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HABIBA NASRIN

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EFFECT OF HOT WATER TREATED WHEAT STRAW ON THE GROWTH AND YIELD OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*)

HABIBA NASRIN

DEPARTMENT OF PLANT PATHOLOGY,
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
SHER-E-BANGLA NAGAR, DHAKA-1207

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The Author

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ABSTRACT

An experiment was carried out to find out the effect of hot water treated wheat straw on the growth and yield of oyster mushroom (*Pleurotus ostreatus*). The highest mycelium running rate (0.69 cm/day) was observed on hot water treatment of wheat straw at 80^0C for 3 hours. The duration (15.33 days) required to complete mycelium running in spawn packet, time (5 days) required from stimulation to primordial initiation, time (3.67 days) from primordial initiation to harvest and time (8.67 days) from stimulation to harvest were found the lowest in case of hot water treatment of wheat straw at 80^0C for 3 hours. Average number of primordia (78.33) was maximal in case of hot water treatment at 100^0C for 2 hours. Oyster mushroom grown on wheat straw treated at 80^0C for 1 hours gave the highest average number of fruiting body/packet (62.33). Average number of effective fruiting body/packet (46.67) was higher in case of hot water treatment at 80^0C for 3 hours. The highest average weight of individual fruiting body (3.97) was found at 100^0C for 1 hour. The highest diameter of stalk (0.90 cm) and diameter of pileus (6.67 cm) and thickness of pileus (0.87 cm) were found at 80^0C for 3 hours. The highest average length of stipe (2.9 cm) was found at 80^0C for 2 hours. Oyster mushroom grown on wheat straw treated at 80^0C for 3 hours gave the highest biological yield (180.71 gm), economic yield (175.23 gm), dry yield (18.85 gm), biological efficiency (108.52 %) and cost benefit ratio (4.67). Among the treatments, wheat straw treated at 80^0C for 3 hours may be recommended as an economically effective treatment to grow oyster mushroom (*P. ostreatus*) cultivation in Bangladesh.

CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	**=**	**Full word**
%	=	Per cent

@	=	At the rate
°C	=	Degree Centigrade
Anon.	=	Anonymous
AOAC	=	Association of Official Analytical Chemist
BARI	=	Bangladesh Agricultural Research Institute
BAU	=	Bangladesh Agricultural University
BBS	=	Bangladesh Bureau of Statistics
CV	=	Coefficient of Variance
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
SAU	=	Sher-e-Bangla Agricultural University
T	=	Treatment
t/ha	=	Ton per Hectare
UNDP	=	United Nation Development Program
$^{w}/_{v}$	=	Weight per Volume
$^{w}/_{w}$	=	Weight per Weight
wt.	=	Weight
BCR	=	Benefit cost ratio
BE	=	Biological efficiency
MRR	=	Mycelium Running Rate
NMDEC	=	National Mushroom Development and Extension Center
MCC	=	Mushroom Culture Centre
mg	=	Milligram
Conc.	=	Concentration

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CHAPTER 1

INTRODUCTION

Oyster mushrooms are large reproductive structures of edible fungi belong to the class of Basidiomycetes or Ascomycetes. Approximately 3 lakh varieties of mushroom are identified in the world. Among them those are fully edible and have no toxic effect are to be considered as edible mushroom. Out of 2000 species of prime edible mushrooms about 80 have been grown experimentally, 20 cultivated commercially and 4-5 produced on industrial scale throughout the world (Chang and Miles, 1988). The vegetative part of mushroom consists of thread like long thin mycelium which under suitable condition forms fruiting body or sporocarps. This fruiting body is used as edible mushroom. Mushroom is a highly nutritious, delicious, medicinal and economically potential vegetable (Alam and Saboohi,2001).

As a vegetable, Mushroom can play an important role to meet the nutritional requirements of the population of Bangladesh. A healthy person requires 200-250g vegetable per day (FAO, 1998). But in Bangladesh only 40-50 g vegetable per day is available to people. To get rid of this situation, it is necessary to increase the production of vegetable which need and huge land areas. Mushroom may be used to reduce shortage of vegetable , since it required minimum land area.

Mushroom fungi have low calorie they are cholesterol free and have certain medicinal properties. Mushroom reduces the diabetic on regular feeding (Anderson and Ward, 1979). It also reduces the serum cholesterol in human bodies which reduces hypertension (Suzuki and Oshima, 1979). Mushroom inhibits the growth of tumor and cancer (Yoshioka, 1975; Mori *et al.,* 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 human diet (Holman, 1976). It is rich in essential minerals and trace elements (Chandha and Sharma 1995). Mushroom reduces serum cholesterol and high blood pressure (Mori, 1986).

There are various types of mushrooms such as oyster mushroom, milky white mushroom, shitake mushroom and button mushroom etc. which are cultivated in our country. Among them, some popular species of oyster mushroom such as *Pleurotus* ostreatus, *P. djamor*, *P. florida, P. cystidiosus* and *P. geesteranus* can be cultivated in our country because the weather and climate of the country is suitable for mushroom cultivation. Though the weather and climate of Bangladesh is suitable for year-round oyster mushroom cultivation but the farmers cannot cultivate the mushroom due to lack of autoclave sterilization of substrate. Without sterilization of spawn packets contaminations occur. In the country mushroom spawn is prepared by using sawdust which is sterilized with autoclave sterilization but corn cobs, rice straw, wheat straw, pulse straw, sugarcane baggage, water hyacinth and tea leaves may be used as substrates after sterilizing them with hot water treatment, drum pasteurization or chemical treatment for spawn production.

Substrate plays an important role in the yield and nutrient content of oyster mushroom. The substrates on which mushroom spawn (Merely vegetative seed materials/ Commercial spawn) is grown, affects the mushroom production (Klingman, 1950). Sarker *et al*. (2007a and 2007b) observed a remarkable variation in nutritional content of oyster mushroom in different substrates. The National Mushroom Development and Extension Centre (NAMDEC), Savar, grows oyster mushroom using rice straw with hot water treatment and sawdust with autoclave sterilization. Farmer of Bangladesh cannot produce mushroom spawn due to high cost autoclave sterilization. Therefore, it is necessary to identify the less expensive sterilization technique without autoclave for mushroom production. If low cost method of sterilization is introduced in place of high cost autoclave sterilization, farmers of Bangladesh will be interested to

grow mushroom in Bangladesh. Hot water treatment of substrate may be an effective alternative of autoclaving.

With this view in mind a research project was designed with the following objective:

1. To find out suitable temperature and time for sterilization of wheat straw for oyster mushroom (*Pleurotus ostreatus*).
2. To determine the effect of sterilization temperature and time on growth and yield of oyster.
3. To find out the cost benefit ratio of the wheat straw based spawn packets.

CHAPTER 2

REVIEW OF LITERATURE

Fekadu Alemu (2014) conducted an experiment to evaluate the growth and yield of *Pleurotus ostreatus* on teff straw. As the result was shown, teff straw was the best quality of media (substrate) for production of oyster mushroom. This due to yielding of high number of primordial was formed which resulted in to fruit body (edible part of mushroom). As conclusion, this study was put the baseline information to cultivate mushrooms other edible mushrooms on this substrate as well as on the other substrate used for cultivation.

Dinesh *et. al* (2013) carried out an experiment to investigate the performance of physical and chemical sterilization of substrates such as sawdust, straw. The physical method of sterilization on the substrate straw yields an average of 416g (w/v) product whereas the substrate sawdust yields an average of 360 g (w/v) product. In chemical sterilization method, the substrate straw yields an average of 371g (w/v) product whereas in the substrate sawdust yields an average of 310 g (w/v) product.

