit, mango, shegun, rain tree and mahagony) + 30% wheat bran

### 3.5 Design and layout of the experiment

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

### 3.6 Preparation of substrates

At first weight of dry sawdust of jackfruit, mango, shegun, rain tree and mahagony 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 primordia appeared 2-4 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

### 3.6.4 Harvesting of mushrooms

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

## 3.7 Data collection:

### 3.7.1 Mycelial growth

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

### 3.7.2 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 $72^0$C temperature for 24 hours and weighed again. The weight of blank envelop was subtracted from both the initial weight. The dry yield was calculated using the following formula (Sarker, 2004):

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

### 3.7.11 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 $100^0$C to $105^0$C 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 $105^0$C 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 $105^0$C for 24 hours. The container was allowed to cool in a desiccator and was weighted. Again, the container was placed in the oven at $105^0$C 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 $105^0$C 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**

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

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

B = ml of NaOH required in the titration of sample

N = Normality of the NaOH

W = Weight of the sample

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

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

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

### 3.8.9 Total fat estimation

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

quantities of ether and the washings were also transferred to the filter paper. The ether in the conical flask was then removed by evaporation, and the flask with the residual was dried in an oven at 80°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 were added to each of all 20 tubes. The tubes were left overnight mixing the contents in the tubes. Covering with the exhaust manifold, the tubes were placed in the digester and the temperature was set to 125°C, turning on the digester, the digestion was continued for 4 hours after boiling started. Every tube was observed to avoid drying.
2. After cooling, the digestion mixture was transferred with distilled water to a 100 ml volumetric flask. Water was added to the flask to make the volume up to the mark.
3. Filtration was performed on a dry filter into a dry bottle, which could be closed

with a screw cap. Keeping the filtrate in the closed bottle Ca, Mg, K, Fe, Mn, Zn, 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,

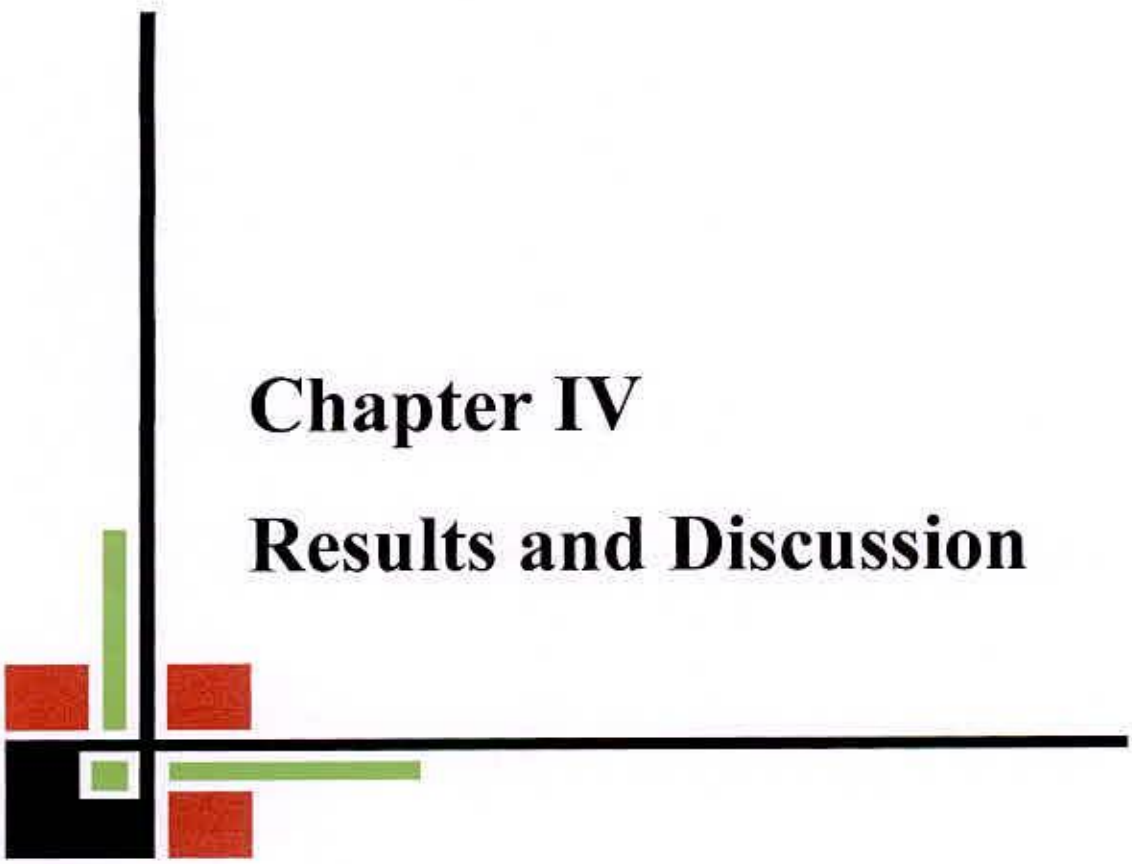
A = weight of $BaSO_4$ g

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

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# RESULTS AND DISCUSSION

The study was conducted to find out the effect of different sawdust on the growth, yield and proximate composition of oyster mushroom (*Pleurotus high-king*). Data on different growth, yield, nutrient composition and mineral content were recorded. The results have been presented and discusses with the help of table and graphs and possible interpretations given under the following headings:

## 4.1 Growth and yield contributing characters

### 4.1.1 Mycelium running rate

Mycelium running rate of oyster mushroom *(Pleurotus high-king)* showed statistically significant variation due to different sawdust under the present trial (Table 1). The highest mycelium running rate was recorded from $T_3$ (0.64 cm) (shegun tree + 30% wheat bran), followed by $T_1$ (Jackfruit sawdust + 30% wheat bran) (0.41 cm), while the lowest mycelium running rate was observed in $T_2$ (0.47 cm) (mango sawdust + 30% wheat bran). Different sawdust showed different mycelium running because of different carbohydrate based on availability and the environment of the spawn. Mycelium running rate varied due to use of different saw dust. Statistically highest mycelium running rate was observed in mixed saw dust. Statistically highest mycelium running rate was observe in mixed saw dust followed jackfruit saw dust and mango saw dust which was statistically similar to the shegun saw dust and followed by mahagony saw dust that also statistically similar to rain tree saw dust. 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.

