STUDY ON SUPPLEMENTATION OF WHEAT BRAN WITH SUGARCANE BAGASSE ON YIELD AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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CERTIFICATE

This is to certify that the thesis entitled **"Study on Supplementation of Wheat Bran with Sugarcane Bagasse 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 **Md. Rostom Ali**, Registration No. **07-2594** 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:June,2009
Dhaka, Bangladesh

Department of Biochemistry
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Dedicated to
My Beloved Parents

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ABSTRACT

The present study was carried out during the period of February to July, 2009 to investigate the performance of different levels of wheat bran as supplement with sugarcane bagasse on the production of oyster mushroom and analysis of their proximate composition. The highest mycelium running rate was observed due to sugarcane bagasse supplemented with wheat bran @ 40% (T_5) (0.96 cm). The highest time from stimulation to primordia initiation was observed in T_1 (11.5 days). The lowest time from primordia initiation to harvest was in the treatment of T4 (3.23 days). The highest average number of primordia/packet (70.67), average number of fruiting body/packet (61.00) was observed in the treatment of T_5. The highest average weight of individual fruiting body was observed in the treatment of T_4 (3.69 g). The highest biological yield (254.7 g), economic yield (243.3 g), dry matter (23.40 g), biological efficiency (87.82%) and cost benefit ratio (8.29) was counted under treatment of T_4. The highest moisture content (90.45 %) was observed in T_5 where as the highest dry matter percentage of (10.07 %) was found in the fruiting body of T_2. The highest content protein (30.31 %), ash (9.15 %) and crud fiber (24.07 %) and the lowest lipid (3.90 %) and carbohydrate (32.57 %) was found in the treatment of T_4. The highest percentage of nitrogen (4.85), potassium (1.39g/mg), calcium (22.08mg), magnesium (20.21mg), sulfur (0.042g/mg), iron (43.11mg) was counted under T_4 but the highest percentage of phosphorus was counted under treatment of T_1 (0.92). Therefore, it can be concluded that sugarcane bagasse supplemented with 30% wheat bran can be further used as a better substrate for oyster mushroom production reducing cost and increasing the yield and nutritional quality.

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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

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ABSTRACT

The present study was carried out during the period of February to July, 2009 to investigate the performance of different levels of wheat bran as supplement with sugarcane bagasse on the production of oyster mushroom and analysis of their proximate composition. The highest mycelium running rate was observed due to sugarcane bagasse supplemented with wheat bran @ 40% (T_5) (0.96 cm). The highest time from stimulation to primordia initiation was observed in T_1 (11.5 days). The lowest time from primordia initiation to harvest was in the treatment of T4 (3.23 days). The highest average number of primordia/packet (70.67), average number of fruiting body/packet (61.00) was observed in the treatment of T_5. The highest average weight of individual fruiting body was observed in the treatment of T_4 (3.69 g). The highest biological yield (254.7 g), economic yield (243.3 g), dry matter (23.40 g), biological efficiency (87.82%) and cost benefit ratio (8.29) was counted under treatment of T_4. The highest moisture content (90.45 %) was observed in T_5 where as the highest dry matter percentage of (10.07 %) was found in the fruiting body of T_2. The highest content protein (30.31 %), ash (9.15 %) and crud fiber (24.07 %) and the lowest lipid (3.90 %) and carbohydrate (32.57 %) was found in the treatment of T_4. The highest percentage of nitrogen (4.85), potassium (1.39g/mg), calcium (22.08mg), magnesium (20.21mg), sulfur (0.042g/mg), iron (43.11mg) was counted under T_4 but the highest percentage of phosphorus was counted under treatment of T_1 (0.92). Therefore, it can be concluded that sugarcane bagasse supplemented with 30% wheat bran can be further used as a better substrate for oyster mushroom production reducing cost and increasing the yield and nutritional quality.

CHAPTER 1

INTRODUCTION

All of us are acquainted with green plants such as trees, flowers and grass, and when we think of plants it is these which we have in mind. But there are other plants which are not green and which do not produce flower or seeds and are often more or less inconspicuous. They are associated with spoiling and decay of food, wood etc. with the growth of their vegetative organ and may produce large fleshy masses. Mushrooms are the member of these groups of plants, the fungi.

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 commercial cultivation 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 are having a long history of use in traditional Chinese Medicine to promote good health and vitality and increasing body's adaptive abilities. Specifically, selected strains of dried mushrooms are used to produce mushrooms capsules and extracts. Mushroom reduces serum cholesterol and high blood pressure (Mori *et al*. 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). Most of the people of Bangladesh have been suffering from malnutrition. Mushroom could substantiate the suffering from malnutrition to some extent. 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.