Soniya *et. al* (2013) carried out an experiment to investigate the performance of *Pleurotus ostreatus* on different substrates such as rice straw, rice straw + wheat straw, rice straw+ paper, sugarcane bagasse and sawdust of alder was investigated. All the substrates except rice straw were supplemented with 10% rice bran. The substrate without supplement was considered as control. The effects of various substrates on mycelial growth, colonization time, primordial appearance time, mushroom yield, biological efficiency (BE), size of the mushroom and chemical composition were analyzed. Among all aspects, rice straw (control) was found as a best substrate with yield (381.85 gm) and BE (95.46%) %) followed by rice plus wheat straw, rice straw plus paper waste for the production of mushroom. The nutritional composition was also better from mushroom fruit grown on rice straw.

Rahman *et. al* (2012 a) investigated the performance of different levels of wheat bran (0, 10, 20, 30 and 40%) as supplement with rice straw on the growth and yield of *Pleurotus ostreatus*. Among the parameters maximum mycelium growth rate in spawn packet (0.70 cm/day), the highest average number of fruiting body/packet (48.70), the highest average weight of individual fruiting body (4.67g), maximum biological yield (218.86g), economic yield (210.61g), dry yield (23.15g) and the highest biological efficiency (117.85%) were found in rice straw supplemented with 30% wheat bran. The lowest time from stimulation to primordia initiation (6.70 days) and the highest average number of primordia/packet (70.63) were found in rice straw supplemented with 20% wheat bran. The lowest time from primordia initiation to harvest was 3.96 days in rice straw supplemented with 10% wheat bran.

Rahman *et. al* (2012 b) determined the effect of different levels of wheat bran (0, 10, 20, 30 and 40%) as supplement with rice straw on the proximate composition of *Pleurotus ostreatus*. The highest moisture content (91.08%) was observed under 20% wheat bran with rice straw treatment where as the highest dry matter percentage (9.85) was observed in 40% wheat bran with rice straw treatment. The highest amount of protein (27.78%) but the lowest amount of lipid (3.24%) and carbohydrate (39.44%) were observed in 30% wheat bran with rice straw treatment. The highest crude fiber (23.38 %) and ash (8.12%) were observed in 10% wheat bran with rice straw treatment. The highest amount of lipid (5.08%) and carbohydrate (48.45%) were observed in 0% wheat bran with rice straw treatment. The highest percentage of phosphorus (0.986), potassium (1.37) and calcium (26.58 mg/100g) were found in 30% wheat bran with rice straw treatment. The highest amount of iron (44.54 mg/100g) and zinc (15.17 mg/100g) were found in 20% wheat bran with rice straw treatment.

Sonali (2012) carried out an experiment to study the growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis. A high production per surface area can be obtained, after picking the spent substrate is still a good soil conditioner. The Mushrooms are good cash crop. The

development of Oyster mushroom (Grey and pink) production methodologies on agricultural waste like Paddy straw and wheat straw gives very high yield as well as the nutritional contain like carbohydrate, protein, ash, calcium, magnesium, crude fibers and lipid were checked.

Fatema *et. al* (2011) carried out an experiment to study the cultivation of *Pleurotus sajor–cajo* on wheat straw, water hyacinth and their combinations. The results obtained revealed that the best response in the form of pin head appearance and productivity of mushroom came from the bags containing wheat straw only (3.1 kg), followed by the3:1 combination of wheat straw + water hyacinth (2.6 kg), 1:1 combination of wheat straw + water hyacinth (1.9 kg), 1:3 combinations of wheat straw + water hyacinth (1.5 kg) and only water hyacinth (0.77 kg) respectively where it took 16, 20, 25, 30 and 40 days for the appearance of pin heads respectively.

Khan *et. al* (2011) conducted an experiment to investigate different sterilization methods viz., Lab autoclave ,Country style autoclave (2hr), Country style autoclave (1hr), Hot water treatment (1/2hr) and Ordinary water (1/2 hr). Oyster mushroom was cultivated on saw dust, wheat straw and rice husk with different treatments which included, wheat straw 50 % + saw dust 50%, saw dust 100 %,wheat straw 50% + rice husk 50% and rice husk 100%. Among the sterilization methods, the significantly effective method was lab autoclave followed by others. It was observed that the *Pleurotus ostreatus* (P-19) gave the maximum yield in the first flush followed by second, third and fourth flush and lab autoclave was recommended one of the best method for the yield improvement of *Pleurotus spp.*

Nuruddin *et. al* (2010) carried out an experiment to investigate the effect of different levels of cow dung (0, 5, 10, 15 and 20%) on yield and proximate composition of *Pleurotus ostreatus* were studied. The highest number of primordia (70.63) and fruiting body (51.92) per packet were observed in rice straw supplemented with 5% level of cow dung. The highest weight of

individual fruiting body (4.71g), biological yield (234.24g), economic yield (227.72g), dry yield (22.83g) per 500 g packet, biological efficiency (140.26%) and benefit cost ratio (5.69) were observed in 10% cow dung. The highest protein content (30.90%), crude fiber (24.03%) and the lowest lipid (3.34%) was found in 10% cow dung.

Deepika Kumari and Varenyam Achal (2008) conducted an experiment to investigate the effect of five different substrates viz. paddy straw, wheat straw, mixture of paddy and wheat straw (in the ratio of 1 : 1), bamboo leaves and lawn grasses on the production of edible Oyster mushroom (*Pleurotus ostreatus*). Wheat straw and a mixture of paddy and wheat straw gave the earliest colonization of fungus. The highest yield of *P. ostreatus* was recorded on wheat straw (29.27 g fresh weight/kg substrate), followed by the combination of paddy and wheat straw (27.96 g fresh weight/kg substrate). Non-enzymatic antioxidant activities were also obtained by estimating vitamins A, C and E. Significant amount of vitamin E was found in both fresh (7.23 mg/g) and dry fruit body (5.93 mg/g) of *P. ostreatus*. All the experiments were carried out in triplicates.

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

Bhatti *et. al* (2007) conducted an experiment to investigate the growth, development and yield of oyster mushroom, as affected by different spawn rates. The oyster mushroom, *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer was

cultivated on wheat straw in polythene bags (containing 500 g wheat straw on dry weight basis per bag) using sorghum grain spawn at different rates. The spawning was done followed by boiling of substrate and sterilization of bags. The bags were kept in mushroom growing room at 25 to 35^{o}C with 80 to 100% humidity under regular white fluorescent light arranged by the tube lights in mushroom growing room (10'x14'x14').The pinheads first appeared 32.33 days after spawning by using 70 g spawn rate per kg on substrate dry weight basis. The minimum period of 4.66 days after pinhead formation for maturation of fruiting bodies was recorded by using 60, 70, 80, 90 and 100 g spawn rate. The minimum period between flushes (6.33 days) was taken by using 20 g spawn rate. The maximum flushes (4.00) were harvested by using 70 g spawn rate. The maximum number of bunches per bag (7.66) were obtained by using 100 g spawn rate. The maximum number of fruiting bodies per bunch (7.30) was observed by using 70 g spawn rate. The maximum yield on fresh weight basis (45.4%) as well as on dry weight basis (4.63%) was also obtained by using 70 g of spawn rate per bag. The results were highly significant from each other. It is concluded that spawning at 70 g per kg on substrate dry weight basis found to be the best dose for obtaining early and high yielding crop of oyster mushroom, with minimum period for maturation of fruiting bodies, maximum number of flushes and fruiting bodies per bag.

Sarker *et al*. (2007 a) carried out an experiment to find out the performance of different cheap agricultural household by products, grasses and weeds as substrate available in Bangladesh. Mycelium growth rate and time required to complete mycelium running in spawn packet varied significantly in different substrates. The minimum duration to complete mycelium running was 17.75 days in waste paper, which differed significantly from that in all other substrates. Significant variation was found in duration from stimulation to primordial initiation, primordial initiation to first harvest and stimulation to first harvest in different 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).

Sarker *et al.* (2007 b) 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.

Namdev *et al.* (2006) determined 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).

