EFFECT OF LIQUID SUPPLEMENT (WUXAL SUPER) ON THE YIELD AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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This is to certify that the thesis entitled **'EFFECT OF LIQUID SUPPLEMENT (WUXAL SUPER) ON THE YIELD AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)'** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE** in **BIOCHEMISTRY**, embodies the result of a piece of bonafide research work carried out by **SONIYA AKTER**, Registration number: **08-02782** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Dated: December, 2014
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DEDICATED
TO
MY BELOVED PARENTS

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The Author

EFFECT OF LIQUID SUPPLEMENT (WUXAL SUPER) ON THE YIELD AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

ABSTRACT

The experiment was conducted to study the effect of different levels of liquid supplement (wuxal super) on the yield and proximate composition of oyster mushroom (*Pleurotus ostreatus*). The experiment considered the following treatments: T_1: Control (without wuxal super); T_2: (wuxal super @ 0.1%); T_3: (wuxal super @ 0.2%); T_4: (wuxal super @ 0.3%); T_5: (wuxal super @ 0.4%). Data on different growth, yield contributing characters, proximate composition of mushroom were recorded and statistically significant variation was observed for different treatments. The Lowest time from stimulation to primordial initiation (3.20 days) and primordia initiation to harvest (3.60 days) was observed in T_4 treatment .The highest number of primordial/packet (71.20) was observed in T_5 treatment while the highest weight of individual fruiting body/packet was observed in T_4 (3.52g). The highest biological yield (261.56 g/packet), economic yield (252.96 g/packet), dry yield (37.34 g/packet) and biological efficiency (186.83%) were recorded in the T_4 treatment. The highest moisture percent was observed in T_3 (88.70%) and the highest dry matter percent was observed in T_4 (13.76%).Among the biochemical attributes the highest content of protein was recorded in T_4 (25.19%). The lowest lipid content was obtained in T_3 (3.17%), the highest percentage of ash was observed in T_4 (8.60%).The highest carbohydrate content was recorded in T_1 (40.15%), whereas the lowest was observed in T_4 (31.83%). The highest percentage of nitrogen (4.03%), potassium (1.98%), calcium (1.97mg/100g), and zinc (15.17mg/100g) was recorded in T_4 treatment. Therefore it can be concluded that wuxal super @ 0.3% was found as an efficient supplement to increase the yield of oyster mushroom.

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LIST OF ABBREVIATED TERMS

ABBREVIATION	FULL NAME
AEZ	Agro-Ecological Zone
et al.	and others
BBS	Bangladesh Bureau of Statistics
cm	Centimeter
^{0}C	Degree Celsius
etc	Etcetera
FAO	Food and Agriculture Organization
MP	Muriate of Potash
m^2	Square meter
UNDP	United Nations Development Program
SAU	Sher-e-Bangla Agricultural University

CHAPTER I

INTRODUCTION

Mushroom is fungi belong to the class Basidiomycetes and order Agaricales in fungal classification. Agaricale order is composed of fungi forming fleshy usually umbrella like bodies. *Pleurotus* species are very much effective in reducing harmful plasma lipids (Alam *et al*., 2007) and thus reduce the chance of atherosclerosis and other cardiovascular and artery- related disorders. These medicinal properties might be due to the presence of some important components in dietary mushrooms. The vitamins of mushrooms are not destroyed by cooking, drying and freezing. It has been used as a food and medicine by different civilizations since immemorial time due to its delicious taste and dietetic qualities. But technology for artificial cultivation of mushroom is recent innovation especially of oyster mushroom. Mushroom has qualities like lowering the blood cholesterol level, warding against cancer and invigorating hair growth. Tewari (1986) reported that the fresh mushroom contains about 85-90% moisture, 3% protein, 4% carbohydrates, 0.3-0.4% fats and 1% minerals and vitamins.

Bangladesh is a thickly populated country and we have to increase intensive use of land for increasing crop production and consider natural resources. In this case mushroom cultivation can be a huge opportunity for increasing crop production per unit area with the vertical use of land. As a vegetable, mushroom can play an important role to meet up the nutritional requirements of the population of our country. It is also a highly nutritious, delicious, medicinal and economically potential vegetable. The Greeks believed that mushrooms provided strength for warriors in battle. The Pharoaphs prized mushrooms as a delicacy and the Romans regarded mushrooms as the "Food of the Gods," which was served only on festive occasions. The Chinese treasured mushrooms as a health food, the "Elixir of life." The Mexican Indians used mushrooms as hallucinogens in religious ceremonies and in witchcraft as well as for therapeutic purposes (Chang and Miles, 1988).

The low calorie and cholesterol free mushroom diets also display certain medicinal properties. Mushroom reduces the diabetic on regular feeding. It also reduces the serum cholesterol in human bodies which reduces hypertension (Suzuki and Oshima, 1979). Mushroom inhibits the growth of tumor and cancer (Mori, 1986). Edible mushrooms have been treated as important tool in modern medicine for their medicinal values (Kovfeen, 2004). Oyster mushroom contains 19-35% protein on dry weight basis as compared to 7.3% in rice, 13.2% in wheat and 25.2% in milk (Chang & Miles, 1988). It contains 4.0% fat having good quantity of unsaturated fatty acids which are essential in our diet (Holman, 1976). It is rich in essential minerals and trace elements (Chandha and Sharma 1995). Mushrooms are source of Niacin (0.3 g) and Riboflavin (0.4 mg). Mushroom is a good source of trypsin enzyme. It is also rich in iron, copper, calcium, potassium, vitamin D and folic acid. Mushrooms are valuable health food which are low in calories, high in vegetable proteins, zinc, chitin, fiber, vitamins and minerals (Alam and Saboohi, 2001). Mushroom reduces serum cholesterol and high blood pressure (Mori, 1986).

There are various types of mushrooms such as oyster mushroom, milky white mushroom, button mushroom etc. which are cultivated in our country. Among them, several species of oyster mushroom are widely cultivated in our country. Oyster mushrooms are the easiest and least expensive commercial mushrooms to grow because they are well known for conversion of crop residues to food protein (Banik and Nandi, 2004). Oyster mushroom (*Pleurotus ostreatus*) is an edible mushroom having excellent fragrant and taste and its cultivation on crop residues is considered as potential source of income, an alternative food production, provision of employment, and for recycling of agricultural wastes. *P. ostreatus* possesses antitumor activity (Yoshioka *et al.*, 1985) and hypoglycaemic effects in experimentally induced diabetic rats (Chorvathova *et al.*, 1993). Only some species of mushrooms are now cultivated in this country and among these *Pleurotus sajor-caju* and *Pleurotus florida* are popular and widely accepted after *Pleurotus ostreatus* (Amin *et al*., 2007).

The enormous increase in our population has necessitated more and more food production through alternate resources such as mushroom as availability of more arable land. In developed countries, mushrooms have become one of the most important horticultural crops (Alam and Saboohi,2001). Mushroom 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. The mushroom cultivation could be a profitable agribusiness also. The spent mushroom substrates can be used as excellent 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.

At present mushroom is cultivated though sawdust supplemented with 40% wheat bran which is most costly. This costly wheat bran is a barrier for the extension of mushroom production in the country. On the other hand some commercial liquid mineral nutrients are available in the market in low cost and rich in concentrated nutrients required for the plants. Therefore, investigation of packets production for high yield and quality mushroom using liquid mineral supplement for the common growers is an urgent demand.

Considering the above all context and situation, the study was undertaken to fulfill the following objectives:

- To find out the suitable level of liquid mineral supplement for better yield of oyster mushroom (*Pleurotus ostreatus*)
- To determine the nutritional status of the produced mushroom by proximate analysis.

CHAPTER II

REVIEW OF LITERATURE

Mushroom grows well in waste materials and the growing materials have to pasteurize by using hot water treatment, autoclaving or through steam circulation or chemical sterilization for preventing contamination. There are many scientific reports on different aspects of mushroom cultivation especially different variety, use of substrate, pasteurization method etc. but still there are major scopes to investigate the effects of different pasteurization method on oyster species. The review includes reports of several investigators which appear pertinent in understanding the problem and which may lead to the explanation and interpretation of results of the present investigation.

A laboratory trial has been conducted by Pervez *et al*. (2009) with formalin, Bavistin and combination of formalin and Bavistin at three different concentrations against identified associated mycoflora of oyster mushroom substrates. The combination of formalin and Bavistin at the highest concentration (400+75 ppm) was found to be the best in inhibiting the radial colony growth of all the identified fungi.

Sangeetha (2007) carried out an experiment to study the effect of organic amendments on yield performance of pink mushroom. The organic amendments viz., groundnut cake powder, neem cake powder, rice bran and black gram powder were added at 3 and 5% levels to mushroom beds as amendments during cultivation. Neem cake at 5% level significantly increased the sporophore production (690.1 g) followed by 3% level (675.3 g). These treatments produce fruiting bodies earlier (10.8 to 11 days) than other amendments tried (11.1 to 12 days). Except neem cake powder and rice bran, all the other amendments had little effect on increasing the yield.

Ali *et al*. (2007) evaluate the influence of pasteurization methods on cotton waste substrate on yield of oyster mushroom (*Pleurotus* spp. Cotton waste subjected to

different methods of pasteurization, namely pasteurization with steam, hot-water treatment and chemical sterilization with formalin, which were compared with control (without pasteurization). Three species of *Pleurotus* i.e. *Pleurotus florida, Pleurotus pulmonarius* and *Pleurotus ostreatus* were selected. Steam pasteurization produced the best results as for as the performances of individual species are concerned, *Pleurotus pulmonarius* completed the mycelial growth in the shortest time. Formalin treatment behaved poorly as the different *Pleurotus* spp, took maximum time to complete mycelial growth. Steam pasteurization technique produced more yield, whereas *Pleurotus florida* behaved better in all the treatments than other species. Substrate was analyzed chemically for N: P: K to determine their contents at different stages. N: P: K contents were increased after the completion of mycelial growth in all the treatments, but were decreased after fructification as the fruiting bodies consumed nutrients for their growth.

