STUDY ON SUBSTRATE CONTAMINATION OF OYSTER MUSHROOM IN BANGLADESH AND THEIR MANAGEMENT THROUGH AGROCHEMICAL ENRICHMENT AND PASTEURIZATION

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CERTIFICATE

This is to certify that thesis entitled, "**S*tudy on substrate contamination of oyster mushroom in Bangladesh and their management through agrochemicals enrichment and substrate pasteurization*"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** IN **PLANT PATHOLOGY,** embodies the result of a piece of *bonafide* research work carried out by **Khadija Akhter** Registration No. **13-05792** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Dated: June, 2017

Place: Dhaka, Bangladesh

Prof. Dr. M. Salahuddin M. Chowdhury
Chairman, Advisory Committee

DEDICATED

TO MY

BELOVED PARENTS,

MY FAMILY & SIBLINGS

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KHADIJA AKHTER

ABSTRACT

Survey and laboratory experiments were carried out during the period of 2013 -2016 to study the prevalence of contamination, effect of micronutrient enrichment and different sterilization techniques of substrates of oyster mushroom in Bangladesh. Survey study on 110 mushroom growers of 59 upazillas in 21 districts revealed that oyster mushroom Po_2 was the most cultivated popular species. Seed crisis and spawn contamination were identified as the main problem of farmers where 30% contamination occurred during incubation, 30% in summer and less than 20% in winter. 52.7% farmers conducted substrate sterilization through hot water for 1 hour. Rice straw (55.7%) and saw dust (21.6%) was mainly used as substrate. *Trchoderma, Rhizopus, Aspergillus, Penicillium, Alternaria, Ceratocytis, Coprinus, Chaetomium sp.* were found to be associated as contaminants where green mold was detected as the major one. Enrichment of substrate with Agrovit plus (1000-5000 ppm), Boron (10000ppm) , 10 ppm $Mncl_2$ showed better result over control where 3000 ppm Agrovit injection in substrate resulted highest yield (316.6g), increased mushroom harvesting time, less contamination. In addition, the rate of carbohydrate, fiber, protein, moisture and mineral were also increased. Use of chemicals viz, Bavistin (75 ppm), Formalin (500 ppm), Surf-xcel (100 ppm), Hydrogen Peroxide (30000 ppm), Chlorox (1000 ppm), Lime (286 ppm), Manganese chloride (100 ppm) found effective in minimizing the contamination of competitor moulds. The highest growth rate (1.3 cm), biological yield (219.6g/packet), biological efficiency (43.3%) was observed while treated substrate packets with Hydrogen per oxide. Pasteurization techniques of the substrates were standardized for oyster mushroom and it was observed that pasteurization of small bags (500g) for 3 hours were found most ideal for less contamination. Efficiency of steam pasteurization and autoclaving were compared at different time duration to control substrate contamination and the result showed that highest growth rate (1.03cm), better yield performance (311.3g) and the highest biological efficiency (66.01%) with minimum contamination were observed while packets steam pasteurized for three hours. Again, performance parameters such as, economic yield (235.2g) and BE (48.2%) was found while packet treated with 80^0C for 2 hours. As such pasteurization of rice straw through steam in steel drum for 3 hours in lieu of other treatments namely hot water treatment, chemical treatment or autoclaved treatment would be a viable and promising technique for substrate pre-treatment that can be adopted to produce a good yield of oyster mushroom in most rural areas, where autoclave sterilization may not be feasible.

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LIST OF SYMBOLS AND ABBREVIATIONS

ABBREVIATION	FULL WORDS
%	Percentage
PO_2	*Pleurotus ostreatus*
PDA	Potato Dextrose Agar
NA	Nutrient Agar
POP	Pink oyster
WS	White snow
PCYS	*Pleurotus cystidiosus*
KB	King'S B medium
DAE	Department of Agricultural Extension
CV	Co efficient of variation
sp	species
Temp.	Temperature
e.g.	Exempli gratia (by way of example)
et al.	and others (at ell)
FAO	Food and Agricultural organization
cm	Centimeter
Mt	Metric ton
SAU	Sher-e-Bangla Agricultural University
J.	Journal
NAMDEC	National Mushroom Development and Extension Centre
DAI	Days after Incubation
@	At the rate of
ml	Milliliter
CRD	Complete Randomized Design
BE	Biological Efficiency
Hclo4	Perchloric Acid
LSD	Least significant difference
df.	Degrees of freedom
g	Gram
ANOVA	Analysis of variances

ppm	parts-per-million
Kg	Kilogram
MDI	Mushroom Development Institute
hr	Hour
H_2O_2	Hydrogen Per Oxide
$MnCl_2$	Mangganese chloride

CHAPTER I

INTRODUCTION

Mushroom is the fleshy large reproductive structure of edible fungi belong to either Ascomycotina or Basidiomycotina typically consisting of a cap (pileus) at the end of a stem arising from an underground mycelium. There are various types of edible mushrooms such as oyster mushroom, milky white mushroom, shitake mushroom, button mushroom, straw mushroom etc. which are cultivated in our country. The Oyster mushroom (*Pleurotus ostreatus)*, first cultivated in Germany as a subsistence measure during World War I (Eger *et al*., 1976) is now grown commercially around the world for food. Mushrooms have been cultivated since ancient time for their nutritional value and flavor especially in the far eastern countries. Mushroom can play an important role to meet up the nutritional requirements of the population of Bangladesh (Amin *et al*., 2007a). Mushrooms are becoming increasingly important and common in human diets, due to their nutritional (Barros *et al*., 2008; Bernas *et al.,* 2006) and medicinal characteristics (Jedinak *et al*., 2010). The nutritional advantages of mushrooms include a low content of calories and a high content of proteins, minerals and dietary fiber (Beluhan and Ranogajec, 2011). Mushroom of *Pleurotus* spp. are rich in medicinal values and useful in preventing disease such as hypertension, hypercholesterolemia (Khatun *et al*., 2007 and Choudhury *et al*., 2008) hyperglycemia and different types of cancer (Nayana and Janardhanan, 2000; Jedinak and Sliva, 2008), some hepatoprotective activity of oyster mushroom (Mishra and Singh, 2010).

Pleurotus mushrooms, commonly known as oyster mushrooms, grow in the wild in tropical, subtropical and temperate regions and are easily artificially cultivated (Akindahunsi and Oyetayo, 2006). Oyster mushrooms (*Pleurotus* spp) are characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. Oyster mushroom can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, baggasse etc.

(Hassan *et al*., 2011) and this has been reported to influence its growth, yield and composition (Iqbal *et al*., 2005; Kimenju *et al*., 2009; Khare *et al*., 2010). Crop residues such as grain crop straw are characterised by the predominance of lignocellulose with cellulose, hemicellulose and lignin as the main components (Yildiz *et al*., 2002; Das and Mukherjee, 2007; Mamiro and Mamiro, 2011; Jonathan *et al.,* 2012). Using such crop residue as a mushroom substrate would subsequently convert them into a more protein-rich biomass and influence the mushroom yields (Mamiro and Mamiro, 2011).There are some differences in the nutrient content of the mushroom cultivated on different substrates (Mabrouk and Ahwanyi, 2008; Akinyele *et al*., 2011; Kulshreshtha *et al*., 2013b). However, this change in nutritional content never found to affect their edibility. Now a day's among various waste materials sawdust and rice straw are most commonly used for mushroom cultivation in Bangladesh. An ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth (Anonymous, 2008). Some studies showed that supplementation with nitrogen source increase the biomass and mushroom's productivity (Curvetto *et al.,* 2002).

Mushroom is now-a-days one of the promising concepts for crop diversification in Bangladesh. The climatic condition of Bangladesh is completely suitable for mushroom cultivation. It does not require any cultivable land. Mushroom cultivation can be a very interesting hobby with delicious results that could easily become a profitable small business, due to the low cost of inputs and high value of the crop. It requires short time, little capital and easy technique for cultivation. This is why all types of people like male and female, youth and old even children and disabled can easily participate in its cultivation. Its cultivation can transfer as a cottage industry and create a good opportunity for export. Therefore, it can generate huge scope of employment opportunities for unemployed people.

The substrates of oyster mushroom are contaminated by various kinds mycoflora, most of them act as competitor moulds thereby spawn run is adversely affected either by competition for food material or through production of toxic substances (Vijay and Sohi, 1987). The ability to quickly identify and control of contamination helps to protect mushroom crops and these common weed molds associated with edible mushrooms can be controlled by several treatments of substrates. The management of

weed mycoflora associated with substrate of mushroom is very difficult because both the host and the parasites are fungi. Various treatments are used for the preparation of substrate for mushroom cultivation to eliminate competitive fungi. These are: steam sterilization/ pasteurization, hot water immersion and chemical treatment (Jaramillo and Alberto, 2013); but these are not always successful. Though the weather and climate of Bangladesh is suitable for year-round oyster mushroom cultivation but the farmers cannot cultivate the mushroom lack of knowledge of sterilization. Without sterilization of substrates, it is not possible to eradicate the contamination of spawn of mushroom

So, research should be conducted to investigate the source and causal organisms and the contamination problem of substrates. In addition, enrichment of micronutrients of substrate has been highlighted by researcher around the world (Royse and Sanchez, 2008a, 2008b; Biswas *et al*., 2009) that enhances the productivity and quality and reduce the contamination. Several strategies of substrates pasteurization are selected as potential prevent the contamination of mushroom substrate. More research work is needed to know more effective pasteurization process to avoid contamination and to determine the influence on yields and growth related characters during production of *P. ostreatus* using different pretreatments.

Considering the above facts the present investigation was undertaken with the following objectives:

(1) To survey on status of mushroom cultivation and contamination with mycoflora of oyster mushroom.

(2) To find out the effect of different substrates, duration of steam pasteurization and size of substrate packet on severity of contamination.

(3) To determine the effect of substrate enrichment with micronutrients on incidence of contaminating mycoflora, production and nutrient status of oyster mushroom.

(4) To find out the effectiveness of chemical sterilization of substrate on contamination and production of oyster mushroom.

(5) To find out the effect of hot water treatment of substrate sterilization to reduce contamination and to improve mushroom production.

CHAPTER II

REVIEW OF LITERATURE

2.1. Oyster mushroom

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.

Kim *et al*., (2002) and Rosado *et al*., (2003) demonstrated that the production of *Pleurotus* spp. mycelial biomass and valuable polysaccharides in submerged liquid fermentation (SLF) depends on the species used, growth parameters, growth timing and their nutritional requirements.

Chowdhury *et al.,* (2011) reported that people have enjoyed mushrooms for their flavor, texture and mystique. Eastern cultures have revered mushrooms as both food and medicine for thousands of years. Among the mushroom kingdom, Oysters are one of the most versatile mushrooms. They are easy to cultivate and common all over the world. The latin name *Pleurotus ostreatus* means "side ways oyster", referring to the oyster-like shape of the mushroom. They are found on hardwoods throughout the world in the spring and fall. The caps usually range between 5 to 25 cm (2 to 10 inches) and are shaped like a fan or an oyster. The caps are rolled into a convex shape when young and will flatten out and turn up as the mushroom ages. They are also very beautiful, coming in a broad spectrum of colors. They can be white, yellow, brown, tan and even pink. They have a unique scent that is often described as sweet like anise or licorice (liquorice).

Uddin *et al*., (2011) conducted an investigation of four species of oyster mushroom: *Pleurotus ostreatus, P. florida, P. sajor-caju* and *P. high king* cultivated in every season (January to December) in Bangladesh to observe the environmental condition for better

production. In all of the selected species of this study, the minimum days required for primordial initiation, and the maximum number of fruiting bodies, biological yield and biological efficiency were found during December to February (14-27 ^{0}C, 70-80% RH). The production was found minimum during the cultivated time August to October.

2.2. Effect on Substrate on mushroom production

Siqueira *et al.,* (2011) utilized banana stalks and Bahia grass as basic starting materials for the production of the mushroom *Pleurotus sajor-caju*. Banana stalks were combined with other waste or supplement products (wheat bran, coast-cross hay, bean straw and cotton textile mill) to obtain different nitrogen concentrations. Since Bahia grass is relatively rich in protein, it was combined with other substrates (banana stalk, coast-cross hay and bean straw) to maintain a substrate nitrogen concentration of about 1.5%. Banana stalks and Bahia grass were both more efficient in the production of the mushroom *P. sajor-caju* when utilized without the addition of other substrates, with biological efficiencies of 74.4% and 74.12%, respectively. When combined with other substrates or grasses, there was a drop in biological efficiency, independent of the concentration of nitrogen.

Yang *et al.*,(2013) cultivated oyster mushroom (*Pleurotus ostreatus*) on rice straw basal substrate, wheat straw basal substrate, cotton seed hull basal substrate and wheat straw or rice straw supplemented with different proportions (15%, 30%, and 45% in rice straw substrate, 20%, 30%, and 40% in wheat straw substrate) of cotton seed hull to find a cost effective substrate. The effect of autoclaved sterilized and non-sterilized substrate on growth and yield of oyster mushroom was also examined. Results indicated that for both sterilized substrate and non-sterilized substrate, oyster mushroom on rice straw and wheat basal substrate have faster mycelial growth rate, comparatively poor surface mycelial density, shorter total colonization period and days from bag opening to primordia formation, lower yield and biological efficiency, lower mushroom weight, longer stipe length and smaller cap diameter than that on cotton seed hull basal substrate. The addition of cotton seed hull to rice straw and wheat straw substrate slowed spawn running, primordial development and fruit body formation. However, increasing the

amount of cotton seed hull can increase the uniformity and white of mycelium, yield and biological efficiency, and increase mushroom weight, enlarge cap diameter and shorten stipe length. Compared to the sterilized substrate, the non-sterilized substrate had comparatively higher mycelial growth rate, shorter total colonization period and days from bag opening to primordia formation. However, the non-sterilized substrate did not gave significantly higher mushroom yield and biological efficiency than the sterilized substrate, but some undesirable characteristics, i.e. smaller mushroom cap diameter and relatively long stipe length.

Odero (2009) tested ten different substrates using plastic bag technology to determine their effect on time to pinning, number of caps, average biological efficiency (ABE), pileus diameter, stipe length and flushing interval. Substrates tested were water hyacinth (*Eichhomia crassipes*), maize cobs (*Zea mays*), coconut fibre (*Cocos nucifera*), finger millet straw (*Seteria microcheata*), banana fibre (Musa sp), sugarcane bagasse (*Saccharum officinarum*), sawdust (Eucalyptus sp), rice straw (*Oryza sativa*), bean straw (*Phaseolus vulgaris*) and wheat straw (*Triticum aestivum*). Supplementation with maize germ, wheat bran and rice bran was done on bean, finger millets, rice and wheat straws at 3% dry weight basis to determine their effect supplementation on the productivity of these substrates. The average biological efficiency (ABE) varied between the ten substrates from 4.0% on sawdust to 106.2% on bean straw and the time to pinning was from 19.6 days on maize cobs to 39.9 days on water hyacinth. Choice of substrate is very important to profitable oyster mushroom cultivation as was observed from the results of this study

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 primordi was formed which resulted in to fruit body (edible part of mushroom).

Sonali (2012) carried out an experiment to study the growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis. The development of Oyster mushroom (Grey and pink) production methodologies on agricultural waste like Paddy

straw and wheat straw gave 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) 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 the 3:1 combination of wheat straw and water hyacinth (2.6 kg), 1:1 combination of wheat straw and water hyacinth (1.9 kg), 1:3 combinations of Wheat straw and water hyacinth (1.5 kg) and only water hyacinth (0.77 kg), where respectively it took 16, 20, 25, 30 and 40 days for the appearance of pin heads.

Kumari and Achal (2008) studied 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) and dry fruit body (5.93 mg/g) of *P. ostreatus*.

Amin *et al*. (2007a) 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%. 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) reported on 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 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 pinheads first appeared 32.33 days after spawning, the maximum number of fruiting bodies per bunch (7.30) ,the maximum flushes (4.00), 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 spawn rate per kg on substrate dry weight basis. It was concluded that spawning at 70 g per kg on substrate dry weight basis found to be the best dose for spawning.

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

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.

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*.

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 primordial, fruiting bodies and amount of fresh weight were found in waste paper 43.75, 31.00 and 94.25g respectively.

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. sajar caju* 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.

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, protein and mineral nutrient contents of *Pleurotus sajor caju* mushroom in Indian subcontinent or similar climatic conditions 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. 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.

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 310.00 k. cal/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 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 31.50 days. The final and third picking was completed in 43.50 days in case of soybean straw which was statistically higher compared to paddy and wheat straw (42.25) and cotton and jowar straw (42.75). The highest yield of 993.00 g/kg was obtained from cotton, followed by soybean straw (935.25 g/kg) and paddy straw (816.0 g/kg). The lowest yield of 445.50 g/kg was recorded in jowar straw.

Ayyappan *et al.*,(2000) used sugarcane trash and coir waste alone and in combination with paddy straw (3:1, 1:1 and 1:3 w/w) for sporophore production of two species of *Pleurotus*. The highest yields of *P. florida* (1395 g) and *P. citrinopileatus* (1365 g) were recorded in a mixture of sugarcane.

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

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

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.

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.

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.

Hoa *et al.*, (2015) conducted to compare the effects of different agro-wastes on the growth and yield of oyster mushrooms *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus* (PC). Seven substrate formulas including sawdust (SD), corncob (CC), sugarcane bagasse (SB) alone and in combination of 80 : 20, 50 : 50 ratio between SD and CC, SD and SB were investigated. The results indicated that different substrate formulas gave a significant difference in total colonization period, characteristics of fruiting bodies, yield, biological efficiency (BE) of two oyster mushrooms PO and PC. Substrates with 100% CC and 100% SB were the most suitable substrate formulas for cultivation of oyster mushrooms PO and PC in which they gave the highest values of cap diameter, stipe thickness, mushroom weight, yield, BE and short stipe length. However, substrate formula 100% CC gave the slowest time for the first harvest of both mushrooms PO and PC (46.02 days and 64.24 days, respectively).

2.3. Contamination of spawn

Adhikari and Jha (2017) studied samples of *Pleurotus ostreatus* and *Pleurotus florida* were collected from three major vegetable market of Kathmandu city which revealed presence of 21 fungi and worked to control of fungal contaminants in mushrooms during the postharvest storage.

Pervez, *et al.,* (2010) carried out the study to identify weed mycoflora associated with *Pleurotus ostreatus* (*Oyster mushroom*) substrate during culture in the spawn packet and to evaluate Formalin and Bavistin (Cabendazim) 50WP against the weed mycoflora. A total of 50 spawn packets colonizing substrate of *Pleurotus ostreatus* were collected randomly at different growth stages Ten weed mycoflora namely *Aspergillus flavus, A. fumigatus, A. niger, A. nidulans, A. terreus, Penicillium citrinum, P. thiersii*, *Penicillium.* sp., *Rhizopus stolonifer* and *Trichoderma harzianum* were found to be associated with the substrate.

Biswas (2014) revealed that the occurrence of seven contaminants in mushroom beds and out of which *Trichoderma harzianum, Penicillium notatum, Sclerotium rolfsii* and *Coprinus* spp. were found to be most dominant fungal contaminants and occurrence was high during June and July (28.4 & 35.8 %) causing maximum loss to mushroom yield. Among the botanicals tested for management of competitors moulds, *Azadirachta indica* (neem) showed its supremacy and exhibited maximum inhibitory effect (54.1 to 71.6 %) against *Aspergillus* spp., *Trichoderma* spp., *Coprinus* spp., and *Penicillium* spp. and was found to be less effective against *Sclerotium rolfsii in vitro* followed by extracts of *Pongamia pinnata* (42.4 to 61.3%). A range of 35.3 to 62.4% reduction in inky caps (*Coprinus sp.*) and 26.3 to 68.4% in green moulds (*Trichoderma* spp) were recorded with different phyto-extracts. The botanicals except *Acacia nilotica* reduced the incidence of competitor moulds (18.18 to 70.91%) in mushroom beds which increase the yield up to 21.3 %. The study will provide the idea of appropriate cultivation time as well as provide an alternative method of surface sterilization.

Sarker *et al.*, (2011) studied that there was a significant difference in percent contamination rate which ranged from 25 to100 % by green mould and other bacteria during cultivation of pretreated saw dust and pasterurized straw with various combination on yield of Oyster Mushroom (*Pleurotus ostreatus*).

Alameda and Mignucci (1998) stated that yield losses due to the associated weed molds on the basidiocarps of oyster mushrooms (*Pleurotus sajor-caju* and *P. ostreatus*) may vary between 10 and 20%.

2.4. Supplementation of substrates

Biswas *et al*., (2009) conducted to find out the effect of wuxal super, a mineral liquid fertilizer containing macro-and micronutrients supplement to sawdust substrate on growth, yield and quality of oyster mushroom (*Pleurotus ostreatus*). Packets were soaked in 0.0, 0.1, 0.2, 0.3 and 0.4% solution of the supplement for 30 minutes. The maximum of 0.45% and 0.35% reduction in days to primordial initiation and time primordia initiation to harvest was obtained with 0.2% which was followed by 0.1 and 0.3%. On the other hand, maximum increase in numbers of primordia, total fruiting bodies and effective fruiting bodies per packet and weight of fruiting bodies was also maximal at 0.2% followed by 0.1%. The highest increases in biological and economic yield and biological efficiency of the mushroom on spawn packet were obtained with 0.2% followed by 0.1 and 0.3% of the supplement. These three treatments also caused considerable increase in protein, lipid, N, P, K and Fe contents of mushroom. The results showed that the most effective level of wuxal super was 0.2%, which was followed by 0.1 and 0.3%. Increase in its concentration more than 0.3% caused adverse effect on growth, yield and nutrient status of mushroom.

Kumar *et al*., (2011), tried eight chemicals viz. salicylic acid (0.1%), ferrous sulphate (1%), copper sulphate (1%), manganese sulphate (1%), potassium sulphate (1%), magnesium sulphate (1%), zinc sulphate (1%) and calcium carbonate (1%) to see their influence on five species of oyster mushroom (*P. sajor caju, P. florida, P. flabellatus, P. fossulatus and P. sapidus*) with respect to radial growth, days for spawn run, number of

fruiting bodies and yield. Maximum radial growth was observed at 6th days in ferrous sulphate and copper sulphate supplemented medium in *P. sajor caju* (8.56 & 9.00 cm), *P. florida* (9.00 & 8.75 cm), *P. flabellatus* (8.26 & 8.50 cm), *P. fossulatus* (8.45 and 8.93 cm) and *P. sapidus (*9.00 and 8.50 cm), respectively. Minimum time was observed for spawn run in *P. sajor caju* (16.33 days), *P. fossulatus* (16.66 days) and *P. sapidus* (15.00 days) supplemented with ferrous sulphate followed by magnesium sulphate (18.00, 16.66 and 16.00 days), respectively. Maximum numbers of fruiting bodies were harvested from *P. sajor caju (*79.95) supplemented with ZnSo4, *P. florida* (77.03) supplemented with salicylic acid, *P. flabellatus* (76.12) supplemented with CuSo4, *P. fossulatus* (79.91) supplemented with MnSo4 and *P. sapidus* (80.62) supplemented with K_2So_4. Yield was harvested significantly well in all the five species, P. sajor caju (602.90 g/kg), P. florida (592.10 g/kg), *P. flabellatus* (566.55 g/kg), *P. fossulatus* (604.00g/kg) and *P. sapidus* (612.12 g/kg) supplemented with ferrous sulphate.

Nunes *et al*., (2012) tested the effect of nitrogen supplementation on the productivity and nutritional composition of *Pleurotus ostreatus* mushrooms. The fungi were grown in various substrates supplemented with urea or rice bran, and the biological efficiency, mineral composition and protein content were evaluated. The growth of *P. ostreatus* in substrates with nitrogen supplementation increases the mushroom's productivity and nutritional value.

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 (*Pleurotus ostreatus*) 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.

Rahman *et al.,* (2012 a) carried out an experiment to investigate 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) reported on 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.

Nuruddin *et al.*, (2010) investigated 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.

Experiments conducted by Baysal *et al.*, (2003) which involved cultivation of *P. ostreatus* on waste paper with addition of chicken manure, peat and rice husks, showed that increasing the amount of rice husks added to the substrate accelerated spawn running, pinhead formation and fruiting body formation.

Sarker (2004) observed that duration from primordial initiation to first harvest of Pink oyster mushroom was significantly lower as compared to control where no supplement was used and the duration required for total harvest of oyster mushroom increased with the level of supplement used.

Khlood and Ahmad (2005) conducted an experiment to study the ability of oyster mushroom (*Pleurotus ostreatus*) $P0_{15}$ strain to grow on live cake mixed with wheat straw. The treatments comprised : 90% straw + 5% wheat bran + 5% gypsum (control); 80% straw + 10% olive cake + 5% wheat bran + 5% gypsum (T_1); 70% straw + 20% olive cake 5% wheat bran + 5% gypsum (T_2); 60% straw + 30% olive cake + 5% wheat bran + 5% gypsum (T_3); 50% straw + 40% olive cake + 5% wheat bran +.5% gypsum (T_4); and 90% olive cake + wheat bran + 5% gypsum (T_5). After inoculation and incubation, transparent plastic bags were used for cultivation. The pinheads started to appear after 3 days and the basidiomata approached maturity 3-7 days after pinhead appearance. The addition of 30% olive cake to the basal growing medium gave the highest yield (400 g/500 g dry substrate), average weight (21.5 g/cap) and average cap diameter (7.05 cm/cap) and BE% (80%). Carbohydrate, protein and fiber contents were high Ash contents were moderate, while fat content was low in the *P. ostreatus* basidiomata. 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.

2.5. Sterilization of substrate

Adhikari and Jha (2017) studied and results exhibited *Aspergillus niger* and *Rhizopus* sp as most abundant contaminants which were treated with different concentrations of essential oils of *Cinnamomum tamala, Mentha spicata, Zanthoxylum armatum* and *Eucalyptus citriodora* using poisoned food technique. All the EOs were found significantly inhibit ($p<0.05$) the growth and spore germination of both test fungi. A strong inhibitory action of cinnamon oil and mentha oil was recorded against *A. niger* and *Rhizopus* sp respectively at a concentration of 20 µl/ml. This clearly suggests that EOs could be an alternative to the synthetic chemicals that are currently used to control fungal contamination in mushroom and extend their shelf life.*Cinnamomum tamala* showed best inhibition effect whereas *Mentha spicata* showed least antifungal effect in controlling *Aspergillus niger* among all four oils. At 20µl/ml oil concentration, *C. tamala* showed highest inhibition (79.30%) followed by *Zanthoxylum armatum* (76.94%), *Eucalyptus citriodora* (76.60%) and *Mentha spicata* (76.56%), respectively. But in case of the spore germination of *Rhizopus* sp, *C. tamala* showed highest inhibition (84.48%) followed by *Zanthoxylum armatum* (78.80%), *C. camphora* (83.35%), *Eucalyptus citriodora* (71.02%) and *Cinnamomum tamala* (69.80%) at 20µl/ml oil concentration respectively.

Mejía and Albertó (2013) carried out an experiment to compare the yields obtained during mushroom production of *P. ostreatus* using different pretreatments (immersion in hot water, sterilization by steam and the use of fungicide) of substrate (wheat straw). Carbendazim treatment produced highest yields (BE: 106.93%) while IHW produced the lowest BE with 75.83%. Sugars, N, P, K and Ca were found in residual water of IHW treatment. It was proved that IHW treatment of substrate reduced yields at least 20% when compared with other straw treatments such as steam, chemical or untreated wheat straw.

Moda *et al*., (2005) investigated cultivation of *P. sajor caju* whether traditional composting and pasteurization processes could be replaced by washed and supplemented (mineral or organic) sugarcane bagasse. In one experiment, fresh sugarcane bagasse was immersed in hot water at 80°C for two hours (control) or washed in fresh water for one

hour using an adapted machine for residue treatment. In another experiment, fresh sugarcane bagasse was washed in fresh water (control), and supplemented with corn grits (organic supplementation), or supplemented with nutrient solution (mineral supplementation). In the first experiment, the washed bagasse presented a average biological efficiency (ABE) of 19.16% with 44% contamination, and the pasteurized bagasse presented a ABE of 13.86% with 70% contamination. In the second experiment, corn grits presented the poorest performance, with a ABE of 15.66% and 60% contamination, while supplementation with the nutrient solution presented a ABE of 30.03%, whereas the control of 26.62%.

Oseni *et al.,* (2012) evaluated substrate pre-treatments by autoclaving at 121°C, hot water dipping (pasteurization) in steel drum at 60°C for 2 h and hot water dipping (pasteurization) in steel drum at 60°C for 3 h. The percent contamination was significantly higher in horse manure compost (70%) compared to sugarcane bagasse (12.5%). The oyster mushroom took significantly less time to colonise the autoclaved sugarcane bagasse (36 days) compared to sugar cane bagasse pasteurised for 2 h (64 days). Autoclaved horse compost manure was fully colonised in 42 days, while those pasteurized with hot water at 2 and 3 h failed to colonize due to heavy contamination by *Trichoderma harzianum* presumably due to insufficient sterilization. Despite the s hortest days to full colonization, there was no significant difference in the yield (410.4 g) and bio-efficiency (82.10%) of autoclaved sugarcane bagasse compared to the yield (301.1 g) and bio-efficiency (60.22%) of sugarcane bagasse pasteurized in hot water for 3 h.

Ali *et al*., (2004) evaluated Different techniques of pasteurization on cotton waste steam, hot water and chemical treatment with Formalin and observed best mycelia growth in three species of oyster mushroom in steam pasteaurization which completed the shortest time .Formalin gave poorer result to complete mycelia growth.

Muhammad Asif *et al*., (.2007) evaluated the influence of pasteurization methods on cotton waste substrate on yield of oyster mushroom (*Pleurotus* spp.). Cotton waste subjected to different methods of pasteurization, namely pasteurization with steam, hot-

water treatment and chemical sterilization with formalin, which were compared with control (without pasteurization). Three species of *Pleurotus* i.e. *Pleurotus florida, Pleurotus pulmonarius* and *Pleurotus ostreatus* were selected. Steam pasteurization produced the best results as for as the performances of individual species are concerned, *Pleurotus pulmonarius* completed the mycelial growth in the shortest time. Formalin treatment behaved poorly as the different *Pleurotus* spp, took maximum time to complete mycelial growth. Steam pasteurization technique produced more yield, whereas *Pleurotus florida* behaved better in all the treatments than other species.

Sánchez *et al*., (2011) studied substrate to grow *Pleurotus ostreatus* pasteurized an alternative treatment by forming a substrate compost pile with sufficient size and moisture to generate the necessary heat for pasteurization. A 1 m3 wooden box with 80 kg dry Pangola grass *Digitaria decumbens* and 2% lime ($Ca(OH)_2$) was adjusted to 55, 60, 65 and 70% moisture. The composting treatment lasted 48 h; subsequently the substrate was cooled to ambient temperature and spawned. The treatment was efficient in preventing other organisms like flies, bacteria and competitor fungi. After two flushes of mushrooms, the biological efficiency was between 70.5 and 88.1% when compared to the control (same substrate steam pasteurized 90ºC, 1 h).

Pervez *et al*., (2012) conducted using different concentrations of H_2O_2 in the substrate of oyster mushroom (*Pleurotus ostreatus*) for controlling associated mycoflora. Spawn packets were treated with five different concentrations of H_2O_2 viz. 5%, 4%, 3%, 2%, 1% and control spawn packet (without using H_2O_2 but using autoclave and laminar air flow). Occurrence of the lowest contamination 3.33% spawn packets were found in case of 3% to 5% H_2O_2 treated spawn packet. Minimum time (18.33 days) was required for completing mycelium running in the spawn packet and the fruiting bodies formations of oyster mushroom was required for control spawn packet nevertheless, 3%, 2% and 1% H_2O_2 treated spawn packets also required statistically similar minimum time. The highest yield of fruiting bodies (680.50g) per spawn packet was observed in 3% H_2O_2 treated spawn packet, which is significantly higher than that of other H_2O_2 treated packets.

Pervez *et al*., (2012) evaluated Formalin, Bavistin and their combination against the identified weed mycoflora at 100, 250 and 500 ppm in vitro following poison food technique. All treatments caused radial mycelium growth inhibition of all isolated weed mycoflora over control. The rate of inhibition corresponded to higher doses of the chemicals. The combine application of Formalin and Bavistin at their highest concentration (500+75 ppm) gave the maximum inhibition of gowth of all the identified fungi.

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 a product average of 416g (w/v) whereas the substrate sawdust yields a product average of 360 g (w/v). In chemical sterilization method, the substrate straw yields a product average of 371g (w/v) whereas in the substrate sawdust yields a product average of 310 g (w/v).

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.*

Pervez *et al.,* (2010) evaluated Formalin and Bavistin (Carbendazim) % 50WP against the weed mycoflora. The combined application of Formalin and Bavistin at there concentration of (500 ppm +75ppm) gave the maximum inhibition of growth of all the identified fungi namely, *Aspergillus flavus, A. fumigatus, A. niger, A. nidulans, A. terreus, Penicillium spp, Rhizopus stolonifer and Trichoderma harzianum* associated with substrate of *Pleurotus ostreatus* (Oyster mushroom).

Ali *et al.*, (2004) evaluated Effect of different pasteurization techniques on mycelial growth of oyster mushroom (*pleurotus spp)* and showed among them steam pasteurization gave maximum mycelial growth which completed in shortest period while Formalin treatment behaved poorly as the species took maximum time to complete their mycelial growth.