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 enormous increase in our population has necessitated more and more food production. In the developed countries, mushrooms have become one of the most important horticultural crops (Alam and Saboohi, 2001). Mushroom is short duration and land saving crop. It needs labor intensive indoor activity, which can help the landless, small and marginal farmers to raise their income, diversify economic activity and create gainful employment, especially for unemployed /under-employed youth and women folk. So it can be welcomed by the poor and marginal farmers. The mushroom cultivation could be a profitable agribusiness. Mushroom farm offer a valuable services to the livestock and poultry sectors by transforming agricultural byproducts. The substrates used for mushroom production are excellent source of organic fertilizers. Mushroom production converts agricultural wastes into a high protein source for human (Labuschagne *et al.* 2000). Our country has resources and potential for large scale production of mushroom both for home consumption and export.

Bangladesh is a developing country with vast population. Our agricultural land is decreasing day by day for the accommodation of large population. So we have to increase intensive use of land for increasing crop production. But it is very difficult

in our country due to natural hazards and other barriers. In this case mushroom cultivation can be a huge opportunity for increasing crop production per unit area with the vertical use of land and thus mushroom cultivation is becoming wide spread among the farmers in the country. It grows independent of sunlight, relatively fast growing, does not require fertile soil since grown on compost or non-compost agro-wastes as wheat and paddy straw, banana leaves, sugarcane bagasse and leaves, wheat barn, rice husk, sawdust etc. and their culture can be concentrated within a relatively small space. No research work on mushroom has yet been done to standardize the production practices of mushroom in our country while countries like India and Pakistan have done much works in this regard.

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

- To determine the yield, biological efficiency and the nutritional status of oyster mushroom
- To select the suitable levels of wheat bran in spawn packets by benefit-cost analysis.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Effect of Substrate

Lozovoi (1981) reported that *Pleurotus spp*. cultivated on a sawdust-based medium produced 20-30% yield by volume on raw substrate in the laboratory, and 10-30% yield by volume on sawdust blocks in the glasshouse. Several strains of P. *ostreatus* were most promising for intensive cultivation.

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.

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.

Substrate is an important item for growing mushroom. It is a kind of media which supports the growth, development and fruiting of mushroom (Chang and Miles. 1988).

Kothandaraman *et al.* (1989) in a study it was reported that in the split or the logs of *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.

Visscher (1989) found that Lucerne meal gave better results than rice bran, which was usually better than straw alone for the production of Oyster mushroom.

Patil (1989) cultivated P. *sajor-caju* on six different substrates, i.e. wheat straw, bajra (*Pennisetunz americana*), maize straw, paddy straw, jower and cotton stick.

The results indicated that all the substrates could be used for commercial cultivation of the oyster mushroom.

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

Stolzer and Grabbe (1991) stated that in the United States, the primary ingredients used for *Pleurotus sp*. production arc chopped wheat straw or cottonseed hulls or mixture thereof. For production on wheat straw, the material is milled to a length of about 2 to 6 cm. The pH of the material is adjusted with limestone to about 7.5 or higher to provide selectivity against *Trichoderma* green mold.

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.

Mathew *et al.* (1991) evaluated *Pleurotus sajor-caju, Pleurotus* citrinopileatus, *Pleurotus florida, Pleurotus* platypus and *Pleurotus ostreatus* for their yield performance on various substrates both for spawn production and cultivation in the plains and in the high ranges of Kerala in the summer and rainy seasons. Sorghum,

wheat and paddy grains were equally good for spawn production. *Pleurotus sajor-caju*, *Pleurotus* citrinopileatus and *Pleurotus florida* were the most suitable species for cultivation in both the plains and the high ranges. These three species were successfully cultivated on paddy Straw. *Eliocharis plantogena* and rubber Wood sawdust, although for commercial cultivation of *Pleurotus sajor-caju* rubber wood sawdust was not rated as an ideal medium.

Bugarski *et al.* (1994) in an experiment studied the strains of Oyster mushrooms NS-16 and NS-11 with the aim to determine the best yielding strain, shortest incubation period and fast fructification. The hybrid H7 was used for check. Three different substrates; Wheat straw, Maize stalks, and Soybean straw were used. First fruiting body appeared on H_7 on 30^{th} to 33^{rd} days depending on substrate.

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%.

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.

Bugarski *et al.* (1995) cultivated two strains of *Pleurotus ostreatus* on six different substrates. Strains NS16 produced mycelium of the best quality on prosomillet and prosomillet + sawdust strain H7 produced the best mycelium on millet + sawdust and wheat + sawdust. NS 16 was grown more rapidly than that of the H7 on all substrates.

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

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.

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 $(NH_4)_2SO_4$ 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.

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.

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%).

Manukovsky *et al.* (1998) reported that *P. florida* was grown on wheat straw. Decreased yields were observed when the residual substrate was mixed with wheat straw. Washing the residual substrate did not result in decreased yields.

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

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

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.

Wani and Sawant (1998) reported that among the various edible fungi, oyster mushroom (*Pleurotus spp.*) has a broad adaptability due to having a wide range of suitable substrates, a simple cultivation technique and minimal cultural requirements. Various substrates on which oyster mushroom can be cultivated are mentioned.