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

Sainos *et al*. (2006) conducted an experiment to determine the mycelial growth, intracellular activity of proteases, laccases and beta -1,3-glucanases and cytoplasmic protein were evaluated in the vegetative phase of *Pleurotus ostreatus* grown on wheat straw and in wheat-grain-based media in Petridishes and in bottles. The productivity of the wheat straw and wheat-grain-based spawn in cylindrical polyethylene bags containing 5 kg of chopped straw was also determined. They observed high activity of proteases and high content of intracellular protein in cultures grown on wheat straw. This suggests that the proteases are not secreted into the medium and that the protein is an important cellular reserve. On the contrary, cultures grown on wheat straw secreted laccases into the medium, which could be induced by this substrate. *Pleurotus ostreatus* grown on media prepared with a combination of wheat straw and wheat grain showed a high radial growth rate in Petridishes and a high level of mycelial growth in bottles. The productivities of wheat straw and wheat-grain-based spawn were similar. Our results show that cheaper and more productive mushroom spawn can be prepared by developing the mycelium on wheat straw and wheat-grain-based substrates.

Zape *et al*. (2006) conducted a study to determine the spawn run, days taken to pin head initiation, yield and biological efficiency of three oyster mushroom species viz. *Pleurotus florida, P. eous* and *P. flabellatus* were grown on wheat straw substrate. Time required for spawn run and pinning was significantly less in *Pleurotus eous* followed by *P. florida*. However, the yield and biological efficiency did not differ significantly but was higher in *P. florida* than *P. flabellatus* and *P. eous*. 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.*

Ancona-Mendex *et al.* (2005) conducted an experiment to grow oyster mushroom (*Pleurotus ostreatus* (Jacq.: Fr.) Kummer 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.

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.

Iqbal *et. al* (2005) conducted an experiment to find out the growth and yield performance of oyster mushroom, *Pleurotus ostreatus* (local & exotic strains) and *P. sajarcaju* on different substrates. Results regarding the time required for completion of spawn running, formation of pin-heads and maturation of fruiting bodies on different substrates showed that in all the three cases, they appeared earlier on sugarcane bagasse followed by cotton waste and the maximum number of flushes were obtained from wheat straw and banana leaves followed by cotton boll locules and cotton waste. Furthermore, the results revealed that the minimum flush to flush interval was obtained on millet followed by wheat straw and sugarcane leaves and the maximum yield percentage on fresh and dry weight basis was obtained from banana leaves followed by paddy and wheat straw.

Khlood and Ahmad (2005) conducted an experiment to study the ability of oyster mushroom (*Pleurotus ostreatus*) P015 strain to grow on live cake mixed with wheat straw. The treatments comprised : 90% straw + 5% wheat bran + 5% gypsum (control); 80% straw + 10% olive cake + 5% wheat bran + 5% gypsum (T1); 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/500 g dry substrate), average weight (21.5 g/cap) and average cap diameter (7.05 cm/cap) and BE% (80%). 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.

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.

Banik and Nandi (2004) carried out an experiment on oyster mushroom for its ease of cultivation, high yield potential as well as its high nutritional value. Laboratory experimentation followed by farm trial with a typical oyster mushroom *Pleurotus sajor- caju* revealed that the yield potential of these mushrooms can be increased significantly when grown on a lignocellulosic crop residue - rice straw supplemented with biogas residual slurry manure in 1:1 ratio as substrate. Residual slurry manures obtained from biogas plants utilising either cattle dung or poultry litter, jute caddis or municipal solid waste as substrates for biogas production were all effective in increasing the yield of *Pleurotus sajor-caju* significantly although to different extents. Disinfection of straw and manure by means of 0.1 % KMnO< 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.

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

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

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^{o} C for spawn running and 17-20^{o} C 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).

Therefore, sawdust is recommended as the best substrate for Oyster mushroom cultivation.

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.

Dhoke *et al.* (2001) studied the effect of different agro-wastes on cropping period and yield of *Pleurotus sajor-caju*. Various plant materials, i.e. soybean, paddy, cotton, wheat and jowar (*Sorghum bicolor*) were used. Cropping period on different substrates was recorded for first, second and third picking. The cropping period for third picking varied from 42.25 to 43.50 days in different substrates. The days required for first picking indicated that soybean straw took 22.00 days to produce first crop of harvestable mushroom while a minimum of 21.25 days were required for paddy and wheat straw. For second picking, jowar and cotton waste took the maximum days of 32.75 days while soybean took the minimum of 31.50days. The final and third picking was completed in 43.50 days in case of soybean straw which was statistically higher compared to paddy and wheat straw (42.25) and cotton and jowar straw (42.75). The highest yield of 993.00 g/kg was obtained from cotton, followed by soybean straw (935.25 g/kg) and paddy straw (816.0 g/kg). The lowest yield of 445.50 g/kg was recorded in jowar straw.

Ayyappan *et al.* (2000) 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.

Upamanya and Rathaiah (2000) conducted an experiment to test the effect of fortification of rice straw with rice bran on the yield and quality of oyster mushroom (*Plelrrotus ostrentus*) in Jorhat, Assam, India. Treatments comprised: (i) addition of rice bran at 5% w/w (weight of rice bran/weight of dry substrate) at the time of spawning and (ii) control (without rice bran). Rice straw fortified with rice bran exhibited a higher yield compared to the control. Rice bran application had no effect on the crude protein content of mushroom but increased the yield by 44% over the control.

Patil and Jadhav (1999) reported that *Pleurotus sajor-caju* was cultivated on cotton, wheat, paddy, sorghum and soyabean straws. 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.

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

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

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 protein content of mushrooms produced was 27.2% on an 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.

Biswas *et al.* (1997) reported that methods including spawning percentage, combinations of paddy straw, wheat straw and supplements, to improve the biological efficiency (BE) of *P. florida* were investigated in Madhya Pradesh, India. Increasing spawning rates reduced the time required for spawn runs. The highest BEs (66.8-101.25%) was observed after the use of the highest spawning percentages. A 1:1 mixture of paddy straw wheat straw promoted a high BE (106.5%); supplementation of this substrate with 5% rice flour also promoted BE (125.75%).

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

Mathew *et al.* (1996) investigated that *Pleurotus sajor-caju*, *Pleurotus citrinopileatus*, *Pleurotus florida*, *Pleurotus platypus* and *Pleurotus ostreatus*

were evaluated for their yield performance on various substrates, both for spawn production and cultivation, in the plains and in the high ranges of Kerala in studies conducted in the summer and rainy seasons. Sorghum, wheat and paddy grains were equally good for spawn production. *Pleurotus sajor-caju*, *Pleurotus citrinopileatus* and *Pleurotus florida* were the most suitable species for cultivation in both the plains and the high ranges. These 3 species were successfully cultivated on paddy straw, *Eliocharis plantogena* [*Eleocharis plantaginea*] and rubber wood [Hevea] sawdust, although for commercial cultivation of *Pleurotus sajor-caju*, rubber wood sawdust was not rated as an ideal medium.

Isik *et al.* (1995) 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 gypsum and 1000 kg of horse manure, 40 kg of chicken food or malt sprout, 7.5 kg of urea, 35 kg of gypsum. 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 and 1000 kg of wheat straw, 65 kg of cotton seed meal or 100 kg of chicken food, 25 kg of urea, 40 kg of molasses and 0 kg of gypsum.

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

(*Acacia arabica*) and Poplar (*Populus alba*) amended with wheat bran and lime were used for spawn preparation.

Badshah *et al.* (1994) mentioned that *Pleurotus ostreatus* and *P. florida* were grown on wheat straw, sugarcane bagasse, corn cobs or sawdust, by mixing 120-130 g of spawn with 2 kg of substrate and placing the mixture in sterilized polyethylene bags which were kept in the dark at 25°C for 2-3 weeks. Once the bags became full of mycelial growth, they were removed, leaving the substrate uncovered. Watering was carried out 2-3 times a day. Fruiting bodies were harvested at maturity. *P. ostreatus* and *P. florida* yields ranged from 49.8 and 277.7 g/2 kg substrate respectively on sawdust, to 432.8 and 420.5 g/ 2 kg substrate respectively, on wheat straw. Controls (grown in the field) yielded only 18.5 and 28.5 g/2 kg substrate for *P. ostreatus* and *P, florida*, respectively. In both species, wheat straw and sugarcane bagasse substrates resulted in the highest mushroom ascorbic acid contents and protein, fat and fiber contents were also affected by substrate. *Pleurotus florida* had higher fat but lower protein contents than *P. ostreatus*.

