EFFECT OF CHEMICAL NUTRIENTS (NPK) ON PRODUCTION AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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DHAKA-1207

DECEMBER, 2014

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Reg. No.: 09-03421

A Thesis

Submitted to the Department of Biochemistry
Sher-e-Bangla Agricultural University, Dhaka
in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE (MS)

IN

BIOCHEMISTRY

SEMESTER: JULY-DECEMBER, 2014

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CERTIFICATE

This is to certify that the thesis entitled,***"Effect of chemical nutrients (NPK) on production and proximate composition of oyster mushroom (pleurotus ostreatus)"***submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in the partial fulfilment of the requirements for the degree of**MASTER OF SCIENCE (M.S.) IN BIOCHEMISTRY,**embodies the result of a piece of *bona fide* research work carried out by**A. K. M. SHALAHUDDIN,** Registration No.**09-03421**under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed during the course of this investigation has been duly acknowledged and style of this thesis have been approved and recommended for submission.

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ACKNOWLEDGEMENT

All praises are due to Almighty Allah, the Great, Gracious and Merciful, Whose blessings enabled the author to complete this research work successfully.

The author like to express his deepest sense of gratitude sincere appreciationto hisrespected supervisor ***Prof. Dr. Kamal Uddin Ahmed****, Department of Biochemistry, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh, for his scholastic guidance, support, encouragement and invaluable suggestions and constructive criticism throughout the study period and gratuitous labor in conducting and successfully completing the research work and in the preparation of the manuscript writing.*

The author also expresses his gratefulness and best regards to respectedCo-Supervisor, ***Prof. Md. Nuruddin Miah****, Department ofBiochemistry,Sher-e Bangla Agricultural University, Dhaka for his scholastic guidance, helpful comments and constant inspiration, inestimatable help, valuable suggestions throughout the research work and preparation of the thesis.*

The author expresses his sincere respect to the Co-ordinator, Department ofBiochemistry,Sher-e-Bangla Agricultural University, Dhaka and Md. Ashraful Islam Pulok, Institute of Seed Technology, SAU for valuable suggestions and cooperation during the study period. The author also expresses heartfelt thanks to all the teachers of the Department ofBiochemistry, SAU, for their valuable suggestions, instructions, cordial help and encouragement during the period of the study.

The author expresses his sincere appreciation to his brother, sisters, relatives, well wishers and friends for their inspiration, help and encouragement throughout the study period.

The Author

EFFECT OF CHEMICAL NUTRIENTS (NPK) ON PRODUCTION AND PROXIMATE COMPOSITION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

ABSTRACT

The study was conducted to the effect of different chemical nutrients (NPK) on the production and proximate composition of oyster mushroom (*Pleurotus ostreatus*). Mother culture of oyster mushroom was used as test crop for this experiment. The experiment consists of three different mixer of chemical nutrients. The experiment considered the following treatments: T_1: Control (0 g NPK in 10 kg straw), T_2: 2 g NPK in 10 kg straw, T_3: 4 g NPK in 10 kg straw and T_4: 6 g NPK in 10 kg straw, in NPK mix N:P:K=2:1:1. Data on different growth, yield contributing characters, proximate composition of mushroom were recorded and statistically significant variation was observed for different treatments. The highest economic yield(267.38 g) was recorded in T_3 treatment, again the lowest economic yield (208.11 g)was observed in T_1. The highest moisture content was found in T_1 (88.57%) treatment, while the lowest moisture content was recorded in T_3(84.12%). The highest protein content was recorded in T_3(25.54%) treatment and the lowest protein content was observed in T_1 (22.45%). The highest lipid content was observed from T_3 (6.43%) treatment, whereas the lowest lipid content was obtained in T_1 (5.47%). The highest carbohydrate content was recorded in T_1 (39.55%), whereas the lowest was observed in T_3(34.83%) treatment. The highest Fe content was recorded in T_3 (525.48 ppm) and the lowest Fe content was observed in T_1 (482.89 ppm). Chemical nutrients (4 g NPK) with 10 kg rice straw performed significantly better on growth, yield, nutrient and mineral content 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

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*., 2007a) 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. Randive (2012) 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 also considering 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 and 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 (*Pleurotusostreatus*) 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

*Pleurotussajor-caju*and *Pleurotusflorida*are popular and widely accepted after *Pleurotusostreatus*(Amin *et al*., 2007a).

The climatic conditions and seasonal diversity of Bangladesh is ideal for the cultivation of the oyster mushroom (Amin *et al.*, 2007).Mushroom production in rural communities can alleviate poverty and improve the diversification of agricultural production (Chang and Mshigeni, 2001). Ourcountryhasresourceandpotential for large scale productionof mushroombothfor homeconsumptionandexport. Mushroom production is greatly affected by pests and diseases. Chemical disinfection is recommended as it is cheaper then steam sterilization(Afyon,1988).

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

- To know the effect of chemical nutrients (NPK) on growth and yield of Oyster mushroom (*Pleurotusostreatus*).
- To know the proximate composition and mineral content of oyster mushroom grown using different chemical nutrients.

CHAPTER II

REVIEW OF LITERATURE

Mushroom grows well in waste materials and the growing materials have to chemical nutrients by using NPK treatment. There are many scientific reports on different aspects of mushroom cultivation especially different variety, use of substrate, chemical nutrients etc. but still there are major scopes to investigate the effects of different chemical nutrients 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.

