EFFECT OF DIFFERENT LEVELS OF COW DUNG WITH SAWDUST ON YIELD AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

Ummey Kulsum

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

December, 2008

EFFECT OF DIFFERENT LEVELS OF COW DUNG WITH SAWDUST ON YIELD AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

Ummey Kulsum
REGISTRATION NO. 00910

MASTER OF SCIENCE (M.S.)
IN
BIOCHEMISTRY

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

December, 2008

EFFECT OF DIFFERENT LEVELS OF COW DUNG WITH SAWDUST ON YIELD AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

By

Ummey Kulsum
REGISTRATION NO. 00910

A Thesis

Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University,
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (M.S.)
IN
BIOCHEMISTRY
SEMESTER: July-December'08

Approved By:

Prof. Md. Shamsul Hoque
Department of Biochemistry
Sher-e-Bangla Agricultural University
Supervisor

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

Md. Nuruddin Miah
Associate Professor and Chairman,
Examination Committee

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

PABX: +88029144270-9
Ext. 205 (Off.) 206 (Res.)
Fax: +88029112649
e-mail: bioc_sau@ymail.com

Ref: **Date:**

CERTIFICATE

This is to certify that the thesis entitled "**EFFECT OF DIFFERENT LEVELS OF COW DUNG WITH SAWDUST ON YIELD AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of ***MASTER OF SCIENCE IN BIOCHEMISTRY*** embodies the result of a piece of *bona fide* research work carried out by **Ummey Kulsum**, Registration No. **00910** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in any other institutes.

I further certify that any help or sources of information, as have been availed during the course of this investigation have duly been acknowledged.

Dated:
Dhaka, Bangladesh

Prof. Md. Shamsul Hoque
Department of Biochemistry
Sher-e-Bangla Agricultural University

Supervisor

Dedicated
To
My Parents

ACKNOWLEDGEMENT

The author is thankful and feels pleasure to express her deepest sense of gratitude to the almighty Allah for immense blessings upon Her for the sound health and successful completion of the thesis.

She expresses her sincere appreciation, heart-felt gratitude and indebtedness to his supervisor Professor Md. Shamsul Hoque, Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka for his scholastic guidance, immeasurable help, valuable suggestions, constructive criticism and encouragement throughout the whole research work and final preparation of this thesis.

Profound gratefulness and abysmal respect to her co-supervisor, Professor Kamal Uddin Ahmed, Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, for his valuable advices, needful suggestions and heart-felt co-operation during the research work and preparation of this thesis.

The author also feels proud to express her abysmal respect and indebtedness to Md. Nuruddin Miah, Associate Professor and Chairman, Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, for his encouragement and constructive comments in preparation of this thesis.

Heart-felt gratefulness and respect to Professor Kamal Uddin Ahmed, Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, for his valuable advices, needful suggestions and heart-felt co-operation during the research work and preparation of this thesis.

Heartiest thanks and gratitude are to all staffs of the Department of Biochemistry, Sher-e-Bangla Agricultural University for their assistance throughout the course of this study and research work.

All intimate friends and well wishers who helped directly or indirectly during the research work and thesis writing are duly acknowledged.

The author expresses his profound gratitude to her beloved parents, and all relatives for their inspiration, blessing and encouragement that facilitates her higher studies.

December, 2008 The Author

LIST OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

%	=	Percent
@	=	At the rate
°C	=	Degree Centigrade
Anon.	=	Anonymous
BARI	=	Bangladesh Agricultural Research Institute
BAU	=	Bangladesh Agricultural University
BBS	=	Bangladesh Bureau of Statistics
cv.	=	Cultivar (s)
DAI	=	Days After Inoculation
DMRT	=	Duncan's Multiple Range Test
e.g.	=	For example
et al.	=	And Others
etc.	=	Etcetera
FAO	=	Food and Agriculture Organization
g	=	Gram
hr	=	Hour (s)
i.e.	=	That is
IRRI	=	International Rice Research Institute
ISTA	=	International Seed Testing Agency
kg	=	Kilogram
LSD	=	Least Significant Difference
no.		Number
PDA	=	Potato Dextrose Agar
SAU	=	Sher-e-Bangla Agricultural University
T	=	Treatment
t/ha	=	Ton per Hectare
UNDP	=	United Nation Development Program
$^{W}/_{V}$	=	Weight per Volume
$^{W}/_{W}$	=	Weight per Weight
wt.		Weight
BCR	=	Benefit cost ratio
BE	=	Biological efficiency
MRR	=	Mycelium Running Rate
NMDEC	=	National Mushroom Development and Extension Center
MCC	=	Mushroom Culture Centre
FAO	=	Food and Agricultural Organization
mg	=	Milligram
Conc.	=	Concentration

EFFECT OF DIFFERENT LEVELS OF COW DUNG ON YIELD AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

By

Ummey Kulsum

ABSTRACT

The present study was carried out at the Biochemistry laboratory and Mushroom Culture House (MCH) of Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, during the month of February to July'09 to determine the effect of different levels of cow dung as supplement with sawdust on the performance of oyster mushroom. All the treatments perform better over control. Mycelium running rate in spawn packet and highest average number of primordia/packet was found to be differed due to different levels of supplements used. The highest average weight of individual fruiting body was observed in sawdust supplemented with cow dung @ 10% (3.69 g). The supplementation of sawdust with cow dung had great effect on biological yield, economic yield and Dry yield, biological efficiency, cost benefit ratio. The highest biological yield (254.7 g), economic yield (243.3 g) and Dry yield (23.40 g) biological efficiency (87.82%), cost benefit ratio (8.29) was counted under sawdust supplemented with cow dung @ 10%. Among the chemical characteristics highest content of protein, ash, crud fiber (24.07) was found in treatment T_4, on the other hand lowest lipid (3.90 %) and carbohydrate (32.57) percentage was counted under treatment T_4. Among the minerals highest amount of nitrogen (4.85), potassium (1.39), calcium (22.08), magnesium (20.21), sulfur (0.042), iron (43.11) and lowest phosphorus (0.92) was counted under sawdust supplemented with cow dung @ 10%. There fore it can be concluded that cow dung @ 10% with saw dust may be an alternative supplement to produce oyster mushroom for better cost benefit management.

CHAPTER 1

INTRODUCTION

Mushroom is defined as a macro fungus with a distinctive fruiting body which can be either epigeous or hypogenous. The macro fungi have fruiting bodies large enough to be seen with the naked eye and to be picked up by hand (Chang and Miles, 1992). Oyster mushrooms are large reproductive structures of edible fungi belong to genus *Pleurotus* under the order Agaricales, the family Tricholomataceae and the class Basidiomycetes. The vegetative parts of the mushrooms mainly consist of threadlike long mycelium which under suitable condition forms fruiting bodies or sporocarps.

The utilization of different fungi as food during the modern time has definitely increased with the acquisition of more knowledge about the edible and poisonous mushrooms and development of cultivation methods of few mushrooms. Of nearly 50,000 valid species of fungi and about more than 2,000 species of prime edible mushrooms about 80 have been grown experimentally and 25 accepted widely as food. However 20 varieties have been brought under cultivation commercially and 4 - 5 produced on industrial scale throughout the world (Chang and Miles, 1988). These are attractive due to their flavor, palatability and nutritive value.

Mushrooms have been considered as a special kind of food since earliest time. According to Chang (1982), the protein value of dried mushrooms has been found to be 30-40% comprising all the essential amino acids. Mushrooms constitute an ideal source of food that reduces body weight. Mushrooms provide more protein per unit area than other crops (Gupta, 1986). 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. 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).

Edible mushrooms have been treated as important tool in modern medicine for their medicinal values (Kovfeen, 2004). Anti-cancer medicine (Leutinan) is produced recently by some chemical companies from the extract (Polysaccharides) of Shitake mushroom (Mori, 1986).

Edible mushrooms are recommended by the FAO, to meet protein requirement of developing countries, the large portion of which depends mainly on cereals. Since, animal proteins are beyond the reach of common people in our country (World Bank; 2004), mushroom can play important role in minimizing that deficiency. For such a potential dish item, works on the nutritive analysis are not available in the country and there is no mushroom based balanced diet charts for the common people as well as for the patient. For this reason the proximate analysis for the oyster mushroom (*Pleurotus ostreatus*) is necessary. In fact, there may be relationship between the nutritional statuses of mushrooms grown on different nutritive substrates. So this is also important to find out the nutritional status of mushroom grown in different substrates, which will help to select mushrooms as a food in balanced diet.

Thus the present study has been carried out to achieve the following objectives:

- To find out the suitability of cow dung as a supplement and its supplementation dose with saw dust for growing Oyster Mushroom (*Pleurotus ostreatus*)
- To determine the nutritional status of produced mushroom by proximate analysis.

CHAPTER 2

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 Oyster mushroom (*Pleurotus ostreatus*) and the effect of different kinds of substrate on mushroom cultivation. The review includes reports of several investigators which appear pertinent in understanding the problem and which may lead to the explanation and interpretation of results of the present investigation.

2.1. Effect of Substrate

Quimio and Sardsud (1981) grew pure cultures of *Pleurotus ostreatus* (Fr.) Kummer in various synthetic and natural media to determine some of its nutritional requirements. Biotin and riboflavin were found to enhance mycelial growth. Fifteen out of 20 carbon compounds used and 20 nitrogen compounds tested were found to support the growth of the mycelium. The optimum carbon/nitrogen ratio using glucose and asparagines ranged from 40:1 to 90:1. Spraying the surface of the culture growing on Sawdust medium with thiamine, biotin, starch and asparagines induced higher fruiting body formation. Molybdenum stimulated both mycelial growth and fruiting body production.

Schmidt (1985) observed that the *Pleurotus* spp. grew well on offents, woodchips, sawdust, wood shavings, barks and leaves or pine needles. Shiitake mushrooms (*Lentinus edodes*) were successfully cultivated on the logs of a conifer, *Cunningbanria lanceolata*. It was observed that the yield of mushroom was related to the essential oil content of the bed logs.