Dhanda *et al.* (1994) conducted an experiment on the use of fermented, semi-fermented and unfermented paddy straw as substrate for *Pleurotus spp*. (oyster mushroom). PAU-4 strain showed early primordia initiation, giving 60% biological efficiency whereas PAU-3 exhibited these effects much earlier with 70% biological efficiency.

Ijaz and Khan (1992) reported that mushroom has been recently introduced in Pakistan. Different species/strains i.e. *Pleurotus sajor-caju*., *P. ostreatus* strain XI, *P. ostreatus* strain 467 and *P. ostreatus* were cultivated on cotton waste. *P. ostreatus* strain XI gave higher (260 g) basidiocarps out of 750 g of substrates per flush. It had 104 percent biological efficiency and 49 percent sustenance potential. In the same manner cotton waste scored maximum yield, biological efficiency and sustenance potential by defeating paddy straw + 25 percent synthetic compost, paddy straw and wheat straw in descending order.

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.

Patil (1989) cultivated *P. sajor-caju* on six different substrates, i.e. wheat straw, bajra (*Pennisetunz americana*), maize straw, paddy straw, jower and cotton stick. The results indicated that all the substrates could be used for commercial cultivation of the oyster mushroom.

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

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

Ramesh and Ansari (1987) evaluated several locally available substrates such as rice straw, banana leaves, saw dust, oil palm refuse, oil palm bunch refuse or grass straw to study conversion efficiency of *Pleurotus sajor-caju*. Rice straw and banana leaves were best substrates, with more than 60% conversion efficiency on dry weight basis. The mean weight of the fruiting body was high (7.1 g) on banana leaves compared to other substrates (2.1-5.0 g). The spawn running time was also less with banana leaves, followed by rice straw, grass

straw, oil palm bunch refuse, sawdust and oil palm waste. *Pleurotus ostreatus* was successfully grown under local conditions utilizing chopped wheat straw, cotton waste, maize cobs or rice straw as bedding material. Wheat straw and cotton waste gave the highest yields with the shortest incubation period; fruiting bodies were appeared after 15-18 days as compared to 4-5 weeks on the other substrates. The first flush gave the highest yield in all treatments and was a gradual decline in the yield of successive flushes.

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

CHAPTER 3

MATERIALS AND METHODS

3.1 Location of experiment

The experiment was carried out at Mushroom Culture House (MCH) of Sher-e-Bangla Agricultural University, Dhaka, during the period from January to June 2013. Maximum and minimum temperature, relative humidity and rainfall during the study period are shown in appendix I.

3.2 Experimental materials

Mother culture of oyster mushroom was collected from National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka. Wheat straw was collected from northern zone of Bangladesh.

3.3 Varietal characteristics of Oyster Mushroom

Oyster mushrooms (*Pleurotus* spp) are 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 different colors viz. white, cream, pink, grey, yellow, light brown etc. If the temperature increases above 32°C, its production markedly decreases.

3.4 Design and layout of the experiment

The experiment was laid out in a Completely Randomized Design (CRD). The experiment considered ten treatments with three replications and three spawn packets in each replication (Appendix II). Wheat straw was sterilized in hot water at 60^0C, 80^0C and100^0C temperature. Each temperature regime was maintained for 1hour, 2 hours and 3 hours.

Treatments:

T_0: control

T_1: Hot water treatment at 60^0C for 1hr

T_2: Hot water treatment at 60^0C for 2hr

T_3: Hot water treatment at 60^0C for 3hr

T_4: Hot water treatment at 80^0C for 1hr

T_5: Hot water treatment at 80^0C for 2 hr

T_6: Hot water treatment at 80^0C for 3 hr

T_7: Hot water treatment at 100^0C for 1 hr

T_8: Hot water treatment at 100^0C for 2 hr

T_9: Hot water treatment at 100^0C for 3 hr

3.5 Preparation and sterilization of substrates

At first dry wheat straw was taken and crushed into small pieces. Then the crushed wheat straw was filled into net bag. The weight of each filled net bag was 20 Kg. Then the straw was soaked in water in a drum and temperature and time were maintained according to the desired treatments. Therefore the straw was taken off from hot water and left on a perforated sieve for removing the excess water for few hours. Then $CaCO_3$ were added with wheat straw substrate @ 1% on dry weight basis. 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%.

3.5.1 Preparation and inoculation of spawn packets

The mixed substrates were filled into 7×11 inch polypropylene bag @ 400 g with 50 g mother spawn into three layers in each bag. The filled polypropylene bags were prepared by using plastic neck and plugged the neck with cotton and covered with brown paper placing rubber band to hold it tightly in place.

3.5.2 Mycelium running in spawn packets

The spawn packets were kept at 20-22°C temperature until the packets become white with the mushroom mycelium. After completion of the mycelium running the rubber band, brown paper, cotton plug and 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.

Plate 1: Mycelium running in spawn packet

Plate 2: Mycelium running complete in spawn packet

3.5.3 Cultivation of spawn packets

Two ends, opposite to each other of the upper position of plastic bag were cut in "D" shape with a blade and opened by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a tea spoon for removing the thin whitish mycelial layer. Then the spawn packets were soaked in water for 15 minutes and invested to remove excess water for another 15 minutes. The packets of each type were placed separately on the floor of culture room and covered with newspaper. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3 times a day. The light around 300-500 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22°C to 25°C. The first primordia

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

Plate 3: Pin head primordia in the spawn packet

Plate 4: Matured fruiting body in the spawn packet

3.5.4 Collection of produced mushrooms

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

3.6 Data collection

3.6.1 Mycelial growth (%):

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

3.6.2 Mycelium running rate (cm/day):

Mycelium running rate (MRR) for each type of substrate was measured after the mycelium colony cross the shoulder of the packet. The linear length was measured at different places of packet using the following formula (Sarker, 2004):

$$\text{MRR} = \frac{L}{N} \text{ cm/day}$$

Where, L= Average length of mycelium running for different places (cm)

N= Number of days

3.6.3 Days required for completing mycelium running:

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

3.6.4 Average number of fruiting body per packet:

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

3.6.5 Average weight of individual fruiting body per packet:

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

3.6.6 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)

3.6.7 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.

3.6.8 Economic yield:

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

3.6.9 Drying of mushrooms:

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

3.6.10 Dry yield:

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

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

3.6.11 Biological efficiency:

Biological efficiency was determined by the following formula:

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

3.6.12 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).

Benefit cost ratio=Profit from production /Cost for production

3.6.13 Cultural operations for subsequent flushes:

After completing the first harvest again the packets were scraped at the place where the ‘D’ shaped cut had been done and were soaked in a bucket for five minutes and then placed in the culture house and water was sprayed regularly. The primordia appeared 9-10 days after first harvest and 7-8 days after second harvest. Water spraying was continued until the mushrooms were ready to be harvested.

3.7 Statistical analysis of data

All the data collected on different parameters were statistically analyzed by following the analysis of variance (ANOVA) technique and mean differences were adjusted by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) using the MSTAT-C computer package program. The mean differences among the treatments were compared by least significant difference (LSD) test at 5% level of significance.

CHAPTER 4

RESULTS

4.1.1 Mycelium running rate (cm/day)

Mycelium running rate per day (MRR) for each type of treatment was measured after the mycelium colony crossed the shoulder of the packet. Mycelium running rate in spawn packet was found to be different due to different time and temperature used. The highest running rate was observed in T_6 (0.69 cm/day) and the lowest running rate of mycelium (0.45 cm/day) was observed in T_0 (Table 1 and Appendix III). Treatment T_6 performed 53.33 % higher mycelium running rate over control treatment (Figure 4.1).