2.1 Effect of chemical nutrients of mushroom

Ogbo and Okhuoya (2009) were conducted to the effect of crude oil contamination which is a perennial problem in the Niger Delta of Nigeria on the yield and nutrient status of Pleurotus tuber-regium was investigated. In this study, after cultivating P. tuber-regium in crude oil contaminated soils to which sawdust, shredded banana leaf blades, NPK fertilizer and poultry litter were added, the yield and nutrient or chemical composition were determined. Crude oil contamination caused significant increase in the size and yield of the mushroom by increasing the pileus and stipe size and also fresh and dry weights showing a fertilizer effect. The addition of sawdust and poultry litter enhanced the fertilizer effect by further significant increases in size and yields of treatments that had them. The addition of NPK and shredded banana leaf blades to crude oil contaminated soils did not enhance the fertilizer effect of crude oil as there was significant decrease in the size and yield of P. tuber-regium in treatments that had them. The nutrient chemical composition of the mushroom was also affected by the presence of the crude oil and supplementation with the various materials. There was a reduction in the moisture and carbohydrate content caused by the addition of poultry litter and sawdust to the contaminated soils and an increase in

the ash, fat, protein and fibre content of the mushroom. On the other hand, the addition of NPK and shredded banana leaf blades caused a reduction in the moisture, protein and carbohydrate content and an increase in the fat, moisture, ash and fibre content. The performance of the fungus in crude oil contaminated substrates can be optimized by the addition of sawdust and poultry litter but not shredded banana leaf blades and NPK. This is evident from the fact that sawdust and poultry litter enhanced growth while shredded banana leaf blades reduced growth in crude oil contaminated soil. The improvement in nutrient status of the mushroom indicates that the fertilizer effect of crude oil also affects the general well being of the fungus.

Bhuyan (2008) conducted an experiment to study the effect of various supplements at different levels with sawdust showed significant effort on mycelium running rate and reduced the required days to complete mycelium running in the spawn packet. The supplementation of sawdust found to be significant in yield and yield contributing characters of oyster mushroom with some extent. The highest biological yield, economic yield, dry yield, biological efficiency (BE) and benefit cost ratio (BCR) of 270.5 g, 266.5 g, 26.34 g, 93.29, 9.57%, respectively was observed in sawdust supplemented with NPK mixed fertilizer (N=0.6%, P=0.3%, K=0.3%).

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. *Pleurotusflorida*, *Pleurotuspulmonarius*and *Pleurotusostreatus*were selected. Steam pasteurization produced the best results as for as the performances of individual species are concerned, *Pleurotuspulmonarius*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 *Pleurotusflorida*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.

Ayodele and Okhuoya (2007) was conducted to three agricultural by-products (oil palm fruit fibre, banana leaves and sawdust) were supplemented with wheat bran, NPK fertilizer and urea at 5 - 20% (w/w) for wheat bran and 0.1, 0.5, 1.0 and 2% (w/w) for NPK and Urea. The supplemented substrates were used to cultivate Psathyrellaatroumbonata. There was sporophore formation in all the substrates supplemented with NPK except at 1% and 2% in oil palm fruit fibre and 2% in banana leaves. The highest yield was on sawdust supplemented with wheat bran at 5%. Urea as supplement did not support sporophore formation in all the substrates except in sawdust but with low yield at 0.5%.

Ancona-Mendex*et al.* (2005) conducted an experiment to grow oyster mushroom (*Pleurotusostreatus* (Jacq.: Fr.) in either maize or pumpkin straw. Samples were taken for each one of the three harvests and analyzed for total nitrogen (N) content and amino acids profile. The substrate had no effect ($P>0.05$) on N content and amino acid profile of the fruits. However, N (g/100 g DM) increased ($P<0.05$) from 4.13 g in the first harvest to 5.74 g in the third harvest. In general, the amino acids tended to be higher on the first harvest samples, but no changes were found ($P>0.05$) in the amino acid profile due to substrate or harvest, except for valine decreasing ($P<0.05$) from 3.96 to 3.15 g/16 g N. Changes in the N content of the fruit could be explained by changes in the stipe and pileus proportions as they had different N content (3.15 and 5.48 + or 0.031 g N/ 100 g DM respectively). The amino acid profile of the mushroom was adequate according to the FAO/WHO/UNU adult human amino acid requirements.

Khlood and Ahmad (2005) conducted an experiment to study the ability of oyster mushroom (*Pleurotusostreatus*) 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%).

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 *Pleurotussajor- 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 *Pleurotussajor-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 *Pleurotussajor-caju* over control, i.e., when disinfection done with hot water.

Maniruzzaman (2004)conducted an experiment to find out the best preparation formulas of horse manure and synthetic compost. Horse manure, wheat straw, gypsum as basic materials and wheat bran, cotton seed meal, sunflower meal, malt sprout, chicken food, molasses, ammonium sulphate, urea as activators were used. The nitrogen content of the starting mixture was brought up 2 in all applications. According to the results, the highest yields with horse manure compost were obtained from the combinations of 1000 kg of horse manure, 50 kg of wheat bran,

3.1 kg of ammonium sulphate, 1.5 kg of urea, 35 kg of gypsum40 kg of chicken food or malt sprout. The highest yields with synthetic compost were obtained from the combinations of 1000 kg of wheat straw, 282 kg of wheat bran, 13 kg of urea, 23.5 kg of ammonium nitrate, 40 kg of molasses, 60 kg of gypsum, 65 kg of cotton seed meal or 100 kg of chicken food.

2.2Growth, yield contributing characters and yield of mushroom

An experiment was carried out by Nuruddin*et al.* (2010) to investigate the effect of different levels of cowdung (0, 5, 10, 15 and 20%) on yield and proximate composition of *Pleurotusostreatus*. 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) 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. They 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) conducted an experiment to determine the effect of five different levels of cowdung (0%, 5%, 10%, 15% and 20%) as supplement with

sawdust on the performance of oyster mushroom. All the treatments performed better over control. The mycelium running rate in spawn packet and the highest number of primordia/packet were found to be differed due to different levels of supplements used. The highest weight of individual fruiting body was observed in sawdust supplemented with cowdung @ 10% (3.69 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%.

Amin *et al*. (2007) carried out an experiment to find out the primordia and fruiting body formation and yield of oyster mushroom (*Pleurotusostreatus*) 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.

Sarker*et al*. (2007a) carried out an experiment to find out the performance of different cheap agricultural household byproducts, grasses and weeds as substrate available in Bangladesh. The minimum duration to complete mycelium running was 17.75 days in waste paper, which differed significantly from that in all other substrates. The minimum duration required from stimulation to first harvest was observed in sugarcane bagasse (6.75 days), which was statistically identical to that in waste paper, wheat straw and sawdust (7.00 days). The number of fruiting body was positively correlated with biological efficiency, biological yield and economic yield of oyster mushroom. The number of fruiting body grown on different substrates differed significantly and the highest number of fruiting body per packet (183.25) was recorded on waste paper, which was significantly higher

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

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).

