EFFECT OF DIFFERENT LEVEL OF LIQUID MINERAL SUPPLEMENT (WUXAL SUPER) ON THE YIELD AND NUTRITIONAL STATUS OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF DIFFERENT LEVEL OF LIQUID MINERAL SUPPLEMENT (WUXAL SUPER) ON THE YIELD AND NUTRITIONAL STATUS OF OYSTER MUSHROOM (*Pleurotus ostreatus*)"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of ***MASTER OF SCIENCE IN BIOCHEMISTRY*** embodies the result of a piece of *bona fide* research work carried out by **Samaren Biswas**, Registration No. 01021 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in any other institutes.

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

Dated:
Dhaka, Bangladesh

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Supervisor

Dedicated to
My
Parents & Teachers those who laid the foundation of my success

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ABSTRACT

The present study was carried out at the Biochemistry laboratory and Mushroom Culture House (MCH)of Biochemistry Department, Sher-e-Bangla Agricultural University, Dhaka, during the month of January to May '09 to investigate the yield performance of oyster mushroom supplemented with different levels of liquid minerals. All the treatments performed significantly over the control. The lowest time from stimulation to primordia initiation **(**5.2 days**) and** primordia initiation to harvest (3.9 days) was observed in T_4 (Wuxal Super @ 0.3%). The highest number of primordia/packet (66.33) was observed in the T_2 and the highest number of fruiting body/packet was observed in T_3 (70.00) while the highest weight of individual fruiting body was observed in T_4 (2.25g). The highest biological yield (129.0 g/packet), economic yield (123.7 g/packet) dry yield (12.73 g/packet) and biological efficiency (44.48%) was recorded in the T_4(Wuxal Super @ 0.3%).

The study also investigate the effect of liquid mineral supplements on nutritional status the highest moisture percent was observed in T_3 (90.44%) the highest dry matter percent was observed in T_4 (10.30%). Among the biochemical attributes the highest content of protein was found in T_4 (23.60%), lowest lipid percentage was counted under T_3 (5.16%), the highest percentage of ash was observed in T_5 (9.60%), the highest percentage of carbohydrate was recorded in T_1 (46.07%) and highest percentage of crud fiber was recorded in T_4 (29.70 %). Therefore there was not much effect of the treatments on biochemical properties of produced mushroom. In case of elemental composition the highest percentage of nitrogen was recorded in the T_4 (3.78) while highest percentage of phosphorus (0.96), potassium (1.44) and iron (43.67) was recorded in T_3 and the the highest amount (mg) of manganese (2.80) and zinc (14.17) was counted under T_5. There fore it can be concluded that wuxal super @ 0.3% is found as a efficient supplement for mushroom production lowering the cost increasing yield.

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LIST OF ABBREVIATIONS

%	=	Percent
@	=	At the rate
°C	=	Degree Centigrade
Anon.	=	Anonymous
BARI	=	Bangladesh Agricultural Research Institute
BAU	=	Bangladesh Agricultural University
BBS	=	Bangladesh Bureau of Statistics
cv.	=	Cultivar (s)
DAI	=	Days After Inoculation
DMRT	=	Duncan's Multiple Range Test
e.g.	=	For example
et al.	=	And Others
etc.	=	Etcetera
FAO	=	Food and Agriculture Organization
g	=	Gram
hr	=	Hour (s)
i.e.	=	That is
IRRI	=	International Rice Research Institute
ISTA	=	International Seed Testing Agency
kg	=	Kilogram
LSD	=	Least Significant Difference

no.		Number
PDA	=	Potato Dextrose Agar
SAU	=	Sher-e-Bangla Agricultural University
T	=	Treatment
t/ha	=	Ton per Hectare
UNDP	=	United Nation Development Program
$^{w}/_{v}$	=	Weight per Volume
$^{w}/_{w}$	=	Weight per Weight
wt.		Weight
BCR	=	Benefit cost ratio
BE	=	Biological efficiency
MRR	=	Mycelium Running Rate
MDEC	=	Mushroom Development and Extension Center
MCC	=	Mushroom Culture Centre
FAO	=	Food and Agricultural Organization
mg	=	Milligram
Conc.	=	Concentration

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CHAPTER 1

INTRODUCTION

Mushroom plants are classified in the group of fungi. They may be seen growing on the trunks of trees, on stumps or logs, coming out of leaf mold on forest floor or in the grass in yards, lawn, field and pasture or along the road sides. The seeding part called the spawn or mycelium consists of fine white threads running all through the material upon which the plant is growing. Therefore, Mushroom is defined as a macrofungus with a distinctive fruiting body which can be either epigeous or hypogeous. The macrofungi have fruiting bodies large enough to be seen with the naked eye and to be picked up by hand (Chang and Miles, 1992).

Oyster mushrooms are large reproductive structures of edible fungi belong to genus *Pleurotus* under the order Agaricales, the family Tricholomataceae and the class Basidiomycetes. The vegetative parts of the mushrooms mainly consist of thread like long mycelium which under suitable condition forms fruiting bodies or sporocarps.

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

Mushrooms are being used as food and medicine since time immemorial. Even, the early men knew the special properties of mushrooms. According to Chang (1982), the protein value of dried mushrooms has been found to be 30-40% comprising all the essential amino acids. Mushrooms constitute an ideal source of food that

reduces body weight. Mushrooms provide more protein per unit area than other crops (Gupta, 1986). Mushrooms are source of Niacin (0.3 g) and Riboflavin (0.4 mg). Mushroom is a good source of trypsin enzyme. It is also rich in iron, copper, calcium, potassium, vitamin D, and folic acid. Mushrooms are valuable health food, which are low in calories, high in vegetable proteins, zinc, chitin, fiber, vitamins and minerals (Alam and Saboohi, 2001). Edible mushrooms are recommended by the FAO, to meet protein requirement of developing countries, the large portion of which depends mainly on cereals. Since, animal proteins are beyond the reach of common people in our country (World Bank; 2004), mushroom can play important role in minimizing that deficiency.

The enormous increase in our population has necessitated more and more food production through alternate resources such as mushroom as the availability of more arable land. In the developed countries, mushrooms have become one of the most important horticultural crops (Alam and Saboohi, 2001). Mushroom needs labor intensive indoor activity, which can help the landless, small and marginal farmers to raise their income, diversify economic activity and create gainful employment, especially for unemployed /under-employed youth and women folk. The mushroom cultivation could be a profitable agribusiness also. The spent mushroom substrates can be used as excellent organic fertilizers. Mushroom production converts agricultural wastes into a high protein source for human (Labuschagne *et al.* 2000). Our country has resources and potential for large scale production of mushroom both for home consumption and export.

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

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

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

CHAPTER 2

REVIEW OF LITERATURE

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

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

Kothandaraman *et al.* (1989) in a study reported that in the split or the logs f *Hevea brasiliensis* was inoculated with spawn of *Pleurotus sajor-caju*, *Pleurotus* citrinopileatus and *Pleurotus florida*. They were covered with polythene sheeting and kept in darkness at around 26°C until mycelium was visible. Rubber tree sawdust was also investigated as a growing medium; it was soaked in water for 24 hours, then dried to about 70% moisture and mixed with 5% $CaCO_3$; in bottles before inoculation. All 3 species began to grow on the logs within 3 days of inoculation and small fruiting bodies appeared 4 days alter spawn running was completed. However, almost all ceased development shortly afterwards; only 5 (*Pleurotus florida*) reached maturity. Mycelia on sawdust ceased to grow alter penetrating to about three-quarters of the depth of the medium. The reason(s) for the failure to develop fully are not yet known but, since rubber wood appears to have no inhibitory activity against *Pleurotus spp*., further studies are proposed.