Veena *et al.* (1998) conducted a preliminary experiment to find out the feasibility of growing *Pleurotus florida* on dried aerial parts of *Cassia hirsuta* (collected in India) in combination with different levels of bagasse (100:0, 75:25, 50:50, 25:75 or 0:100). Equal amounts of these substrates (1:1 proportion) gave highest yield (313 fruiting bodies) and the highest bioefficiency (65.94%).

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.

Permana *et al.* (2000) reported that Sugarcane bagasse supplemented with soyabean meal or wheat bran served as valuable substrate for production of *Pleurotus sajor-caju*, *P. eiyngii* and *Agrocybe aegerita*. Comparable lignin degradation, fruiting body yield and increase in vitro digestibility, as obtained with other traditional substrates, were achieved. Judging from the above, sugarcane industries could source further benefits through recycling of bagasse in the production of mushroom substrate and animal feed, which can be commercialized. The resulting compost can further be incorporated in the soil to boost the fertility

status of tropical soils. Appropriate technology for this bioconversion is a subject for which further studies are needed.

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.

Panneerselvam *et al.* (2000) conducted an experiment by *Pleurotus sajor-caju* using a standard polypropylene bag method containing coffee and cherry husk 500 g dry weight basis, 1.5 kg wet weight basis), which had been subjected to hot water treatment for 15-20 min. The substrate was inoculated with spawn (5%, wet weight basis) using a triple layer spawning technique, and kept at room temperature. Fruiting bodies were observed 14-15 days after inoculation, with a mean yield of 238.4 g/bag.

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.

Baysal *et al.* (2003) conducted an experiment to spawn running, pin head and fruit body formation and mushroom yield of Oyster mushroom (*Pleurotus ostreatus*) on waste paper supplemented with peat, chicken manure and husk rice (90+10; 80+20 W:W). The fastest spawn running (mycelia development) (15.8 days), pin head formation (21.4 days) and fruit body formation (25.6 days) and the highest yield (350.2 g) were realized with the substrate composed of 20% rice husk in weight. In general, increasing the ratio of rice husk within the substrate accelerated spawn running, pin head and fruit body formation and resulted increased mushroom yields, while more peat and chicken manure had a negative effect on growing.

Vyas *et al.* (2002) found that the yield of *Pleurotus florida* cultured on wheat straw supplemented with wheat bran, rice bran or neem cake under laboratory condition

maintaining 26°C temperature and 80-85 % relative humidity and found that wheat straw supplemented with neem cake was the most favorable substrate for *florida* production.

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.

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.

Maniruzzaman (2004) in his study used wheat, maize, rice and sawdust for the production of spawn in Oyster mushroom and loud that substrate rice was the best for spawn production of Oyster mushroom.

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.

Ramjan (2006), in study found that high concentration of IAA is effective for mycelial growth and mustard straw performed best as a substrate for the production of fruiting bodies of oyster mushroom.

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*.

Sterilized chicken manure was recommended as supplement by Vijoy and Upadhyay (1989) for *Pleurotus sajor-caju* and *Pleurotus flabellantus* production.

Cressewll *et al.* (1990) tested the toxicity of B and Cu to cultivated mushroom in two experiments. They found that the tissue B was buffered around 7 mg/kg and Cu around 73 mg/kg. The results indicate that the levels of B and Cu that are normally contributed to mushroom composed from poultry litter are unlikely to cause significant losses in mushroom production.

Various oilseed meal and cakes, powdered pulse, wheat and rice bran etc. have also been tried in India (Bahukhandi, 1990).

Royse *et al.* (1991) found that yields of *Pleurotus sajor-caju* strain 537 from the substrate supplemented with the commercial nutrient were 1.7-fold higher than yields from non-supplemented substrate. As the supplement level increased from 6 to 12%, the mushroom yields increased. The yields ranged from 3.56 kg/m^2 for non-supplemented substrates to 7.36 kg/m^2 for substrate supplemented (12% DW) with formaldehyde soybean meal.

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 the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, from February, 2009 to July, 2009.

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: Sugarcane bagasse supplemented with wheat bran @ 0% (Control)
- T_2: Sugarcane bagasse supplemented with wheat bran @ 10%
- T_3: Sugarcane bagasse supplemented with wheat bran @ 20%
- T4: Sugarcane bagasse supplemented with wheat bran @ 30%
- T_5: Sugarcane bagasse supplemented with wheat bran @ 40%

3.3. Preparation of packets

Spawn packets using different levels of supplements were prepared separately. With spawn preparing substrate; different supplements (at the different rate on dry weight basis) and $CaCO_3$ (1 g 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%. The mixed substrates were filled into 9×12 inch polypropylene bag @ 500 g. The filled polypropylene bags were prepared by using bamboo neck and plugged the neck with cotton and covered with brown paper placing rubber band to hold it tightly in place.

3.4. 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.

3.5. 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 2-4 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

3.6. Collection of produced mushrooms

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

3. 7. Data collection

3.7.1. Days required for completing mycelium running: Days required from inoculation to completion of mycelium running were measured.

3.7.2. 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.

3.7.3. 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.

3.7.4. 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.