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).

Obodai*et al.* (2003) evaluated eight lignocellulosic by-products as substrate, for cultivation of the oyster mushroom. *Pleurotusostreatus* (Jacq. ex. fr.) Kummer. The yields of mushroom on different Substrates were 183.1, 151.8, 111.5, 87.5, 49.5, 23.3, 13.0 and 0.0 g for composted Sawdust of *Triplochitonscleroxylon*, 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 (*Pleurotusostreatus*) 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 *Pleurotussajor-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.

Chang*et al.* (1981) conducted an experiment to test the effect of fortification of rice straw with rice bran on the yield and quality of oyster mushroom (*Plelrrotusostrentus*) 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 *Pleurotussajor-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 *Pleurotussajor-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 (*Pleurotussajor-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).

2.3 Proximate composition of mushroom

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 *Pleurotusostreatus*. 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. *Pleurotusflorida, P. eous*and *P. flabellatus* were grown on wheat straw substrate. Time required for spawn run and pinning was significantly less in *Pleurotuseous* 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 (*Pleurotusostreatus*) 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 (*Pleurotusostreatus* 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 (*Pleurotussajor-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 *Pleurotussajor-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 *Pleurotussajorcaju* 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 *Pleurotussajorcaju* mushroom in Indian subcontinent or similar climatic conditions.

Manzi*et al.* (2001) analyzed fresh and processed mushrooms (*Agaricusbisporus*, *Pleurotusostreatus* 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 *Pleurotusostreatus* and Boletus group. On an average, a serving (100 g) of mushroom will supply 9 to 40% of the recommended of dietary fiber.

Chang*et al.* (1981) conducted an experiment to test the effect of fortification of rice straw with rice bran on the yield and quality of oyster mushroom (*Plelrrotusostrentus*) 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 *Pleurotussajor-caju*, *Pleurotusplatypus* and *Pleurotuscitrinopileatus* respectively.

Murugesan*et al.* (1995) cultivated mushroom P. *sajor-caju* (Fr.) Sing, on water hyacinth (*Elchhornicrassipe*). 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. *Pleurotussajor-caju* contained 41.26% crude protein when cultivated on rice straw and 29 % when cultivated on wheat straw.

CHAPTER III

MATERIALS AND METHODS

The study was conducted during the period from March, 2015 to June, 2015 to study the effect of different chemical nutrients (NPK) on the production and proximate composition of oyster mushroom (*Pleurotusostreatus*). The chapter includes a brief description of the location of experimental site, climate condition, materials used for the experiment, design of the experiment, preparation of substrates, preparation of spawn packets, cultivation of spawn packet, collection of produced mushrooms, proximate analysis of the mushrooms, data collection and data analysis procedure. The details materials and methods are presented below under the following headings-

3.1 Experimental site

The experiment was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. 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, Savar, Dhaka.

3.3 Varietal characteristics of Oyster Mushroom

Oyster mushroom is *Pleurotusostreatus*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

The experiment consists of four different fertilizer treatments. The experiment considered the following treatments:

T_1: Control (0 g NPK in 10 kg straw)

T_2: 2 g NPK in 10 kg straw

T_3: 4 g NPK in 10 kg straw

T_4: 6 g NPK in 10 kg straw

Here, N:P:K=2:1:1

3.5 Design and layout of the experiment

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

3.6 Preparation of substrates

At first 10 kg weight of dry rice straw was taken. Then the rice straw was soaked in water over night with NKP fertilizer as per treatment. All processed 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 water.

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 "D" shape with a blade and opened by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a tea spoon for removing the thin whitish mycelial layer. Then the spawn packets were soaked in water for 5 minutes and inverted 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 temperature of culture house was maintained 22^0C to 25^0C. The first primordia appeared 2-4 days after D-shaped cutting depending upon the type of substrate. The harvesting time also varied depending upon the composition of chemical nutrients (NPK).

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 (2004). Mushrooms were harvested by twisting to uproot from the base.

3.7 Data collection

3.7.1Time from stimulation to primordia initiation

Days required from stimulation to primodia initiation were recorded.

3.7.2Time required to complete mycelium running

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

3.7.3Time from primordia initiation to harvest

Days required from primodia initiation to harvest were recorded.

3.7.4Average number of primordia packet^{-1}

Number of primordia was recorded.

3.7.5Average number of fruiting body packet^{-1}

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

3.7.6Average weight of individual fruiting body packet^{-1}

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.

3.7.7 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)

3.7.8 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.

3.7.9Economic 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.

3.7.10Dry 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):

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

3.7.11 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).

3.8 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 find out approximate composition.

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. Then the constant weight was redacted from the fresh weight to determine moisture content. The moisture content was expressed as percent of the fresh fruiting bodies.

3.8.3 Determination of dry matter

A clean container (dish or beaker) was place in an oven at 105^0C 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 constant weight of dry sample is known as dry matter.

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.

3.8.5Determination 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

3.8.6 Totallipid estimation

Lipid 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:

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

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

3.8.7Determination 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):

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

3.8.8Total 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).

3.8.9Determination 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.

3.9 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, N and P

The sample was digested with nitric acid to release of Ca, Mg, K, Fe, S, Zn, Nand P. Ca, Mg, Fe, S and Zn were determined by atomic absorption spectrophotometer, K was determined by flame photometry, N 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 $CaC1_3$-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 ofFe and Zn elements were measured by atomic absorption spectrophotometer (AAS) directly in the undiluted filtrate.

3.9.2.7 Calculations: For Ca, Mg, K, P

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

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

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

c = g sample weighed into the digestion tube

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

For Zn and Fe

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

Zn and Fe measured on atomic absorption spectrophotometer

c = g sample weighed into the digestion tube

3.9.2.8 Estimation of N

50 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette to volume with water and mixed.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.