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

Ali *et al*. (2004) conducted an experiment at the Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Different techniques of

pasteurization including control, hot water treatment, steam pasteurization and chemical sterilization with formalin were applied to cotton waste to evaluate optimum method for best mycelia growth of three species of oyster mushroom. Results showed that steam pasteurization gave maximum mycelia growth which completed in shortest period of time. Formalin treatment behaved poorly as the species took maximum time to complete their mycelial growth.

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

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

Khan *et al.* (1991) used sawdust to prepare compost for spawn running amended with lime and different combinations of wheat chaff, wheat bran, paddy straw and cotton waste. Sawdust from *D. sisso* was the most suitable for spawn preparation and all types of sawdust amended with cotton waste were found to give optimum conditions for spawn running.

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

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

Nuruddin *et al.* (2010) reported that oyster mushroom gave the highest number of primordia (70.63) and fruiting body (51.92) were observed in rice straw supplemented with 5% level of cowdung. The highest weight of individual fruiting body (4.71g), biological yield (234.24g), economic yield (227.72g), dry yield (22.83g), biological efficiency (140.26%) and benefit cost ratio (5.69) were observed in rice straw supplemented with 10% level of cowdung.

Ali *et al.* (2010) reported that the highest mycelium growth rate (0.96 cm/day), the highest average number of primordia/packet (70.67), average number of fruiting body/packet (61.00) were observed in sugarcane bagasse supplemented with 40% wheat bran as supplement with sugarcane bagasse. The lowest time from primordia initiation to harvest (3.23 days) and the highest average weight of individual fruiting body (3.69 g) were observed in 30% level of wheat bran. The highest biological yield (254.7 g/500 g wet substrate), economic yield (243.3 g), dry matter (23.40 g), biological efficiency (87.82%) and benefit cost ratio (8.29) also observed in 30% level of wheat bran.

Kulsum *et al.* (2009) reported that oyster mushroom gave the highest weight of individual fruiting body was observed in sawdust supplemented with cowdung @ 10% (3.69 g). The supplementation of sawdust with cowdung had remarkable effect on biological yield, economic yield, the dry yield, biological efficiency and cost benefit ratio. The highest biological yield (217.7 g), economic yield (213g), dry yield (21.27g) biological efficiency (75.06%) and cost benefit ratio (8.41) were observed due to sawdust supplemented with cowdung @ 10%.

Bhuyan (2008) reported that supplementation of sawdust found to be significant in yield and yield contributing characters of oyster mushroom with some extent. The highest biological yield, economic yield, dry yield, biological efficiency (BE) and benefit cost ratio (BCR) respectively was observed in sawdust supplemented with NPK mixed fertilizer (N=0.6%, P=0.3%, K=0.3%).

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

Namdev *et al.* (2006) conducted a study to determine the effect of different straw substrates on spawn growth and yield of oyster mushroom. The number of days required for spawn run was significantly less (14 days) in case of gram straw, parthenium straw, sugarcane straw and wheat straw, compared with 20 days for sunflower stalk, mustard straw and paddy straw. Yield was very poor on parthenium straw (95 g/500 g dry substrates) and it was highest on paddy straw (666 g/500 g), followed by wheat straw and mustard straw (427 and 400 g/500 g respectively).

Khlood and Ahmad (2005) conducted an experiment to study the ability of oyster mushroom (*Pleurotus ostreatus*) P015 strain to grow on live cake mixed with wheat straw. The treatments comprised: 90% straw + 5% wheat bran + 5% gypsum (control); 80% straw + 10% olive cake + 5% wheat bran + 5% gypsum (T_1); 70% straw + 20% olive cake 5% wheat bran + 5% gypsum (T_2); 60% straw + 30% olive cake + 5% wheat bran + 5% gypsum (T_3); 50% straw + 40% olive cake + 5% wheat bran +.5% gypsum (T_4); and 90% olive cake + wheat bran + 5% gypsum (T_5). After inoculation and incubation, transparent plastic bags were used for cultivation. The pinheads started to appear after 3 days and the basidiomata approached maturity 3-7 days after pinhead appearance. The addition of 30% olive cake to the basal growing medium gave the highest yield (400 g/500 g dry substrate), average weight (21.5 g/cap) and average cap diameter (7.05 cm/cap) and BE% (80%).

Habib (2005) tested different substrates such as sawdust, sugarcane bagasse, rice straw, wheat straw and waste paper for the production of oyster mushroom in polypropylene bag. Different substrates significantly affected the number of primordia, number of fruiting bodies and amount of fresh weight or yield. This experiment revealed that the highest number of primordia and fruiting bodies were found in waste paper 43.75 and 31.00 respectively. The highest amount of fresh weight was also found in waste paper 94.25 g.

Shah *et al.* (2004) carried out an experiment to investigate the performance of Oyster mushroom on the following substrates: 50 % sawdust + 50 % wheat straw, 75 % sawdust + 25 % leaves, 50 % wheat straw + 50 % leaves, 100 % sawdust, 100 % wheat straw and 100 % leaves. The temperature was kept at 25^0C for spawn running and 17-20^0C for fruiting body formation. The time for the completion of mycelial growth, appearance of pinheads and maturation of fruiting bodies on different substrates were recorded. The number of fruiting bodies and the biological efficiency of substrates were observed. The results show that spawn running took 2-3 weeks after inoculation, while small pinhead-like structures formed 6-7 days after spawn running. The fruiting bodies appeared 3-6 weeks after pinhead formation and took 27-34 days later after spawn inoculation. Sawdust at 100 % produced the highest yield (646.9 g), biological efficiency (64.69 %) and the number of fruiting bodies (22.11).

Banik and Nandi (2004) carried out an experiment on oyster mushroom for its ease of cultivation, high yield potential as well as its high nutritional value. Laboratory experimentation followed by farm trial with a typical oyster mushroom *Pleurotus sajor- caju* revealed that the yield potential of these mushrooms can be increased significantly when grown on a lignocellulosic crop residue - rice straw supplemented with biogas residual slurry manure in 1:1 ratio as substrate. Residual slurry manures obtained from biogas plants utilising either cattle dung or poultry litter, jute caddis or municipal solid waste as substrates for biogas production were all effective in increasing the yield of *Pleurotus sajor-caju* significantly although to different extents. Disinfection of straw and manure

by means of 0.1 % $KMnO_4$ plus 2 % formalin solution in hot water caused 42.6 % increase in yield of *Pleurotus sajor-caju* over control, i.e., when disinfection done with hot water.

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 183.1g, 151.8g, 111.5g, 87.5g, 49.5g, 23.3g, 13.0g and 0.0 g for composted Sawdust of *Triplochiton scleroxylon*, Rice straw, Banana leaves, Maize stover, Corn husk, Rice husk, Fresh Sawdust and Elephant grass respectively. The biological efficiency (BE) followed the same pattern and ranged from 61.0%, for composted Sawdust to 50.0% for elephant grass. Based on the yield and BE of the substrates tested, Rice straw appeared to be the best alternate substrate for growing oyster mushroom.

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

Dhoke *et al.* (2001) studied the effect of different agro-wastes on cropping period and yield of *Pleurotus sajor-caju* the experiments carried out in Prabhani and Maharashtra in India. Various plant materials, i.e. soybean, paddy, cotton, wheat and jowar (*Sorghum bicolor*) were used. Cropping period on different substrates was recorded for first, second and third picking. The cropping period for third picking varied from 42.25 to 43.50 days in different substrates. The days required for first picking indicated that soybean straw took 22.00 days to produce first crop

of harvestable mushroom while a minimum of 21.25 days were required for paddy and wheat straw. For second picking, jowar and cotton waste took the maximum days of 32.75 days while soybean took the minimum of 31.50 days. The final and third picking was completed in 43.50 days in case of soybean straw which was statistically higher compared to paddy and wheat straw (42.25) and cotton and jowar straw (42.75). The highest yield of 993.00 g/kg was obtained from cotton, followed by soybean straw (935.25 g/kg) and paddy straw (816.0 g/kg). The lowest yield of 445.50 g/kg was recorded in jowar straw.

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

Upamanya and Rathaiah (2000) conducted an experiment to test the effect of fortification of rice straw with rice bran on the yield and quality of oyster mushroom (*Plelrrotus ostrentus*) in Jorhat, Assam, India. Treatments comprised: (i) addition of rice bran at 5% w/w (weight of rice bran/weight of dry substrate) at the time of spawning and (ii) control (without rice bran). Rice straw fortified with rice bran exhibited a higher yield compared to the control.

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.

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

Zhang-Ruihong *et al.* (1998) cultivated oyster mushroom (*P. sajor-caju*) on rice and wheat straw without nutrient supplementation. The effects of straw size reduction methods and particle sizes spawn inoculation level and types of substrate (rice straw vs. wheat straw) on mushroom yield, biological efficiency and substrate degradation were determined. The 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.

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.