3.7.5. 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.

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

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

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

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

3.7.8. 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).

3.8. Drying of mushrooms

The collected fruiting bodies of the mushroom are transferred to the laboratory. Therefore data are collected on different parameter. After collection of the data the fruiting bodies are dried in the sun separately treatment wise. In the time of drying the stipe and the pileus are separated for better drying.

3.9. Proximate analysis of the mushrooms

3.9.1. 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 fruits or vegetables.

3.9.2. 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.

3.9.3. Determination of Crude Fiber

Ten gram of moisture and fat-free sample was taken in a beaker and 200 ml of boiling 0.255N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The

mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker, and 200 ml of boiling 0.313N NaOH added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100°C and weighed (We) in an electric balance *(KEY]: JY-2003; China).* The crucible was heated in a muffle furnace *(Nebertherm: Mod-L9/11/c6; Germany)* at 600°C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber.

Therefore,

Crude fiber (g/l00g sample) = [100-(moisture + fat)] x (We-Wa) / Wt. of sample (Raghuramulu *et al.*, 2003).

3.9.4. Lipid estimation

Fat was estimated as crude ether extraction of the dry materials. 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:

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

3.9.5. Total Carbohydrate Estimation:

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

Carbohydrate (g/l00g sample) = 100 - [(Moisture + Fat + Protein + Ash + Crude Fiber) g/100g] (Raghuramulu *et al., 2003)*

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

3.9.7. Determination of total Nitrogen

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

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

Where,

a = ml HCI measured 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 mushroom used for the analysis

3.9.8. Determination of total sulphur

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

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

Where, A = weight of $BaSO_4$ g

M = amount of solution transferred to beaker for precipitation of $BaSO_4$ (ml)
W = weight of sample in g

3.9.9. 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 spectrophotometry, K was determined by flame photometry, and P was determined by spectrophotometry.

3.10. 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) and Randomized Complete Block Design (RCBD) method. 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 levels of supplements used. The highest running rate was observed in T_5 (0.96 cm) and the lowest running rate of mycelium was observed in T_1 (0.72 cm). The other treatments varied significantly over control (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 (11.5 days). The other treatments varied significantly in terms of time from stimulation to primordia initiation (Table 1). But the lowest time from stimulation to primordia initiation was in the treatment T_3 (7.17 days). 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 sugarcane bagasse 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_4 (3.23 days) and the highest time from primordia initiation to harvest was observed in the treatment T_1 (5.17days) followed by T_5 (5.16 days). The other treatments were statistically similar (Table 1). The result of the present findings keeps 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 wheat bran with sugarcane bagasse on mycelial growth, 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	0.72e	11.5a	5.17a
T_2	0.78d	7.83b	4.2b
T_3	0.87c	7.17b	4.1b
T_4	0.91b	7.57b	3.23c
T_5	0.96a	7.3b	5.16a
CV (%)	0.71	5.16	3.01
Level of significance	**	**	**
LSD (0.05)	0.028	1.169	0.358

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: Sugarcane bagasse (Controlled)
T_2: Sugarcane bagasse + Wheat bran (10%)
T_3: Sugarcane bagasse + Wheat bran (20%)
T_4: Sugarcane bagasse + Wheat bran (30%)
T_5: Sugarcane bagasse + Wheat bran (40%)

4.4. Average Number of Primordia

The highest average number of primordia/packet was observed in the treatment T_5 (70.67) followed by T_4 (69.00) and the lowest average number of primordia/packet was in the treatment T_1 (48.00). The other treatments varied significantly over control (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 fruting body

The highest average number of fruiting body/packet was observed in the treatment T_5 (61.00) followed by T_4 (58.00) and the lowest average number of fruiting body /packet was in the treatment T_1 (41.00). 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 weighs of individual fruiting body (g)

Supplementation of sugarcane bagasse with levels of wheat bran had great effect on average weight of individual fruiting body. The average weight of individual fruiting body in different treatment ranged from 3.06 g to 3.69 g. The highest average weight of individual fruiting body was observed in the treatment T_4 (3.69 g) and the lowest average weight of individual fruiting body was in the treatment T_1 (3.06 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 comparatively 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 wheat bran with sugarcane bagasse 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	48.00d	41.00d	3.06d
T_2	59.00c	51.00c	3.33b
T_3	66.00b	53.00c	3.33b
T_4	69.00a	58.00b	3.69a
T_5	70.67a	61.00a	3.15c
CV (%)	1.49	1.80	0.91
Level of significance	**	**	**
LSD (0.05)	2.55	2.60	0.087

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

4.7. Biological Yield (g)

The supplementation of sugarcane bagasse with wheat bran had great effect on biological yield. The highest biological yield was counted under treatment T_4 (254.7 g) and the lowest biological yield was counted under T_1 (147.0 g). The other treatments varied significantly as compared with 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 sugarcane bagasse with wheat bran increases the economic yield over control. The highest economic yield was recorded under treatment T_4 (243.3 g) and the lowest economic yield was counted under T_1 (142.0 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 sugarcane bagasse responded significantly in terms of dry yield with the different levels of supplement (wheat bran). The dry yield of mushroom was maximum under the treatment T_4 (23.40 g) and the lowest dry yield was counted under T_1 (14.13 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 g to 29.98 g, which was more or less similar to this study.