3.9.2.9 Determination of total sulphur

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

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

Where,

A = weight of $BaSO_4$ g

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

W = weight of sample in g

3.10 Statistical analysis

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

CHAPTER IV

RESULTS AND DISCUSSION

The study was conducted to find out the effect of chemical nutrients (NPK) on production and proximal composition of oyster mushroom (*Pleurotusostreatus*). 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 characters

4.1.1 Time required to complete mycelium running

Time required to complete mycelium running of oyster mushroom varied significantly due to different chemical nutrients (Table 1). The highest time required to complete mycelium running was recorded from T_1 (20.40 days) while, the lowest time required to complete mycelium running was found in T_3 (16.20 days).

4.1.2Time from stimulation to primordia initiation

Different chemical nutrients showed statistically significant variation in terms of time from stimulation to primordia initiation of oyster mushroom (Table 1). The highest time from stimulation to primordia initiation was observed in T_1 (3.50 days) treatment which was statistically similar with T_2 (3.20 days) whereas, the lowest time from stimulation to primordia initiation was obtained in T_3 (2.70 days) treatment. Sarker (2004) observed that duration of primordia initiation to oyster mushroom was significantly lower as compared to control.

4.1.3Time from primordia initiation to harvest

Statistically significant variation was recorded in terms of time from primordia initiation to harvest of oyster mushroom due to chemical nutrients (Table 1). The highest time from primordia initiation to harvest was attained in T_1 (4.60 days) treatment. On the other hand, the lowest time from primordia initiation to harvest was found in T_4(3.50 days) which was statistically identical with T_3 (3.60 days) treatment. Ali *et al*. (2004) reported that chemical nutrients 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. Mahjabin*et al*. (2011) reported minimum days (13.25) required for completion of mycelial growth was observed in chemical nutrients whereas the highest days (31.75) required for mycelial growth was observed in 5 g NKP in 10 kg straw.

4.1.4Average number of primordiaper packet

Average number of primordiaper packet of oyster mushroom varied significantly due to chemical nutrients (Table 1). The highest average number of primordiaper packet was recorded in T_3 (74.40) treatment while, the lowest average number of primordiaper packet was observed in T_1 (63.40) treatment. Pathan*et al*. (2009) that 5 g NKP in 10 kg straw was the best in relation to studied characters. Amin (2004) in his experiment found that the highest number of primordia of oyster mushroom was found in nutrients paddy straw but lowest was found in control treatment.

4.1.5Average number of fruiting body per packet

Chemical nutrients showed statistically significant differences in terms of fruiting body per packet of oyster mushroom (Table 1). The highest average number of fruiting body per packet was observed in T_3 (65.40) which was statistically similar with T_4(62.40) and T_2(62.00) treatment whereas, the lowest average number of fruiting body per packet was found in T_1 (56.30) treatment. Sarker*et al*. (2011)

reported that the number of effective fruiting bodies was the highest (21.00) in autoclaved N, P and K (2:1:1) and it was the lowest (7.50) in autoclaved N, P and K (1:1:1).

Table 1. Effect of different chemical nutrients (NPK) on growth 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 primordiapacket^{-1}	Average number of fruiting body packet^{-1}
T_1	20.40 a	3.50 a	4.60 a	63.40 c	56.30b
T_2	18.40 b	3.20 ab	4.10 b	69.40 b	62.00 a
T_3	16.20 c	2.70 bc	3.60 c	74.40 a	65.40 a
T_4	17.80 b	3.00 b	3.50 c	70.10 b	62.40 a
$LSD_{(0.05)}$	1.101	0.485	0.455	4.283	5.132
CV(%)	3.32	4.24	3.00	4.36	5.47

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability
T_1: Control (0 g NPK in 10 kg straw)
T_2: 2 g NPK in 10 kg straw
T_3: 4 g NPK in 10 kg straw
T_4: 6 g NPK in 10 kg straw

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 chemical nutrients (Figure 1). The highest average weight of individual fruiting body was attained in T_3 (3.61 g) treatment which was statistically similar with T_4 (3.45 g) treatment. On the other hand, the lowest average weight of individual fruiting body was found in T_1 (2.83 g) and closely followed by T_3 (3.18 g) treatment. Pathan*et al*. (2009) that 6 g NPK was the best in relation to studied characters.

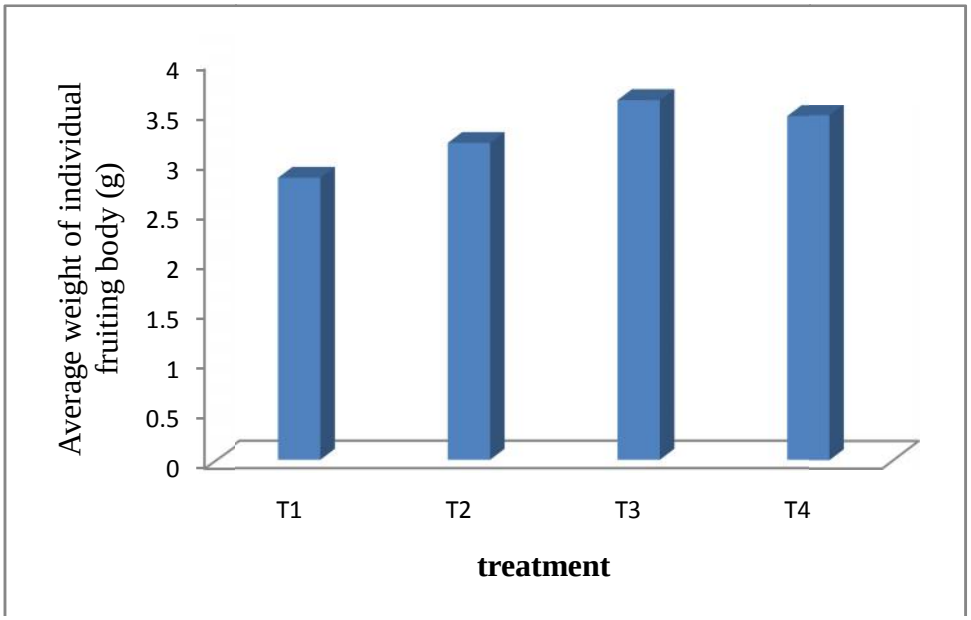

Figure 1. Effect of chemical nutrients (NPK) on average weight of individual fruiting body of oyster mushroom