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

Payapanon *et al.* (1994) mentioned that suitable amount of rice bran added to saw dust medium means the maximum yield of Neuron's florida at optimum production cost. Therefore, investigation on the addition of rice bran to saw dust medium, which consist of 100% saw dust, 0.5% $CaCO_3$ and 0.2% $MgSO_4.7H_20$ by weight was conducted, for the experiment rice bran 0, 1, 5, 10 and 15% respectively was used. *P. florida* was cultivated in plastic bags. Sawdust medium with 10% provided maximum yield. However, the yields obtained by addition of 5, 10 and 15% of rice bran were not significantly different. Yield of *P. florida* cultivation in the saw dust medium with 0% and 1% rice bran were not significantly different. Nevertheless, these were significantly different when compared with the higher rates of rice bran. The recommendation of the appropriate amount of rice bran to be added in the sawdust medium should be 5-10%.

Murugesan *et al.* (1995) cultivated mushroom P. *sajor-caju* (Fr.) Sing, on water hyacinth (*Elchhorni crassipe*). They compared water hyacinth with other conventional substrates paddy straw. Total yields for 20 bags of the two substrates were 15.0 and 10.5 kg respectively, although the time taken to reach the pin-head stage was longer on the water hyacinth substrate (17 days in water hyacinth and 10 days in paddy straw). The high yield on water hyacinth was attributed to the C: N ratio (24.3 compared with 53.5) and low lignin content (9% compared with 17%) of this substrate. Use of water hyacinth would provide a cheap substrate and a means of eradicating a troublesome aquatic weed.

Adamovie *et al.* (1996) stndied the impact of *Pleurotus ostreatus* mushroom enzymes on Wheat straw degradation in laboratory conditions. Chopped and pressured pasteurized straw with 24 dry matters was seeded with a mushroom mycelium. Chemical of the straw were done after 15, 30, 45, 60, 90 and 120 days

upon seeding. The mushroom collection was done four times. After seedling crude fiber, NDF and ADF decreased from 46.93 to 32.40 from 82.42 to 58.5 and From 56.12 to 41.17 respectively. A similar tendency was found for hemlicelluloses and celluloses, while it was not so expressed in lignin. The ash content increased from 0.26 to 9.78. The obtained results show that the mushroom enzymes degraded a substantial part of straw dry matter. The effects were most notable on the lignocellulosic complex. This enabled a successful use of the straw as a substrate for mushroom growth.

Pattana-Pulpium (1996) investigated that mushroom is fungus which grow in natural material such as wood or agricultural waste. The nutrients that stimulate the growth of this mold are present in those materials. The purpose of the study is to study the optimum condition for growth of abalone mushroom; *Pleurotus ostreatus* No 510 and *Pleurotus ostreatus* No 515 in plastic bag. From the experiments, the good growth of *Pleurotus ostreatus* No 510 and *Pleurotus ostreatus* No 515 could be seen in the following mixtures, saw dust, rice bran 7 g, $CaCO_3$ 2 g (NH4)2SO4 0.5 g. and saw dust 100 g, rice bran 5 g, $CaCO_3$ 1 g $(NH_4)_2SO_4$ 0.5 g plus Corn meal 4 g. respectively. The optimum pit and moisture content of the mixture are 6.5, 6.0 and 75, 65 percentage respectively. The optimum temperature of each is 30°C.

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

Kalita *et al.* (1997) studied the growth of *Pleurotus sajor-caju* in polyethylene hag on different combinations of substrates viz. only rice straw, rice straw plus rice husk mixture (1:1 v/v), water hyacinth, chopped banana leaves, areca nut husk and sugarcane bagasse. They found that only rice straw, rice straw plus rice husk mixture and areca nut husksubstrafes completed spawn running comparatively within short time (12-14 days.) but other substrates took longer time.

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

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

Obodai *et al.* (2000) mentioned that Seasonal effects on spawn run period, time for first appearance of fruiting bodies, number of flushes, morphological characteristics of the first flush and biological efficiency of 7 strains of oyster mushroom (*Pleurotus eous, Pleurotus ostreatus* and *Pleurotus sajor-caju*), grown

on composted sawdust of T. scleroxylon in Ghana, were studied. *P. eons* strain EM-1 and *Pleurotus sajor-caju* strain ST-6 gave the best yield and biological efficiencies in the wet and dry seasons, respectively. The spawn run period, mycelia growth density and the first appearance of fruiting bodies were not season-dependent.

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

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

Zhang-RuiHong *et al.* (1998.) cultivated of Oyster mushroom (*P. sajor-caju*) on rice and wheat straw without nutrient supplementation was studied. The effects of straw size reduction methods and particle sizes spawn inoculation level, and types of substrate (rice straw vs. wheat straw) on mushroom yield, biological efficiency, and substrate degradation were determined. The protein content of mushrooms produced was 27.2% on average. The dry matter loss of the substrate after

mushroom growth varied from 30.1 to 44.3%. Yields were higher from substrates which had been ground-up to 2.5 cm lengths; further size reductions lowered yields. Mushroom cultivation is a highly efficient method for disposing of agricultural residues as well as producing nutritious human food.

Khan *et al.* (2001) investigated the different aspects of the cultivation of Oyster mushroom on industrial wastes to push it as a new biotechnology and as a commercial crop in Pakistan. They found that after spawn running, pinhead formation took 7-8 days and sporocarps formed after 10-12 days. Cotton waste recorded the highest yield of 198.67 g. Wheat straw yielded 129.253 g, paper waste + wheat straw yielded 58.95 g and paper waste alone recorded on yield. The best mycelium growth was observed in cotton waste substrate. The average time taken for complete spawn running was 17 days. The second best mycelium growth was on wheat straw, where the average time for spawn running was 19 days. In paper waste, the average time for spawn running was 22 days. However, the average time taken for completion of spawn running on paper waste + wheat straw was 20 days. The differences among the phase of mycelium growth and their interaction with substrate were statistically significant.

Ayyappan *et al.* (2000) studied the effect of ventilation tubes on *P. citrinopileatus* and *Pleurotus florida*. Poly (vinyl chloride) tubes (45 cm long) were kept at the center of bags while filling the bags with spawn and straw. After filling, the top of the end bag was tied, keeping the tubes slightly producing. Equidistant holes (2, 5 or 8) of varying diameter were made on the tubes at opposite directions (total of 4, 10, or 16 holes). The use of ventilated tubes shortened the maturation period of *P. citrinopileatus* and *P. florida*. Ventilated tubes with a diameter of 10 mm and. 16 holes gave the greatest reduction in days to maturity and the highest sporophore yield (720 and 735 g).

Khan *et al.* (2002) evaluated the sawdust of shisham (*Dalbergia sissoo*) supplied alone or in combination with 10% cotton seed hulls, 5% horse dung, 5% wheat

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

Obodai *et al.* (2003) evaluated eight lignocellulosic by-products as substrate, for cultivation of the Oyster mushroom. *Pleurotus ostreatus* (Jacq. ex. fr.) Kummer. The yields of mushroom on different Substrates were 183.1, 151.8, 111.5, 87.5, 49.5, 23.3, 13.0 and 0.0 g for composted Sawdust of *Triplochiton scleroxylon*, Rice straw, Banana leaves, Maize stover, Corn husk, Rice husk, Fresh Sawdust and Elephant grass respectively. The biological efficiency (BE) followed the same pattern and ranged from 61.0%, for composted Sawdust to 50.0% for elephant grass. The Yield of mushroom was positively correlated to cellulose (r^2 = 0.6). Lignin (r^2 =0.7) and fiber (r^2 = 0.7) contents of the substrates. Based on the yield and BE of the substrates tested, Rice straw appeared to be the best alternate substrate for growing oyster mushroom.