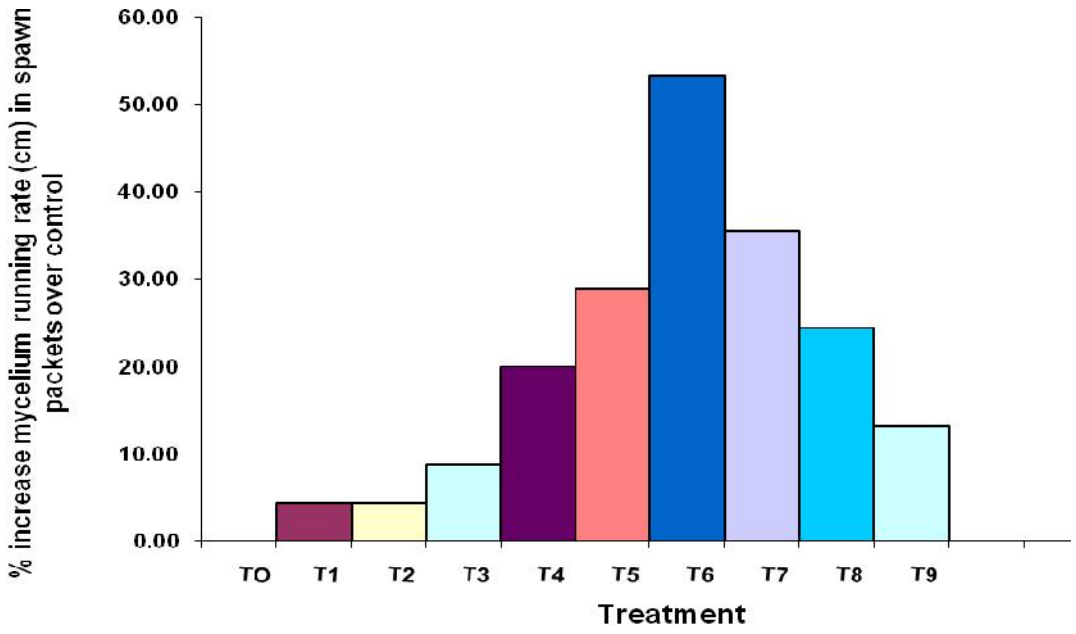

Figure 1. Effect of hot water treatment of wheat straw on percent increase mycelium running rate in spawn packets (cm)of oyster mushroom (*Pleurotus ostreatus*)

4.1.2 Days required to complete mycelium running in spawn packets

Days required completing mycelium running in spawn packet ranged 15.33 to 23.67 days on different time and temperature (Table 1). Significantly the lowest days to complete mycelium running was recorded on T_6 (15.33). Days for completing the mycelium running on other treatments were statistically similar

and significantly lower as compared to control. Maximum days required to complete mycelium running was on T_0 (23.67) which was statistically insignificant with T_1 (22.67) and T_2 (22.67).

4.1.3 Time from stimulation to primordial initiation (Days)

Hot water treatment of wheat straw showed great effect on time from stimulation to primordial initiation. The lowest time (5 days) required from stimulation to primordial initiation was observed in the treatment T_6 where wheat straw was treated at 80^{o}C for 3 hours and the highest time (8.33 days) from stimulation to primordial initiation was observed in the treatment T_0 (control) that was statiscally similar with T_1 (8.0 days) (Table 1 and Appendix III).

4.1.4 Time from primordial initiation to harvest (days)

The lowest time (3.67 days) from primordial initiation to harvest was recorded in the treatment T_6 and the highest time (6.67 days) from primordial initiation to harvest was observed in the treatment T_0 followed by T_1 (6.33 days) (Table 1 and Appendix III).

4.1.5 Time from stimulation to harvest (days)

The highest time (14.67 days) from stimulation to harvest was observed in the treatment T_0 & T_1 and the lowest time (8.67days) from stimulation to harvest was in the treatment T_6. The other treatments differed significantly from control (Table 1).

Table 1. Effect of hot water treated wheat straw on the mycelium growth and primordia initiation of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Mycelium running rate in spawn packets (cm)	Days required to complete mycelium running	Time from stimulation to primordial initiation (days)	Time from primordia initiation to harvest (days)	Time from stimulation to harvest (days)
T_O	0.45 g	23.67 a	8.33 a	6.67 a	14.67 a
T_1	0.47 fg	22.67 ab	8.0 a	6.33 ab	14.67 a
T_2	0.47 fg	22.67 ab	7.67 ab	5.67 bc	13.33 b
T_3	0.49 efg	21.33 bc	7.0 bc	5.33 cd	12.33 bc
T_4	0.54 cde	19.67 de	7.0 bc	5.33 cd	12.33 bc
T_5	0.58 bc	18.33 ef	6.0 d	4.33 ef	10.33 d
T_6	0.69 a	15.33 g	5.0 e	3.67 f	8.67 e
T_7	0.61 b	17.33 f	6.33 cd	4.33 ef	10.67 d
T_8	0.56 bcd	18.67 ef	6.67 cd	4.67 de	11.33 cd
T_9	0.51 def	20.67 cd	7.0 bc	5.0 cde	12.00 c
LSD (0.05)	0.05425	1.469	0.7298	0.7155	1.064
CV (%)	4.74	4.27	6.17	8.13	5.16

In a column the figures having a common letter(s) do not differ significantly.

Treatments:

T_0: Control

T_1: Hot water treatment at 60^0C for 1hour

T_2: Hot water treatment at 60^0C for 2 hours

T_3: Hot water treatment at 60^0C for 3 hours

T_4: Hot water treatment at 80^0C for 1 hour

T_5: Hot water treatment at 80^0C for 2 hours

T_6: Hot water treatment at 80^0C for 3 hours

T_7: Hot water treatment at 100^0C for 1hour

T_8: Hot water treatment at 100^0C for 2 hours

T_9: Hot water treatment at 100^0C for 3 hours

4.2.1 Average number of primordia/packet

The lowest average number of primordia/packet was observed from the treatment T_0 (67.67) and the highest average number of primordia /packet was

found in the treatment T_8 (78.33) which was statistically similar T_2 (77.67), T_9 (77.67), T_6 (77.33) and T_7 (75.33) (Table 2 and Appendix IV).

4. 2.2 Average number of fruiting body/packet

The highest average number (62.33) of fruiting body/packet was observed in the treatment T_4 and the lowest average number (47.67) of fruiting body/packet was in the treatment T_0 (Table 2 and Appendix IV). Treatment T_4 produced 30.75 % higher average number of fruiting body/packet over control treatment (Figure 4.2).

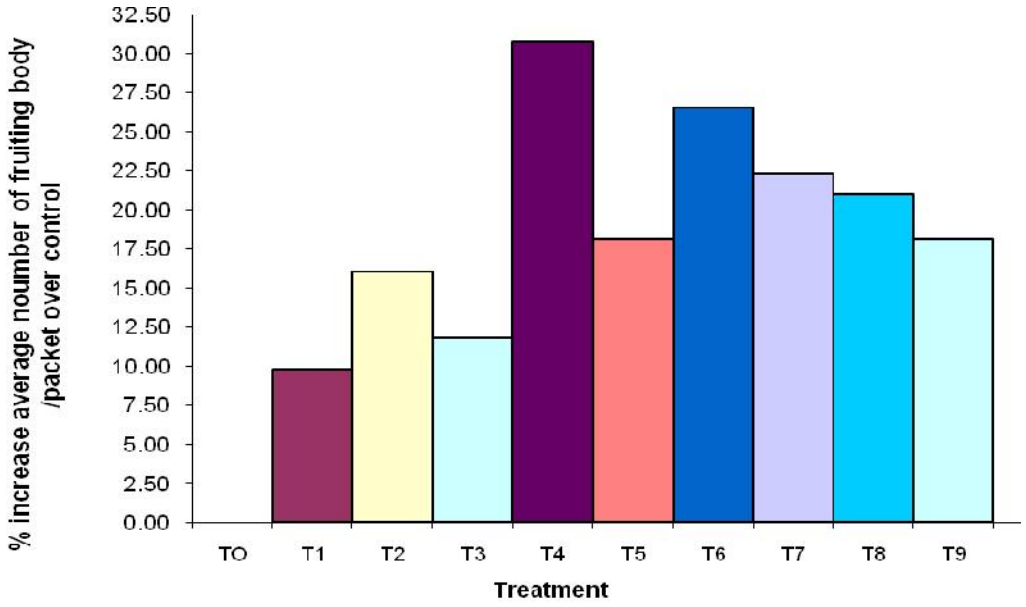

Figure 2. Effect of hot water treatment of wheat straw on percent increase average number of fruiting body /packet of oyster mushroom (*Pleurotus ostreatus*)

4. 2.3 Average number of effective fruiting body/packet

The highest average number (46.67) of effective fruiting body/packet was observed in the treatment T_6 followed by T_4 (44.67) and the lowest average number (35.33) of effective fruiting body/packet was in the treatment T_0 (35.33).