4.1.7 Length of stipe

Length of stipe of oyster mushroom varied significantly due to chemical nutrients (Table 2). The highest length of stipe was observed in T_3 (2.68 cm) treatment which was statistically similar with T_4 (2.56 cm) treatment whereas, the lowest length of stipe was found in T_1 (1.82 cm). 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 chemical nutrients (Table 2). The highest diameter of stipe was found in T_3 (1.16 cm) treatment and closely followed by T_4 (1.09 cm), while the lowest diameter of stipe was attained in T_1 (0.96 cm) treatment and closely followed by T_2 (1.03 cm) treatment. Amin (2002) 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

Chemical nutrients showed statistically significant variation in terms of diameter of pileus of oyster mushroom (Table 2). The highest diameter of pileus was recorded in T_3 (6.87 cm) treatment and closely followed by T_4 (6.72 cm) and T_2 (6.51 cm) treatment, whereas the lowest diameter of pileus was observed in T_1 (6.03 cm) treatment. Pathan*et al*. (2009) that NKP was the best in relation to studied characters. 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 NKP (2:1:1).

4.1.10 Thickness of pileus

Significant difference was recorded in terms of thickness of pileus of oyster mushroom due to chemical nutrients (Table 2). The highest thickness of pileus was observed in T_3 (0.83 cm) treatment which was closely followed with T_4(0.77 cm) treatment. On the other hand, the lowest thickness of pileus was found in T_1 (0.67 cm) treatment which was closely followed by T_2 (0.71 cm) treatment. Sarker*et al*. (2011) reported the thickness of pileus ranged from 0.47 to 0.55 cm respectively and the highest was found in NKP (2:1:1).

Table 2. Effect of chemical nutrients (NPK) 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	1.82 c	0.96 d	6.03 d	0.67 d
T_2	2.22 b	1.03 c	6.51 c	0.71 c

T_3	2.68 a	1.16 a	6.87 a	0.83 a
T_4	2.56 a	1.09 b	6.72b	0.77 b
$LSD_{(0.05)}$	0.378	0.047	0.138	0.025
CV(%)	5.36	4.36	3.26	5.47

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

T_1: Control (0 g NPK in 10 kg straw)

T_2: 2 g NPK in 10 kg straw

T_3: 4 g NPK in 10 kg straw

T_4: 6 g NPK in 10 kg straw

4.2 Yield parameter

4.2.1 Biological yield

Chemical nutrients showed statistically significant variation in terms of biological yield of oyster mushroom (Table 3). The highest biological yield was found in T_3 (282.36 g) treatment which was statistically identical with T_4(274.90 g) treatment whereas, the lowest biological yield was recorded in T_1 (226.49 g) treatment which was closely followed by T_2 (265.38 g) treatment. Marimuthu*et al.* (1994) reported earlier that neem cake at 5% level increased the yield of *P. citrinopileatus, P.sajor-caju* and *P. pathypus* by 26-49, 24-79 and 16%, respectively compared with control.

4.2.2 Economic yield

Statistically significant variation was recorded in terms of economic yield of oyster mushroom due to chemical nutrients (Table 3). The highest economic yield was recorded in T_3 (267.38 g) treatment which was statistically identical with T_4(255.75 g) and T_2 (239.62 g) treatment, again the lowest economic yield was observed in T_1 (208.11 g) treatment. Sarker*et al*. (2011) recorded the highest economic yield in autoclaved NPK at 2:1:1 ratio respectively.

4.2.3 Dry yield

Dry yield of oyster mushroom varied significantly due to chemical nutrients (Table 3). The highest dry yield was observed in T_3 (41.90 g) treatment and

closely followed by T_4 (37.29 g) treatment whereas, the lowest dry yield was found in T_1 (23.00 g) treatment which was closely followed by T_2 (30.36 g) treatment. Pathan*et al*. (2009) that NKP soaking was the best in relation to studied characters. Kulsum*et al.*(2009) found that the highest dry yield was 21.27 g due to 6 g NKP socking.

4.2.4 Benefit cost ratio

Benefit cost ratio of oyster mushroom showed statistically significant variation due to chemical nutrients under the present trial (Table 3). Data revealed that the highest benefit cost ratio was observed in T_3 (5.17) treatment and closely followed by T_4 (4.85) treatment, whereas the lowest benefit cost ratio in T_1 (4.03) treatment which was closely followed by T_2 (4.69) treatment. Sarker*et al*. (2007a) mentioned the performances of substrates were significantly differed based on benefit cost ratio and the highest of 6.50 withNKP socking wheat straw.

Table 3. Effect of chemical nutrients (NPK) on the yield parameter and benefit cost ratio of oyster mushroom

Treatments	Biological yield (g)	Economic yield (g)	Dry yield (g)	Benefit cost Ratio
T_1	226.49 c	208.11 c	23.00 d	4.03d
T_2	265.38b	239.62 b	30.36 c	4.69c
T_3	282.36 a	267.38 a	41.90 a	5.17 a
T_4	274.90ab	255.75ab	37.29 b	4.85 b
$LSD_{(0.05)}$	15.18	18.035	1.027	0.240
CV(%)	4.32	5.46	4.69	3.34

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

T_1: Control (0 g NPK in 10 kg straw)

T_2: 2 g NPK in 10 kg straw

T_3: 4 g NPK in 10 kg straw

T_4: 6 g NPK in 10 kg straw

4.3 Proximate composition

4.3.1Moisture

Statistically significant variation was recorded in terms of moisture content of oyster mushroom due to chemical nutrients (Table 4). The highest moisture content was found in T_1 (88.95%) treatment which was statistically identical with T_2 (87.33 %) treatmentwhereas, the lowest moisture content was recorded in T_3 (84.33%) treatment which was statistically identical with T_4(85.42%) 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%.