Sanchez (2010) reported that substrate used for the oyster mushroom cultivation do not require sterilization, but only pasteurization, which is less expensive to diminish the damages produced by different pathogens (bacteria, moulds or insect pests) on mushroom development and yield.

Diana *et al*., (2006) recommended disinfection of the substratum before spawning, which should only destroy the competitive fungi and not the useful micro organisms.

2.6. Nutritional composition

Hoa *et al*., (2015) conducted to compare the effects of different agro-wastes on nutritional composition of oyster mushrooms *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus* (PC). Seven substrate formulas including sawdust (SD), corncob (CC), sugarcane bagasse (SB) alone and in combination of 80 : 20, 50 : 50 ratio between SD and CC, SD and SB were investigated. The results indicated that different substrate formulas gave a significant difference in nutritional composition and mineral contents of two oyster mushrooms PO and PC. The results showed that increasing CC and SB reduced C/N ratio, and enhanced some mineral contents (Ca, P, and Mg) of substrate formulas. The increased amount of CC and SB of substrate formulas enhanced protein, ash, mineral contents (Ca, K, Mg, Mn, and Zn) of fruiting bodies of both mushrooms. Substrates with 100% CC and 100% SB were the most suitable substrate formulas for cultivation of oyster mushrooms PO and PC in which they gave the highest values protein, fiber, ash, mineral content (Ca, K, and Mg). It is also found that the C/N ratio of substrate formulas has close correlation with total colonization period, mushroom weight, yield, BE and protein content of mushroom PO and PC.

Zape *et al*., (2006) studied in analyzing the physico-chemical composition of dehydrated fruit bodies of *Pleurotus* species and 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.*

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.

Rahman *et al.,* (2012 b) analyzed nutritional compositions of different strains of *Pleurotus cystidiosus* (PC) in comparison *to Pleurotus ostreatus* (PO_2) on sawdust. The available carbohydrate content was highest in PO_2 (40.3 g/100 g) followed by PC_4, PC_2, PC_1 and lowest in PC_3 (34.3 g/100 g). The fiber content was highest in PO_2 (26.48 g/100g) followed by PC_2, PC_3, PC_1 and PC_4. The protein content was in PO_2 (24.03 g/100 g). The Lipid contents was in PO2 (2.567 g/100g) and the ash content was 7.27g/100g. The highest nitrogen (N) content was found in PO_2 (3.700%). The phosphorous (P) content was found in PO2 (1.553%). The highest potassium (K) content was found in PO_2 (3.700%) followed by PC_2 (3.567%) and PC_3 (3.567%). The lowest potassium (K) content was found in PC_1 (3.103%) and PC_4 (3.103%). The calcium (Ca) content was found in PO_2 (23.87 mg/100g) preceded by PC_2 (24.15 mg/100g).The magnesium (Mg) was found in PO_2 (20.29 mg/100g).The iron (Fe) content was found in PO_2 (29.61 mg/100g).The copper (Cu) content was found in PO_2 (30.10 mg/100g). The zinc (Zn) content was found in PO_2 (21.31 mg/100g).

Ashraf *et al*., (2013) compared the effect of different agricultural wastes on growth and yield of mushroom production, three species of *Pleurotus* viz. *P. sajor-caju* (V_1), *P. ostreatus* (V_2), and *P. djmor* (V_3) were grown on three different substrates cotton waste (T_1), wheat straw (T_2) and paddy straw (T_3). The fastest spawn running, primordial initiation, harvesting stage, maximum number of fruiting bodies and maximum yield was observed in T1 took minimum number of days T_3 showed maximum yield in 1st flush showing no significant differences with treatment T_1 whereas T_1 took maximum yield in 2nd flush and 3rd flush. *P. djmor* showed the highest percentage of dry matter (17.23%)

and moisture content was found high in *P. sajor-caju* (87.37%). *P. ostreatus* and *P. sajor-caju* showed the maximum protein (27.23%) and fiber (26.28%) contents. The ash contents were found maximum *P. sajor-caju* (9.08%).The highest fat and carbohydrate contents were found in *Pleurotus djmor* (3.07%) and *P. djmor* (37.69) respectively.

Baysal (2003) investigated paper waste supplemented with rice husk, chicken manure and peat for *Pleurotus ostreatus* cultivation. Highest yield for fresh weight was recorded as 350.2 grams in the substrate containing 20% rice husk.

CHAPTER III

MATERIALS AND METHODS

A series of six experiments were conducted to achieve objective of the study. Duration of the study was 2013 to 2016. If other location is not mentioned, most of the research works of all experiments were conducted in mushroom culture house (MCH) and plant pathology laboratory of Sher-e-Bangla Agricultural University (SAU), Dhaka. Materials and methods of the study have been described under each of the experiments.

3.1. Survey on status of mushroom cultivation and contamination with mycoflora in Bangladesh

3.1.1. Objectives

The survey was conducted to collect information about present status of mushroom cultivation and contamination of spawn packet as well as fruiting bodies with mycoflora; and to identify the mycoflora contaminating spawn packet of oyster mushroom in the farms of different districts of Bangladesh.

3.1.2. Survey duration, area and selection of mushroom growers

The survey was conducted during 2014-2015. The locations of survey were mushroom centers of Department of Agricultural Extension (DAE) of the Peoples' Republic of Bangladesh and mushroom farms in 21 districts of the country (Figure 1). A total of 110 mushroom growers were selected from 110 farms including mushroom centers in different districts (Table 1).

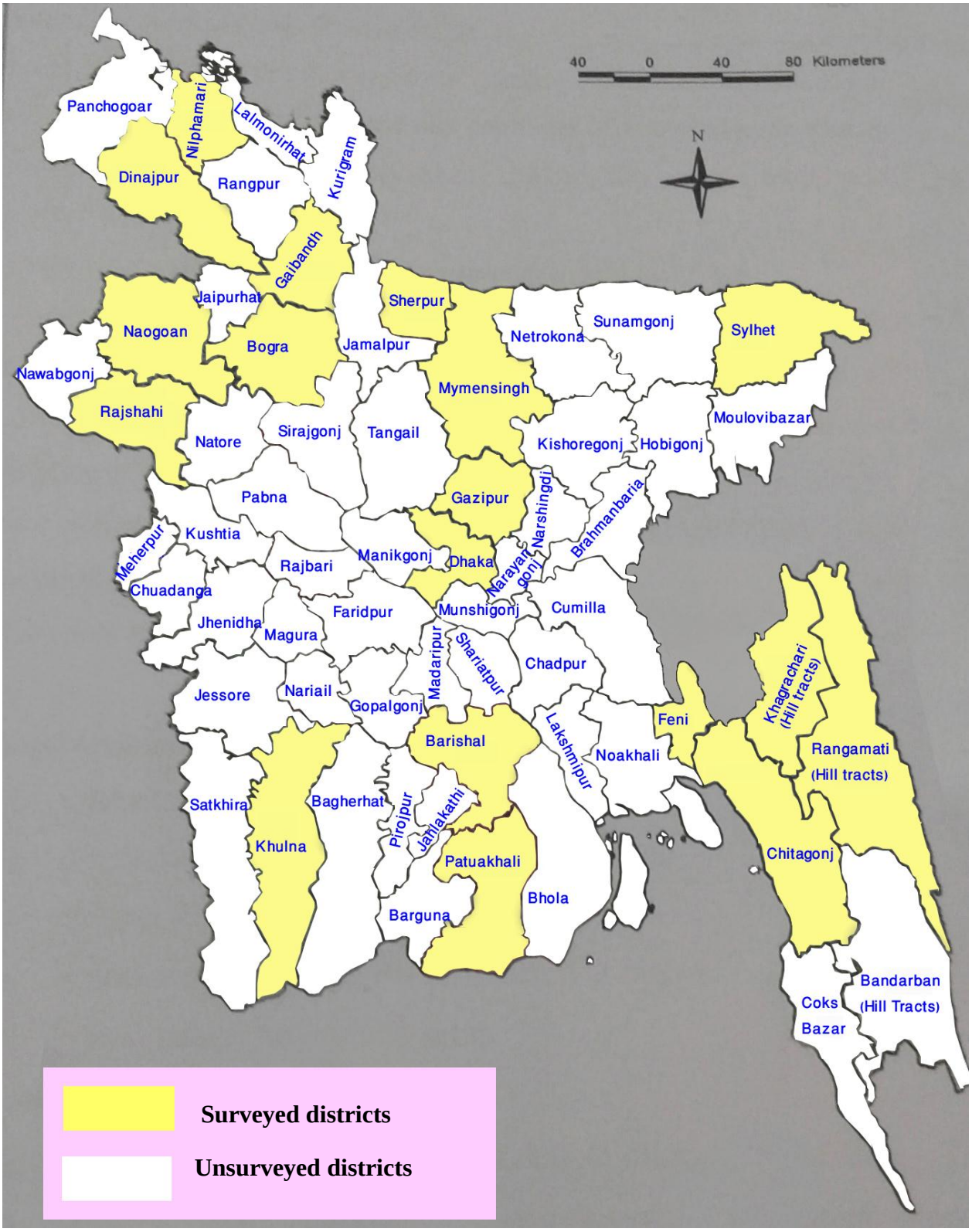

Figure 1. Survey area of mushroom farms /centers in Bangladesh map

Table 1. List of surveyed area of mushroom farms /centers in Bangladesh

Sl. No.	District(s)	Name of centre/ farm(s)/proprietors	Number (s) of mushroom farm
01	Dhaka	Mushroom Culture House,SAU, Dhaka Dainty Mushroom centre, Mirpur, Dhaka Khadem mushroom centre, Khilgaon, Dhaka. Marry mushroom, Dhakhin Khan Jashim, mushroom, Dhaka Kanunj protein, Uttarkhan Rani mushroom, Dakhhin Khan	07
02	Savar	NAMDEC , Sobhanbag, Savar DD mushroom, Savar, Dhaka Paradise Agrofarm, savar, Dhaka Organic Agrofarm, ganda, Savar Tahsan, Dagarmora Kulsum mushroom Mollah, Joyapara Holy mushroom, Ashulia Golapi, Savar Munni mushroom, savar A.k. Mushroom Jahangir baul, Savar Zaman , savar	13
03	Gazipur	World mushroom Bhuyan mushroom Voawal mushroom M.R.C.L.Multifarm	04
04	Mymensingh	Md. Edri sullah Hossain Winner Mushroom Usuf Hannan Mia Sayed	05

Table 1. (Cont'd)

Sl. No.	District(s)	Name of centre/ farm(s)	Number (s) of mushroom farm
05	Noakhali	Lakhipur Mushroom Prakalpa Noakhali Mushroom Jahir pathan, Chhagalnaya Ujjibon Centre, sonaimuri Sajedamushroom centre, Chhagalnaya Asa mushroom Centre, Sunaimuri	06
06	Bogra	Mansur Ali, Bagura sadar Aklima Mushroom Centre Khairul Islam, rajshahi sadar Sajib, Bagura sadar Fazlul haque, atria Shafiqul Islam, Bagira Sadar Hasan Mushroom, kahalo Raju, Bogura Mijanur rahman, Gaibandha Surovi, Bagura sadar Rehana,Bagura Sadar Kajal, Sonatala Labu Mia, Sonatala Alamin, sajahanpur Based, Bagura Sadar Aklima, Bagura Sadar	16
07.	Dinajpur	Nasrin Khurshid Jahan, sadar Laki, Nayanpur Nahina Akhter, sadar Arnaba Mushroom, Mirjapur, Sadar Israt Jahan, Mirjapur Anjumanara, Trishbnadar Mamtaj Lavli	08
08	Comilla	Mohoshin, Muradnagar Khidmah, Choddogram Chowdhory mushroom & Strawberry, Haziganj	03
09	Bandorban	Mushroom Sub Centre Sabura, Gudarpara Sanuara, Gudarpara Samud Khatun Tipu, sadar Juni, sadar Kallan Barua, Sadar	07

Table 1. (Cont'd)

Sl. No.	District(s)	Name of centre /propriter/ farm(s)	Number (s) of mushroom farm
10	Rangamati	Mushroom sub-centre Janani Mushroom Centre, Sada Sagar Mushroom, sadar Anika, rangamati, sadar Jewel Mushroom, Sadar Sanchita chakma Anjana Dey, sadar Yasin Centre, Sadar	08
11	Sylhet	Mushroom Garden Yakob Ali Mostak	03
12	Cox's Bazar	Sahidul haq Kajal Rupban, Sadar Laila begum, Sadar Dildar Begum Tanjila Alam, Sadar Nur akter Nasima, Adarsha gram, Lutfunnesa, adarsha gram, Sadar Nurnahar, adarsha gram, Sadar Nilofa Yasmin, adarsha gram, Sadar Morshida, Sadar	11
13	Chittagong	Mahiuddin Jenin, Hathazari Usouf, Sitakundu Himel barua, Patia	04
14	Khulna	Rabiul mushroom farm, Magura Shadul Islam Nizamuddin Adarsa manna mushroomi Makka-Madina	05
15	Khagrachhari	Ataur Rahman Hmmam Rekhanjali chakma Suman mushroom	03
16	Nilphamari	Fatema Mushroom Hafijur Rahman	02
17	Barisal	kamrul mushroom Joy	02

Table 1. (Cont'd)

Sl. No.	District(s)	Name of centre /propriter/ farm(s)	Number (s) of mushroom farm
18	Rajshahi	Md. Khairul Islam Rangdhonu	02
19	Naogaon	Md. Fazlul Haque	01
20	Gaibandha	Md.Mijanur rahman	01
21	Patuakhali	Mother mushroom	01
Total			**110**

3.1.3. Collection of contaminated spawn packet and isolation of contaminating mycoflora from spawn as well as infected fruiting bodies

To identify causes of contamination, contaminated spawn packets were collected during the visit of surveyed farms and centers. Collected specimens were preserved in the laboratory at 4^{o}C. Isolation of contaminating mycoflora causing spoilage of spawn packets and fruiting bodies were done following appropriate methodology (Dhingara and Sinclair, 1995).

The mycoflora associated substrates were isolated following dilution plate techniques (Dhingara and Sinclair, 1995). One gram of substrate sample was taken in a test tube containing 9 ml sterile water and stirred thoroughly for 5 minutes to obtain 1:10 suspension of the substrate. One milliliter of the suspension was transfer to another test tube containing 9 ml sterile water and shaken thoroughly to obtain 10^{-1} dilution. Similarly 10^{-2}, 10^{-3} and 10^{-4} dilutions of the substrate suspension were prepared. After dilution, 1 ml 10^{-4} diluted substrate suspension was placed in the center of 90 mm PDA plates and spread over whole surface of PDA. After inoculation, the plates were incubated in an incubator at 25 ± 1^{0}C temperature for 7-10 days (Dhingara and Sinclair, 1995).

The mycoflora associated fruiting bodies were isolated following tissue planting method on moist sterilized blotting paper (Dhingara and Sinclair, 1995). The diseased fruiting bodies in spawn packets were collected and cut into small pieces (5 mm). The pieces were surface sterilized with sodium hypochloride4 solution for 5 minute. The sterilized pieces were rinsed thrice and soaked with sterilized filter paper. The pieces were placed on sterilized blotting paper in sterilized Petri dishes and incubated in an

incubator at 25±1^{0}C for 7 days. The individual colony of mycoflora grew on the PDA plate as well as filter paper were transfer to fresh PDA plates and allowed to and preserved the culture plates in a refrigerator at 4ºC (Dhingara and Sinclair, 1985).

3.1.4. Purification and identification of contaminating mycoflora

Pure culture of the each mycoflora was prepared following hyphal tip culture technique (Hyakumachi, 1994). Semi-permanent slides of each fungus were prepared and observed under compound microscope. The morphological characteristics of individual fungi were recorded and compared with appropriate key book like CMI description of fungi to identify each fungus (Barnett, 1972). Identified fungi were grown in PDA slants and preserved in refrigerator at 4^{0}C.

3.1.5. Data collection and data analysis

Data were recorded on the following parameters using a pre-tested questionnaire (Appendix I).

i. Demographic information of the farmers
ii. Name of mushroom species and strains cultivated by growers
iii. Mushroom species and strains of oyster mushroom cultivated and seasons of cultivation
iv. Preparation and sources of mother culture and mother spawn
v. Spoilage of mother spawns due to contamination
vi. Problems of mushroom cultivation
vii. Methods of substrate sterilization used by farmers
viii. Farmers response about contamination during incubation and cultivation during summer and winter
ix. Types of mushroom house and sources of water for growing mushroom
x. Number and size of spawn packet prepared for cultivation of mushroom
xi. Number of harvest and yield of mushroom per spawn pawn packet
xii. Marketing of mushroom and activities.
xiii. Farmers' idea about contamination, contaminating fungi and symptoms of contamination of spawn and fruiting bodies

xiv. Activities of mushroom farmers performed
xv. Various problems identified by mushroom famers
xvi. Contaminating fungi in spawn packets
xvii. Cock's comb symptoms of fruiting bodies
xviii. Abnormal fruiting bodies

The data collected during survey based on questionnaire were analyzed following SPSS computer program.

3.2 Effect of different substrates, duration of pasteurization and size of substrate packet on severity of contamination in substrate packet

3.2.1. Objective, location and duration of experiment

The experiment was conducted to find out the effect of different substrates, duration of sterilization and size of substrate packet on severity of contamination. The experiment was conducted during September to December 2014 in MCH and plant pathology laboratory of SAU.

3.2.2. Preparation of substrate, spawn packet, pasteurization of substrate packet

Four types of substrates were used in the experiment. These were rice straw, saw dust, waste paper and mixture of the three materials. Rice straw and waste paper were cut into small pieces (4 mm length) and soaked in water for overnight before substrate preparation. After draining the excess water content of the final mixture was adjusted to 65% (w/w) was filled into polyethylene bags at 500 and 1000 g/bag. The packets were pasteurized for 2, 3, 4, 5 and 6 hours with steam in a steel drum. The drum was filled with water at a level of 10.0 to 12.5 cm from the bottom and heated for the selected period. Mouth of spawn packets were plugged with absorbent cotton inserting through plastic neck fixed in the mouth. The packets were incubated in the MCH having 25^0C ambient temperature and 75% RH. Diffused day light and proper ventilation were maintained in the MCH.

3.2.3. Incubation of spawn packet

After preparation of substrate in polyethylene bags, the packets were pasteurized in a hot water drum. After pasteurization, the spawn packets were placed on iron shelves and incubated in the MCH. The packets were observed regularly with 10 days interval to record prevalence of contaminating fungi. Data on prevalence (%) of contamination and severity of contamination were recorded and expressed in percentage computed based on total number of packets checked the number of contaminated packets.

3.2.4. Design of experiment and Data collection and data analysis

The experiment was laid out in a 4x5x2 factorial experiment in completely randomized design (CRD), with 5 replications. The first factor was substrate with 4 levels (rice straw, saw dust, waste paper and a mixed substrate containing rice straw, saw dust, waste paper), the second factor was duration of pasteurization with 5 levels (2, 3, 4, 5 and 6 hours) and the third factor was size of spawn packet with 2 levels (500g and 1000g).

3.2.5. Data collection and data analysis

Data on severity of contamination were recorded starting from 30 days after incubation (DAI) and continued up to 60 DAI with 10 days interval. The area of mycelium covered by contaminating fungi in spawn packet was graded based on a 0-5 scale, where 0= 0% coverage, 1=<0-20%, 2=<20-40%, 3=<40-60%, 4=<60-80% and 5=<80-100% coverage by mycelium of contaminating fungi.

The severity of infection was calculated using the following formula:

Prevalence of contamination (%) = (Sum of the total scoresx100) ÷ (total number of observation x maximum grade in the scale) (Biswas, 2014).

Collected data were analyzed according to Gomez and Gomez (1984) using MSTAT-C computer program. Means were compared using LSD test following the same computer package.

3.3. Effect of substrate enrichment with micronutrient on oyster mushroom production and nutritional status

3.3.1. Objective, duration and location

The duration of the experiment was April-August 2016 and the location was the MCH of SAU and Plant Pathology Laboratory of SAU.

3.3.2. Preparation of substrate

Rice straw was used as the basic material to prepare the substrate. Rice straw was selected because it was identified as the most suitable to grow oyster mushroom (Mondal *et al*., 2010; Stamets, 1993). Fifteen kilogram of rice straw was taken, cut into small (4 mm) pieces soaked in water and sundried to have 65% moisture content. $CaCO_3$ was mixed with rice straw at 2.0% (w/w) before pouring into the substrate bag. Rice straw was poured into 22x26 cm polyethylene bag at 500 g per bag, which served as substrate packets. A plastic neck was inserted into the mouth of polyethylene bag and tight rubber band. The mouth was closed with absorbent cotton, covered with brown paper and further tight with rubber band. The substrate packets so prepared were pasteurized in a pasteurization drum following standard procedures.

3.3.3. Inoculation and incubation of spawn packet

The mother spawn (seed) of oyster mushroom (*P. ostreatus* strain-4) was collected from MDI. The spawn packets were inoculated with the mother spawn through the mouth under sterile condition in a laminar air flow. After inoculation, the spawn packets were incubated in the MCH under dark condition at 20 ± 2^0C temperature for mycelium running. After completion of mycelium running, the spawn packets were transferred to the MCH. The rubber band, brown paper, cotton plug, and plastic neck of the mouth were removed and the mouth of polyethylene bag was tight with rubber band. A“D” shaped opening was made on the shoulder of the spawn packets. The surface was scraped with tea spoon for removing the thin whitish mycelium layer.

3.3.4. Enrichment of substrate with micronutrient

The substrate in spawn packet was enriched with micronutrient solution. The solution was prepared with a formulated product of Global Agrovit Ltd containing micronutrients in the form of ETDA Fe: ≥4%, Delta Cu: ≥1.5, ETDA Zn: ≥1.5, Mo: 0.1, B : ≥0.5 and MgO: ≥9.0. The product was mixed with water at 1000, 2000, 3000, 4000 and 5000 ppm. In addition to those micronutrients boron and manganese were also used in the experiment. Solutions of boron and $MnCl_2$was prepared to supply boron and Mn. Concentration of elements was used at 1000 ppm and 10 ppm, respectively. Each concentration and/or micronutrient represented a treatment. So, there were altogether 8 treatments including a control (pure water treatment).

3.3.5. Application of enrichment

The micronutrient solutions were applied following two methods viz. immersion and injection. In immersion method, the spawn packets were immersed for soaking in micronutrients solution for 20 minutes. The packets under control were soaked in plain water for same period. In injection method, the spawn packets were soaked in plain water for 20 minutes. Excess water was released by placing on plastic net. The suspension was injected into water soaked spawn packet at 10 ml/packet. Application of micronutrient was done after every harvest by injection.

3.3.6. Cultivation of spawn and harvesting

The spawn packets were transferred to MCH, placed on wooden shelves and covered with newspaper. The relative humidity of culture house was maintained at 80-90% RH and temperature 20-25^{0}C. The light was around 200-300 lux and ventilation of culture house was maintained uniformly. During growing period, water was sprayed regularly over newspaper on spawn packets.

3.3.7. Harvesting

Matured fruiting bodies were identified by observing curial margin of the cap as described by Amin (2002). Mushrooms were harvested by twisting to uproot from the base. The fruiting body was collected up to 7th flush during the harvesting period.

3.3.8. Design of experiment

The experiment was laid out following a completely randomized design with 3 replications. Each spawn packet used in the experiment represented a replication.

3.3.9. Data collection

Data on the following parameters were recorded using standard procedures with some modification as described below:

Incidence of spawn packet contamination

Incidence of contamination in spawn packet with mycoflora was determined following the procedures as described earlier.

The spawn packets were checked and number of contaminated spawn packet was identified. The incidence (%) of contamination was computed using the following formula: Incidence of contamination = (Number of contaminated spawn packet ÷ Total number of spawn packets checked) x 100.

Days to mycelium running, primordia initiation, first harvest and final harvest

Days required for completion of mycelium running in the spawn packet, first harvest and final harvest were recorded.

Number of primordia and fruiting bodies

Average number of primordia and well-developed fruiting bodies per packet was recorded. Dry and pinheaded fruiting bodies were discarded but tiny fruiting bodies were included in counting.

Fresh weight of individual fruiting body

Fresh weight of well-developed and mature fruiting bodies were harvested. Average weight individual fruiting body (g/fruiting body) was calculated dividing the total weight of fruiting bodies per packet by total number of fruiting bodies per packet.

Dimension of fruiting bodies

Three fruiting bodies were selected and length of pileus of three randomly selected fruiting bodies was measured. Diameter of stipe and thickness were also measured. 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 pileus (cm)

Biological yield

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

Economic yield

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

Dry weight of fruiting

The harvested fruiting bodies were dried in the sun separately as per treatment. During drying the stipe and pileus were separated. About 50 g randomly selected mushroom sample was taken in brown paper envelop. Weight of fresh mushroom along brown paper envelop was recorded. The mushroom in envelop was oven dry at 75ºC temperature for 24 hours and dry weight of mush room along with envelop was taken again.

Biological efficiency

Biological efficiency was computed using the formula, Biological efficiency (%) = {Total biological yield (g) ÷ Total dry weight (g)} x 100.

3.3.10. Proximate analysis of the mushrooms

Mushrooms grown from the spawn were collected packet wise and all the wastes and dusts were removed from the fruiting body. The proximate analysis of the mushroom of

total experiment was conducted with the determination of moisture, dry matter, crude fiber, total fat, total carbohydrate, total ash, protein and determination of mineral content.

Total carbohydrate estimation

The content of the available carbohydrate was determined by the following equation: Carbohydrate (g/100g sample) = 100-[(moisture + fat + protein + Ash + Crude fiber) g/100g] (Ashraf *et al*., 2013; Raghuramulu *et al.,* 2003)

Determination of protein

Crude protein content was obtained by multiplying the total nitrogen value by the conventional factor (Khan *et. al*, 2013; Chang & Buswell, 2003) The percentage of protein in the sample was calculated by the following equation: Crude Protein (%)=% N X (Khan *et. al,* 2013).

Total nitrogen was determined by following a series of procedure such as digestion, distillation of Samples, titration and the calculation was done by the following formula:

% N in the supplied fibre sample = (a x MHCl - b x MNaOH) x 1.401/c

Where, a = ml HCI measured into the conical task in the distill (usually 20.00ml),

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

MHCl = molarity of the HCI measured into the conical flask,

MNaOH = molarity of the NaOH used for titration,

c = g powder of mushroom used for the analysis.

Total fat estimation

Crude fat was estimated by extracting the dry materials with diethyl ether solvent. The solvent was removed by using rotator evaporate (Raghuramulu, *et al,*. 2003). The percentage of crude fat content was calculated by the following equation:

Crude Fat (%) = Weight of other extract/Weight of sample X 100

Determination of total ash

To determine total ash, 1g of the sample was weighed accurately into a crucible and placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5-6 hours at 600 °C. It was then cooled in a dessicator and weighed. (Khan *et. al*, 2013; Raghuramulu *et al., 2003*) The ash content was calculated by the following equation:

Ash (%) = Weight of ash/weight of fresh sample X 100

Determination of crude fiber

To determine crude fiber, 10g of moisture and fat-free sample was heated at 80°C for about 0.5h with 200mL 0.25N sulphuric acid solution. The boiling medium volume was kept constant by frequent addition of hot water. The mixture was then filtered through a muslin cloth and the residue washed with hot water to get it free from acid. The residue was then treated with 200mL 0.32N Na (OH) solution and again washed with hot water, alcohol and ether to remove the alkali. It was then transferred to a crucible, dried overnight at 80-100°C and weighed (We) on electric balance. The crucible was then heated in a muffle furnace at 600°C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We−Wa) represents the weight of crude fiber (Raghuramulu *et al.,* 2003; Khan *et al*, 2013).

Crude Fiber (g/100) = [100- (moisture + fat)] X (We−Wa)/ Weight of sample

Determination of moisture and dry matter

Moisture content was determined by keeping weighed quantity of sample in a thermostat controlled oven at 105ºC for 6 hours (Sarker, 2004 ;Raghuramulu *et al.,* 2003). The dry weight of each sample was taken on an electric balance. The percentage of the moisture content and dry mater was then calculated by the following formula:

Moisture (%) = Initial weight−Final weight/Weight of sample X 10 Dry Matter (%) =100−Moisture (%)

3.3.11. Analysis of minerals

The amount of minerals was determined by Atomic Absorption Spectrophotometer (analyticjenanovAA 400P; Germany), flame photometry (PFP7; Germany) and spectrophotometer (HALO BD-20S; Germany).A sub sample of mushroom weighing 0.5 g was transferred into a dry, clean 100 ml digestion vessel (Figure 2). Ten ml of di-acid (HNO_3: $HClO_4$ in the ratio 2:1) mixture was added to the flask. After leaving for a while, the flasks were heated at a temperature slowly raised to 200^0C. Heating were stopped when the dense white fumes of $HClO_4$ occurred. The content of the flask were boiled until they were become clean and colorless. After cooling, the content was taken into a 100 ml volumetric flask and the volume was made up to the mark with de-ionized water. P, K and S were determined from this digest by using different standard methods.

Figure 2. Digestion chamber

Figure 3. Atomic absorption spectrometry (HG-AAS)

To determine phosphorus content mushroom samples were digested by di-acid (Nitric acid and Per chloric acid) mixture and P content in the digest was measured by blue color development (Olsen *et al.*, 1954). Phosphorus in the digest was determined by using 1 ml sample from 100 ml digest by developing blue color with reduction of phosphomolybdate complex and the color intensity were measured colorimetrical at 660 nm wavelength and readings were calibrated with the standard P curve.

To determine potassium content 10 ml of digest mushroom sample were taken and diluted 50 ml volume to make desired concentration so that the flame photometer reading of samples were measured within the range of standard solutions. The concentrations were measured by using standard curves.

The digested S was determined by developing turbidity by adding acid solution (20 ppm S as K_2SO_4 in 6N HCl) and $BaCl_2$ crystals. The intensity of turbidity was measured by spectrophotometer at 420 nm wavelengths (Hunter *et al*., 1984).

To determine the elements (Cu, Zn, Fe, Mn) were determined from the same digest. Samples were analyzed for total expected minerals by hydride generated atomic absorption spectrometry (HG-AAS) using matrix matched standard (Figure 3). Certified reference materials were carried through the digestion and analyzed as part of the quality assurance/quality control protocol. Fe and Mn in water was measured by AAS using air-acetylene flame and matrix matched standards. 100 ppm stock solution was prepared from respective element salt. For Zn, $ZnSo_4.7H_2o$, for Fe $Feso_4.7H_2o$, for Cu $CuSo_4.5H_2o$ and for Mn, $Mncl_2\ 4H_2o$ Then the concentration of then 0, 1, 2, 4, 8 ppm prepared for standard curve. The absorbance of standard solution were taken in Atomic Absorption Spectrophotometer (AAS) The concentration of Zn in digested samples of mushroom were determined by using respective standard curve and standard curve was prepared by the machine.

The concentration obtained from AAS was multiplicated by dilution factor and actual concentration of mushroom sample was determined.

3.3.12. Statistical analysis of data

Recorded data of the experiment was analyzed following CRD using MSTAT-C computer program and means were separated following LSD test using the same computer program.

3.4. Evaluation of chemicals to sterilize substrate against contaminating fungi and production of oyster mushroom

An experiment was conducted to find out the effectiveness of chemical sterilization of substrate on contamination and production of oyster mushroom. The experiment was conducted during June to September 2016 and in the MCH of SAU. The chemicals tested were (T_1) Bavistin 50 wp (Carbendazim) at 75 ppm, (T_2) Fomalin (40% formaldehyde) at 500 ppm, (T_3) Alkalanized lime (hydrated calcium hydroxide) at 286 ppm, (T_4) Hydrogen per oxide at 30000 ppm, (T_5) Chlorox (52.5% NaOCl) 1000 ppm, (T_6) Surf –X- cel at 100 ppm, (T_7) Manganese chloride ($MnCl_2$) and (T_0) untreated control. The chemicals were evaluated both *in-vitro* and *in-vivo*.

3.4.1. In-vitro evaluation of chemicals against mushroom contaminating fungi

The *in-vitro* evaluation of the chemicals was conducted following poison food technique as described Dhingra and Sinclair (1995). Stock solution of the chemicals was prepared in water. PDA medium was prepared by mixing water extract 200 g pilled potato slices, 20 g glucose, 17 g agar and distilled water to make volume 1000 ml. The mixture was poured into 500 ml conical flasks and sterilized after mixing with the chemical. Stock solutions of all chemical were prepared in sterile water. Requisite quantity of stock solutions of the test chemicals was added to the PDA. The mixture of stock solution of chemicals and PDA were mixed thoroughly for proper amendment. Amended PDA was sterilized by autoclaving (at 120˚C under 1.1kg/cm^2 for 20 minutes) and poured into 90 mm sterilized Petri dishes at 20 ml/dish. Petri plates having PDA without any chemical served as a control. After solidification of PDA, the plates were inoculated by placing one 5 mm mycelium blocks of 6 days old culture of *A. niger, A. flavus, Rhizopus, Trichoderma*, *Penicillium* and *Sclerotium*. In in-vitro test culture of *P. ostreatus* was also included to find out response of a mushroom fungus to the test chemicals. The inoculated plates were incubated at room temperature (30±2ºC) until the mycelium reached the rim of Petri dishes. Petri dishes with un-amended PDA served as control. The mycelial inhibition was calculated using the formula {(dc-dt)÷dc}x100, where dc=diameter of colony and dt=diameter of colony in amended plates.