The other treatments differed significantly in terms of average number of primordia/packet (Table 2 and Appendix IV).

4.2.4 Average weight of individual fruiting body (g)

The highest average weight (3.97) of individual fruiting body was observed in the treatment T_7 which was followed by treatment T_6 (3.88) and the lowest average weight of individual fruiting body was found in the treatment T_0 (2.31).The other treatments differed significantly in terms of average weight of individual fruiting body (Table 2 and Appendix IV).

Table 2. Effect of hot water treated wheat straw on the primordia and fruiting body of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Average no of primordia /packet	Average no of fruiting body /packet	Average no of effective fruiting body/packet	Average wt of individual fruiting body (g)

T_O	67.67 c	47.67 d	35.33 g	2.31 d
T_1	70.00 bc	52.33 cd	38.00 f	3.70 bc
T_2	77.67 a	55.33 bc	39.67 ef	3.75 bc
T_3	73.33 abc	53.33 cd	40.67 de	3.84 ab
T_4	70.33 bc	62.33 a	44.67 ab	3.61 c
T_5	72.33 abc	56.33 abc	44.00 bc	3.85 ab
T_6	77.33 a	60.33 ab	46.67 a	3.88 ab
T_7	75.33 ab	58.33 abc	43.00 bc	3.98 a
T_8	78.33 a	57.67 abc	44.00 bc	3.83 ab
T_9	77.67 a	56.33 abc	42.33 cd	3.85 ab
LSD (0.05)	6.502	6.679	2.145	0.1879
CV (%)	5.12	6.95	2.99	3.03

In a column the figures having a common letter(s) do not differ significantly.

Treatments:

T_0: Control

T_1: Hot water treatment at 60^0C for 1hour

T_2: Hot water treatment at 60^0C for 2 hours

T_3: Hot water treatment at 60^0C for 3 hours

T_4: Hot water treatment at 80^0C for 1 hour

T_5: Hot water treatment at 80^0C for 2 hours

T_6: Hot water treatment at 80^0C for 3 hours

T_7: Hot water treatment at 100^0C for 1hour

T_8: Hot water treatment at 100^0C for 2 hours

T_9: Hot water treatment at 100^0C for 3 hours

4.3.1 Diameter of pileus

The highest average diameter of pileus was observed in the treatment T_6 (6.67 cm) followed by T_7 (6.60 cm) and the lowest average diameter of pileus was in the treatment T_0 (5.50 cm). The other treatments differed significantly in terms of average diameter of pileus (Table 3 and Appendix V).

4.3.2 Thickness of pileus

The highest average thickness of pileus was observed in the treatment T_6 (0.87 cm) and the lowest average thickness of pileus was in the treatment T_0 (0.70 cm). The other treatments differed significantly in terms of average thickness of pileus (Table 3 and Appendix V).

4.3.3 Length of stipe

The highest average length of stipe was observed in the treatment T_5 (2.89 cm) and the lowest average length of stipe was in the treatment T_0 (2.70 cm). There was no statistical significant difference among the treatments in terms of average length of stipe (Table 3 and Appendix V).

4.3.4 Diameter of stipe

The highest average diameter of stipe was observed in the treatment T_6 (0.90 cm) and the lowest average diameter of stipe was in the treatment T_0 (0.76 cm). The other treatments differed significantly in terms of average diameter of stipe (Table 3 and Appendix V).

Table 3. Effect of hot water treated wheat straw on the dimension of fruiting body of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Diameter of pileus (cm)	Thickness of pileus (cm)	Length of stipe (cm)	Diameter of stipe (cm)
T_O	5.50 e	0.70 d	2.70	0.76 g
T_1	5.73 de	0.72 cd	2.75	0.81 f

T_2	5.77 de	0.75 c	2.69	0.84 e
T_3	6.03 cd	0.77 bc	2.80	0.86 de
T_4	6.17 c	0.81 ab	2.81	0.85 e
T_5	6.50 ab	0.84 a	2.89	0.87 cd
T_6	6.67 a	0.87 a	2.87	0.90 a
T_7	6.60 ab	0.84 a	2.87	0.88 abc
T_8	6.30 bc	0.83 a	2.90	0.89 ab
T_9	6.03 cd	0.83 a	2.83	0.87 bcd
LSD (0.05)	0.3116	0.05425	0.1627	0.01715
CV (%)	2.96	3.03	3.41	0.51

In a column the figures having a common letter(s) do not differ significantly.

Treatments:

T_0: Control

T_1: Hot water treatment at 60^0C for 1hour

T_2: Hot water treatment at 60^0C for 2 hours

T_3: Hot water treatment at 60^0C for 3 hours

T_4: Hot water treatment at 80^0C for 1 hour

T_5: Hot water treatment at 80^0C for 2 hours

T_6: Hot water treatment at 80^0C for 3 hours

T_7: Hot water treatment at 100^0C for 1hour

T_8: Hot water treatment at 100^0C for 2 hours

T_9: Hot water treatment at 100^0C for 3 hours

4.4.1 Biological yield (g)

The highest biological yield was recorded from the treatment T_6 (180.71 g) and the lowest biological yield was recorded from T_0 (81.72 g). The rest of the treatments differed significant as compared to control (Table 4 and Appendix VI).

4.4.2 Economic yield (g)

The highest economic yield was recorded from the treatment T_6 (175.23 g) and the lowest economic yield was recorded from T_0 (76.31 g). The economic yield of the rest of the treatments differed statistically compared to control (Table 3 and Appendix VI). Treatment T_6 produced 129.63 % higher economic yield from control treatment (Figure 4.3).

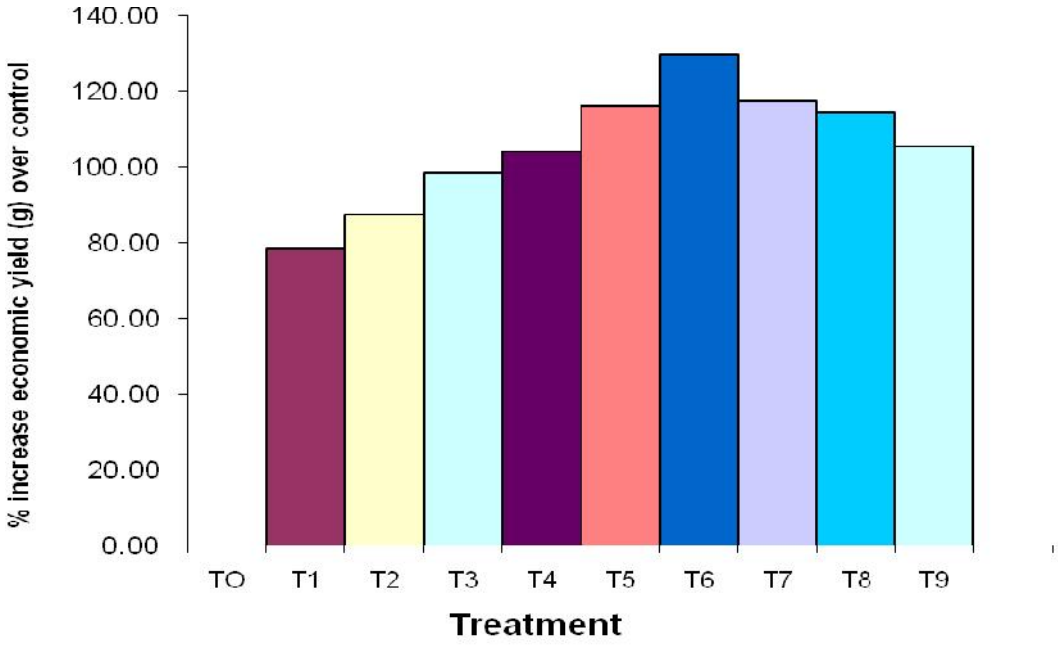

Figure 3. Effect of hot water treatment of wheat straw on percent increase economic yield (g) of oyster mushroom (*Pleurotus ostreatus*)