Al Amin (2004) in his experiment revealed that the highest number of primordia of Oyster mushroom was found in sterilized paddy straw at first flush; whereas the lowest was obtained with saw dust.

Shah *et al.* (2004) an experiment carried out to investigate the cultivation of Oyster mushroom on different substrates. Sawdust produced highest yield, biological

efficiency and number of fruiting bodies, recommended as the best substrate for Oyster mushroom cultivation.

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

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

Khlood-Ananbeh *et al.* (2005) conducted an experiment to study the ability of Oyster mushroom (*Pleurotus ostreatus*) P015 strain to grow on live cake mixed with wheat straw. The treatments comprised : 90% straw + 5% wheat bran + 5% gypsum (control); 80% straw + 10% olive cake + 5% wheat bran + 5% gypsum (Ti); 70% straw + 20% olive cake 5% wheat bran + 5% gypsum (T2); 60% straw + 30% olive cake + 5% wheat bran + 5% gypsum (T3); 50% straw + 40% olive cake + 5% wheat bran +.5% gypsum (T4); and 90% olive cake + wheat bran + 5% gypsum (T5). After inoculation and incubation, transparent plastic bags were used for cultivation. The pinheads started to appear after 3 days, and the basidiomata

approached maturity 3-7 days after pinhead appearance. Several growth parameters including primordial induction and fructification period, earliness, average weight of individual basidiomata, average yield for each treatment, diameter of the pileus and biological efficiency percentage (BE%) were examined and proximate analyses for protein, crude fat, crude fiber, ash, carbohydrates, mineral and moisture contents were performed. The addition of 30% olive cake to the basal growing medium gave the highest yield (400 g/500g dry substrate), average weight (21.5 g/cap), and average cap diameter (7.05 cm/cap) and BE% (80%). Carbohydrate, protein and fibre contents were high in the *P. ostreatus* basidiomete. Ash contents were moderate, while fat content was low. For mineral contents in the mushrooms the trend was the same in all treatments. The K and P contents were high compared to the other minerals in all treatments, sodium was moderate while both Mg and Ca were found at low concentrations (Mg was relatively higher than Ca). Fe and Zn were relatively high compared to Cu and Mn which had very low concentrations.

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

Lim *et al.* (1997) analyzed the cost and return of *Volvariella* and *Pleurotus* mushroom production and found the ROI of 89.16 percent and 51.93 percent respectively. This indicates that mushroom production is economically feasible. The feasibility of low input mushroom production for upland farmers in reforested areas under the closed canopy high-diversity forest faming system was determined. Agricultural and tree wastes were tested and utilized for spawn and mushroom production. Findings showed that among 10 agricultural/tree wastes tested, mung

bean pods, kakawate and cassava leaves, log sawdust, and ipil-ipil leaves, sugarcane bagasse with rice bran, and water hyacinth can be used as alternative substances for *Volvariella* spawn production. Local isolate (VISCA) of *Volvariella volvacea* gave higher yield (2263.65g) compared with *Volvariella* (1574.80 g) isolates from BIOTECH College, Laguna, Philippines. This fruited well in the closed-canopy area than when cultivated in the open area. *Pleurotus* yield was higher (209.60 g/bag) inside mushroom house under closed-canopy area than when grown inside mushroom house in relatively open area (198.54 g).

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

Performance of five species of *Pleurotus* grown on cotton seed hulls, wheat, rice and maize straw was evaluated. The crude protein content of the fruiting bodies was varied with different substrates. *Pleurotus sajor-caju* contained 41.26% crude protein when cultivated on rice straw and 29% when cultivated on wheat straw. Those cultivated on rice and maize straw contained 17 amino acids but oystin was lacking in those cultivated on cottonseed husks or wheat straw. The total amino acid and essential amino acid contents in the fruiting bodies grown on the different substrates like rice straw, maize straw, and cotton seed husks were also found very significantly (Qin, 1989).

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

Sharma, B. B. (2004) conducted a laboratory experiment to study the effects of different organic amendments (gram dal powder, Lathyrus dal powder, moong dal powder and soyabean dal powder) on the growth and yield of the oyster mushroom, Pleurotus djamor. Chopped rice and wheat straw served as substrates. Observations were recorded on the number of days for spawn run, number of days for the initiation of primordia formation and yield. The effect of supplements did not differ significantly for the time taken for spawn run and initiation of pinhead stage. P. djamor yield was significantly influenced by the different supplements on both substrates. The addition of 2% moong dal powder resulted in the highest yield in both substrates while the lowest yield was recorded when Lathyrus dal powder was added to the substrates. Rice straw supplemented with gram dal powder showed a significantly lower yield than even the untreated control.

Desrumaux *et al.* (2005) in a series of 4 tests studied the application of cleaned flax-shives (central part of the flax stem, rich in lignin) in the oyster mushroom [Pleurotus sp.] substrate with regard to the oyster mushroom growing cycle and yield. The tested straw/flax-shive mixtures were 100/0, 90/10, 80/20 and 70/30 (dry weight). The flax-shives were moistened and added to the straw before pasteurization. The addition of the flax-shives induced a 1st flush that came 1-2 days earlier than the 1st flush on the straw substrate. This phenomenon was not repeated in the 2nd flush. On average, the yield increased due to the addition of the flax-shives by respectively 9, 10 and 6%. Though, this yield-increase was only noticed in 2 of the 4 tests, the other tests showed a small yield decrease and a status quo. The tests showing no or a negative result were both performed with cultivar Sylvan HK35, the tests showing a yield increase were performed with cultivars Amycel 3000 and Mycelia 2191. The influence of the genotype is not excluded, but has to be proved through further research.

Meera Pandey and Tewari (2004) found the spores of *P. florida* are known to cause respiratory allergies in workers. This is one of the major drawbacks in the cultivation of this species. The completely sporeless mutant available at the Indian Institute of Horticultural Research (IIHR) has not yet been commercialized due to its inferior sporophore morphology. Hence, this mutant was utilized as one of the parents for the development of low-spore-count strains with commercial agronomic traits suitable for the market. Among the 85 matings evaluated, 8 matings, which showed significantly low spore count and desirable agronomic traits for commercial cultivation, were selected. The yield of 2 of the selections was similar to the normal sporulating parent but lower than that of the sporeless mutant of IIHR.

Subrata Biswas and Singh (2004) in An experiment found the production efficacy of 3 oyster mushrooms (Pleurotus sajor-caju, P. flabellatus and P. florida) using 2 kinds of rice straws (Aush and Aman) following different disinfection methods, i.e. fume disinfection (fuming the overnight (18-20 h) water-soaked small pieces of cut straws with boiled water for 2 h), chemical disinfection (treating the cut straws in a solution of 20 litres water, 25 ml formalin and 3 g Bavistin [carbendazim] mixture overnight) and without disinfection (only by water soaking the cut straws overnight prior to mushroom bed preparation). The mushrooms grew well under in-house conditions of Tripura, India, during August 2003-February 2004. On the other hand, P. sajor-caju gave the highest yield of fruit bodies with biological efficacies of 52.5 and 108.6% on Aush and Aman substrates, respectively. In addition, all 3 species gave higher yield on Aman than Aush. Furthermore, the yield of mushroom with chemical disinfected straw substantiated the yield observed with fume disinfected straw. The straw without disinfection showed the lowest yield and was almost negligible in Aush.