Suprapti (1987) in an experiment found that wood waste such as sawdust, leaves of legume plants such as turi (*Sesbabia grandiflora* pers.) and lamtoro gung (*Leucaena leucocephala* kam.) used as substrates for oyster mushroom cultivation. The substrate consisted of rubber wood sawdust mixed with turi or lamtoro gung leaves containing 5%, 10%, 15% and 20%, by W/V with distilled water. The highest production was found from sawdust substrate mixed with 10% turi leaf.

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*. Rice straw and banana leaves were best substrates, with more than 60% conversion efficiency on dry weight basis. The mean weight of the fruiting body was high (7.1 g) on banana leaves compared to other substrates (2.1-5.0 g). The spawn running time was also less with banana leaves, followed by rice straw, grass straw, oil palm bunch refuse, sawdust and oil palm waste. *Pleurotus ostreatus* was successfully grown under local conditions utilizing chopped wheat straw, cotton waste, maize cobs or rice straw as bedding material. Wheat straw and cotton waste gave the highest yields with the shortest incubation period; fruiting bodies were appeared after 15-18 days as compared to 4-5 weeks on the other substrates. The first flush gave the highest yield in all treatments, and was a gradual decline in the yield of successive flushes.

Balasubramanyam (1988) used the willow dust (a waster from cotton textile mills comprising short fiber, seed hulls, leaf fragments, etc.) as a growing medium for *Pleurotus sajor-caju*. It was first soaked in water and calcium carbonate and calcium sulfate were added at the rate of 4 and 2%, respectively. About 600 g of mushrooms per kg willow dust were obtained in 25 days.

Kothandaraman *et al.* (1989) in a study reported that in the split or the logs f *Hevea brasiliensis* was inoculated with spawn of *Pleurotus sajor-caju*, *Pleurotus* citrinopileatus and *Pleurotus florida*. They were covered with polythene sheeting

and kept in darkness at around 26°C until mycelium was visible. Rubber tree sawdust was also investigated as a growing medium; it was soaked in water for 24 hours, then dried to about 70% moisture and mixed with 5% $CaCO_3$; in bottles before inoculation. All 3 species began to grow on the logs within 3 days of inoculation and small fruiting bodies appeared 4 days alter spawn running was completed. However, almost all ceased development shortly afterwards; only 5 (*Pleurotus florida*) reached maturity. Mycelia on sawdust ceased to grow alter penetrating to about three-quarters of the depth of the medium. The reason(s) for the failure to develop fully are not yet known but, since rubber wood appears to have no inhibitory activity against *Pleurotus spp*., further studies are proposed.

Gupta (1989) found that the fruiting 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.

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

Triratna *et al.* (1991) conducted an investigation into the suitability sawdust and available agricultural wastes as substrates for commercial bag cultivation of the mushroom (*Ganoderma lucidum*). Sawdust from *Hevea brasiliensls*, *Diplerocarpus alalus, Penlacnie suavis* and *Tectona grandis* were used to prepare the substrate. *H. brasiliensis* sawdust gave optimum mycelial growth.

Payapanon *et al.* (1994) mentioned that suitable amount of rice bran added to saw dust medium means the maximum yield of Neuron's florida at optimum production cost. Therefore, investigation on the addition of rice bran to saw dust medium, which consist of 100% saw dust, 0.5% $CaCO_3$ and 0.2% $MgSO_4.7H_20$ by weight

was conducted, for the experiment rice bran 0, 1, 5, 10 and 15% respectively was used. *P. florida* was cultivated in plastic bags. Sawdust medium with 10% provided maximum yield. However, the yields obtained by addition of 5, 10 and 15% of rice bran were not significantly different. Yield of *P. florida* cultivation in the saw dust medium with 0% and 1% rice bran were not significantly different. Nevertheless, these were significantly different when compared with the higher rates of rice bran. The recommendation of the appropriate amount of rice bran to be added in the sawdust medium should be 5-10%.

Badshah *et al.* (1994) mentioned that *Pleurotus ostreatus* and *P. florida* were grown on wheat straw, sugarcane bagasse, corn cobs or sawdust, by mixing 120-130 g of spawn with 2 kg of substrate and placing the mixture in sterilized polyethylene bags which were kept in the dark a 25°C for 2-3 weeks. Once the bags became full of mycelial growth, they were removed, leaving the substrate uncovered. Watering was carried out 2-3 times a day. Fruiting bodies were harvested at maturity. *P. ostreatus* and *P. florida* yields ranged from 49.8 and 277.7 g/ 2kg substrate, respectively, on sawdust, to 432.8 and 420.5 g/ 2kg 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*.

Sarawish (1994) found no significantly difference in either the growth of mycelium or the yield of straw mushroom on kaptok residue, chopped-dried water hyacinth, chopped-dried banana stem or chopped-dried water hyacinth, chopped-dried banana stem chopped-dried rice straw as a main substrate.

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.

Adamovie *et al.* (1996) stndied the impact of *Pleurotus ostreatus* mushroom enzymes on Wheat straw degradation in labotatory conditions. Chopped and pressured pasteurized straw with 24 dry matters was seeded with a mushroom mycelium. Chemical of the straw were done after 15, 30, 45, 60, 90 and 120 days upon seeding. The mushroom collection was done four times. After seedling crude fiber, NDF and ADF decreased from 46.93 to 32.40 from 82.42 to 58.5 and From 56.12 to 41.17 respectively. A similar tendency was found for hemlicelluloses and celluloses, while it was not so expressed in lignin. The ash content increased from 0.26 to 9.78. The obtained results show that the mushroom enzymes degraded a substantial part of straw dry matter. The effects were most notable on the lignocellulosic complex. This enabled a successful use of the straw as a substrate for mushroom growth.

Pattana-Pulpium (1996) investigated that mushroom is fungus which grow in natural material such as wood or agricultural waste. The nutrients that stimulate the growth of this mold are present in those materials. The purpose of the study is to study the optimum condition for growth of abalone mushroom; *Pleurotus ostreatus* No 510 and *Pleurotus ostreatus* No 515 in plastic bag. From the experiments, the good growth of *Pleurotus ostreatus* No 510 and *Pleurotus ostreatus* No 515 could be seen in the following mixtures, saw dust, rice bran 7 g, $CaCO_3$ 2 g (NH4)2SO4 0.5 g. and saw dust 100 g, rice bran 5 g, $CaCO_3$ 1 g $(NH_4)_2SO_4$ 0.5 g plus Corn meal 4 g. respectively. The optimum pit and moisture

content of the mixture are 6.5, 6.0 and 75, 65 percentage respectively. The optimum temperature of each is 30°C.

Jadhav *et al.* (1996) reported that Oyster mushroom (*Pleurotus sajor-caju*) was cultivated on wheat straw, paddy straw, stalks 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).

Pani *et al.* (1997) mentioned that wheat bran, rice bran, sawdust, black gram [Vigna rrnmgo] husk, apple pomace, maize cobs and grains of wheat, maize and ragi [*Eleusine coracana*] were evaluated as supplements to improve the sporophore yield of 3 species of *Pleurotus*. Supplementing straw with cereal grains and bran significantly increased yield of all 3 species. The highest yields of *P, solar-cap*' (92.0% biological efficiency, BE), *P. jlahclialus* (75.0% BE) and *P. florida* (93.3% BE) were obtained in response to wheat grain supplementation. Apple pomace, sawdust, maize cobs and black gram husks appeared to be poor supplements.

Kalita *et al.* (1997) studied the growth of *Pleurotus sajor-caju* in polyethylene hag 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 husksubstrafes completed spawn running comparatively within short time (12-14 days.) but other substrates took longer time.

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

Krishnamoorthy (1997) cultivated oyster mushrooms *Pleurotus citrinopileatus* and *P. sajor-caju*) on paddy straw with 1 of 15 different organic supplements at 2% of the wet weight of substrate. Neem cake increased the yield of P. citrinopileatus and P. *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 increased yields compared with the control. Importantly, mushrooms harvested from amended paddy straw did not differ in flavor and taste compared with control.

Biswas *et al.* (1997) reported that methods, including spawning percentage, combinations of paddy straw and 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%).

Yildiz *et al.* (1998) mentioned that this study was conducted on the growth and cultivation of *Pleurotus ostreatus* var. *salignus* on local cellulosic wastes. The highest and lowest yields for 100 g material (70% moisture) were obtained with peanut straw (24.8 g) and with sorghum straw (11.3 g), respectively. Protein, pilus/stip, sporophore weight, 4 dry material, N and C in highest amounts were obtained with peanut straw. The lowest mushroom weight and pilus/stip ratio were obtained with sorghum, whereas the lowest protein, N and dry material weight were obtained with wheat straw. In all the *P. osrrealiir* var. *saligrllus* cultivated on

peanut and sorghum straw, the most abundant nutrients were protein, potassium and carbon. These results are discussed in relation to the prospect of cultivating *P. ostreatus* var. *salignus* in Diyarbakir, Turkey..

Yoshizawa *et al.* (1998) reported that the best mixtures were 3:1 and 1:1 beech/softwood and smoke treated sugi sawdust gave better shiitake fruiting body yields than non-smoke treated sugi and smoke treated karamus. In the sawdust-based cultivation of shiitake using smoke-heated sugi or karamus sawdust in the same ratios with beech as above, yields of fruiting bodies were similar in the various media mixes and with smoke-heated or non-heated softwood sawdust. The results suggest that smoke-heated sawdust cultivation.

Obodai *et al.* (2000) mentioned that Seasonal effects on spawn run period, time for first appearance of fruiting bodies, number of flushes, morphological characteristics of the first flush and biological efficiency of 7 strains of oyster mushroom (*Pleurotus eous, Pleurotus ostreatus* and *Pleurotus sajor-caju*), grown on composted sawdust of T. scleroxylon in Ghana, were studied. *P. eons* strain EM-1 and *Pleurotus sajor-caju* strain ST-6 gave the best yield and biological efficiencies in the wet and dry seasons, respectively. The spawn run period, mycelia growth density and the first appearance of fruiting bodies were not season-dependent.