4.10. Biological efficiency

The highest biological efficiency of 87.82% was calculated in treatment T_4 and the lowest biological efficiency of 50.69 % was calculated from T_1 (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 Madan, 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. Cost benefit ratio

The highest cost benefit ratio was calculated in treatment T_4 (8.29) and the lowest cost benefit ratio 6.09 was calculated from T_1. 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 wheat bran with sugarcane bagassee 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	147.0d	142.0d	14.13d	50.69d	6.09e
T_2	196.3c	192.3c	19.37c	67.70c	7.21c
T_3	219.7b	214.7b	21.13b	75.75b	7.67b
T_4	254.7a	243.3a	23.40a	87.82a	8.29a
T_5	222.7b	215.0b	20.53b	76.78b	6.72d
CV (%)	0.93	0.44	2.10	0.93	0.43
Level of significance	**	**	**	**	**
LSD (0.05)	5.32	2.42	1.13	1.83	0.087

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

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 89.93 % to 90.45 %. The highest moisture percent was observed in treatment T_5 (90.45 %) followed by T_4 (90.38 %). The other treatment was varied significantly over control. But the lowest moisture percent was observed in T_2 (89.93 %) followed by T_1 (90.05 %) (Table 4). The result of the present study keep in with the findings of previous workers (Rahman, 1994; Moni , 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 wheat bran.

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 10.07 % to 9.55 %. The highest dry matter percentage was observed in treatment T_2 (10.07 %) which was followed by T_4 (9.62 %) and T_1 (9.95 %). The other treatment was varied significantly over control but the lowest dry matter percentage was in T_5 (9.55 %) (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 11.40-31.30% (w/w) in the mushroom grown on sugarcane bagasse with different levels of wheat bran. The highest content of protein was found in treatment T_4 (30.31 %) and the lowest protein was found in T_1 (11.40 %). The

other treatments 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_4 (3.90 %) and the highest lipid percentage was counted under T_1 (6.16 %). The rest of the treatments varied significantly over control (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 *et al*., (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_4 (9.15) and the lowest percentage of ash was in the treatment T_1 (7.05). The other treatments were statistically different 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.* In the present study the ash content is as high as 12.80 may be due to the newly introduced varieties.

4.12.6. Carbohydrate

The lowest percentage of carbohydrate was counted under treatment T_4 (32.57) and the highest carbohydrate percentage was counted under T_1 (55.22). The rest of the treatments were statistically different but differed significantly 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_4 (24.07) and the lowest crud fiber percentage was counted under T_1 (20.17). The rest of the treatments were statistically different but varied significantly 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 22.87g/100g to 23.29g/100g of fiber in *Pleurotus spp.*

Table 4. Effect of different levels of wheat bran with sugarcane bagassee on chemical composition of oyster mushroom (*Pleurotus ostreatus*)

Treatment	Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	CHO (%)	Crud fiber (%)
T_1	90.05bc	9.95ab	11.40e	6.16a	7.05e	55.22a	20.17d
T_2	89.93c	10.07a	22.50d	5.75b	8.20d	42.49b	21.06c
T_3	90.16b	9.84b	24.30c	4.15d	8.75b	39.65c	23.15b
T_4	90.38a	9.62c	30.31a	3.90e	9.15a	32.57e	24.07a
T_5	90.45a	9.55c	27.13b	4.43c	8.55c	36.85d	23.03b
CV (%)	0.23	2.14	0.35	1.39	0.44	0.15	0.18
Level of significance	**	**	**	**	**	**	**
LSD (0.05)	0.17	0.173	0.23	0.19	0.087	0.171	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: Sugarcane bagasse (Controlled)
T_2: Sugarcane bagasse + Wheat bran (10%)
T_3: Sugarcane bagasse + Wheat bran (20%)
T_4: Sugarcane bagasse + Wheat bran (30%)
T_5: Sugarcane bagasse + Wheat bran (40%)

4.12.8. Effect on elemental content

4.11.8.1. Nitrogen

The highest percentage of nitrogen content (g/100gm) was counted under treatment T_4 (4.85) and the lowest nitrogen percentage was counted under T_1 (1.82). The rest of the treatments were statistically and significantly varied 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 content (g/mg/100gm) was counted under treatment T_1 (0.92) which was followed by T_2 (0.88). The rest of the treatments were statistically similar (Table 5) but the lowest phosphorus percentage was counted under T_3 and T_5 (0.82). 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 sugarcane bagasse based substrates.