Biswas *et al.* (1997) reported that methods including spawning percentage, combinations of paddy straw, wheat straw and supplements, to improve the biological efficiency (BE) of *P. florida* were investigated in Madhya Pradesh, India. Increasing spawning rates reduced the time required for spawn runs. The highest BEs (66.8-101.25%) was observed after the use of the highest spawning percentages. A 1:1 mixture of paddy straw wheat straw promoted a high BE

(106.5%); supplementation of this substrate with 5% rice flour also promoted BE (125.75%).

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

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

Nuruddin *et al.* (2010) carried out an experiment to investigate the effect of different levels of cowdung (0, 5, 10, 15 and 20%) on yield and proximate composition of *Pleurotus ostreatus*. The highest protein content (30.90%), crude fiber (24.03%) and the lowest lipid (3.34%) were found in 10% cowdung.

Ali *et al.* (2010) conducted an experiment to investigate the performance of different levels of wheat bran (0, 10, 20, 30 and 40 %) as supplement with sugarcane bagasse on the yield and proximate compositions of oyster mushroom were studied. The highest content of protein (30.31 %), ash (9.15 %) and crude fiber (24.07 %) and the lowest content of lipid (3.90 %) and carbohydrate (32.57 %) were recorded in 30% wheat bran.

Kulsum *et al.* (2009) conducted an experiment to determine the effect of five different levels of cowdung (0%, 5%, 10%, 15% and 20%) as supplement with sawdust on the performance of oyster mushroom. Among the chemical characteristics highest content of protein (31.30%), ash (8.41%), crude fiber (24.07%), the lowest lipid (3.44%) and carbohydrate (32.85%) were observed due to sawdust supplemented with cowdung @ 10%. Among the minerals the highest amount of nitrogen (5.01%), potassium (1.39%), calcium (22.15%), magnesium (20.21%), sulfur (0.043%), iron (43.4%) and the lowest phosphorus (0.92) were observed due to sawdust supplemented with cowdung @ 10%.

Bhuyan (2008) conducted an experiment to study the effect of various supplements at different levels with sawdust showed significant effort on mycelium running rate and reduced the required days to complete mycelium running in the spawn packet. The supplementation of sawdust found to be significant in yield and yield contributing characters of oyster mushroom with some extent. Sawdust supplemented with different levels has a profound effect on chemical composition of oyster mushroom. Sawdust supplemented at different substrate found to be significant with mineral content of the fruiting body.

Sarker *et al.* (2007b) found that remarkable difference in nutrient content of oyster mushroom was observed in respect of different substrates. Wide variation was recorded in the protein content of fruiting body. On dry weight basis, the highest protein content (11.63%) was observed in fruiting body grown on sugarcane bagasse. The 2nd highest protein (11.00%) was observed in that grown on wheat straw and water hyacinth. The lowest protein (7.81%) was observed in that grown on rice straw. Mushrooms are good source of minerals. Maximum of 18400 ppm Ca was found in mushroom which was grown on wheat straw. On other substrates its content varied from 1600 ppm to 18400 ppm. The content of Fe in the mushroom grown on different substrates varied from 92.09 ppm to 118.40 ppm. The highest Fe content was found in waste paper cultured oyster mushroom and lowest on water hyacinth.

Zape *et al*. (2006) conducted a study to determine the spawn run, days taken to pin head initiation, yield and biological efficiency of three oyster mushroom species viz. *Pleurotus florida, P. eous* and *P. flabellatus* were grown on wheat straw substrate. Time required for spawn run and pinning was significantly less in *Pleurotus eous* followed by *P. florida*. In analyzing the physico-chemical composition of dehydrated fruit bodies of *Pleurotus* species revealed that among different species *P. eous* was rich in protein (33.89%), moderate in fat (3.10%), carbohydrate (32.60%) and ash (8%) followed by *P. florida*. However, *P. flabellatus* was rich in crude fibre, carbohydrate and ash but low in protein and fat content as compare to *P. eous* and *P. florida.*

Khlood and Ahmad (2005) conducted an experiment to study the ability of oyster mushroom (*Pleurotus ostreatus*) P015 strain to grow on live cake mixed with wheat straw. The treatments comprised: 90% straw + 5% wheat bran + 5% gypsum (control); 80% straw + 10% olive cake + 5% wheat bran + 5% gypsum (T_1); 70% straw + 20% olive cake 5% wheat bran + 5% gypsum (T_2); 60% straw + 30% olive cake + 5% wheat bran + 5% gypsum (T_3); 50% straw + 40% olive cake + 5% wheat bran +.5% gypsum (T_4); and 90% olive cake + wheat bran + 5% gypsum (T_5). Carbohydrate, protein and fiber contents were high in the *P.*

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

Ancona-Mendex *et al.* (2005) conducted an experiment to grow oyster mushroom (*Pleurotus ostreatus* in either maize or pumpkin straw. Samples were taken for each one of the three harvests and analyzed for total nitrogen (N) content and amino acids profile. The substrate had no effect on N content and amino acid profile of the fruits. However, N (g/100 g DM) increased from 4.13 g in the first harvest to 5.74 g in the third harvest. In general, the amino acids tended to be higher on the first harvest samples, but no changes were found in the amino acid profile due to substrate or harvest, except for valine decreasing from 3.96 to 3.15 g/16 g N. Changes in the N content of the fruit could be explained by changes in the stipe and pileus proportions as they had different N content (3.15 and 5.48 + or 0.031 g N/100 g DM respectively). The amino acid profile of the mushroom was adequate according to the FAO/WHO/UNU adult human amino acid requirements.

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

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

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

Upamanya and Rathaiah (2000) conducted an experiment to test the effect of fortification of rice straw with rice bran on the yield and quality of oyster mushroom (*Plelrrotus ostrentus*) in Jorhat, Assam, India. Treatments comprised: (i) addition of rice bran at 5% w/w (weight of rice bran/weight of dry substrate) at

the time of spawning and (ii) control (without rice bran). They reported that rice bran application had no effect on the crude protein content of mushroom.

Zhang-Ruihong *et al.* (1998) cultivated oyster mushroom (*P. sajor-caju*) on rice and wheat straw without nutrient supplementation. The protein content of mushrooms produced was 27.2% on an average.

Ragunathan *et al.* (1996) investigated that the fruiting bodies of oyster mushroom were rich in nutrients such as carbohydrate, protein, amino nitrogen and minerals and low fat content. The moisture content of the fruiting bodies ranged from 84.70 to 91.90% and the carbohydrate content ranged from 40.6 to 46.3%, the crude protein content ranged from 31.9 to 42.5 %, 26.92 to 38.8%, and 30.0 to 42.5% in *Pleurotus sajor-caju*, *Pleurotus platypus* and *Pleurotus citrinopileatus* respectively.

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.

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

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during the period from August to December 2014 to study the effect of liquid supplement (wuxal super) on the yield and proximate composition of oyster mushroom (*Pleurotus ostreatus*). The chapter includes a brief description of the location of experiment, soil and climate condition, materials used for the experiment, design of the experiment, preparation of substrates, preparation of packets, cultivation of spawn packet, collection of produced mushrooms, proximate analysis of the mushrooms, data collection and data analysis procedure which are presented below under the following headings-

3.1 Experimental location

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

3.2 Planting materials

Mother culture of Oyster mushroom (mushroom seed) was collected from National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka.

3.3 Varietal characteristics of Oyster Mushroom

Oyster mushroom is *Pleurotus ostreatus* that has a light to dark whitish colored cap depending upon the strain and growing conditions. Primordia and young mushrooms are light white but become less intensely colored as the mushroom matures. Oyster mushroom is characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. Their fruiting

bodies are shell or spatula shaped with white color. If the temperature increases above 32^0C, its production markedly decreases.

3.4 Treatment of the experiment

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

T_1: Control (without Wuxal Super)

T_2: Wuxal Super @ 0.1%

T_3: Wuxal Super @ 0.2%

T_4: Wuxal Super @ 0.3%

T_5: Wuxal Super @ 0.4%

3.5 Design and layout of the experiment

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

3.6 Preparation of substrates

At first weight of dry rice straw was taken. Rice straw was boiled thereafter the rice straw was taken off from water and left on a perforated sieve for removing the excess water for few hours. All rice straw was dried in sun. Then $CaCO_3$ @ 1% on dry weight basis were added with spawn preparing substrate. The measured materials were taken in a plastic bowl and mixed thoroughly by hand and moisture was increased by adding wuxal super at different rate.

3.6.1 Preparation of spawn packets and inoculation of mother spawn

The mixed substrates were filled into 9×12 inch polypropylene bag @ 400g with 75g mother spawn per packet. The packets were kept at 20-25^0C temperature until the packets become white with the mushroom mycelium. The filled polypropylene bags were prepared by using plastic neck and plugged the neck with cotton and placed rubber band to hold it tightly in place. After completion of the mycelium

running the rubber band, brown paper, cotton plug and plastic neck of the mouth of spawn packet were removed and the mouth was wrapped tightly with rubber band. Then these spawn packets were transferred to the culture house.

3.6.2 Cultivation of spawn packet

Two ends, opposite to each other of the upper position of plastic bag were cut in "Rectangular" shape with a blade and opened by removing the plastic. Then the spawn packets were soaked in water for 5 minutes and invested to remove excess water for another 15 minutes. The packets of each type were placed separately on the shelf of culture room and covered with newspaper. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3 times a day. The light around 300-500 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22^0C to 25^0C. The first primordia appeared 2-4 days after Rectangular-shaped cutting depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

3.6.3 Harvesting of 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 Mycelial growth

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

3.7.2 Time required for completing mycelium running

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

3.7.3 Average number of fruiting body per packet

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

3.7.4 Average weight of individual fruiting body per packet

Average weight of individual fruiting body was calculated by dividing the total weight of fruiting body per packet by the total number of fruiting body per packet.