Labuschagne, *et al.* (2000) found that main raw material for *Pleurotus ostreatus* (oyster mushroom) cultivation is wheat straw. Estimation of straw biodegradability from 15 different spring wheat cultivars under irrigation in South Africa was determined using linear discriminant analysis to discriminate or group the 15 cultivars by combining chemical analysis and in vitro enzymatic hydrolysis. Significant differences ($P < 0.01$) were found between ash, nitrogen, reducing sugars, anthrone reactive-carbohydrates, water-soluble dry matter and oyster mushroom yields.

Peng-JinTorng *et al.* (2000) studied the effect of sawdust (from Trema orientalis) substrates containing different percentages of rice bran on the production of fruiting bodies by *Pleurotus eryngii* (stains ATCC 36047 and Holland 150). The average yield, biological efficiency (BE) and production efficiency (PE) of ATCC 36047 increased significantly with increasing supplementation of the substrate with rice bran (0-47.95%). In Holland 150, BE and PE were highest with 38.08% rice bran, but decreased significantly when rice bran supplementation increased to 47.95%. It was concluded that ATCC 36047 and Holland 150 could be grown commercially in substrates containing 48 and 38% rice bran, respectively.

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

Zhang-RuiHong *et al.* (1998.) cultivated of Oyster mushroom (*P. sajor-caju*) on rice and wheat straw without nutrient supplementation was studied. 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 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.

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 on 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* in 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.

Ayyappan *et al.* (2000) studied the effect of ventilation tubes on *P. citrinopileatus* and *Pleurotus florida*. Poly (vinyl chloride) tubes (45 cm long) were kept at the center of bags while filling the bags with spawn and straw. After filling, the top of the end bag was tied, keeping the tubes slightly producing. Equidistant holes (2, 5 or 8) of varying diameter were made on the tubes at opposite directions (total of 4, 10, or 16 holes). The use of ventilated tubes shortened the maturation period of *P.*

citrinopileatus and *P. florida*. Ventilated tubes with a diameter of 10 mm and. 16 holes gave the greatest reduction in days to maturity and the highest sporophore yield (720 and 735 g).

Khan *et al.* (2002) evaluated the sawdust of shisham (*Dalbergia sissoo*) supplied alone or in combination with 10% cotton seed hulls, 5% horse dung, 5% wheat bran or 5% cowdung for the productivity of *P. ostreatus*. Sisham sawdust combined with 10% cotton seed hulls proved the best for spawn running with a mean 13.35 days, followed by Sisham sawdust combined with 5% horse dung, 5% wheat bran, 5% cowdung and Shisham sawdust alone. Maximum yield was obtained by Shisham + 10% cotton seed hulls while minimum yield was obtained by Shisham sawdust alone. Six different substrates, viz. cotton waste, sawdust of popular, Kikar, Pine, Shisham and Diar, were also evaluated for the productivity of *P. ostreatus*. Cotton waste proved the best for spawn running with a mean of 8.15 days followed by popular, pine, kikar and Shisham, while Diar showed no spawn running. Yield was highest on cotton waste followed by popular, Shisham, Kikar axed Pine, while Diar gave no yield.

Ivan *et al.* (2003) investigated the myceliation rate, mycelial vigor and estimated biomass of *Lentinus edodes* (Berk.) pegler and spawn production using sugarcane bagasse supplemented with rice bran and sugarcane molasses as substrate in different ratio. The 25 and 30 % rice bran proportions exhibited highest mycelial vigor.

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

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

Al 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.

Shah *et al.* (2004) an experiment carried out to investigate the cultivation of Oyster mushroom on different substrates. Sawdust produced highest yield, biological efficiency and number of fruiting bodies, recommended as the best substrate for Oyster mushroom cultivation.

Scherba *et al.* (2004) considered the lack of a large scale production system for sowing mycelium slowing down production of mushrooms in Belarus. The possibility of production of sowing mycelium in conditions of deep fermentation allowing a significant decrease of growing time of mycelium is discussed. Investigations were conducted to study growing habits and fructification of *Pleurotus ostreatus* strains No 42 and 186 on sterile and pasteurized rye straw. Diagrams are presented on the effect of methods of treatment of the substrate, application methods (surface application or mixing with the substrate) and quantity of spawn (1, 5 and 10% of the mass of the raw substrate) on mushroom yield. Diagrams are included on the effect of the method of application of spawn on speed of growing of mushrooms. Evidence was obtained of advantages of using liquid spawn material compared with grain spawn.

Mukesh *et al.* (2004) evaluated different substrates, i.e. wheat, chickpea, pea, sugarcane bagasse and water hyacinth, for production of Oyster mushroom (*Pleurotus sajor-caju*), during 2002-03 in Uttar Pradesh, India. The period of spawn run on water hyacinth straw (21 days) was longer than other straws during

January to April when minimum temperature varied from 13.93 to 20.62 degrees C and average temperature varying from 23.57 to 28.16 degrees C. The relative humidity during these period varied from 76.38 to84.34 percent (morning) and 57.54 to 63.33 percent (evening). The number of days taken for pin head initiation on water hyacinth straw (25 days) was also longer compared to other straws. The biological efficiency of mushroom was higher on wheat straw (8.1.00) followed by chickpea (80.00), pea (77.77), water hyacinth (67.77) and sugarcane bagasse (66.67). Biological efficiency started declining with an increase in temperature (25+or-2 observed in wheat straw (810 g).

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 hodies 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.

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 primordial and fruiting bodies were found in waste paper 43.75 and 31.00 respectively. The highest amount of fresh weight was also found in waste paper 94.25 g.

Khlood-Ananbeh *et al.* (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 (Ti); 70% straw + 20% olive cake 5% wheat bran + 5% gypsum (T2); 60% straw + 30% olive cake + 5% wheat bran + 5% gypsum (T3); 50% straw + 40% olive cake + 5% wheat bran +.5% gypsum (T4); and 90% olive cake + wheat bran + 5% gypsum (T5). 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/500g dry substrate), average weight (21.5 g/cap), and average cap diameter (7.05 cm/cap) and BE% (80%). Carbohydrate, protein and fibre 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.

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

Reyes *et al.* (1994) conducted a study with the objective of utilizing selected agro industrial wastes for the production of *Pleurotus sajor-caju* (Fr.) Singer. Five agro-industrial wastes were evaluated singly or in combination with other substrates. Three parts coarse materials (cotton wastes, rice straw and rice hull) and two are fine materials (sawdust and rice bran). Three parts of coarse materials were combined with every one part of fine materials. Results of the experiment revealed that composted substrates generally produced more and heavier basidiocarps and relatively high biological efficiency compared to the uncomposted materials. *Pleurotus sajor-caju* grown in composted rice hull + rice straw + cotton waste with a ratio of 3:3:3 produced the heaviest basidiocarp (80.54 g) with a biological efficiency of 26.84 percent.

Lim *et al.* (1997) analyzed the cost and return of *Volvariella* and *Pleurotus* mushroom production and found the ROI of 89.16 percent and 51.93 percent respectively. This indicates that mushroom production is economically feasible. The feasibility of low input mushroom production for upland farmers in reforested areas under the closed canopy high-diversity forest faming system was determined. Agricultural and tree wastes were tested and utilized for spawn and mushroom production. Findings showed that among 10 agricultural/tree wastes tested, mung bean pods, kakawate and cassava leaves, log sawdust, and ipil-ipil leaves, sugarcane bagasse with rice bran, and water hyacinth can be used as alternative substances for *Volvariella* spawn production. Local isolate (VISCA) of *Volvariella volvacea* gave higher yield (2263.65g) compared with *Volvariella* (1574.80 g) isolates from BIOTECH College, Laguna, Philippines. This fruited well in the closed-canopy area than when cultivated in the open area. *Pleurotus* yield was higher (209.60 g/bag) inside mushroom house under closed-canopy area than when grown inside mushroom house in relatively open area (198.54 g).

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.

Chang *et al.* (1981) reported that the Fruit bodies mushrooms contained 82.5-92.2% of moisture, 4.30-50.7% of carbohydrate, 26.6-34.1% crude protein and 1.1-8.0% fat.

Performance of five species of *Pleurotus* grown on cotton seed hulls, wheat, rice and maize straw was evaluated. 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. Those cultivated on rice and maize straw contained 17 amino acids but oystin was lacking in those cultivated on cottonseed husks or wheat straw. The total amino acid and essential amino acid contents in the fruiting bodies grown on the different substrates like rice straw, maize straw, and cotton seed husks were also found very significantly (Qin, 1989).

The fruit 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 bruit bodies ranged 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 (Ragunathan *et al.* 1996).

Fujihara *et al.* (2000) found that the nitrogen content of Fruit bodies cultivated on sawdust medium was closely related to that in the medium. Nitrogen content of the sawdust medium was related to amino acid, nucleic acid and chitin contents. No significant relation between lentinic acid in fruit bodies and nitrogen content in the medium was observed. The amount of lentinic acid in fruit bodies cultivated onsawdust medium containing rice bran and corn bran was about two times that cultivated on Okara-added medium. Nitrogen content of fruit bodies was affected by nitrogen sources present in the medium. High levels of nitrogen in sawdust medium should decrease carbohydrates in fruit bodies cultivated on the medium, thus making the fruit too soft for eating.

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

CHAPTER 3

MATERIALS AND METHODS

3.1. Location of experiment

The experiment was carried out at the, Biochemistry laboratory and Mushroom Culture House (MCH) of Biochemistry Department, Sher-e-Bangla Agricultural University, Dhaka, during February'09 to July'09.

3.2. Experiments and treatments

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

Treatments used:

- T_1: Sawdust supplemented with cowdung @ 0% (Control)
- T_2: Sawdust supplemented with cowdung @ 5%
- T_3: Sawdust supplemented with cowdung @ 10%
- T_4: Sawdust supplemented with cowdung @ 15%
- T_5: Sawdust supplemented with cowdung @ 20%

3.3. Preparation of substrates

Spawn packets using different levels of supplements were prepared separately. With spawn preparing substrate (sawdust); different levels of supplements (cow dung) and $CaCO_3$ (1g per packet) was added. The measured materials were taken in a plastic bowl and mixed thoroughly by hand and moisture was increased by adding water. Moisture was measured by using the moisture meter and adjusted the moisture content at 50%.