4.4.3 Dry yield (g)

The maximum dry yield of mushroom was recorded from the treatment T_6 (18.85 g). The lowest dry yield was recorded from T_0 (8.13 g). The other treatments differed statistically compared to control (Table 4 and Appendix VI).

4.4.4 Biological efficiency

The highest biological efficiency 108.52 % was observed in the treatment T_6 and the lowest biological efficiency 49.05 % was observed from T_2. The rest of the treatments varied significantly from control (Table 4 and Appendix VI). Treatment T_6 produced 121.24 % higher biological efficiency from control treatment (Figure 4.4).

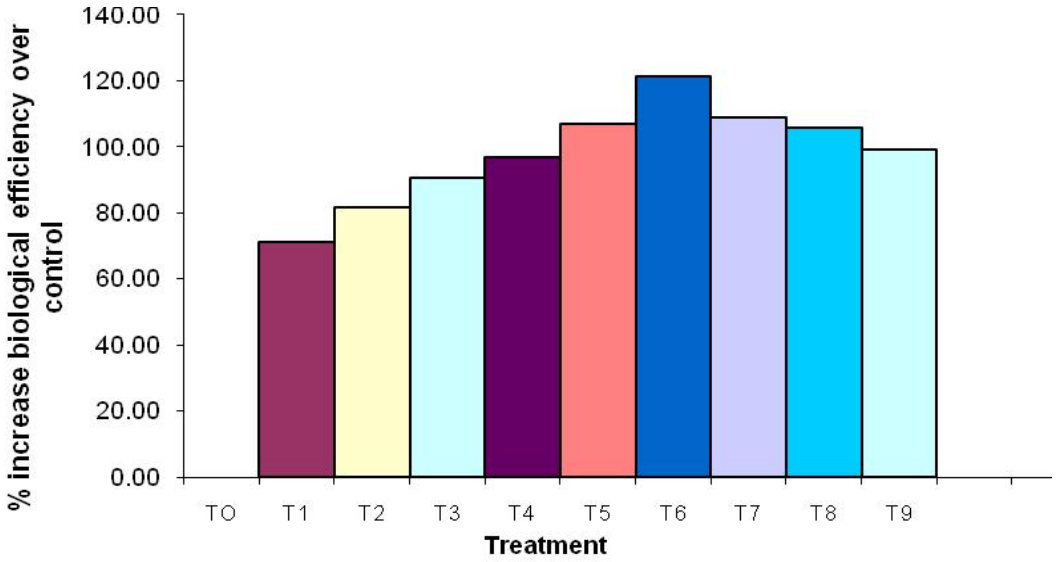

Figure 4. Effect of hot water treatment of wheat straw on percent increase biological efficiency of oyster mushroom (*Pleurotus ostreatus*)

4.4.5 Benefit cost ratio

The highest benefit cost ratio 4.67 was calculated from the treatment T_6 and the lowest benefit cost ratio 2.03 was calculated from T_0 treatment. The rest of the treatments varied significantly from control (Table 4 and Appendix VI).

Table 4. Effect of hot water treated wheat straw on the yield, biological efficiency and benefit cost ratio of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)	Benefit cost ratio
T_O	81.72 g	76.31 h	8.133 g	49.05 g	2.03 h
T_1	140.0 f	136.21 g	14.00 f	84.04 f	3.633 g
T_2	148.41 ef	142.92 fg	14.58 ef	89.11 ef	3.81 fg
T_3	155.83 de	151.51 ef	15.57 de	93.51 de	4.04 ef
T_4	160.81 cd	155.62 de	16.05 d	96.51 cd	4.15 de

T_5	169.23 bc	164.71 bc	17.27 bc	101.50 bc	4.39 bc
T_6	180.71 a	175.23 a	18.85 a	108.52 a	4.67 a
T_7	170.72 b	165.91 b	17.32 b	102.51 b	4.42 b
T_8	168.31 bc	163.42 bcd	17.18 bc	101.0 bc	4.36 bcd
T_9	162.72 bcd	156.81 cde	16.26 cd	97.63 bcd	4.18 cde
LSD (0.05)	8.453	8.856	1.035	5.076	0.2365
CV (%)	3.20	3.47	3.89	3.20	3.44

In a column the figures having a common letter(s) do not differ significantly.

Treatments:

T_0: Control

T_1: Hot water treatment at 60^0C for 1hour

T_2: Hot water treatment at 60^0C for 2 hours

T_3: Hot water treatment at 60^0C for 3 hours

T_4: Hot water treatment at 80^0C for 1 hour

T_5: Hot water treatment at 80^0C for 2 hours

T_6: Hot water treatment at 80^0C for 3 hours

T_7: Hot water treatment at 100^0C for 1hour

T_8: Hot water treatment at 100^0C for 2 hours

T_9: Hot water treatment at 100^0C for 3 hours

CHAPTER 5

DISCUSSION

The oyster mushroom, *Pleurotus ostreatus*, is cultivated worldwide. It is one of the most appreciated mushrooms due to its high nutritional value. Immersion of the substrate in hot water is one of the most popular and worldwide sterilization technique used for mushroom farmers. It is cheap and easy to implement.

The effect of hot water treatment of wheat straw showed significant effect on mycelium running rate of oyster mushroom that reduced the required days to

complete mycelium running in the spawn packet compared to the control treatment. During this study it was observed that completion time of mycelium running in spawn packet 15.33 to 23.67 days and fruiting body formation 8.67 to 14.67 days was required for different time and temperature. Among the treatment lowest time was 15.33 days for completion of mycelium running and 8.67 days for fruiting body formation which was found at 80^0C for 3 hours. Similar result was previously reported by Shah *et al*. (2004), where spawn running took 2 weeks for its completion on saw dust. Khan, (2009) reported that Pleurotus ostreatus took 24-25 days for completion of 100% spawn running on wheat straw. These findings was practically supported by Muhammad Hussain *et al*. (2001) who stated that 17 to 18 days required for full spawn running, 23 to 24 days for pinhead formation, and 26 to 27 days for fruiting body formation in autoclave sterilization for 1 hour. The duration of mycelium running was 2 weeks where paddy straw was sterilized at 100^0C for 1 hour (Diana *et al*., 2006). On the other hand findings of this study partially supported by Siddhant *et al*. (2014) who found that spawn run and primodial development 14 daysand 15 days.They obtained 340g yield from three flushes
and biological efficiency was 68% where wheat grain was treated in boiling water for 1 hour.

Khan *et al*. (2011) used wheat straw 50%+ saw dust 50% that was sterilized at 100^0 C for half an hour and observed 17.5 days required for spawn running and 22days required for development of fruiting body.

