EFFECT OF DIFFERENT SAWDUST ON THE GROWTH, YIELD AND PROXIMATE COMPOSITION OF *Pleurotus sajor-caju.*

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CERTIFICATE

This is to certify that the thesis entitled **"Effect of Different Sawdust on the Growth, Yield and Proximate Composition of *Pleurotus sajor-caju.*"** 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 bonafide research work carried out by **Akikun Nesa Brinti,** Registration No. **13-05759** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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Abstract

The experiment consists of six different type of sawdust as- T_1: Controlled (Mixture of sawdust) with 30% wheat bran and 1% lime; T_2: Mango sawdust with 30% wheat bran and 1% lime; T_3: Mahogony sawdust with 30% wheat bran and 1% lime; T_4: Jackfruit sawdust with 30% wheat bran and 1% lime; T_5: Teak sawdust with 30% wheat bran and 1% lime and T_6: Rain tree sawdust with 30% wheat bran and 1% lime. 30% wheat bran was taken as basal substrate. The highest economic yield (269.40 g) was recorded from T_2, whereas the lowest economic yield (228.15g) was observed in T_5. The highest benefit cost ratio (5.22) was found from T_2, and the lowest benefit cost ratio (4.05) was attained in T_5. The lowest dry matter content (10.45%) was found from T_5, whereas the highest dry matter content (14.77%) was recorded in T_2. The highest protein content (30.88%) was recorded from T_2, while the lowest protein content (25.25%) was observed in T_5. The highest lipid content (4.25%) was found from T_6. The highest carbohydrate (39.54%) was observed from T_5, whereas the lowest carbohydrate content (34.03%) was observed in T_2. The highest amount of Nitrogen content (4.94%) was attained from T_2, whereas the lowest Nitrogen content (4.04%) was found in T_5. The highest P (0.95%), K (1.28%), Ca (1.97 mg/100mg), Mg (0.738 mg/100mg), iron (513.48 mg/100mg) was attained from T_2, whereas the lowest P (90.72%), K (1.12%), Ca (1.77 mg/100mg), Mg (0.673mg/100mg), iron content (492.08 mg/100mg) was observed in T_5. The highest amount of zinc (16.32%) was observed from T_3, whereas the lowest zinc content (14.36%) was recorded in T_5. In this experiment, T_2: Mango sawdust supplemented with 30% wheat bran and 1% lime performed better in respect of different growth, yield and nutrient composition and mineral content of *Pleurotus sajor-caju*. Therefore, T_2: Mango sawdust supplemented with 30% wheat bran and 1% lime can be recommended for farmer level *Pleurotus sajor-caju* mushroom cultivation.

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LIST OF ABBREVIATED TERMS

ABBREVIATION	FULL NAME
AEZ	Agro-Ecological Zone
et al.	and others
BBS	Bangladesh Bureau of Statistics
cm	Centimeter
^{0}C	Degree Celsius
etc	Etcetera
FAO	Food and Agriculture Organization
LSD	Least Significant Difference
CV	Coefficient of Variation
m^2	Square meter
SAU	Sher-e-Bangla Agricultural University
ppm	Parts per million
BARI	Bangladesh Agricultural Research Institute
NAMDEC	National Mushroom Development and Extension Center
BMD	Bangladesh Metrological Department
BCR	Benefit Cost Ratio
BE	Biological Efficiency
w/v	Weight per Volume
w/w	Weight per Weight
MCC	Mushroom Culture Centre
MRR	Mycelium Running Rate

CHAPTER I

INTRODUCTION

Mushroom is fleshy, spore-bearing fruiting body of a fungus, typically produced above ground on soil or on its food source. Most mushroom-producing fungi are members of the phylum Basidiomycota or Ascomycota. The vegetative part of mushroom consists of thread like long thin mycelium which under suitable condition forms fruiting body or sporocarps. This fruiting body is used as edible mushroom. Mushrooms had long been used for medicinal and food purposes since decades(Suzuki and Oshima,1979). **Mushrooms are low in calories, high in fiber, and contain many important vitamins and minerals.** Some have medicinal properties such as complex carbohydrates that strengthen the immune system. There is a common saying that “Medicines and foods have a common origin” (Kaul, 2001). Out of 2000 species of prime edible mushrooms about 80 have been grown experimentally, 20 cultivated commercially and 4-5 produced on industrial scale throughout the world (Chang and Miles, 1988). Mushroom a food of high quality, flavour and nutrition value have high content of protein, low content of fat (4%), vitamins (B1, B2, C, niacin, biotin etc), minerals (P, Na, K, Ca) and high content of fibers and carbohydrates (Peter, 1991). The Greeks believed that mushroom provided strength for warriors in battle and Romans regarded mushroom as the “Food of the Gods”. Which was served only on festive occasions. The Chinese treasured mushrooms as a health food, the "Elixir of life." The Mexican Indians used mushrooms as hallucinogens in religious ceremonies and in witchcraft as well as for therapeutic purposes (Chang & Miles, 1988). Iman Bukhari (Ra) quoted from holly verse of Prophet Mohammed (S) that “Mushrooms originated from the extract of Manna (the holy Devine food) and it curse eye diseases”.

Bangladesh is a developing country and our agricultural land is decreasing day by day due to accommodation of large population. A healthy person requires 200-250 gm/day. But in our country, on an average, we get only 40-50 gm vegetable per day. To increase the production of vegetable and huge amount of land is required for this purpose. But we are in lack of sufficient land to cultivate vegetable. So,

we have to increase intensive use of land for increasing crop production also considering natural hazards and other calamities. In this case mushroom cultivation can be a huge opportunity for increasing crop production because mushroom requires very small amount of land per unit area with the vertical use of land. As a vegetable, Mushroom can play an important role to meet up the nutritional requirements of the population of our country. Mushrooms have been considered as a special kind of food since the earliest time. Mushroom is a highly nutritious, delicious, medicinal and economically potential vegetable. Mushrooms are used for chronic catarrh diseases of the breast and hinges, mushroom lower the cholesterol level of blood, improves circulation, remedy for night sweating in tuberculosis, rheumatism, gout, jaundice, dropsy, intestinal worms and have anti-tumor, anti-viral and anti-cancer agents (Alam,2007) .

The low calorie and cholesterol free mushroom diets also display certain medicinal properties. Mushroom reduces the diabetic on regular feeding (Anderson and Ward, 1979). It also reduces the serum cholesterol in human bodies which reduces hypertension (Suzuki and Oshima, 1979). Mushroom inhibits the growth of tumor and cancer (Mori, 1986). Edible mushrooms have been treated as important tool in modern medicine for their medicinal values (Kovfeen, 2004). Oyster mushroom contains 19-35% protein on dry weight basis as compared to 7.3% in rice, 13.2% in wheat and 25.2% in milk (Chang & Miles, 1988). It contains 4.0% fat having good quantity of unsaturated fatty acids which are essential in our diet (Holman, 1976). It is rich in essential minerals and trace elements (Chandha and Sharma 1995). Mushrooms are source of Niacin (0.3 g) and Riboflavin (0.4 mg). Mushroom is a good source of trypsin enzyme. It is also rich in iron, copper, calcium, potassium, vitamin D and folic acid (Rahman, 1994). Mushrooms are valuable health food, which are low in calories, high in vegetable proteins, zinc, chitin, fiber, vitamins and minerals (Alam and Saboohi, 2001). Mushroom reduces serum cholesterol and high blood pressure (Mori, 1986). With increasing population and conventional agricultural methods we cannot cope with the food problem. Once, our staple food was rice and fish. At that time we could meet our protein need from fish as well as energy from rice. In

the last decades the fish production decreased and we had to meet our protein need from vegetable source i.e. pulse. But now days this is also much expensive and now we should find out an alternative source of protein as well as other food materials. Mushroom can help us in this aspect. Edible mushrooms are recommended by the FAO as food, to meet protein requirement of developing countries, the large proportion of which depends mainly on cereals (World Bank, 2004). The history of mushroom cultivation is very recent in Bangladesh; its consumption is increasing rapidly in this country.There are various types of mushrooms such as oyster mushroom, milky white mushroom, button mushroom etc. which are cultivated in our country. Among them, several species of oyster mushroom is widely cultivated in our country. Oyster mushroom is an edible mushroom having excellent flavor and taste. In Bangladesh, oyster mushrooms are most popular and four different species of this mushroom like *Pleurotus ostreatus, P. sajor-caju , P. florida,* and *P. high king.* Cultivation of *Pleurotus sajor-caju* has several advantages that can be easily exploited by farmers for cultivation. *P. Sajor-caju* are commercially cultivated all over the year by using sawdust and/or rice straw as main substrate. This mushroom is very easy to cultivate. It does not need any composting like button mushroom. *Pleurotus sajor-caju* is 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. Cultivation of *P. sajor-caju* mushroom has increased tremendously throughout the world because of their abilities to grow at a wide range of temperature and harvested all over the year. Substrate plays an important role in the yield and nutrient content of oyster mushroom.