4.12.8.3. Potassium

The highest percentage of potassium content (g/100gm) was counted under treatment T_4 (1.39) and the lowest potassium percentage was counted under T_1 (1.12) which was followed by T_2 (1.18). The rest of the treatments were statistically similar 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 content (mg/100g) was counted under treatment T_4 (22.08) and the lowest calcium percentage was counted under T_1 (20.20) which was followed by T_2 (20.82 cm). 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 content (mg/100g) was counted under treatment T_4 (20.21) and the lowest magnesium percentage was counted under T_1 (18.13) which was followed by T_2 (18.87). 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_4 (0.042) and the lowest sulfur percentage was counted under T_1 (0.013) (Table 5).

4.12.8.7. Iron

The highest percentage of iron content (g/100g) was counted under treatment T_4 (43.11) which was followed by T_3 (42.40) and the lowest iron percentage was counted under T_1 (40.53). The rest of the treatments were statistically similar 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 wheat bran with sugarcane bagassee on elemental contents of oyster mushroom (*Pleurotus ostreatus*)

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (%)
T_1	1.82e	0.92a	1.12d	20.20d	18.13d	0.013	40.53c
T_2	3.60d	0.88b	1.18c	20.82c	18.87c	0.019	41.84b
T_3	3.88c	0.82c	1.26b	21.15b	19.40b	0.037	42.40ab
T_4	4.85a	0.83c	1.39a	22.08a	20.21a	0.042	43.11a
T_5	4.34b	0.82c	1.28b	21.06b	19.23b	0.035	42.27b
CV (%)	0.37	2.17	0.83	0.2	0.41	3.63	0.66
Level of significance	**	**	**	**	**	NS	**
LSD (0.05)	0.027	0.027	0.026	0.151	0.212	0.028	0.76

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: Sugarcane bagasse (Controlled)
T_2: Sugarcane bagasse + Wheat bran (10%)
T_3: Sugarcane bagasse + Wheat bran (20%)
T_4: Sugarcane bagasse + Wheat bran (30%)
T_5: Sugarcane bagasse + Wheat bran (40%)

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 wheat bran as supplement with sugarcane bagasse 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 levels of supplements used. The highest running rate was observed in T_5 (0.96 cm) and the lowest running rate of mycelium was observed in T_1 (0.72 cm). The other treatments varied significantly over control (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 (11.5 days). The other treatments varied significantly in terms of time from stimulation to primordia initiation (Table 1). But the lowest time from stimulation to primordia initiation was in the treatment T_3 (7.17 days). The lowest time from primordia initiation to harvest was in the treatment T_4 (3.23 days) and the highest time from primordia initiation to harvest was observed in the treatment T_1 (5.17 days) followed by T_5 (5.16 days). The other treatments were statistically similar (Table 1).

The highest average number of primordia/packet was recorded in the treatment T_5 (70.67) followed by T_4 (69.00) and the lowest average number of primordia/packet was in the treatment T_1 (48.00). The other treatments varied significantly over control (Table 2). The highest average number of fruiting body/packet was observed in the treatment T_5 (61.00) followed by T_4 (58.00) and the lowest

average number of fruiting body/packet was in the treatment T_1 (41.00). The other treatments were statistically and significantly varied over the control in terms of average number of primordia/packet (Table 2). Supplementation of sugarcane bagasse with levels of wheat bran had great effect on average weight of individual fruiting body. The average weight of individual fruiting body in different treatment ranged from 3.06 g to 3.69 g. The highest average weight of individual fruiting body was observed in the treatment T_4 (3.69 g) and the lowest average weight of individual fruiting body was in the treatment T_1 (3.06 g). The other treatments varied significantly over control in terms of average weight of individual fruiting body (Table 2).

The supplementation of sugarcane bagasse with wheat bran had great effect on biological yield. The highest biological yield was determined under treatment T_4 (254.7 g) and the lowest biological yield was counted under T_1 (147.0 g). The other treatments varied significantly as compared with control in terms of biological yield (Table 3). The supplementation of sugarcane bagasse with wheat bran increases the economic yield over control. The highest economic yield (g/packet) was recorded under treatment T_4 (243.3 g) and the lowest economic yield was counted under T_1 (142.0 g). The other treatments varied significantly over control (Table 3). The dry yield of the oyster mushroom, grown on sugarcane bagasse responded significantly in terms of dry yield with the different levels of supplement (wheat bran). The dry yield of mushroom was maximum under the treatment T_4 (23.40 g) and the lowest dry yield was counted under T_1 (14.13 g). The other treatments varied significantly over control (Table 3).

The highest biological efficiency of 87.82% was calculated in treatment T_4 and the lowest biological efficiency of 50.69 % was calculated from T_1 (Table 3). The other treatments varied significantly over control. The highest cost benefit ratio was calculated in treatment T_4 (8.29) and the lowest cost benefit ratio 6.09 was calculated from T_1. The other treatments differed significantly in terms of cost benefit ratio (Table 3).