3.4.2. *In-vivo* evaluation of chemicals for substrate sterilization against contaminating fungi and production of oyster mushroom

For *in vivo* evaluation of the chemicals, the inocula of the contaminating fungi were multiplied in water soaked chickpea in conical flasks. Water soaked chickpea was inoculated with 5 mm mycelium blocks cut from 6 days old pure culture. After colonization, sterilized distilled water was poured into the flasks and shaken well with hands to release the spores. The conidial suspension was filtered through cheesecloth to remove mycelium fragments. Concentration of conidial suspension was adjusted to 10^7 spores per milliliter using a Haemacytometer.

Substrate was prepared with rice straw as basic material following the procedures as described earlier. Air dried substrate was inoculated with the contaminating fungi by spraying 100 ml spore suspension per kilogram of substrate. Inoculated substrate was incubated for 7 days at room temperature for colonization of contaminants and air dried. The substrates were treated with the chemicals by deeping in freshly prepared solutions having required concentration (ppm) for 24 hours. Spawn packets were prepared with the substrate following the procedures described earlier. Spawn packets were inoculated with culture of oyster mushroom strain-4 and incubated in the MCH under the conditions mentioned earlier.

3.4.3. Design of experiment, data collection and data analysis

The experiment was laid out in CRD with 5 replications. The data on the following parameters were collected following the procedures as mentioned under earlier experiments. For data analysis MSTAT-C computer program was used. Means were compared following LSD test with the same computer program. Data collected were :

(i) Radial colony diameter and percent inhibition colony growth of the selected contaminating fungi

(ii) Prevalence (%) of contaminated fungi in spawn packet

(iii) Mycelium run rate in spawn packet

(iv) Number of primordia per spawn packet

(v) Number of fruiting bodies per spawn packet

(vi) Diameter and thickness of pileus

(vii) Number of flushes and prevalence of contaminated fruiting bodies

(viii) Fresh weight of individual fruiting body

(ix) Biological efficiency

3.5. Effect of pasteurization of substrate with steam on contamination and production of oyster mushroom

3.5.1. Objective, duration, location,

The experiment was conducted to determine the effect of steam sterilization of substrate in a pasteurizing steel drum on contamination of spawn packet and production of oyster mushroom. Duration of the experiment was April to June, 2015 and the location was MCH and plant pathology laboratory of SAU.

3.5.2. Treatments

The substrate packets were prepared using rice straw at 500 g per packet. The substrates were steam sterilized in a steel drum at 80ºC for 2 hr (T_1), 3 hr (T_2), 4 hr (T_3), 5 hr (T_4) and 6 hr (T_5). For comparison of efficacy of the drum another treatment (T_6) was maintained where substrate was sterilized in an autoclave for 90 minutes at 121ºC and under 1.1 kg/cm^2 pressure. A control (T_0) was maintained where substrate was not sterilized.

3.5.3. Preparation of substrate

Rice straw was used as the basic materials of substrate. Rice straw was sun dried and cut into small piece, spread on wooden desk and inoculated with spore suspension of contamination fungi. For preparation of spore suspension, *Aspergillus*, *Penicilium* and *Trichoderma* were grown in PDA pates, spores were collected in sterilized water and spore suspensions were prepared. For inoculation, spore suspensions were sprayed over pieces of straw and air dried. Inoculated rice straw was sterilized with steam at par selected treatments.

3.5.4. Growing of oyster mushroom, collection of bacterial blight infected fruiting bodies and isolation of causal bacterium

Oyster mushroom was grown in the MCH of SAU following the methods described earlier under different experiments. Fruiting bodies infected with bacterial blight were collected during growing period and preserved in a refrigerator at 4ºC.

Nutrient agar (NA) medium was prepared following the procedure of Schaad *et al.*(1988). To prepare 1000 ml NA medium 15 g bactoagar was taken in a conical flask containing 1000 ml distill water. Exactly 5 g peptone and 3 g beef extract were added to the flask. The mixture was shaken vigorously for few minutes and autoclaved at 121ºC for 20 minutes under 1.1 kg/cm^{2}pressure.

Collected diseased fruiting bodies were washed under running tap water and cut into small pieces. The pieces were surface sterilized by dipping in 5% $NaOCl_2$ solution for 2-3 minutes and ringed with sterile distill water for three times. After surface sterilization, the cut pieces were kept for 30 minutes in 2 ml sterilized water for bacterial oozing to prepare a stock bacterial suspension. The stock suspension was diluted with water to have bacterial concentration of 10^1 to 10^4. Exactly 0.01 ml of each dilution was spreaded over NA plat. Spreading was done with sterilized glass rod. The inoculated NA plates was incubated in an incubator at 30ºC. The plates were checked after 24-48 hours to observe growth of bacteria. Bacterial colonies grew from the pieces of fruiting bodies were streaked on fresh NA plates. Pure colonies of bacteria were selected and transferred to fresh NA plates for preservation.

3.5.5 Design of experiment, data collection and data analysis

The experiment was laid out in CRD with five replications. Data were collected on the following parameters and analyzed using MSTAT-C computer program:

(i) Incidence of contamination of spawn packets during incubation
(ii) Incidence of contamination of spawn packets during cultivation
(iii) Severity of contamination of spawn packet
(iv) Bacterial blotch or yellowing of fruiting bodies
(v) Days required for completion of mycelium

(vi) Mycelium run rate

(vii) Days to formation of primordia

(viii) Number of primordia

(ix) Diameter of pileus

(x) Biological and economic yield, dry weight, biological efficiency

3.6. Effect of substrate sterilization with hot water on contamination and yield of oyster mushroom

3.6.1. Objective, duration and location of the experiment

The experiment was conducted to determine the effect of hot water sterilization of substrate on contaminating fungi and yield of oyster mushroom. The experiment was conducted during June to April 2015 and in the plant pathology laboratory and MCH of SAU.

3.6.2. Treatments

The experiment was laid out following 3x3 factorial design with 5 replications. The first factor was level of temperature and the second factor was duration of sterilization. Rice straw was used to prepare substrate; Substrate was inoculated with contaminating fungi following the procedures as mentioned earlier. Inoculated rice straw was sterilized with hot water following the procedures. The levels of temperature of hot water were 60ºC (T_1), 80ºC (T_2) and 100ºC (T_3). Under control (T_0) substrate inoculated with contaminating fungi was used without any treatment. The durations of sterilization tested in the experiment was 1 hr (D_1), 2 hr (D_2) and 3 hr (D_3) under different levels of temperature.

There were 9 (3x3) treatment combinations. The treatment combinations were T_1D_1, T_1D_2, T_1D_3, T_2D_1, T_2D_2, T_2D_3, T_3D_1, T_3D_2, T_3D_3. A control (T_0) was maintained where substrate was not sterilized.

3.6.3. Experimental substrates, procedures of spawn packet preparation, inoculation, incubation, cultivation and harvesting of mushroom

The materials used in the experiment, procedures of spawn packet preparation, inoculation, incubation, cultivation and harvesting were the same as mentioned under experiment 5. Preparation of inoculum of different contaminating fungi, inoculation of

substrate with spore suspension of contaminants was the same as mentioned under experiment 5. Before inoculation with contaminants the substrate was pasteurized.

3.6.4. Sterilization of inoculated substrates with hot water

The substrate packets were prepared using rice straw at 500 g per packet. The substrate was inoculated with contaminating fungi and sterilized with hot water at 60, 80 and 100ºC temperature for 1, 2 and 3 hours. Hot water treated substrates were placed on sieves for few minutes to remove excess water.

3.6.5. Data collection and data analysis

Data were collected on the following parameters:

i. Incidence of contamination in spawn packets during incubation
ii. Contamination severity
iii. Mycelium run rate
iv. Days required for completion of mycelium running
v. Effect of temperature x duration of sterilization of substrate on contamination
vi. Days required for primordia formation, first harvest and total harvest
vii. Days required to primordia formation, first harvest and total harvest
viii. Interaction effect of temperature and duration of hot water sterilization of substrate on days required for primordia initiation, first harvest and total harvest
ix. Interaction effect of temperature and duration of hot water sterilization days required for primordia initiation, first harvest and total harvest
x. Effect of duration of hot water sterilization of substrate on biological yield
xi. Interaction effectof level of temperature and duration of hot water sterilization on biological yield of a mushroom
xii. Effect of hot water sterilization of substrate on dry yield, economic yield and biological efficiency

Design of the experiment was followed used in previous exponent. Procedures of data collection data analysis were the same as used in experiment 5.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Experiment 1. Survey on status of mushroom cultivation and contamination with mycoflora in Bangladesh

Data collected during survey have been described under the following headings.

4.1.1. Mushroom species of mushroom cultivated and different seasons of cultivation in Bangladesh

Among the mushroom farms visited during survey in different districts of Bangladesh, the maximum 73.0% farms cultivated oyster mushroom, 17% reishi mushroom, 5% straw mushroom, 3.0% milky white and 2.0% button mushroom (Figure 4).

The farmers reported that 72.0 % farmers cultivated oyster mushroom in both winter and summer seasons, 26.0% cultivated only in winter and 2.0% only in summer (Figure 5). In summer, only strain PO_2 of oyster mushroom was cultivated above 51.2% and in winter above 36.4%. Strains PCYS (*Pleurotus cystidiosus*), HK (High king) and WS (White snow) of oyster mushroom were reported to grow well in both winter and summer. Only POP (pink oyster mushroom) was cultivated 25% in winter and 6.7% in summer whereas, PO_2 and POP were cultivated together 18.5% in winter. PO_2, POP, WS, PCYS, and HK were 11.4% in summer and 7.1% in winter. WS, PCYS, HK with PO_2 were cultivated by 19.1% in summer, WS, PCYS, and HK with POP were cultivated by 6.0% in winter (Table 2).

So, it has been found that different species of mushrooms are cultivated in different seasons of Bangladesh. The findings indicate that different species and strains of mushroom are suitable to cultivate in the country. Among these $PO_2$of oyster mushroom is very popular to farmers and consumers. Other researchers also reported that cultivation of oyster mushroom is gaining importance in tropical as well as

subtropical regions due to its simple way of cultivation and high bio efficiency (Singh *et al.*, 1990).

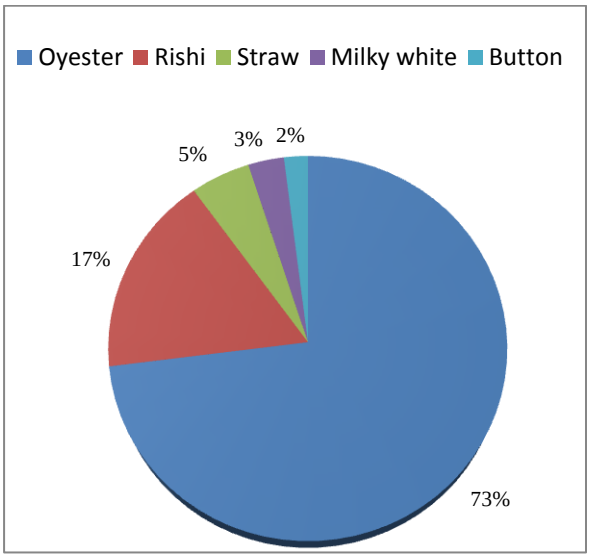

Figure 4. Percentages of production of different mushroom species in Bangladesh

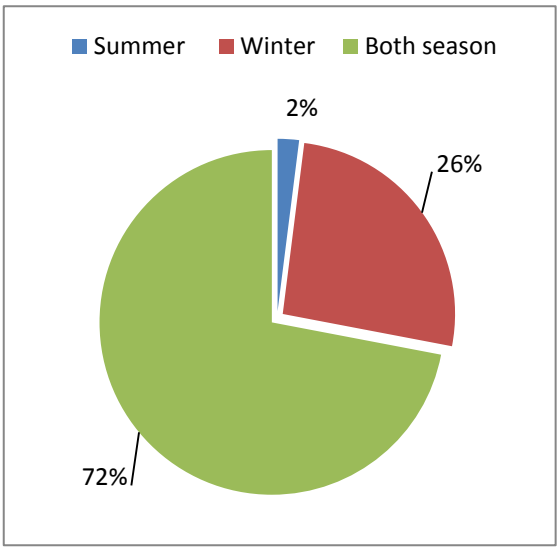

Figure 5. Seasons of cultivation of mushroom in Bangladesh

Table 2. Different strains of oyster mushroom cultivated by farmers of Bangladesh in summer and winter

Strains of oyster mushroom and other species	% Famers cultivated oyster mushroom in two winter seasons	
	Summer	Winter
PO_2	51.2	36.45
POP	6.71	25.06
PO_2+WS+PCYS+HK	19.1	6.0
PO_2+POP+WS+HK	11.4	7.1
POP+WS+ PYCS	7.7	-
POP+PYCS	2.9	-
POP+HK+PCYS	1.0	-
POP+WS	-	6.9
PO_2+POP	-	18.5

PO_2 –*P. ostreatus 2*, HK- High King, WS-White snow, Pop-pink oyster, *PCYS-P.cystidiosus*, FLO- *P. florida*,- *Not cultivated*

4.1.2. Sources and preparation of mother culture and mother spawn

The highest 51.3% farmers produced their own mother culture from tissue of fruiting bodies, while 25.6% farmers used either tissue or another mother culture. The lowest 23.1% farmers produced mother culture from previous mother culture or mother spawn (Figure 6). Pure culture is grown on PDA from tissues of fruiting body and then transferred onto grain or plant parts for media preparation of mother culture.

The maximum of 49.0% mushroom farmers collected mother spawn from mushroom content followed by 23.0% farmers produced by them and 10.0% collected from private enterprises. The lowest 12.0% farmers prepared either themselves or collected from private enterprises or mush centers preparation of spawn for cultivation of mushroom (Figure 7).

The highest 40.0% farmers used wheat grains as the medium for production of mother spawn, 28.0% used paddy grains, 15.0 % saw dust with straw, 10% saw dust mixed with wheat bran and 5.0% used millets (Figure 8).

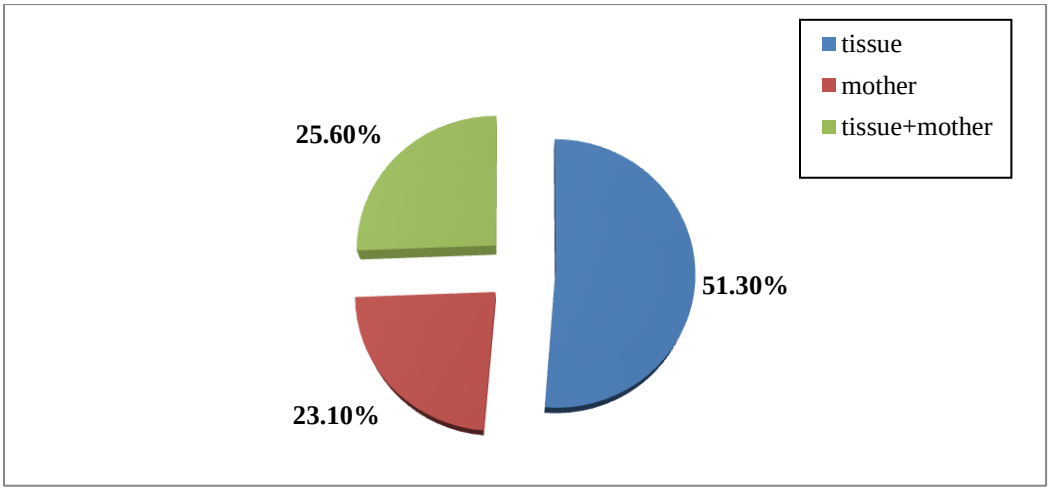

Figure 6. Fungal sources of mother culture used by farmers

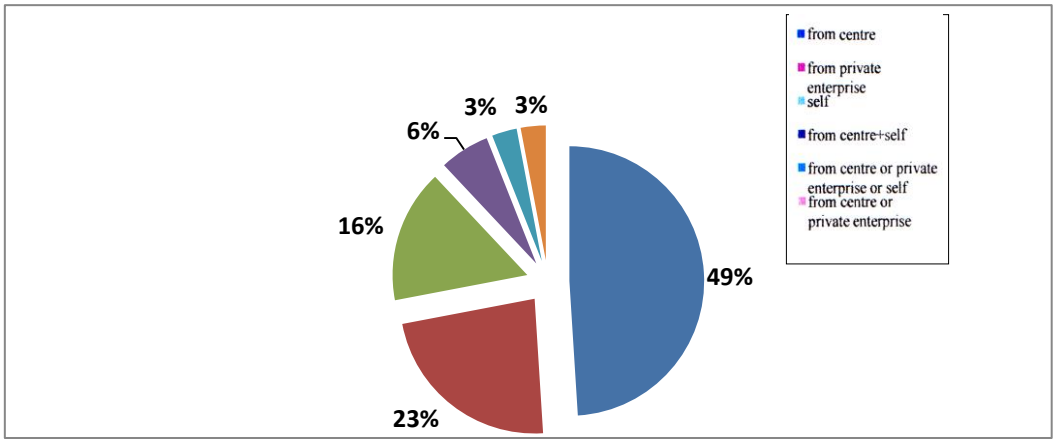

Figure 7. Different sources for collection of mother cultures

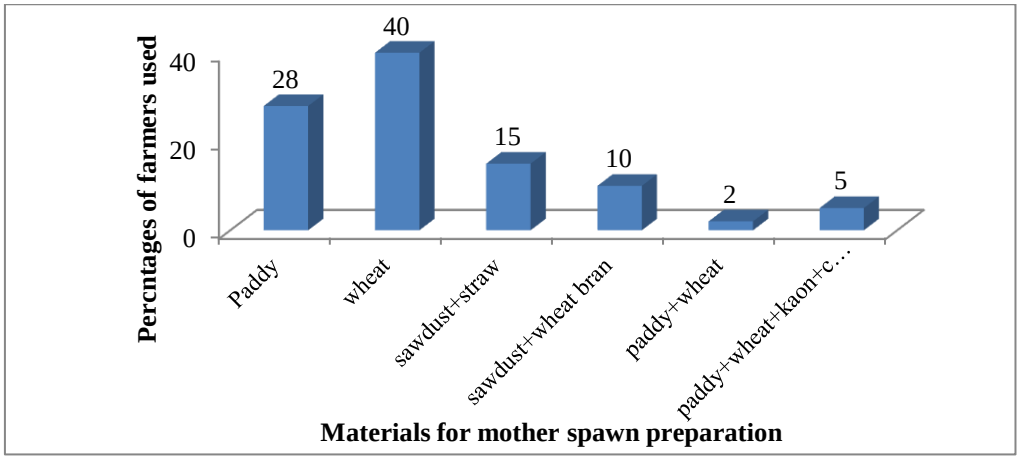

Figure 8. Materials used for mother spawn preparation

4.1.3. Spoilage of mother spawns due to contamination

The lowest 10.0% spoilage was reported by the maximum of 28.33% farmers, 20.0% by 27.0%, 30% spoilage reported by 18.9%, 40% by 40% farmers and 50% by 2.7% farmers and above 2.7% spoilage was reported by more than 50% farmers (Figure 9).

4.1.4. Information about substrates for oyster mushroom cultivation

Among 110 mushroom growers selected for data collected, the maximum of 55.7% used rice straw, 21.6% used saw dust, 15.1% used rice straw mixed with saw dust, 3.4% used wheat straw, another 3.4% used wheat straw mixed with saw dust and the minimum 1.1% famers used sugarcane baggase as substrates (Figure 10).

Among 110 mushroom growers selected for data collected, the highest 55.8% used different kind of supplements with substrates to enhance nutrient contents and vigority of mushroom and 44.2% did not used any kind of supplement (Figure11). The maximum of 50.8% farmers used rice bran as a supplement followed by 20.8% used combination of rice bran and wheat bran, 18.7% used wheat bran alone and 10.3% farmers used combination of rice bran, wheat bran and rice husk (Figure 12).

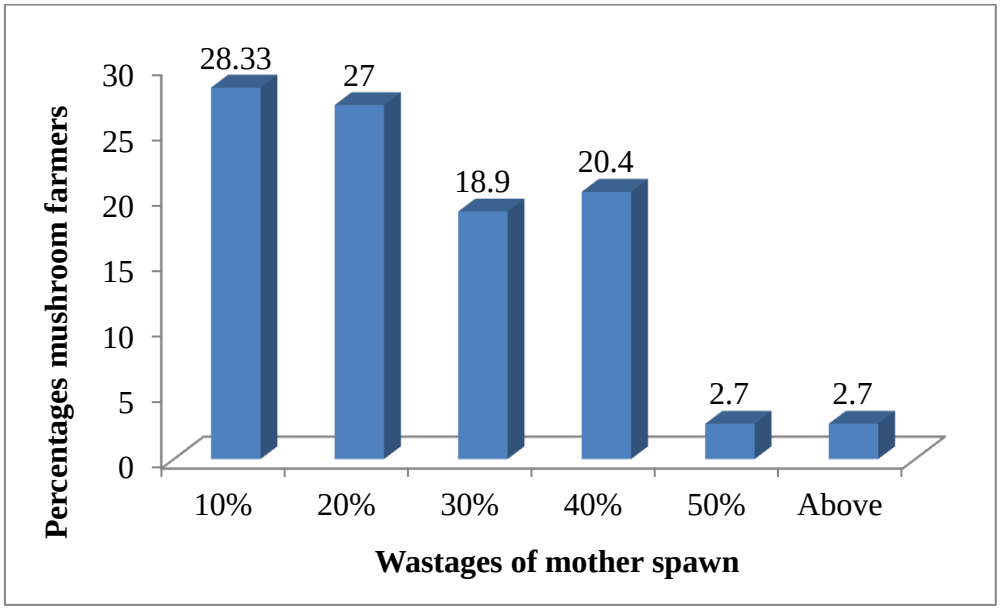

Figure 9. Wastages of mother spawn of mushroom

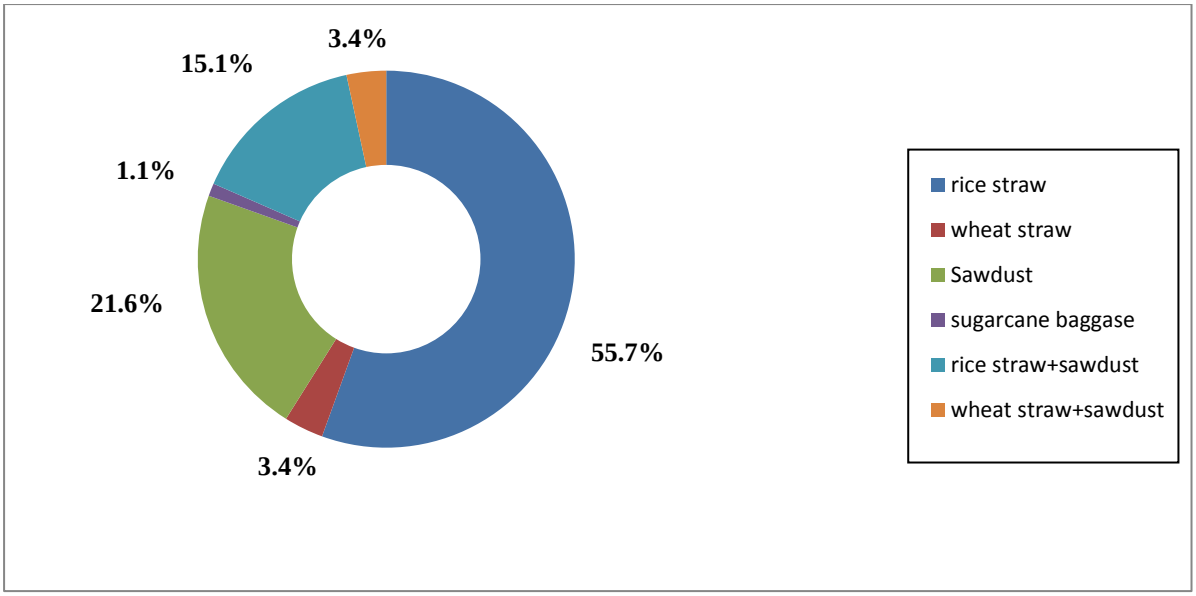

Figure 10. Substrates for oyster mushroom cultivation

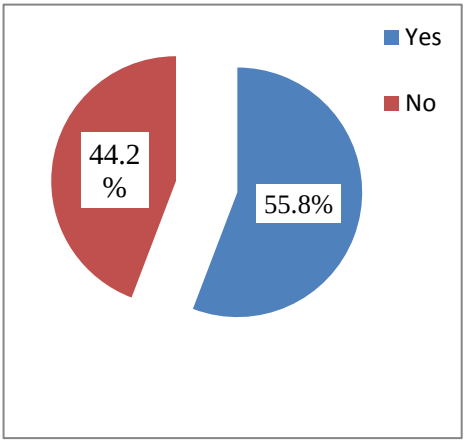

Figure11. Perceptions of farmers about used supplements to prepare substrate of mushroom

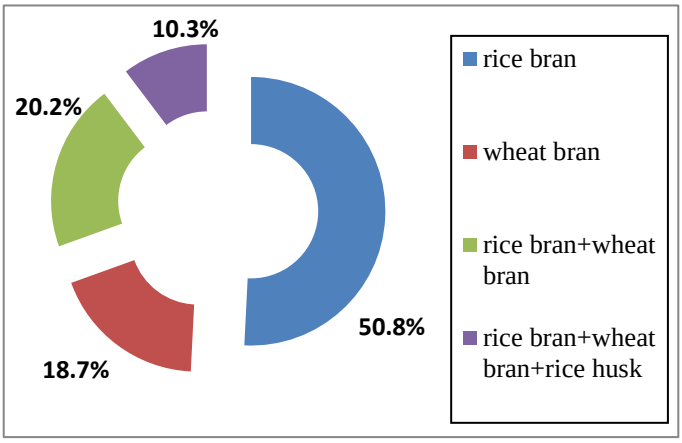

Figure 12. Different supplements used with substrates

4.1.5. Methods of substrate sterilization used by farmers

Methods of sterilization or pasteurization, duration of sterilization or pasteurization and percentage of farmers used different methods are shown in Figure 13. During survey it was found that 52.7% farmers pasteurized substrates of mushroom using hot water treatments for one hour, 26.7% using autoclave for one hour, 8.6% using steam pasteurization in drum for one hour, 1.2% for 5 hours, 1.5% using sundry and 7.0% growers pasteurized the substrates in NAMDEC steam oven for 2 hours while 2.3% for 6 hours. NAMDEC steam oven is special type oven for pasteurization of substrate developed by NAMDEC (Figure 13).

4.1.6. Types of mushroom house and sources of water for growing mushroom

Generally, five types of mushroom houses were reported to be used for cultivation. These were bamboo house, mud house, thin shed, hut and brick building. The maximum of 57.3% mushroom were cultivated in bamboo made houses, 26.2% in tin shed, 6.8% in brick made buildings 5.8% in hut and 3.8% in mud houses (Figure 14).
The sources of water used for irrigation were tap, tube, pond and well. The water sources were found to be used by 48.6, 44.8, 4.8 and 1.9% mushroom farmers, respectively (Figure 15).

4.1.7. Number and size of spawn packet prepared for cultivation of mushroom

The number of spawn packets prepared by 34.0, 12.0, 21.0, 9.0, 8.0, 10 and 7% mushroom farmers were 500, 500-1000, 1000-1500, 1600-2500, 2500-3500 and more than 4500, respectively (Figure 16). The maximum of 47.2% farmers prepared 0.5kg, 40.2% prepared 1.0kg, 9.8% prepared 2.0kg, and 2.0% farmers prepared 4.0kg size spawn packets (Figure 17).

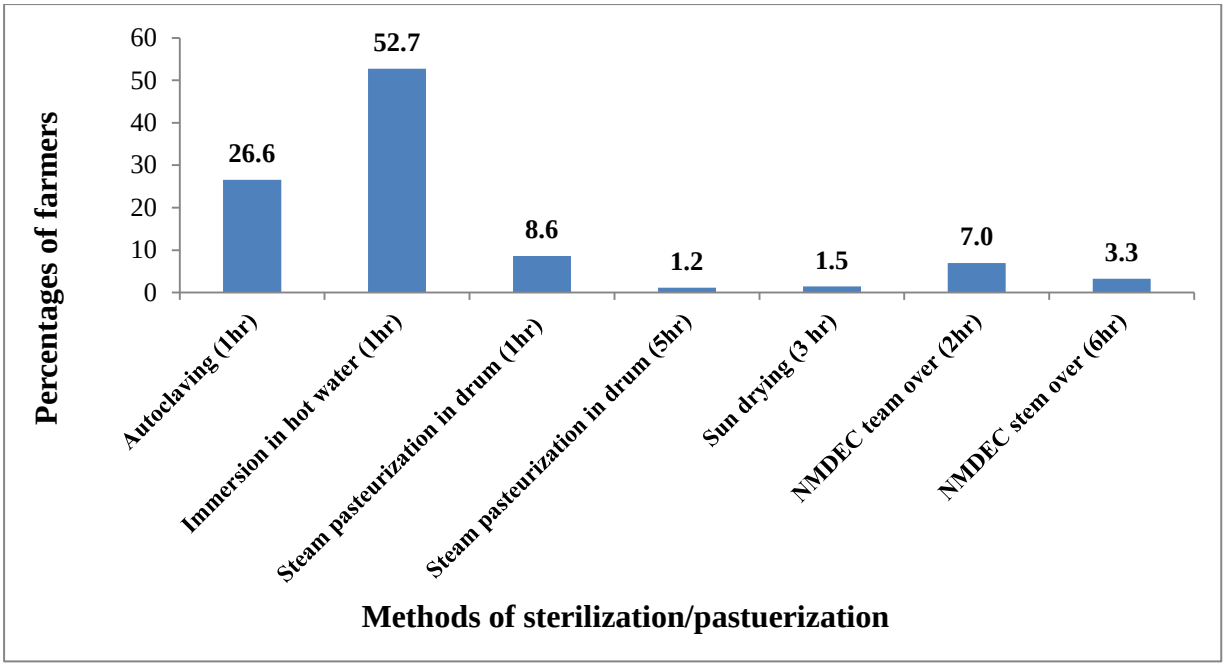

Figure13. Methods of sterilization/pasteurization of mushroom substrates in survey areas of Bangladesh (NAMDEC= National Mushroom Development and Extension Center)

4.1.8. Number of harvest and marketing of mushroom

During entire growing period, mushroom was harvested 2, 3, 4, 5 and 6 times by 3.2, 5.7, 32.1, 40.6, and 20.6% farmers, respectively during summer (Figure 14). During winter 1.1, 66.7, 23.7, 5.4 and 1.0% farmers harvested 2, 3, 4, 5 and 6 times, respectively (Figure15). According to the survey, 78.0% farmers marketed their mushroom in fresh form, 21.6 in dry form and 10.9% marketed in powder form (Figure 16).

4.1.9. Different activities of mushroom farmers performed

During survey, it has been reported that 65.6% farmers prepared spawn packets and cultivated mushroom, 21.6% farmers prepared commercial spawn packets. The activities of other 4.1, 3.0, 3.8 and 1.9% mushroom farmers were marketing of mushroom; spawn preparation or commercial packets cultivation; only spawn preparation and marketing; and research, production and training and marketing, respectively (Figure 17).