The substrates on which mushroom spawn (Merely vegetative seed materials) is grown, affects the mushroom production (Klingman, 1950). *Pleurotus sajor-caju* 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 sajor-caju.* 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:

- To improve the yield of *Pleurotus sajor-caju.*
- To prepare suitable substrate based spawn packets.
- To find out the physio-chemical characteristics of *P. sajor-caju* grown on different sawdust.
- To find out cost benefit ratio of suitable sawdust based spawn packets.

CHAPTER II

REVIEW OF LITERATURE

A number of literatures relating to the performance of different substrate on mushroom cultivation are available but performances on same substrate with same supplements in different level are not available. The review of literature given below is based on the present information about the performance of *Pleurotus sajoe-caju* and the effect of different kinds of substrate on mushroom cultivation. The review includes report 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.

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 *et al*. (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*.

Bano *et al*. (1981) and Bisaria *et al*. (1987) have assessed the minerals and heavy metals content in *Pleurotus spp.*

Patil *et al*. (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 *et al*. (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.

Gupta (1989) found that the fruting bodies appeard 12-15 days after the bags were removed and the first crop was harvested 2-3 days later on wheat straw and *Pleurotus sajor-caju* can be successfully cultivated in both hot and spring seasons.

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 3.56 kg/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, 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%).

Krishnamoorthy (1997) cultivated oyster mushroom *Pleurotus citrinopileatus* and *Pleurotus sajor-caju* on paddy straw with 1 of 15 different organic suppliments at 2% of the wet weight of substrate. Neem cake increased the yield of *Pleurotus citrinopileatus* and *Pleurotus sajor-caju* by 48.7 and 75.0% respectively, compared with the control. Red gram husk, green gram husk and black gram husk also significantly increase yield compare with control. Importantly, mushroom harvested from amended paddy straw did not differ in flavor and taste compare with control.

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 (1999) 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.

S. T. Chang, P. G. Miles (1988) finds out the minerals content of some edible mushroom species.

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. 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).

Sarker *et al.* (2007b) found that remarkable difference in nutrient content of oyster mushroom was observed in respect of different substrates. Wide variation was recorded in the protein content of fruiting body. On dry weight basis, the highest protein content (11.63%) was observed in fruiting body grown on sugarcane bagasse. The 2nd highest protein (11.00%) was observed in that grown on wheat straw and water hyacinth. The lowest protein (7.81%) was observed in that grown

on rice straw. Mushrooms are good source of minerals. Maximum of 18400 ppm Ca was found in mushroom which was grown on wheat straw. On other substrates its content varied from 1600 ppm to 18400 ppm. The content of Fe in the mushroom grown on different substrates varied from 92.09 ppm to 118.40 ppm. The highest Fe content was found in waste paper cultured oyster mushroom and lowest on water hyacinth.

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.

Ibekwe *et al.* (2008) cultivated oyster mushroom (*Pleurotus ostreatus*) on different carbohydrate substrates (millet, corn, rice and rye). Millet gave the highest mycelia yield while rye gave the lowest. Further growth on different concentrations of millet extract showed that 1.00 mg\ml concentration of millet extract gave an optimum mycelia growth of 4.00 mg\ml. Cultivation of the mushroom on different nitrogen sources namely, lime beans extract, soybeans (Glycine max) and vigna species (Brown beans) showed that soybean gave the highest mycelia yield while lima beans gave the lowest yield. Optimum mycelia yield was also achieved at pH 6.5. this study shows that given the right substrate and optimal environmental conditions, oyster mushroom can be mass-produced to the meet the nutritional requirement of the Nigerian populace.

Kulsum *et al.* (2009) conducted an experiment to determine the effect of five different levels of cowdung (0%, 5%, 10%, 15% and 20%) as supplement with sawdust on the performance of oyster mushroom. All the treatments performed better over control. The mycelium running rate in spawn packet and the highest number of primordia/packet were found to be differed due to different levels of supplements used. The highest weight of individual fruiting body was observed in sawdust supplemented with cowdung @ 10% (3.69g). The supplementation of sawdust with cowdung had remarkable effect on biological yield, economic yield, the dry yield, biological efficiency and cost benefit ratio. The highest biological yield (217.7 g), economic yield (213g), dry yield (21.27g) biological efficiency

(75.06%) and cost benefit ratio (8.41) were observed due to sawdust supplemented with cowdung @ 10%. Among the chemical characteristics highest content of protein (31.30%), ash (8.41%), crude fiber (24.07%), the lowest lipid (3.44 %) and carbohydrate (32.85%) were observed due to sawdust supplemented with cowdung @ 10%. Among the minerals the highest amount of nitrogen (5.01%), potassium (1.39%), calcium (22.15%), magnesium (20.21%), sulfur (0.043%), iron (43.4%) and the lowest phosphorus (0.92) were observed due to sawdust supplemented with cowdung @ 10%.

The oyster mushroom, *Pleurotus sajor-caju* (Fr.) Singer was cultivated by Pathan *et al*. (2009) on fresh, dried and chopped sugarcane leaves in polythene bags at 400 g per bag. The spawning was done at 50 g/bag, followed by soaking and boiling of substrate. The pinheads were noted first (11.25 days after spawning) in case of soaking till it starts boiling followed by soaking and boiling for 15 and 75 minutes. Minimum period for maturation of fruiting bodies (5.25 days) was in case of soaking and boiling for 30 minutes and soaking and boiling for 75 minutes. The minimum period between flushes (4.25 days) was recorded in soaking and boiling for 30 minutes, followed by soaking and boiling for 75 and 90 minutes. The maximum number of flushes (2.75) was recorded in case of soaking and boiling for 75 minutes followed by 90 and 60 minutes. The maximum fresh yield (61.75%) was obtained in case of soaking and boiling for 75 minutes, followed by soaking and boiling for 90 and 60 minutes. Data revealed that 75 minutes soaking was the best in relation to yield and other studied characters.

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 cowdung (0, 5, 10, 15 and 20%) on yield and proximate composition of *Pleurotus ostreatus*. The highest number of primordia (70.63) and fruiting body (51.92) were observed in rice straw supplemented with 5% level of cowdung. The highest weight of individual fruiting body (4.71g), biological yield (234.24g), economic yield (227.72g), dry yield (22.83g), biological efficiency (140.26%) and benefit cost ratio (5.69) were observed in rice straw supplemented with 10% level of cowdung. The highest protein content (30.90%), crude fiber (24.03%) and the lowest lipid (3.34%) were found in 10% cow dung.

CHAPTER III

MATERIALS AND METHODS

The study was conducted during the period from August-December, 2014 to study the effect of different sawdust on the growth, yield and proximate composition of *Pleurotus sajor-caju*. The chapter includes a brief description of the location of experiment, 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.

3.1. Location of Experiment

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

3.2. Experimental materials

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

3.3. Varietal characteristics of *Pleurotus sajor-caju*

Pleurotus sajor-caju 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 . *Pleurotus sajor-caju* mushroom also popular in the name of gray oyster mushroom. If the temperature increases above 32^0C, its production markedly decreases.

3.4 Treatments of the experiments

Two different experiments with six treatments with three replications were conducted to achieve the desired objectives. The experiments were as follows:

Experiment 1: Effect of different sawdust substrates on yield contributing character of *Pleurotus sajor-caju* mushroom.