3.7.5 Dimension of fruiting body (stipe and pileus)

Length of the pileus of three randomly selected fruiting bodies was measured using a slide calipers. Diameter of stipe, diameter and thickness of pileus were also measured.

a. Length of stipe (cm)
b. Diameter of stipe (cm)
c. Diameter of pileus (cm)
d. Thickness of pileus (cm)

3.7.6 Biological yield

Biological yield per 400 g packet was measured by weighing the whole cluster of fruiting body without removing the lower hard and dirty portion.

3.7.7 Economic yield

Economic yield per 400 g packet was recorded by weighing all the fruiting bodies in a packet after removing the lower hard and dirty portion.

3.7.8 Drying of mushrooms

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

3.7.9 Dry yield

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

$$\text{Dry yield (g/400g packet)} = \text{Economic yield} \times \frac{\text{Oven dry weight of sample(g)}}{\text{Fresh weight of sample(g)}}$$

3.7.10 Biological efficiency

Biological efficiency was determined by the following formula:

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

3.7.11 Benefit cost ratio:

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

3.7.12 Cultural operations for subsequent flushes

After completing the first harvest again the packets were scraped at the place where the 'Rectangular' shaped cut had been done and water was sprayed regularly. The primordia appeared 9-10 days after first harvest and 7-8 days after second harvest. Water spraying was continued until the mushrooms were ready to be harvested.

3.8 Proximate analysis of the mushrooms

3.8.1 Collection of the samples

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

3.8.2 Determination of Moisture

About 10-20 g of each sample were weighed into separated and weighed petridishes and dried in an oven at 100^0C to 105^0C till the weight of the petridishes with their contents was constant. The moisture content was determined by redacting the constant weight from the fresh weight and then expressed in percentage.

3.8.3 Determination of dry matter

A clean container (dish or beaker) was place in an oven at 105^0C overnight. The container was allowed to cool in a desiccator and was weighed. The sample was kept into the container and weighed with the sample. The container was placed in the oven at 105^0C for 24 hours. The container was allowed to cool in a desiccator and was weighted. Again, the container was placed in the oven at 105^0C for 2 hours. It was cooled in a desiccator and weighed again. Repeat drying, cooling and weighing were continued until the weight became constant. The dried sample was stored in an airtight container. The moisture content of the sample was calculated and dry matter was determined by redacting the moisture content from fresh sample.

3.8.4. Grinding

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

3.8.5 Determination of total ash

One gram of the sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5-6 hours at 600°C. It was then cooled in a desiccator and weighed. To ensure completion of ashing, the crucible was then heated in the muffle furnace for 1h, cooled and weighed. This was repeated till two consecutive weights were

the same and the ash was almost white or grayish white in color. Then total ash was calculated as following equation (Raghuramulu *et al.*, 2003):

$$\text{Ash content (g/l00 g sample)} = \frac{\text{Weight of ash}}{\text{Weight of sample taken (g)}} x100$$

3.8.6 Determination of crude fiber

Ten gram of moisture and fat-free sample was taken in a beaker and 200 ml of boiling 0.255 N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a Moslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker and 200 ml of boiling 0.313 N NaOH was added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a Moslin cloth and the residue was washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100°C and weighed (We) in an electric balance *(KEY: JY-2003; China)*. The crucible was heated in a muffle furnace *(Nebertherm: Mod-L9/11/c6; Germany)* at 600°C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber (Raghuramulu *et al.*, 2003).

Therefore,

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

3.8.7 Total carbohydrate estimation

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

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

3.8.8 Determination of protein

The Protein contents of the fruiting bodies of the mushrooms were determined by the standard Micro-kjeldhal procedure. According to this method total nitrogen contents of the samples were estimated and protein contents were finding out by multiplying by 6.25 to the total nitrogen values. The total nitrogen was determined by the Kjeldahl methods, which depends upon the conversion of protein nitrogen into ammonium sulfate, by digestion ammonia liberated from the ammonium sulfate by making the solution alkaline were distilled into known volume of a standard acid, which was then back titrated.

Reagents

a) Concentrated sulfuric acid

b) Digestion Mixture: Potassium sulfate : Copper sulfate (98 : 2 w/w)

c) 40% Sodium hydroxide in distilled water

d) N/10 Sulfuric acid and N/10 Sodium hydroxide

e) 0.1% methyl red indicator: 0.1 g of the indicator was dissolved in 60 ml of alcohol and the volume was made 100ml with the distilled water

Procedure

Weighed dried sample 2.0 g, 5.0 g of the digestion mixture, 25 pieces of the glass beads, and 25ml of concentrated sulfuric acid were taken in a Kjeldahl flask. The content of the flask was digested in a flame chamber until the total content became clear. The digested materials were quantitatively transferred into a one liter flat-bottomed flask and the volume was made up to about 400ml with distilled water. Then about 40% NaOH and some pumice stone were added to prevent bumping, and distilled immediately in the distillation chamber of the Kjeldahl apparatus. The distillation was continued till its volume diminished to one-half of the initial. The distillate was collected in a receiver containing 100ml of N/10 sulfuric acid containing 2/3 drops of methyl red indicator. The liberated

ammonia absorbed in the sulfuric acid solution was titrated against standard (N/10) NaOH solution.

Calculation

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

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

B = ml of NaOH required in the titration of sample

N = Normality of the NaOH

W = Weight of the sample

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

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

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

3.8.9 Total fat estimation

Fat was estimated as crude ether extraction of the dry materials. The dried sample (about 5.0 g) was weighed into a conical flask and plugged with fat free cotton. The flask was then placed in an electric shaker and extracted with anhydrous ether for about 16 hours. The ether extract was filtered into another weighed conical flask. The flask containing the original ether extract was washed 4 to 5 times with small quantities of ether and the washings were also transferred to the filter paper. The ether in the conical flask was then removed by evaporation, and the flask with the residual was dried in an oven at 80^0C to 100^0C, cooled in a dessicator and weighed. The result was expressed as follows:

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

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

3.9 Estimation of minerals

3.9.1 Equipments

For elementary composition analysis the equipment were used as electric balance, desiccators, atomic absorption apectrophotometer (AAS), spectrophotometer, porcelain crucible, beaker and flame photometer etc.

3.9.2 Determination of Ca, Mg, K, Fe, S, Zn and P

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

3.9.2.1 Digestion

1. 0.500 g of dried sample was taken into each of 18 nitrogen digestion tubes. The two remaining tubes were kept blanks. 5 ml nitric acid were added to each of all 20 tubes. The tubes were left overnight mixing the contents in the tubes. Covering with the exhaust manifold, the tubes were placed in the digester and the temperature was set to 125°C, turning on the digester, the digestion was continued for 4 hours after boiling started.
2 After cooling, the digestion mixture was transferred with distilled water to a 100 ml volumetric flask water added to make the volume up to the mark.
3 Filtration was performed on a dry filter into a dry bottle, which could be closed with a screw cap. Keeping the filtrate in the closed bottle Ca, Mg, K, Fe, Mn, Zn, S, Cu and P were determined in the filtrate.

3.9.2.2 Estimation of Ca

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

3.9.2.3 Estimation of Mg

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

3.9.2.4 Estimation of K

10 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette to volume with water and mixed. The content of K was measure by flame photometer.

3.9.2.5 Estimation of P

5 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette. 30 ml water was added, mixed and then 10 ml ammonium molybdate-ascorbic acid solution was added to volume with water and mixed. After 15 minutes, the absorbance was measured on a spectrophotometer at 890 nm.

3.9.2.6 Estimation of Fe and Zn

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

3.9.2.7 Calculations: For Ca, Mg, K, P

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

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

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

c = g sample weighed into the digestion tube

If an additional dilution is made before the transfer to the 50 ml volumetric flask, the result is multiplied with the dilution factor. But the above elements were in trace. So addition of dilution was not to be performed.

For Zn and Fe

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

Zn and Fe measured on atomic absorption spectrophotometer

c = g sample weighed into the digestion tube

3.10 Statistical analysis

The data obtained for different parameters were statistically analyzed to find out the significance of the difference among the treatment. All the data collected on different parameters were statistically analyzed by Duncan's Multiple Range Test (DMRT). The mean values of all the characters were evaluated and analysis of variance was performing by the 'F' test. The significance of the difference among the treatments means was estimated by the least significant difference (LSD) test at 5% level of probability (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS AND DISCUSSION

The experiment was conducted to find out the effect of liquid supplement (wuxal super) on the yield and proximate composition of oyster mushroom (*Pleurotus ostreatus*). Data on different growth, yield contributing characters, proximate composition of mushroom were recorded. The results have been presented and discussed with the help of table and graphs and possible interpretations given under the following headings:

4.1 Growth and yield contributing characters

4.1.1 Time required to complete mycelium running

Mycelium running rate in spawn packet was found to be differed due to different levels of supplements used. The highest time required to complete mycelium running was recorded from T_1 (21.60 days) which was statistically similar with T_2 (20.40 days) while, the lowest time required to complete mycelium running was found in T_4 (14.60 days) which was statistically similar with T_3 (14.80 days) and T_5 (16.60 days). Mahjabin *et al*. (2011) reported the highest days (31.75) required for mycelial growth in sugarcane baggase, whereas minimum days (13.25) required for completion of mycelial growth in pasteurized rice straw substrate.