**Table1. Effect of different sawdust on the growth and yield contributing characters of oyster mushroom (*Pleurotus high-king*).**

| Treatments | Mycelium running rate in spawn packets (cm) | Time from stimulation to primordia initiation (days) | Average number of primordia per packet | Average weight of individual fruiting body (g) | Time from primordial initiation to harvest (days) | Average Number of fruiting body per packet |
|---|---|---|---|---|---|---|
| $T_1$ | 0.48 c | 6.80 a | 150.0 a | 4.00 a | 4.29 a | 79.77 a |
| $T_2$ | 0.47 c | 4.70 c | 140.0 a | 2.99 c | 3.73 ab | 50.00 b |
| $T_3$ | 0.64 a | 6.21 ab | 161.7 a | 3.26 bc | 4.16 a | 80.00 a |
| $T_4$ | 0.55 b | 5.73 b | 136.7 a | 3.04 c | 3.06 c | 83.33 a |
| $T_5$ | 0.48 c | 5.86 b | 166.0 a | 3.20 bc | 3.36 bc | 63.00 ab |
| $T_6$ | 0.49 c | 6.66 a | 166.0 a | 3.45 b | 3.19 bc | 57.33 b |
| LSD (0.05) | 0.05 | 0.67 | 35.71 | 0.33 | 0.59 | 18.75 |
| Level of significance | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| CV(%) | 6.15 | 6.15 | 12.80 | 5.53 | 8.96 | 6.13 |

In column means having similar letter does not vary significantly at 0.05 level of probability.

$T_1$: Jackfruit sawdust + 30% wheat bran

$T_2$: Mango sawdust + 30% wheat bran

$T_3$: Shegun + 30% wheat bran

$T_4$: Mahagony sawdust + 30% wheat bran

$T_5$: Rain tree sawdust + 30% wheat bran

$T_6$: Mixed sawdust (Jackfruit, mango, shegun, mahagony, rain tree) + 30% wheat bran

### 4.1.2 Time from stimulation to primordia initiation

There was significant variation in terms of time from stimulation to primordia initiation of oyster mushroom *(Pleurotus high-king)* due to different sawdust (Table 1). The highest time from stimulation to primordia initiation was found from $T_1$ (6.80 days), which was statistically similar with $T_5$ (5.86 days) and followed by $T_4$ (5.73 days) and $T_3$ (6.21 days), whereas the lowest time from stimulation to primordia initiation was recorded in $T_2$ (4.70 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 Time from primordia initiation to harvest

Data revealed that time from primordia initiation to harvest of oyster mushroom *(Pleurotus high-king)* was statistically significant, due to different sawdust (Figure 1). The highest time from primordia initiation to harvest was recorded from $T_1$ (4.29 days), which was followed by $T_6$ (3.19 days), $T_5$ (3.36 days) and $T_2$ (3.73 days), respectively and there was no significant difference. On the other hand, the lowest time from primordia initiation to harvest was found in $T_4$ (3.06 days). Similar results were also reported by Khan *et al.* (2001); Dhoke *et al.* (2001); Royse, (2002). Khan *et al.* (2001) reported that time from primordia initiation to harvest vary from 3-5 days. Dhoke *et al.* (2001) found significant effect of different agro-wastes on that time from primordia initiation to harvest of oyster mushroom and the days required for the final picking complete from 2.25 to 3.50 days depending on different substrates.

### 4.1.4 Average number of primordia per packet

Average number of primordia per packet of oyster mushroom *(Pleurotus high-king)* varied significantly due to different sawdust under the present trial (Table 1). The maximum average number of primordia per packet was observed from $T_6$ (166.0),

which was followed by $T_5$ (166.0), again the minimum average number of primordia per packet was found in $T_4$ (136.7) which was followed by $T_2$ (140.0). 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 primordia 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 primordia on different substrates. Bhuyan (2008) found similar findings when he growing oyster mushroom on saw dust supplemented with different levels of cow dung.

### 4.1.5 Average number of fruiting body per packet

Significant variation was observed in case of average number of fruiting body per packet of oyster mushroom *(Pleurotus high-king)* (Figure 2). The maximum average number of fruiting body per packet was recorded from $T_4$ (83.33), which was statistically similar with $T_3$ (80.00) and followed by $T_1$ (79.77), while the minimum average number of fruiting body per packet was observed in $T_2$ (50.00). Which was similar to $T_5$ (63.00) respectively and was not statistically different. This variation might be due to variation among the sawdust. The result of the present study found similar with the previous findings of Yoshida *et al.* (1993); Sarker (2004), Bhuyan (2008). Yoshida *et al.* (1993) reported that the number of fruiting bodies was lower, but increased when the substrates was mixed with different supplements. Sarker (2004) found that the number of fruiting body increased with the levels of supplement and continued up to a certain range and decline thereafter. Bhuyan (2008) in a same type of experiment found similar results.

### 4.1.6 Average weight of individual fruiting body

Statistically variation was observed in case of average weight of individual fruiting body of oyster mushroom *(Pleurotus high-king)* for different sawdust under the present trial (Table 1). The highest average weight of individual fruiting body was found from $T_1$ (4.0 g), which was followed by $T_6$ (3.45g). On the other hand, the

lowest average weight of individual fruiting body was found in $T_2$ (2.99 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).

### 4.1.7 Length of stipe

Length of stipe of oyster mushroom *(Pleurotus high-king)* showed statistically significant variation and that might be due to different sawdust under the present trial (Table 2). The longest length of stipe was recorded from $T_6$ (2.67 cm) followed by with $T_1$ (2.53 cm), while the shortest length of stipe was found in $T_5$ (1.76 cm) which was followed by $T_4$ (1.93 cm). Ahmed (1998) reported significant effects of various substrates on length of stalk. Habib (2005) found that the length of stipe of oyster mushroom on different substrates varied from 1.93cm to 2.97cm.