Treatments used:

T_1: Controlled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

T_2: Mango (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime

T_3: Mahogony (*Swietenia mahagony*) sawdust supplemented with 30% wheat bran and 1% lime

T_4: Jackfruit (*Artocarpus heterophyllus*) sawdust supplemented with 30% wheat bran and 1% lime

T_5: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime

T_6:Rain tree (*Albizia saman*) sawdust supplemented with 30% wheat bran and 1% lime

Experiment 2: Effect of different sawdust substrates on proximate composition analysis of *Pleurotus sajor-caju* mushroom.

Treatments used:

T_1: Controlled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

T_2: Mango (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime

T_3: Mahogony (*Swietenia mahagony*) sawdust supplemented with 30% wheat bran and 1% lime

T_4: Jackfruit (*Artocarpus heterophyllus*) sawdust supplemented with 30% wheat bran and 1% lime

T_5: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime

T_6:Rain tree (*Albizia saman*) sawdust supplemented with 30% wheat bran and 1% lime

3.5. Design and layout of the experiment

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

3.6. Sterilization procedure

In the laboratory, all instruments, glassware and culture media were sterilized by autoclaving strictly for maintaining sterility.

3.6.1. Sterilization of culture media

The bottles containing the media and also spawn packets were autoclaved with 15 PSI at 121^0C for 1-2 hours. The culture media were allowed to be cold under normal condition after autoclaving.

3.6.2. Sterilization of glassware and instruments

Beakers, test tubes, conical flasks, measuring cylinders flat bottles pipettes, metallic instruments like forceps, scalpels, needles and spatulas, petridishes, culture tubes, nanoabsorbent cotton and brown paper were sterilized in the autoclave at 121^0C for 1 hours at 1.5 kg/cm^2 pressure.

3.6.3. Sterilization of culture room and transfer area

The culture room of the laboratory was cleaned by gently washing with detergent followed by 70% ethyl alcohol regularly. Before inoculation, laminar airflow cabinet was sterilized using ultra violet light for 30 min keeping blower active.

3.6.4. Precautions to ensure sterile condition

All inoculation measures were carried out in the laminar airflow cabinet to avoid contamination. The cabinet was expose on the UV light for 30 min before use. All the instruments and equipments used were sterilized with alcohol before use.

3.7. Production of *Pleurotus sajor-caju* mushroom

To produce *Pleurotus sajor-caju* mushroom following steps were undertaken

3.7.1. Preparation of PDA media:

At first, 250 g potatoes were washed, peeled and slice to prepare 1000ml PDA media. Then peeled and slice potatoes were bolied in water to make them soft and also filtered through a cheese cloth and further water was added to get 1000 ml media. After adding 18 g agar and 20 g dextrose, it was heated and stirred for about 45 min. Then 10 ml media was taken into each of the test tubes and mouths of test tube were plugged with cotton and brown paper. After that all the test tubes were sterilized in an autoclave for 20 minutes at 121^0C for 1 hour at 1.5 kg/cm^2 pressure and kept in slanting position for having maximum space for the organism in pure culture to proliferate.

3.7.2. Tissue culture

To obtain pure culture, a small piece of tissue was collected from the fruting body of *Pleurotus sajor-caju* mushroom and placed on the sterilized PDA medium under aseptic condition in a laminar air flow cabinet. It was then kept for 7-10 days in an incubator under 25^0C for sufficient mycelial growth. These pure culture were used for the entire experiment.

3.7.3. Preparation of mother spawn

Mother culture substrate was prepared by using sawdust. Sawdust was sieved and sun dried. The mother culture substrate was prepared by sawdust and wheat bran in 2:1 ratio with 0.1% calcium carbonate (Ruhul Amin, 2002). Then it was mixed thoroughly with hands and maintained 55% moisture content by adding sufficient water. Then 200 gm of mixture was packed tightly 18*25 cm polypropylene (PP) bag. Each of the bags was 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. The packets were sterilized for 1 hour at 121^0C for 1 hours at 1.5 kg/cm^2 pressure in an autoclave and kept them for cooling. Then inoculums from pure

culture were placed aseptically to the mother spawn packets. The packets after inoculation were again plugged with cotton and were kept at 20-22^{0}C for spawn run. The whole packet containing substrate became white due to fungal mycelia proliferation within 15-20 days and thus ready for spawning the substrate.

3.7.4. Preparation of substrates

Spawn packets using different sawdust, wheat bran, $CaCo_3$ in ratio 69:30:1 respectively and moisture should be maintained. 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%.

3.7.5. Preparation of spawn packets

The mixed substrates were filled into 10×12 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.

3.7.6. 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.

3.7.7. Cultivation of spawn packet

Two ends, opposite to each other of the upper position of plastic bag were cut in "D" shape with a blade and opened by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a 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 22^0C to 25^0C. 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.

3.7.8. Harvesting of mushrooms

Pleurotus sajor-caju 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.

3.8. Data collection

Data were collected on the following parameters

3.8.1. 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.

3.8.2. 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):

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

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

N= Number of days

3.8.3. Days required for completing mycelium running

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

3.8.4. Time from stimulation to primordial initiation (days):

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

3.8.5. Time from stimulation to primordial initiation to harvest (days):

Time required from stimulation to primordial initiation to harvest (days) were recorded.

3.8.6. Average number of primordia per packet:

Number of primordial per packet was recorded.

3.8.7 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.

3.8.8. Average number of effective fruiting body per packet:

Number of well-developed fruiting body was recorded. Tiny fruiting bodies were discarded from counting.

3.8.9. 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.

3.8.10. Dimension of fruiting body (stipe and pileus)

Thickness of the pileus of three randomly selected fruiting bodies was measured using a slide calipers. Thickness of pileus and length of stripe were also measured.

a. Thickness of pileus (cm)
b. Length of stripe (cm)

3.8.11. Biological yield

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

3.8.12. Economic yield

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

3.8.13. 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.

3.8.14. Dry yield

About 50 g of randomly selected mushroom sample was taken in a paper envelop and was weighed correctly. The mushroom was oven dried at 72^0C temperature for 24 hours and weighed again. The weight of blank envelop was subtracted from both the initial weight. The dry yield was calculated using the following formula (Sarker, 2004):

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

3.8.15. 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.

3.8.16. Biological efficiency

Biological efficiency was determined by the following formula (Ahmed, 1998):

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

3.8.17. 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). Market price of raw mushroom is 150-200tk/kg in Bangladesh.

3.9. Proximate analysis of the mushrooms

3.9.1. Collection of the samples

Mushrooms grown from the spawn were collected packet wise and all the wastes and dusts were removed from the fruiting body. Therefore they are ready to be analyzed.

3.9.2. Determination of Moisture

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

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

3.9.3. Determination of dry matter

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

% Dry matter = 100- % moisture content

3.9.4. Grinding

The dried plant materials were cut into small pieces with a knife or scissor. The sample was grinded in a plant grinder fitted with a suitable screen. If the grinding takes a long time, the sample will absorb moisture and it is necessary to dry the sample again in the oven at 105^0C overnight.

3.9.5 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/l00 g sample) = Wt of ash × 100/Wt of sample taken (Raghuramulu *et al.*, 2003)

3.9.6 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.

Therefore,

Crude fiber (g/l00 g sample) = [100-(moisture + fat)] x (We-Wa)/Wt. of sample.

(Raghuramulu *et al.*, 2003).

3.9.7 Total carbohydrate estimation

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

Carbohydrate (g/l00 g sample) = 100 - [(Moisture + Fat + Protein + Ash + Crude Fiber) g/100 g] (Raghuramulu *et al.*, 2003)

3.9.8. Total Fat estimation

Fat was estimated as crude by the ethereal extraction of the deied mushroom using the method that reported by (Raghuramulu *et al.*, 2003). The dried sample (about 5g) was weighted into a conical flask and plugged with fat free cotton. The flask was then placed in an electric shaker and extracted with anhydrous ether for about 16 hours. The ether extract was filtered into another weighted conical flask. The flask containing the original ether extract was washed 4 to 5 times with small quantities of ether and then 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 too 100°C, cooled in a desiccators and weighted. The resuit was expressed as follows:

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

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

3.10. Determination of approximate composition of mineral content

3.10.1. The following Equipments were used

Electric balance, Muffle furnace, Oven, desiccator, Atomic Absorption Spectrophotometer (AAS), Grinding machine, Porcelain crucible, Beaker and Flame photometer etc.