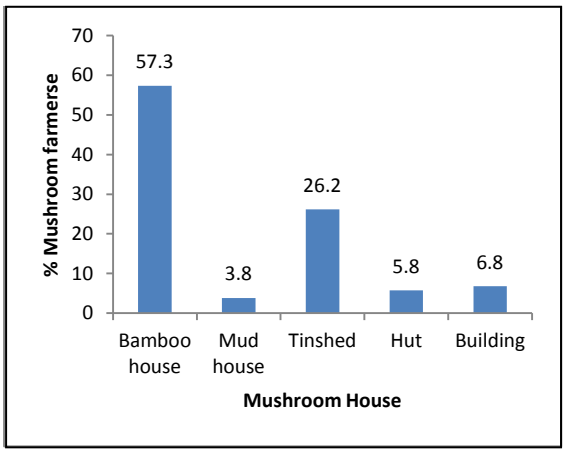

Figure 14. Types of mushroom house used by different percentage of mushroom famers

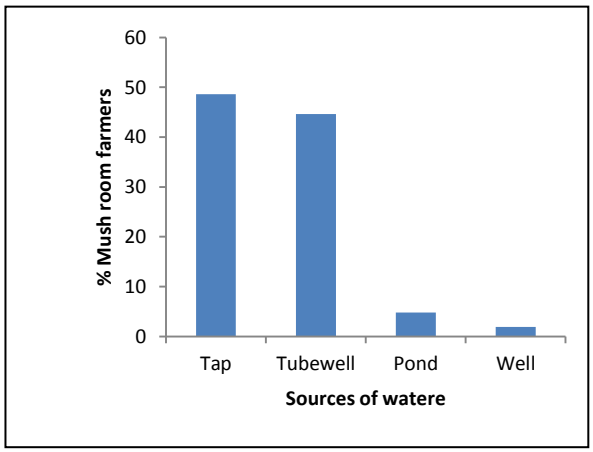

Figure15.Different sources of water used by different percentage of mushroom famers

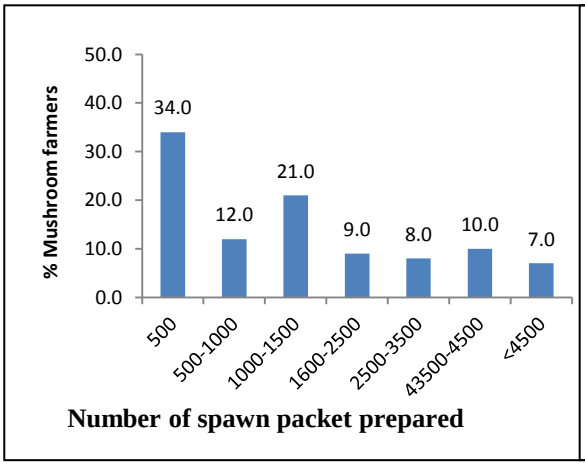

Figure16. Number of spawn packet prepared by farmers to cultivate mushroom

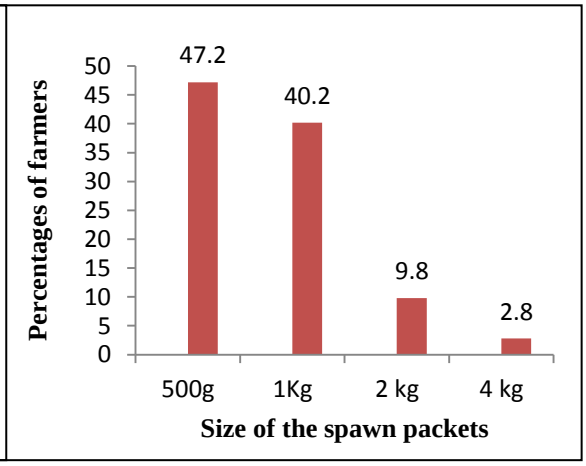

Figure 17. Size of spawn packets prepared by farmers to cultivate mushroom

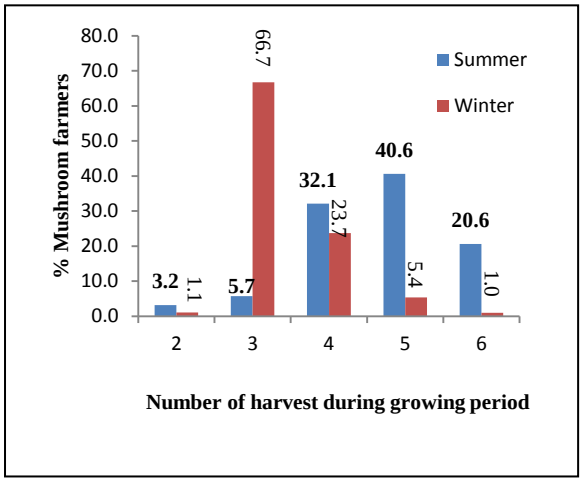

Figure 18. Number of harvest of mushroom during growing period in summer and winter

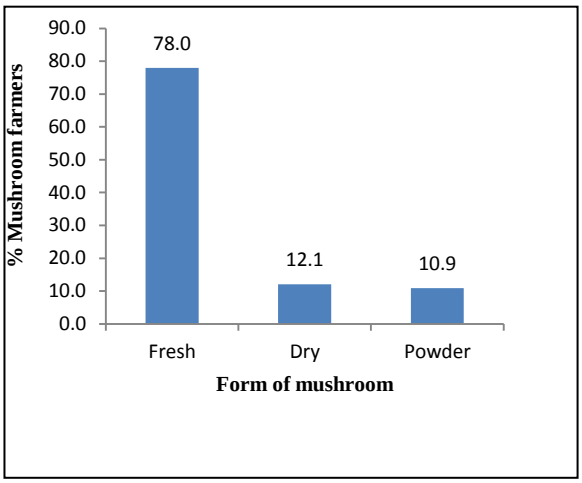

Figure 19. Three forms of mushroom for marketing by the farmers

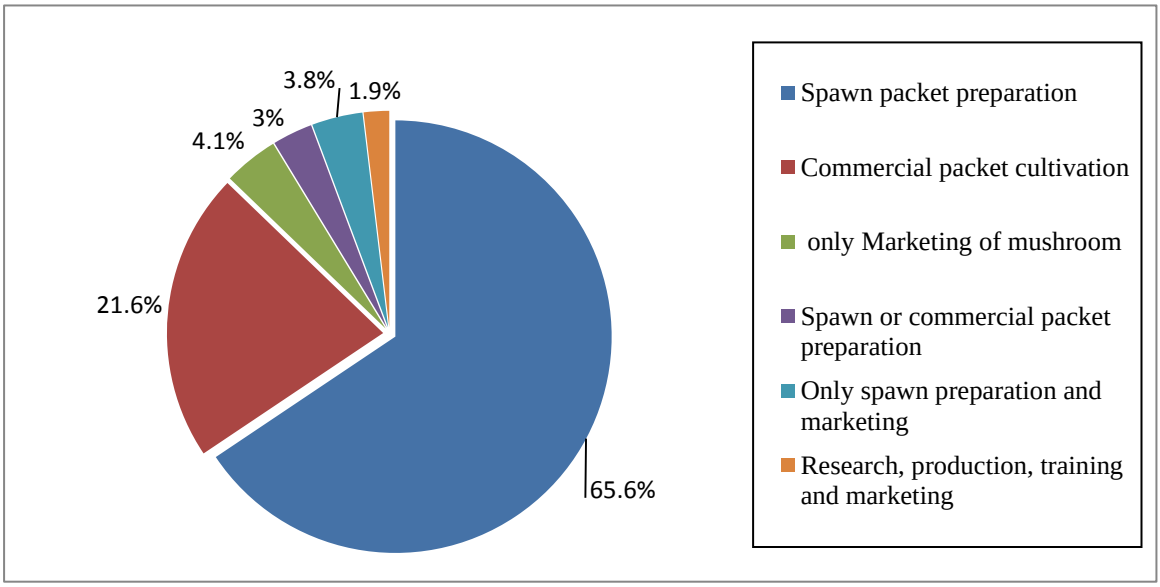

Figure 20. Various activities performed by mushroom farmers

4.1.10. Farmers response about contamination during cultivation in summer and winter

The maximum of 46.3% followed by 27.4, 9.5, 8.4, 5.3 and 3.2% farmers reported >10, 10, 20, 30 and 40% spoilage of spawn packets during incubation in summer (Figure 21).

Less than 10, 10, 20, 30 and 40% spoilage of spawn packet during cultivation was recorded by 47.1, 27.6, 14.9, 8.0 and 2.3% mushroom farmers during cultivation in winter (Figure 22).

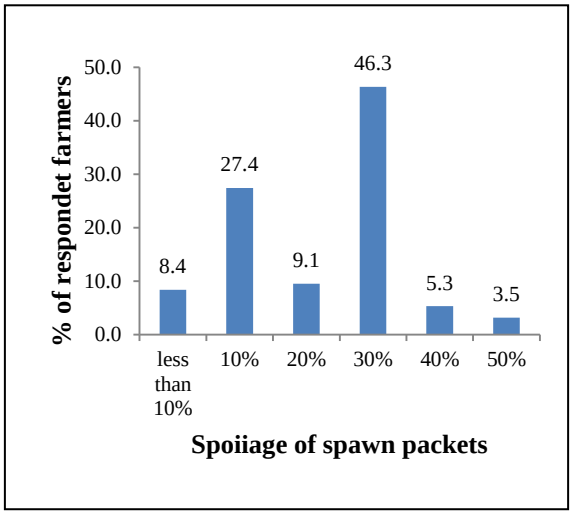

Figure 21. Percentage of farmers reported spoilage of spawn packets during cultivation in summer

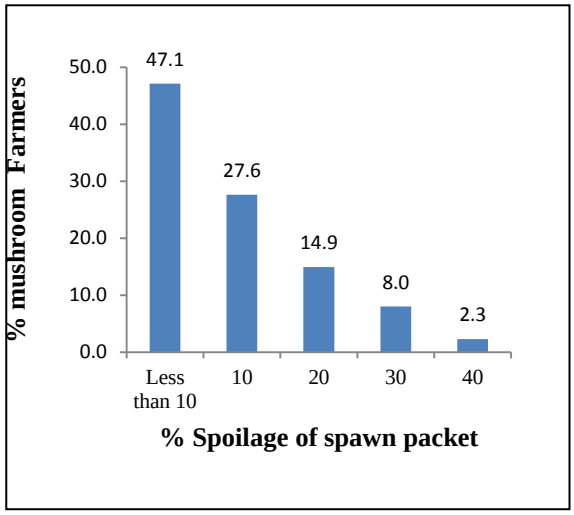

Figure 22. Percentage of farmers reported spoilage of spawn packets during cultivation in winter

4.1.11. Farmers' idea about contamination, contaminating fungi and symptoms of contamination of spawn and fruiting bodies

Among the selected farmers 94.9% mushroom farmers expressed their concern about spoilage of mother spawn due to contaminations and 5.1% farmers did not have idea about contamination of spawn packets (Figure 23).

The survey revealed that the highest 67.0% farmers did not know name of the contaminants, whereas 24.0% farmers knew name of contaminants and symptoms developed due to attack of *Trichoderma*, 7.0% science literate farmers and service holder of mushroom centers reported the name of *Trichoderma*, *Aspergillus* and *Penicillium*as contaminating fungi. The lowest 2.0% farmers knew about green mold disease of spawn packet (Figure 24).

Among 110 mushroom farmers, 13.2% reported only black, 17.9% reported only green, maximum 35.7% farmers noticed black or green, 4.8% reported green or brown, 17.0% reported black, green or yellow, 8.5% reported green or yellow, 1.8% noticed white and 1.1.% farmers reported no change in color of spawn packets due to contamination with different types of microorganisms (Figure 25).

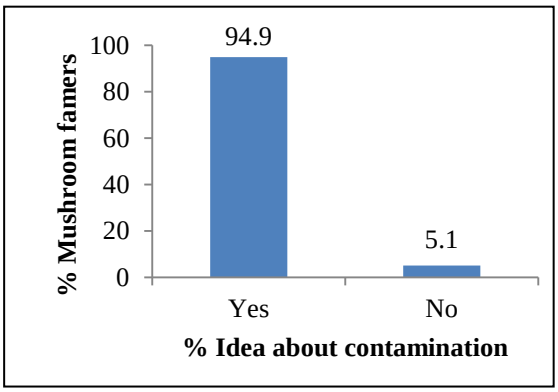

Figure 23. Idea of mushroom farmers about contamination of spawn packet.

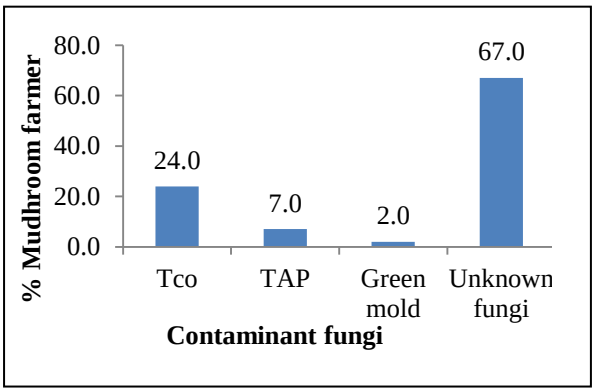

Figure 24. Fungal contaminating fungi reported by mushroom farmers survey area (Tco=*Trichoderma*, TAP=*Trichoderma+Aspergillus+Penicillium*)

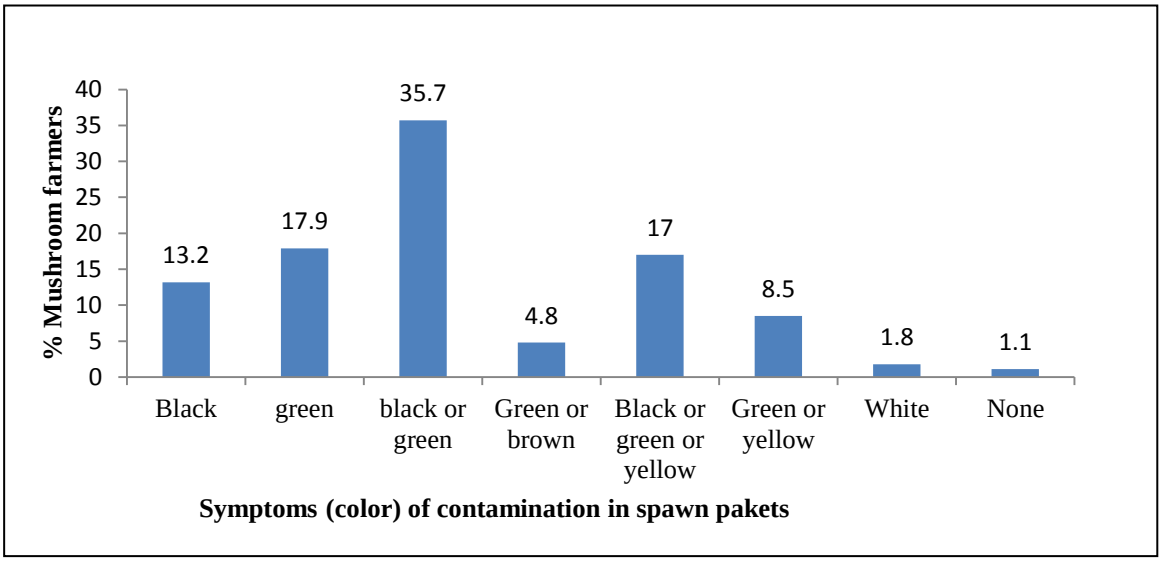

Figure 25. Symptoms in the forms of color on spawn packets due to contamination by different types of microorganism

Among 110 mushroom farmers, 20.8% farmers reported curling, 20.8% noticed brown, 13.8% noticed small size, 11.7% reported less number, 18.5% noticed curling, small size and less number, 6.8% famers observed black and 8.6% famers observed

curling , brown, small size and less number of fruiting bodies due to contamination with different types of microorganism (Figure 26).

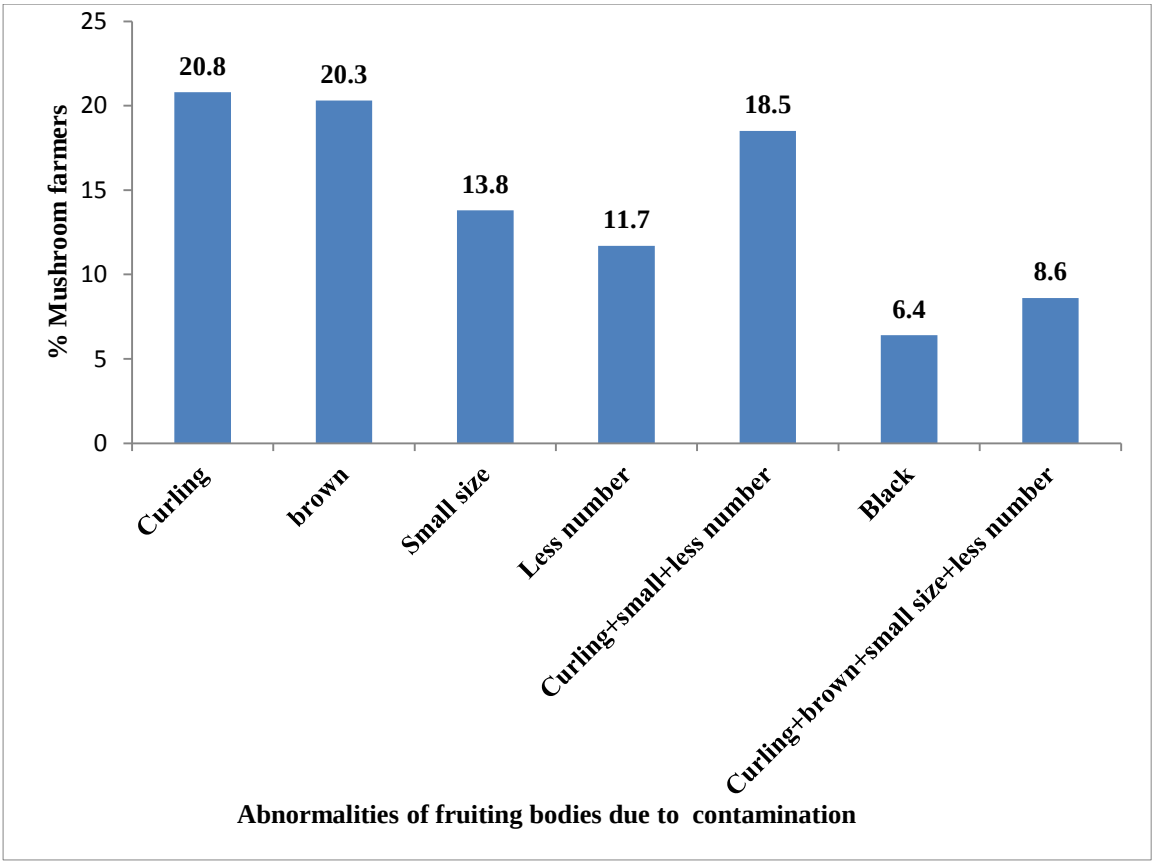

Figure 26. Symptoms of contamination on spawn packets due to contamination by different microorganisms

4.1.12. Various problems identified by mushroom famers

In surveyed area, seed crisis and spawn packet contamination were identified as important problems of mushroom cultivation by 58.0% famers, seed crisis was identified by 25.0% famers, only seed contamination was identified by 16.0% farmers, marketing problem was identified by 4.0%, and varietal problem reported by 3.0% mushroom famers (Figure 27).

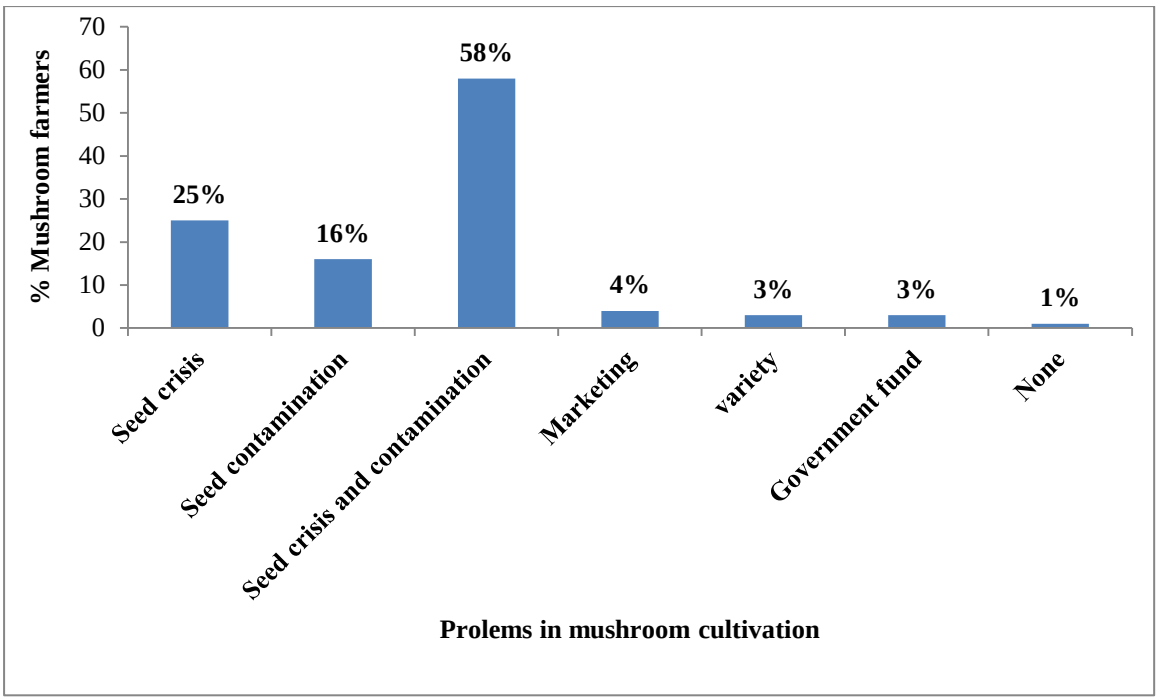

Figure 27. Miscellaneous problems faced by mushroom farmers during cultivation

4.1.13. Discussion

The information obtained from survey on selected mushroom farmers supported by the recommendation of some previous researchers, which are presented here. Shah *et.al*, (2004) reported that the mushroom cultivation is a profitable agri-business and oyster (*Pleurotus ostreatus*) mushroom is an edible mushroom having excellent flavor and taste. Roye (2002) stated that among the edible mushroom species, oyster mushroom (*Pleurotus* spp.) is the second ranking mushroom in the world. But, in case of cultivation, oyster mushroom secured the highest position in Bangladesh. Nwanze *et al.*, (2005) stated that environmental factors such as temperature, O_2, Co_2, humidity, light, p^H affect the mycelial growth in the spawn. *Pleurotus* spp grows in wide range

of temperature (15-30^{0}C) which also varies from species to species (Sarker *et al.*, 2008). Uddin *et al*., (2011) reported that winter (temperature zone 14-27^{0}C with relative humidity (70-80%) is suitable for better production and biological efficiency of *Pleurotus* spp in Bangladesh. Amin (2004) revealed that Oyster mushrooms are ideally suitable for cultivation under both temperate and tropical climatic conditions and can be harvested all over the year (Some of oyster mushrooms are adaptable both in summer and winter season. In Bangladesh, summer is the most long lasting season and it is possible to cultivate many kinds of oyster varieties in this season. So, it is possible to mitigate the needs of mushroom all year round, which was corroborated with present study. Klingman (1950) reported that the spawn is the vegetative mycelium from a selected mushroom grown on a convenient medium or substrate. As per Mbogoh (2011), spawn is pure culture of mycelium grown on a solid substrate such as cereal grain. It is the mushroom seed, comparable to the seed of crop plants. It serves as the planting material in mushroom cultivation. Different materials are used to multiply the mother spawn (seed) for large scale production. viz. cereal grains such as wheat (Elhami*et.al*., 2008; Chang 2009; Stanely, 2010), rye (Chang, 2009) rice (Oei, 1996), millet sorghum, white maize (Stanely, 2010) were reported to use as media for mother spawn production. Kumbhar (2012) reported that mycelium of *P. eous* had marked preference for cereal grains over pulses and crop residues and ragi grains took only six days for mycelial colonization followed by maize, pearl millet, sorghum, wheat and paddy grains while pulses did not allowed growth of the fungal mycelium. Munser *et al*., (2012) reported that wheat substrate was the best substrate for spawn production of oyster mushroom followed by rice and wheat bran. Sofi *et al*., (2014) evaluated wheat grains, barley maize and millets for spawn production and reported maximum growth rate in the corn (38.60mm) and minimum in millet (26.80mm) after 12 days of inoculation. From survey report, it was noticed that in Bangladesh, oyster mushrooms are most popular and different species of this mushroom like *Pleurotus ostreatus*, *P. florida*, *P. sajor-caju* and *P. high king* are commercially cultivated all over the year by using sawdust and/or rice straw as main substrate, which was also reported by Amin *et al*., (2007). Maniruzzaman (2004) in his study found that substrate rice straw was the best for spawn production of oyster mushroom. Sarker *et al.*,(2007) tested waste paper, wheat straw, rice straw, sugarcane bagasse and *Saccharam spontaneum* as the substrates of *Pleurotus ostreatus* and indicated possibility of their commercial use. Substrate selection depends on the

availability or costs or area for mushroom production in Bangladesh. Different substrates can, therefore, be recommended per region depending on local availability of agricultural wastes (Cohen *et al.* 2002). The supplementation of the substrates with various sources of organic nitrogen, such as wheat bran, rice bran, maize waste water, soya cake powder and rice, has increased the biological efficiency of various species of basidiomycetes (Loss *et al.,* 2009; Moonmoon *et al.,* 2011). Siqueira *et al.,* (2012) proposed a number of different methods for substrate pasteurization or sterilization viz. autoclaving (axenic), axenic and inoculation with thermophilic microorganisms, rapid substrate steam treatment between 80^0 and 100 °C for several hours, pasteurization at 72 °C for four or five days and pasteurization by substrate steam treatment for several days (60 °C) in a tunnel.

Tewari (1986) reported that the mushroom fungus requires a considerable amount of water for fruiting bodies formation, due to the high content of water in mushrooms. Sarker *et al.***,** (2007) reported that time required from stimulation to primordia initiation, stimulation to first harvest, total harvest, the number of fruiting bodies, biological efficiency and economic yield of *Pleurotus ostreatus* were influenced significantly by frequency of watering. López *et al*., (1995) determined the quality of water for pasteurizing substrate (coffee wastes) of *P. ostreatus* cultivation. They found that residual water could become highly polluted. As a consequence, this factor may have affected mushroom yield.

Contaminants are one of the major problems in mushroom spawn production. Various microorganismse.g, bacteria, actinomycetes, yeast and fungi affect spawn preparation, sometimes leading to total spawn spoilages (Biserka, 1972). The 'wet spot' or rottening of spawn was first reported by Stoller (1962). Mazumder and Rathaiah (2001) found *Trichoderm aharzianum, Aspergillus* spp and *Pencillium* spp as the three most dominant fungal contaminants during spawn production in oyster mushroom. Mazumder *et al*., (2005) isolated and identified eight fungal and one bacterial contaminant from naturally contaminated spawn of oyster mushroom. They observed month wise variation in spawn contamination and found that the combination was highest during the monsoon season (28.57%) followed by pre-monsoon (21.9%). They also reported that paddy grain based spawn recorded significantly lowest (15.00%) contamination as compared to wheat grain based (30.0%) spawn.

4.1.14. Experiment 4.1.ii .Study on prevalence of contaminants from collected contaminated spawn packets from survey area

4.1.15. Collection of contaminated spawn packets

Contaminated spawn packets and diseased fruiting bodies were collected from Mushroom Development Institute (former NAMDEC), Savar and different mushroom farms of Bangladesh.

4.1.16. Contaminants found in spawn

Different types of fungus contamints were found in contaminated spawn packets. Some were green colored, some were blackish, some were yellowish, some packets were brown, in some packets incomplete colonization or lack of mycelium running. *Trichoderma* sp, *Penicillium sp* and *Aspergillus flavus* were detected from these contaminated spawn packets. Among these moulds, *Trichoderma* was found in most of the contaminated packets largely and significantly.

4.1.17. *Trichoderma sp*

Green colour growth of mycelium was observed in substrate packet (Plate 1 A-E) due to heavy sporulation of causal agent. *Trichoderma* is a cellulolytic filamentous fungus, the spores were round and globular in shape, the phialides of *T. harzianum* tended to cluster (Plate 2A-D). *Trichoderma* was often mistaken for *Penicillium* or *Aspergillus* molds (and vice versa), which was difficult to distinguish from the mushroom mycelium, being that all three looked very similar on the surface of spawn packets and are not easy to identify apart without the microscopic analysis, especially before sporulation.

A spawn packet caused 2% contamination by *Trichoderma* sp., which partially sporulation of *Pleurotus* was observed but primordia or fruiting bodies were not formed from the packet (Plate 3C and D).

Plate 1. *Trichoderma* contaminated spawn packets (oyster)

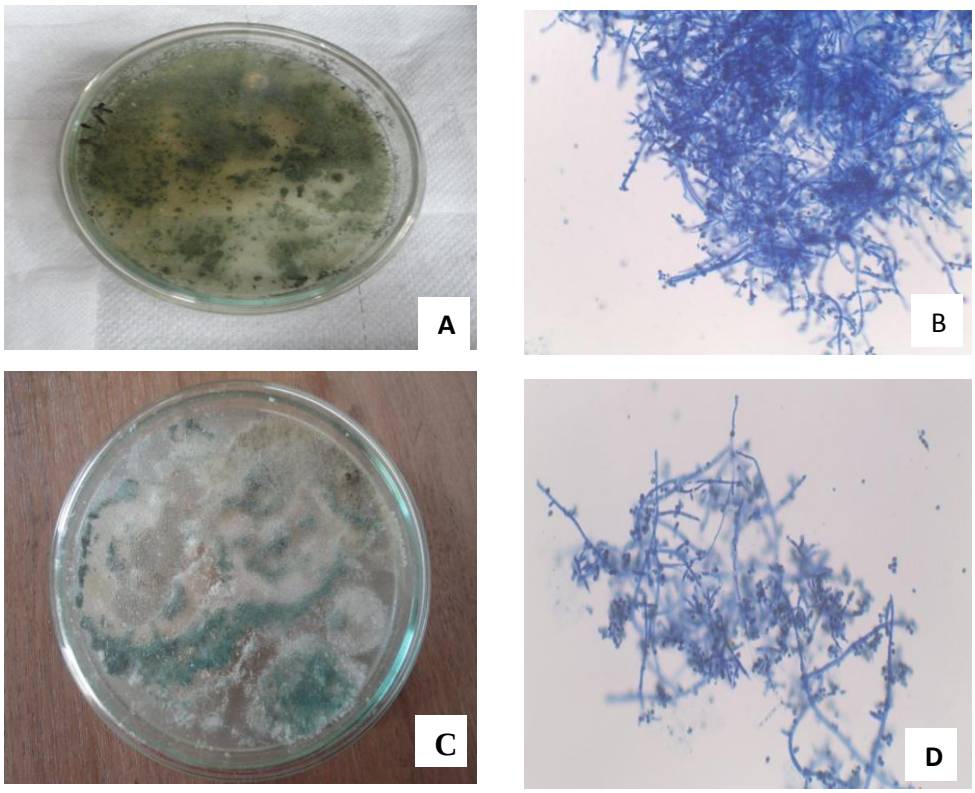

Plate 2. Pure culture and pathogenic structure of *Trichoderma* isolated from contaminated spawn

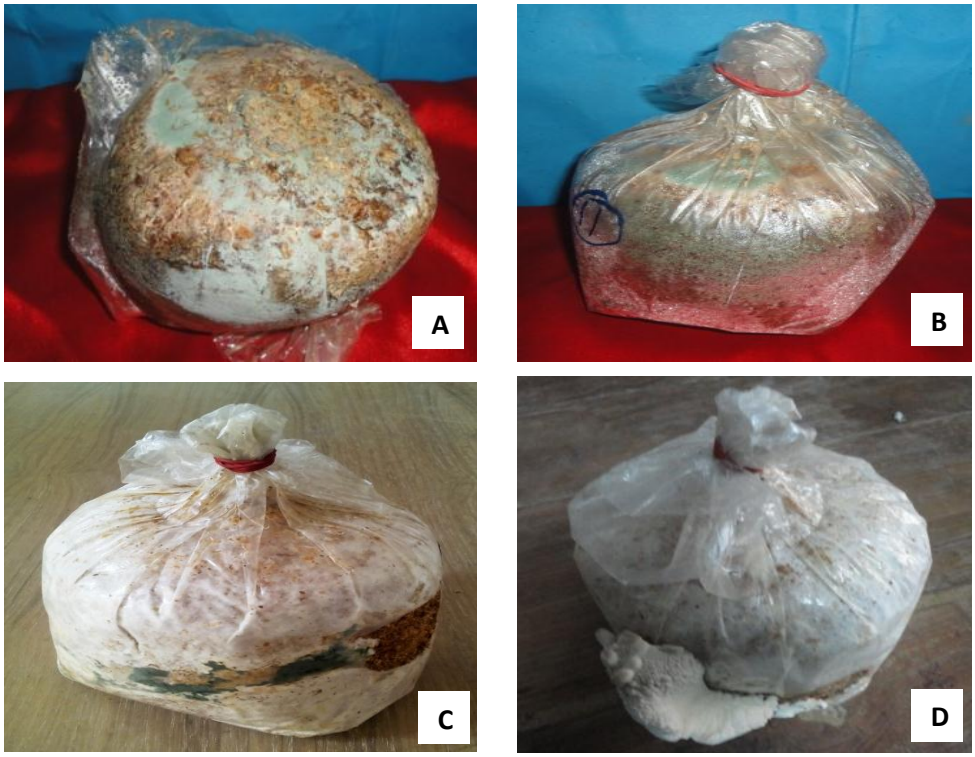

Plate 3. A B. Green mold contaminated packets, C D Partial contaminated spawn

4.1.18. *Penicillium sp*

Initially, *Penicillium* appeared as a green-gray colored powder on the substrates of oyster mushroom and turned black as timed passed, it is called blue green mold (Plate 4. A-D). Pure culture of *Penicillium* was prepared on PDA from collected contaminated spawn (Plate5 A-D). The multiple branching of conidiophore ends in a group of phialides which bear the long conidial chains, the entire structure resembles like a brush (Plate 5.C-D).

4.1.19.*Aspergillus* spp.

Aspergillus was found in the contaminated the spawn packets in the growing house. *Aspergillus niger* produced black colored spores so it was called black mold (Plate 6 A-F). Pure culture of *Aspergillus niger* was prepared from contaminated spawn (7A) and the shape was similar to an onion flower stalk and their spores had inside the globose head on these outer surface (7B), *Aspergillus flavus* (Plate 7 E-G).

4.1.20. *Rhizopus sp*

Rhizopus also resulted in white mycelial growth with black pin head on the surface of the mushroom substrates, later turned on pale brown or black colour (7 C). It produced white foamy and fluffy white mycelial growth with black pin head on PDA media. Rhizoids were observed attached with the substrates, Sporangiophores arise from the rhizoid, bearing brown or black, globose shaped sporangia at the end (Plate 7 D).