4.1.2 Time from stimulation to primordia initiation

The lowest time from stimulation to primordial initiation (3.20 days) was observed in T_4 and the highest time from stimulation of primordia initiation (6.20 days) was in T_1 (control). The rest of the treatments were statistically different and varied significantly over control in terms of time from stimulation of primordial initiation (Table 1). Sarker (2004) observed that duration of primordia initiation to oyster mushroom was significantly lower as compared to control.

Table 1. Effect of different levels of liquid supplement (wuxal super) on growth and yield contributing characters of oyster mushroom

Treatments	Time required to complete mycelium running (days)	Time from stimulation to primordia initiation (days)	Time from primordia initiation to harvest (days)	Average number of primordia /packet	Average number of fruiting body/ packet
T_1	21.60 a	6.20 a	5.10 a	63.40 d	45.00 d
T_2	20.40 b	4.80 b	3.80 c	66.40 c	48.40 b
T_3	14.80 d	3.90 c	3.90 c	56.60 e	35.60 e
T_4	14.60 e	3.20 e	3.60 d	70.40 b	55.84 a
T_5	16.60 c	3.60 d	4.40 b	71.20 a	46.20 c
LSD (0.05)	0.08420	0.1684	0.1684	0.2063	0.8336
CV(%)	0.25	2.06	2.15	0.17	0.70

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 5% level of probability

T_1: Wuxal Super @ 0% (Control)

T_2: Wuxal Super @ 0.1%

T_3: Wuxal Super @ 0.2%

T_4: Wuxal Super @ 0.3%

T_5: Wuxal Super @ 0.4%

4.1.3 Time from primordia initiation to harvest

Statistically significant variation was recorded in terms of time from primordia initiation to harvest of oyster mushroom due to different levels of liquid supplement (Table 1). The highest time from primordia initiation to harvest was attained in T_1 (5.10 days) followed by T_5 (4.40 days) and the lowest time was required in T_4 (3.60 days).The other treatments were statistically similar but varied significantly over control in terms of time required from primordia initiation to harvest.The result of the present study keeps in with the result of Shelly (2008), Gupta (1989) and Khan *et al*. (2001).They separately found that the crop was harvested 2-3 days later from primordial initiation.A similar result was also founded by Ahmed (2008).

4.1.4 Average number of primordia per packet

Average number of primordia per packet of oyster mushroom varied significantly due to different levels of liquid supplement (Table 1). The highest average number of primordia per packet was recorded in T_5 (71.20) followed by T_2 (66.40) whereas the lowest number of primordial per packet was in the T_3 (56.60).The other treatments were statistically similar but differed significantly in terms of Average no of primordial per packet (Table 1).The findings of the present study matches with the study of Ahmed (2008) who reported significantly different numbers of primordial on different oyster mushroom varieties.Al-amin (2004) also found different no. of primordial in different treatments.

4.1.5 Average number of fruiting body per packet

The highest average number of fruiting body per packet was observed in T_4 (55.84) which was statistically similar with T_2 (48.40), T_5 (46.20), and T_1 (45.00) treatment respectively, whereas the lowest average number of fruiting body per packet was found in T_3 (35.60) treatment.The findings of the present study matches with the study of Shelly (2008) who found approximately 70-90 fruiting body per packet in different oyster mushroom varieties.

4.1.6 Average weight of individual fruiting body

Significant variation was observed in terms of average weight of individual fruiting body of oyster mushroom due to different levels of liquid supplement (Figure 1). The highest average weight of individual fruiting body was attained in T_3 (6.29 g) treatment which was statistically similar with T_5 (5.01 g) .On the other hand, the lowest average weight of individual fruiting body was found in T_2 (4.45 g) followed by T_1 (4.49 g) and closely followed by T_4 (4.53 g) treatment. Alam *et al*. (2007) reported that the individual weight of fruiting body 3.86g with 50ppm of NAA

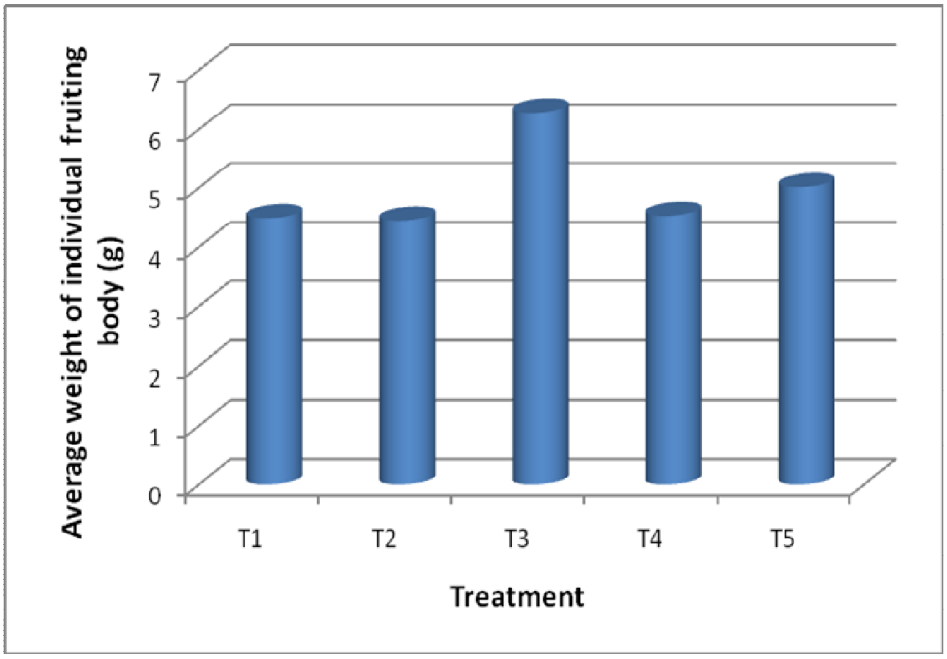

Figure 1. Effect of different levels of liquid supplement (wuxal super) on average weight of individual fruiting body of oyster mushroom

4.1.7 Length of stipe

Length of stipe of oyster mushroom varied significantly due to different levels of liquid supplement (Table 2). The highest length of stipe was observed in T_1 (3.20 cm) followed by T_5 (3.15 cm) and closely followed by T_2 (3.10 cm) , whereas the lowest length of stipe was found in T_3 (1.82 cm) which was closely followed by T_4 (2.16 cm) and they were statistically similar. Sarker *et al*. (2011) reported that length of stalk ranged from 1.80 to 2.57 cm which was similar to the findings of this experiment.

4.1.8 Diameter of stipe

Statistically significant variation was recorded in terms of diameter of stipe of oyster mushroom due to different levels of liquid supplement (Table 2). The highest diameter of stipe was found in T_3 (1.15 cm) treatment was statistically similar with T_4 (1.08 cm) and T_5 (1.03 cm) treatment while the lowest diameter of stipe was attained in T_2 (0.95 cm) treatment which was statistically similar with T_1 (0.98 cm) . Ahmed (1998) reported significant effects of various substrates on diameter of stalk. Habib (2005) found that stipe of oyster mushroom on different substrates varied from 0.74 cm to 1.05 cm.

4.1.9 Diameter of pileus

Diameter of pileus under different treatments showed significant difference (Table 2). The highest diameter of pileus was recorded in T_3 (5.87 cm).There were no significant difference among the rest of the treatments but the lowest diameter of pileus was obtained in T_1 (5.30 cm). Sarker *et al*. (2011) reported the diameter pileus ranged from 5.66 to 7.44 cm. The highest diameter of pileus (7.44 cm) was found in sundry sawdust with pasteurized straw (1:2).

Table 2. Effect of different levels of liquid supplement (wuxal super) on the dimension of fruiting body of oyster mushroom

Treatments	Length of stipe (cm)	Diameter of stipe (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1	3.20 a	0.98 cd	5.30 c	0.40 d
T_2	3.10 a	0.95 d	5.40 bc	0.48 c
T_3	1.82 c	1.15 a	5.87 a	0.63 b
T_4	2.16 b	1.08. b	5.48 bc	0.68 a
T_5	3.15 a	1.03 bc	5.65 ab	0.61 b
LSD (0.05)	0.1031	0.05954	0.3368	0.02663
CV(%)	1.96	2.87	3.25	3.19

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 5% level of probability

T_1: Wuxal Super @ 0% (Control)

T_2: Wuxal Super @ 0.1%

T_3: Wuxal Super @ 0.2%

T_4: Wuxal Super @ 0.3%

T_5: Wuxal Super @ 0.4%

4.1.10 Thickness of pileus

Significant difference was recorded in terms of thickness of pileus of oyster mushroom due to different levels of liquid supplement (Table 2). The highest thickness of pileus was observed in T_4 (0.68 cm) treatment which was closely followed with T_3 (0.63cm) and T_5 (0.61 cm).On the other hand, the lowest thickness of pileus was found in T_1 (0.40 cm) treatment which was closely followed by T_2 (0.48cm). Sarker *et al*. (2011) reported the thickness of pileus ranged from 0.47 to 0.55 cm respectively and the highest was found in sundry sawdust with pasteurized straw (1:2).

4.1.11 Biological yield

Different levels of liquid supplement showed statistically significant variation in terms of biological yield of oyster mushroom (Table 3). The highest biological yield was found in T_4 (261.56 g) treatment which was statistically identical with T_5 (242.20 g), T_3 (234.74 g), treatment, whereas the lowest biological yield was recorded in T_1 (212.11 g) treatment which was closely followed by T_2 (225.34 g) treatment.The findings of the present study more or less matches with the study of Ruhul Amin (2004),Bhuyan (2008).They found that the biological yield of oyster mushroom varied with different supplement used.