Mukesh *et al.* (2004) tested different substrates, i.e. wheat, chickpea, pea, sugarcane bagasse and water hyacinth, were evaluated for production of oyster mushroom (Pleurotus sajor-cajo), during 2002-03 in Uttar Pradesh, India. The

period of spawn run on water hyacinth straw (21 days) was longer than other straws during January to April when minimum temperature varied from 13.93 to 20.62 degrees C and average temperature varying from 23.57 to 28.16 degrees C. The relative humidity during these period varied from 76.38 to 84.34 percent (morning) and 57.54 to 63.33 percent (evening). The number of days taken for pin head initiation on water hyacinth straw (25 days) was also longer compared to other straws. The biological efficiency of mushroom was higher on wheat straw (81.00) followed by chickpea (80.00), pea (77.77), water hyacinth (67.77) and sugarcane bagasse (66.67). Biological efficiency started declining with an increase in temperature (25 +or-2 degrees C) and decrease in relative humidity (80+or-2 percent). The highest yield was observed in wheat straw (810 g).

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

Shnyreva (2002) tested sixteen wild and 11 commercial cultivars of the oyster mushroom, Pleurotus ostreatus that were divided into 3 intersterile groups corresponding to the biological species using di-mon matings. All the isolates and cultivars tested exhibited selective growth on different substrates, as well as biodestructive efficiency. Lignocellulose destructive activity was higher in the cultivars than in the isolates. Majority of the wild cultivated and commercial

cultivars of the oyster mushroom were grouped into the II intersterile group, which is characterized by massive fruiting bodies and high productivity.

Banik and Nandi (2004) found popularity of oyster mushroom is increasing for its ease of cultivation, high yield potential as well as its high nutritional value. Laboratory experimentation followed by farm trial with a typical oyster mushroom Pleurotus sajor caju revealed that the yield potential of these mushrooms can be increased significantly when grown on a lignocellulosic crop residue - rice straw supplemented with biogas residual slurry manure in 1:1 ratio as substrate. Residual slurry manures obtained from biogas plants utilising either cattle dung, poultry litter, jute caddis or municipal solid waste as substrates for biogas production were all effective in increasing the yield of Pleurotus sajor caju significantly although to different extents. Disinfection of straw and manure by means of 0.1% KMnO< sub>4</ sub> plus 2% formalin solution in hot water caused 42.6% increase in yield of Pleurotus sajor caju over control, i.e., when disinfection done with hot water. In addition to increased yield, the above treatments caused significant increase in protein content, reduction in carbohydrate and increase in essential mineral nutrients in mushroom sporophores. Thus, it is concluded from the study that supplementation of rice straw with biogas residual slurry manure has strong impact in improving the yield potential, protein and mineral nutrient contents of Pleurotus sajor caju mushroom in Indian subcontinent or similar climatic conditions.

CHAPTER 3

MATERIALS AND METHODS

3.1. Location of experiment

The experiment was carried out at the, Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, during March'09 to July'08. The environmental condition of the experimental location was given in appendix 1.

3.2. Experiments and treatments

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

Treatments used:

- T_1: Wuxal Super @ 0 %
- T_2: Wuxal Super @ 0.1%
- T_3: Wuxal Super @ 0.2%
- T_4: Wuxal Super @ 0.3%
- T_5: Wuxal Super @ 0.4%

3.3. Preparation of spawn packet

Spawn packets were prepared using sawdust and $CaCO_3$ (1g per packet). The measured materials were taken in a plastic bowl and mixed thoroughly by hand and moisture was increased by adding water. Moisture was measured by using the moisture meter and adjusted the moisture content at 65%. The mixed substrates were filled into 7×11 inch polypropylene bag @ 500 g. The filled polypropylene bags were finally completed using plastic neck and plugged the neck with cotton and covered with brown paper and rubber band to hold it tightly in place.

3.4. Sterilization, inoculation and mycelium running in spawn packets

Therefore the packets were sterilized about 1 hrs in 121ºC and 1.5 kg/cm^2 pressures and then these were kept for cooling. After cooling, 5g mother spawn were inoculated into the packets in the laminar airflow cabinet and were kept in the incubation chamber at 20-22°C temperature until the packets become white with the mushroom mycelium. After completion of the mycelium running the rubber band, brown paper, cotton plug and plastic neck of the mouth of spawn packet were removed and the mouth was wrapped tightly with rubber band. Than this spawn packets were transferred to the culture house.

3.5. Application of the Wuxal super

Three spawn packets each of which represented a replication were used for each treatment. The spawn packets were opened by cutting "D" shape with a blade and opened by removing the plastic sheet after which they are soaked in wuxal super solution (3L) in a bucket for half an hour and squeezed for removal of excess water.

3.6. Cultivation of spawn packet

The packets of each type were placed separately on the shelf of culture room and covered with news paper. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3 times a day. The light around 300-500 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22°C to 25°C. The first primordia appeared 3-4 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

3.7. Collection of produced mushrooms

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

There fore the collected mushrooms are transferred to the laboratory and data are collected on different parameters.

3.8. Data collection

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

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

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

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

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

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

3.8.6. Biological efficiency: Biological efficiency was determined by the following formula:

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

3.9. Drying of mushrooms

The fruiting bodies are dried in the sun treatment wise separately. In the time of drying the stipe and the pileus are separated for better drying.

3.10. Proximate analysis of the mushroom

3.10.1. Moisture

About 10g of the fresh mushroom sample of each treatment were weighed, into separated and weighed petridishes and dried in an oven at 100°C to 105°C till the weight of the petridishes with their contents was constant. The moisture content was expressed as percent of the fresh mushroom fruiting bodies and calculated with the following equation.

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

3.10.2. Dry matter:

The dry matter content of the mushroom sample was calculated by deducting of the percent moisture of each sample from 100.

There fore,

$$\text{\% dry matter} = 100 - \text{\%moisture content}$$

3.10.3. Determination of Crude Fiber

Crud fiber was determined according to the methods described by Raghuramulu *et al.*, 2003 using the following formula.

$$\text{Crude fiber (g/l00g sample)} = \frac{[100 - (\text{moisture} + \text{fat})] \times (\text{We - Wa})}{\text{Wt. of sample taken}}$$

Where,

We =Initial weight before placing in Muffel furnace

Wa =Final weight after removing from Muffel furnace

3.10.4. Total Lipid estimation

Total lipid was estimated by the method described by Folch *et al.* 1957. 5g of mushroom sample was taken with 50 ml phosphate buffer and homogenated. 5 ml of the homogenated sample was taken with 50 ml of chloroform:methanol (2:1) mixture and lipid content was determined.

3.10.5. Total Carbohydrate Estimation

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

% Carbohydrate = 100 - [(Moisture + Fat + Protein + Ash + Crude Fiber)]

3.10.6. Determination of Total Ash

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

Ash content (%) = Wt of ash × 100 / Wt of sample taken

3.10.7. Determination of total Protein

The total protein was estimated by multiplying total nitrogen with 6.25.(Reff.AOAC/02)

3.10.8. Determination of total Nitrogen

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

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

Where,

a = ml HCI taken into the conical flask (usually 20.00 ml)

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

M_{HCl} = Molarity of the HCl

M_{NaOH} = Molarity of the NaOH

c = g of mushroom powder used for the analysis

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

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

3.11. Statistical analysis of data

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

CHEPTER 4

RESULTS & DISCUSSION

4.1. Time required from stimulation to primordia initiation (days)

The lowest time from stimulation to primordia initiation (5.2 days) was observed in T_4 and the highest time from stimulation to primordia initiation (8.2 days) was in T_1 (control). The rest of the treatments were statistically different and varied significantly over control in terms of time from stimulation to primordia initiation (Table 1). The findings of the present study corroborate with the findings of Gupta (1989), Kothandaraman *et al. (1989)*. The findings also match with the findings of Patra and Pani (1995) who found that the fruiting bodies appeared 4-8 days after the stimulation. Khan *et al.* (2001) reported that after spawn running pinhead formation took 7-8 days and may be harvested after 10-12 days.