### 4.1.8 Diameter of stipe

Different sawdust showed statistically significant differences in terms of diameter of stipe of oyster mushroom *(Pleurotus high-king)* (Table 2). The highest diameter of stipe was found from $T_6$ (1.12 cm) followed by $T_1$ (1.11 cm), whereas the lowest diameter of stipe was recorded in $T_5$ (0.87 cm) which was followed by $T_4$ (0.91 cm). Ahmed (1998) reported significant effects of various substrates on diameter of stalk. Habib (2005) found that stipe of oyster mushroom on different substrates varied from 0.74 cm to 1.05 cm.

**Table 2. Effect of different sawdust on the dimension of fruiting body of oyster mushroom (*Pleurotus high-king*).**

| Treatments | Length of stipe (cm) | Diameter of stipe (cm) | Diameter of pileus (cm) | Thickness of pileus (cm) |
|---|---|---|---|---|
| $T_1$ | 2.53 ab | 1.11 b | 6.51 a | 0.81 a |
| $T_2$ | 2.45 b | 1.02 c | 6.03 b | 0.74 b |
| $T_3$ | 2.11 c | 1.06 d | 5.76 c | 0.72 b |
| $T_4$ | 1.93 d | 0.91 e | 5.24 d | 0.79 b |
| $T_5$ | 1.76 e | 0.87 f | 5.19 d | 0.62 c |
| $T_6$ | 2.67 a | 1.12 a | 6.85 a | 0.82 a |
| LSD (0.05) | 0.133 | 0.032 | 0.251 | 0.062 |
| Level of significance | 0.05 | 0.05 | 0.05 | 0.05 |
| CV(%) | 7.11 | 3.96 | 6.03 | 5.65 |

In column means having similar letter does not vary significantly at 0.05 level of probability.

$T_1$: Jackfruit sawdust + 30% wheat bran

$T_2$: Mango sawdust + 30% wheat bran

$T_3$: Shegun + 30% wheat bran

$T_4$: Mahagony sawdust + 30% wheat bran

$T_5$: Rain tree sawdust + 30% wheat bran

$T_6$: Mixed sawdust (Jackfruit, mango, shegun, mahagony, rain tree) + 30% wheat bran

### 4.1.9 Diameter of pileus

Diameter of pileus of oyster mushroom *(Pleurotus high-king)* varied significantly might be due to different sawdust under the present trial (Table 2). The highest diameter of pileus was recorded from $T_6$ (6.85 cm), which was statistically similar with $T_1$ (6.51 cm) and followed by $T_2$ (6.03 cm) and $T_3$ (5.76 cm), again the lowest diameter of pileus was found in $T_5$ (5.19 cm) which was statistically similar with $T_4$ (5.24 cm). Ahmed (1998) reported significant effects of various substrates on diameter of pileus. He also found that lower diameter of pileus produced the lowest yield and concluded that the diameter of pileus increased the quality and yield of mushroom and highest dry yield from mango sawdust. Habib (2005) found that the diameter of pileus ranged from 4.85 cm to 8.95 cm.

### 4.1.10 Thickness of pileus

There was statistically significant variation in terms of thickness of pileus of oyster mushroom *(Pleurotus high-king)* might be due to different sawdust (Table 2). The highest thickness of pileus was observed from $T_6$ (0.82 cm), which was statistically similar with $T_1$ (0.81 cm) and followed by $T_4$ (0.79 cm), $T_2$ (0.74 cm) and $T_3$ (0.72 cm) and. On the other hand, the lowest thickness of pileus was found in $T_5$ (0.62 cm). Ahmed (1998) reported significant effects of various substrates on thickness of pileus. He found that lower thickness of pileus produced the lowest yield and concluded that the thickness of pileus increased with the quality and yield of mushroom and highest dry yield also recorded by using mango sawdust in his earlier experiment. Habib (2005) found that thickness of the pileus ranged from 0.45cm to 0.70 cm due to different substrates.

### 4.1.11 Biological yield

Biological yield of oyster mushroom *(Pleurotus high-king)* showed statistically significant variation might be due to different sawdust under the present trial (Table 3). The highest biological yield was recorded from $T_1$ (297.0 g), which was statistically similar with $T_4$ (297.0 g). While is the lowest biological yield was recorded in $T_5$ (280.4g). The result of the present study found similar with the previous studies (Chowdhury *et al.*, 1998; Amin *et al.*, 2007 and Dhoke *et al.*, 2001).

Amin *et al.* (2004) found the highest biological yield 247.3 g/packet. Chowdhury *et al.* (1998) examined the effects of adding different supplements to substrates for growing oyster mushrooms (*Pleurotus sajor-caju*) and found adding 5% supplements gave the highest yield of oyster mushroom. Dhoke *et al.* (2001) found significant effect of different agro-wastes on yield of oyster mushroom. Baysal *et al.* (2003) found the highest yield of Oyster mushroom (*Pleurotus ostreatus*) with the substrate composed of 20% rice husk in weigh.

### 4.1.12 Economic yield

Economic yield of oyster mushroom *(Pleurotus high-king*) grown on different sawdust showed statistically significant variation also (Table 3). The highest economic yield was recorded from $T_4$ and $T_1$ (296.7 g), which was statistically similar with $T_2$ (293.3 g) and followed by $T_3$ (290.0 g), whereas the lowest economic yield was observed in $T_5$ (280.0 g). The finding of this experiment was also supported by the earlier findings of Baysal *et al.* (2003) and Amin *et al.* (2007). Amin *et al.* (2007) found that the trend of economic yield corresponded with different supplements at different level. Baysal *et al.* (2003) found the highest yield of oyster mushroom (*Pleurotus ostreatus*) with the substrate composed of 20% rice husk in weight. Appreciable variations in economic yield also observed at different levels of supplements under different substrate-supplement combinations. Payapanon *et al.* (1994) mentioned that suitable amount of supplements added to sawdust medium maximized economic yield of oyster mushroom at optimum production cost. Sarker (2004) found appreciable variations in economic yield also observed at different levels of supplements under different substrate-supplement combinations. Bhuyan (2008) observed that the yield of *Pleurotus ostreatus* responded with the levels of supplements used with sawdust and increased with the level of supplementation and declined thereafter.