3.10.2. 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.

3.10.2.1. Reagents

a) Concentrated sulfuric acid

   98% H_2SO_4, specific gravity 1.84.

b) Catalyst Mixture

   Crush and mixed 100g potassium sulphate (K_2SO_4) and 100g Copper (II) sulphate ($CuSO_4.5H_2O$) in a motor.

c) 33% Sodium hydroxide

Can be procured as a solution or prepared by dissolving 16.67g NaOH in water in a 5 I volumetric flask. After complete dissolution, the flask is filled to volume with water and the content is mixed.

d) 0.0500 M Sodium hydroxide

Transfer the content of 1 vial sodium hydroxide (4.0g) to a 2 L volumetric flask filled to with water and mixed.

e) 0.0500M hydrocholoric acid

Transfer the content of 1 vial hydrochloric acid (3.645 g) to a 2 L volumetric flask filled to with water and mixed.

f) Methyl red- methylene blue indicator solution

Dissolve 0.667 g methyl red in 500ml 96% ethanol. Also dissolve 0.625 g methylene blue in 500 ml 96% ethanol. Mixed equal volumes of the two solution.

3.10.2.2. Digestion

Step 1: The digester was turned on to reach the digestion temperature (390°C) by the time the samples were ready for digestion.

Step 2: 20 clean and dry digestion tubes were placed in the digestion rack. Every 0.20 g sample was taken in each of 18 tubes. The 2 remaining tubes served as blanks.

Step 3: 1g catalyst mixture and 5ml conc. H_2SO_4 were added to each tube included the blanks.

Step 4: The rack with the tubes was put beside the digester and place the exhaust manifold on the tubes. All the stoppers were properly inserted into the tubes. Then the exhaust pump were started to open the regulating valve fully.

Step 5: The tubes were placed in the digester at 390°C. After about 5 min the suction rate was reduced by almost closing the regulating valve. Digestion was continued for 2 hours.

Step 6: Turning off the digester, the rack with the tubes was removed and placed it besides the digester for cooling. Suction was continued for 5 min; the exhaust manifolds were removed from the tubes and turned off the exhaust pump.

3.10.2.3. Distillation

Some water was distilled following the appropriate distillation procedure and distilled was received in a conical flask for further use 20.00 mi .0500 M HCl was taken from a burette into a conical flask and placed the flask on the platform in the distilled. 25 ml water was added carefully to one of the digestion tubes from the digestion rack. The addition of water was done carefully as the mixture become very hot. The tube was placed in the left hand side of the distilled around the plastic tube. To touch the plastic tube with the hands was avoided. The tube was tightening properly against the upper rubber adapter and then the safety door was closed. 25 ml 33% NaOH was dispensed gently into the digestion tube by pulling the alkali handle to it's down position and was released it. The steam valve was opened by pulling it to its down position and the timer was set to 3 min. When the alarm sounds, the distillation was continued for about 30 sec by lowering the platform with the receiver flask. Closing the steam valve by pushing it up, the safety door was opened and the receiver flask was replaced with another flask containing 20.00 ml 0.05 M HCl. The platform was then pushed up. The content in the flask which was removed from the distilled titrated with 0.05 M NaOH as described below. Following the above procedure all of the digestion tubes were distilled.

3.10.2.4. Titration

4 drops of indicator solution were added to the content in the flask and titrate with 0.05 NaOH until the color was changed from violet to green.

3.10.2.5. Calculation

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

Where,

a = ml HCl measured into the conical task in the distill (usually 20.00 ml)

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

M_{HCl} = Molarity of the HCl measured into the conical flask

M_{NaOH}= Molarity of the NaOH used for titration

c= g of mushroom powder used for the analysis

3.10.3. Determination of total protein:

The total protein was estimated by multiplying total nitrogen with 6.25 (Reff. Standard Micro-Kjeldhal procedure of AOAC,1975)

3.10.4. Determination of Ca, Mg, K, Fe, Zn and S

The sample was digested with nitric acid to release of Ca, Mg, K, Fe, Zn and S. Ca, Mg, Fe, Co, Mo, Se and Zn were determined by atomic absorption spectrophotometer, K was determined by flame photometry and P by spectrophotometer using the following formula.

3.10.4.1. Digestion

Step 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.

Step 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.

Step 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, Zn, and S were determined in the filtrate.

3.10.4.2 Estimation of Ca

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

3.10.4.3 Estimation of Mg

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

3.10.4.4 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.

3.10.4.5 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.

3.10.4.6 Estimation of Fe, Zn and S

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

3.10.4.7 Calculations

For Ca, Mg, K

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

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 Fe, Zn, S

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

Zn and Fe measured on atomic absorption spectrophotometer

c = g sample weighed into the digestion tube

3.11. Statistical analysis of data

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

The study was conducted to find out the effect of different sawdust on the production and proximate composition of *Pleurotus sajor-caju*. The results have been presented and discussed with the help of table and graphs and possible interpretations given under the following headings:

Experiment 1: Effect of different sawdust substrates on yield and yield contributing character of *Pleurotus sajor-caju*

4. 1. Effect on mycelium growth

4.1.1. Effect of different sawdust substrates on mycelium running rate in spawn (cm)

Mycelium running rate per day (MMR) for each type of substrates was measured after the mycelium colony crossed the shoulder of the packet. The linear length was measured at different places of packet. Statistically significant variation was recorded in terms of mycelium running rate per day (MMR) of *Pleurotus sajor-caju* due to different sawdust substrate used (Table 1). The highest running rate was observed in T_2 (0.77 cm) followed by T_3 (0.70 cm) and the lowest mycelium running rate was observed in T_5 (0.59 cm) followed by T_4 (0.62 cm). Different sawdust showed different mycelium running because of different carbohydrate based on availability and the environment of the spawn. The present findings found more or less similar with the previous workers. Khan *et al.* (1991) reported that sawdust amended with different organic supplement like wheat chaff, wheat bran, paddy straw, cotton waste etc. provided suitable condition for spawn running. Sarker (2004) found that the mycelium running rate of oyster mushroom

greatly influenced with the supplement of wheat barns in different levels. Bhuyan (2008) also found similar result as found in the present experiment.

4.1.1.2 Effect of different sawdust substrates on time from stimulation to primordial initiation (Days)

Time from stimulation to primordial initiation varied significantly due to different sawdust used (Table 1).The highest time from stimulation to primordial initiation was observed in T_5 (7.62days) followed by T_4 (7.33 days) , whereas lowest time from stimulation to primordial initiation was shown in T_2 (5.64 days) followed by T_3 (5.76 days). The result of present findings keeps in with with the findings of previous scientists (Gupta 1989, Sarkar 2004, Ruhul Amin, 2007, Bhuyan, 2008). Gupta(1989) found that the fruting bodies appeared 12-15 days after the bags were removed and the first crop was harvested 2-3 days later on wheat straw and *Pleurotus sajor-caju* can be successfully cultivated in both hot and spring seasons. Sarkar, (2004) observed that duration from primordial initiation to first harvest of oyster mushroom was significantly lower as compared to control where no supplement was used and the duration required for total harvest of oyster mushroom increased with the level of supplement used. In the present study, the time required for total harvest also decreased with the level of supplements increased compared to rice straw alone. Ruhul Amin *et al.* (2007) found significant differences among the level of supplements used for preparing the substrates. Bhuyan (2008) also found similar effect as found in the present study.

4.1.1.3 Effect of different sawdust substrates on time from primordial initiation to harvest (Days)

Statistically significant variation was recorded in terms of time from primordial initiation to harvest of *Pleurotus sajor-caju* due to different sawdust substrate used (Table 1). The lowest time from primordial initiation to harvest was in the treatment T_2 (3.26 days) followed by T_3 (3.33 days) and the highest time primordial initiation to harvest was observed in the treatment T_4 (4.33days) followed by T_1 (3.82 days).The result of present findings keeps in with the

findings of previous scientists (Khan *et al.* 2001, Dhoke *et al.* 2001, Royse, 2001). Khan *et al*. (2001) reported that after spwan running pinhead formation took 7-8 days and fruting body formed after 3-5 days, sporocarps may be harvested after 10-12 days. Dhoke *et al.* (2001) found significant effect of different agro-waste on the yield of mushroom. The days required for first picking varied from 11.25-12.00 days and the final picking varied from 42.25-43.50 days depending on different substrates. Royse (2001) found as the spwan rate increased the number of days to production decreased.