4.2. Time required from primordia initiation to harvest (days)

The lowest time from primordia initiation to harvest was observed in the T_4 (3.9 days) and the highest time (5.1 days) was required in T_1 followed by T_5 (4.2 days). The other treatments were statistically similar but varied significantly over control in terms of time required from primordia initiation to harvest. The result of the present study keeps in with the result of Shelly (2008), Gupta (1989) and Khan *et al.* (2001). They separately found that the first crop was harvested 2-3 days later from primordia initiation. A similar result was also founded by Ahmed (2008).

4.3. Number of primordia /packet

The highest number of primordia/packet was observed in the T_2 (66.33) followed by T_5 (61.00) whereas the lowest number of primordia/packet was in the T_3 (56.00). The other treatments were statistically similar but differed significantly in terms of Average no of primordia/packet (Table 1). The findings of the present study matches with the study of Ahmed (2008) who reported significantly different numbers of primordia on different oyster mushroom varieties. Al-amin (2004) also found different no. of primordia in different traetments.

4.4. Number of fruiting body/packet

The highest number of fruiting body/packet was observed in T_3 (70.00) and the lowest number of fruiting body/packet was recorded in T_1 (55.00). Other treatments were statistically similar but differed significantly over control in terms of number of primordia/packet (Table1). The findings of the present study matches with the study of Shelly (2008) who found approximately 70-90 fruting body per packet in different oyster mushroom varieties.

4.5. Number of effective fruiting body/packet

The highest number of effective fruiting body/packet was observed in T_1 (41.00) followed by T_4 (40.00). The lowest number of effective fruiting body/packet was recorded in T_5 (36.33). The other treatments were statistically similar but differed significantly in terms of number of effective fruiting body/packet (Table 1). The findings of the present study matches with the study of Yoshida *et al.* (1993) who reported that the number of effective fruiting bodies was lower, but increased when the substrates was mixed with different supplements. The comparative similar findings were also found by Adamovie *et al.* (1996) and Ruhul Amin 2007, Ahmed, 2008.

Table1. Performance of different levels of commercial liquid mineral supplement (wuxal super) on time required from stimulation to primordia initiation, primordia initiation to harvest, number of primordia /packet, number of fruiting body /packet, number of effective fruiting body/packet of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Time required from stimulation to primordia initiation (days)	Time required from primordia initiation to harvest (days)	Number of primordia /packet	Number of fruiting body /packet	Number of effective fruiting body/packet
T_1	8.2a	5.1a	57.00bc	46.67ab	41.00a
T_2	7.2b	4.1bc	66.33a	48.00a	37.00bc
T_3	6.7c	4.1bc	56.00c	45.00b	37.67bc
T_4	5.2e	3.9c	57.33bc	48.67a	40.00ab
T_5	6.5d	4.2b	61.00b	41.00c	36.33c
CV (%)	1.08	1.96	2.57	2.01	2.93
LSD	0.194	0.229	4.20	2.52	3.08
Level of significance	**	**	**	**	**

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

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

Legend:

T_1: Wuxal Super @ 0% (Control)`
T_2: Wuxal Super @ 0.1%
T_3: Wuxal Super @ 0.2%
T_4: Wuxal Super @ 0.3%
T_5: Wuxal Super @ 0.4%

4.6. Weight of individual fruiting body (g)

The highest weight of individual fruiting body was observed in T_4 (2.25 g) and the lowest weight of individual fruiting body was in T_1 (1.39 g) followed by T_2 (1.94 g). The other treatments were statistically similar (Table 2). The result of the present study revels that waxal super increased the weight of the fruiting body up to a certain concentration and declined there after. The result of the present study did not matches with the study of Alam *et al*. (2007) who reported the individual weight of fruiting body 3.86g with 50ppm of NAA but in the present study the weight of individual fruiting body ranged from 2.55g-2.84g. This might be due to the environmental conditions, or due to different supplements used.

4.7. Dimension of fruiting body (cm)

4.7.1(Length of stalk).

Length of stalk under different treatments showed significant difference. The highest length of stalk was obtained in T_1 (3.71 cm) followed by T_5 (3.65cm) & T_2 (3.61cm) and the lowest length was found in T_3 (3.30cm) followed by T_4 (3.45cm). The highest diameter of stalk was obtained in T_3 (0.9 cm). The lowest diameter of stalk was obtained in T_1 (0.72 cm) followed by T_2 (0.77cm). The rest of the treatments were statistically similar (Table 2). The findings of the present study matches with the study of Habib (2005) who found that the diameter of pileus ranged from 4.85 cm to 8.95 cm and thickness of the pileus ranged from 0.45cm to 0.70cm due to different substrates and Sarker *et al., 2007* reported that the thickness of pileus ranged from 0.50 to 0.80 cm in case oyster mushroom.

4.7.2(Pileus Diameter).

Diameter of pileus under different treatments showed significant difference (Table 2). The highest diameter of pielus was obtained in T_3 (5.60cm). There were no significant differences among the rest of the treatments but the lowest diameter of pileus was obtained in T_1 (5.10cm). The thickness of the pileus in all the treatments showed significant differences although the highest thickness was observed in T_3 (0.48cm) and the lowest was in T_1 (0.40cm) followed by T_4 (0.42cm). The rest of the treatments were statistically different and varied significantly over control. The findings of the present study were matched with the study of Habib (2005) and sarker *et al.*, 2007. Both of two mentioned that the stalk length of *Pleorotus spp.* on different substrate varied from 1.93cm to 2.97cm and the diameter ranged from 0.74cm to 1.05cm.

Table 2. Performance of different levels of commercial liquid mineral supplement (wuxal super) on weight of individual fruiting body and dimension of fruiting bodies of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Weight of individual Fruiting body (g)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1	1.39c	3.71a	0.727d	5.10b	0.40c
T_2	1.49c	3.61ab	0.77c	5.33b	0.43bc
T_3	2.06b	3.30c	0.90a	5.60a	0.48a
T_4	2.25a	3.45bc	0.80b	5.35b	0.42c
T_5	1.90b	3.65a	0.81b	5.39b	0.45b
CV (%)	3.32	1.76	1.26	1.69	2.77
LSD	0.173	0.173	0.027	0.245	0.027
Level of significance	**	**	**	**	**

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

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

Legend:

T_1: Wuxal Super @ 0% (Control)
T_2: Wuxal Super @ 0.1%
T_3: Wuxal Super @ 0.2%
T_4: Wuxal Super @ 0.3%
T_5: Wuxal Super @ 0.4%

4.8. Biological yield (g)

The highest biological yield was recorded in the T_4 (129.0 g/packet) which were significantly higher as compared to all the treatments. The lowest biological yield was recorded in T_1 (79.00 g/packet) followed by T_2 (99.0 g/packet). The rest of the treatments were statistically similar (Table 3). The findings of the present study more or less matches with the study of Ruhul Amin (2004), Bhuyan (2008). They found that the biological yield of *Pleurotus ostreatus* varied with different supplement used.