4.3.2Dry matter

Chemical nutrients varied significantly in terms of dry matter content of oyster mushroom (Table 4). The highest dry matter content was found from T_3 (15.67 %) treatment which was statistically identical with T_4(14.58%) treatment and the lowest dry matter content was recorded in T_1 (11.05 %) treatmentwhich was statistically identical with T_2(12.67 %) treatment. The result of the present study matches with the findings of previous one that reported by Kulsum*et al.*(2009), they revealed that the dry matter percentage of the fruiting body was ranged from 9.40 to 9.98.

4.3.3Protein content

Protein content of oyster mushroom showed statistically significant due to chemical nutrients (Table 4). The highest protein content was recorded in T_3 (25.54%) treatment which was statistically identical with T_4(25.49%) treatment. On the other hand, the lowest protein content was observed in T_1 (22.45%) treatment which was statistically identical with T_2 (23.07%)treatment. Zhang-

Ruihong*et al.* (1998) reported the protein content of mushrooms produced was 27.2% on an average.

4.3.4Lipid content

Statistically significant variation was recorded in terms of lipid content of oyster mushroom due to chemical nutrients (Table 4). The highest lipid content was observed from T_3 (6.23%) treatment and closely following by T_4(5.97 %), T_2 (5.83 %) treatment whereas, the lowest lipid content was obtained in T_1 (5.47%). The result of the present study was found more or less similar with the findings of Alam*et al.* (2007a) who reported 4.30 to 4.41% lipids in oyster mushroom.

4.3.5Ash

Chemical nutrients varied significantly in terms of ash content of oyster mushroom under the present trial (Table 4). The highest ash content was found in T_3 (8.76%) treatment, again the lowest ash content was recorded in T_1 (7.64%) 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.* (2007b) reported 8.28 to 9.02% ash in *Pleurotus spp.* which was also higher than the findings of present study.

4.3.6Carbohydrate

Statistically significant variation was recorded in terms of carbohydrate content of oyster mushroom due to chemical nutrients (Table 4). The highest carbohydrate content was recorded in T_1 (41.96%) treatment which was statistically identical with T_2 (38.98%) treatment whereas, the lowest was observed in T_3 (33.42 %) treatment which was closely followed with T_4 (35.94%)treatment. The findings of the present study were not supported by the study of Kulsum*et al.*(2009)who found that carbohydrate content was ranged from 32.85 to 56.38% which showed a high rate of variation. Ragunathan*et al.* (1996) recorded the carbohydrate content ranged from 40.6 to 46.3%. Chang *et al.* (1981) reported that the fruiting

bodies of mushrooms contained 40.30-50.7% carbohydrates which were also than the findings of this study.

4.3.7Crude fiber

Crude fiber content of oyster mushroom showed statistically significant variation due to chemical nutrients (Table 4). The highest crude fiber content was found in T_3 (25.85 %) treatment which was closely following by T_4(24.26 %) and T_2(24.17 %) treatment, while the lowest ash content was obtained in T_1 (22.48%) treatment. The findings of the present study corroborate with the study Alam*et al*. (2007a) 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.

Table 4. Effect of chemical nutrients (NPK) on proximate nutrient composition of oyster mushroom

Treatments	Moisture (%)	Dry matter(%)	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)	Crude fiber (%)
T_1	88.95 a	11.05 b	22.45 b	5.47 c	7.64 d	41.96 a	22.48 c
T_2	87.33 a	12.67 b	23.07 b	5.83 b	7.95 c	38.98 b	24.17 b
T_3	84.33 b	15.67 a	25.54 a	6.43 a	8.76 a	33.42 d	25.85 a
T_4	85.42 b	14.58 a	25.49 a	5.97 b	8.34 b	35.94 c	24.26 b
$LSD_{(0.05)}$	2.294	2.436	1.284	0.305	0.285	1.386	1.501
CV(%)	5.32	4.27	4.27	5.48	3.95	3.57	4.98

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

T_1: Control (0 g NPK in 10 kg straw)

T_2: 2 g NPK in 10 kg straw

T_3: 4 g NPK in 10 kg straw

T_4: 6 g NPK in 10 kg straw

4.4 Mineral content

4.4.1Nitrogen (N)

Statistically significant variation was recorded in terms of N content of oyster mushroom due to chemical nutrients (Table 5). The highest N content was recorded in T_3 (4.09%) treatment which was closely followed by T_4(3.92 %) and T_2(3.83 %) treatment. On the other hand, while the lowest N content was observed in T_1 (3.47 %) treatment. 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.

4.4.2Phosphorus (P)

Different chemical nutrients showed significant differences in terms of P content of oyster mushroom (Table 5). The highest P content was observed in T_3 (1.49 %) treatment which was statistically identical with T_4(1.44 %) and T_2(1.40 %)treatment, while the lowest P content was found in T_1 (1.33 %) treatment. 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.

4.4.3Potassium (K)

Significant variation was recorded in terms of K content of oyster mushroom due to chemical nutrients (Table 5). The highest K content was recorded in T_3 (2.69 %) treatment which was statistically identical with T_4(2.60 %) treatment whereas, the lowest K content was observed in T_1 (2.34%) treatment which was closely followed by T_2 (2.51 %) treatment. 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.* (2007a) also found 1.3% potassium in oyster mushroom which was smaller than the findings of present study.

4.4.4Calcium (Ca)

Different chemical nutrients varied significantly in terms of Ca content of oyster mushroom (Table 5). The highest Ca content was observed in T_3 (1.96%) treatment which was statistically identical with T_4(1.95 %) and T_2(1.92%)

treatment and the lowest Ca content was recorded in T_1 (1.73%) treatment. Sarker*et al.* (2007b) reported maximum of 18400 ppm Ca was found in mushroom which was grown on wheat straw. Alam*et al.* (2007a) who found 22.15 to 33.7 mg/100 g calcium in different oyster mushroom varieties.

4.4.5Magnesium (Mg)

Statistically significant variation was recorded in terms of Mg content of oyster mushroom due to chemical nutrients (Table 5). The highest Mg content was found in T_3 (0.79%) whereas, the lowest Mg content was recorded in T_1 (0.67%) treatment which was closely followed by T_2 (0.72 %) and T_4 (0.71%) treatment. Sarker (2004) also found 0.21% magnesium in oyster mushroom which was smaller than the findings of this experiment.