4.1.12 Economic yield

The highest economic yield was recorded in T_4 (252.96 g) treatment which was statistically identical with T_5 (231.68 g), T_3 (224.03 g) treatment, again the lowest economic yield was observed in T_1 (201.97 g) treatment which was closely followed by T_2 (215.33 g) treatment. The findings of the present study matches with the Alam *et al*. (2008) who found that the trend of economic yield corresponded with different nutrient content in the substrate.

Table 3. Effect of different levels of liquid supplement (wuxal super) on the yield, biological efficiency and benefit cost ratio of oyster mushroom

Treatments	Biological yield (g)	Economic yield (g)	Dry yield (g)	Benefit cost ratio
T_1	212.11 e	201.97 e	28.66 c	4.14 a
T_2	225.34 d	215.33 d	26.85 d	4.56 a
T_3	234.74 c	224.03 c	25.32 e	4.76 a
T_4	261.56 a	252.96 a	37.34 a	4.89 a
T_5	242.20 b	231.68 b	33.94 b	4.92 a
LSD (0.05)	0.05954	0.8250	0.8525	0.8811
CV(%)	0.01	0.18	2.63	10.06

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 5% level of probability

T_1: Wuxal Super @ 0% (Control)

T_2: Wuxal Super @ 0.1%

T_3: Wuxal Super @ 0.2%

T_4: Wuxal Super @ 0.3%

T_5: Wuxal Super @ 0.4%

4.1.13 Dry yield

The highest dry yield was observed in T_4 (37.34 g) treatment which was statistically identical with T_5 (33.94 g), and T_1 (28.66 g) treatment, whereas the lowest dry yield was found in T_3 (25.32 g) treatment which was closely followed by T_2 (26.85 g) treatment. The findings of present study matches with the study of Sarkar *et al*. (2007) who found the range of dry yield from 4.28 to 39.98 g per packet.

4.1.14 Biological efficiency

The highest biological efficiency was obtained in T_4 (186.83%) treatment which was statistically identical with T_5 (173%), T_3 (167.67%), treatment, again the lowest biological efficiency was found in T_1 (151.51%) treatment which was closely followed by T_2 (160.96%) treatment. In the present study the biological efficiency increases with level of supplement increased but declined thereafter. This may be due to application of supplement once through water. But the yield and biological efficiency may be increased using this supplement as basal dose during packet preparation.

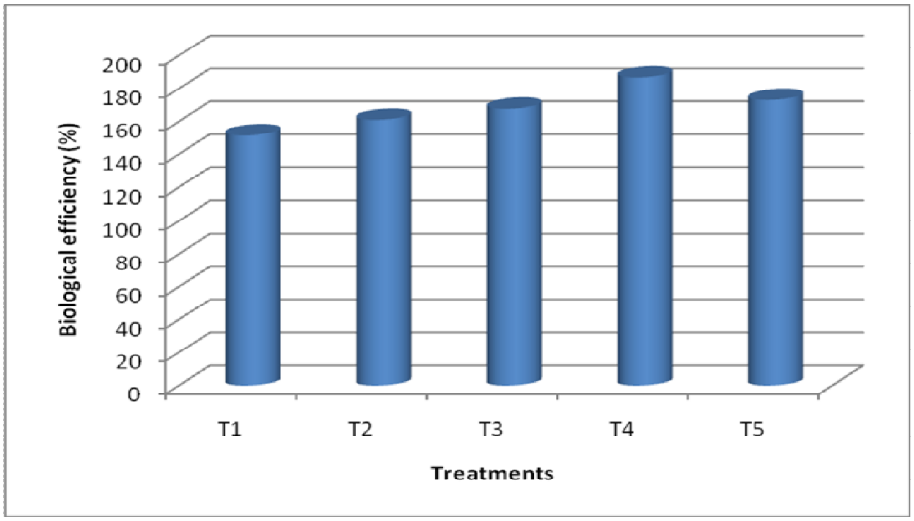

Figure 2. Effect of different levels of liquid supplement (wuxal super) on biological efficiency of oyster mushroom

4.1.15 Benefit cost ratio

Data revealed that the highest benefit cost ratio was observed in T_5 (4.92) treatment which was statistically identical with T_4 (4.89), T_3 (4.76), whereas the lowest benefit cost ratio in T_1 (4.14) treatment which was closely followed by T_2 (4.56) treatment. Sarker *et al*., (2007) mentioned the performances of substrates were significantly differed based on benefit cost ratio and the highest of 6.50 with wheat straw.

4.2 Proximate composition

4.2.1 Moisture

Different levels of liquid supplement varied significantly in terms of moisture content of oyster mushroom (Table 4). The highest moisture content was found in T_3 (88.70%) treatment which was statistically identical with T_2 (87.53%) treatment, while the lowest moisture content was recorded in T_4 (85.24%) treatment which was statistically similar with T_5 (85.35%), and T_1 (85.81%), treatment. The findings of the present experiment corroborate with the Ragunathan *et al.* (1996) where they showed that the moisture content of the fruiting bodies ranged from 84.70 to 91.90%.

4.2.2 Dry matter

Different levels of liquid supplement varied significantly in terms of dry matter content of oyster mushroom (Table 4). The highest dry matter content was found from T_4 (13.76%) treatment which was statistically identical with T_5 (13.65%), and T_3 (10.3%), whereas the lowest dry matter content was recorded in T_1 (13.52%) treatment. Kulsum *et al.* (2009), they revealed that the dry matter percentage of the fruiting body was ranged from 9.40 to 9.98.

Table 4. Effect of different levels of liquid supplement (wuxal super) on proximate nutrient composition of oyster mushroom

Treatments	Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)	Crude fiber (%)
T_1	85.81b	13.52 b	20.06 c	6.38 a	7.42 b	40.15 a	25.79 d
T_2	87.53 a	11.46 b	22 bc	4.1 ab	7.94 ab	39.58 a	26.38 cd
T_3	88.70 a	10.30 ab	23.31 b	3.17 b	8.25 a	37.52 b	27.75 ab
T_4	85.24 b	13.76 a	25.19 a	5.84 ab	8.60 a	31.83 c	28.04 a
T_5	85.35 b	13.65 ab	24.75 a	5.77 ab	8.36 a	33.26 c	27.15 bc
LSD (0.05)	1.574	1.005	2.053	0.9711	0.8250	1.633	0.8608
CV(%)	0.97	5.32	4.98	8.81	5.41	2.38	1.69

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 5% level of probability

T_1: Wuxal Super @ 0% (Control)

T_2: Wuxal Super @ 0.1%

T_3: Wuxal Super @ 0.2%

T_4: Wuxal Super @ 0.3%

T_5: Wuxal Super @ 0.4%

4.2.3 Protein content

Protein content of oyster mushroom showed statistically significant due to different levels liquid supplement (Table 4). The highest protein content was recorded in T_4 (25.19%) treatment which was statistically identical with T_5 (24.75%), and T_3 (23.31%) treatment. On the other hand, the lowest protein content was observed in T_1 (20.06%) treatment which was statistically similar with T_2 (22.00%) treatment. Zhang-Ruihong *et al.* (1998) reported the protein content of mushrooms produced was 27.2% on an average.

4.2.4 Lipid content

Statistically significant variation was recorded in terms of lipid content of oyster mushroom due to different levels of liquid supplement (Table 4). The highest lipid content was observed from T_1 (6.38%) treatment.The rest of the treatments were statistically similar in respect to percentage of lipid content (Table 4).But the lowest percentage of lipid was counted in T_3 (3.17%) treatment. The result of the present study was found more or less similar with the findings of Alam *et al.* (2007) who reported 4.30 to 4.41% lipids in oyster mushroom.

4.2.5 Ash

Different levels of liquid supplement varied significantly in terms of ash content of oyster mushroom under the present trial (Table 4). The highest ash content was found in T_4 (8.60%) treatment and the lowest ash content was recorded in T_1 (7.42%) treatment . The findings of the present study was supported by the study of Kulsum *et al.* (2009) who found that ash content was ranged from 6.58 to 8.41%. Alam *et al.* (2007) reported 8.28 to 9.02% ash in *Pleurotus spp.* which was also higher than the findings of present study.

4.2.6 Carbohydrate

Statistically significant variation was recorded in terms of carbohydrate content of oyster mushroom due to different levels of liquid supplement (Table 4). The highest carbohydrate content was recorded in T_1 (40.15%) treatment which was statistically identical with T_2 (39.58%) and T_3 (37.52%) treatment, whereas the lowest was observed in T_4 (31.83%) treatment which was closely followed with T_5 (33.26%) treatment. The findings of the present study matches with were the study of Chang *et al.* (1981) reported that the fruiting bodies of mushrooms contained 40.30-50.7% of carbohydrates. The result is also supported by Alam *et al.* (2007) who found 39.82 to 42.83% of carbohydrates in oyster mushroom .

4.2.7 Crude fiber

Crude fiber content of oyster mushroom showed statistically significant variation due to different levels of liquid supplement (Table 4). The highest crude fiber content was found in T_4 (28.04%) treatment and the lowest crude fiber counted under T_1 (25.79%) followed by T_2 (26.38%).The rest of the treatments were statistically similar (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.* Manzi *et al.* (2001) reported that on an average, a serving (100 g) of mushroom will supply 9 to 40% of the recommended of dietary fiber which was also differ from the present study.