Plate 4. *Penicillium* contaminated spawn (A-D)

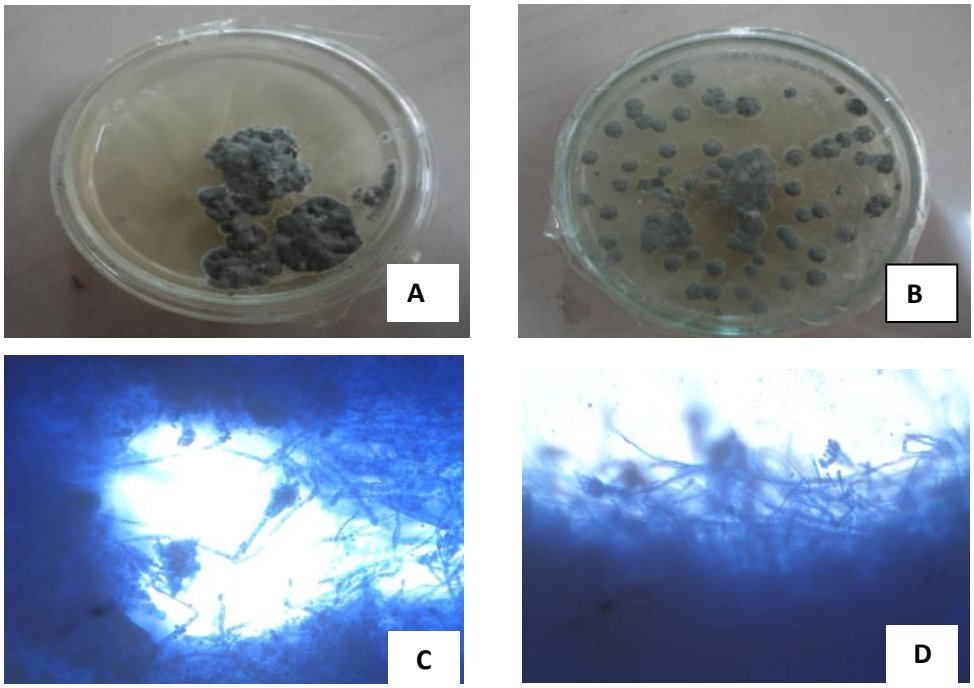

Plate 5. A-B. Pure Culture of *Penicillium*:, C-D. Microscopic Structure of *Penicillium*

Plate 6. Black mold contaminated spawn plate (A-F)

Plate 7.A. Pure Culture of *Aspergillus niger*, **B.** Microscopic stuructrure of *Aspergillus niger* **C.** Pure culture of *Rhizopus*,**D.** microscopic sturucture *Rhizopus*, **E-F**. Pure Culture of *Aspergillus flavus*, ***G***. microscopic sturucture of *Aspergillus flavus.*

4.1.21. Stroma

Whitedense layer of mycelium were observed on the substrates of the packets, these were called stroma (Plate 8 AB). Fruiting bodies were not formed in these packets. Sharma *et al.*, (2007) reported that stroma are related to the genetic character of the spawn but are sometimes induced if spawn is mishandled or exposed to harmful chemicals or certain detergents during preparation, storage, transit or at the farm (These may be caused due to low-quality degenerating mushroom strain or mycelial growth in spawn. It might be due to a high concentration of carbon dioxide or high temperature and low humidity of mycelial growth inside the packet.

4.1.22. Horn-like symptoms on substrates of oyster mushroom

Black, hard, sharp, black gram seed like structures were observed on the contaminated spawns of oyster mushroom (Plate 8 C D).

Plate 8. A and B. Stroma, C-D Showing horn-like structures inside the spawn.

4.1.23.*Coprinus spp*

Coprinus was found in the both substrate pile (Plate 9 A-B) and inside the substrate packets (Plate 10 C-F). *Coprinus spp* was found on substrates in clusters and these were slender, long stipe, bell-shaped mushrooms during the fructification stage, the fruiting bodies of different *Coprinus*species were immerged from spawn packet of untreated or at 60^0C for 2or 3 hours treated packets during hot water treatment. In *Coprinus lagopus* firstly cream coloured cap covered with scales was appeared and later turned to blueish- black. After several days ink caps were decayed and form a blackish slimy mass due to autodigestion. The caps were different shapes, size 1.5-5 cm, conical, finally with recurved margin, split and curled over on itself, completely white. As the mushroom matures, the shape of the cap becomes more conical or convex, and finally flattens out, with edges curved upward. Some are bluish black bell shaped and the stipe is slender, long and grown in clusters through the cutting portion. A cluster of young fruit bodies of *Coprinus micacious* emerged from spawn packets, the gills are initially white but rapidly matured and become brown.

Plate 9. A-B. *Corprinus micacious* producuced in the substrate (saw dust), *C. Coprinus micacious* inside the spawn. E-F. *Coprinus Lagopus* contaminated packets

4.1.24. *Chaetomium* sp

Chaetomium was identified from a yellowish white contaminated mushroom packet collected from mushroom development Institute. The fungus consisting of a grayish white mycelial growth was observed on the surface of spawn (Plate 10 A), which later produces perithecia. Perithecia of *C. olivaceum* are superficial, opaque, globose, thin, membranous with an apical tuft of dark bristles of setae (Plate 10 B). It is called olive green mould.

4.1.25. Needle mushroom

Needle mushroom was found in the substrate (rice straw) pile and in the experiment it was grown as weed mushroom. The stalk was long, succulent and pileus (cap) was small ball shaped. It was called Enokitake mushroom (Plate 10 C).

4.1.26.*Alternata alternata*

Conidiophores dark, simple, muriform conidia bearing a simple chain of conidia were observed in the microscope (Plate10 D).

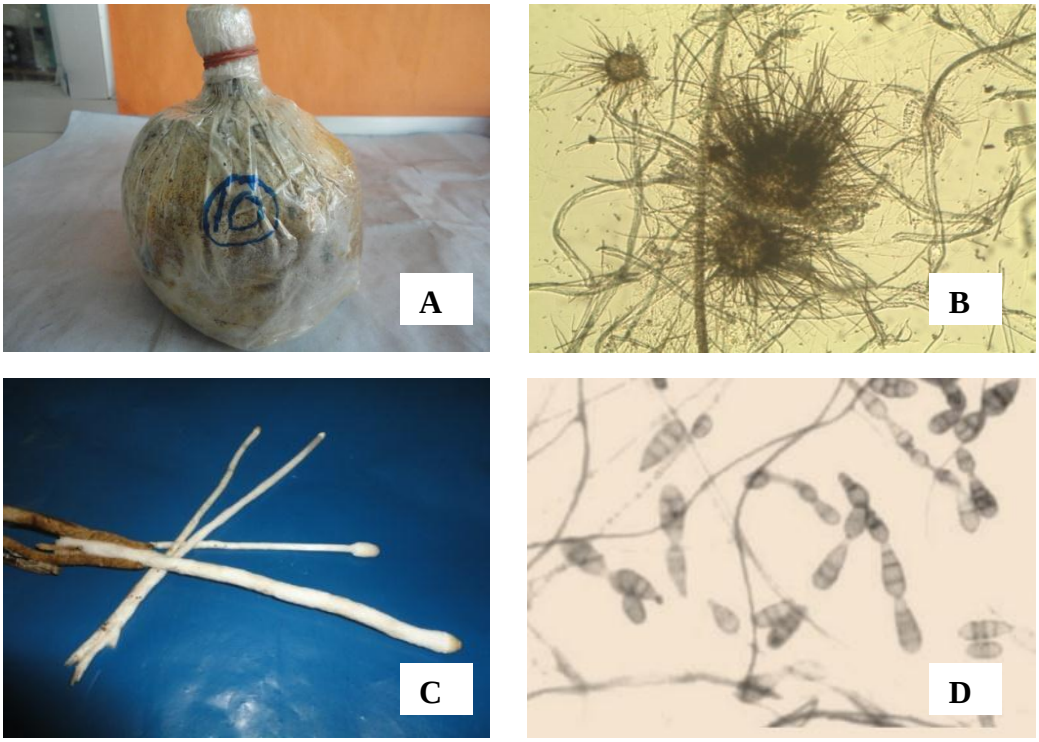

Plate 10. **A.** Contaminated Packet by olive green mold **B.** *Chaetomium Sp.* under compound microscope, **C**. Enokitake mushroom (Needle mushroom), **D.** *Alternaria alternate*

4.1.27. *Ceratocystis sp*

Ceratocystis was identified from sawdust packets and cultured on PDA plate (Plate 11A and B). Conidiophores branched, consisting of upper portion with penicillate branches lower portion dark but variable in shade, upper branches hyaline, phialides slender, conidia hyaline, ovoid, held together in rather large heads by mucilaginous substances (11B). Conidial stage *Ceratocystis* sp. and perfect stage *Leptographium* sp.

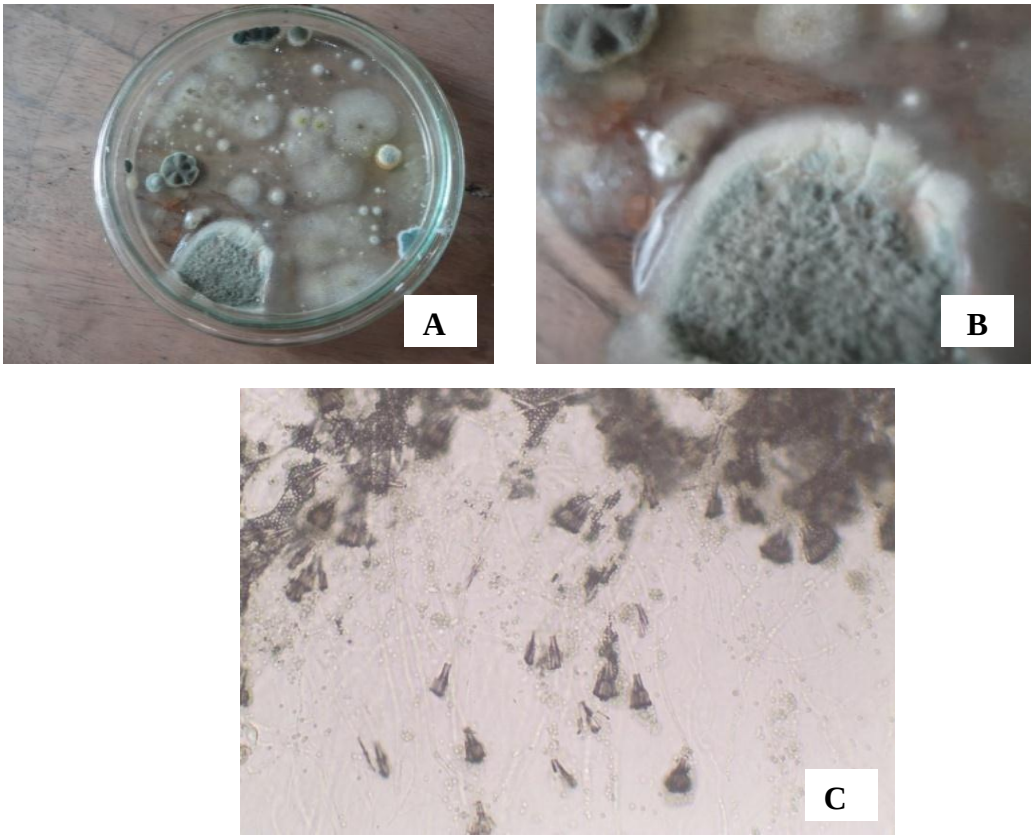

Plate11. AB. Culture of *Ceratocystis*sp., C. Pathogenic structure of C*eratocystis sp*

4.1.28. Cock's comb symptoms of fruiting bodies of oyster mushroom

Different abnormal fruiting bodies were observed due to different physiological disorder in the environments during cultivation of *Pleurotus*. Blister like raised, fluffy growths were found on the fruiting bodies of oyster mushroom, these outgrowths on mushroom caps are called cock's comb (Plate 12 A-D). It was caused due to effect of excess heat (Information of www.infonet-biovision.org).

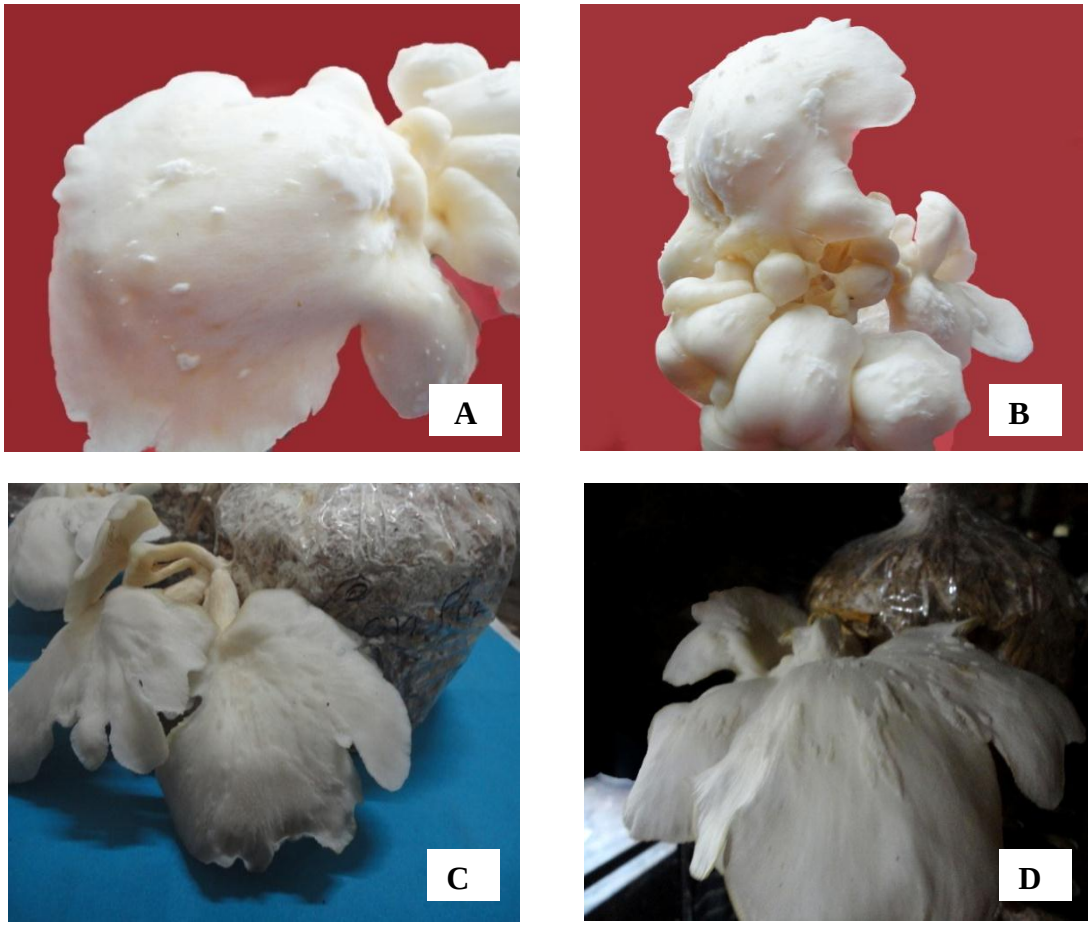

Plate12. Cock's comb symptoms of fruiting bodies of *Pleurotus ostreatus* (PO_2) from SAU mushroom culture house

4.1.29. Abnormal fruiting bodies and observation of symptoms

Curling, shrivel, wrapped and twisted the pileus of mushroom fruiting body (cap) (Plate 13 A-E). It may be caused due to high temperature. Soft, pale young pin heads and a cluster of little brown coloured caps of fruiting bodies (Plate13 F) collected from the contaminated spawn packets. It may cause due to infection of *Trichoderma* sp. during cultivation of oyster mushroom. Distortion was observed in pileus of mushroom fruiting body (Plate 13 G), it may be caused due to excess watering. Thick stipe, small caps were also observed in fruiting body of oyster mushroom and the pileus turned into black colour (Plate 13H), it may be caused due to excessively high carbon dioxide level on the initial stage of growth. Similar abnormalities were also recorded in information of www.infonet-biovision.org.

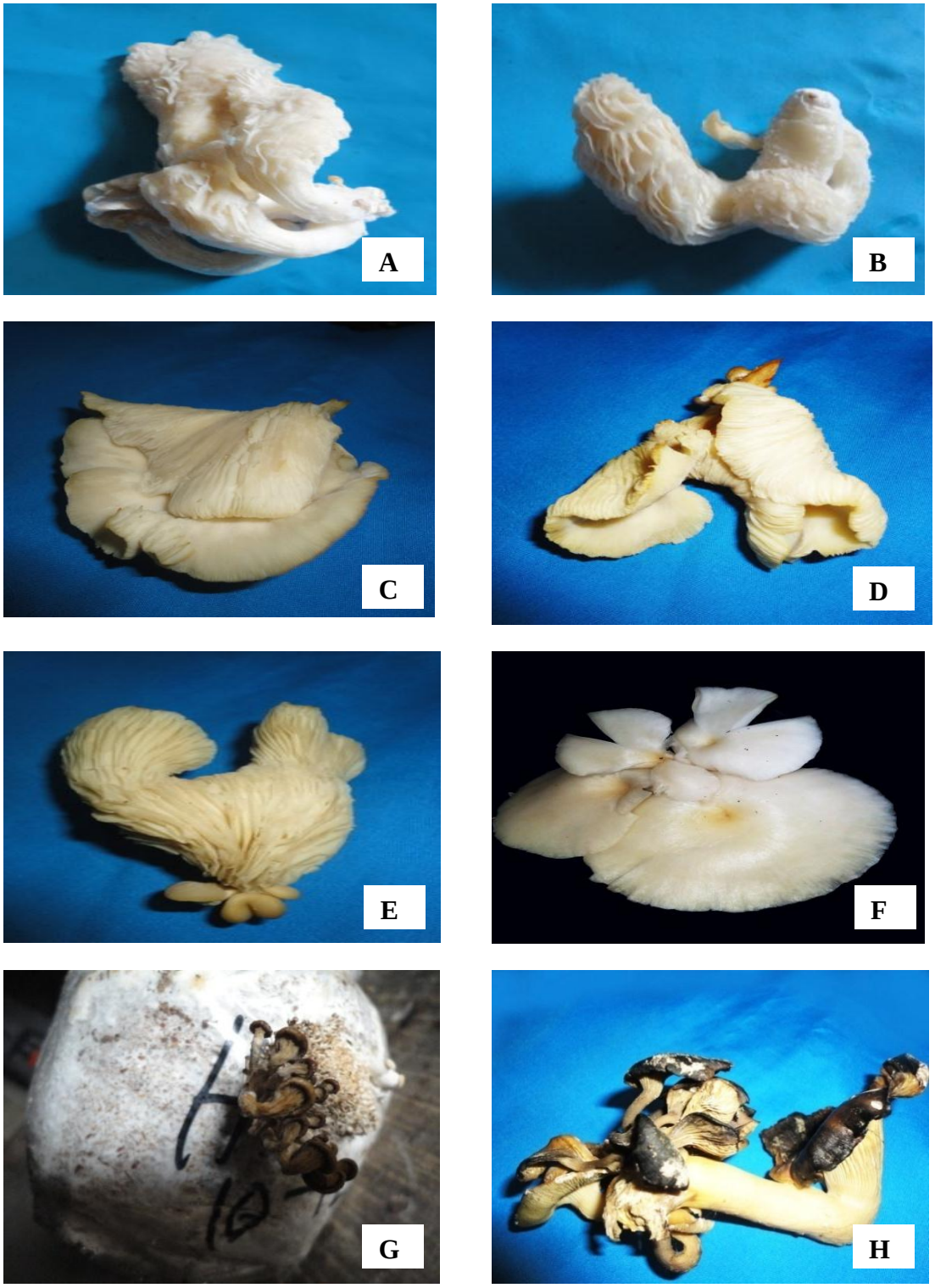

Plate 13. Showing different abnormalities on fruiting bodies

4.1.30. Discussion

Several contaminants were isolated from contaminated substrates during survey and more or less similar findings have been reported by previous scientists. Pervez *et al.,* (2010) reported weed mycoflora namely *Aspergillus* spp*, Penicillium* spp., *Rhizopus stolonifer* and *Trichoderma harzianum* were found to be associated with the substrate of oyster mushroom at different growth stages. Choi *et al*., (2010) also isolated and identified *Trichoderma, Pleuroticola*, *Penicillium crustosum, Mucor racemosus* f. *racemosus, Aspergillus tubingensis* from *Pleurotus ostreatus* substrates for molecular and morphological characterization. Sharma *et al., (*2007) reported that one of the most common and destructive diseases in mushroom cultivation is the green mould which was mainly caused by different species of *Trichoderma, Penicillium and Aspergillus,* which was observed in the present experiment. Hang and Miles (1989) and Chang (2008) reported that green mould competes with the mushroom for space, nutrients as well as causing chemical alteration of the substrate, which hinders mushroom development. Kligman (1950) was the first to report the presence of *Trichoderma* in mushroom compost. Samuels *et al.*, (2002) also reported similar dimensions for *Trichoderma* spp. isolated from *Agaricus bisporus*. Choi *et al.,* (2003) reported that the conidia of *Trichoderma* spp. are ellipsoidal and ovoid and that the phialides were lageniform and bowling pin shaped. Chen and Moy (2004) have stated that parameters of mushroom cultivation, such as the sources of carbon and nitrogen, high relative humidity, warm temperatures, a fluctuation of these factors, and the absence of light during spawn run are ideal environmental conditions for moulds as well, which can easily lead to a contamination. Komon-Zelazowska *et al.*,(2007) reported that, *Trichoderma spp* infection in edible basidiomycetes have been known for a long time. *T. harzianum* was recognized as causing the most severe problems from contaminated spawn in the study. Hatvani *et al.*, (2007) noticed that, serious cases of green mold diseases in *P. ostreatus* in mushroom farms were detected in South Korea, Italy, Hungary and Romania. The present findings of this experiment were also supported by the findings of previous researchers. Won (2000) and Largeteau-Mamoun *et al.,(*2002) reported that *Trichoderma* produce whitish mycelia indistinguishable from those of the mushrooms during spawn run, therefore it is difficult to recognize the infection at this stage.

Yu (2002) and Woo *et al.*,(1999) also observed that *Trichoderma* species are present at the initial phase of substrate preparation, then they disappear with the pasteurization, but they can be found again in the substrate after spawning (inoculation with *Pleurotus*), during spawn run (incubation phase) and the harvesting cycles. Kredics *et al*.,(2010) reported that, *Trichoderma* competes efficiently for space and nutrients, produces extracellular enzymes, toxic secondary metabolites and volatile organic compounds, which results in drastically crop losses. Biswas (2014) reported that, contamination of various mould fungi occur during the growth and postharvest stages of mushroom, which adversely affect the mushroom yield and its shelf life. Similar results, that *Trichoderma harzianum* is characterized by an aggressive, white mycelium that grows over substrate of mushrooms, causing a soft decay and masses of spores that eventually form are emerald green, were described according to http://www.ppath.cas.psu.edu/ Mush Grow Info/ Trichoderma% 20 Green% 20 Mold.html.

Similar morphological characters about *Penicillium* spp. were reported by Jo *et al*., (1999). Choi *et al.,* (2010) reported that, *Penicillium* competes for preoccupancy with green spores and inhibits the formation of fruiting bodies of the mushrooms spawn packets. Samson *et al.,* (2006) found similar morphological structures of *Aspergillus* of the present experiment. Substrates of oyster mushroom were used for frequent habitats, spores and mycelia contaminated the surface and inside the mushroom spawn packets and inhibit the formation of fruiting bodies of mushroom. *Aspergillus flavus* produced a yellow to yellowish green colour (http: // www. shroomery. org / index. Php / par / 26841)spores inside the spawn packets (Plate 50 E and F). Pegler (1977) also characterized that the stem is whitish in color, hollow, hairy (flocculose) over the whole surface but especially at lower part and becomes smooth (glabrous) with age, size 2.5-7.5 x 0.3-0.6 cm, narrowing towards top. Amandeep *et al.,* (2014) reported that Pileus campanulate to applanate, finally plano–concave with revolute margin, umbonate; stipe 0.2–0.35 cm broad; lamellae subdistant, moderately broad; basidiospores 10–13.6 × 6.8–9.3 mμ.*Coprinus lagopus* belonging to the family Coprinaceae is a delicate and short-lived weed fungus of which the fruit bodies last only a few hours before dissolving into a black ink, a process called deliquescence. Hossain *et al,* (2010) reported that *Coprinus lagopus*, a common weed fungus for mushrooms was found to occur in a mushroom industry for spawn production of

Pleurotus ostreatus in Bangladesh and also stated that proper attention and care to be paid for appropriate pasteurization and sterilization of the substrate used for developing spawn. *Chaetomium* might be appeared due to improper pasteurization accompanied by high temperatures in the absence of adequate fresh air (Sharma *et al.*, 2007).The first evidence of the occurrence of *C. olivaceum* in India was provided by Gupta *et al*., (1975) at the mushroom farm at Chail, Kasauli and Taradevi. Another species, *C. globosum*, was later reported from mushroom farms in Delhi and Mussorie (Thapa *et al*., 1979).

4.2. Experiment 2. Effect of different substrates, duration of pasteurization and size of substrate packet on severity of contamination in substrate packet

Normally mushroom famers of Bangladesh harvested mushroom within 30-60 days after incubation (DAI). Therefore, data on contamination severity was recorded during this period of incubation. The substrate packets prepared with only waste paper in any experiment were free from contamination irrespective of stage of data collection and it is not shown in any table or figure.

4.2.1. Contaminating mycoflora

Trichoderma sp., *Rhizopus* sp., *Coprinus* spp., *Aspergillus* sp. were identified as contaminants from substrate packets and their description, photographic structures and pure culture were the same as described in experiment 4.1. Williams *et al.*,(2003) reported that in preferred conditions moulds exhibited fast growth, therefore they can compete for space and nutrients more effectively than the mushrooms, furthermore, they are able to produce toxic secondary compounds, extracellular enzymes as well as volatile organic compounds, which can result in a drastically decrease in production or even entire crops can be wiped out.

4.2.2. Contaminated rice straw Packets

In case of 500g rice straw packets, poor growth with 5-10% contaminants was observed at 60 DAI (Plate 14 A-C). In case of 1000g rice straw packets, intermediate growth with 40% contaminants was observed at 60 DAI (Plate 15A). Abundant growth with 100% contamination was observed at 60 DAI in treatment combination $S_1B_1T_5$ (Plate 15B).

4.2.3. Contaminated saw dust packets

Contaminating mycoflora in substrate packets containing 500g sawdust showed poor growth and the severity was 2% (Plate 18A) at 40 DAI and 8% (Plate 16B) at 60 DAI. Abundant growth of microorganisms, 60% was observed in 1000g sawdust packet (Plate 16C) at 60 DAI.

4.2.4. Contaminated mixed packets of substrates

In case of 2 hours pasteurized500g mixed packets, 40% contamination was observed at 60 DAI (Plate 17 A). In case of 1000g mixed packets, abundant growth of microorganism (60% contamination) was observed at 60 DAI for 2 hours steam pasteurization bags (Plate17 B).

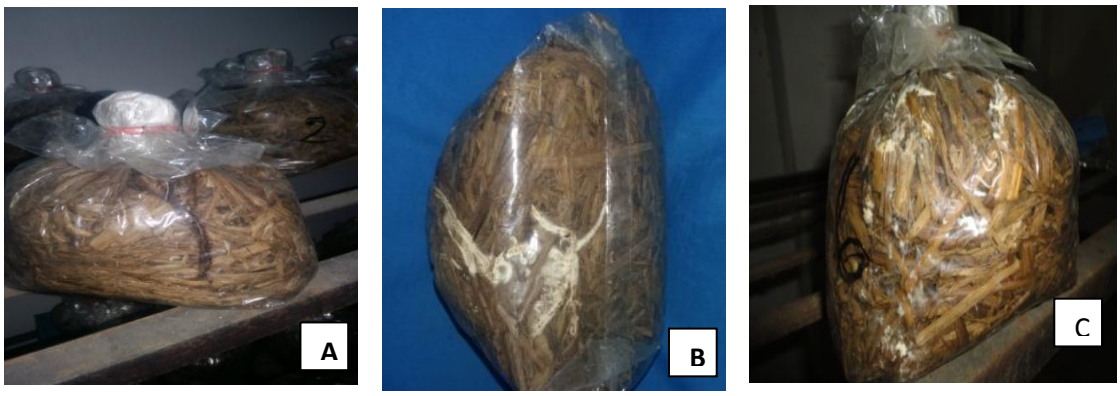

Plate14. A. Poor growth,5% contamination, **B**.10% *Coprinus* contamination and **C.** 10% contamination

Plate 15. Contaminated rice straw packets (1000g): **A.** intermediate growth 40% contaminated at 60 DAI; **B.** Left (*Rhizopus*) and Right:*Aspergillus* abundant growth, 100% contamination.

Plate 16. Contaminated sawdust packet (500g): A. poor growth, 2% Contaminated at 40 DAI; B. poor growth, 8% at 60 DAI; C. 60% abundant growth 1000 g saw dust packet

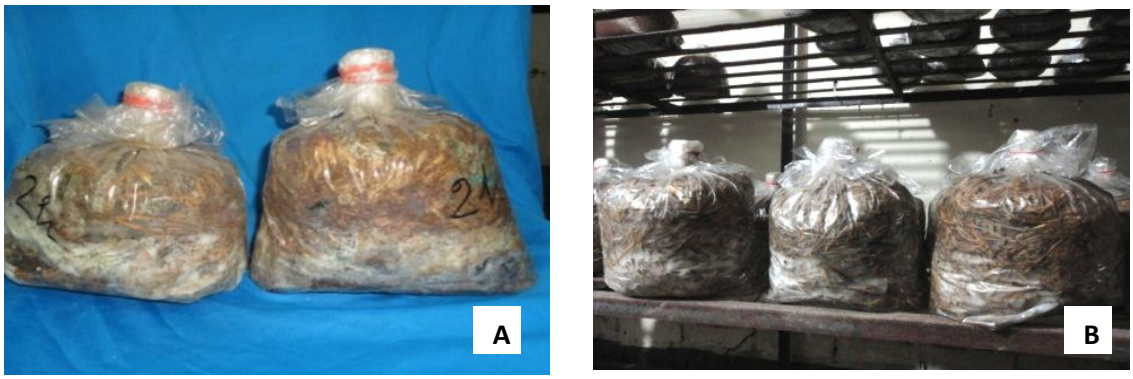

Plate 17. **A.** Intermediate growth: 40% Contaminated mixed packets (500g);
B. Abundant growth: 60% Contaminated mixed packets (1000g) at 60 DAI.

4.2.5. Main effect of different substrates on severity of contamination

The substrate packets prepared with only rice straw, only saw dust and mixed substrate containing rice straw, saw dust and waste paper were contaminated with mycoflora. The lowest severity of contamination was recorded from saw dust followed by rice straw at all stages of data collection. The maximum contamination was found in mixed substrate (rice straw+sawdust+waste paper) at all days after incubation. The severity of contamination increased gradually with gradual progress of days after incubation (DAI). The effect of rice straw, saw dust and mixed substrate on contamination severity was significantly different (Table 3).

4.2.6. Main effect of duration of pasteurization on contamination severity

The main effect of duration of pasteurization on severity of contamination was significant. Irrespective of duration of pasteurization, the severity of contamination increased gradually with increasing DAI. Significantly the lowest severity of contamination was recorded when the substrate packets was pasteurized for 3 hours followed by duration of 4 hours at 30 and 60 DAI. At 40 and 50 DAI, severity of contamination in substrate packets pasteurized for 3 and 4 hours was statistically similar but significantly higher compared other duration of pasteurization. At all stages of data collection significantly the highest severity was found in packets pasteurized for 2 hour followed by 6 hours. So, 3 hours were noted as the best duration for substrate packet pasteurization (Table 4).

4.2.7. Main effect of substrate packet size on severity of contamination

The severity of contamination increased gradually with the progress of days after incubation. Significantly the highest severity of contamination was recorded from 1000g substrate packets and the lowest in 500g packets (Table 5).

Table 3. Effect of different substrates on severity of contamination in substrate packets recorded 30-60 days after incubation with 10 days interval

Substrate	Contamination severity (%) at days after inoculation			
	30DAI	40DAI	50DAI	60DAI
Rice straw	6.55b (2.559)	14.07b (3.751)	18.69b (4.323)	21.88b (4.678)
Saw dust	3.28c (1.812)	5.31c (2.304)	8.24c (2.870)	10.49c (3.240)
Mixture of substrates	9.30a (3.050)	21.07a (4.590)	22.66a (4.760)	26.73a (5.170)

Values within the same column with a common letter(s) do not differ significantly at less than 5% level of significance.

**Figures within parentheses are squire root transformed values ($\sqrt{(x+0.5)}$), where x=original value.

Table 4. Effect of duration of pasteurization of substrates with steam on contamination severity of substrate packets recorded up to 60 days after inoculation with 10 days interval

Duration of pasteu-rization (hours)	Contamination Severity (%)			
	30DAI	**40DAI**	**50DAI**	**60DAI**
2	6.39a (2.527)	9.98a (3.159)	15.42a (3.926)	18.58a (4.310)
3	2.00d (1.416)	3.47d (1.864)	4.95d (2.224)	5.58e (2.405)
4	2.66c (1.630)	3.74d (1.934)	5.13d (2.266)	6.30d (2.510)
5	3.03b (1.742)	4.56c (2.136)	6.40c (2.530)	8.48c (2.912)
6	3.14b (1.771)	8.08b (2.842)	11.76b (3.429)	12.97b (3.602)

*Values within the same column with a common letter(s) do not differ significantly at less than 5% level of significance.
**Figures within parentheses are squired root transformed values (√(x+0.5)), where x=original value.

Table 5. Effect of spawn packet size on **c**ontamination severity

Amount of substrate (g)	Contamination Severity (%)			
	30DAI	**40DAI**	**50DAI**	**60DAI**
500	2.69b (1.642)	4.01b (2.004)	5.99b (2.449)	6.99b (2.645)
1000	3.97a (1.993)	7.67a (2.770)	10.89a (3.301)	13.33a (3.651)

*Values within the same column with a common letter(s) do not differ significantly at less than 5% level of significance.
**Figures within parentheses are squired root transformed values (√(x+0.5)), where, x=original value.