It was observed that hot water treatment at 80 ^{0}C for 3 hours produced higher yield as well as higher biological efficiency. The optimum efficiency was 108.52% and yield 175.23g. Partially similar result was found by Oseni *et al*. (2012) where they obtained yield 301.1 g and bio-efficiency 60.22% in oyster mushroom cultivation when sugarcane baggag were sterilized at 60^0C for 2 hours. Hernández et al. (2003) developed an alternative method of sterilization where stacking the substrate for 2 to 3 days to allow for self-heating without an

external energy source. The highest biological efficiency 112 and 62% was recorded for self-heating and alkaline immersion. (Barrios&Espinoza *et al.*2009).Chohan *et al*. (2001) found that the highest yields 772.5 g from cotton straw which were treated with formalin 750 ppm + Bavistin 75 ppm and one hour laboratory autoclaving sterilization. This findings was similar with Caral Dinesh *et al*.(2013) used two substrate and sterilization was done by hot water treatment and chemical treatment where they found wheat straw was the best compared the chemical sterilization. They obtained 460g and 360g yield from wheat straw and saw dust that sterilized by hot water respectively. Dey (2006) recorded 10-360g yield and 2-72% biological efficiency from three flushes.

The findings of the present study clearly indicated that wheat straw sterilization with hot water at 80^{o}C for 3 hours gave the best performance regarding all the parameter studied. It could be used as method of sterilization of oyster mushroom growing substrate.

CHAPTER 6

SUMMARY

The present study was conducted with ten treatments and each treatment with three replications to investigate the effect of hot water treated wheat straw on the growth and yield of oyster mushroom (*Pleurotus ostreatus*).

The effect of hot water treatment of wheat straw showed significant effect on mycelium running rate of oyster mushroom that reduced the required days to complete mycelium running in the spawn packet compared to control treatment. The highest mycelium running rate (0.69 cm) was observed in hot water treatment of wheat straw at 80^0C for 3 hours. The lowest days (15.33) required to complete mycelium running in spawn packet, the lowest time (5 days) required from stimulation to primordial initiation, the lowest time (3.67 days) from primordial initiation to harvest and the lowest time (8.67 days) from stimulation to harvest were found in hot water treatment of wheat straw at 80^0C for 3 hours. The effect of hot water treatment of wheat straw found to be significant in yield contributing characters of oyster mushroom with some extent. Average number of primordia (78.33) was higher in case of hot water treatment of wheat straw at 100^0C for 2 hours. Oyster mushroom grown on wheat straw treated at 80^0C for 1 hours gave the best result with the highest average number of fruiting body/packet (62.33). Average number of effective fruiting body/packet (46.67) was higher in case of hot water treatment of wheat straw at 80^0C for 3 hours. Average weight of individual fruiting body (3.97) was higher in case of hot water treatment of wheat straw at 100^0C for 1 hour. The highest diameter of stipe (0.90 cm), diameter of pileus (6.67 cm) and thickness of pileus (0.87 cm) were in case of wheat straw treated at 80^0C for 3 hours. The highest average length of stipe (2.9 cm) was found wheat straw treated at 80^0C for 2 hours. Oyster mushroom grown on wheat straw treated at 80^0C for 3 hours gave the best result with the highest biological yield (180.71 gm), economic yield (175.23 gm), dry yield (18.85 gm), biological efficiency (108.52 %) and benefit cost ratio (4.67). Considering all the parameters wheat straw treated at

80^0C for 3 hours is more or less feasible for oyster mushroom (*Pleurotus ostreatus*) cultivation in Bangladesh.

Conclusion

Hot water treatment of wheat straw at 80^0C for 3 hours, found to be contributed significantly in yield and yield contributing characters with some extent. Hot water treatment of wheat straw at 80^0C for 3 hours provided the best results with the highest yield, biological efficiency and benefit cost ratio (BCR). Hot water treatment of wheat straw at 100^0C for 2 hours performed the best in terms of average number of primordia. Hot water treatment of wheat straw at 80^0C for 1hours gave the best results with the highest average number of fruiting body/packet.

Recommendations

In this experiment more than one treatment performed better in respect of yield and yield contributing characters. Hot water treatment of wheat straw at 80^0C for 3 hours can be recommended as an economically beneficial method of sterilization since it gave the highest yield. Further work is needed to justify the result.

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APPENDICES

Appendix I. Monthly temperature, relative humidity and rainfall of the experimental site during the period from January to June, 2013

Month	Temperature (°C)		Relative humidity (%)	Rain fall (mm)
	Minimum	Maximum		
January, 2011	11.00	15.00	90.15	00.00
February, 2011	17.74	22.25	85.60	0710
March, 2011	25.89	31.42	69.15	06.40
April, 2011	29.00	32.10	75.00	57.50
May, 2011	27.42	31.33	76.15	250.10
June, 2011	29.15	32.00	64.10	377.50

Source: Bangladesh Meteorological Department, Agargaon, Dhaka

Appendix II. Experimental layout for the study

Ọ Ọ Ọ T_0	Ọ Ọ Ọ T_1	Ọ Ọ Ọ T_2	Ọ Ọ Ọ T_3	Ọ Ọ Ọ T_4	Ọ Ọ Ọ T_5	Ọ Ọ Ọ T_6	Ọ Ọ Ọ T_7	Ọ Ọ Ọ T_8	Ọ Ọ Ọ T_9
Ọ Ọ Ọ T_7	Ọ Ọ Ọ T_6	Ọ Ọ Ọ T_5	Ọ Ọ Ọ T_9	Ọ Ọ Ọ T_8	Ọ Ọ Ọ T_3	Ọ Ọ Ọ T_4	Ọ Ọ Ọ T_2	Ọ Ọ Ọ T_1	Ọ Ọ Ọ T_0
Ọ Ọ Ọ T_0	Ọ Ọ Ọ T_1	Ọ Ọ Ọ T_2	Ọ Ọ Ọ T_3	Ọ Ọ Ọ T_4	Ọ Ọ Ọ T_7	Ọ Ọ Ọ T_8	Ọ Ọ Ọ T_9	Ọ Ọ Ọ T_5	Ọ Ọ Ọ T_6

Legend

Ô: Mushroom spawn packet

Appendix III. Analysis of variance on data with the effect of hot water treated wheat straw on the mycelium growth and primordia initiation of oyster mushroom (*Pleurotus ostreatus*)

Source of variance	Degrees of freedom	Mean square of				
		Mycelium running rate in spawn packets (cm)	Days required to complete mycelium running	Time from stimulation to primordial initiation (days)	Time from primordia initiation to harvest (days)	Time from stimulation to harvest (days)
Replication	2	0.004	5.733	6.700	2.433	16.533
Treatment	9	0.017*	21.144 **	2.893 **	2.607**	10.774**
Error	18	0.001	0.733	0.181	0.174	0.385

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

Appendix IV. Analysis of variance on data with the effect of hot water treated wheat straw on the primordia and fruiting body of oyster mushroom (*Pleurotus ostreatus*)

Source of variance	Degrees of freedom	Mean square of			
		Average no of primordia /packet	Average no of fruiting body /packet	Average no of effective fruiting body/packet	Average wt of individual fruiting body (g)
Replication	2	140.700	136.900	28.933	0.183
Treatment	9	43.778 *	52.593*	35.130**	0.703**
Error	18	14.367	15.159	1.563	0.012

Appendix V. Analysis of variance on data with the effect of hot water treated wheat straw on the dimension of fruiting body of oyster mushroom (*Pleurotus ostreatus*)

Source of variance	Degrees of freedom	Mean square of			
		Length of stipe (cm)	Diameter of stipe (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Replication	2	0.006	0.011	0.183	0.000
Treatment	9	0.018^{NS}	0.005**	0.460**	0.010**
Error	18	0.009	0.000	0.033	0.001

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

Appendix VI. Analysis of variance on data with the effect of hot water treated wheat straw on the yield, biological efficiency and benefit cost ratio of oyster mushroom (*Pleurotus ostreatus*)

Source of variance	Degrees of freedom	Mean square of				
		Biological yield (gm)	Economic yield (gm)	Dry yield (gm)	Biological efficiency (%)	Benefit cost ratio
Replication	2	12.044	17.470	0.608	4.347	0.012
Treatment	9	2334.723**	2338.492**	26.265 **	841.167**	1.664**
Error	18	24.283	26.655	0.364	8.755	0.019

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