4.4.6Iron (Fe)

Fe content of oyster mushroom showed statistically significant variation due to chemical nutrients (Table 5). The highest Fe content was recorded in T_3 (525.48 ppm) treatment which was closely followed by T_4(510.81 ppm) treatment. On the other hand, the lowest Fe content was observed in T_1 (482.89 ppm) treatment. 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.* (2007a) who found that iron content of different oyster mushroom varieties ranged from 33.45 to 43.2 ppm.

4.4.7Sulphur (S)

Statistically significant variation was recorded in terms of S content of oyster mushroom due to chemical nutrients (Table 5). The highest S content was found in T_4 (0.33%) treatment which was statistically identical with T_2(0.32%), T_3(0.32 %) treatment, whereas the lowest S content was recorded in T_1 (0.28%) treatment. The findings of the present study were supported with the findings of Alam*et al.*

(2007a) who recorded 0.238 to 0.321% of sulphur from their earlier study in oyster mushroom varieties.

4.4.8Zinc (Zn)

Different chemical nutrients showed statistically significant differences in terms of Zn content of oyster mushroom (Table 5). The highest Zn content was observed in T_3 (15.54%) treatment which was statistically identical with T_2(15.32 %) and T_4(15.22 %) treatment whereas, the lowest Zn content in T_1 (14.02%) treatment. The results of the present study have the similarity with the study of Alam*et al.*(2007b) found from their earlier experiment that zinc content of different oyster mushroom ranged from 16 to 20.9%. Sarker*et al.* (2007a) found 30.92 ppm zinc in oyster mushroom.

Table 5. Effect of chemical nutrients (NPK) on the mineral contents of oyster mushroom

Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	S (%)	Zn (%)
T_1	3.47 c	1.33 b	2.34 c	1.73 b	0.67 c	482.89 c	0.28 b	14.02 b
T_2	3.83 b	1.40 ab	2.51 b	1.92 a	0.71 b	498.86 b	0.32 a	15.32 a
T_3	4.17 a	1.49 a	2.69 a	1.96 a	0.79 a	525.48 a	0.32 a	15.54 a
T_4	3.92 b	1.44 a	2.60 a	1.95 a	0.72 b	510.81 b	0.33 a	15.22 a
$LSD_{(0.05)}$	0.278	0.108	0.128	0.145	0.014	15.84	0.012	0.759
CV(%)	5.22	3.78	5.95	4.95	4.46	3.35	5.28	3.79

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

T_1: Control (0 g NPK in 10 kg straw)

T_2: 2 g NPK in 10 kg straw

T_3: 4 g NPK in 10 kg straw

T_4: 6 g NPK in 10 kg straw

CHAPTER V

SUMMARY AND CONCLUSION

The study was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from March to June, 2015 to study the effect of different chemical nutrients (NPK) on the production and proximate composition of oyster mushroom (*Pleurotusostreatus*). The experiment consists of four different type of sawdust as- T_1: Control (0 g NPK in 10 kg straw), T_2: 2 g NPK in 10 kg straw, T_3: 4 g NPK in 10 kg straw and T_4: 6 g NPK in 10 kg straw. 10 kg straw was taken as basal substrate. The experiment was laid out in single factor Completely Randomized Design. Data on different growth, yield and nutrient composition and mineral content were recorded and significant variation was recorded for different studied parameter.

The highest time from stimulation to primordial initiation (3.50 days) was found from T_1, whereas the lowest time from stimulation to primordial initiation (2.70 days) was recorded in T_3.The highest time required to complete mycelium running (20.40 days) was found from T_1, whereas the lowest time required to complete mycelium running (16.20 days) was recorded in T_3. The highest time from primordial initiation to harvest(4.60 days) was attained from T_3 and the lowest time from primordial initiation to harvest(350 days) was found in T_4. The maximum average number of primordial per packet (74.40) was observed from T_3, again the minimum average number of primordial perpacket (63.40) was found in T_1. The maximum average number of fruiting bodyper packet (65.40) was recorded from T_3, while the minimum average number of fruiting body perpacket (56.30) was observed in T_5. The highest average weight of individual fruiting body (3.68 g) was attained from T_3 and the lowest average weight of individual fruiting body (2.90 g) was found in T_1. The longest length of stipe (2.68 cm) was recorded from T_3, while the shortest length of stipe (1.82 cm) was

found in T_1. The highest diameter of stipe (1.16 cm) was found from T_3, whereas the lowest diameter of stipe (0.96 cm) was recorded in T_1. The highest diameter of pileus (6.87 cm) was recorded from T_3, again the lowest diameter of pileus (6.03 cm) was found in T_1. The highest thickness of pileus (0.83 cm) was observed from T_3, and the lowest thickness of pileus (0.67 cm) was found in T_1. The highest biological yield (282.36 g) was attained from T_3, while the lowest biological yield (226.49 g) was recorded in T_1. The highest economic yield (267.38 g) was recorded from T_3, whereas the lowest economic yield (208.11 g) was observed in T_1. The highest dry yield (19.34 g) was observed from T_3, while the lowest dry yield (14.36 g) was attained in T_1. The highest benefit cost ratio (5.17) was found from T_3, and the lowest benefit cost ratio (4.03) was attained in T_1.

The highest moisture content (88.57%) was observed from T_1, while the lowest moisture content (84.12%) was found in T_3. The lowest dry matter content (11.05%) was found from T_1, whereas the highest dry matter content (15.67%) was recorded in T_3. The highest protein content (25.54%) was recorded from T_3, while the lowest protein content (22.45%) was observed in T_1. The highest lipid content (6.43%) was found from T_3, again the lowest ash content (5.47%) was recorded in T_1. The highest ash content (8.76%) was recorded from T_3 and the lowest ash content (7.64%) was found in T_1. The highest carbohydrate (39.55%) was observed from T_1, whereas the lowest carbohydrate content (34.83%) was observed in T_3. The highest crude fiber (25.85%) was recorded from T_3 and the lowest crude fiber content (22.48%) was found in T_1.