4.3 Mineral content

4.3.1 Nitrogen (N)

Statistically significant variation was recorded in terms of N content of oyster mushroom due to different levels of liquid supplement (Table 5). The highest N content was recorded in T_4 (4.03%) treatment and the lowest N content was observed in T_1 (3.21%).The rest of the treatments were statistically different (Table 5). Moni *et al*. (2004) reported that on dry matter basis, the percentage of nitrogen 18.46 to 27.78% which was much higher than the findings of this experiment.

4.3.2 Phosphorus (P)

The highest P content was observed in T_3 (0.98%) treatment and the lowest P content was found in T_1 (0.85%) treatment. The rest of the treatments were statistically similar in respect to percent phosphorous content (Table 5). Kulsum *et al.* (2009) also found that phosphorus content was ranged from 0.84 to 0.92% which was smaller than the findings of this experiment.

4.3.3 Potassium (K)

Significant variation was recorded in terms of K content of oyster mushroom due to different levels of liquid supplement (Table 5). The highest K content was recorded in T_4 (1.98%) treatment and the lowest percentage was recorded in T_1 (1.48%) treatment. The rest of the treatments were statistically similar in recpect to percent potassium content (Table 5). The findings of the present study similar with the study of Chang *et al.* (1981) who reported that the fruiting bodies of *Pleurotus* contained 1.43 to 1.88 mg/g of K on dry weight basis. Sarker *et al.* (2007) also found 1.3% potassium in oyster mushroom which was smaller than the findings of present study.

Table 5. Effect of different levels of liquid supplement (wuxal super) on the mineral contents of oyster mushroom

Treatments	N (%)	P (%)	K (%)	Ca (mg/100gm)	Mg (mg/100gm)	Fe (mg/100gm)	Mn (mg/100gm)	Zn (mg/100gm)
T_1	3.21 e	0.85 bc	1.48 e	1.67 d	0.573 a	41.52 d	2.25 d	13.02 c
T_2	3.52 d	0.83 c	1.54 d	1.87 c	0.608 a	46.23 b	2.35 c	15.15 ab
T_3	3.73 c	0.98 a	1.69 c	1.90 bc	0.621 a	48.52 a	2.66 b	15.12 b
T_4	4.03 a	0.87 b	1.98 a	1.97 a	0.627 a	48.67 a	2.75 a	15.17 a
T_5	3.96 b	0.83 c	1.85 b	1.95 ab	0.629 a	43.55 c	2.68 b	15.15 ab
LSD (0.05)	0.05954	0.03261	0.05954	0.05954	0.08420	0.8250	0.04210	0.03261
CV(%)	0.72	2.26	1.57	1.38	7.99	0.96	0.33	0.11

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 5% level of probability

T_1: Wuxal Super @ 0% (Control)

T_2: Wuxal Super @ 0.1%

T_3: Wuxal Super @ 0.2%

T_4: Wuxal Super @ 0.3%

T_5: Wuxal Super @ 0.4%

4.3.4 Calcium (Ca)

The highest Ca content was observed in T_4 (1.97mg/100g) treatment which was statistically identical with T_5 (1.95mg/100g), T_3 (1.90mg/100g), and T_2 (1.87mg/100g), and the lowest Ca content was recorded in T_1 (1.67mg/100g) treatment. Sarker *et al.* (2007) reported maximum of 18400 ppm Ca was found in mushroom which was grown on wheat straw. Alam *et al.* (2007) who found 22.15 to 33.7 mg/100 g calcium in different oyster mushroom varieties.

4.3.5 Magnesium (Mg)

Statistically significant variation was recorded in terms of Mg content of oyster mushroom due to different levels of liquid supplement (Table 5). The highest Mg content was found in T_5 (0.629mg/100g) treatment which was statistically identical with T_4 (0.627mg/100g) and T_3 (0.621mg/100g), whereas the lowest Mg content was recorded in T_1 (0.573mg/100g) treatment which was closely followed by T_2 (0.608mg/100g) treatment and they were statistically identical. Sarker *et al.* (2004) also found 0.21% magnesium in oyster mushroom which was smaller than the findings of this experiment.

4.3.6 Iron (Fe)

Fe content of oyster mushroom showed statistically significant variation due to different levels of liquid supplement (Table 5). The highest Fe content was recorded in T_3 (48.67mg/100g) treatment and the lowest Fe content was observed in T_1 (41.52mg/100g) treatment.The rest of the treatments were statistically different over control in respect to percent of iron content. Sarker *et al.* (2007b) reported that content of Fe in the mushroom grown on different substrates varied from 92.09 ppm to 118.40 ppm. The result of the present study found iron higher than the value found by Alam *et al.* (2007) who found that iron content of different oyster mushroom varieties ranged from 33.45 to 43.2 ppm.

4.3.7 Manganese (Mn)

Statistically significant variation was recorded in terms of Mn content of oyster mushroom due to different levels of liquid supplement (Table 5). The highest Mn

content was found in T_4 2.75mg/100g and the lowest amount was counted under T_1 2.25mg/100g followed by T_2 2.12 mg/100g .The rest of the treatments were statistically similar with the study of Alam *et al.* (2007) who recorded 2.7 to 2.87 mg per 100g of Mn in different oyster mushroom varieties.

4.3.8 Zinc (Zn)

Different levels of liquid supplement showed statistically significant differences in terms of Zn content of oyster mushroom (Table 5). The highest Zn content was observed in T_4 (15.17mg/100g) treatment and the lowest Zn content in T_1 (13.02mg/100g) treatment. The results of the present study have the similarity with the study of Alam *et al.* (2007) found from their earlier experiment that zinc content of different oyster mushroom ranged from 16 to 20.9ppm. Sarker *et al.* (2007a) found 30.92 ppm zinc in oyster mushroom.

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka during the period from August to December 2014 to study the effect of liquid supplement (wuxal super) on the yield and proximate composition of oyster mushroom (*Pleurotus ostreatus*)**.** Mother culture of oyster mushroom was used as test crop for this experiment. The experiment considered the following treatments: T_1: Control (without wuxal super); T_2: Wuxal Super @ 0.1% ; T_3: Wuxal Super @ 0.2% ; T_4: Wuxal Super @ 0.3% ; T_5: Wuxal Super @ 0.4% . The experiment was laid out in single factor Completely Randomized Design (CRD). Data on different growth, yield contributing characters, proximate composition of mushroom were recorded and statistically significant variation was observed for different treatments.

The highest time required to complete mycelium running was recorded from T_1 (21.60 days) while, the lowest time required to complete mycelium running was found in T_4 (14.60 days). The highest time from stimulation to primordia initiation was observed in T_1 (6.20 days), whereas the lowest was obtained in T_4 (3.20 days) treatment. The highest time from primordia initiation to harvest was attained in T_1 (5.10 days) and the lowest time was found in T_4 (3.60 days). The highest average number of primordia per packet was recorded in T_5 (71.20), while the lowest was observed in T_3 (56.60) treatment. The highest average number of fruiting body per packet was observed in T_4 (55.84), whereas the lowest was found in T_1 (45.00) treatment. The highest average weight of individual fruiting body was attained in T_3 (6.29 g) and the lowest average weight of individual fruiting body was found in T_2 (4.45 g) treatment. The highest length of stipe was observed in T_1 (3.20cm), whereas the lowest length of stipe was found in T_3 (1.82 cm) . The highest diameter of stipe was found in T_3 (1.15 cm) treatment, while the lowest was

attained in T_2 (0.95 cm) treatment. The highest diameter of pileus was recorded in T_3 (5.87 cm), whereas the lowest diameter of pileus was observed in T_1 (5.30 cm) treatment. The highest thickness of pileus was observed in T_4 (0.68 cm) treatment and the lowest thickness of pileus was found in T_1 (0.40 cm) treatment. The highest biological yield was found in T_4 (261.56 g), whereas the lowest biological yield was recorded in T_1 (212.11 g) treatment. The highest economic yield was recorded in T_4 (252.96 g) treatment, again the lowest economic yield (201.97 g) was observed in T_1. The highest dry yield was observed in T_4 (37.34 g) treatment, whereas the lowest dry yield was found in T_3 (25.32 g). The highest biological efficiency was obtained in T_4 (186.83%), again the lowest biological efficiency was found in T_1 (151.51%) treatment. The highest benefit cost ratio was observed in T_5 (4.92), whereas the lowest was found in T_1 (4.14) treatment.

The highest moisture content was found in T_3 (88.70%) treatment, while the lowest moisture content was recorded in T_4 (85.24%). The highest dry matter content was found from T_4 (13.76%), whereas the lowest dry matter content was recorded in T_3 (10.3%) treatment. The highest protein content was recorded in T_4 (25.19%) treatment and the lowest protein content was observed in T_1 (20.06%). The highest lipid content was observed from T_1 (6.38%) treatment, whereas the lowest lipid content was obtained in T_3 (3.17%). The highest ash content was found in T_4 (8.60%) treatment, again the lowest ash content was recorded in T_1 (7.42%) . The highest carbohydrate content was recorded in T_1 (40.15%), whereas the lowest carbohydrate content was observed in T_4 (31.83%) treatment.

The highest crude fiber content was found in T_4 (28.04%) treatment, while the lowest crude fiber content was obtained in T_1 (25.79%). The highest N content was recorded in T_4 (4.03%) treatment and the lowest N content was observed in T_1 (3.21%) treatment. The highest P content was observed in T_3 (0.98%) treatment, while the lowest P content was found in T_1 (0.85%) treatment. The highest K content was recorded in T_5 (1.98%) treatment, whereas the lowest K content was observed in T_1 (1.48%). The highest Ca content was observed in T_4 (1.97mg/100g) and the lowest Ca content was recorded in T_1 (1.67mg/100g)

treatment. The highest Mg content was found in T_5 (0.629mg/100g) treatment, whereas the lowest Mg content was recorded in T_1 (0.573mg/100g) . The highest Fe content was recorded in T_4 (48.67mg/100g) and the lowest Fe content was observed in T_1 (41.52mg/100g) treatment. The highest Mn content was found in T_4 (2.75 mg/100g) treatment, whereas the lowest Mn content was recorded in T_1 (2.25 mg/100g). The highest Zn content was observed in T_4 (15.17mg/100g) treatment and the lowest Zn content was recorded in T_1 (13.02mg/100g) treatment.