4.9. Economic yield (g)

The highest economic yield was recorded in T_4 (123.7 g/packet) and the lowest economic yield was under T_1 (71.0 g/packet). The rest of the treatments were statistically different (Table 3). The findings of the present study matches with the study of Alam *et al.,* (2008) who found that the trend of economic yield corresponded with different nutrient content in the substrate.

4.10. Dry yield (g)

The maximum dry yield of mushroom was in T_4 (12.73 g/packet). The lowest dry yield was recorded under T_1 (7.07 g/packet). The rest of the treatments were statistically different (Table 3). The findings of the present study matches with the study of Sarker *et al.* (2007) who found the range of dry yield from 4.28 to 29.98 g/packet.

4.11. Biological efficiency

The highest biological efficiency of 44.48% was calculated in T_4 and the lowest biological efficiency of 27.24% was calculated from T_1 which was followed by T_2 (34.14). The rest of the treatments were statistically similar (Table 3). The findings of the present study does not match with the study of Obodai *et al.* (2003), Ahmed (2008), Bhuyan (2008). All of them found biological efficiency (BE) followed a pattern and ranged from 61.0% to 90.0%. But in the present study the biological efficiency increases with the level of supplement increased but declined there after. This may be due to application of supplement once through water. But the yield and biological efficiency may be increased using this supplement as basal dose during the packet preparation.

Table 3. Performance of different levels of commercial liquid mineral supplement (wuxal super) on yield and biological efficiency of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Biological yield (g/packet)	Economic yield (g/packet)	Dry yield (g/packet)	Biological efficiency (%)
T_1	79.0d	71.00e	7.07d	27.24d
T_2	99.0c	91.33d	9.10c	34.14c
T_3	115.3b	106.3c	10.17bc	39.77b
T_4	129.0a	123.7a	12.73a	44.48a
T_5	116.3b	111.3b	11.20b	40.12b
CV (%)	1.12	0.51	1.50	1.11
LSD	3.30	1.42	1.31	1.13
Level of significance	**	**	**	**

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

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

Legend:

T_1: Wuxal Super @ 0% (Control)
T_2: Wuxal Super @ 0.1%
T_3: Wuxal Super @ 0.2%
T_4: Wuxal Super @ 0.3%
T_5: Wuxal Super @ 0.4%

4.12. Effect on proximate composition

4.12.1. Moisture (%)

The moisture percentage of the fruiting body shows little significant difference. The moisture percent ranged from 89.70% to 90.44%. The highest moisture percent was observed in T_3 (90.44%). The rest of the treatments were statistically similar (Table 4) but the lowest was in T_4 (89.70%). The findings of the present study keep in with the study of Rahman (1994) who observed more or less 90% moisture in the mushroom *Pleurotus ostreatus*. Moni *et al.* (2004) found 88.15 to 91.64% moisture in oyster mushroom. The data does not support by Alam *et al* (2007) who reported 87 to 87.5% moisture in *Pleurotus spp.*, this might be due to the excess moisture or relative humidity in the environment.

4.12.2. Dry matter (%)

The dry matter percentage of the fruiting body shows no significant differences. The dry matter percent of fruiting body ranged from 9.56% to 10.30% (Table 4). The highest dry matter percent was observed in T_4 (10.30%). The rest of the treatments were statistically similar but the lowest was in T_3 (9.56%). The result matches with Rahman (1994), Ahmed (2008) who reported 10 to 13% dry matter in *Pleurotus spp* growing on different substrates.

4.12.3. Protein (%)

All the treatments contain a great amount of protein. The content of protein varied from 12.60%-23.60% (w/w) in the mushroom grown (Table 4). The highest content of protein was found in T_4 (23.60%) and the lowest content of protein was in T_1 (12.60%). The rest of the treatments were statistically different. Chang *et al.* (1981) who reported that the fruit bodies mushrooms contained 26.6-34.1% crude protein. Zhang-RuiHong *et al.* (1998) also found 27.2% protein content on average. Moni *et al.* (2004) found 18.46 to 27.78% crude protein in oyster mushroom. Zaman (2004) recorded 7.85-8.81% protein in oyster mushroom.

4.12.4. Lipid (%

The highest lipid percentage was counted under T_1 (6.90%). The rest of the treatments were statistically similar in respect to percent lipid content (Table 4). But the lowest lipid percentage was counted under T_3 (5.16%). The findings of the present study has similarity with the study of Moni *et al.* (2004) found 1.49 to 1.90% crude fats, in oyster mushroom. The data also supported by Alam *et al.* (2007) and Ahmed (2008) who reported 4.30 to 4.41% lipids in *Pleurotus spp.*

4.12.5. Ash (%)

The highest percentage of ash was observed in T_5 (9.60%) and the lowest percentage of ash was in T_1 (7.70 %). The rest of the treatments were statistically different (Table 4). The findings of the present study are supported by the study of Khlood-Ananbeh *et al.* (2005) who reported ash contents were moderate in the fruiting bodies. Alam *et al* (2007) reported 8.28 to 9.02% of ash in *Pleurotus spp.*

4.12.6. Carbohydrates (%)

The highest percentage of carbohydrate was recorded in T_1 (46.07%) and the lowest carbohydrate percentage was recorded in T_4 (31.77%). The rest of the treatments differed significantly over control in respect to percent carbohydrate content (Table 4). The findings of the present study matches with the study of Chang *et al.* (1981) reported that the fruit bodies mushrooms contained 40.30-50.7% of carbohydrates. the result is also supported by Alam *et al.* (2007) who found 39.82 to 42.83% of carbohydrates in *Pleurotus spp.*

4.12.7. Crud fiber (%)

The highest percentage of crud fiber was recorded in T_4 (29.70 %) and the lowest crud fiber percentage was counted under T_2 (27.70 %) followed by T_1 (26.73). The rest of the treatments were statistically similar (Table 4). The findings of the present study corroborate with the study Alam *et al.* (2007), Ahmed (2008) who reported.87g/100g to 23.29g/100g of fiber in *Pleurotus spp.*

Table 4. Chemical composition of oyster mushroom (*Pleurotus ostreatus*) as effected by different levels of commercial liquid mineral supplement (wuxal super)

Treatments	Moisture (%)	Dry Matter (%)	Protein (%)	Lipid (%)	Ash (%)	CHO (%)	Crud fiber (%)
T_1	90.05b	9.953a	12.60e	6.90a	7.70e	46.07a	26.73d
T_2	90.04b	9.963a	15.20d	5.40b	8.10d	43.60b	27.70c
T_3	90.44a	9.563b	19.63c	5.16b	8.40c	38.39c	28.41b
T_4	89.70b	10.30a	23.60a	5.73b	9.20b	31.77e	29.70a
T_5	89.94b	10.06a	22.30b	5.33b	9.60a	34.43d	28.33b
CV (%)	0.15	1.36	0.57	12.81	1.78	0.48	1.19
LSD	0.367	0.367	0.287	1.91	0.2873	1.890	0.367
Level of significance	NS	NS	**	*	**	**	**

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

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

Legend:

T_1: Wuxal Super @ 0% (Control)
T_2: Wuxal Super @ 0.1%
T_3: Wuxal Super @ 0.2%
T_4: Wuxal Super @ 0.3%
T_5: Wuxal Super @ 0.4%

4.12.8. Effect on mineral content

4.12.8.1. Nitrogen (%)

The highest percentage of nitrogen was recorded in the T_4 (3.78) and the lowest nitrogen percentage was counted under T_1 (2.02). The rest of the treatments were statistically different (Table 5). The findings of the present study matches with the study of Moni *et al.* (2004) who analyzed for various nutritional parameters and found 4.22 to 5.59 % of nitrogen on dry matter basis in fruiting bodies of oyster mushroom. Ahmed (2008) found similar percentage of nitrogen in *Pleurotus* spp.