The moisture content (g/100g) of the fruiting body shows significant difference. The moisture percent ranged from 89.93 % to 90.45 %. The highest moisture percent was obtained in treatment T_5 (90.45 %) followed by T_4 (90.38 %). The other treatment was varied significantly over control. But the lowest moisture percent was observed in T_2 (89.93 %) followed by T_1 (90.05 %) (Table 4). The dry matter percentage of the fruiting body shows significant difference. The dry matter percent of fruiting body ranged from 10.07 % to 9.55 %. The highest dry matter percentage was obtained in treatment T_2 (10.07 %) which was followed by T_4 (9.62 %) and T_1 (9.95 %). The other treatment was varied significantly over control but the lowest dry matter percentage was in T_5 (9.55 %) (Table 4).

All the treatments contain a considerable amount of protein. The content of (g/100g) protein varied from 11.40-31.30% (w/w) in the mushroom grown on sugarcane bagasse with different levels of wheat bran. The highest content of protein was found in treatment T_4 (30.31 %) and the lowest protein was found in T_1 (11.40 %). The other treatments varied significantly over control in respect to protein content (Table 4). The lowest lipid content was determined under treatment T_4 (3.90 %) and the highest lipid percentage was counted under T_1 (6.16 %). The rest of the treatments varied significantly over control (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 highest content (g/100g) of ash was observed in the treatment T_4 (9.15) and the lowest percentage of ash was in the treatment T_1 (7.05). The other treatments were statistically different but differed significantly in terms of percentage ash content (Table 4). The lowest percentage of carbohydrate was counted under treatment T_4 (32.57) and the highest carbohydrate percentage was counted under T_1 (55.22). The rest of the treatments were statistically different but differed significantly over control in respect to percent carbohydrate content (Table 7). The highest percentage of crud fiber was counted under treatment T_4 (24.07) and the lowest crud fiber percentage was counted under T_1 (20.17). The rest of the

treatments were statistically different but varied significantly over control in respect to percent crud fiber content (Table 4).

The highest content (g/100g) of nitrogen was estimated under treatment T_4 (4.85) and the lowest nitrogen percentage was counted under T_1 (1.82). The rest of the treatments were statistically and significantly varied over control in terms of percent nitrogen content (Table 5). The highest content of phosphorus was estimated under treatment T_1 (0.92) which was followed by T_2 (0.88). The rest of the treatments were statistically similar (Table 5) but the lowest phosphorus percentage was counted under T_3 and T_5 (0.82). The highest percentage of potassium was counted under treatment T_4 (1.39) and the lowest potassium percentage was counted under T_1 (1.12) which was followed by T_2 (1.18). The rest of the treatments were statistically similar and varied significantly in respect to percent potassium content (Table 5). The highest content (mg/100g) of calcium was determined under treatment T_4 (22.08) and the lowest calcium percentage was counted under T_1 (20.20) which was followed by T_2 (20.82). The rest of the treatments were statistically similar but differed significantly over control in respect to percent calcium content (Table 5).

The highest content (g/100g) of magnesium was determined under treatment T_4 (20.21) and the lowest magnesium percentage was counted under T_1 (18.13) which was followed by T_2 (18.87). 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_4 (0.042) and the lowest sulfur percentage was counted under T_1 (0.013) (Table 5). The highest content (mg/100g) of iron was estimated under treatment T_4 (43.11) which was followed by T_3 (42.40) and the lowest iron percentage was counted under T_1 (40.53). The rest of the treatments were statistically similar in respect to percent iron content (Table 5).

REFERENCES

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.

Bahukhandi, D. (1990). Effect of various treatments on paddy straw on yield of some cultivated species of *Pleurotus*, Indian Phytopath.. 43: 471-472.

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.

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.

Bugarski, D., D. Gvozdenovic., A. Takae and J. Cervenski. (1994). Yield and yield components of different strains of oyster mushroom. Savremena poljoprivreda (Yugoslavia). 42(1): 314-318.

Bugarski, D., Gvozdcnovic, D. and Takac, A. (1995). Effect of strain and substrate on development of oyster mushroom mycelium. Selekcija-I-Semenarstvo. 2(2):239-241.

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.

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.

Cresswell, G. C., N. G. Nair and .1. C. Evans. (1990). Effect of boron and copper contaminants in poultry manure on the growth of the common mushroom. *Agaricus bioporus*. Australian .J. Exptl. Agric. 30(5): 707-712.

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.

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.

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.

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.

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, 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.

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

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.

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.

Maniruzzaman, M. (2004). Influence of media composition and growth regulators on mycelial growth and spawn production of three mushroom species. MS Thesis, Department of Biotechnology, BAU, Mymensingh.

Manukovsky, N. S., V. S. Kovalev and I. V. Uribovskaya. (1998). Utilization of substrate when growing oyster mushroom *Pleurotus florida* Fovose. Mikologiya-i-Fitopatologiya, 32(6):43-46.

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

Mathew. J., R. Kothandaraman and K.J. Thresiama. (1991). Cultivation of Oyster mushrooms on rubber processing factory waste- A possible solid waste utilization method. Indian Mushrooms. Proc. National Symposium on Mushrooms. Thiruvananthapuram. pp. 97-99.