3.4. Preparation of packets

The mixed substrates were filled into 7"×11" 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.

3.5. Sterilization, inoculation and mycelium running in spawn packets

Therefore the packets were sterilized about 1 hrs and then these were kept for cooling. After cooling, 5g mother spawn were inoculated into the packets in the laminar airflow cabinet and were kept at 20-22°C temperature until the packets become white with the mushroom mycelium. After completion of the mycelium running the rubber band, brown paper, cotton plug and bamboo neck of the mouth of spawn packet were removed and the mouth was wrapped tightly with rubber band. Than this spawn packets were transferred to the culture house.

A. Immediately after inoculation

B. After 7 days of inoculation

C. After 10 days of inoculation

D. After 22 days of inoculation

Plate 1. Mycelium running stages of oyster mushroom in spawn packet after inoculation

3.6. 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 news paper. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3 times a day. The light around 300-500 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22°C to 25°C. The first primordia appeared 3-5 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

3.7. Harvesting of produced mushrooms

Oyster mushrooms matured within 2-3 days after primordia initiation. The matured fruiting body was identified by cural margin of the cap, as described by Ruhul Amin, 2002. Mushrooms were harvested by twisting to uproot from the base. There fore the harvested mushroom were taken to the laboratory.

A. Pin head primordia in the spawn packet

B. Young fruiting body in the spawn packet

C. Matured fruiting body in the spawn packet

D. Fruiting body harvested from the spawn packet

Plate 2. Primordia and fruiting body of produced oyster mushroom

3.8. Data collection

Data were collected on the following parameters.

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

3.8.2. Average weight of individual fruiting body/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.

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

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

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

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

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

$$\text{Biological efficiency} = \frac{\text{Total biological weight (g)}}{\text{Total weight substrate usesd (g)}} \times 100.$$

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

3.9. Drying of mushrooms

The collected fruiting bodies of the mushroom are transferred to the laboratory. The data the fruiting bodies were dried in the sun separately treatment wise. During the time of drying the stipe and the pileus are separated for better drying.

A. Mushroom Samples kept for grinding immediately after removing form oven

B. Prepared ground sample preserved for further analysis

Plate 3. Drying and grinding of mushroom sample for biochemical analysis

3.10. Proximate analysis of the mushrooms

3.10.1. Moisture

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

3.10.2. Dry matter

A clean container (dish or beaker) was place in an oven at 105ºC overnight. The container was allowed to cool in a desiccator and was weighted. The sample was kept into the container and weighted with the sample. The container was placed in the oven at 105°C for 24 hours. The container was allowed to cool in a desiccator and was weighted. Again, the container was placed in the oven at 105°C for 2 hours. It was cooled in a desiccator and weighted again. Repeat drying, cooling and weighing was continued until the weight becomes constant. The dried sample was stored in an airtight container. The moisture content of the sample was calculated.

.

3.10.3. Determination of Crude Fiber

Crude fiber was determined according to the method described by (Raghuramulu *et al.*, 2003) by the following formula.

Crude fiber (g/l00g sample) = [100-(moisture + fat)] x (We-Wa) / Wt. of sample

3.10.4. Fat estimation

Fat was estimated as crude ether extraction of the dry materials according to the method described by Rahman (1994). The dried sample (about 5.0 g.) was weighted into a conical flask and plugged with fat free cotton. The flask was then placed in an electric shaker and extracted with anhydrous ether for about 16 hours. The ether extract was filtered into another weighed conical flask. The flask containing the original ether extract was washed 4 to 5 times with small quantities of ether and the washings were also transferred to the filter paper. The ether in the

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

$$\text{Fat contents (g) per 100g of dried sample} = \frac{\text{weight of ether extract} \times \text{Percentage of dried sample}}{\text{Weight of the dried sample taken}}$$

3.10.5. Total Carbohydrate Estimation:

The content of the available carbohydrate was determined with the method described by (Raghuramulu *et al., 2003)* using the following equation:

Carbohydrate (g/l00g sample) = 100 - [(Moisture + Fat + Protein + Ash + Crude Fiber) g/100g]

3.10.6. 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 dessicator 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/l00g sample) = Wt of ash × 100 / Wt of sample taken
(Raghuramulu et al., 2003)

3.10.7. Determination of total Nitrogen

Total nitrogen was determined by a micro kjaldhal apparatus in the traditional method and calculated using the following formula.

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

Where,

a = ml HCI taken into the conical flask

b = ml NaOH used for titration

M_{HCl} = Molarity of the HCI

M_{NaOH} = Molarity of the NaOH

c = g powder of jute Mushroom used for the analysis

3.10.8. Determination of total Sulphur

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

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

Where, A = weight of $BaSO_4$ g

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

W = weight of mushroom sample in g

3.10.9. Determination of Ca, Mg, K, Fe, and P

The sample was digested with nitric acid to release of Ca, Mg, K, Fe, and P. Ca, Mg, Fe, were determined by atomic absorption spectrophotometer, K was determined by flame photometer, and P was determined by spectrophotometry using the following formulas.

For Ca, Mg, K, P

$$\text{mg per 100 gm} = \frac{a \times 25000}{b \times c}$$

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

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

c = g sample weighed into the digestion tube

For Fe

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

Where, d = mg/L Fe, measured on atomic absorption spectrophotometer
c = g sample weighed into the digestion tube

3.11. Statistical analysis of data

The experiment was laid out in single factor CRD (Complete Randomized Design). The experiment considered 5 treatments with 3 replications and 1 spawn packets in each replication. The data for the characters considered in the present experiments were statistically analyzed following the Complete Randomized Design (CRD). The analysis of variance was conducted and means were compared following least significant difference (LSD) test at 1% and 5% level of probability for interpretation of results (Gomez and Gomez, 1984).

CHEPTER 4

RESULTS AND DISCUSSION

4.1. Mycelium Running Rate (cm)

Mycelium running rate per day (MRR) 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. Mycelium running rate in spawn packet was found to be differed due to different supplements used. The highest running rate was observed in T_4 (0.71 cm) and the lowest running rate of mycelium was observed in T_1 (0.50 cm). The other treatments were statistically similar (Table 1). The present findings corroborated with the findings of previous workers (Khan *et al.*, 1991; Kalita *et al.*, 2001; Sarker, 2004; Bhuyan, 2008). 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. Kalita *et al.* (2001) reported that time taken for completion of spawn running may required to 17 days from 22 days by use of different substrates. Sarker (2004) found that the mycelium running rate of oyster mushroom greatly influenced with the supplement of wheat brans in different levels. Bhuyan (2008) also found similar result as found in the present experiment.

4.2. Time from stimulation to primordia initiation (Days)

The time from stimulation to primordia initiation ranged from 5.1 days to 7.1 days. The highest time from stimulation to primordia initiation was observed in T_1 (7.01 days). The lowest time from stimulation to primordia initiation was in the treatment T_4 (5.01 days). The other treatments varied significantly in terms of time from stimulation to primordia initiation (Table 1). The result of the present findings keeps in with the findings of previous scientists (Sarker, 2004, Ruhul Amin, *et al.* 2007; Bhuyan, 2008). Sarker, (2004) observed that duration from primordia initiation to first harvest of oyster mushroom was significantly lower as compared to control where no supplement was used and the duration required for

total harvest of oyster mushroom increased with the level of supplement used. In the present study, the time required for total harvest also decreased with the levels of supplements increased compared to sawdust 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.

4.3. Time from primordia initiation to harvest (days)

The lowest time from primordia initiation to harvest was in the treatment T_3 (3.2 days) and the highest time from primordia initiation to harvest was observed in the treatment T_1 (5.27days). The other treatments were statistically similar (Table 1). The results of the present findings keep in with the findings of previous scientists (Khan *et al.*, 2001; Dhoke *et al.*, 2001; Royse, 2002). Khan *et al.* (2001) reported that after spawn running pinhead formation took 7-8 days and fruiting body formed after 3-5 days, sporocarps may be harvested after 10-12 days. Dhoke *et al.* (2001) found significant effect of different agro-wastes on yield of oyster mushroom. The days required for first picking varied from 11.25-12.00 and the final picking complete from 42.25 to 43.50 days depending on different substrates. Royse, (2002) found, as the spawn rate increased the number of days to production decreased.

Table 1. Effect of different levels of cow dung with sawdust on mycelium running rate in spawn packets, time from stimulation to primordial initiation (days) and time from primordial initiation to harvest (days) of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Mycelium running rate in spawn packet (cm)	Time from stimulation to primordial initiation (days)	Time from primordia Initiation to harvest (days)
T_1	0.50c	7.1a	5.3a
T_2	0.63b	6.1b	4.1b
T_3	0.64b	5.2c	3.2c
T_4	0.71a	5.1c	4.1b
T_5	0.65b	5.2c	4.3b
CV (%)	1.32	1.45	2.52
Level of significance	**	**	**
LSD (0.05)	0.031	0.229	0.287

Means followed by same letter significantly different at 1% or 5% level of significance.

^{NS} Not significant * Significant at 5% level; ** Significant at 1% level

Legend:

T_1: Sawdust (Controlled)
T_2: Sawdust + Cow dung (5%)
T_3: Sawdust + Cow dung (10%)
T_4: Sawdust + Cow dung (15%)
T_5: Sawdust+ Cow dung (20%)

4.4. Average number of primordia

The highest average number of primordia/packet was observed in the treatment T_3 (73.21) followed by T_4 (66.70) and the lowest average number of primordia/packet was in the treatment T_1 (57.40). The other treatments were statistically similar (Table 2). The result of the present findings keeps in with the findings of previous scientists (Ahmed, 1998; Dey, 2006; Bhuyan, 2008). Ahmed (1998) reported significantly different number of primordia on different substrates. Dey (2006) found that the number of primordia and the average yield significantly varied with the substrates used in production of oyster mushroom. Bhuyan (2008) found similar findings growing oyster mushroom on saw dust supplemented with different levels of cow dung.