**Table3. Effect of different sawdust on the yield, biological efficiency and benefit cost ratio of oyster mushroom (*Pleurotus high-king*).**

| Treatments | Biological yield (g) | Economic yield (g) | Dry yield (g) | Biological efficiency (%) | Benefit cost ratio |
|---|---|---|---|---|---|
| $T_1$ | 297.0 a | 296.7 a | 30.67 a | 64.77 a | 4.04 ab |
| $T_2$ | 293.6 a | 293.3 a | 31.33 a | 60.68 a | 3.88 b |
| $T_3$ | 290.3 a | 290.0 a | 29.67 a | 44.36 a | 3.88 b |
| $T_4$ | 297.0 a | 296.7 a | 30.67 a | 63.52 a | 4.13 a |
| $T_5$ | 280.4 a | 280.0 a | 29.33 a | 60.03 a | 3.89 ab |
| $T_6$ | 287.1 a | 286.7 a | 31.33 a | 64.50 a | 3.99 ab |
| LSD (0.05) | 19.76 | 19.84 | 3.238 | 25.06 | 0.25 |
| Level of significance | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| CV(%) | 3.73 | 3.75 | 5.83 | 4.65 | 3.47 |

In column means having similar letter does not vary significantly at 0.05 level of probability.

$T_1$: Jackfruit sawdust + 30% wheat bran

$T_2$: Mango sawdust + 30% wheat bran

$T_3$: Shegun + 30% wheat bran

$T_4$: Mahagony sawdust + 30% wheat bran

$T_5$: Rain tree sawdust + 30% wheat bran

$T_6$: Mixed sawdust (Jackfruit, mango, shegun, mahagony, rain tree) + 30% wheat bran

### 4.1.13 Dry yield

Statistically significant variation was recorded in terms of dry yield of oyster mushroom *(Pleurotus high-king)* due to different sawdust (Table 3). The highest dry yield was observed from $T_6$ (31.33 g), which was statistically similar with $T_2$ (31.33 g) and followed by $T_1$ (30.67 g) and $T_4$ (30.67 g), respectively. On the other hand, the lowest dry yield was attained in $T_5$ (29.33 g) which was statistically similar with $T_3$ (29.67 g). The result of the present study was supported by the study of previous researcher Sarker *et al.* (2007) who found the range of dry yield ranged from 4.28 to 29.98 g/packet of *Pleurotus ostreatus* grown on different substrate. Kulsum *et al.* (2009) found that the highest dry yield was 21.27 g due to sawdust. Ahmed (1998) observed similar result in case of dry yield and it was related with the diameter of pileus.

### 4.1.14 Biological efficiency

Different sawdust showed statistically significant variation for biological efficiency of oyster mushroom *(Pleurotus high-king)* under the present trial (Table 3). The maximum biological efficiency was recorded from $T_1$ (64.77%), which was statistically similar with $T_6$ (64.50%) and followed by $T_4$ (63.52%) and $T_2$ (60.68%), again the lowest biological efficiency was observed in $T_3$ (44.36%). Kalita *et al.* (1997); Shen and Royse (2001); Obodai *et al.* (2003) and many other researchers reported earlier similar findings from their experiment. Kalita *et al.* (1997) observed biological efficiency for different substrates ranged from 35.2 to 60.9%. Obodai *et al.* (2003) found biological efficiency (BE) followed a pattern and ranged from 61.0% to 80.0%. But Biswas *et al.* (1997) found supplementation of substrate promoted biological efficiency (125.75%). Shen and Royse (2001) found supplements combined with basal ingredient results better mushroom quality as well as Biological efficiency.

### 4.1.15 Benefit cost ratio

Benefit cost ratio of oyster mushroom *(Pleurotus high-king)* varied significantly due to different sawdust (Table 3). The highest benefit cost ratio was found from $T_4$ (4.13), which was statistically similar with $T_1$ (4.04) and followed by $T_6$ (3.99). On the other hand, the lowest benefit cost ratio was recorded in $T_2$ and $T_3$ (3.88) which were

statistically similar with $T_5$ (3.88). The present findings found similar with the findings of previous research. Lim *et al.* (1997) analyzed the cost and return of *Volvariella* and *Pleurotus* mushroom production and found the BCR of 8.9 and 5.1, respectively. Ahmed (1998) also observed the benefit cost ratio of 7.32, 23.78 and 16.23 in case of *Pleurotus sajor-caju*. The cause of these variations between the results of this study might be due to consideration of other costs involved in the production of oyster mushroom or might be due to measuring system. Sarker *et al*, (2007) mentioned the performances of substrates were significantly differed based on benefit cost ratio. They reported the highest cost benefit ratio of 6.50 with wheat straw.

## 4.2 Proximate composition

### 4.2.1 Moisture

Moisture content of oyster mushroom *(Pleurotus high-king)* showed statistically significant variation in different treatment (Table 4). The highest moisture content (98.38%) was observed from $T_5$, which was statistically similar (90.31%) to $T_4$ and followed (90.18% and 90.10%) by $T_3$ and $T_1$, while the lowest moisture content (90.02%) was found in $T_2$ which was statistically similar (90.14%) with $T_6$. The result of the present study found more or less similar with the study of previous researchers (Moni *et al.*, 2004; Alam *et al.*, 2007 and Rahman, 1994). Moni *et al.* (2004) cultivated the oyster mushroom (*Pleurotus sajor-caju*) on paddy straw, banana leaves, sugarcane baggase, water hyacinth, betel nut husk and he found moisture content varied from 88.15 to 91.64%. Bhuyan (2008) found no significant differences among the mushrooms produced in sawdust supplemented with wheat bran.