Conclusion

From the above discussion, it was observed that treatment T_4 (Wuxal Super @ 0.3%) with rice straw performed significantly better on growth, yield, nutrient and mineral content of oyster mushroom (*Pleurotus ostreatus*) compare to the other treatments under the study.Therefore farmers can grow mushrooms in this rate of supplementation. But further investigation is needed to justify whether Wuxal Super increases the yield as basal supplement at the time of incorporation with rice straw to prepare spawn packet.

REFERENCES

Ali, M.R., Hoque, M.S., Ahmed, K.U. and Rahman, M.H. (2010). Effect of Wheat Bran Supplements with Sugarcane Bagasse on the Yield and Proximate Composition of Oyster Mushroom (*Pleurotus ostreatus*). *Bangladesh J. Mushroom*. **4**(2): 21-26.

Amin, S.M.R. (2002). Performance of different Oyster mushroom (*Pleurotus* spp) varieties. M.S. Thesis. Bangabundhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur. 72 p.

Amin, S.M.R., Sarker, N. C., Khair, A. and Alam, N. (2007). Detection of Novel Supplements on Paddy Straw Substrates on Oyster Mushroom Cultivation. *Bangladesh J. Mushroom*. **1**(2): 18-22.

Ancona-Mendex, M.L., Sandoval, C., Belmar-Casso, R. and Capetilo-Leal, C.M. (2005). Effect of substrate and harvest on the amino acid profile of oyster mushroom (*Pleurotus ostreatus*). *J. Food Comp. Analysis*. **18**(5): 447-450.

Alam, N., Khan, A., Hossain, M.S., Amin S.M.R. and Khan, L.A. (2007). Nutritional Analysis of dietary Mushroom *Pleurotus florida* Eger *and Pleurotus sajorcaj (*Fr.) Singer. *Bangladesh J. Mushroom*. **1**(2): 1-7.

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

Ali, M.A., Hussain, S., Nawaz, R., Ahsan, A. and Siddiq, M. (2004). Effect of Pasteurization techniques on mycelial growth of Oyster Mushroom, Pleurotus SPP. *J. Agric Res*.. **42**(2): 201-205.

Ali, M.A., Mehmood, M.I., Nawaz, R., Hanif, M.A. and Wasim, R. (2007). Influence of substrate pasteurization methods on the yield of OYSTER mushroom (*Pleurotus species*). *Pakistan J. Agri. Sci*., **44**(2): 300-303.

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

Balakrishna, J., N. Earanna, K. S. Shetty. (2001). Sunflower plant waste can be a new substrate for oyster mushroom production. Mysore Journal of Agricultural Scinces. 35(3): 203-205.

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

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

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

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

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

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.

Chorvathoba, V., Bobek, P., Ginter, E. & Klavanova, J. 1993. Effect of the oyster fungus on glycemia and cholesterolemia in rats with insulin depended diabetes. *Physol. Res*. **42**: 175-179.

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

Dravininkas, A. 1997. Investigation of the technological parameters of *Pleurotus* cultivation. Zemes Ukio Inzinerija, Mokslo Darbai. 29(1): 73-86.

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

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

Holman, R. I. (1976). Sigficance of essential fatty acids in human nutrition, in Lipids, Vol. 1.Paoletti, R., Poscellati, G.and Jasina, G., Eds, Raven press, New York. PP. 215.

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

Jiskani, M. M., M. A. Pathan and K. H. Wagan. 1999. Yield performance of oyster mushroom, *Pleurotus florida* (strain Pk-401) on different substrate, Pak. J. Agri., Agri. Engg., Vet. Sc. 15 (2): 26-29.

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

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

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

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

Mahjabin, T., Moonmoon, M., Kakon, A.J., Shamsuzzaman, K.M., Haque, M.M. and Khan, A.S. (2011). Effect of different media, pH and temperature on mycelial growth and substrates on yield of *Pleurotus djamor. Bangladesh J. Mushroom*. **5**(2): 31-38.

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

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

Mathew, A.V., Mathai, G. and Suharban, M. (1996). Performance evaluation of five species of *Pleurotus* (Oyster mushroom) in Kerela. *Mushroom Res*. **5**(9): 9-12.

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

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

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

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

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

Obodai, M., Okine, C. and 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 Microbio. and Biotech.,* **30**(3): 146-149.

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

Pani, B. K. and S. R. Das. 1998. Effect of pretreatment of substrate on the yield of oyster mushroom *Pleurotus sajor-caju* (Fr. Singer). Journal of Mycopathological Research. 36(2): 113-114.

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

Patil, M.B. and Jadhav, V.T. (1999). Studies on productivity of oyster mushroom on different agro-wastes under Marathwada condition. *J. Maharashtra Agril. Univ.,* **24**: (2) 162-163.

Patra, A.K. and Pani, B.K. (1995). Yield response of different species of Oyster mushroom (*Pleurotus spp*.) to paddy straw. *Current Agril. Res.,* **8**: 11-14.

Pervez, Z., Bhuiyan, M.K.A. and Islam, M.S. (2009). In vitro control of associated mycoflora of oyster mushroom substrates by the application of fungicides. Bangladesh Res. Pub. J., 2(4): 737-741.

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

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

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

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

Sangeetha, M.T.A.A. (2007). Influence of organic amendments on the yield of pink mushroom (*Pleurotus eous*) variety APK 1. *Mushroom Res*., **16**(1): 49-50

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

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

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

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

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

Suzuki, S. and Oshima, S. (1979). Influence of Shiitake (*Lentenus edodes*) on human scrum cholesterol. Mushroom Sci. 9 (I): 463. Thangamuthu, P. 1990. Food from sugarcane waste. *SISSTA-sugar. J.*, 16(2): 45-50.

Yoshioka, Y., Tabeta, R., Saito, H., Uehara, N. & Fukoaka, F. 1985. Antitumor polysaccharides from *P. ostreatus* (Fr.) Quel. Isolation and structure of a β-glucan. *Carbohydrate res*. **140**: 93-100.

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

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

Ziombra, M., Z. Fiedorw. 1998, Influence of substrate and pasteurization on *Pleurotus eryngii* yield. Ekologiczne aspektly produkeji agrodniczej, Poznan, Poland, 17-18 listopada, Poznanium, ogrodnictwo. 27: 373-376.

Ziombra, M., Z. Fiedorw. 1998, Influence of substrate and pasteurization on *Pleurotus eryngii* yield. Ekologiczne aspektly produkeji agrodniczej, Poznan, Poland, 17-18 listopada, Poznanium, ogrodnictwo. 27: 373-376.

APPENDICES

Appendix I. Analysis of variance of the data on growth and yield contributing characters of oyster mushroom due to different pasteurization method

Source of variation	Degrees of freedom	Mean square					
		Time required to complete mycelium running	Time from stimulation to primordial initiation (days)	Time from primordial initiation to harvest (days)	Average number of primordial per packet	Average number of fruiting body per packet	Average weight of individual fruiting body (g)
Between	6	38.381**	0.590*	1.390**	62.733**	54.628**	0.338**
Within	28	1.343	0.214	0.186	10.929	15.695	0.031

** Significant at 0.01 level of probability;

Appendix II. Analysis of variance of the data on the dimension of fruiting body of oyster mushroom due to different pasteurization method

Source of variation	Degrees of freedom	Mean square			
		Length of stipe (cm)	Diameter of stipe (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Between	6	0.410**	0.030**	0.464**	0.015**
Within	28	0.025	0.002	0.012	0.001

** Significant at 0.01 level of probability;

Appendix III. Analysis of variance of the data on the yield, biological efficiency and benefit cost ratio of oyster mushroom due to different pasteurization method

Source of variation	Degrees of freedom	Mean square				
		Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)	Benefit cost ratio
Between	6	1505.088**	1391.239**	5.456**	142.493**	0.526**
Within	28	196.344	173.227	0.880	18.589	0.065

** Significant at 0.01 level of probability;

Appendix IV. Analysis of variance of the data on proximate nutrient composition of oyster mushroom due to different pasteurization method

Source of variation	Degrees of freedom	Mean square						
		Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)	Crude fiber (%)
Between	6	11.234*	14.456*	3.956*	0.076*	0.178**	18.456**	3.123*
Within	28	5.034	4.892	1.309	0.023	0.069	1.452	1.003

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Appendix V. Analysis of variance of the data on the mineral contents of oyster mushroom due to different pasteurization method

Source of variation	Degrees of freedom	Mean square							
		N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	S (%)	Zn (%)
Between	6	0.156*	0.028*	0.032*	0.027*	0.001*	395.78*	0.001	1.246*
Within	28	0.053	0.009	0.014	0.011	0.0001	153.781	0.0001	0.448

* Significant at 0.05 level of probability

List of plates

Plate 1: Sterilized rice straw in the spawn packet

Plate 2: Prepared packets placed for mycelium growth

Plate 3: Pin head primordial in the spawn packet

Plate 4: Young fruiting body in the spawn packet

Plate 5: Matured fruiting body in the spawn packet