4.5. Average number of fruiting body

The highest average number of fruiting body/packet was observed in the treatment T_3 (60.42) and the lowest average number of fruiting body /packet was in the treatment T_1 (37.43). The other treatments were statistically and significantly varied over control in terms of average number of primordia/packet (Table 2). The result of the present findings keeps in with the findings of previous scientists (Yoshida *et al.*, 1993; Al Amin, 2004; Sarker, 2004, Bhuyan, 2008). Yoshida *et al.* (1993) reported that the number of fruiting bodies was lower, but increased when the substrates was mixed with different supplements. Al Amin (2004) reported that the number of primordia grown on different substrates differed significantly. Sarker, (2004) found that the number of primordia increased with the levels of supplement and continued up to a certain range and decline there after. In the present study the average number of fruting body in creased up to 10 % of cow dung used as supplement and decreased there after. Bhuyan (2008) in a same type of experiment found similar results.

4.6. Average weight of individual fruiting body (g)

Supplementation of sawdust with levels of cow dung had great effect on average weight of individual fruiting body. The average weight of individual fruiting body in different treatment ranged from 2.99 g to 2.68 g. The highest average weight of individual fruiting body was observed in the treatment T_4 (2.99 g) and the lowest average weight of individual fruiting body was in the treatment T_1 (1.91 g). The other treatments varied significantly over control in terms of average weight of individual fruiting body (Table 2). The present study matches with the study of the previous scientists (Sarker, 2004; Sarker *et al*. 2007; 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. Sarker *et al.* (2007) reported the individual weigh of fruiting body ranged from 1.33-1.59g, which was more or less similar to this study. Bhuyan (2008) found significant effect of supplementation on the weigh of fruiting body but he found comperatively higher weigh of individual fruiting body ranged from (5.02g to 7.01g), which may be due to environmental conditions or growing season.

Table 2. Effect of different levels of cow dung with sawdust on the yield contributing characters of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Avg. no of primordia/packet	Avg. no of fruiting body/packet	Avg. wt of individual fruiting body (g)
T_1	57.40d	37.43e	1.91d
T_2	63.83c	45.50d	2.68c
T_3	73.21a	60.42a	2.97a
T_4	66.70b	49.40b	2.99a
T_5	63.83c	48.20c	2.83b
CV (%)	0.08	0.17	1.48
Level of significance	**	**	**
LSD (0.05)	0.150	0.229	0.123

Means followed by same letter significantly different at 1% or 5% level of significance.

^{NS} Not significant * Significant at 5% level; ** Significant at 1% level

Legend:

T_1: Sawdust (Controlled)
T_2: Sawdust + Cow dung (5%)
T_3: Sawdust + Cow dung (10%)
T_4: Sawdust + Cow dung (15%)
T_5: Sawdust+ Cow dung (20%)

4.7. Biological Yield (g)

The supplementation of sawdust with cow dung had great effect on biological yield. The highest biological yield was counted under treatment T_3 (217.7 g) and the lowest biological yield was counted under T_1 (109.7 g). The other treatments varied significantly as compared with the control in terms of biological yield (Table 3). 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. Baysal *et al.* (2003) found the highest yield of Oyster mushroom (*Pleurotus ostreatus*) with the substrate composed of 20% rice husk in weigh. Ruhul Amin *et al.* (2007) found the highest biological yield 247.3g/packet. He also found that the trend of economic yield corresponded with different supplements at different level.

4.8. Economic Yield (g)

The supplementation of sawdust with cow dung increases the economic yield over control. The highest economic yield was recorded under treatment T_3 (213.0 g) and the lowest economic yield was counted under T_1 (104.0 g). The other treatments varied significantly over control (Table 3). Payapanon *et al.* (1994) mentioned that suitable amount of supplements added to sawdust medium maximized economic yield of oyster mushroom at optimum production cost. Sarker (2004) found appreciable variations in economic yield also observed at different levels of supplements under different substrate-supplement combinations. Bhuyan (2008) observed that the yield of *Pleurotus ostreatus* responded with the levels of supplements used with sawdust and increased with the level of supplementation and declined there after.

4.9. Dry yield

The dry yield of the oyster mushroom, grown on sawdust responded significantly in terms of dry yield with the different levels of supplement (cow dung). The dry yield of mushroom was maximum under the treatment T_3 (21.27 g) and the lowest dry yield was counted under T_5 (10.23 g). The other treatments varied significantly over control (Table 3). The result of the present study corroborates with Ahmed (1998) who observed significant effects of various substrates on diameter and length of stalk also diameter and thickness of pileus. He also found that lower diameter of pileus produced the lowest yield and concluded that the diameter of pileus increased the quality and yield of mushroom and highest dry yield from mango sawdust. Sarker *et al*. (2007) found the range of dry yield from 4.28 to 29.98, which was more or less similar to this study.

4.10. Biological efficiency

The highest biological efficiency of 75.06% was calculated in treatment T_3 and the lowest biological efficiency of 37.82% was calculated from T_5 (Table 3). The other treatments varied significantly over control. The present findings keep in with the findings of previous workers (Biswas *et al.*,1997; Patrabansh and Madasn, 1999; Kalita *et al.*, 1997; Shen and Royse, 2001; Obodai *et al.*, 2003). Biswas *et al.* (1997) found supplementation of substrate promoted Biologycal Efficiency (125.75%). Patrabansh and Madan (1997) reported the similar result in growing *Pleurotus sajor-caju.* Kalita *et al.* (1997) observed biological efficiency for different substrates ranged from 35.2 to 60.9 %. Shen and Royse (2001) found supplements combined with basal ingredient results better mushroom quality as well as Biological efficiency. Obodai *et al.* (2003) found biological efficiency (BE) followed a pattern and ranged from 61.0% to 80.0%.

4.11. Benefit Cost Ratio

The highest cost benefit ratio was calculated in treatment T_3 (7.46) and the lowest cost benefit ratio 3.80 was calculated from T_1 (control). The other treatments differed significantly in terms of cost benefit ratio (Table 3). The present findings keep in with the findings of previous workers (Lim *et al.*, 1997; Ahmed, 1998; Sarker *et al*, 2007). Lim *et al.* (1997) analyzed the cost and return of *Volvariella* and *Pleurotus* mushroom production and found the ROI of 8.9 and 5.1, respectively. Ahmed (1998) also observed the benefit cost ratio of 73.2, 23.78 and 16.23 in case of *Pleurotus sajor-caju*. The cause of these variations between the results of this study might be due to consideration of other costs involved in the production of oyster mushroom or might be due to measuring system. Sarker *et al*, (2007) mentioned the performances of substrates were significantly differed based on benefit cost ratio. They reported the highest cost benefit ratio of 6.50 with wheat straw.

Table 3. Effect of different levels of cow dung with sawdust on the yield, biological efficiency and cost benefit ratio of oyster mushroom (Pleurotus ostreatus)

Treatments	Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)	Cost benefit ratio
T_1	109.7e	104.0e	10.23e	37.82e	3.80d
T_2	171.0d	161.0d	15.13d	58.97d	6.53c
T_3	217.7a	213.0a	21.27a	75.06a	8.41a
T_4	200.0b	194.0b	19.10b	68.97b	7.46b
T_5	181.0c	171.0c	16.20c	62.41c	6.41c
CV (%)	1.65	0.62	0.49	1.65	0.60
Level of significance	**	**	**	**	**
LSD (0.05)	7.964	2.873	0.212	2.749	0.123

Means followed by same letter significantly different at 1% or 5% level of significance.

^{NS} Not significant * Significant at 5% level; ** Significant at 1% level

Legend:

T_1: Sawdust (Controlled)
T_2: Sawdust + Cow dung (5%)
T_3: Sawdust + Cow dung (10%)
T_4: Sawdust + Cow dung (15%)
T_5: Sawdust+ Cow dung (20%)

4.12. Effect on proximate composition

4.12.1. Moisture

The moisture content of the fruiting body shows significant difference. The moisture percent ranged from 90.60 to 90.01. The highest moisture percent was observed in treatment T_2 (90.60) followed by T_5 (90.52). The other treatments were statistically similar but the lowest moisture was in T_3 (90.01) (Table 4). The result of the present study keep in with the findings of previous workers (Rahman, 1994; Moni *et al.*, 2004; Alam *et al*, 2007, Bhuyan, 2008). Rahman (1994) observed more or less 90% moisture in the mushroom *Pleurotus ostreatus*. Moni *et al.* (2004) found 88.15 to 91.64% moisture. Alam *et al.* (2007) reported 87 to 87.5% moisture in oyster mushrooms grown on different substrates. Bhuyan (2008) found no significant differences among the mushrooms produced in sawdust supplemented with cow dung.

4.12.2 Dry matter

The dry matter percentage of the fruiting body shows significant difference. The dry matter percent of fruiting body ranged from 9.98 to 9.40. The highest dry matter percentage was observed in treatment T_3 (9.98) which was followed by T_4 (9.85) and T_1 (9.84). The other treatments were statistically similar but the lowest dry matter percentage was in T_2 (9.40) (Table 7). The result of the present study matches with the findings of previous scientists. Bhuyan (2008) found no significant differences among the treatments when cow dung used as supplement. But in this study there was significant differences found among the treatments. This may be due to different levels of cultural practices.

4.12.3. Protein

All the treatments contain a considerable amount of protein. The content of protein varied from 11.31-31.30% (w/w) in the mushroom grown on sawdust with different levels of cow dung. The highest content of protein was found in treatment T_3 (31.30%) which was followed by T_4 (27.70 %) and the lowest protein was found in T_1 (11.31 %). The other treatments statistically similar but varied

significantly over control in respect to protein content (Table 4). The result of the present study corroborates with the study of Chang *et al.* (1981) who reported that the fruit bodies of oyster mushrooms contained 26.6-34.1% protein. Zhang-RuiHong *et al.* (1998) found the protein content of oyster mushroom was 27.2% on an average.