**Table 4. Effect of different sawdust on proximate nutrient composition of oyster mushroom (*Pleurotus high-king*).**

| Treatments | Moisture (%) | Dry matter (%) | Ash (%) | Carbohydrate (%) | Crude fiber (%) | Protein (%) | Lipid (%) |
|---|---|---|---|---|---|---|---|
| $T_1$ | 90.10 a | 9.90 a | 12.40 a | 39.93 b | 17.39 c | 25.33 a | 3.86 b |
| $T_2$ | 90.02 a | 9.98 a | 8.06 c | 42.23 a | 21.49 b | 21.43 e | 4.24 b |
| $T_3$ | 90.18 a | 9.82 ab | 8.73 bc | 39.52 b | 22.20 ab | 24.00 d | 4.15 ab |
| $T_4$ | 90.31 a | 9.69 b | 9.66 b | 41.61 a | 18.41 c | 26.24 b | 4.45 b |
| $T_5$ | 90.38 a | 9.62 b | 8.41 c | 36.85 c | 23.29 a | 25.11 a | 4.21 ab |
| $T_6$ | 90.14 a | 9.86 ab | 9.00 bc | 39.13 b | 21.37 b | 25.94 bc | 3.51 b |
| LSD (0.05) | 0.42 | 0.24 | 0.98 | 1.40 | 1.67 | 0.78 | 1.25 |
| Level of significance | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| CV(%) | 0.26 | 1.35 | 5.80 | 1.93 | 4.45 | 1.73 | 16.97 |

In column means having similar letter does not vary significantly at 0.05 level of probability.

$T_1$: Jackfruit sawdust + 30% wheat bran

$T_2$: Mango sawdust + 30% wheat bran

$T_3$: Shegun + 30% wheat bran

$T_4$: Mahagony sawdust + 30% wheat bran

$T_5$: Rain tree sawdust + 30% wheat bran

$T_6$: Mixed sawdust (Jackfruit, mango, shegun, mahagony, rain tree) + 30% wheat bran

### 4.2.2 Dry matter

Different sawdust showed statistically significant variation in terms of dry matter content of oyster mushroom *(Pleurotus high-king)* (Table 4). The lowest dry matter content was obtained from $T_5$ (9.62%), which was followed by $T_4$ (9.69%), whereas the highest dry matter content was recorded in $T_2$ and $T_1$ (9.98% & 9.90%) which was statistically similar with $T_6$ (9.86%). The result of the present study matches with the findings of previous one that reported by Kulsum *et al.* (2009), they revealed that the dry matter percentage of the fruiting body was ranged from 9.40 to 9.98 due to sawdust supplemented with different levels of cow dung. Bhuyan (2008) found no significant differences among the treatments when cow dung used as supplement. But in this study there was significant differences found among the treatments. This might maybe due to different levels of cultural practices.

### 4.2.3 Protein content

Statistically significant variation was found in terms of protein content of oyster *(Pleurotus high-king)* mushroom due to different sawdust under the present trial (Figure 3). The highest protein content was recorded from $T_4$ (26.24%), which was statistically similar to $T_6$ (25.94%) and followed by $T_1$ (25.33%) and $T_5$ (25.11%), while the lowest protein content was observed in $T_2$ (21.43%) which was statistically similar with $T_3$ (24.00%). The results of the present study was supported by the the findings of previous workers (Chang *et al.*, 1981; Moni *et al.*, 2004 and Zhang-Ruihong *et al.*, 1998). Chang *et al.* (1981) reported that the fruiting bodies of mushrooms contained 26.6-34.1% crude protein. Moni *et al.* (2004) cultivated the oyster mushroom (*Pleurotus sajor-caju*) and found that the percentage of crude protein varied from 18.46 to 27.78% respectively. Zhang-Ruihong *et al.* (1998.) found the protein content of mushroom was 27.2% on an average.

### 4.2.4 Lipid content

Lipid content of oyster mushroom *(Pleurotus high-king)* showed statistically significant variation due to different sawdust under the present trial (Figure 4). The highest lipid content was found from $T_4$ (4.45%), which was statistically similar to $T_2$ (4.24%) and $T_5$ (4.21%) and followed by $T_3$ (4.15%) and $T_1$ (3.86%), again the lowest lipid content was recorded in $T_6$ (3.51%). The results of the present study was found more or less similar with the findings of Alam *et al.* (2007) who reported 4.30 to 4.41% lipids in oyster mushroom grown on different substrates. Kulsum *et al.* (2009) also found that lipid content was ranged from 3.44 to 5.43% due to sawdust supplemented with different levels of cowdung which is more or less similar to the present study.

### 4.2.5 Ash

Data revealed that the amount of ash of oyster mushroom *(Pleurotus high-king)* showed statistically significant variation due to use of different sawdust (Table 4). The highest ash content was recorded from $T_1$ (12.40%), which was statistically similar to $T_4$ (9.66%) and followed by $T_6$ (9.00%) and $T_3$ (8.73%). On the other hand, the lowest ash content was found in $T_2$ (8.06%) followed by $T_5$ (8.41%). The findings of the present study was supported by the study of Kulsum *et al.* (2009) who found that ash content was ranged from 6.58 to 8.41% due to sawdust supplemented with different levels of cow dung. Khlood and Ahmad (2005) reported that ash contents were moderate in the fruiting bodies. Alam *et al.* (2007) reported 8.28 to 9.02% ash in *Pleurotus spp.*

### 4.2.6 Carbohydrate

Different amount of carbohydrate content of oyster mushroom *(Pleurotus high-king)* was recorded under the present trial (Table 4). The highest carbohydrate was observed from $T_2$ (42.23%), which was statistically similar to $T_4$ (41.61%), $T_1$ (39.93%) and $T_3$ (39.52%), whereas the lowest carbohydrate content was observed in $T_5$ (36.85%) which was statistically similar with $T_6$ (39.13%). The findings of the present study are supported by the study of Kulsum *et al.* (2009) who found that carbohydrate content was ranged from 32.85 to 56.38 % due to sawdust supplemented with different levels of cow dung. Chang *et al.* (1981) reported that the fruiting bodies of mushrooms contained 40.30-50.7% carbohydrates.