Table 1. Effect of sawdust substrates on mycelial growth of *Pleurotus sajor-caju*

Treatments	Mycelium running rate in spawn packets (cm)/dad	Time from stimulation to primordia initiation (days)	Time from primordia initiation to harvest (days)
T_1	0.66 b	6.21b	3.82a
T_2	0.77a	5.64c	3.26c
T_3	0.70 b	5.76b	3.33b
T_4	0.62 b	7.33a	4.33a
T_5	0.59 c	7.62a	3.67a
T_6	0.64b	6.00a	3.58b
CV (%)	6.87%	0.72%	6.71%
LSD (0.05)	0.083	0.084	0.441

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

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime

4.1.2 Effect on yield contributing characters and yield

4.1.2.1 Effect of different sawdust substrates on average no. of primordia/packet

Statistically significant variation was found in terms of average number of primordia per packet of *Pleurotus sajor-caju* due to different sawdust used (Table 2).The highest average number of primordia per packet was observed from T_2 (75.33), which was followed by T_3 (73.67), again the lowest average number of primordia per packet was found in T_5 (62.33) followed by T_6 (65.67) .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.

4.1.2.2 Effect of different sawdust substrates on average number of fruiting body / packet

Statistically significant variation was found in terms of average number of fruiting body per packet of *Pleurotus sajor-caju* due to different sawdust used (Table 2).The highest average number of fruiting body per packet was recorded from T_2(61.33) followed by T_3 (59.67) and T_1(55.67). And the lowest average number of fruiting body per packet was observed in T_5 (51.95) followed by T_4 (53.26).The result of the present findings keeps in with the findings of previous scientists (Yoshida *et al.* 1993, Al amin, 2004; Sarker 2004, Bhuyan 2008). Yoshida *et al.* (1993) reported that the number of fruiting bodies was lower, but increased when the substrates was mixed with different supplements. Amin (2004) reported that the number of primordial grown on different substrates differ significantly. 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.

4.1.2.3 Effect of different sawdust substrates on average no. of effective fruting body/ packet

Statistically significant variation was found in terms of average number of effective fruiting body per packet of *Pleurotus sajor-caju* due to different sawdust used (Table 2).The highest average no of effective fruting body /packet was observed in the treatments T_2 (17.67) followed by T_3 (15.25) and the lowest average no of effective fruiting body /packet was observed in the treatments T_5 (11.65) followed by T_4 (12.33). The findings of the present study matches with the study of Yoshida *et al.* (1993) who reported that the number of effective fruting body were lowest, but increase when the substrates was mixed with different supplements. The comparative similar findings were also found by Adamovie *et al.* (1996), Ruhul Amin (2007) and Ahmed (1998).

4.1.2.4 Effect of different sawdust substrates on average weight of individual fruiting body (g)

Statistically significant variation was found in terms of average weight of individual fruiting body of *Pleurotus sajor-caju* due to different sawdust used (Table 2). Different sawdust substrates had great effect on average weight of individual fruiting body. The average weight of individual fruiting body in different treatments ranged from 4.45g to 2.95g.The highest average weight of individual fruiting body was found from T_2 (4.94g), which was followed by T_6 (4.92g). On the other hand, the lowest average weight of individual fruiting body was found in T_5 (4.39g) which was followed by T_3 (4.43g). 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), which may be due to environmental conditions or growing season.

4.1.2.5 Effect of different sawdust substrates on average length of stipe

Statistically significant variation was found in terms of average length of stipe of *Pleurotus sajor-caju* due to different sawdust used (Table 2). The longest length of stipe was recorded from T_3 (2.69cm) followed by with T_2 (2.55cm), while the shortest length of stipe was found in T_5 (1.78 cm) followed by T_4 (1.95cm). The findings of the present study matches with the study of Habib (2005) and Sarkar *et al.* (2007). Both of two mentioned that the stripe length of *Pleurotus spp.* On different substrate varied from 1.93 cm to 2.97cm and diameter range from 0.74cm to 1.05cm.

4.1.2.6 Effect of different sawdust substrates on average thickness of pileus

Different sawdust showed statistically significant differences in terms of thickness of pileus. The average thickness of pileus in different treatment range from 0.09 to 0.70 cm. All the treatments were statistically similar (Table 2). The highest thickness of pileus was found from T_2 (0.81cm) followed by T_3 (0.80cm), whereas the lowest thickness of pileus was recorded in T_5 (0.64 cm) and T_4 (0.72cm). The findings of present study matches with the study of Habib (2005) who found that the thickness of pileus ranged from 0.45cm to 0.70cm due to different substrates and Sarkar *et al.* (2007) reported that the thickness of pileus ranged from 0.05cm to 0.80 cm in case of oyster mushroom.

Table 2. Effect of different sawdust on yield attributes of *Pleurotus sajor- caju*

Treatments	**Average no. of primordial/ packet**	**Average no. of Fruiting body/packet**	**Average weight of individual fruiting body(g)**	**Average length of stripe (cm)**	**Average thickness of pileus (cm)**
T_1	70.00 b	55.67b	4.71b	2.12b	0.79b
T_2	75.33 a	61.33a	4.94a	2.55b	0.81a
T_3	73.67 b	59.67a	4.43c	2.69a	0.80a
T_4	68.33b	53.26c	4.67b	1.95c	0.72b
T_5	62.33c	51.95d	4.39c	1.78c	0.64c
T_6	65.67b	52.43c	4.92a	2.47b	0.74b
CV (%)	0.61%	0.52%	1.44%	2.30%	6.41%
LSD(0.05)	0.763	0.517	0.099	0.094	0.087

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

T_1: Controlled (Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime

Figure 1

4.1.3.1 Effect of different sawdust substrates on biological yield

Biological yield of *Pleurotus sajor-caju* mushroom varied significantly due to different sawdust used under the present trial (Table 3). Different sawdust substrates had great effect on biological yield. The highest biological yield was recorded from T_2 (276.65 g), which was statistically similar with T_3 (272.55 g) followed by T_1 (268.15 g), while the lowest biological yield was recorded in T_5 (234.82 g) followed byT_4 (256.60g). The result of the present study found similar with the of 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 weight.

4.1.3.2 Effect of different sawdust substrates on economic yield

Economic yield of *Pleurotus sajor-caju* grown on different sawdust showed statistically significant variation (Table 3). The highest economic yield was recorded from T_2 (269.40 g), followed by T_3 (264.80 g), whereas the lowest economic yield was observed in T_5 (228.15 g) which was followed by T_4 (248.15 g). The findings of this experiment 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.

4.1.3.3 Effect of different sawdust substrates on Dry yield

Different sawdust showed statistically significant variation in terms of dry yield of *Pleurotus sajor-caju* mushroom (Table 3). The highest dry yield was observed from T_2 (39.79 g), followed by T_3 (36.17 g) .On the other hand, the lowest dry yield was attained in T_5 (23.84g) which was statistically similar with T_4 (30.84 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 24.28 to 29.98 g/packet of *Pleurotus* grown on different substrate. Kulsum *et al.* (2009) found that the highest dry yield was 21.27 g due to sawdust. Ahmed (1998) observed that the diameter of pileus increased the quality and yield mushroom and highest dry yield from mango sawdust.

4.1.3.4 Effect of different sawdust substrates on Biological efficiency

Statistically significant variation was observed in terms of biological efficiency of *Pleurotus sajor-caju* mushroom due to different pasteurization method (Table 3). The highest biological efficiency was recorded from T_2 (158.09%), which was statistically similar with T_3 (155.74%) and followed by T_6 (151.58%) and T_1(153.22%), again the lowest biological efficiency was observed in T_5 (134.18%) which was statistically similar with T_4 (146.63%). 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 135.2 to 160.9%. Biswas *et al.* (1997) found supplementation of substrate promoted biological efficiency

(125.75%). Shen and Royse (2001) found supplements combined with basal ingredient results better mushroom quality as well as Biological efficiency.