4.12.4. Lipid

The lowest lipid percentage was counted under treatment T_3 (3.44) followed by T_2 (3.47) and the highest lipid percentage was counted under T_1 (5.43). The rest of the treatments were statistically similar (Table 4). The result of the present study showed that the lipid content of the mushroom decreased as the supplements added with the substrates. The result of the present study keep in with the findings of Chang *et al.* (1981) who found 1.1-8.0 lipid in oyster mushroom varieties. Moni, (2004) found 1.49 to 1.90% crude fats in oyster mushroom, Alam *et al.* (2007) reported 4.30 to 4.41% lipids in oyster mushroom grown on different substrates.

4.12.5. Ash

The highest percentage of ash was observed in the treatment T_3 (8.41) and the lowest percentage of ash was in the treatment T_1 (6.58). The other treatments were statistically similar but differed significantly in terms of percentage ash content (Table 4). The findings of the present study are supported by the study of Khlood-Ananbeh *et al.* (2005) who reported ash contents were moderate in the fruiting bodies. Alam *et al* (2007) reported 8.28 to 9.02% of ash in *Pleurotus spp.*

4.12.6. Carbohydrate

The lowest percentage of carbohydrate was counted under treatment T_3 (32.85) and the highest carbohydrate percentage was counted under T_1 (58.38). The rest of the treatments were statistically similar but differed over control in respect to percent carbohydrate content (Table 7). The findings of the present study does not match with the study of Chang *et al.* (1981) reported that the fruit bodies

mushrooms contained 40.30-50.7% of carbohydrates. But it was supported by Alam *et al.* (2007) who found 39.82 to 42.83% of carbohydrates in *Pleurotus spp.*

4.12.7. Crud fiber

The highest percentage of crud fiber was counted under treatment T_3 (24.01) followed by T_2 (23.64) and the lowest crud fiber percentage was counted under T_1 (20.31). The rest of the treatments were statistically similar but varied over control in respect to percent crud fiber content (Table 4). The findings of the present study corroborate with the study Alam *et al* (2007) reported .87g/100g to 23.29g/100g of fiber in *Pleurotus spp.*

Table 4. Effect of different levels of cow dung with sawdust on chemical composition of oyster mushroom (*Pleurotus ostreatus*)

Treatment	Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	CHO (%)	Crud fiber (%)
T_1	90.16b	9.84a	11.31d	5.43a	6.58b	56.38a	20.31d
T_2	90.60a	9.40b	25.66c	3.47c	8.13a	39.10b	23.64ab
T_3	90.01b	9.98a	31.30a	3.44c	8.41a	32.85c	24.01a
T_4	90.15b	9.85a	27.70b	4.26b	8.38a	37.80b	21.86c
T_5	90.52a	9.48b	25.93c	4.33b	8.1a	39.24b	22.40bc
CV (%)	0.11	1.04	1.27	1.43	1.64	2.38	1.24
Level of significance	**	**	**	**	**	**	**
LSD (0.05)	0.27	0.274	0.849	0.15	0.86	1.84	1.46

Means followed by same letter significantly different at 1% or 5% level of significance.

^{NS} Not significant * Significant at 5% level; ** Significant at 1% level

Legend:

T_1: Sawdust (Controlled)
T_2: Sawdust + Cow dung (5%)
T_3: Sawdust + Cow dung (10%)
T_4: Sawdust + Cow dung (15%)
T_5: Sawdust+ Cow dung (20%)

4.12.8. Effect on elemental content

4.12.8.1. Nitrogen

The highest percentage of nitrogen was counted under treatment T_3 (5.01) followed by T_4 (4.43) and the lowest nitrogen percentage was counted under T_1 (1.81). The rest of the treatments were statistically similar but varied significantly over control in terms of percent nitrogen content (Table 5). The rest of the treatments were statistically similar in respect to percent nitrogen content (Table 5). 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.

4.12.8.2. Phosphorus

The highest percentage of phosphorus was counted under treatment T_1 (0.92) and the lowest phosphorus percentage was counted under T_4 and T_5 (0.83). The rest of the treatments differed statistically in respect to percent phosphorus content (Table 5). The findings of the present study does not match with the study of Chang *et al.* (1981) who reported that the fruiting bodies of *Pleurotus* contained 5.87 to 8.40 mg/g of P on dry weigh of fruiting bodies. This may be due to the system of measurement. But Sarker *et al.* (2007) found 0.97% phosphorus, in oyster mushroom grown on sawdust based substrates.

4.12.8.3. Potassium

The highest percentage of potassium was counted under treatment T_3 (1.39) and the lowest potassium percentage was counted under T_1 (1.12). The rest of the treatments were statistically different and varied significantly in respect to percent potassium content (Table 5). The findings of the present study confirms by the study of Chang *et al.* (1981) reported that the fruiting bodies of *Pleurotus* contained 1.432 to 1.88 mg/g of K on dry weigh of fruiting bodies. Sarker *et al.* (2007) also found 1.3% potassium, in oyster mushroom grown on sawdust based substrates.

4.12.8.4. Calcium

The highest percentage of calcium was counted under treatment T_3 (22.15) and the lowest calcium percentage was counted under T_1 & T_5 (20.17). The rest of the treatments were statistically similar but differed significantly over control in respect to percent calcium content (Table 5). The findings of the present study matches with the study of Alam *et al.* (2007) who found 22.15 to 33.7 mg/100g of calcium in different oyster mushroom varieties. Sarker *et al.* (2007) found 2400ppm calcium, in oyster mushroom grown on sawdust based substrates.

4.12.8.5. Magnesium

The highest percentage of magnesium was counted under treatment T_4 (20.50) and the lowest magnesium percentage was counted under T_1 (18.15). The rest of the treatments were statistically similar but differed significantly over control in respect to percent magnesium content (Table 5). The findings of the present study corroborates with the study of Alam *et al.* (2007) found 13.4 to 20.22 mg/100g of magnesium in different oyster mushroom varieties.

4.12.8.6. Sulfur

There was no statistical difference among the treatments in terms of percent sulfur content. But the highest percentage of sulfur was counted under treatment T_3 (0.043) and the lowest sulfur percentage was counted under T_1 (0.012) (Table 5).

4.12.8.7. Iron

The highest percentage of iron was counted under treatment T_2 (9.98) & T_3 (43.4) and the lowest iron percentage was counted under T_1 (40.5). The rest of the treatments were statistically different over control in respect to percent iron content (Table 5). The findings of the present study matches with the findings of Alam *et al.* (2007) found 33.45 to 43.2 mg/100g of iron in different oyster mushroom varieties. Sarker *et al.* (2007) found 92.09 ppm to 118.40 ppm iron, in oyster mushroom grown on sawdust based substrates.

Table 5. Effect of different levels of cow dung with sawdust on elemental contents of oyster mushroom (*Pleurotus ostreatus*)

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (%)
T_1	1.81d	0.92a	1.12d	20.17c	18.15c	0.012	40.5c
T_2	4.12c	0.85bc	1.35b	21.15b	19.40b	0.037	43.4a
T_3	5.01a	0.88b	1.39a	22.15a	20.21ab	0.043	43.4a
T_4	4.43b	0.84c	1.25c	21.06b	20.50a	0.035	43.1a
T_5	4.15c	0.84c	1.15d	20.17c	19.30b	0.024	42.1b
LSD (0.05)	1.20	1.86	0.87	0.86	0.35	3.14	0.57
Level of significance	**	**	**	**	**	NS	**
CV (%)	0.123	0.031	0.030	0.287	1.007	0.087	1.24

Means followed by same letter significantly different at 1% or 5% level of significance.

^{NS} Not significant * Significant at 5% level; ** Significant at 1% level

Legend:

T_1: Sawdust (Controlled)
T_2: Sawdust + Cow dung (5%)
T_3: Sawdust + Cow dung (10%)
T_4: Sawdust + Cow dung (15%)
T_5: Sawdust+ Cow dung (20%)

CHEPTER 5

SUMMARY AND CONCLUSION

The present study was carried out at the Biochemistry laboratory and Mushroom Culture House (MCH) of Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, during the month of February to July'09 to investigate the performance of different levels of cow dung as supplement with straw for the production of oyster mushroom and analysis of their proximate composition.

Mycelium running rate per day (MRR) 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. Mycelium running rate in spawn packet was found to be differed due to different supplements used. The highest running rate was observed in T_4 (0.71 cm) and the lowest running rate of mycelium was observed in T_1 (0.50 cm). The other treatments were statistically similar (Table 1). The time from stimulation to primordia initiation ranged from 5.1 days to 7.1 days. The highest time from stimulation to primordia initiation was observed in T_1 (7.01 days). The lowest time from stimulation to primordia initiation was in the treatment T_4 (5.01). The other treatments varied significantly in terms of time from stimulation to primordia initiation (Table 1). The lowest time from primordia initiation to harvest was in the treatment T_3 (3.2 days) and the highest time from primordia initiation to harvest was observed in the treatment T_1 (5.27days). The other treatments were statistically similar (Table 1).

The highest average number of primordia/packet was observed in the treatment T_3 (73.21) followed by T_4 (66.70) and the lowest average number of primordia/packet was in the treatment T_1 (57.40). The other treatments were statistically similar (Table 2). The highest average number of fruiting body/packet was observed in the treatment T_3 (60.42) and the lowest average number of fruiting body /packet was in the treatment T_1 (37.43). The other treatments were statistically and significantly varied over control in terms of average number of primordia/packet

(Table 2). Supplementation of sawdust with levels of cow dung had great effect on average weight of individual fruiting body. The average weight of individual fruiting body in different treatment ranged from 2.99 g to 2.68 g. The highest average weight of individual fruiting body was observed in the treatment T_4 (2.99) and the lowest average weight of individual fruiting body was in the treatment T_1 (1.91) The other treatments varied significantly over control in terms of average weight of individual fruiting body (Table 2).