### 4.2.7 Crude fiber

Statistically significant variation was recorded in term of crude fiber content of oyster mushroom *(Pleurotus high-king)* showed due to different sawdust (Table 4). The highest crude fiber was recorded from $T_5$ (23.29%), which was statistically similar with $T_3$ (22.20%) and followed by $T_2$ (21.49%) and $T_6$ (21.37%) and they were statistically similar. On the other hand, the lowest crude fiber content was found in $T_1$ (17.39%) which was statistically similar to $T_4$ (18.41%). The findings of the present study corroborate with the study Alam *et al.* (2007) reported 22.87g/100g to 23.29g/100g of fiber in *Pleurotus spp.*

**Table 5. Effect of different sawdust on the mineral contents of oyster mushroom (*Pleurotus high-king*).**

| Treatments | Ca (%) | Mg (mg %) | Fe (ppm) | S (mg %) | Zn (mg %) |
|---|---|---|---|---|---|
| $T_1$ | 25.06 b | 18.04 ab | 345.53 d | 0.286 b | 24.97 b |
| $T_2$ | 29.84 ab | 15.22 ab | 512.77 a | 0.286 b | 28.31 a |
| $T_3$ | 26.20 ab | 19.17 ab | 251.21 e | 0.243 c | 27.59 a |
| $T_4$ | 26.83 ab | 21.46 a | 338.56 d | 0.323 a | 23.57 b |
| $T_5$ | 31.63 a | 13.58 c | 480.64 b | 0.307 a | 21.50 c |
| $T_6$ | 27.73 ab | 16.38 bc | 401.21 c | 0.318 a | 21.18 c |
| LSD (0.05) | 6.20 | 4.34 | 8.22 | 0.01 | 1.54 |
| Level of significance | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| CV(%) | 12.24 | 13.80 | 5.31 | 5.71 | 3.46 |

In column means having similar letter does not vary significantly at 0.05 level of probability.

$T_1$: Jackfruit sawdust + 30% wheat bran

$T_2$: Mango sawdust + 30% wheat bran

$T_3$: Shegun + 30% wheat bran

$T_4$: Mahagony sawdust + 30% wheat bran

$T_5$: Rain tree sawdust + 30% wheat bran

$T_6$: Mixed sawdust (Jackfruit, mango, shegun, mahagony, rain tree) + 30% wheat bran

## 4.3 Mineral content

### 4.3.1 Calcium (Ca)

Calcium content of oyster mushroom *(Pleurotus high-king)* showed statistically significant variation due to different sawdust used under the present trial (Table 5). The highest amount of calcium was observed from $T_5$ (31.63%) which was followed by $T_2$ (29.84%), whereas the lowest calcium content was observed in $T_1$ (25.06%) which was statistically similar with $T_3$ (26.20%). 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.

### 4.3.2 Magnesium (Mg)

Variation was observed in terms of magnesium content of oyster mushroom *(Pleurotus high-king)* due to different sawdust under the present trial (Table 5). The highest amount of magnesium was attained from $T_4$ (21.46%) which was followed by $T_3$ (19.17%) and $T_1$ (18.04%). On the other hand, the lowest magnesium content was found in $T_5$ (13.58%) which was followed by $T_2$ (15.22%). Sarker *et al.* (2004) also found 0.21% magnesium in oyster mushroom grown on sawdust based substrates.

### 4.3.3 Iron (Fe)

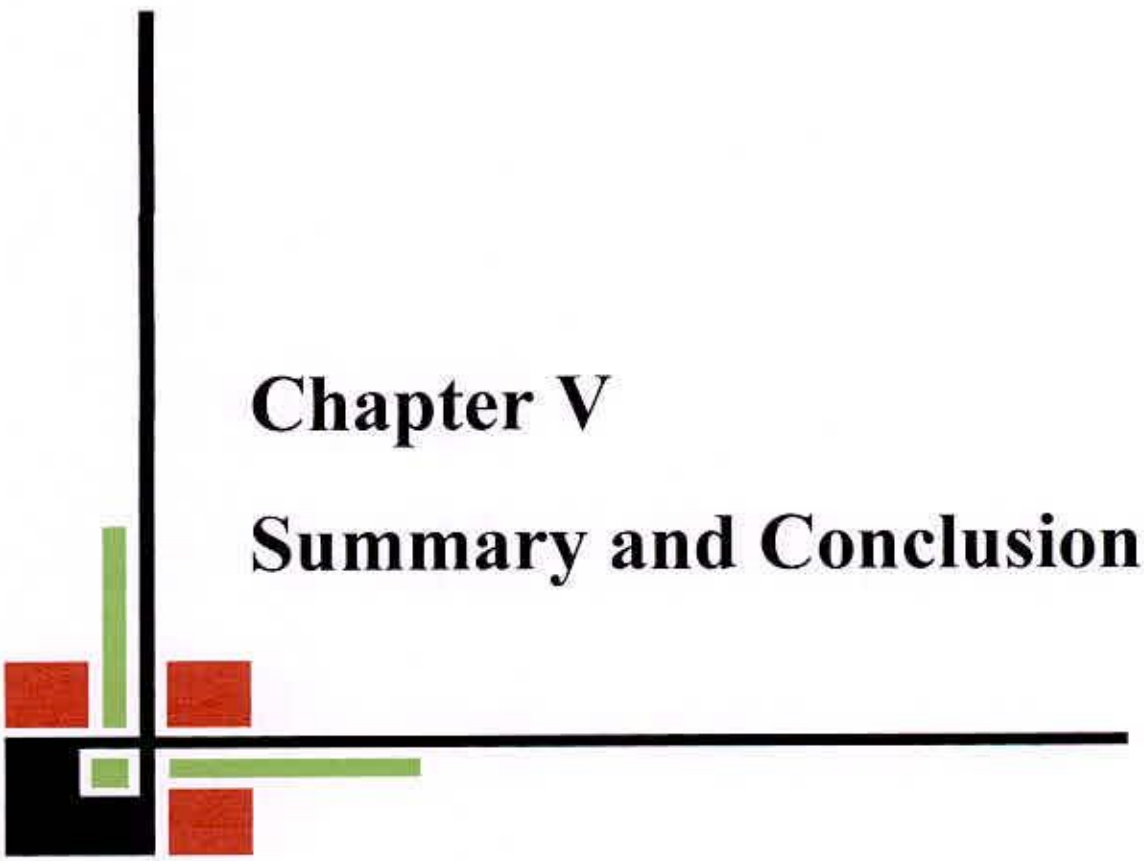
Iron content of oyster mushroom *(Pleurotus high-king)* showed statistically significant variation due to use of different sawdust under the present trial (Table 5). The highest amount of iron was recorded from $T_2$ (512.77 ppm) which was followed by $T_5$ (480.64 ppm), whereas the lowest iron content was observed in $T_3$ (251.21 ppm) which was followed by $T_4$ (338.56 ppm) and $T_1$ (345.52 ppm). The result of the present study found iron higher than the value found by Alam *et al.* (2007) who found that iron content of different oyster mushroom varieties ranged from 33.45 to 43.2 ppm. Kulsum *et al.* (2009) also found that iron content was ranged from 40.5 to 43.4 ppm due to sawdust supplemented with different levels of cow dung.