4.12.8.2. Phosphorus (%)

The highest percentage of phosphorus was recorded in T_3 (0.96) and the lowest percentage of phosphorus was recorded in T_2 (0.85). The rest of the treatments were statistically similar in respect to percent phosphorus content (Table 5). The findings of the present study does not match with the study of Chang *et al.* (1981) who reported that the fruiting bodies of *Pleurotus* contained 5.87 to 8.40 mg/g of P on dry weigh of fruiting bodies. This may be due to the system of measurement. But Sarker *et al.* (2007) and Ahmed (2008) found around 0.97% phosphorus, in oyster mushroom grown on sawdust based substrates.

4.12.8.3. Potassium (%)

The highest percentage of potassium was recorded in T_3 (1.44) and the lowest percentage was observed in T_1 (1.32). The rest of the treatments were statistically similar in respect to percent potassium content (Table 5). The findings of the present study confirms by the study of Chang *et al.* (1981) who reported that the fruiting bodies of *Pleurotus* contained 1.432 to 1.88 mg/g of K on dry weigh of fruiting bodies. Sarker *et al.* (2007) and Ahmed (2008) also found about 1.3% potassium, in oyster mushroom grown on sawdust based substrates.

4.12.8.4. Iron (%)

The highest percentage of iron was recorded in T_3 (43.67) and the lowest iron percentage was counted under T_4 (40.00). The rest of the treatments were

statistically different over control in respect to percent iron content (Table 5). The findings of the present study matches with the findings of Alam *et al.* (2007) found 33.45 to 43.2 mg/100g of iron in different oyster mushroom varieties. Sarker *et al.* (2007) found 92.09 ppm to 118.40 ppm iron, in oyster mushroom grown on sawdust based substrates.

4.12.8.5. Manganese (mg)

There was no significant difference among the varieties tested in respect to amount (mg) of Magnesium content (Table 5). The highest amount (mg) of manganese was counted under T_5 (2.80) and the lowest amount was counted under T_1 (2.20) followed by T_2 (2.17). The rest of the treatments were statistically similar. The findings of the present study keep in with the study of Alam *et al.* (2007) found 2.7 to 2.87 mg/100g of manganese in different oyster mushroom varieties. Sarker *et al.* (2007) found 0.21% magnesium, in oyster mushroom grown on sawdust based substrates.

4.12.8.6. Zinc (mg)

The highest amount (mg) of zinc was counted under variety T_5 (14.17) and the lowest amount was counted under T_3 (13.10). The rest of the treatments were statistically similar (Table 5). The result of the present study matches with the study of Alam *et al.* (2007) found 16 to 20.9 mg/100g of zinc in different oyster mushroom varieties. Sarker *et al.* (2007) found 30.92ppm zinc, in oyster mushroom grown on sawdust based substrates.

Table 5. Elemental contents of different popular oyster mushroom (*Pleurotus ostreatus*) as effected by different levels of commercial liquid mineral supplement (wuxal super)

Treatments	N (%)	P (%)	K (%)	Fe (%)	Mn (mg)	Zn (mg)
T_1	2.02e	0.91ab	1.32b	41.00d	2.20c	14.00a
T_2	2.43d	0.85b	1.37ab	41.67c	2.17c	13.87a
T_3	3.14c	0.96a	1.42a	43.67a	2.51b	13.10b
T_4	3.78a	0.91ab	1.38ab	40.00e	2.64b	14.10a
T_5	3.57b	0.94ab	1.41ab	43.00b	2.80a	14.17a
CV (%)	0.54	3.73	0.82	1.51	2.04	1.28
LSD	0.027	0.087	0.087	0.548	0.1501	0.482
Level of significance	**	**	**	**	**	**

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

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

Legend:

T_1: Wuxal Super @ 0% (Control)
T_2: Wuxal Super @ 0.1%
T_3: Wuxal Super @ 0.2%
T_4: Wuxal Super @ 0.3%
T_5: Wuxal Super @ 0.4%

CHEPTER 5

SUMMARY AND CONCLUSION

The present study was carried out at the Biochemistry laboratory and Mushroom Culture House (MCH) of Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, during the month of January to May ’09 to investigate the performance of oyster mushroom growing on liquid mineral supplemented substrates and analysis of their proximate composition.

The lowest time from stimulation to primordia initiation (5.2 days) was observed in T_4 and the highest time from stimulation to primordia initiation (8.2 days) was in T_1 (control). The rest of the treatments were statistically different and varied significantly over control in terms of time from stimulation to primordia initiation (Table 1). The lowest time from primordia initiation to harvest was observed in the T_4 (3.9 days) and the highest time (5.1 days) was required in T_4 followed by T_5 (4.2 days). The other treatments were statistically similar but varied significantly over control in terms of time required from primordia initiation to harvest. The highest number of primordia/packet was observed in the T_2 (66.33) followed by T_5 (61.00) whereas the lowest number of primordia/packet was in the T_3 (56.00). The other treatments were statistically similar but differed significantly in terms of Average no of primordia/packet (Table 1).

The highest number of fruiting body/packet was observed in T_3 (70.00) and the lowest number of fruiting body/packet was recorded in T_1 (55.00). Other treatments were statistically similar but differed significantly over control in terms of number of primordia/packet (Table1). The highest number of effective fruiting body/packet was observed in T_1 (41.00) followed by T_4 (40.00). The lowest number of effective fruiting body/packet was recorded in T_5 (36.33). The other

treatments were statistically similar but differed significantly in terms of number of effective fruiting body/packet (Table 1). The highest weight of individual fruiting body was observed in T_4 (2.25) and the lowest weight of individual fruiting body was in T_1 (1.39) followed by T_2 (1.94). The other treatments were statistically similar (Table 2). The result of the present study revels that waxal super increased the weight of the fruiting body up to a certain concentration and declined there after.

Length of stalk under different treatments showed significant difference. The highest length of stalk was obtained in T_1 (3.71 cm) followed by T_5 (3.65cm) & T_2 (3.61cm) and the lowest length was found in T_3 (3.30cm) followed by T_4 (3.45cm). The highest diameter of stalk was obtained in T_3 (0.9 cm). The lowest diameter of stalk was obtained in T_1 (0.72 cm) followed by T_2 (0.77cm). The rest of the treatments were statistically similar (Table 2). Diameter of pileus under different treatments showed significant difference (Table 2). The highest diameter of pielus was obtained in T_3 (5.60cm). There were no significant differences among the rest of the treatments but the lowest diameter of pileus was obtained in T_1 (5.10cm). The thickness of the pileus in all the treatments showed significant differences although the highest thickness was observed in T_3 (0.48cm) and the lowest was in T_1 (0.40) followed by T_4 (0.42cm). The rest of the treatments were statistically different and varied significantly over control.