4.1.3.5 Effect of different sawdust substrates on Benefit cost ratio

Different sawdust showed statistically significant variation in terms of benefit cost ratio of *Pleurotus sajor-caju* mushroom (Table 3). The highest benefit cost ratio was found from T_2 (5.22), which was statistically similar with T_3 (5.15) and followed by T_1 (4.87) and T_6 (4.67). On the other hand, the lowest benefit cost ratio was recorded in T_5 (4.05) which was statistically similar with T_4 (4.33). 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.

Table 3. Effect of different sawdust on the yield of (*Pleurotus sajor-caju*)

Treatments	Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)	Benefit cost ratio
T_1	268.15b	262.55 b	26.84 b	153.22 b	4.87 b
T_2	276.65 a	269.40 a	27.79 a	158.09 a	5.22 a
T_3	272.55 b	264.80 b	26.17b	155.74b	5.15 a
T_4	256.60 c	248.90 c	25.47 c	146.63 c	4.33b
T_5	234.82 d	228.15 d	23.84 d	134.18 c	4.05 c
T_6	265.28 c	258.34 b	26.69 b	151.58 b	4.67 b
CV (%)	0.03%	0.02%	0.20%	0.12%	1.32%
LSD (0.05)	0.163	0.097	0.091	0.135	0.113

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

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime

4.1.3.6 Relation between average number of fruiting body and economic yield (g)

The highest average number of fruiting body was recorded under treatment T_2 and that was 61.33 and highest economic yield was recorded under treatment T_2 and that was (269.4 g) respectively and the lowest average number of fruiting body was recorded under treatment T_5 and that was 44.56 followed by T_4 (48.25) lowest economic yield was under T_5 (228.15).

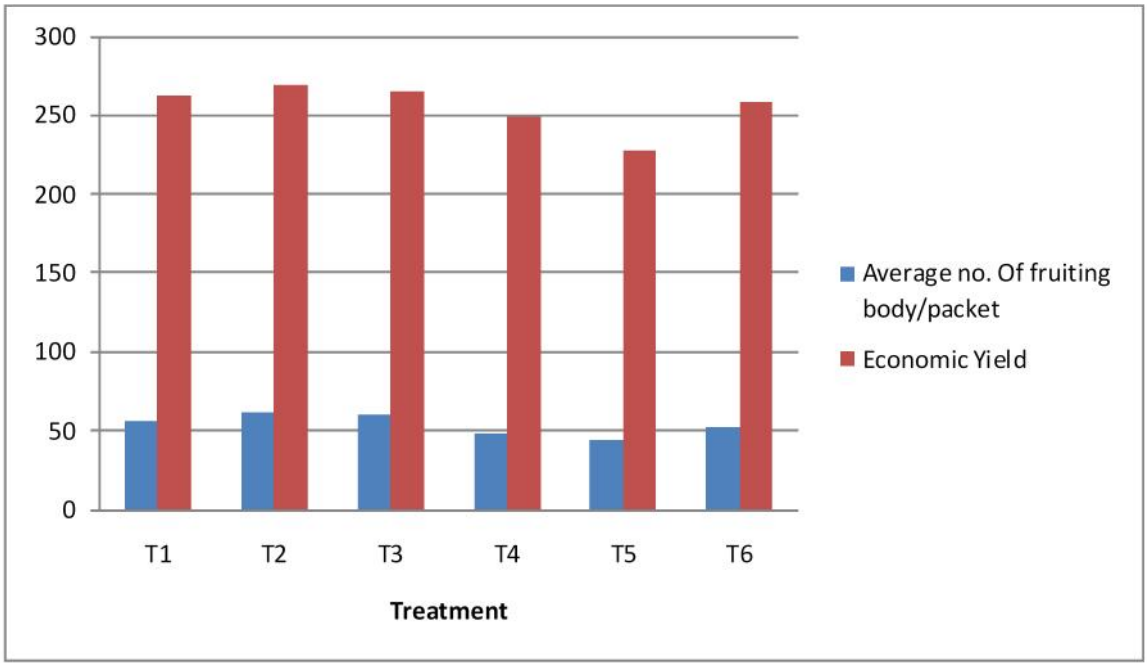

Fig 2. Effect of different sawdust on relation between average numbers of fruiting body/ packet and economic yield(g)

4.2 Experiment 2: Effect of different sawdust substrates on Proximate analysis of *Pleurotus sajor-caju*

4.2.1 Effect on proximate composition of *Pleurotus sajor-caju* mushroom that pppproduced in different treatment of this experiment.

4.2.1.1 Moisture content

Moisture content showed statistically significant variation in different treatment (Table 4). The highest moisture content was observed from T_5 (89.55%), which was statistically similar to T_6 (88.12%) and followed by (87.11%) T_1, while the lowest moisture content was found in T_2 (85.23%) which was statistically similar with T_4 (85.75%). 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.

4.2.1.2 Dry matter

Different sawdust showed statistically significant variation in terms of dry matter content (Table 4). The lowest dry matter content was obtained from T_5 (10.45%), which was followed by T_6 (11.88%), whereas the highest dry matter content was recorded in T_2 (14.77%) which was statistically similar with T_4 (14.65%). 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 be due to different levels of cultural practices.

4.2.1.3 Protein content

Different sawdust showed statistically significant variation in terms of protein content (Table 4). All the treatment contains a considerable amount of protein. The highest protein content was recorded from T_2 (30.88%), followed by T_3 (29.31%), while the lowest protein content observed in T_5 (25.25%) followed by T_4 (28.94%). 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.

4.2.1.4 Lipid content

Significant difference was observed in terms of lipid content of *Pleurotus sajor-caju* mushroom due to different sawdust (Table 4). The highest lipid content was found from T_6 (4.25%), followed by T_3 (3.75%), the lowest lipid content was recorded in T_5 (3.43%) followed by T_4(3.46%). 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 cow dung which is more or less similar to the present study.

4.2.1.5 Carbohydrate

Different amount of carbohydrate content was recorded under the present trial (Table 4). The highest carbohydrate was observed from T_5 (39.54%), followed by T_6 (36.13%), whereas the lowest carbohydrate content was observed in T_2 (34.03%) followed by T_1 (35.76%). The finding of the present study does not match with the study of Chang *et al.* (1981) reported that the fruiting bodies of mushrooms contained 40.30-50.7% carbohydrates. But it was supported by Alam *et al*. (2007) who found 39.82 to 42.83% of carbohydrates in *Pleurotus spp.* 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.

4.2.1.6 Crude fiber

Statistically significant variation was recorded in term of crude fiber content showed due to different sawdust (Table 4). The highest crude fiber was recorded from T_2 (23.28%), which was statistically similar with T_4 (23.22%). On the other hand, the lowest crude fiber content was found in T_6 (21.98%). The findings of the present study corroborate with the study Alam *et al*. (2007) reported 22.87g/100g to 23.29g/100g of fiber in *Pleurotus spp.*

4.2.1.7 Ash

Statistically significant variation was recorded in term of ash content showed due to different sawdust (Table 4). The highest ash content was recorded from T_1 (8.64%), which was statistically similar to T_2 (8.13%). On the other hand, the lowest ash content was found in T_2 (7.12%) followed by T_5 (7.55%). 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.*

Table 4. Effect of different sawdust on proximate nutrient composition of (*Pleurotus sajor-caju*)

Treatments	Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Crud fiber (%)	Ash (%)
T_1	87.11 b	12.89 c	29.31b	3.57c	22.67 b	8.64 a
T_2	85.23 c	14.77 a	30.88a	3.67c	23.28 a	7.12 d
T_3	86.34 b	13.66 b	29.88b	3.75b	22.34 b	8.04 b
T_4	85.75 c	14.65 a	28.94c	3.46d	23.22 a	7.87 c
T_5	89.55 a	10.45d	25.25d	3.43d	23.05a	7.55 c
T_6	88.12a	11.88 c	29.50b	4.25a	21.98 c	8.13 a
CV (%)	0.06%	0.35%	0.09%	1.46%	0.50%	0.78%
LSD (0.05)	0.098	0.084	0.043	0.102	0.206	0.113

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

T_1: Controlled (Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime

Figure: 3

Different sawdust substrates had an effect on the approximate composition of oyster mushroom which was shown in the following figure.