### 4.3.4 Sulphur (S)

Statistically significant variation was observed in terms of sulphur content of oyster mushroom *(Pleurotus high-king)* due to use of different sawdust under the present trial (Table 5). The highest amount of sulphur was found from $T_4$ (0.323%) which was statistically similar to $T_6$ (0.318%) and $T_5$ (0.307%), while the lowest sulphur content was recorded from $T_3$ (0.243%) which was followed by $T_1$ (0.286%) and $T_2$ (0.286%). The findings of the present study were supported with the findings of Alam *et al.* (2007) who recorded 0.238 to 0.321% of sulphur from their earlier study in oyster mushroom varieties.

### 4.3.5 Zinc (Zn)

Different sawdust showed statistically significant variation in terms of zinc content of oyster mushroom *(Pleurotus high-king)* (Table 5). The highest amount of zinc was obtained from $T_2$ (28.31%) which was followed by $T_3$ (27.59%), whereas the lowest zinc content was recorded in $T_6$ (21.18%) which was followed by $T_5$ (21.50%). 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 oyster mushroom ranged from 16 to 20.9%. Sarker *et al.* (2007a) found 30.92 ppm zinc in oyster mushroom grown on sawdust based substrates.



# Chapter V

# Summary and Conclusion

# CHAPTER V

## SUMMARY AND CONCLUSION

The study was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka - 1207 during the period from June to December, 2015 to evaluate the performance of different sawdust on the growth, yield and proximate composition of oyster mushroom *(Pleurotus high-king)*. The experiment consists of six different type of sawdust as- $T_1$: Jackfruit sawdust + 30% wheat bran; $T_2$: Mango sawdust + 30% wheat bran; $T_3$: Shegun sawdust + 30% wheat bran; $T_4$: Mahagony sawdust + 30% wheat bran; $T_5$: Rain tree sawdust + 30% wheat bran and $T_6$: Mixed sawdust (Jackfruit, mango, shegun, mahagony and rain tree) + 30% wheat bran. 30% wheat bran was taken as basal substrate. 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 (0.64 cm) was recorded from $T_3$, while the lowest mycelium running rate (0.47 cm) was observed in $T_2$. The highest time from stimulation to primordial initiation (6.80 days) was found from $T_1$, whereas the lowest time from stimulation to primordial initiation (4.70 days) was recorded in $T_2$. The highest time from primordial initiation to harvest (4.29 days) was attained from $T_1$ and the lowest time from primordial initiation to harvest (3.06 days) was found in $T_4$. The maximum average number of primordia/packet (166.0) was observed from $T_5$ and $T_6$, again the minimum average number of primordia/packet (136.7) was found in $T_4$. The maximum average number of fruiting body/packet (83.33) was recorded from $T_4$, while the minimum average number of fruiting body/packet (50.00) was observed in $T_2$. The highest average weight of individual fruiting body (4.0 g) was attained from $T_1$ and the lowest average weight of individual fruiting body (2.99 g) was found in $T_2$. The longest length of stipe (2.67 cm) was recorded from $T_6$, while the shortest length of stipe (1.76 cm) was found in $T_5$. The highest diameter of stipe (1.12 cm) was found

from $T_6$, whereas the lowest diameter of stipe (0.87 cm) was recorded in $T_5$. The highest diameter of pileus (6.85 cm) was recorded from $T_6$, again the lowest diameter of pileus (5.19 cm) was found in $T_5$. The highest thickness of pileus (0.82 cm) was observed from $T_6$, and the lowest thickness of pileus (0.62 cm) was found in $T_5$. The highest biological yield (297.0 g) was attained from $T_1$ and $T_4$ while the lowest biological yield (280.4 g) was recorded in $T_5$. The highest economic yield (296.7 g) was recorded from $T_1$ and $T_4$ whereas the lowest economic yield (280.0 g) was observed in $T_5$. The highest dry yield (31.33 g) was observed from $T_6$, while the lowest dry yield (29.33 g) was attained in $T_5$. The maximum biological efficiency (64.77%) was recorded from $T_1$, again the lowest biological efficiency (41.36%) was observed in $T_3$. The highest benefit cost ratio (4.13) was found from $T_4$, and the lowest benefit cost ratio (3.89) was attained in $T_5$.The highest moisture content (90.38%) was observed from $T_5$, while the lowest moisture content (90.02%) was found in $T_2$. The lowest dry matter content (9.62%) was found from $T_5$, whereas the highest dry matter content (9.98%) was recorded in $T_4$ and $T_2$. The highest protein content (26.24%) was recorded from $T_4$, while the lowest protein content (21.43%) was observed in $T_2$. The highest lipid content (4.45%) was found from $T_4$, again the lowest lipid content (3.51%) was recorded in $T_6$. The highest ash content (12.40%) was recorded from $T_1$. The highest carbohydrate (42.23%) was observed from $T_2$, whereas the lowest carbohydrate content (36.85%) was observed in $T_5$. The highest crude fiber (23.29%) was recorded from $T_5$, and the lowest crude fiber content (17.39%) was found in $T_1$. The highest amount of iron (512.77 ppm) was attained from $T_2$, whereas the lowest iron content (251.21 ppm) was observed in $T_3$. The highest amount of sulphur (0.323%) was found from $T_4$, while the lowest sulphur content (0.243%) was attained in $T_3$. The highest amount of zinc (28.31%) was observed from $T_2$, whereas the lowest zinc content (21.18%) was recorded in $T_6$.