The highest biological yield was recorded in the T_4 (129.0 g/packet) which were significantly higher as compared to all the treatments. The lowest biological yield was recorded in T_1 (79.00 g/packet) followed by T_2 (99.0 g/packet). The rest of the treatments were statistically similar (Table 3). The highest economic yield was recorded in T_4 (123.7 g/packet) and the lowest economic yield was under T_1 (71.0 g/packet). The rest of the treatments were statistically different (Table 3). The dry yield of mushroom was maximum in T_4 (12.73 g/packet). The lowest dry yield

was recorded under T_1 (7.07 g/packet). The rest of the treatments were statistically different (Table 3). The highest biological efficiency of 44.48% was calculated in T_4 and the lowest biological efficiency of 27.24% was calculated from T_1 which was followed byT_2 (34.14). The rest of the treatments were statistically similar (Table 3).

The moisture percentage of the fruiting body shows little significant difference. The moisture percent ranged from 89.70% to 90.44%. The highest moisture percent was observed in T_3 (90.44%). The rest of the treatments were statistically similar (Table 4) but the lowest was in T_4 (89.70%). The dry matter percentage of the fruiting body shows no significant differences. The dry matter percent of fruiting body ranged from 9.56% to 10.30% (Table 4). The highest dry matter percent was observed in T_4 (10.30%).

Protein is the most important constituent of food material. All the treatments contain a great amount of protein. The content of protein varied from 12.60%-23.60% (w/w) in the mushroom grown (Table 4). The highest content of protein was found in T_4 (23.60%) and the lowest content of protein was in T_1 (12.60%). The rest of the treatments were statistically different. The highest lipid percentage was counted under T_1 (6.90%). The rest of the treatments were statistically similar in respect to percent lipid content (Table 4). But the lowest lipid percentage was counted under T_3 (5.16%). The highest percentage of ash was observed in T_5 (9.60%) and the lowest percentage of ash was in T_1 (7.70 %). The rest of the treatments are statistically different (Table 4). The highest percentage of carbohydrate was recorded in T_1 (46.07%) and the lowest carbohydrate percentage was recorded in T_4 (31.77%). The rest of the treatments differed significantly over control in respect to percent carbohydrate content (Table 4).

The highest percentage of crud fiber was recorded in T_4 (29.70 %) and the lowest crud fiber percentage was counted under T_2 (27.70 %) followed by T_1 (26.73). The rest of the treatments were statistically similar (Table 4). The highest percentage of nitrogen was recorded in the T_4 (3.78) and the lowest nitrogen percentage was counted under T_1 (2.02). The rest of the treatments were statistically different (Table 5). The highest percentage of phosphorus was recorded in T_3 (0.96) and the lowest percentage of phosphorus was recorded in T_2 (0.85). The rest of the treatments were statistically similar in respect to percent phosphorus content (Table 5).

The highest percentage of potassium was recorded in T_3 (1.44) and the lowest percentage was observed in T_1 (1.32). The rest of the treatments were statistically similar in respect to percent potassium content (Table 5). The highest percentage of iron was recorded in T_3 (43.67) and the lowest iron percentage was counted under T_4 (40.00). The rest of the treatments were statistically different over control in respect to percent iron content (Table 5). There was no significant difference among the varieties tested in respect to amount (mg) of Magnesium content (Table 5). The highest amount (mg) of manganese was counted under T_5 (2.80) and the lowest amount was counted under T_1 (2.20) followed by T_2 (2.17). The rest of the treatments were statistically similar. The highest amount (mg) of zinc was counted under variety T_5 (14.17) and the lowest amount was counted under T_3 (13.10). The rest of the treatments were statistically similar (Table 5).

There fore it can be concluded that Wuxal Super @0.4% with saw dust increases the yield and biological efficiency of oyster mushroom. Therefore farmers can grow mushrooms in this rate of supplementation. But further investigation is needed to justify whether Wuxal Super increases the yield as basal supplement at the time of incorporation with saw dust to prepare spawn packet.

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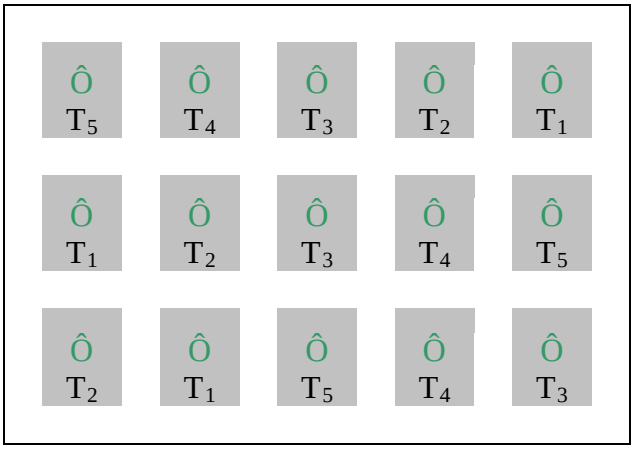
APPENDICES

Appendix 1. Experimental layout



Legend
Ô: Mushroom Packet

Appendix 2. Primordia and fruiting body of produced oyster mushroom

Primordia

Fruiting body

Harvested Fruiting body for taking data

Appendix 3. Analysis of the variance of the data on time required from stimulation to primordia initiation, primordia initiation to harvest, number of primordia /packet, number of fruiting body /packet, number of effective fruiting body/packet, weight of individual fruiting body of produced oyster mushroom supplemented with commercial liquid mineral (wuxal super)

Source	Time required from stimulation to primordia initiation (days)	Time required from primordia initiation to harvest (days)	Number of Primordia /packet	Number fruiting body /packet	Number effective fruiting body/ packet
Replication	0.069	0.029	13.267	1.267	2.600
Treatment	3.543**	0.579**	54.1**	28.1**	12.067**
Error	0.005	0.007	2.350	0.850	1.267

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

Appendix 4. Analysis of the variance of the data on dimension of fruiting bodies of produced oyster mushroom supplemented with commercial liquid mineral (wuxal super)

Source	Weight of individual Fruiting body (g)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Replication	0.010	0.001	0.001	0.018	0.0001
Treatment	0.408**	0.086**	0.012**	0.126**	0.003**
Error	0.004	0.004	0.0001	0.008	0.00012

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

Appendix 5. Analysis of the variance of the data of yield and biological efficiency of produced oyster mushroom supplemented with commercial liquid mineral (wuxal super)

Source	Biological yield (g/packet)	Economic yield (g/packet)	Dry yield (g/packet)	Biological efficiency (%)
Replication	1.867	7.267	0.009	0.222
Treatment	1114.4**	1231.57**	13.75**	132.5**
Error	1.450	0.267	0.023	0.171

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

Appendix 6. Analysis of the variance of the data on chemical composition of different popular oyster mushroom (*Pleurotus* spp.) varieties of produced oyster mushroom supplemented with commercial liquid mineral (wuxal super)

Source	Moisture (%)	Dry Matter (%)	Protein (%)	Lipid (%)	Ash (%)	CHO (%)	Crud fiber (%)
Replication	0.030	0.099	0.593	1.690	0.050	0.008	0.002
Treatment	0.21**	65.47**	1.46**	108.4**	3.53**	1.85**	1.68**
Error	0.018	0.011	0.534	0.476	0.018	0.011	0.000

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

Appendix 7. Analysis of the variance of the data on elemental contents of different popular oyster mushroom (*Pleurotus* spp.) varieties of produced oyster mushroom supplemented with commercial liquid mineral (wuxal super)

Source	N (%)	P (%)	K (%)	Fe (%)	Mn (mg)	Zn (mg)
Replication	0.005	0.001	6.067	0.004	0.021	0.413
Treatment	0.005**	0.005**	6.6**	0.229**	0.561**	1.658**
Error	0.001	0.0001	0.400	0.003	0.031	0.059

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