The highest amount of nitrogen content (4.17%) was attained from T_3, whereas the lowest nitrogen content (3.47%) was found in T_1.The highest amount of phosphorus content (1.49%) was attained from T_3, whereas the lowest phosphorus content (1.33%) was found in T_1. The highest amount of potassium (2.69%) was attained from T_3, again the lowest phosphorus content (2.34%) was found in T_1. The highest amount of calcium (1.96%) was observed from T_3, whereas the lowest calcium content (1.73%) was observed in T_1. The highest amount of magnesium (0.79%) was attained from T_3 and the lowest magnesium content

(0.67%) was found in T_1. The highest amount of iron (525.48 ppm) was attained from T_3, whereas the lowest iron content (482.89 ppm) was observed in T_1. The highest amount of sulphur (0.33%) was found from T_4, while the lowest sulphur content (0.28%) was attained in T_1. The highest amount of zinc (15.54%) was observed from T_3, whereas the lowest zinc content (14.02%) was recorded in T_1.

Conclusion

From the above discussion, it was observed that treatment T_3 [4 g NPK in 10 kg straw], among the treatments performed significantly better on growth, yield, nutrient and mineral content of oyster mushroom (*Pleurotusostreatus*).

Recommendations

In this experiment, mixed different chemical nutrients (NKP) performed better in respect of different growth, yield and nutrient composition and mineral content of oyster mushroom. Therefore, 4 g NPK in 10 kg straw can be recommended for farmer level oyster mushroom cultivation.

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APPENDICES

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

Month (2015)	Air temperature (^{0}c)		Relative humidity (%)	Rainfall (mm)	Sunshine (hr)
	Maximum	Minimum			
March	33.0	20.6	81	119	5.0
April	34.8	24.4	80	209	5.4
May	35.5	26.4	81	222	6.9
June	34.8	25.0	83	245	6.8

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

Appendix II. Analysis of variance of the data on growth characters of oyster mushroom due to different chemical nutrients (NPK)

Source of variation	Degrees of freedom	Mean square					
		Time required to complete mycelium running	Time from stimulation to primordial initiation	Time from primordial initiation to harvest	Average number of primordial per packet	Average number of fruiting body per packet	Average weight of individual fruiting body
Between	4	28.335**	0.590*	1.390**	56.384**	68.859**	0.368**
Within	18	1.274	0.328	0.480	12.924	13.485	0.026

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

Appendix III. Analysis of variance of the data on the dimension of fruiting body of oyster mushroom due to different chemical nutrients (NPK)

Source of variation	Degrees of freedom	Mean square			
		Length of stipe	Diameter of stipe	Diameter of pileus	Thickness of pileus
Between	4	0.608**	0.042**	0.564**	0.023**
Within	18	0.005	0.001	0.005	0.001

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

Appendix IV. Analysis of variance of the data on the on the yield parameter and benefit cost ratio of oyster mushroom due to different chemical nutrients (NPK)

Source of variation	Degrees of freedom	Mean square			
		Biological yield	Economic yield	Dry yield	Benefit cost Ratio
Between	4	2108.994**	1733.932**	8.824**	0.656**
Within	18	133.026	147.997	0.995	0.056

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

Appendix V. Analysis of variance of the data on proximate nutrient composition of oyster mushroom due to different chemical nutrients (NPK)

Source of variation	Degrees of freedom	Mean square						
		Moisture	Dry matter	Protein	Lipid	Ash	Carbohydrate	Crude fiber
Between	4	11.234*	14.456*	3.956*	0.076*	0.178**	18.456**	3.123*
Within	18	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 VI. Analysis of variance of the data on the mineral contents of oyster mushroom due to different chemical nutrients (NPK)

Source of variation	Degrees of freedom	Mean square							
		N	P	K	Ca	Mg	Fe	S	Zn
Between	4	0.156*	0.028*	0.032*	0.027*	0.001*	395.78*	0.001	1.246*
Within	18	0.053	0.009	0.014	0.011	0.0001	153.781	0.0001	0.448

** Significant at 0.01 level of probability;

* Significant at 0.05 level of probability.

LIST OF PLATES

Plate 1. Mushroom bag preparation

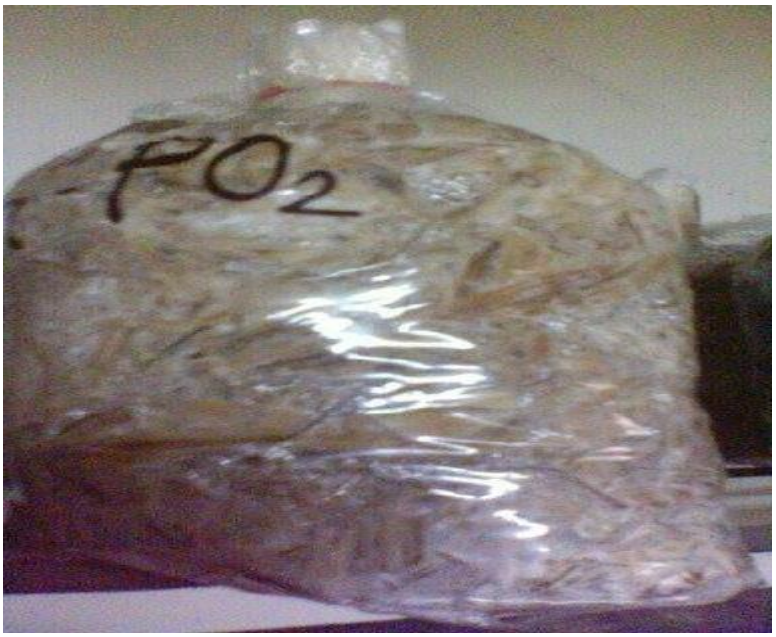

Plate 2. Prepared mushroom bag

Plate 3. Mushroom Spawn packet in cold environment for primordial initiation

Plate 4. Primordial initiation and growth of primordial

Plate 5. Produced mushroom in spawn packet

Plate 6. Dry mushroom after harvesting mushroom