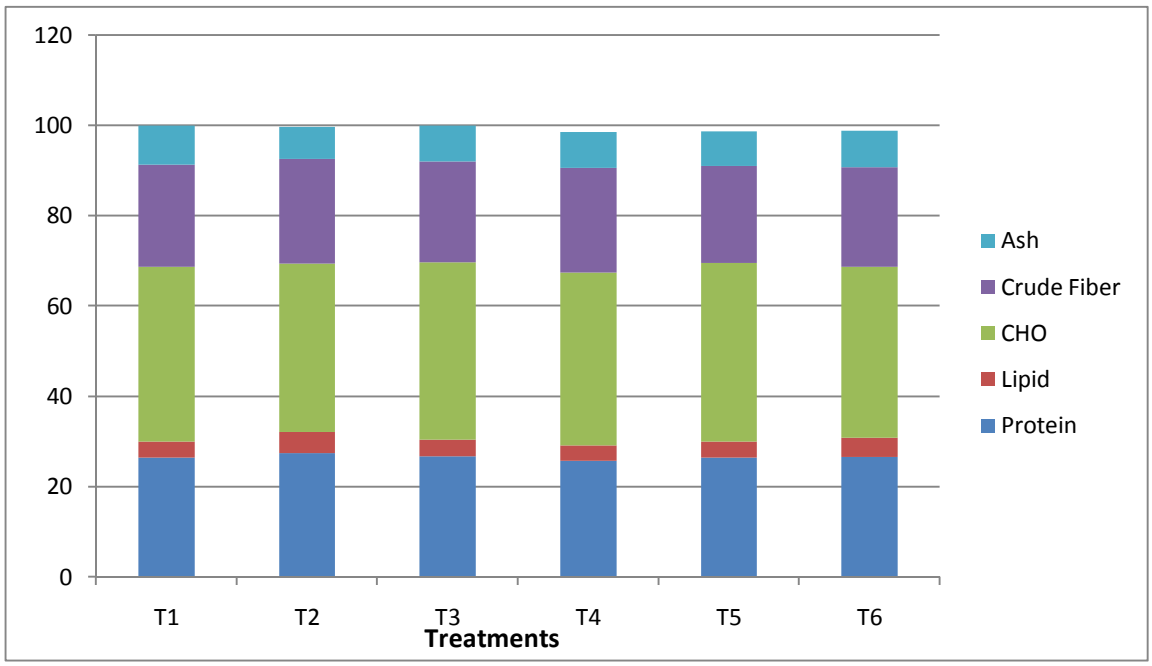

Fig.4. Effect of different sawdust substrates on proximate composition analysis of dry matter of *Pleurotus sajor-caju*

4.2.2 Effect on major mineral content

4.2.2.1 Effect on Nitrogen (N)

Statistically significant variation was recorded in terms of Nitrogen content due to different sawdust used (Table 5). The highest amount of nitrogen content was recorded in T_2 (4.94%) which was followed by T_3 (4.78%), whereas the lowest in T_5 (4.04%) which was statistically similar with T_4 (4.63%). The findings of the present study matches with the study of Moni *et al* .(2004) who analyzed for various nutritional parameters and found 4.22 to 5.59 % of nitrogen on dry matter basis in fruiting bodies of oyster mushroom.

4.2.2.2 Effect on Phosphorus (P)

Statistically significant variation was recorded in terms of phosphorus content due to different sawdust used (Table 5). The highest amount of phosphorus content was recorded in T_2 (0.95%) which was followed by T_1 (0.89%), whereas the lowest in T_5 (0.72%) which was statistically similar with T_4 (0.75%). The findings of the present study match with the study of Sarker *et al.* (2007) who found 0.97% phosphorus in oyster mushroom grown on sawdust based substrates. Kulsum *et al.* (2009) also found that phosphorus content was ranged from 0.84 to 0.92% due to sawdust supplemented with different levels of cow dung.

4.2.2.3 Effect on Potassium (K)

Statistically significant variation was recorded in term of Potassium content showed due to different sawdust (Table 5). The highest amount of potassium was attained from T_2 (1.35%) which was statistically similar with T_1 (1.33%), the lowest phosphorus content was found in T_5 (1.21%) followed by T_4 (1.22%). The findings of the present study similar with the study of Chang et al. (1981) who reported that the fruiting bodies of Pleurotus contained 1.432 to 1.88 mg/g of K on dry weight basis. Sarker *et al.* (2007) also found 1.3% potassium in oyster mushroom grown on sawdust based substrates.

4.2.2.4 Effect on Calcium (Ca)

statistically significant variation showed due to different sawdust used under the present trial (Table 5). The highest amount of calcium was observed from T_2 (1.97%) which was followed by T_3 (1.94%), whereas the lowest calcium content was observed in T_5 (1.77%) which was followed by T_1 (1.91%). Alam *et al.* (2007) who found 22.15 to 33.7 mg/100 g calcium in different oyster mushroom varieties. Sarker *et al.* (2007) also found 2400 ppm calcium in oyster mushroom grown on sawdust based substrates.

4.2.2.5 Effect on Magnesium (Mg)

Variation was observed in terms of magnesium content due to different sawdust under the present trial (Table 5)The highest amount of magnesium was attained from T_2 (0.738%) which was followed by T_1 (0.727%) .On the other hand, the lowest magnesium content was found in T_4 (0.658%) which was followed by T_6 (0.673%). *Sarker et al.* (2004) also found 0.21% magnesium in oyster mushroom grown on sawdust based substrates.

4.2.2.6 Effect on Iron (Fe)

Iron content 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 (513.48ppm) which was followed by T_1 (502.12ppm), whereas the lowest iron content was observed in T_5 (492.08 ppm) which was followed by T_4 (495.69 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 mg/100g. Sarker *et al.* (2007) found 92.09 ppm to 118.40 ppm iron in oyster mushroom grown on sawdust based substrates.

4.2.2.7 Effect on Zinc (Zn)

Different sawdust showed statistically significant variation in terms of zinc content (Table 5). The highest amount of zinc was obtained from T_3 (16.32%) which was followed by T_2 (15.75%), whereas the lowest zinc content was recorded in T_5 (14.36%) which was followed by T_4 (14.49%). 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.

4.2.2.8 Sulphur (S)

Statistically significant variation was recorded in terms of S content due to different sawdust substrates (Table 5). The highest S content was found in T_2 (0.389%) which was statistically identical with T_1 (0.385%), whereas the lowest S content was recorded in T_5 (0.275%) treatment which was statistically similar with T_6 (0.323%). 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.

Table 5. Effect of sawdust substrate on mineral contents of oyster mushroom (*Pleurotus sajor-caju*)

Treatment	N (%)	P (%)	K (%)	Fe (mg/100mg)	Zn (mg/100mg)	S (mg/100mg)
T_1	4.69 b	0.89 a	1.33ab	502.12bc	15.64 a	0.385 a
T_2	4.94 a	0.95 a	1.35a	513.48 a	15.75 b	0.389 a
T_3	4.78 b	0.86bc	1.28b	497.86c	16.32 a	0.328 b
T_4	4.63c	0.75 d	1.22c	495.69d	14.49 b	0.365 b
T_5	4.04 d	0.72 d	1.21c	492.08ab	14.36 c	0.275d
T_6	4.72 c	0.82c	1.36a	508.89 b	15.22 b	0.323 c
CV (%)	1.69%	1.91%	2.98%	0.16%	0.28%	1.42%
LSD (0.05)	.142	0.074	0.070	1.488	0.078	0.009