## Conclusion

From the above discussion, it was observed that treatment $T_4$: Mahagony sawdust + 30% wheat bran, among the treatments performed significantly better on growth, yield, nutrient and mineral content of oyster mushroom (*Pleurotus high-king)*.

## Recommendations

In this experiment, Mahagony sawdust + 30% wheat bran performed better in respect of different growth, yield and nutrient composition and mineral content of oyster mushroom (*Pleurotus high-king)*. Therefore, $T_1$: Jackfruit + 30% wheat bran substrate can be recommended for farmer level oyster mushroom (*Pleurotus high-king)* cultivation.

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# Appendices

# APPENDICES

**Appendix 1. Monthly record of air temperature, relative humidity, rainfall, and sunshine (average) of the experimental site during the period from June to December 2015.**

| Month (2015) | *Air temperature ($^0$C) | | *Relative humidity (%) | *Rainfall (mm) | *Sunshine (hr) |
|---|---|---|---|---|---|
| | Maximum | Minimum | | | |
| June | 32 | 26 | 79 | 500 | 5.7 |
| July | 31 | 26 | 79 | 600 | 6.7 |
| August | 31 | 26 | 78 | 450 | 8.2 |
| September | 31 | 26 | 78 | 430 | 8.1 |
| October | 31 | 24 | 72 | 150 | 7.8 |
| November | 29 | 19 | 66 | 25 | 4.2 |
| December | 26 | 14 | 63 | 00 | 3.1 |

* Monthly average,

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

**Appendix 2: Analysis of variance on data with the effect of sawdust substrate on mycelium growth of oyster mushroom (*pleurotus high-king*).**

| Source of variance | Degree of freedom | Mean square of | | | |
|---|---|---|---|---|---|
| | | mycelium running rate in spawn packet | time from stimulation to primordia initiation(days) | Average number of primordia per packet | Average weight of individual fruiting body (g) |
| Replication | 2 | 0.000 | 0.198 | 871.056 | 0.028 |
| Treatment | 5 | 0.013* | 1.744* | 514.189$^{NS}$ | 0.405* |
| Error | 10 | 0.001 | 0.136 | 385.322 | 0.034 |

NS Not significant, * significant at 5% level

**Appendix 3:** **Analysis of variance on data with the effect of sawdust substrate on Different growth of oyster mushroom (*pleurotus highking*).**

| Source of variance | Degree of freedom | Mean square of | | | | |
|---|---|---|---|---|---|---|
| | | Biological yield (g) | Economic yield (g) | Dry yield (g) | Biological efficiency (%) | Benefit cost ratio |
| Replication | 2 | 37.582 | 38.889 | 3.167 | 246.026 | 0.089 |
| Treatment | 5 | $123.930^{NS}$ | $125.556^{NS}$ | $2.100^{NS}$ | $179.825^{NS}$ | 0.033* |
| Error | 10 | 117.954 | 118.889 | 3.167 | 189.682 | 0.019 |

NS Not significant. * Significant at 5% level

**Appendix 4:** **Analysis of variance on data with the effect of sawdust substrate on proximate nutrient composition of oyster mushroom (*pleurotus highking*)**

| Source of variance | Degree of freedom | Mean square of | | | | |
|---|---|---|---|---|---|---|
| | | Moisture (%) | Dry matter (%) | Ash (%) | Carbohydrate (%) | Crude fiber (%) |
| Replication | 2 | 0.101 | 0.008 | 0.224 | 0.489 | 1.595 |
| Treatment | 5 | $0.239^{NS}$ | 0.053* | 7.439* | 11.040* | 15.76* |
| Error | 10 | 0.055 | 0.018 | 0.296 | 0.593 | 0.847 |

NS Not significant, * significant at 5% level

**Figures:**

**Fig1: Varietal characteristics of Oyster Mushroom (*Pleurotus high-king*).**

**Fig2: Preparation of spawn packets.**

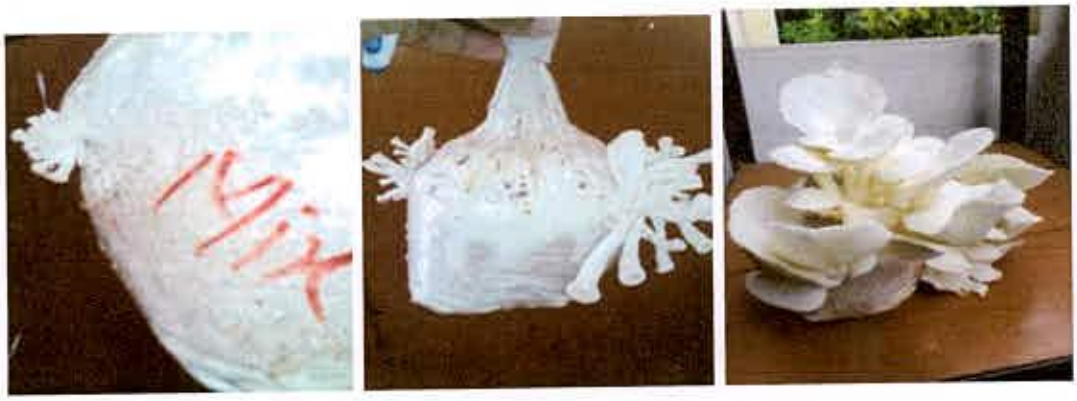

**Fig3: Cultivation of spawn packet.**

**Fig4: Economic yield.**

**Fig5: Drying of mushrooms.**