4.2.8. Interaction effect of substrate and duration of pasteurization on contamination

Interaction effect of substrate and duration of pasteurization was significant. Substrate packets prepared with only rice straw, only sawdust and the mixed substrate (rice straw+sawdust+waste paper) were contaminated with mycoflora observed during 30-60 DAI with 10 days interval. The severity of contamination under different treatment combinationsranged 0.00-19.98, 1.93-29.16, 4.20-51.41 and 2.86-57.15 at 30, 40, 50 and 60 DAI, respectively. At 30 DAI, saw dust pasteurized for 3 hours (S_2T_2) and mixed substrate containing waste paper pasteurized for 3 hour (S_3T_2) did not show any contamination. The lowest contamination was found in sawdust pasteurized for 6 hours (S_2T_5) followed by S_2T_4, S_3T_3 and S_3T_5. The highest contamination was recorded from treatment combination S_3T_1 followed by S_1T_4, S_1T_5, S_2T_3, S_3T_5 and

S_1T_1.At 40 DAI, the lowest severity was recorded from treatment combination S_3T_2 followed by S_2T_2, S_2T_5, S_3T_3, S_3T_5 and S_2T_4. The highest severity was found in treatment combination S_3T_1followed by S_1T_4, S_3T_5, S_2T_1, S_1T_3 and S_2T_3.At 50 DAI, the lowest severity was recorded from treatment combination S_2T_2 followed by S_2T_5, S_3T_2, S_3T_3, S_2T_4, S_3T_5, S_3T_3 and S_1T_2. The highest severity was found in treatment combination S_1T_5 followed by S_3T_1, S_2T_1, S_1T_4, S_3T_5 and S_2T_2 (Table 6).

At 60 DAI, the lowest severity was recorded from treatment combination S_2T_2 followed by S_3T_2, S_2T_5, S_2T_4, S_3T_5, S_3T_3, S_1T_2 and S_1T_1. The highest severity was found in treatment combination S_1T_5 followed by S_3T_1, S_2T_1, S_1T_4, S_3T_5 and S_1T_3. At 60 DAI, rice straw pasteurized for 3 hours showed the lowest severity of contamination, which was statistically similar to 2 hours. The severity of contamination increased gradually with increased duration of pasteurization from 3 to 6 hours. The highest severity was recorded when pasteurization was done for 6 hours followed by 5 and 4 hours. The severity for three higher durations was significantly different. In sawdust, the lowest severity of contamination was found at 3 hours of duration, which was statistically similar to 5 and 6 hours. The highest severity was recorded at 2 hours of duration followed by 4 hours. In mixed substrate, the lowest severity was also recorded from the duration 3 hours. However, the severity at 3, 4 and 5 hours was statistically similar. The highest severity was recorded from 2 hours duration followed by 6 hours (Table 6 and Figure 28).

Table 6. Interaction effect of substrate and duration of pasteurization on contamination severity at 20-60 DAI with 10 days interval

Substrate x duration of pasteurization	Contamination severity (%)			
	30DAI	**40DAI**	**50DAI**	**60DAI**
Rice straw x 2 hr (S_1T_1)	6.00d (2.45)	7.95f (2.82)	10.36g (3.25)	11.42g (3.38)
Rice straw x 3 hr(S_1T_2)	3.39f (1.84)	5.43g (2.33)	6.45h (2.54)	10.37gh (3.22)
Rice straw x 4 hr (S_1T_3)	4.88e (2.21)	12.39de (3.52)	14.75f (3.84)	15.44f (3.93)
Rice straw x 5 hr (S_1T_4)	10.82b (3.29)	20.43b (4.52)	23.14d (4.81)	27.88d (5.28)
Rice straw 6 hr (S_1T_5)	8.94c (2.99)	30.80a (5.55)	51.41a (7.17)	57.15a (7.56)
Saw dust x 2 hr (S_2T_1)	6.15d (2.48)	13.76cd (3.71)	25.60c (5.06)	33.41c (5.78)
Saw dust x 3 hr (S_2T_2)	0.00 j (0.95)	1.82j (1.35)	2.53k (1.59)	2.86l (1.69)
Saw dust x 4 hr (S_2T_3)	9.18c (3.03)	11.36e (3.37)	14.14f (3.76)	16.40f (4.05)
Saw dust x 5 hr (S_2T_4)	1.79i (1.34)	2.86hi (1.69)	4.33i (2.08)	6.55j (2.56)
Saw dust x 6 hr (S_2T_5)	1.54 i (1.24)	1.93j (1.39)	3.39j (1.84)	4.41k (2.10)
Mixed substrate x 2 hr (S_3T_1)	19.98a (4.47)	29.16a (5.40)	44.49b (6.67)	54.17 b (7.36)
Mixed substrate x 3 hr (S_3T_2)	0.00 j (0.94)	1.77j (1.33)	4.20i (2.05)	4.20k (2.05)
Mixed substrate x 4 hr (S_3T_3)	2.66g (1.63)	2.66i (1.63)	6.35h (2.52)	9.55h (3.09)
Mixed substrate x 5 hr (S_3T_5)	2.28h (1.51)	3.35h (1.83)	6.25h (2.50)	8.24i (2.87)
Mixed substrate x 6hr (S_3T_5)	5.90d (2.43)	14.14c (3.76)	17.98e (4.24)	19.71e (4.44)

*Values within the same column with a common letter(s) do not differ significantly at less than 5% level of significance.

**Figures within parentheses are squired root transformed values ($\sqrt{(x+0.5)}$), where x=original value

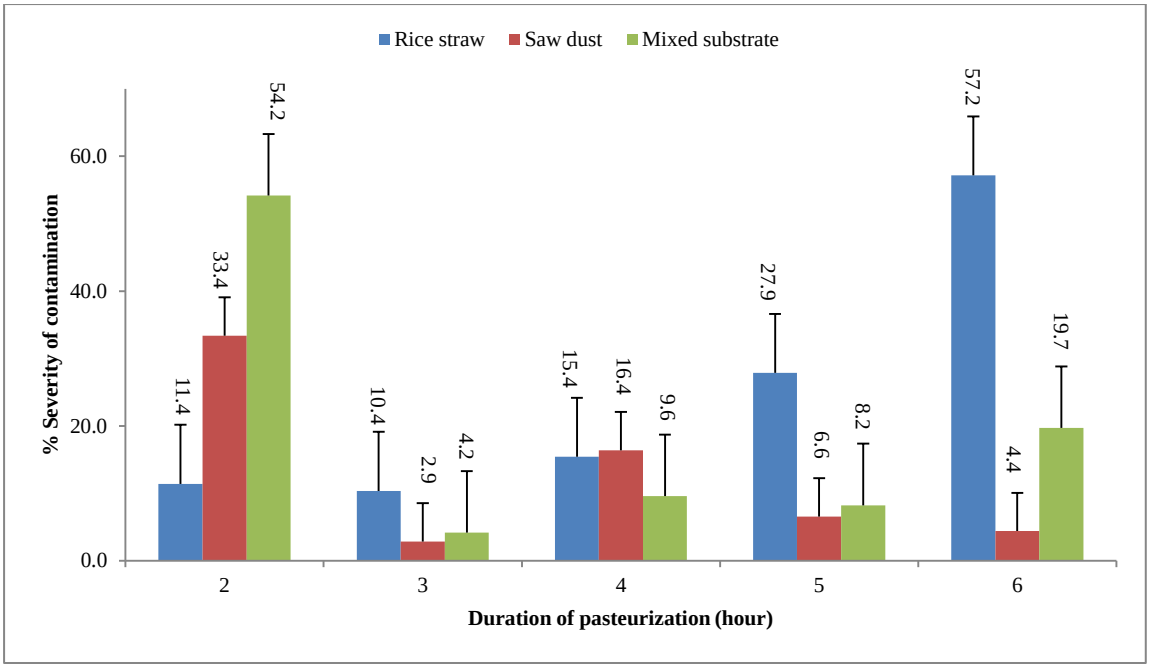

Figure 28. Severity of contamination in substrate packet containing different substrates and pasteurized for 2-6 hours at 60 DAI

4.2.9. Interaction effect of substrates and bag size on contamination severity

At all stages of data collection, the maximum severity of contamination was found in rice straw at 1000g/packet followed by mixed substrate at 500g and sawdust at 1000g. At 30 DAI, the lowest severity was recorded when sawdust was used at 500g followed by mixed substrate at 1000g and rice straw at 500g/packet. At 40 DAI, significantly the lowest severity was recorded from sawdust at 500g followed by rice straw at 500g and mixed substrate 1000g/packet. At 50 and 60 DAI, the minimum severity of contamination was recorded from sawdust at 500g followed by rice straw at 500g and mixed substrate at 1000g. The differences of three treatment combinations were significantly different (Table 7 and Figure 29)

Table 7. Effect of interaction of substrate and size of substrate bag (substrate quantity) on contamination severity at 30-60 DAI

Substrate x bag size	Severity of contamination (%)			
	30DAI	40DAI	50DAI	60DAI
Rice straw at 500g	3.34d (1.83)	5.31d (2.30)	7.38e (2.72)	8.62e (2.93)
Rice straw at 1000g	10.82a (3.29)	27.02a (5.20)	35.16a (5.930)	41.23a (6.42)
Saw dust at 500g	1.97f (1.40)	3.24e (1.80)	4.97f (2.23)	6.74f (2.59)
Saw dust at 1000g	4.92c (2.22)	7.88c (2.80)	12.32c (3.51)	15.08c (3.88)
Mixed substrateat 500g	6.92b (2.63)	10.31b (3.21)	17.14b (4.14)	18.75b (4.33)
Mixed substrate at1000g	3.09e (1.76)	5.62d (2.37)	9.30d (3.05)	12.89d (3.59)

*Values within the same column with a common letter(s) do not differ significantly at less than 5% level of significance.

**Figures within parentheses are squire root transformed values ($\sqrt{(x+0.5)}$), where x=original value

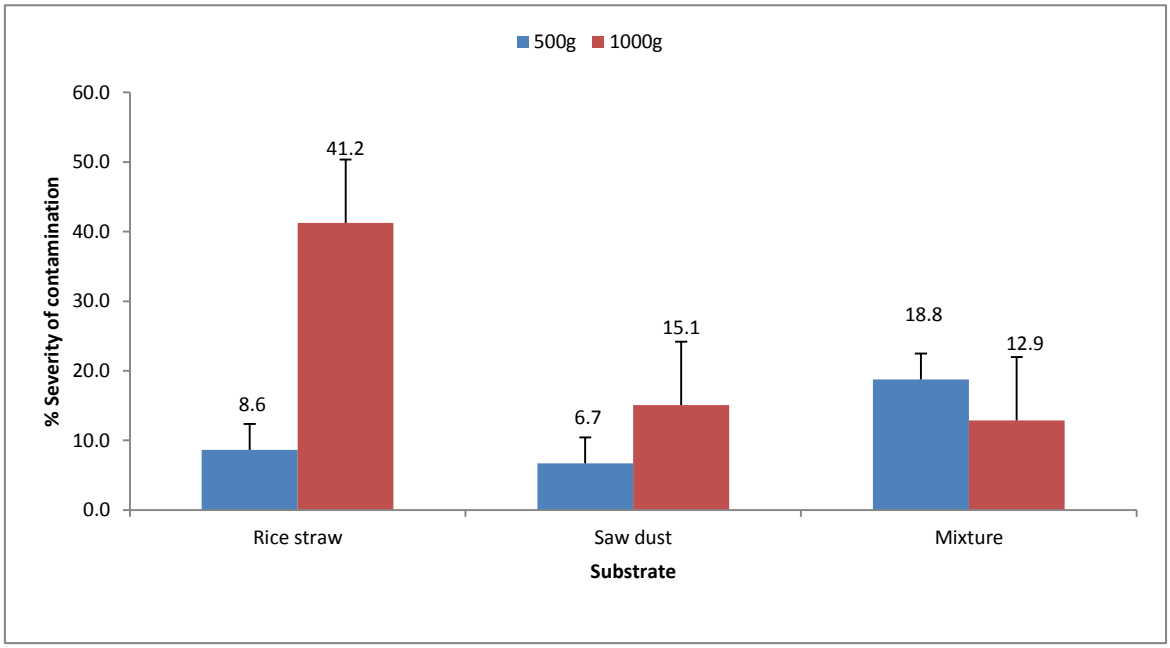

Figure 29. Severity of contamination in substrate packet containing 500 and 1000g of substrate at 60 DAI

4.2.10. Interaction effect of substrate packet size and duration of pasteurization of substrate on contamination severity

At 30 DAI, the severity of contamination in 500 g substrate packets pasteurized for 4, 5 and 6 hours were statistically similar but significantly lower compared to other treatment combinations. The highest severity was recorded from 1000g substrate pasteurized for only 2 hours followed by 500g substrate pasteurized for 2 hours and 1000g substrate pasteurized for 5 or 6 hours. At 40 DAI, significantly the lowest severity of contamination was found in 500g packets pasteurized for 3 hours. The severity of contamination in 500g packets pasteurized for 4 and 5 hours was statistically similar significantly compared other treatment combinations except 500g packet pasteurized for 3 hours. The highest severity was recorded from 1000g packets pasteurized for 6 hours followed by 500g packets pasteurized for 2 hours and 1000g packets pasteurized for 4 or 5 hours. At 50 DAI, severity of contamination ranged 5.15- 18.40% showing the maximum in 1000g packets pasteurized for 2 hours and the minimum was found in 500g packet pasteurized for 2 hours (Table 8).

At 60 DAI, the severity of contamination ranged 2.69-22.37%. The lowest severity was recorded in 500g packet pasteurized for 3 hours followed by 500g pasteurized for 4 hours and 5 hours, 1000g for 3 hours and 500g pasteurized for 6 hours. The highest severity was found in 1000g packet pasteurized for 2 hours followed by 1000g packet pasteurized for 6 hours and 500g packet pasteurized for 2 hours. Irrespective packet size, the severity was the maximal when pasteurized for 2 hours and the lowest when pasteurization was done for 3 hours. Increase of duration of pasteurization after 3 hours caused gradual increase in severity. This happens may be due to destruction of nutrients in substrate (Figure 30).

Table 8. Interaction effect of substrate packet size and duration of pasteurization on severity of contamination in substrate packet

Substrate packet X Duration of pasteurization	**Contamination severity (%)**			
	30DAI	**40DAI**	**50DAI**	**60DAI**
500g x 2 hour	5.90b **(2.43)	9.86b (3.14)	12.67c (3.56)	15.13c (3.89)
500g x3 hour	1.10g (1.05)	1.42f (1.19)	2.13g (1.46)	2.69i (1.64)
500g x 4 hour	2.40f (1.55)	3.13e (1.77)	5.15f (2.27)	5.57h (2.36)
500g x5 hour	2.43f (1.56)	3.03e (1.74)	4.88f (2.21)	6.00h (2.45)
500g x6 hour	2.59f (1.61)	4.67d (2.16)	7.45e (2.73)	8.24f (2.87)
1000g x2 hour	6.86a (2.62)	10.05b (3.17)	18.40a (4.29)	22.37a (4.73)
1000g x3 hour	2.89e (1.70)	4.37d (2.09)	5.12f (2.26)	7.02g (2.65)
1000g x4 hour	3.17d (1.78)	6.40c (2.53)	5.24d (2.98)	9.99e (3.16)
1000g x5 hour	3.69c (1.92)	6.35c (2.52)	8.12de (2.85)	11.36d (3.37)
1000g x6 hour	3.72c (1.93)	12.39a (3.52)	16.97b (4.12)	18.75b (4.33)

*Values within the same column with a common letter(s) do not differ significantly at less than 5% level of significance.

**Figures within parentheses are squired root transformed values ($\sqrt{(x+0.5)}$, where x=original value

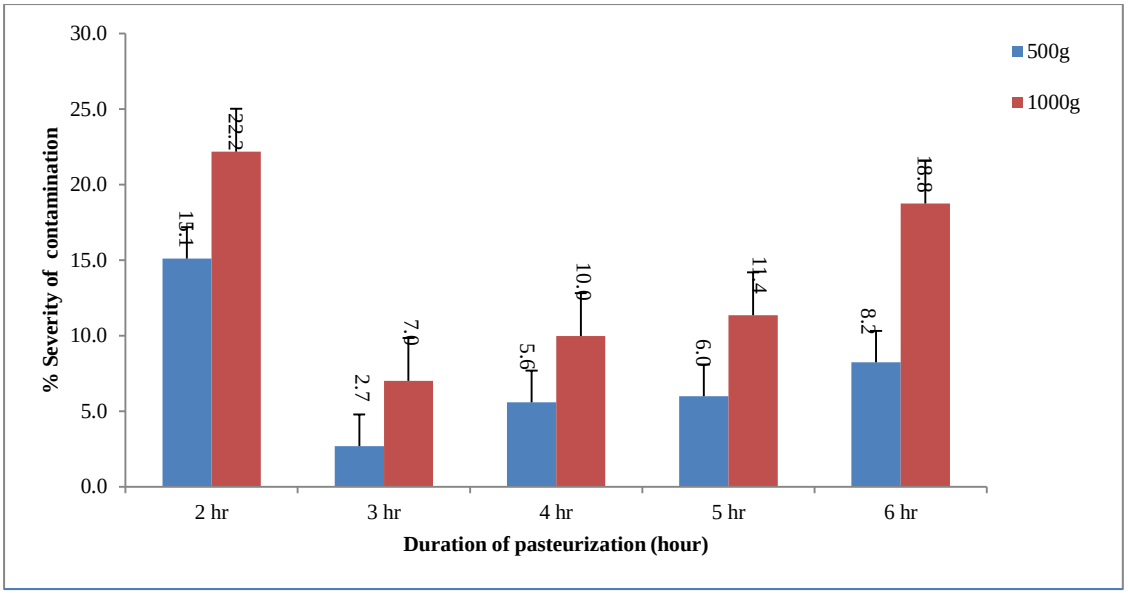

Figure 30. Severity of contamination in 500 and 1000g substrate packet pasteurized for 2-6 hours at 60 DAI

4.2.11. Interaction effect of substrate, packet size and pasteurization duration on contamination severity

Interaction effect of three factors namely substrate (four levels), polyethylene packet size (two sizes) and duration of pasteurization (five levels) on severity of contamination was significant and ranged 0-100% at 60 days after incubation (Table 9). There was no contamination in substrates containing waste paper and those data were not shown in table or graphs. The highest severity of contamination (100%) was observed in 500g mixed substrate bags pasteurized for 2 hours ($S_3B_1T_1$) followed by $S_3B_1T_1$ (61.3%), $S_2B_2T_1$ (54.2%), $S_1B_2T_4$ (49.2%) and $S_1B_2T_1$ (39.7%). Mixed substrate at 1000g and pasteurized for 3 hours ($S_3B_2T_2$) was free from contamination. The lowest severity ranged 2.4, 2.6, 2.8, 2.8, 2.8, 3.1 and 4.8 in treatment combinations $S_1B_1T_2$, $S2B_1T_3$, $S_2B_1T_2$, $S_1B_1T_3$, $S_2B_1T_5$, $S_1B_2T_5$ and $S3B_2T_4$, respectively. Therefore, treatment combination $S_1B_1T_2$, $S2B_1T_3$, $S_2B_1T_2$, $S_1B_1T_3$, $S_2B_1T_5$, $S_1B_2T_5$ and $S3B_2T_4$ may be considered as better treatment compared other treatment combination with three factors (Table 8 and Figure 31).

Table 9. Interaction effect of substrate (S), packet size (B) and duration of pasteurization (T) on severity of contamination in substrate packet at 60 DAI

Combination of substrate* packet size**	Duration of pasteurization***				
	T_1	T_2	T_3	T_4	T_5
S_1B_1	7.9 n****	2.4p	2.8p	12.4kl	26.1f
S_1B_2	15.7ij	39.7e	22.9g	49.7d	100.0a
S_2B_1	17.7hi	2.8p	2.6p	13.6jk	3.1p
S_2B_2	54.2bc	6.0no	19.5h	12.3kl	2.8p
S_3B_1	61.3b	11.5kl	15.8ij	6.4mn	15.8ij
S_3B_2	47.5d	0.0q	10.4 l	4.8 o	24.2fg

*****Substrate: S_1=Rice straw, S_2=Saw dust, S_3=Mixture of rice straw, saw dust and waste paper;

**Size of substrate packet: B_1=500g bag, B_2=1000g

***Duration of pasteurization $T_{1=}$ 2hour, T_2 = 3hour, T_3= 4 hour, $T_{4=}$ 5 hour, $T_{5=}$ 6 hour

****Data were analyzed after squired root transformed values ($\sqrt{(x+0.5)}$, and those within same column as well row having a common letter(s) do not differ significantly.

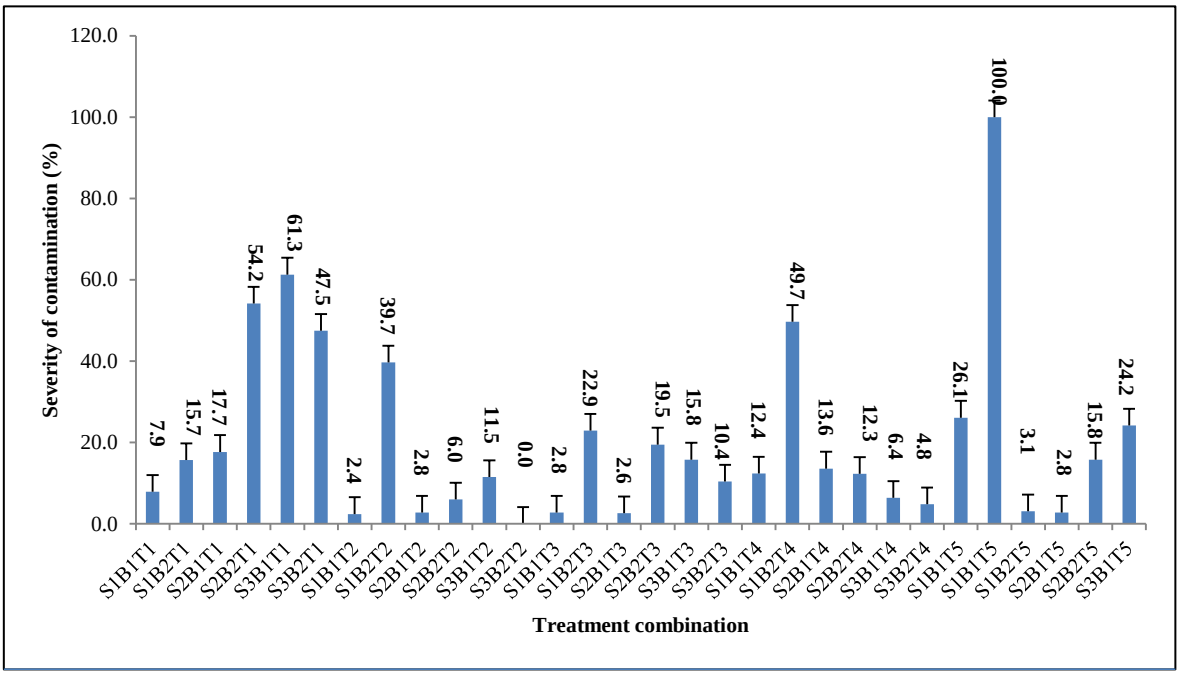

Figure 31. Interaction effect of substrate (S), packet size (B) and duration of pasteurization (T) on severity of contamination in substrate packet at 60 DAI

4.2.12. Discussion

Hassan *et al*., (2011) reported that oyster mushroom can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, bagasse etc. and this has been reported to influence its growth, yield and composition (Iqbal *et al*., 2005; Kimenju *et al*., 2009; Khare *et al*., 2010). Iqbal *et al*., (2005) reported 37 days for full colonization completion in exotic strains of *P. ostreatus* on sugarcane bagasse. Dhoke *et al.*,(2001) also found significant effect of different agro-wastes on yield of oyster mushroom. However, an ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth (Anonymous, 2008). In the present investigation occurrence of seven contaminants i.e. *Trichoderma* sp., *Penicillium* sp., *Aspergillus niger*, *Coprinus* sp., *Rhizopus*sp., *Alternaria* sp. and *Ceratocystis* sp were recorded to be associated with substrates. Mejía and Albertó (2013) reported that the low contamination might have occurred due to quality of a substrate. In present findings, rice straw promoted maximum contamination due to contain more protein and starch content and pore space, which helps to growth and penetration of mycelium of microorganisms. Studies conducted by Tan (1981) revealed that spawn-running took two to three weeks, when cotton waste was used for the cultivation of *Pleurotus ostreatus* and related species. Similarly, Leong (1980) confirmed that a fast growing strain of *Pleurotus florida* took 9-12 days for spawn-running on cotton waste when held at 20 - 30°C. Bernardi *et al.,* (2007) reported that the productivity and biological efficiency will vary according to different strains and various kinds of substrates used. Namdev *et al.* (2006) observed that the number of days required for spawn run was significantly less than 14 days to 20 days in different straw substrates (gram straw, parthenium straw, sugarcane straw and wheat straw, sunflower stalk, mustard straw and paddy straw). This confirms the finding of Mandeel *et al*., (2005) that B.E is highly affected by the quality of the spawn of the cultivated mushroom strain. Different substrates have been used to grow *Pleurotus* sp. with BE values varying from 32.10 - 79.18% (Dhanda *et al*., 1994).

Contamination of spawn, identification of major contaminants and comparison of different methods for the management of these contaminants has been worked out by Mazumder and Rathaiah (2001), Mazumder *et al*., (2005), Kurtzman (2010) and Kumar (2015) and their results agreed with the result of the present experiment.

Mazumder and Rathaiah (2001) found *Trichoderma harzianum*, *Aspergillus* spp. and *Penicillium* spp. As the three most dominant fungal contaminants during spawn production in oyster mushroom. Mazumder *et al*., (2005), isolated and identified eight fungal contaminants and one bacterial contaminant (*Bacillus brevis*) from severely contaminated spawn bearing wet spot symptom from the naturally contaminated paddy grain base spawn and reported that contamination on paddy was significantly lower (15.00%) than the wheat grain (30.00%) They also reported that 'wet spot' symptom development decreased with increasing number of boiling and autoclaving treatments.

This concurred with the findings of Kurtzman (2010), who reported several causes of mushroom substrate contamination that was insufficient pasteurization could be attributed to the contamination of mushroom. Similarly, this agreed with Kalberer (1974) who observed that the horse manure compost was a poor substrate for the growing oyster mushrooms and insufficient pasteurization (2 or 3 h at 60^0C) could also be attributed to the contamination for the growing oyster mushrooms. So, all the horse manure compost bags were heavily contaminated by green mould and thus abandoned. Kumar (2015) reported that Percentage contamination varied significantly 12.66-20.66% depending on different spawn substrates.

Diana *et al*., (2006) recommended disinfection of the substratum before spawning, which should only destroy the competitive fungi and not the useful microorganisms. Chang, (2008) reported that the substrates for cultivating edible mushrooms e.g. *Pleurotus ostreatus*, has been reported to require varying degrees of pre-treatment in order to promote growth of the mushroom mycelium to the exclusion of other microorganisms. Sanchez (2010) reported that, substrate used for the oyster mushroom cultivation do not require sterilization, but only pasteurization, which is less expensive to diminish the damages produced by different pathogens (bacteria, moulds or insect pests) on mushroom development and yield. Staments (1993) commented that pasteurization selectively kills temperature sensitive microorganisms. The population left intact presents little competition to the mushroom mycelia for initial period giving ample opportunity for the mushroom mycelium to colonize. But, Quimio *et al*.,(1990) observed that substrate sterilization is not ideal since both beneficial and harmful organisms in the substrate are killed.

In this experiment the contamination differed may be due to different substrates and inadequate time pasteurization could also be attributed to the contamination of the substrates. Ponmurugan *et al*., (2007) obtained full colonization in *Pleurotus ostreatus* in 17 - 20 days on different substrates. Dhoke *et al.,* (2001) found that the days required for first picking varied from 11.25-12.00 and the final picking completed from 42.25 to 43.50 days depending on different substrates. Gupta (1989) found that the first crop harvest was required 2-3 days after primordial initiation. Baysal *et al*., (2003) and Sarker *et al*., (2007) reported almost similar results depending on substrates. In the present study, considering the pasteurization time, in all treatments it was observed that two (2) hours steam pasteurization was not enough to control contamination and three or four hours gave the better result. The ability of a sterilization method to eliminate substrate contaminants is shown by the presence or absence of contaminants in the substrate after sterilization, spawning and incubation.

It might be due to amount of substrate is high, so the contamination rate is high. The amount of substrates is a matter of discussion as it affects the yield largely (Zhang *et al.,* 2002). Jebunnahar *et al.*,(2007) reported that the yield of button mushrooms have increased with increasing the amount of substrates. Khan *et al.*,(2012) reported that, the highest biological efficiency 84.80 % was found in 250 g rice straw and the lowest biological efficiency 54.26 % was found in 750g rice straw used as substrate for the cultivation of *Pleurotus salmoneostramineus*. Amin *et al.*,(2008) who reported that the biological efficiency for the cultivation of oyster mushroom was highest in 500g rice straw but in this experiment the highest biological efficiency. Amin (2002) also reported that the number of effective fruiting bodies ranged from 97.25 to 27.75 from different amount rice straw was used for the cultivation of *Pleurotus salmoneo-stramineus,* this variation may occur due to the impact of the amount of rice straw and other strain.

Among the treatments, it was revealed that higher contamination was observed in large sized substrates bags might be due larger surface area of substrates having more space for mycelial growth of contaminants. Tinoco *et. al.,* (2001) however found that larger the surface area and pore of substrates, more is the mycelial growth rate of fungi. This confirms the finding of Mandeel *et al*., (2005) that B.E is highly affected by the quality of the spawn of the cultivated mushroom strain. Different substrates

have been used to grow *Pleurotus* sp. with BE values varying from 32.10 - 79.18% (Dhanda *et al*., 1994). Sarker *et al*.,(2007 a) who found the range of dry yield ranged from 4.28 to 29.98 g/packet of *Pleurotus ostreatus* grown on different substrates. 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 (Haque, 2004). Ahmed (1998) also reported that significant variation was observed on different number of primordia on different pretreated substrates while the lowest was recorded from untreated packets.

Experiment 4.3. Effect of substrate enrichment with different micronutrients on contamination and production of oyster mushroom (*Pleurotus ostreatus*)

4.3.1. Effect of substrate enrichment with micronutrient on spawn packet contamination

During first harvest, incidence of contamination with mycoflora was absent at every concentrations of each micro nutrient. The lowest contamination of 10% was found Agrovit Plus 5000 ppm and $MnCl_2$ 10 ppm enriched packets during third harvest, Agrovit Plus 2000 ppm during fourth harvest and Agrovit Plus 1000, 4000 ppm and 5000 ppm during fifth harvest. Maximum 30% contamination was recorded at control (0 ppm) and Boron 10000 ppm during fifth harvest (Plate 18). In other treatment (3000 ppm Agrovit Plus) the substrate packets were also free from contamination (Table 10).

4.3.2. Main effect of substrate enrichment with micronutrient at different concentrations of four parameters

4.3.2.1. Days to mycelium running

The days required for completion of mycelium running in spawn packet was the lowest in Agrovit Plus at 5000 ppm followed by 0 ppm and 1000 ppm. The effect of three treatments on this parameter was statistically similar but significantly lower compared to other treatments. The maximum 32.70 days was required for completion of mycelium running in spawn packet enriched with $MnCl_2$ at 10 ppm followed by Agrovit Plus 3000 ppm, 2000 ppm and Boron 10000 ppm. Their differences were significant (Table 10).

4.3.2.2. Days to primordia formation

The minimum of 5.90 days was required for primordia formation at Agrovit Plus at 0 ppm (control), which was statistically similar to Agrovit Plus at 1000, 2000ppm and 5000 ppm. The maximum 10.40 days was required for the formation of primordia in spawn packet enriched with Boron at 10000 ppm followed by Agrovit Plus 4000 and 3000 ppm and $MnCl_2$ 10 ppm (Table 10).

4.3.2.3. Days required for primordial formation to first harvest

The lowest of 5.10 days was required for first harvest in substrate enriched with Agrovit Plus at 1000 ppm, which was statistically similar to Agrovit Plus 3000, 4000 and 5000 ppm. The maximum of 11.6 days was required from primordia formation to first harvest of mushroom in substrate enriched with $MnCl_2$ at 10 followed by control and Agrovit Plus 5000 ppm. Their differences were significant. Effect of Agrovit Plus at 2000 and 5000 ppm on this parameter was not significant (Table 10).

4.3.2.4. Days required for total harvest

Days required for total harvest was the lowest under control (0 ppm) followed by $MnCl_2$ 10 ppm, boron 10000 ppm, Agrovit Plus at 3000 and 5000 ppm. Their differences were significant. Effect of Agrovit 2000 and 4000 ppm on total harvest was not significantly different (Table 10).

4.3.3. Effect of two application methods of micronutrient solutions on days to mycelium running, primordia formation, first harvest and total harvest

Effect of two application methods of micro nutrient solutions into spawn packets for substrate enrichment on days require for mycelium running, primordia formation, primordia to fist harvest and total harvest are summarized in Table 11. The four parameters were 23.75, 7.35, 7.45 and 71.43 days, respectively when spawn packets were enriched by dipping in micronutrient solution, and 24.02, 7.22, 6.38 and 79.93 days, respectively days when the micronutrient solutions were injected into water soaked spawn packets.