The supplementation of sawdust with cow dung had great effect on biological yield. The highest biological yield was counted under treatment T_3 (217.7 g) and the lowest biological yield was counted under T_1 (109.7 g). The other treatments varied significantly as compared with control in terms of biological yield (Table 3). The supplementation of sawdust with cow dung increases the economic yield over control. The highest economic yield was recorded under treatment T_3 (213.0 g) and the lowest economic yield was counted under T_1 (104.0 g). The other treatments varied significantly over control (Table 3). The dry yield of the oyster mushroom, grown on sawdust responded significantly in terms of dry yield with the different levels of supplement (cow dung). The dry yield of mushroom was maximum under the treatment T_3 (21.27 g) and the lowest dry yield was counted under T_5 (10.23 g). The other treatments varied significantly over control (Table 3).

The highest biological efficiency of 75.06% was calculated in treatment T_3 and the lowest biological efficiency of 37.82% was calculated from T_5 (Table 3). The other treatments varied significantly over control. The highest cost benefit ratio was calculated in treatment T_3 (7.46) and the lowest cost benefit ratio 3.80 was calculated from T_1. The other treatments differed significantly in terms of cost benefit ratio (Table 3).

The moisture content of the fruiting body showed significant difference. The moisture percent ranged from 90.60 to 90.01. The highest moisture percent was observed in treatment T_2 (90.60) followed by T_5 (90.52). The other treatments

were statistically similar but the lowest moisture was in T_3 (90.01) (Table 4). The dry matter percentage of the fruiting body shows significant difference. The dry matter percent of fruiting body ranged from 9.98 to 9.40. The highest dry matter percentage was observed in treatment T_3 (9.98) which was followed by T_4 (9.85) and T_1 (9.84). The other treatments were statistically similar but the lowest dry matter percentage was in T_2 (9.40) (Table 7). All the treatments contain a considerable amount of protein. The content of protein varied from 11.31-31.30% (w/w) in the mushroom grown on sawdust with different levels of cow dung. The highest content of protein was found in treatment T_3 (31.30%) which was followed by T_4 (27.70) and the lowest protein was found in T_1 (11.31 %). The other treatments statistically similar but varied significantly over control in respect to protein content (Table 4). The lowest lipid percentage was counted under treatment T_3 (3.44) followed by T_2 (3.47) and the highest lipid percentage was counted under T_1 (5.43). The rest of the treatments were statistically similar (Table 4).

The highest percentage of ash was observed in the treatment T_3 (8.41) and the lowest percentage of ash was in the treatment T_1 (6.58). The other treatments were statistically similar but differed significantly in terms of percentage ash content (Table 4). The lowest percentage of carbohydrate was counted under treatment T_3 (32.85) and the highest carbohydrate percentage was counted under T_1 (58.38). The rest of the treatments were statistically similar but differed over control in respect to percent carbohydrate content (Table 7). The highest percentage of crud fiber was counted under treatment T_3 (24.01) followed by T_2 (23.64) and the lowest crud fiber percentage was counted under T_1 (20.31). The rest of the treatments were statistically similar but varied over control in respect to percent crud fiber content (Table 4).

The highest percentage of nitrogen was counted under treatment T_3 (5.01) followed by T_4 (4.43) and the lowest nitrogen percentage was counted under T_1 (1.81). The rest of the treatments were statistically similar but varied significantly over control in terms of percent nitrogen content (Table 5). The highest percentage of phosphorus was counted under treatment T_1 (0.92) and the lowest phosphorus

percentage was counted under T_4 and T_5 (0.83). The rest of the treatments differed statistically in respect to percent phosphorus content (Table 5). The highest percentage of potassium was counted under treatment T_3 (1.39) and the lowest potassium percentage was counted under T_1 (1.12). The rest of the treatments were statistically different and varied significantly in respect to percent potassium content (Table 5).

The highest percentage of calcium was counted under treatment T_3 (22.15) and the lowest calcium percentage was counted under T_1 & T_5 (20.17). The rest of the treatments were statistically similar but differed significantly over control in respect to percent calcium content (Table 5). The highest percentage of magnesium was counted under treatment T_4 (20.50) and the lowest magnesium percentage was counted under T_1 (18.15). The rest of the treatments were statistically similar but differed significantly over control in respect to percent magnesium content (Table 5).

There was no statistical difference among the treatments in terms of percent sulfur content. But the highest percentage of sulfur was counted under treatment T_3 (0.043) and the lowest sulfur percentage was counted under T_1 (0.012) (Table 5). The highest percentage of iron was counted under treatment T_2 (9.98) & T_3 (43.4) and the lowest iron percentage was counted under T_1 (40.5). The rest of the treatments were statistically different over control in respect to percent iron content (Table 5).

Therefore it can be concluded that sawdust supplemented with 10% cow dung is better for growing oyster mushroom and can be used by the farmers for growing mushroom at a low cost.

REFERANCES

Adamovie, M.; G. Grubic; I. Milenkovic, R. Jovanovic, R. Protic, L. Sretenovic and L. Stoicevic. (1996). Biodegradation of wheat straw achieved during *Pleurotus ostreatus* mushroom production. J. Sc. Agril. Res. 57(3-4): 79-88.

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

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

Alam Nuhu, Asaduzzaman Khan, Md. Shahdat Hossain, S.M. Ruhul Amin and Liakot Ali Khan. (2007). Nutritional Analysis of dietary Mushroom *Pleurotus florida* Eger *and Pleurotus sajorcaj* Fr.) Singer. Bangladesh Journal of Mushroom. 1(2): 1-7.

Alam, S. M. and R. Saboohi. (2001). Importance of mushroom, http://www.mushroomworld.com

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

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

Balasubramanym, R. H. (1988). Mushroom Crop on willow-dust. Indian J. of microbiology, 28 (1-2): 131-132.

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

Bhuyan, M. H. M. B. U. (2008). Study on Preparation of Low Cost Spawn Packets for the Production of Oyster Mushroom (Pleurotus Ostreatus) and its Proximate Analysis. M.S. Thesis, Department of Biochemistry, SAU, Dhaka-1207.

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

Chang, S. T. and S. R. Miles. (1992). Oyster Mushroom Cultivation. The Chinese Univ. Hongkong, 26(5):66-68.

Chang, S.T. (1982). Cultivation of *Volvariella* Mushrooms in Southeast Asia, In: Trop. Mushrooms; Biological Nature and Cultivation Methods. The Chinese Univ. Press, Hong Kong. PP 135-156.

Chang, S.T. and Miles, P.G. (1988). Edible Mushroom and their cultivation. CRC Press, Inc. Boca Raton, Florida U.S.A. pp. 27, 83, 88.

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

Chowdhury, A. K., B. N. Panja and S. K. Laha. (1998). Organic supplements for better yield of oyster mushroom. J. Interacademicia B.C.K.V., India. 2(1-2): 116-117.

Dey, R.C. (2006). Mycelial Growth and Oyster Mushroom Production with Different Hormone and Media Composition. M. S. Thesis, Department of Biotechnology, BAU, Mymensingh.

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

Fujihara, S., A. Kasuga, T. Sugahara. K. Hashimoto, Y. Kiyomizu. T. Nakazawa and Y. Aoyagi. (2000). Nitrogen content of shiitake mushroom (*Lenlimus edodes* (Berk.) Sing.) cultivated on sawdust medium and dependence on that in the medium. J. Japanese Soc. Food Sci. Technol. 47(3): 191-196.

Gomez, K. A. and A. A. Gomez. (1984). Statistical procedures for agricultural research. John Wiley & Sons, Inc. New York.

Gupta, J.H. (1989). Yield potentiality of oyster mushroom on wheat straw under natural room temperatures, during March-April and September-October at Saharanpur. Progressive Horticulture. 21(1-2): 184.

Gupta, R.S. (1986). Mushroom Cultivation. Indian Hort. 31(1): 11.

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

Ivan, H.R.; Antonio, C.M.; Jose, O.M. and Jose. C.B. (2003). Supplementation of sugarcane bagasse with rice bran and sugarcane molasses for shiitake (*Lentinula edodes*) spawn production. Brazil J. Microb. 34: 151.

Jadhav A. B; 13agal P. K. and Jadhav S. W. (1996). Effects of different substrates on
yield of oyster mushroom. J. Maharashtra Agril. Univ. 21: (3) 424-426.

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

Kalita, P., Mazumder, N., Kalita, P. (2001). Performance of Oyster mushroom (*Pleurotus spp.*) on certain plant wastes. Horticultural Res. Stat. Assam Agricultural University, Assam, India. J. the Agricultural Sci. Society of North East India. 14(2): 221-224.

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

Khan, S.M,. Javed, N. Khan, S.M. Khan, S.M. Javed, N., Khan, S.M. (2002). Studies on cultivation of Oyster mushroom (*Pleurotus spp.*) on different forest wastes. Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. Integrated Plant Disease Management. Proceedings of 3rd National Conference of Plant Pathology, NARC, Islamabad, October, 2001. 144-147.

Khan, S.M., Mirza, J.H. and Khan, M.A. (1991). Studies on Shiitake mushroom (*Lentinula edodes*). Proc. 13th Int'l. Con. Sci. Culti. Edible Fungi. Dublin, Irish Republic. pp 503-508 Khlood, A. Ahmad, A. (2005

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

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

Kothandaraman, R., K. Joseph, J. Mathew and K. Jayarathnam. (1989). Mushroom cultivation on rubber wood wastage: a new approach. Rubber Board Bulletin. 25(2):17-18.

Kovfeen, C. (2004). Economic Times. http://www.techno-preneur.net

Krishnamoorthy; A. S. (1997). Influence of organic supplements on yield and protein content of oyster mushroom., Regional Research Station, Tamil Nadu Agril. Univ. Madras Agril. J. India, 84(10):604-606.

Labuschagne, P. M., A. Eicker, T.A.S. Aveling, S. deMeillon and M. F. Smith. (2000). Influence of wheat cultivars on straw quality and *Pleurotus ostreatus* cultivation. J. Bioresource Tech. 71(1):71-75.

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

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

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

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

Mukesh, S.K.; Das, S. R. and Pani, B. K. (2004). Effect of pretreatment of substrate on the yield of oyster mushroom (*Pleurotus sajor-caju* (Fr.) Singer). J. Mycopathological - Res. Bhubaneswar, India, 36 (2): 113-114.