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

Mori, K.; Toyomasu, T.; Nanba, H. and Kuroda, H. 1986. Antitumor activities of edible mushrooms by Oral administration, Proc. Int. L. Sym. Scientific and Technical aspect of cultivation edible fungi. The Penna. State Univ. USA. pp. 772-773.

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.

Paerna, D. and A. Paerna. (1998) Cultivation of oyster mushroom alternative direction of production in agriculture. Transactions of the Estonian Agril. Univ. (Estonia). 196:94-96.

Pani, B. K., A. K. Mohanty. (1998). Utilization of water hyacinth as an alternative substrate for Oyster mushroom cultivation. Crop Res. Hisar. 15(2-3): 294-296.

Patil, B.D. (1989) Studies on cultivation of (*Pleurotus sajor-cuju* (Fr.) Sing on different substrate. J. of the Moharashtra Agril. Univ. 14(2): 156-158.

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.

Permana I. G.; Flachowsky G.; Meulen L.; Zadrazil F.; Griensven L; Van J. L. D. (2000). Use of sugarcane bagasse for mushroom and animal feed production. Science and cultivation of edible fungi. Proceedings of the 15th International Congress on the Sci. and Cul. of Edible Fungi, Maastricht, Netherlands, 7:385-391.

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.

Ramjan, M. A. (2006). Effect of Growth regulators on Mycelial Growth and Different Substrates on the growth and Yield of Oyster Mushroom. M. S. Thesis, Department of Biotechnology, BAU, Mymensingh.

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

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).

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.

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

Ruhul Amin, S.M., M.M. Rahman, M.M. Hossain, M. Moynul Haque and Nirod Chandra Sarker. (2007). Effect of Different Substrates on the Growth and Yield of Five Selected Oyster Mushroom. Bangladesh Journal of Mushroom. 1(2): 39-43.

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.

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.

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

Stolzer, S. and Grabbe, K. (1991). Mechanisms of substrate selectivity in the cultivation of edible fungi. Mushroom Sci. 13(1): 141-146.

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

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

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.

Veena, S, S. Vijakumar, J. H. Kulkarni, V. Savalgi and V. Savalgi. (1998). Cultivation of Oyster mushroom on common weed in combination with bagasse. India. Karnataka J. Agril. Sci., 1998, 11(3): 693-695.

Vijoy, B. and R. C. Upadhyay. (1989) Chicken manure as a new nitrogen supplement in oyster mushroom cultivation. Indian J. Mycol. Plant Pathol. 19: 297-298.

Visscher, H.R. (1989). Oyster mushroom substrate more than straw alone? Champignon culture. 33(8): 417-425.

Vyas, N.L., Manjit, S. and Singh, M. (2002).Yield performance of *Pleurotus florida* (oyster mushroom) on supplemented substrates in arid zones. J. Eco. Physiol. India. 5(3-4): 149-151.

Wani, P. V, and D. M. Sawant. (1998). Oyster - A mushroom of broad adaptability: an overview. J. Maharashtra Agril. Univ. India, 23(3): 230-237.

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. Pin head primordia in the spawn packet as shown in the experimental plot

Appendix 2. Young fruiting body in the spawn packet

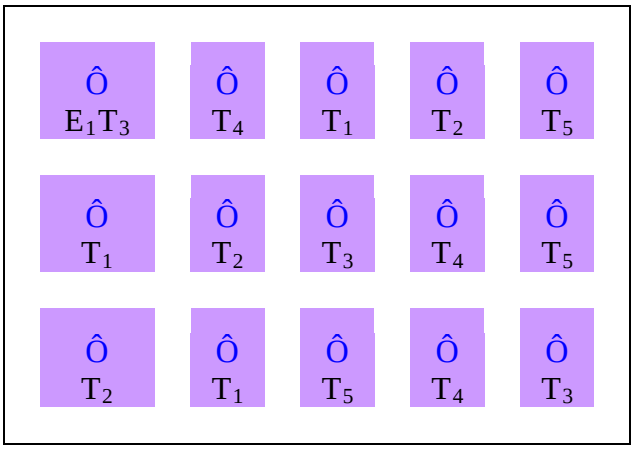
Appendix 3. Matured fruiting body in the spawn packet

Appendix 4. Fruiting body harvested from the spawn packet

Appendix 5. Experimental Plot

Appendix 6. Taking Biological Yield in the Laboratory

Appendix 7. Experimental layout



Legend
Ô: Mushroom Packet

Appendix 8. 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 sugarcane bagasse supplemented with different levels of wheat bran

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 9. 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 sugarcane bagasse supplemented with different levels of wheat bran

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 10. Analysis of the variance of the data of yield and biological efficiency and cost benefit ratio of the mushroom produced on sugarcane bagasse supplemented with different levels of wheat bran

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 11. Analysis of the variance of the data on chemical composition of the mushroom produced on sugarcane bagasse supplemented with different levels of wheat bran

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 12. Analysis of the variance of the data on elemental contents of the mushroom produced on sugarcane bagasse supplemented with different levels of wheat bran

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