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

T_1: Controlled (Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime

Figure 5 and 6

CHAPTER V

SUMMARY AND RECOMMENDATION

The study was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka during the period from January – July, 2014 to evaluate the performance of different sawdust on the growth, yield and proximate composition of *Pleurotus sajor-caju*. The experiment consists of six different type of sawdust as- T_1: Controlled (Mixture of sawdust)with 30% wheat bran and 1% lime; T_2: Mango sawdust with 30% wheat bran and 1% lime; T_3: Mahogony sawdust with 30% wheat bran and 1% lime; T_4: Jackfruit sawdust with 30% wheat bran and 1% lime; T_5: Teak sawdust with 30% wheat bran and 1% lime and T_6: Rain tree sawdust with 30% wheat bran and 1% lime. 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 (0.77 cm) was recorded from T_2, while the lowest mycelium running rate (0.59 cm) was observed in T_5. The highest time from stimulation to primordial initiation (7.62 days) was found from T_5, whereas the lowest time from stimulation to primordial initiation (5.64 days) was recorded in T_2. The highest time from primordial initiation to harvest (4.33 days) was attained from T_4 and the lowest time from primordial initiation to harvest (3.26 days) was found in T_2. The maximum average number of primordia/packet (75.33) was observed from T_2, again the minimum average number of primordia/packet (62.33) was found in T_5. The maximum average number of fruiting body/packet (61.33) was recorded from T_2, while the minimum average number of fruiting body/packet (44.56) was observed in T_5. The highest average number of effective fruting body/packet (17.67) was recorded T_2, while the minimum average number of effective fruiting body/packet (11.65) was observed in T_5.The highest average weight of individual fruiting body (4.45 g) was attained from $T_2$and the lowest average weight of

individual fruiting body (2.67 g) was found in T_5. The longest length of stipe (2.69 cm) was recorded from T_3, while the shortest length of stipe (1.78 cm) was found in T_5. The highest thickness of pileus (0.81 cm) was observed from T_2, and the lowest thickness of pileus (0.64 cm) was found in T_5. The highest biological yield (276.65 g) was attained from T_2, while the lowest biological yield (234.82 g) was recorded in T_5. The highest economic yield (269.40 g) was recorded from T_2, whereas the lowest economic yield (228.15g) was observed in T_5. The highest dry yield (27.79 g) was observed from T_2, while the lowest dry yield (23.84 g) was attained in T_5. The maximum biological efficiency (158.09%) was recorded from T_2, again the lowest biological efficiency (134.18%) was observed in T_5. The highest benefit cost ratio (5.22) was found from T_2, and the lowest benefit cost ratio (4.05) was attained in T_5.

The highest moisture content (89.55%) was observed from T_5, while the lowest moisture content (85.23%) was found in T_2. The lowest dry matter content (11.45%) was found from T_5, whereas the highest dry matter content (14.77%) was recorded in T_2. The highest protein content (30.88%) was recorded from T_2, while the lowest protein content (25.25%) was observed in T_5. The highest lipid content (4.25%) was found from T_6, again the lowest ash content (3.43%) was recorded in T_5. The highest carbohydrate (39.54%) was observed from T_5, whereas the lowest carbohydrate content (34.03%) was observed in T_2. The highest crude fiber (23.28%) was recorded from T_2, and the lowest crude fiber content (21.54%) was found in T_5. The highest ash content (8.64%) was recorded from T_2, and the lowest ash content (7.12%) was found in T_5.

The highest amount of Nitrogen content (4.94%) was attained from T_2, whereas the lowest Nitrogen content (4.04%) was found in T_5.The highest amount of phosphorus content (0.95%) was attained from T_2, whereas the lowest phosphorus content (0.72%) was found in T_5. The highest amount of potassium (1.35%) was attained from T_2, again the lowest potassium content (1.21%) was found in T_5. The highest amount of calcium (1.97mg/100mg) was observed from T_2, whereas the lowest calcium content (1.77 mg/100mg) was observed in T_5. The highest amount of magnesium (0.738 mg/100mg) was attained from T_2 and the lowest magnesium

content (0.721 mg/100mg) was found in T_5. The highest amount of iron (513.48 mg/100mg) was attained from T_2, whereas the lowest iron content (492.08 mg/100mg ppm) was observed in T_5. The highest amount of zinc (16.32%) was observed from T_3, whereas the lowest zinc content (14.36 mg/100mg) was recorded in T_5. The highest amount of sulphur (0.389%) was found from T_2, while the lowest sulphur content (0.275 mg/100mg) was attained in T_5

Recommendations

In this experiment, T_2: Mango sawdust supplemented with 30% wheat bran and 1% lime performed better in respect of different growth, yield and nutrient composition and mineral content of *Plurotus sajor-caju*. Therefore, T_2: Mango sawdust supplemented with 30% wheat bran and 1% lime can be recommended for farmer level *Plurotus sajor-caju* mushroom cultivation.

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APPENDICES

Appendix I. Monthly record of air temperature, relative humidity, rainfall, and sunshine (average) of the experimental site during the period from August to December 2014

Month (2014)	Air temperature (^{0}c)		Relative humidity (%)	Rainfall (mm)	Sunshine (hr)
	Maximum	Minimum			
August	36.0	23.6	81	319	4.0
September	34.8	24.4	81	279	4.4
October	26.5	19.4	81	22	6.9
November	25.8	16.0	78	00	6.8
December	22.4	13.5	74	00	6.3

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

Appendix II. Analysis of variance on data with the effect of sawdust substrates on mycelium growth of *Pleurotus sajor-caju* mushroom.

Source of variation	Degrees of freedom	Mean square of		
		Mycelium running rate in spawn packets(cm)	Time from stimulation to primordial initiation (days)	Time from primordial initiation to harvest (days)
Replication	2	0.004	0.003	0.063
Treatment	5	0.012*	2.119**	0.667**
Error	10	0.002	0.002	0.059

** Significant at 1% level of probability; * Significant 5% level of probability

Appendix III. Analysis of variance on data with the effect of sawdust substrates on yield attributes of *Pleurotus sajor-caju* mushroom.

Source of variation	Degrees of freedom	Mean square of					
		Average number of primordia/ packet	Average number of fruiting body/packet	Average no. of effective fruiting body/packet	Average weight of individual fruiting body	Average length of stripe	Average thickness of pileus
Replication	2	0.580	0.099	0.003	0.001	0.001	0.002
Treatment	5	71.166**	127.034**	14.296**	0.591**	0.396**	0.012**
Error	10	0.176	0.078	0.006	0.003	0.003	0.002

** Significant at 1% level of probability; * Significant 5% level of probability

Appendix IV. Analysis of variance on data with the effect of sawdust substrates on the yield, biological efficiency and cost benefit ratio of *Pleurotus sajor-caju* mushroom.

Source of variation	Degrees of freedom	Mean square of				
		Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)	Benefit cost ratio
Replication	2	0.001	0.0002	0.003	0.008	0.001
Treatment	5	685.031**	677.356**	8.570**	355.141**	0.636**
Error	10	0.008	0.003	0.002	0.005	0.004

** Significant at 1% level of probability; * Significant 5% level of probability

Appendix V. Analysis of variance on data with the effect of sawdust substrates on proximate composition of *Pleurotus sajor-caju* mushroom.

Source of	Degrees	Mean square of

variation	of freedom							
		Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	CHO (%)	Crud fiber (%)	Ash (%)
Replication	2	0.003	0.004	0.011	0.001	0.061	0.146	0.042
Treatment	5	7.739*	4.703*	0.882**	0.749**	2.308**	1.424**	0.811**
Error	10	0.003	0.002	0.001	0.003	0.035	0.013	0.004

** Significant at 1% level of probability; * Significant 5% level of probability

Appendix VI. Analysis of variance on data with the effect of sawdust substrates on minerals content of *Pleurotus sajor-caju* mushroom.

Source of variation	Degrees of freedom	Mean square of							
		N(%)	P(%)	K (%)	Ca(%)	Mg(%)	Fe(ppm)	Zn(%)	S (%)
Replication	2	0.020	0.009	0.003	0.001	0.001	1.055	0.018	0.001
Treatment	5	0.287**	0.022**	0.013**	0.016**	0.003**	200.432**	1.743*	0.006*
Error	10	0.006	0.002	0.001	0.002	0.001	0.669	0.002	0.001

** Significant at 1% level of probability; * Significant 5% level of probability

Appendix VII. Experimental Area

Fig: Experimental Area

Appendix VIII: List of Plate

Plate 1: Preparation of sawdust substrates

Plate 2: Preparation of sawdust substrates packet of prescribed quantity

Plate 3: Prepared packet

Plate 4: Mycelium growth in spawn packet

Plate 5: Primordia in the spawn packet

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Plate 8: Mushroom collection and data gathering

Plate 9: Drying Mushroom

Plate 10: Autoclave used in sterilization plate