Murugesan, A. G., Vijayalakshmi, G. S. Sukumaran, N. Mariappan, C. (1995). Utilization of water hyacinth for oyster mushroom cultivation. Biorcsourcc-Technology. 51(1):97-98.

Obodai M.; Sawyerr L. C. B. and Johnson P. N. T. (2000). Yield of seven strains of oyster mushrooms (*Pleurotus* spp.) grown on composted sawdust of *Triplochiton scleroxylon*. Trop. Sci. 40(2):95-99.

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

Pani B. K.; Panda S.N. and Das S. R. (1997). Effect of organic supplementation of substrate on the yield of some species of oyster mushroom. Environment and Ecology. 15(3): 609-611.

Patrabansh, S. and R. Madan. (1997). Mineral content of the fruiting bodies of *Pleurotus sajor-caju* (Fr.) Singer cultivated on different kinds of Biomass. Acta Blotechnologica India, 19 (2): 101-109

Pattana Pulpium (1996). Optimum condition for *Pleurotus ostreatus* in plastic bag. King Mongkut' s Inst. Tech. pp. 233-234.

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

Peng-Jin Torng, Lee-Chien Ming, Tsai-Yin Fung, J. T. Peng, C. M. Lee and Y. F. Tsai. (2000). Effect of rice bran on the production of different king Oyster mushroom strains during bottle cultivation. J. Agril. Res. China. 49(3): 60-67.

Qin. S. X. (1989). Effects of different cultivation materials on nutritive composition of *Pleurotus* fruiting bodies. Edible fungi of China. 3:12-13.

Quimio, T. H. and U. Sardsud. (1981) Nutritional requirements of *Pleurotus ostreatus* (Fr.). Philippine Agriculturist. 64(1): 79-89.

Raghuramulu, N., Madhavan, N. K. & Kalyanasundaram, S. 2003. A Manual of Laboratory Techniques. National Institute of Nutrition. Indian Council of Medical Research, Hyderabad-500 007, India. pp: 56-58.

Ragunathan. R., R. Gurusamy, M. Palniswamy and K. Swaminathan. (1996). Cultivation of *Pleurotus* spp. on various agro-residues. Food Chem. 55(2): 139-144.

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

Ramesh, C. R. and M. N. Ansari. (1987). Substrate evaluation for cultivation of Oyster mushroom *Pleurotus sajor-caju* (Fr.) Sing. Andamans J. of the Andamans Sci. Assoc. 3(2): 110-112 (cited from Hort. abst. 569(2). 1105. 1986).

Reyes, R. G., A. D. Encarnacion and E. A. Abella. (1994). Utilization or selected agro-industrial wastes for mushroom (*Pleurotus sajor-caju*(Fr.) Singer) production. CLSU Sc. J. 14(1): 9-23.

Royse, D. J. (2002). Influence of spawn rate and commercial delayed release nutrient levels on *Pleurotus cornucopiae* (oyster mushroom) yield, size and time to production. Appl. Microbiol. Biotechnol. 58 (4): 527-531.

Ruhul Amin, S. M. (2002). Performance of different Oyster mushroom (*Pleurotus* spp) varieties. M.S. Thesis. Bangabundhu Sheikh Mujibur Rahman Agricultural University. Salna, Gazipur.

Ruhul Amin, S. M. (2002). Performance of different Oyster mushroom (*Pleurotus* spp) varieties. M.S. Thesis. Bangabundhu Sheikh Mujibur Rahman Agricultural University. Salna, Gazipur.

Ruhul Amin, S.M., Nirod Chandra Sarker, Abul Khair and Nuhu Alam. (2007). Detection of Novel Supplements on Paddy Straw Substrates on Oyster Mushroom Cultivation. Bangladesh Journal of Mushroom. 1(2): 18-22.

Sarawish, W. (1994). Study on using local materials are main substrate for the straw mushroom spawn production. proc. 11th Rajamangala Inst. of Technol. Seminar. pp. 73-80.

Sarker, N. C. (2004). Oyster mushroom (*Pleurotus ostreatus*) Production Technology Suitable for Bangladesh and its Nutritional and Postharvest Behavior. Phd Thesis. Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur.

Sarker, N.C., M.M. Hossain, N. Sultana, I.H. Mian, A.J.M. Sirazul Karim and S.M. Ruhul Amin. (2007). Impect of different Substrates on Nutrient Content of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer. Bangladesh Journal of Mushroom. 1(2): 35-38.

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

Scherba, V.V., Osadchaya, O.V., Truchonovec, V.V. (2004). Growth habits and propagation of Oyster mushroom on vegetative substrates using liquid sowing mycelium.Vestsi-Natsyyanal-nai-Akademii-Navuk-Belarusi-Seryya-Biyalagichnykh-Navuk. 2:87-89.

Schmidt. O. (1985). Investigations on mushroom culture on wood wastes. Champignon, 291: 22-29.

Shah, Z. A., M. Ashraf and M. Ishtiaq. (2004). Comparative Study on Cultivation and Yield Performance of Oyster Mushroom (*Pleurotus ostreatus*) on Different Substrates (Wheat Straw, Leaves, Saw Dust). Pakistan J. of Nutrition 3 (3): 158-160.

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

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

Triratna, S., Thaithatgoon, S. and Gawgla, M. (1991). Cultivation of *Ganoderma.lucidum* in sawdust bags. Proc. 13th Int'l Cong. Sci. Culti. Edible Fungi. Dublin, Irish Republic. pp 567-572.

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

Yildiz, A.; Karakaplan, M. and Aydin, F. (1998). Studies on *Pleurotus ostreatus* cultivation, proximate composition, organic and mineral composition of carpophores. Food-chem. Oxford : Elsevier Sci. Ltd. 61 (1/2): 127-130.

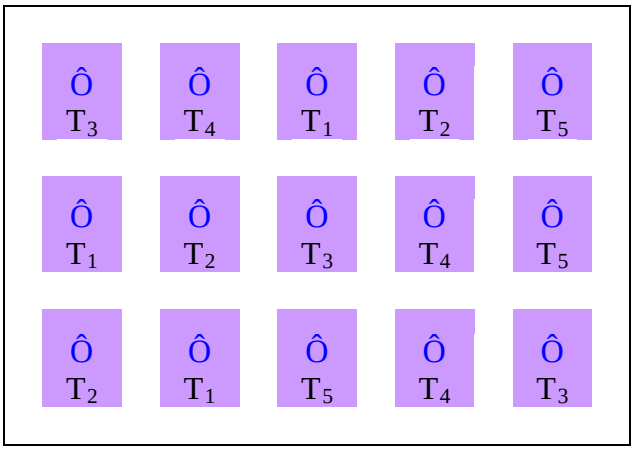
Yoshida, N., T. Takahashi, T. Nagao and J. Chen. (1993). Effect of edible mushroom (*Pleurotus ostreatus*) cultivation on *in vitro* digestibility of wheat straw and sawdust substrate. J. Japanese Soc. Grassland Sci. 39(2): 177-182.

Yoshizawa, N., T. Itoh, M. Ohnishi, F. Ishiguri, M. Ando, S. Yokota, M. Sunagawa and T. ldei. (1998). Mushroom cultivation using smoke-heated softwood sawdust. Bulletin of the Utsunomiya Univ. Forests, 34: 69-79.

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

APPENDICES

Appendix 1. Experimental layout



Legend
Ô: Mushroom Packet

Appendix 2. Analysis of the variance of the data on mycelium running rate, time required from stimulation to primordia initiation, primordia initiation to harvest of the mushroom produced on sawdust supplemented with different levels of cow dung

Source	Mycelium running rate in spawn packets (cm)	Time required from stimulation to primordia initiation (days)	Time required from primordia initiation to harvest (days)
Replication	0.00012	0.089	0.089
Treatment	0.017*	2.176**	1.626**
Error	0.0001	0.007	0.011

^{NS} Not significant * Significant at 5% level; ** Significant at 1% level

Appendix 3. Analysis of the variance of the data on, number of primordia/packet, number of fruiting body/packet, weight of individual fruiting body of the mushroom produced on sawdust supplemented with different levels of cow dung

Source	Number of Primordia /packet	Average number of fruiting body /packet	Average weight of individual fruiting body (g)
Replication	0.063	0.019	0.004
Treatment	98.105**	205.592**	0.601**
Error	0.003	0.007	0.002

^{NS} Not significant * Significant at 5% level; ** Significant at 1% level

Appendix 4. Analysis of the variance of the data of yield and biological efficiency and cost benefit ratio of the mushroom produced on sawdust supplemented with different levels of cow dung

Source	Biological yield (g/packet)	Economic yield (g/packet)	Dry yield (g/packet)	Biological efficiency (%)	Benefit cost ratio
Replication	13.867	0.600	0.005	1.650	0.001
Treatment	5071.6**	5139.9**	52.98**	603.052**	8.886**
Error	8.450	1.100	0.006	1.007	0.002

^{NS} Not significant * Significant at 5% level; ** Significant at 1% level

Appendix 5. Analysis of the variance of the data on chemical composition of the mushroom produced on sawdust supplemented with different levels of cow dung

Source	Moisture (%)	Dry Matter (%)	Protein (%)	Lipid (%)	Ash (%)	CHO (%)	Crud fiber (%)
Replication	0.005	0.005	0.043	0.008	0.413	0.225	0.003
Treatment	0.197**	0.197**	175.39**	1.989**	239.85**	6.588**	1.744**
Error	0.010	0.010	0.096	0.003	0.454	0.285	0.010

^{NS} Not significant * Significant at 5% level; ** Significant at 1% level

Appendix 6. Analysis of the variance of the data on elemental contents of the mushroom produced on sawdust supplemented with different levels of cow dung

Source	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (%)
Replication	0.001	0.0001	0.0001	0.0001	0.003	0.001	0.005
Treatment	4.492*	0.003*	0.042*	0.0002*	2.036**	2.528*	4.513**
Error	0.002	0.0001	0.0001	0.0001	0.001	0.011	0.135

^{NS} Not significant * Significant at 5% level; ** Significant at 1% level