Table 10. Effect of substrate enriched with micronutrient at different concentrations on incidence of contamination in spawn packets

Substrates enriched with different concentration of micronutrient	Incidence of contamination (%)				
	1st Harvest	2nd harvest	3rd harvest	4th harvest	5th Harvest
Micronutrient 0 ppm	-	30	-	-	-
Agrovit Plus 1000 ppm	-	-	-	-	10
Agrovit Plus 2000 ppm	-	-	-	10	-
Agrovit Plus 3000 ppm	-	-	-	-	-
Agrovit Plus 4000 ppm	-		-	-	10
Agrovit Plus 5000 ppm	-	-	10	-	10
Boron 1000 ppm	-	-	-	-	30
$MnCl_2$ 10 ppm	-	-	10	-	-

- = Contamination absent

Plate 18. The minimum of 10% contamination of spawn packet (A and B) and poorly primodia formation (C)

Table 11. Effect of micronutrient at different concentrations on days required for mycelium running, primordia formation to total harvest

Substrates enriched with different concentration of micronutrient	Days required for			
	Mycelium running	**Primordia formation**	**Primordia to 1st harvest**	**Total harvest**
Micronutrient 0 ppm	20.4 e*	5.9 d	5.1 d	91.5 a
Agrovit Plus 1000 ppm	20.3 e	6.2 d	6.9 c	86.5 b
Agrovit Plus 2000 ppm	25.0 c	7.2 c	5.4 d	79.5 c
Agrovit Plus 3000 ppm	28.0 b	8.7 b	5.4 d	86.7 b
Agrovit Plus 4000 ppm	22.7 d	6.1 d	5.3 d	90.9 a
Agrovit Plus 5000 ppm	20.0 e	10.4 a	6.7 c	62.5 d
Boron 1000 ppm	22.0 d	6.7 c	11.6 a	57.9 e
$MnCl_2$ 10 ppm	32.7 a	7.1 c	8.9 b	49.9f

*Values within the same column with a common letter (s) do not differ significantly (P=0.05).

Table 12. Effect of micronutrient application methods on days required for mycelium colonization to harvesting

Treatment method	**Days required for**			
	Mycelium running	**Primordia formation**	**Primordia to 1st harvest**	**Total harvesting**
Deeping in nutrient solution	23.75	7.35	7.45 a	71.43 b
Injection of solution	24.02	7.22	6.38 b	79.93 a

*Values within the same column with a common letter (s) do not differ significantly (P=0.05).

4.3.4. Interaction effect of substrate enrichment with micronutrient and methods of application

Interaction effect of substrate enrichment with micronutrients at different concentrations and application methods of their solutions on days required for completion of mycelium running, primordia formation, primordia formation to first harvest and total harvest are summarized in Table 13.

4.3.4.1. Days to mycelium running

Days to complete mycelium running in spawn packets having substrate enriched with Agrovit Plus at 1000 ppm (E_1) was 20.0 under deeping (M_1) and 20.8 under injection (M_2) methods of application of micronutrient solution, which were statistically similar to days to complete the mycelium running due to substrate enrichment with Agrovit Plus at 2000, 3000, 5000 ppm and $MnCl_2$ at 10 ppm. The maximum days to complete the mycelium running were 33.8 under M_1 and 31.6 under M_2, which were followed by Agrovit Plus at 4000 ppm, Boron 10000 ppm and $MnCl_2$ at 10 ppm. The influence of two application methods of micronutrient solution on days to complete mycelium running was not significantly different under control as well as each treatment with micronutrient.

4.3.4.2. Days to primordia formation

The minimum of 5.8 and 6.0 days required for primordia formation respectively under deeping (M_1) and injection (M_2) methods of application of Agrovit Plus solution at 1000 ppm (E_1) were statistically similar. The Effect of Agrovit Plus 1000 ppm was statistically similar to Agrovit Plus 2000, 3000 ppm and $MnCl_2$. Significant the highest 10.4 days was required for primordial formation in substrate without enrichment followed by enrichment with Agrovit Plus 4000, Agrovit Plus 5000 ppm.

4.3.4.3. Days required from primordia formation to first harvest

The days required for primordia formation to first harvest of mushroom fruiting bodies grew in substrate enriched with Agrovit Plus at 1000, 3000, 4000 and 5000 ppm under two application methods (deeping and injection) ranged 5.0-7.0. The interaction effect of the factor micronutrient and factor application method on this parameter was not significantly different. Significantly higher days to primordia formation to first harvest were found in substrate without micronutrient enrichment irrespective of application methods compared to other treatments except $MnCl_2$. Under $MnCl_2$, abnormally long day was required for primordia formation of first harvest.

4.3.4.4. Days required for total harvest

The maximum of days required for total harvest was 95.5 and 97.6 under respectively deeping and injection methods of application of Agrovit Plus at 3000 ppm. The days to total harvest recorded from Agrovit Plus at 3000 ppm was statistically similar to Agrovit Plus Agrovit Plus at 1000, 4000 and 5000 ppm irrespective of application method. The lowest required days to final harvest were 45.5 and 54.6 found in substrate without enrichment which was statistically similar with $MnCl_2$ under deeping method (Table 13).

Table 13. Interaction effect of substrate enrichment with micronutrients at different concentrations (E) and method of application (M) on days required for completing of mycelium running, primordia formation, primordia to first harvest and total harvest

Treatment combination	Days required for			
	Mycelium running	Primordia formation	Primordia to 1st harvest	Total harvest
E_0xM_1*	33.8 a***	10.4 a	9.0 b	45.2 g
E_0**xM_2	31.6 b	10.4 a	8.8 b	54.6 f
E_1xM_1	20.0 j	6.0 fg	5.0 f	85.4 b
E_1xM_2	20.8ij	5.8 g	5.2 ef	85.8 b
E_2xM_1	20.6ij	6.4 efg	6.8 cd	77.8 c
E_2xM_2	20.0 j	6.0 fg	7.0 c	73.2 d
E_3xM_1	19.8 j	6.4 efg	5.0 f	95.2 a
E_3xM_2	20.2 j	5.8 g	5.8cdef	97.6 a
E_4xM_1	27.2 d	8.4 b	5.2 ef	86.0 b
E_4xM_2	28.80 c	9.0 b	5.6cdef	94.4 a
E_5xM_1	22.0 ghi	7.2 cd	5.2 ef	87.4 b
E_5xM_2	23.4 fg	7.2 cd	5.4 def	87.4 b
E_6xM_1	25.2 e	7.4 c	6.6 cde	63.0 e
E_6xM_2	24.8 ef	6.8 cde	6.8 cd	62.0 e
E_7xM_1	21.4 hij	6.6 def	16.8 a	53.4 f
E_7xM_2	22.6 gh	6.8 cde	6.4cdef	62.4 e

*E_0= Substrates without enrichment, E_1= Substrates enriched with Agrovit Plus 1000 ppm, $E_{2=}$ substrates enriched with Agrovit Plus 2000 ppm, $E_{3=}$ Substrates enriched with Agrovit Plus 3000 ppm, $E_{4=}$ substrates enriched with Agrovit Plus 4000 ppm, $E_{5=}$ substrates enriched with Agrovit Plus 5000 ppm, E_6= substrates enriched with Boron 10000 ppm, E_7=substrates enriched with $MnCl_2$ 10ppm.

**M_1 = Application of micronutrient solution by deeping and M_2 = Application of micronutrient solution by injection

***Values within the same column with a common letter (s) do not differ significantly (P=0.05)

4.3.5. Biological yield

4.3.5.1. Yield in different flushes

Enrichment of substrate with micronutrient caused significant increase in mushroom production in every flush as well as total yield compared to control. Total yield of five flushes was 542.8, 442.6, 340.7, 258.7 and 178.4 g/packet under first, second, third, fourth and fifth flushes, respectively. The total biological yield were 85.3, 278.3, 245.6, 277.1, 231.7, 241.2, 214.8 and 189.2 g/packet under control E_0, E_1, E_2, E_3, E_4, E_5, E_6 and E_7, respectively. Significantly the highest yield was obtained with E_1 under 1st and 2nd flush and with E_3 under 3rd, 4th and 5th flushes. The yield decreased gradually with the progress of harvesting time showing maximum yield at first and lowest at fifth flush. Differences in yield under different flushes were significant (Table 14). Out of two application methods of micronutrient solution, injection gave higher biological yield compared to deeping method in every flush as well as total (Figure 32).

Table 14. Biological yield of oyster mushroom under different micronutrient at different concentrations used for substrate enrichment and harvested from first to fifth flushes

Substrates enriched with different concentration of micronutrient	**Biological yield (g/spawn packet)**					
	1st flush	**2nd flush**	**3rdflush**	**4thflush**	**5thflush**	**Total**
Substrates without enrichment (E_0)	28.2 d	24.8 d	21.1 f	9.9e	1.3 e	85.3h
Agrovit Plus 1000 ppm (E_1)	90.5 a	74.0 a	48.7 b	37.4 b	27.7 b	278.3a
Agrovit Plus 2000 ppm (E_2)	79.4b	61.0 b	43.3 cd	34.1 c	27.5 b	245cd
Agrovit Plus 3000 ppm (E_3)	80.1 b	66.1 b	54.9 a	40.8 a	35.2 a	277.1ab
Agrovit Plus 4000 ppm (E_4)	60.1 c	51.1 c	50.1 b	42.7 a	27.7 b	231.7cd
Agrovit Plus 5000 ppm (E_5)	76.0 b	61.9 b	45.3 c	35.1 bc	22.9 c	241.2bc
Boron 10000 ppm (E_6)	62.9 c	52.7 c	41.0 d	34.7 c	23.8 c	214.8a
$MnCl_2$ 10 ppm (E_7)	65.6 c	51. c	36.3 e	24.0 d	12.3 d	189.2e
Total	542.8	442.6	340.7	258.7	178.4	

*Values within the same column with a common letter(s) are not significantly different.

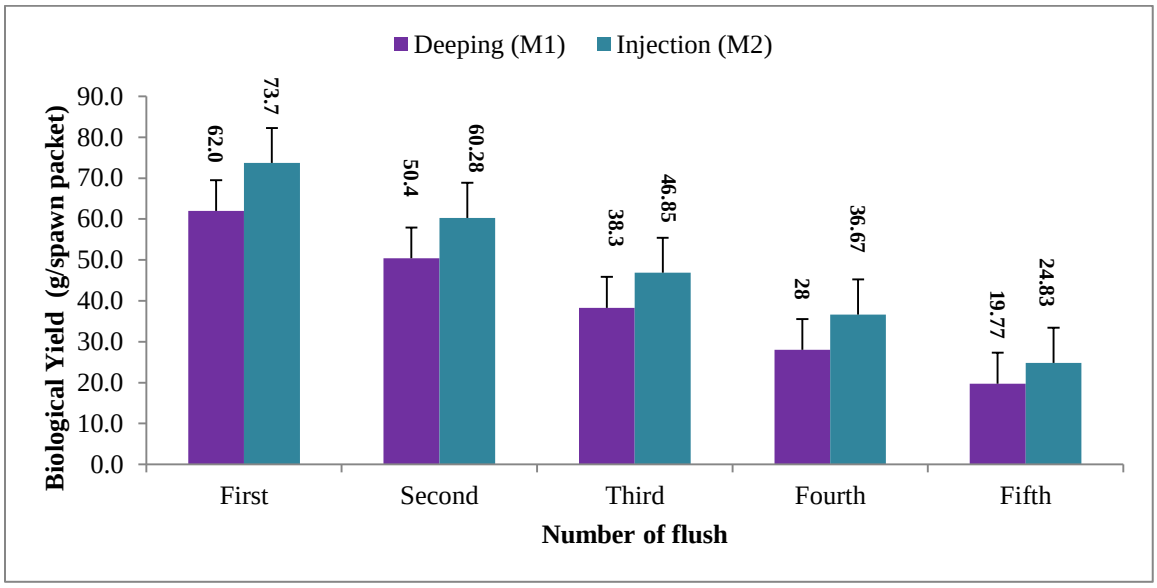

Figure 32. Biological yield of oyster mushroom obtained with different micronutrients from first to fifth flushes

[E_0= Substrates without enrichment, E_1= Substrates enriched with Agrovit Plus 1000 ppm, $E_{2=}$ substrates enriched with Agrovit Plus 2000 ppm, $E_{3=}$ Substrates enriched with Agrovit Plus 3000 ppm, $E_{4=}$ substrates enriched with Agrovit Plus 4000 ppm, $E_{5=}$ substrates enriched with Agrovit Plus 5000 ppm, E_6= substrates enriched with Boron 10000 ppm, E_7=substrates enriched with $MnCl_2$ 10ppm].

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4.3.5.2. Interaction effect of substrate enrichment with micronutrient and methods of application on biological yield

The highest biological yield was obtained at first flush which was decreased gradually with the progress of flushes showing the lowest yield at fifth flush. In first flush, the biological yield under treatment combinations E_2xM_2, E_3xM_1, E_3xM_2, E_4xM_1 and E_4xM_2 was 90.4, 94.2, 89.6, 92.6 and 88.4g/packet, respectively. Effect of those treatment combinations on biological yield was statistically similar but significantly higher compared to all other treatment combinations, which gave statistically similar biological yield (Table 15).

In second flush, the maximum yield 77.6 g/packet was obtained with E_3xM_1, which was statistically similar to the yield obtained with the treatment combinations E_4xM_1, E_3xM_2

and E_3xM_1. The effect of those four treatment combination on this parameter was statistically similar but significantly higher compared to all other treatments. Except control, the lowest yield of 43.0 and 46.2 g/packet were recorded from E_7M_1 and E_7M_2, which were statistically similar to E_1M_2, E_5M_2, E_6M_1, E_6M_2 (Table 15).

At third flush, significantly the highest biological yield of 65.2 g/packet was obtained with E_3xM_2. The second highest yield was recorded from E_3xM_1 which was statistically similar to the yield from E_5xM_2. Except control, the minimum of 31.2 and 32.4 g/packet found in treatment combinations E_7xM_1 and E_7xM_2, respectively (Table 15).

In fourth flush, the highest biological yield was found in E_3M_1which was statistically similar to E_3M_2 and E_5M_2.Under other treatment combination, except control, biological yield ranged 21.8-38.4 g/packet (Table 15).

In fifth flush, treatment combination E_3xM_2 gave the significantly highest biological yield of 41.0 g/packet. Other treatment combinations yielded 10.8-32.0 g/packet mushroom (Table 15).

The maximum total biological yield was obtained with E_3M_2 followed E_3M_1, E_4M_1, E_3M_2, E_2M_2. The treatment combination yielded 316.6, 310.4, 282.0, 278.8 and 268.4 g/packet. Except control, the lowest yield of 172.0 and 176.0 g/packer was observed under E_7M_2 and E_7M_1, respectively (Table15).

Table 15. Interaction **e**ffect of different micronutrients with different concentrations and two enrichment methods on biological yield recorded from first to fifth flushes

Interaction effect treatment combination	Biological yield (g/spawn packet)					
	1st flush	**2nd flush**	**3rd flush**	**4th flush**	**5th flush**	**Total**
E_1**xM1**	70.6 b	58.0 cde	44.6 de	35.0 bc	29.4 bc	237.6e
E_1xM_2	56.2 b	48.0 def	45.2 de	38.4 b	26.0 cde	213.8f
E_2xM_1	68.4 b	57.0 cde	42.4 ef	30.4 d	24.0 de	222.2f
E_2xM_2	90.4 a	65.0 bc	44.2 de	37.8 b	31.0 b	268.4 c
E_3xM_1	94.2 a	77.6 a	58.2 b	48.4 a	32.0 b	310.4a
E_3xM_2	89.6 a	74.2 ab	65.2 a	46.6 a	41.0a	316.6a
E_4xM_1	92.6 a	75.0 ab	47.6 cd	38.2 b	28.6 bc	282.0b
E_4xM_2	88.4 a	73.0 ab	49.8 c	36.6 bc	26.8 cd	274.6bc
E_5xM_1	65.2 b	59.0 cd	41.4 ef	26.0 e	10.8 f	202.4 g
E_5xM_2	64.0 b	54.2 cdef	55.0 b	47.0 a	29.4 bc	249.6 d
E_6xM_1	61.0 b	53.8 cdef	41.6 ef	33.2 cd	24.4 de	214.0 f
E_6xM_2	64.8 b	51.6 def	40.4 f	36.2 bc	23.2 e	216.2 f
E_7xM_1	66.0 b	43.0 f	31.2 g	22.0 f	13.8 f	176.0 h
E_7xM_2	57.8 b	46.2 ef	32.4 g	21.8 f	13.8 f	172.0 h
E_0xM_1*	27.4 c	24.0 g	19.4 h	6.6 h	0.0 g	77.4 j
E_0xM_2	29.0 c	25.6 g	22.8 h	13.2 g	2.6 g	93.2 i

[*E_0= Substrates without enrichment, E_1= Substrates enriched with Agrovit Plus 1000 ppm, $E_{2=}$ substrates enriched with Agrovit Plus 2000 ppm, $E_{3=}$ Substrates enriched with Agrovit Plus 3000 ppm, $E_{4=}$ substrates enriched with Agrovit Plus 4000 ppm, $E_{5=}$ substrates enriched with Agrovit Plus 5000 ppm, E_6= substrates enriched with Boron 10000 ppm, E_7=substrates enriched with $MnCl_2$ 10ppm].

**M_1 = Application of micronutrient solution by deeping and M_2 = Application of micronutrient solution by injection

***Values within the same column with a common letter (s) do not differ significantly (P=0.05)

4.3.6. Economic yield, dry weight and biological efficiency

4.3.6.1. Main effect of micronutrient concentration for substrate enrichment

Main effect of micronutrients used at different concentration for substrate enrichment on economic yield, dry weight and biological efficiency is significant as shown in Table 16. Enrichment of substrate with different micronutrients at different concentrations significantly increased economic yield, dry weight and biological efficiency over control. Under different micronutrients with different concentrations, the ranges of economic yield, dry weight and biological efficiency were 197.2-299.3 g/packet, 45.0-77.3 g/packet and 40.66-62.0%, respectively. Significantly the highest economic yield was obtained with Agrovit Plus at 3000 ppm followed by Agrovit Plus at 2000 ppm. The lowest economic yield of 82.6 g/spawn packet was recorded from control. The highest dry weight and biological efficiency were obtained with Agrovit Plus at 3000 ppm followed by Agrovit Plus 4000 ppm and the lowest dry weight and biological efficiency were found under control.

4.3.6.2. Main effect of inoculation methods for substrate enrichment

The economic yield, dry weight and biological efficiency of oyster mushroom were 207.6 g/packet, 49.1 g/packet and 42.97% under deeping method and 259.7 g/packet, 51.88 g/packet and 53.4% under injection method, respectively. Generally, all parameter were significantly higher under injection method of spawn packets compared to inoculation method (Table 17).

4.3.6.3. Interaction effect of micronutrient concentration and method of enrichment

Interaction effect of enrichment with micronutrients with their concentration (E_0 to E_7) and methods of enrichment (M_1-M_2) on economic yield, dry weight and biological efficiency was significant. The economic yield ranged 75.40 to 355.0g/packet, dry weight ranges 17.0 - 80.4 g/packet and the biological efficiency ranged from 15.52 to 73.24% under different treatment combinations. Enrichment of substrate with micronutrients applied following deeping and injection methods significantly increased above three parameters over control (Table 18). The highest economic yield of oyster mushroom was

observed at E_3M_2 followed by E_4 M_2, E_3M_1 and E_4M_1, while the lowest economic yield was showed under $MnCl_2$ (Table 18). Almost similar trends were observed in case of dry weight and biological efficiency.

Table 16. Main effect of micronutrients with concentrations used for substrate enrichment on economic yield, dry weight and biological efficiency of oyster mushroom

Substrates enriched with different concentration of micronutrient	Economic yield (g/pk)	Dry weight (g/pk)	Biological efficiency (%)
Substrates without enrichment(E_0)	82.60 f	18.0 f	17.0 f
Agrovit Plus 1000 ppm (E_1)	256.7 cd	49.4 d	52.9 c
Agrovit Plus 2000 ppm (E_2)	287.3 ab	55.1 c	59.2 ab
Agrovit Plus 3000 ppm (E_3)	299.3 a*	77.3 a	62.0 a
Agrovit Plus 4000 ppm (E_4)	264.5bc	60.1b	54.5 bc
Agrovit Plus 5000 ppm (E_5)	252.0cd	56.0c	51.8 cd
Boron 10000 ppm (E_6)	229.6 d	42.8 e	47.2 d
$MnCl_2$ 10 ppm (E_7)	197.2 e	45.0 e	40.6 e

*Values within the same column with a common letter (s) do not differ significantly (P=0.05)

Table 17. Effect of application methods of micronutrients for substrate enrichment on yield contributing character of oyster mushroom

Application method micronutrient enrichment	Economic yield (g/packet)	Dry weight (g/packet)	Biological efficiency (%)
Deeping (M_1)	207.6 b	49.05 b	42.97 b
Injection method (M_2)	259.7 a	51.88 a	53.42 a

*Values within the same column with a common letter(s) do not differ significantly (P=0.05)

Table 18. Interaction **e**ffect of different micronutrients with different concentrations and two enrichment methods on yield contributing characters

Interaction effect treatment combination	Economic yield (g/packet)	Dry weight (g/packet)	Biological efficiency (%)
E_1*xM_1**	251.4 de***	59.80 c	52.00 de
E_1xM_2	231.2 e	47.40 f	47.76 e
E_2xM_1	230.2 e	51.40 e	47.60 e
E_2xM_2	283.2 cd	74.20 b	58.36 bcd
E_3xM_1	303.8 bc	73.00 b	62.84 bc
E_3xM_2	355.0 a	46.00 f	73.24 a
E_4xM_1	294.8 bc	54.20 de	61.20 bc
E_4xM_2	323.2 ab	80.40 a	66.44 ab
E_5xM_1	216.0 e	52.20 e	44.56 e
E_5xM_2	272.8 cd	44.80 f	56.00 cd
E_6xM_1	225.6 e	40.80 g	47.12 e
E_6xM_2	233.6 e	44.00 fg	47.32 e
E_7xM_1	178.4 f	47.20 f	36.76 f
E_7xM_2	174.0 f	19.00 h	35.84 f
E_0xM_1**	75.40 g	17.00 h	15.52 g
E_0xM_2	89.80 g	56.00 d	18.64 g

[*E_0= Substrates without enrichment, E_1= Substrates enriched with Agrovit Plus 1000 ppm, $E_{2=}$ substrates enriched with Agrovit Plus 2000 ppm, $E_{3=}$ Substrates enriched with Agrovit Plus 3000 ppm, $E_{4=}$ substrates enriched with Agrovit Plus 4000 ppm, $E_{5=}$ substrates enriched with Agrovit Plus 5000 ppm, E_6= substrates enriched with Boron 10000 ppm, E_7=substrates enriched with $MnCl_2$ 10ppm].

**M_1 = Application of micronutrient solution by deeping of spawn and M_2 = Application of micronutrient solution by injection

***Values within the same column with a common letter (s) do not differ significantly (P=0.05)

4.3.7. Effect of substrate enrichment with micronutrients on yield parameter

4.3.7.1. Main effect of enrichment with micronutrient on number of primordia and fruiting body, average weight, diameter of pileus and length of stipe

Enrichment of substrate with micronutrients significantly increased number of primordia, fruiting bodies, average weight of fruiting bodies and diameter of pileus over control. Under different enrichment (except control) the parameters ranged 119.5-169.4, 46.9-80.6, 4.75-55.7g, 3.1-4.9 cm and 1.65-4.2 cm, respectively. To increase number of fruiting bodies, Agrovit Plus 3000 ppm most effective followed by 1000 and 4000 ppm.

The heaviest fruiting body was obtained with Agrovit Plus 5000 ppm followed by 2000 and 1000 ppm. The maximum pileus diameter was recorded from Agrovit 5000 ppm followed by 3000 ppm. The least effective enrichment was $MnCl_2$ 10 ppm to form primordia and fruiting bodies. The lowest weight of fruiting body was observed under the enrichment Agrovit 1000 ppm followed by $MnCl_2$. The diameter of pileus was minimal in the substrate enriched with $MnCl_2$ which was statistically similar to boron 10000 ppm (Table 19).

4.3.7.2. Main effect of enrichment method

Between two enrichment methods of substrate, number of primordia and effective fruiting bodies of oyster mushroom were significantly higher in injection method compared to deeping method. On the other hand, average weight of fruiting was higher in case of average weight of fruiting body. Effect of two enrichment methods on diameter of pileus was statistically similar (Table 20)

4.3.7.3. Interaction effect of enrichment and its methods on fruiting body and pileus

Interaction effect of enrichment with micronutrients (E_0-E_7) and method of application by deeping (M_1) and injection (M_2) on number of primordia and number of effective fruiting bodies was significant. The highest number of primordia (223) was obtained from spawn packet enriched with Agrovit Plus 3000 ppm following deeping method and maximum number of fruiting body (90.8) by injection method of enrichment. The lowest numbers of primordia (78.4) fruiting bodies (25.4) were recorded from control where enrichment method was injection (Table 21). The highest average weight of fruiting bodies was found in Agrovit Plus 4000 ppm applied following injection method. The maximum diameter of pileus was recorded from Agrovit 3000 ppm applied by injection followed by 3000 ppm enriched by deeping method. The lowest diameter was observed under control (Table 21).

Table 19. Effect of enrichment of substrates with micronutrients at different concentration on four yield contributing characters

Substrates enriched with different concentration of micronutrient	Number of		Mean of	
	Primordia	Effective fruiting body	Weight fruiting body (g)	Diameter of pileus (cm)
Agrovit Plus 1000 ppm (E_1)	169.4 a	70.90 b	4.41 e	4.42 bc
Agrovit Plus 2000 ppm (E_2)	149.7 cd	55.10 d	5.02 b	4.58 b
Agrovit Plus 3000 ppm (E_3)	152.8 bc	80.60 a	3.83 g	4.91 a
Agrovit Plus 4000 ppm (E_4)	156.9 b	65.90 c	4.05 f	3.34 d
Agrovit Plus 5000 ppm (E_5)	140.8 e	48.30 f	5.73 a	4.96 a
Boron 10000 ppm (E_6)	145.9 de	52.00 e	4.89 c	4.18 c
$MnCl_2$ 10 ppm (E_7)	119.5 f	46.90 f	4.75 d	4.18 c
Substrates without enrichment (E_0)	91.10 g	26.90 g	3.19 h	3.05 e

*Values within the same column with a common letter(s) do not differ significantly (P=0.05)

Table 20. Effect of micronutrient application methods on yield contributing characters and dimension of fruiting body

Methods of enrichment with micronutrient	Number of		Mean	
	Primordia	Effective fruiting bodies	Weight of fruiting bodies (g)	Diameter of pileus (cm)
Deeping (M_1)	115.1 b	49.88 b	4.534 a	4.24
Injection (M_2)	166.4 a	61.78 a	4.435 b	4.165

*Values within the same column with a common letter(s) do not differ significantly (P=0.05)

Table 21. Interaction effect of enrichment of substrates by different micronutrients on yield contributing characters and dimension of fruiting bodies

Interaction effect treatment combination	Number of primordia	Effective fruiting bodies	Mean weight of fruiting bodies (g)	Diameter of pileus (cm)
E_1**xM1**	115.8 f*	70.40 c	3.98 fg	4.52 bc
E_1xM_2	113.0 f	59.60 e	4.15 f	4.32 bcd
E_2xM_1	104.6 g	50.80 g	4.71 d	4.68 bc
E_2xM_2	194.8 b	59.40 e	5.28 b	4.48 bc
E_3xM_1	223.0 a	64.80 d	4.75 d	5.22 a
E_3xM_2	192.6 b	90.80 a	3.67 h	5.30 a
E_4xM_1	141.2 d	77.00 b	4.10 fg	3.64 e
E_4xM_2	172.6 c	60.00 e	6.16 a	3.04 f
E_5xM_1	139.0 d	51.20 g	5.29 b	4.70 b
E_5xM_2	142.6 d	72.20 c	3.95 g	4.52 bc
E_6xM_1	126.8 e	48.80 g	5.23 b	4.34 bcd
E_6xM_2	165.0 c	55.20 f	4.54 e	4.02 d
E_7xM_1	102.0 g	42.60 h	5.04 c	4.08 d
E_7xM_2	137.0 d	36.60 i	4.46 e	4.28 cd
E_0xM_1	78.40 h	25.40 j	3.08 j	3.44 e
E_0xM_2	103.8 g	28.40 j	3.28 i	2.66 g

[*E_0= Substrates without enrichment, E_1= Substrates enriched with Agrovit Plus 1000 ppm, $E_{2=}$ substrates enriched with Agrovit Plus 2000 ppm, $E_{3=}$ Substrates enriched with Agrovit Plus 3000 ppm, $E_{4=}$ substrates enriched with Agrovit Plus 4000 ppm, $E_{5=}$ substrates enriched with Agrovit Plus 5000 ppm, E_6= substrates enriched with Boron 10000 ppm, E_7=substrates enriched with $MnCl_2$ 10ppm].

**M_1 = Application of micronutrient solution by deeping and M_2 = Application of micronutrient solution by injection

***Values within the same column with a common letter (s) do not differ significantly (P=0.05)

4.3.8. Discussion

Enrichment of substrate by micronutrients increased the yield, yield attributes, quality and nutrient contents of mushroom over control in the present study. Additional nutrients can be easily used by fungi because the absorption of these molecules is more energetically efficient than synthesizing the molecules, which allow the fungi to obtain more energy for mycelial growth and mushroom formation. The supplements are materials mostly enriched with carbohydrates, proteins, amino acid, macro- and micronutrient (Royse and Sanchez, 2008a; Royse *et al*., 2008). The present findings corroborated with the findings of previous workers. The substrate can also be

supplemented with additional N sources, such as wheat bran, oat bran, copra cake (from spent coconut), rice bran, sorghum or millet in order to obtain quality mushrooms and additives such as gypsum, limestone and chalk can function as pH buffers in a substrate (Kwon and Kim 2004a and 2004b). Stamets (2005) stated that nutritional composition of substrates is crucial in determining mycelia growth initiation. Royse (2002) found as the spawn rate was increased, the number of days to production and the time required for total harvest decreased with the levels of supplements increased compared to sawdust alone.

The result of the present study corroborates with the study of previous researchers, Biswas *et al*. (2009) reported that the maximum of 0.45% and 0.35% reduction in days to primordial initiation and time primordia initiation to harvest was obtained with 0.2% wuxul super supplementation (well balanced NPK liquid fertilizer supplemented with a full complement of macro- and micronutrients) which was followed by 0.1 and 0.3%. Biswas *et al*., (2016) also recoded biological yield (368.18g) from mango tree sawdust supplemented with 30% wheat bran of oyster mushroom *(Pleurotus ostreatus)* spawn. Chowdhury *et al.,* (1998) examined the micronutrients effects of adding different supplements to substrates for growing oyster mushrooms (*Pleurotus sajor-caju*) and found adding 5% supplements gave the highest yield of oyster mushroom. Baysal *et al.,* (2003) found the highest yield (350.2 g) of oyster mushroom (*Pleurotus ostreatus*) with the substrate supplemented with 20% rice husk in weight. Sarker *et al.,* (2008) observed that number of fruiting bodies of oyster mushroom ranged from 20 to 98.25/ packets on wheat straw supplemented with different levels of wheat and rice bran.

Royse and Sanchez, (2008a and 2008b) and Royse *et al.,* (2008) enriched substrates with carbohydrates, proteins, amino acid, macro- and micronutrient. They found that all of the supplements increase yield and quality of mushroom (Royse and Sanchez, 2008a and 2008b). Biswas *et al.,*(2009) stated that the highest economic yield (224.3g) and biological efficiency (79.54%) of the mushroom on spawn packet were obtained with 0.2% wuxul super supplementation followed by 0.1 and 0.3% of the supplement. MC Substradd products are tuned on the nutritional needs of mushroom and complete the deficits in the compost of essential nutrients like nitrogen, carbohydrates or trace-

elements. It gives higher yield (up to 30%) and offers a direct economical benefit for every mushroom grower. Royse and Sanchez (2008b) tested 16 supplements of mushroom compost, including five crystalline amino acids, one amino acid blend, one egg white and four hydrolyzed proteins, Micromax (a micronutrient containing nine minerals) and four commercial supplements to find out their effect on mushroom yield and biological efficiency.

The present findings keep in with the findings of previous workers, as, Biswas *et al.*,(1997) found supplementation of substrate promoted biological efficiency (125.75%). Mandeel *et al*,. (2005) cultivated *Pleurotus* spp. on various lignocellulosic wastes supplemented with fresh chicken manure, where, the highest biological efficiency (BE) was noted on cardboard with both *P. columbinus* (134 %) and *P. ostreatus* (117 %). The supplementation of the substrates with various sources of organic nitrogen, such as wheat bran, rice bran, maize waste water, soya cake powder and rice, has increased the BEs of various species of basidiomycetes (Loss *et al.*, 2009, Moonmoon *et al.*, 2011). Nunes *et.al.*, (2012) stated that the BE increase can be due to the high availability of water in substrate additional with rice bran, since addition of rice bran decreases the granulometry of substrate, which improve the moisture retention (Özçelik & Peksen, 2007). Biswas *et al*.,(2009) Significantly the highest diameter of stalk (0.90 cm) and pileus and weight of individual fruiting body (2.84g) of mushroom was obtained with 0.2% wuxal super.

Biswas *et.al*.,(2016) recoded biological yield (368.18 g), economic yield (360.68 g), dry yield (35.15 g) and weight of individual fruiting body (5.60 g) from mango tree sawdust supplemented with 30 % wheat bran of oyster mushroom *(Pleurotus ostreatus)* spawn. Yoshida *et al.* (1993) reported that the number of fruiting bodies was increased when the substrates was mixed with different supplements. Sarker (2004) found that the number of primordia increased with the levels of supplement and continued up to a certain range and declined thereafter.

4.3.9. Effect of enrichment of substrates with micronutrients on nutritional composition of oyster mushroom

4.3.9.1. Effect on carbohydrate content

The carbohydrate content varied from 34.20-52.30% regarding micronutrient enrichment in different concentration. The highest carbohydrate content was found in untreated substrate (E_0) and the lowest was recorded from Agrovit Plus 2000 ppm enriched packets (Table 22).

4.3.9.2. Effect on protein content percentage

Mushrooms are considered to be a good source of digestible proteins. The protein content differed significantly 17.5-25.6% in all treatments of injected micronutrients packets. The highest was obtained from 0.4% Agrovit plus (T_4) injected packets followed by T_2 and T_3 while, the lowest was found in 0.5% Agrovit plus (T_5) treated packets (Table 22).

4.3.9.3. Effect on lipid content

The Lipid content ranged from 1.3-3.8% depending on different micronutrients and their concentration. The highest lipid content was 3.8 % Agrovit plus 5000 ppm enriched packets (E_5) followed by E_1, T_7 and E_2 and the lowest was observed in substrate enriched with Agrovit plus 4000 ppm (E_4) packets (Table 22).

4.3.9.4. Effect on total ash content

The highest ash content (9.9%) was obtained from Agrovit plus 2000 ppm (E_2) enriched spawn statistically similar to E_1 and E_3. The lowest ash content (6.7%) was found in Boron enriched substrate (E_6) which was statistically similar to untreated control (E_0) regarding micronutrient enrichment in different concentration (Table 22).

4.3.9.5. Effect on total fiber content

The total fiber content varied from 10.8 to 30% among all enrichment. The highest was obtained from Agrovit Plus 4000 ppm injected into the substrate and the lowest was observed under control. The fiber content in E_1 (Agrovit Plus 1000 ppm), E_2 (Agrovit Plus 2000 ppm), E_3 (Agrovit Plus 3000 ppm) were statistically similar. The fiber content in Agrovit Plus 5000 ppm (24.2%), $Mncl_2$10 ppm (24.0%) and Boron 10000 ppm (25.0%) enriched substrate were statistically identical (Table 22).

4.3.9.6. Effect on dry matter content (%)

The highest percentage of dry matter (14.16%) was recorded from Boron10 ppm followed by control (13.74%) and Agrovit Plus 3000 ppm treated packets (13.71%), whereas, the lowest (8.79) was recorded from Agrovit Plus 1000 ppm enriched packets. The dry matter content in 0Agrovit Plus 4000 ppm (12.40%), Agrovit Plus 5000 ppm (12.42%) and $Mncl_2$10 ppm (12.78%) treated packets were statistically similar (Table 22).

4.3.9.7. Effect on moisture content

The moister content ranged from 85.84-91.21% depending on enrichment with different micronutrients and their concentrations. The highest moisture content was recorded from Agrovit plus 1000 ppm supplemented packets and the lowest was observed in Boron 10000 ppm enriched packets (Table 22).

Table 22. Effect of enrichment of substrates with micronutrients on nutritional composition of oyster mushroom

Enrich-ments	Carbohydrate content (%)	Protein content (%)	Lipid content (%)	Total ash content (%)	Total fiber content (%)	Dry matter content (%)	Moisture content (%)
E_{1*}	35.9 e*	22.8 d	3.50 b	9.8 a	28.0 b	8.790 f	91.2a
E_2	34.2 f	23.9 b	3.00 d	9.9 a	29.0 ab	10.11 e	89.9 b
E_3	37.5 d	25.6 a	1.30 h	5.6 d	30.0 a	12.40 d	87.6 c
E_4	37.5 d	23.1 c	1.90 g	8.9 ab	28.6 b	13.71 b	86.3 de
E_5	47.3 b	17.5 g	3.80 a	7.2 c	24.2 c	12.42 d	87.6 c
E_6	48.0 b	18.4 f	2.90 e	6.7 c	24.0 c	14.16 a	85.8 e
E_7	44.4 c	19.0 e	3.30 c	8.3 b	25.0 c	12.78 c	87.2 cd
E_0	52.3 a	16.6 h	2.40 f	6.9 c	21.8 d	13.74 b	86.3 de

*E_0= Substrates without enrichment, E_1= Substrates enriched with Agrovit Plus 1000 ppm, $E_{2=}$ substrates enriched with Agrovit Plus 2000 ppm, $E_{3=}$ Substrate enriched with Agrovit Plus 3000 ppm, $E_{4=}$ substrate enriched with Agrovit Plus 4000 ppm, $E_{5=}$ substrates enriched with Agrovit Plus 5000 ppm, E_6= substrate enriched with Boron 10000 ppm, E_7=substrate enriched with $MnCl_2$ 10 mm.

**Means within the same column with a common letter (s) do not differ significantly (P=0.05)

4.3.10. Effect of enrichment of substrates with micronutrients on mineral contents of oyster mushroom

4.3.10.1. Effect on potassium content

The highest content of potassium (2.67 mg/g) was observed under control (E_0) and the lowest (1.03 mg/g) was observed under Agrovit plus 4000 ppm (E_4). The content of potassium in E_3 (2.00 mg/g) and E_5 (1.98mg/g) E_6 (1.14 mg/g) and E_7 (1.21 mg/g) were statistically similar (Table 23).

4.3.10.2. Effect on Phosphorus

The highest percentage of phosphorus (1.78mg/g) was observed inT_4 followed by T_3,T_6 and T_2, whereas the lowest phosphorus percentage (0.80mg/g) was observed under 0.1% Agrovit plus (T_1), statistically similar with T_5 (Table 23).The content of phosphorus (0.96 mg/g) in Boron and unenriched packets were statistically identical.

4.3.10.3. Effect on Zinc content

Content of Zn varied from 9.57 mg/100 g to 13.40 mg/100g in mushroom. The highest amount of zinc was observed under treatment Agrovit plus4000 ppm (E_4) was statistically similar to E_3, E_6 and the lowest amount was observed under control (Table 23).

4.3.10.4. Effect on copper content

The highest percentage of copper content (31.28 mg/100g) was observed under treatment Agrovit Plus 4000 ppm (E_4) and the lowest copper content (20.72mg/100g) was observed under 0.5% Agrovit Plus 5000 ppm (E_5) (0.15 mg/g) (Table 23).

4.3.10.5. Effect on iron (mg/g) content

In present study, iron content in mushrooms was found to be in a range of 27.55-46.87 mg/g (Table 23). Iron content was found to be relatively high (46.87 mg%) in E_4 followed by E_1 and E_2 and low in E_0 in the basis of dry weight.

4.3.10.6. Effect on Manganese

Manganese content in mushrooms ranged 0 .03 - 0.94 mg/100g. Manganese content was the highest in Agrovit Plus 1000 ppm enriched packets (E_1) and the lowest in Agrovit Plus 4000 ppm enriched packets (Table 23).

Table 23. Effect of enrichment with micronutrients on mineral contents of oyster mushroom

Enrichments	Potassium (mg/g)	Phosphorus (mg/g)	Zinc (mg/g)	Copper (mg/g)	Iron (mg/g)	Manganese (mg/g)
E_{1*}	1.75 c**	0.80 e	12.55 c	22.20 e	43.20 b	0.94 a
E_2	1.65 d	1.07 c	12.28 d	30.15 b	42.16 b	0.83 b
E_3	2.67 a	1.78 a	13.40 a	31.28 a	46.87 a	0.03 f
E_4	2.00 b	1.67 b	13.36 a	30.19 b	33.80 e	0.82 b
E_5	1.98 b	0.82e	10.98 e	20.72 g	34.89 d	0.22 e
E_6	1.14 e	1.10 c	12.66 b	27.98 c	28.67 f	0.71 d
E_7	1.21 e	0.96 d	10.20 f	22.70 d	40.30 c	0.74 c
E_0	1.03 f	0.96 d	9.57 g	21.01 f	27.55 g	0.74 c

[*E_0= Substrates without enrichment, E_1= Substrates enriched with Agrovit Plus 1000 ppm, $E_{2=}$ substrates enriched with Agrovit Plus 2000 ppm, $E_{3=}$ Substrates enriched with Agrovit Plus 3000 ppm, $E_{4=}$ substrates enriched with Agrovit Plus 4000 ppm, $E_{5=}$ substrates enriched with Agrovit Plus 5000 ppm, E_6= substrates enriched with Boron 10000 ppm, E_7=substrates enriched with $MnCl_2$ 10ppm].

**Means within the same column with a common letter (s) do not differ significantly (P=0.05)

4.3.11. Discussion

Mushroom can play an important role to meet up the nutritional requirements of the population of Bangladesh (Amin *et al*., 2007). The nutritional advantages of mushrooms include a low content of calories and a high content of proteins, minerals and dietary fiber (Beluhan and Ranogajec, 2011). Mushrooms are a good source of protein, vitamins and minerals (Khan *et al*., 2013). The efficiency of mushroom species in producing food protein in the form of biomass or fruiting bodies from different wastes lies in their ability to degrade waste via secretion of a variety of hydrolyzing and oxidizing enzymes (Kuforiji and Fasidi 2008 and Zhu *et al*., 2013). The result of the present study more or less similar to the findings of previous researchers. Dundar *et al.*, (2008) found that the carbohydrate content of *P. sajor-caju*, *P. ostreatus* and *P. eryngii* are 37.72, 37.87 and 39.85 (g/100 g dried matter), respectively. Mona *et al*., (2009) investigated nutritional analysis and enzyme activities of *Pleurotus ostreatus* cultivated on *citrus limonium* and *Carica papaya* wastes and they concluded that fruit bodies containing 26.0-31.5% digestible protein, 20.9 -33.0% total soluble carbohydrates and 2.0-5.9% fat (on dry basis).

Biswas *et al.* (2009) reported that the moisture and dry matter content of oyster mushroom grown under different levels of wuxal super varied from 89.5 to 90.23 % and 9.77 to 10.46% respectively. Beluham and Ranogajec (2011) reported mushrooms are a potential source of total carbohydrates in the range of 42.62-66.78 g/100g and protein in the range of 27.95-38.89 g/100g and very low fat contents 1.34-6.45g/100g depending upon the species of mushroom. Zahid *et al.,* (2010) studied nutritional status, where, crude protein, total lipids, available carbohydrates, dietary fiber, total carbohydrates, moisture, total solids and ash content in mushrooms were found to be in the ranges of 3.22-4.83, 0.41-1.05, 4.2-6.37, 0.58-1.11,4.82-7.48, 85.95-90.07, 9.93-14.05 and 0.98-2.3 g/100g of fresh edible portion, respectively on the basis of different species of oyster mushroom.

Ashraf *et al*., (2013) showed crude protein was ranged 24.83%-27.23%, fiber content showed ranges between 22.03%-26.28, ash contents in three varieties of oyster mushroom were found 6.76-9.08%, the carbohydrate content (36.74%), the total fat content (2.37%), protein (27.23%) and fiber contents (26.28%) was found in *P. ostreatus* depending upon the different substrates. Ashraf *et al*., (2013) observed that the percentage of dry matter and the moisture content varied from 12.63-17.23% and 82.77-87.37% among the interaction of different oyster species and substrates. Kalač, P. and Svoboda, L. (2000) reported that minerals in diet are essential for metabolic reactions, healthy bone formation, transmission of nerve impulses, regulation of water, and salt balance. The finding of the present work matches with the study of Sarker *et al.,* (2007) who found 0.97% phosphorus and 1.3% potassium in oyster mushrooms grown on sawdust based substrates. Ali *et al.,*(2007) also showed, a significant change in potassium percentage before and after treatments of substrate. Potassium contents decreased after treatment. Maximum contents (1.096%) of potassium were observed in substrate, before subjecting to different treatments. Minimum (0.52%) were determined in substrate after the treatment. However, potassium contents increased after the completion of mycelial growth. Ali *et al.,* (2007) reported that, maximum phosphorus contents (0.64%) were observed in substrate after the completion of mycelial growth, while minimum (0.16%) were determined before and after treatments. Increase in phosphorus contents, after the

completion of mycelial growth, might be due to acidity produced through CO_2 released through respiration of *Pleurotus* species which resulted in the formation of H_2CO_3. Lee *et al.* (2009) found that Potassium were 25.7, Cu 14.6, Fe 1.3, Zn 18 and Mn 0.7 (g/100 g dried matter) in *P. eryngii* in supplemented substrate (pine sawdust (23%)+ concob (29%)+ rice bran (18%)+ beet pulp (4%)+ wheat bran (14%)+ cottonseed hull (4%)+ shell powder (4%)+ dehydrated beverage by-product from soybean (14%).

Sarker *et al.,* (2007 b) found 30.92 ppm zinc in oyster mushroom grown on sawdust based substrates. Similar result was observed the value of Cu 3.2 (mg/ kg) by Ahmed *et al.*, (2013) and Copper 0.65 mg/g by Deepalakshmi (2014) in case of Po_2 in their study. The result of the present study matches with the study of Alam *et al.,* (2007) who found 16-20.9 mg% of zinc in different Oyster mushroom varieties. The finding of the present work differ with the study of Deepalakshmi and Mirunalini (2014) that reported the 55-65 mg/g of iron on dry weight of fruiting bodies. The result also differs by the study of Ahmed *et al.* (2013) who found 421 g/Kg Iron in Oyster (Po_2). Biswas *et al*., (2009) observed that the highest P content (0.96%), K (1.42%) and Fe content (43.60%) at 0.2% wuxal super (A mineral combination) treated packets. This is similar to the report of Deepalakshmi and Mirunalini (2014) that Manganese content of *Pleurotus ostreatus* ranged between 0.5-3 mg/100 g dried mushrooms, but differs with the result of Ahmed (1998), who reported 2.5mg/ kg.

Experiment 4.4.Evaluation of chemicals to sterilize substrate against contaminating fungi and production of oyster mushroom

4.4.1. In-vitro evaluation of chemicals against mushroom contaminating fungi

4.4.1.1.Radial colony growth of fungi on chemical amended PDA

Results of the preliminary *in vitro* test conducted to determine effectiveness of seven chemicals tested against mycelium growth of *P. ostreatus* and six contaminating fungi such as *Penicillium* sp., *Aspergillus niger*, *Trichoderma* sp., *Sclerotium* sp., *Rhizopus* sp. and *Aspergillus flavus* are summarized in Table 24.

4.4.1.2. Growth of *Pleurotus ostreatus*

Pleurotus ostreatus is an edible mushroom fungus. In the *in vitro* test, colony diameter of *P. ostreatus* was 57.0 mm under control. Under the amendment with Bavistin 50 WP, Hydrogen peroxide and Chlorox increased the mycelium growth to 90 mm and increase was 57.9% over control. Except $MnCl_2$, other chemicals also increased the mycelium growth by 26.7-42.0%. Only $MnCl_2$ inhibited mycelium growth by 3.6% (Table 24 and Plate 19).

4.4.1.3. Growth of *Penicillium* sp.

Under control, the mycelium growth of *Penicillium* sp., a contaminating fungus, covered the whole petridish up to the rim with mycelium, where the radial diameter of colony growth was 90 mm. In Petri dishes containing PDA amended with chemicals inhibited the growth of the fungus. The range of inhibition of radial mycelium growth was18.7-92.2%. The highest growth inhibition was recorded from H_2O_2 followed by Formalin, Alkalanized hydrated lime and Bavistin. The lowest inhibition was observed under the amendment with $MnCl_2$ followed by Surf x-cel (Table 24 and Plate 20).

4.4.1.4. Growth of the contaminating fungus, *Aspergillus niger*

Under control, radial colony diameter of *A. niger* was 90 mm. It was increased to 57.8 mm under Surf x-cel and 53.5 mm under $MnCl_2$. The radial colony diameter under those two chemicals and control was statistically similar. The range of inhibition of radial

mycelium growth was 35.7-98.5%. The highest growth inhibition was recorded from Chlorox followed by H_2O_2Bavistin, Formalin and Alkalanized hydrated lime. The lowest inhibition was observed under the amendment with Surf x-cel followed by $MnCl_2$ (Table 24 and Plate 21).

4.4.1.5. Growth of the contaminating fungus, *Trichoderma* sp.

The radial growth of *Trichoderma* sp. was 90 mm under control, which mean that the whole plate was covered by the mycelium of the contaminating fungus under control. Amendment of PDA with each chemical inhibited the radial mycelium growth significantly over control. The range of inhibition under six chemicals was 22.2-92.4% over control. The highest inhibition was obtained with H_2O_2 followed by Bavistin and Chlorox. The lowest inhibition was observed under $MnCl_2$ followed by Surf x-cel and Alkalanized lime (Table 24 and Plate 22).

4.4.1.6. Growth of the contaminating fungus, *Sclerotium* sp

Radial mycelium growth *Sclerotium* sp. under control was 90 mm. Due to amendment of PDA with Alkalanized lime, $MnCl_2$, Bavistin and Surf x-cel, the radial growth of colony increased to 90.0, 75.3, 65.2 and 55.8 mm, respectively. The results indicate that these chemicals are not toxic to *Sclerotium* sp. The highest inhibition was observed with Chlorox followed by $H_2O_2$and Formalin. The lowest inhibition was obtained under $MnCl_2$ followed by Bavistin and Surf x-cel (Table 24 and Plate 23)

4.4.1.7. Growth of the contaminating fungus, *Rhizopus sp*

Radial colony growth of *Rhizopus* sp on PDA without amendment (control), amended with Bavistin and $MnCl_2$ was 90.0 mm. Amendment of PDA with other four chemicals caused significant reduction in radial growth of the fungus to 4.0-73.4 mm (Table 24). Photographs of radial growth on PDA in Petri dishes clearly showed the effectiveness of seven chemicals (Plate 24).

4.4.1.8. Growth of the contaminating fungus, *Aspergillus flavus*

The radial colony diameter of *A. flavus* was 90 mm under control. Amendment of PDA with all chemicals significantly reduced the colony diameter. The reduction was 18.5 to 93.9% over control. The highest inhibition was obtained with H_2O_2 and the lowest was obtained with Surf x-cel (Table 24). Photographs of radial growth on PDA in Petri dishes clearly showed the effectiveness of seven chemicals (Plate 25).

Table 24. *In vitro* mycelium growth of *P. ostreatus* and six contaminating fungi in petridishes containing PDA amended with chemicals

Chemicals with dose (ppm)	Mycelium growth *in vitro* (within parentheses percentage and without mm)						
	P. ostreatus	*Penicillium* sp.	*A. niger*	*Trichoderma* sp.	*Sclerotiums* p.	*Rhizopus* sp.	*A. flavus*
Bavistin 75 ppm (T_{1*})	90.0 a** (+57.9)***	19.2 c (-78.7)	8.0 b (-91.1)	6.20 e (-92.1)	65.2 a (27.3)	90.0 a (0.0)	6.5 d (-92.8)
Formalin 100 ppm (T_2)	77.0 c (+35.0)	11.2 d (-87.6)	9.0 b (-89.9)	25.0 d (-72.0)	11.3 c (86.5)	4.5 d (-95.0)	21.1 c (-76.6)
Alkalanized lime (T_3)	81.5 b (+42.9)	15.0 d (-83.3)	9.5 b (-89.4)	27.7 d (-69.2)	90.0 a (+0)	15.3 c (-83.0)	23.5 c (-73.9)
H_2O_2 3% (T_4)	90.0 a (+57.9)	7.0 e (-92.2)	2.4 b (-97.2)	6.0 e (-92.4)	5.5 c (92.9)	4.0 d (-95.6)	5.5d (-93.9)
Chlorox (T_5)	90.0 a (+57.9)	22.0 c (-75.5)	1.3 b (-98.5)	9.1 e (-89.8)	4.0 c (94.6)	4.0 d (-95.6)	24.8 c (-72.4)
Surfx-cel (T_6)	72.2 d (26.7)	23.4 c (-73.92)	57.8 a (+35.7)	63.0 c (-30.0)	55.8 ab (37.6)	73.4 b (-18.4)	73.3 b (-18.5)
$MnCl_2$ 100 ppm(T_7)	55.0 F (-3.6)	61.0 b (-32.2)	53.5 a (40.5)	70.0 b (-22.2)	75.3 a (16.17)	90.0 a (0.0)	21.8 c (-75.8)
Control (T_0)	57.0 f	90.0 a	90.0 ab	90.0 a	90.0 bc	90.0 a	90.0 a

*T_1= Bavistin (75 ppm) for 24 hr T_2=Formalin (500 ppm) for 24 hr, $T_{3=}$Alkalanized with hydrated lime for 36 hr, T_4=Hydrogen peroxide (3%), T_5=Chlorox, $T_{6-=}$Surf x-cel and $T_{7=}$ Manganese chloride (100 ppm) and T_0 =Control

**Means of the original values within the same column having a common letter(s) do not differ significantly P=0.05).

***Values within parentheses are percentages [(value under treatment-value under control)÷(value under control)x{100}]

- = Growth inhibited, + = Growth enhanced

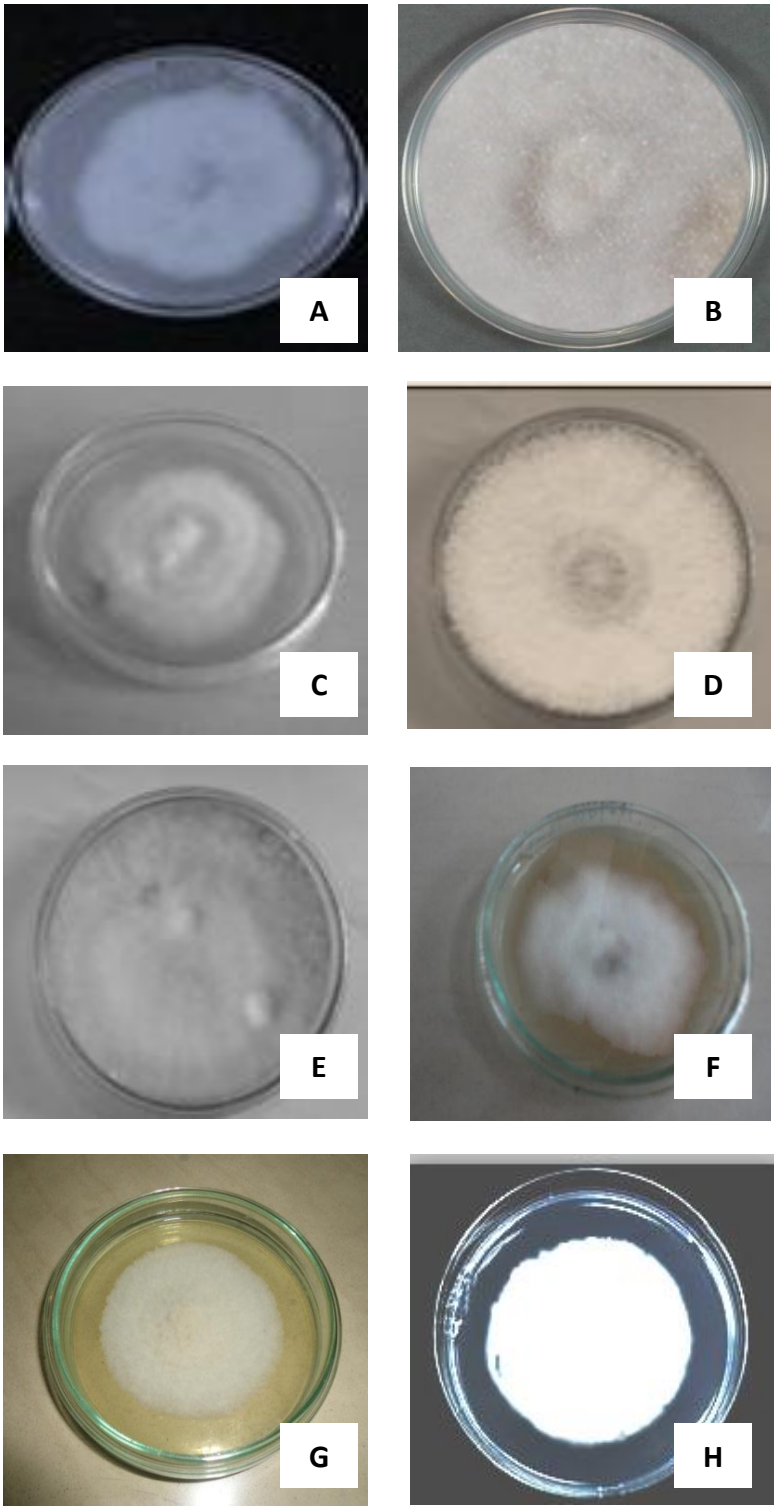


Plate 19. Radial mycelium growth of oyster mushroom (*Pleurotus ostreatus*) on PDA amended with six chemicals (A-G):
A. Bavistin, **B.** Formalin, **C**. Alkalanized lime, **D.** Hydrogen peroxide, **E**. Chlorox, **F.** Surf x-cel, **G.** Manganese chloride, **H.** Control

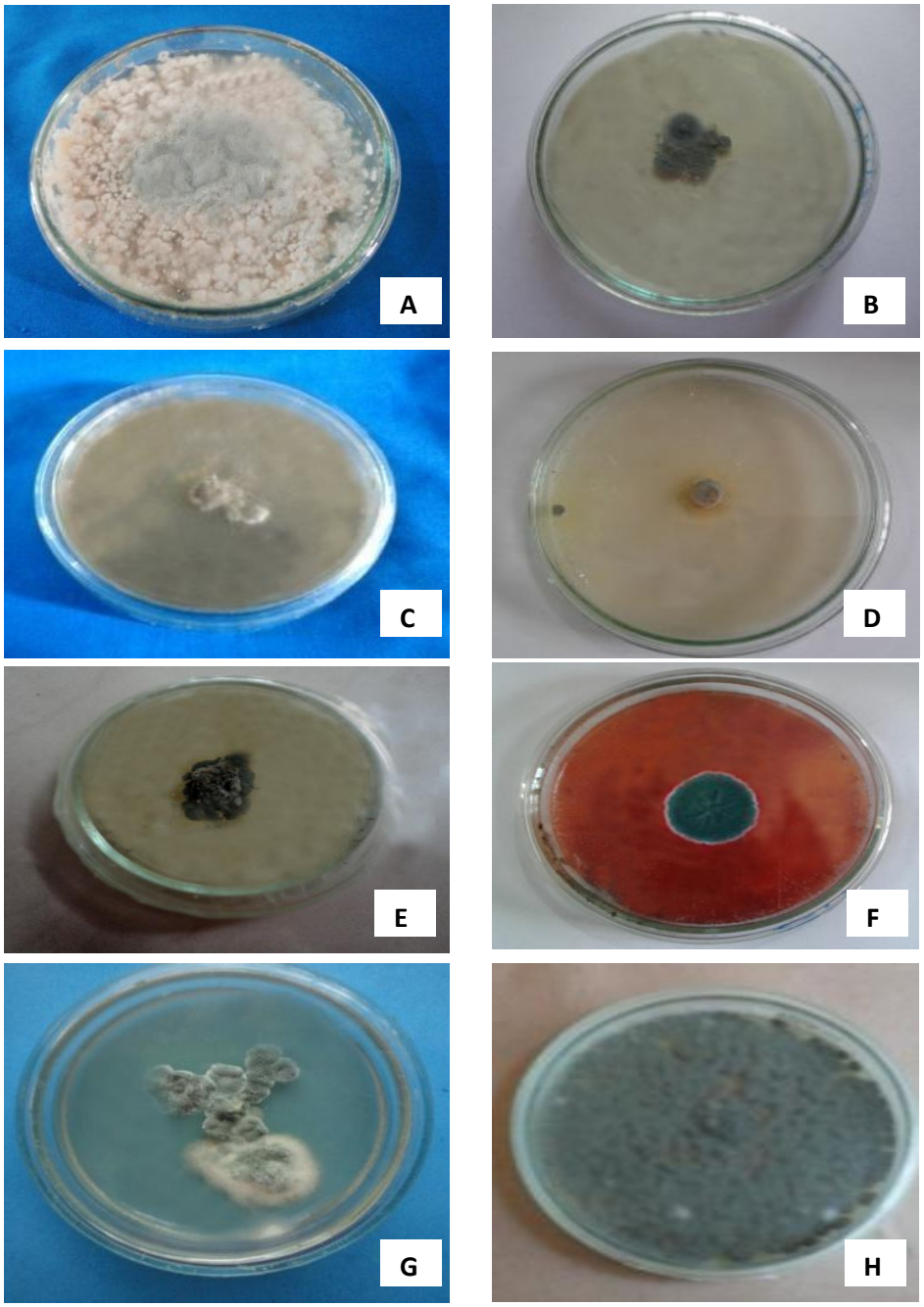


Plate 20. Radial mycelium growth of *Penicillium* sp. on PDA amended with six chemicals (A-G):
A. Bavistin, **B.** Formalin, **C**. Alkalanized lime, **D.** Hydrogen peroxide, **E**. Chlorox, **F.** Surf x-cel, **G.** Manganese chloride, **H.** Control

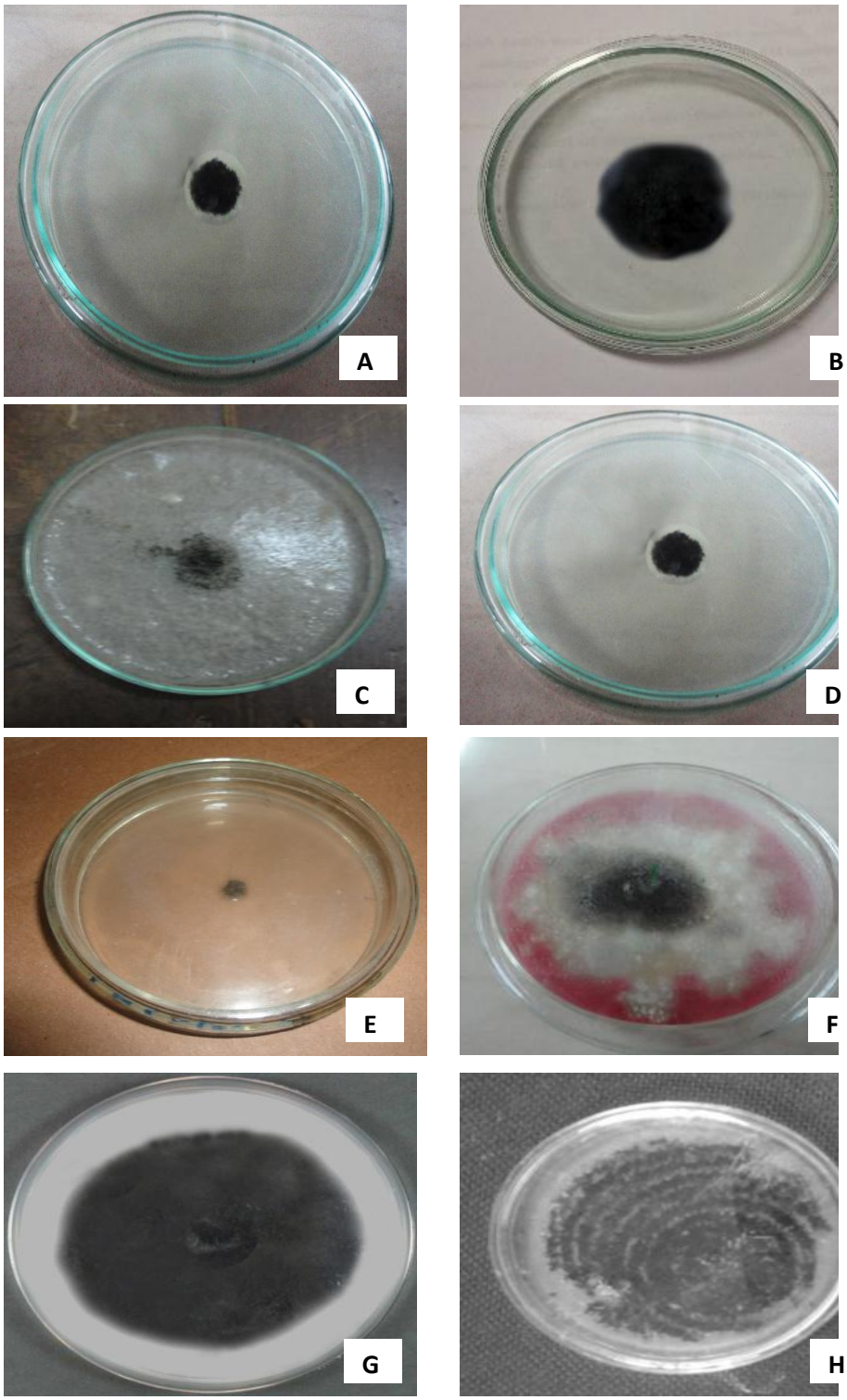


Plate 21. Radial mycelium growth *Aspergillus niger* on PDA amended with six chemicals (A-G):
A. Bavistin, **B.** Formalin, **C**. Alkalanized lime, **D.** Hydrogen peroxide, **E**. Chlorox, **F.** Surf x-cel, **G.** Manganese chloride, **H.** Control

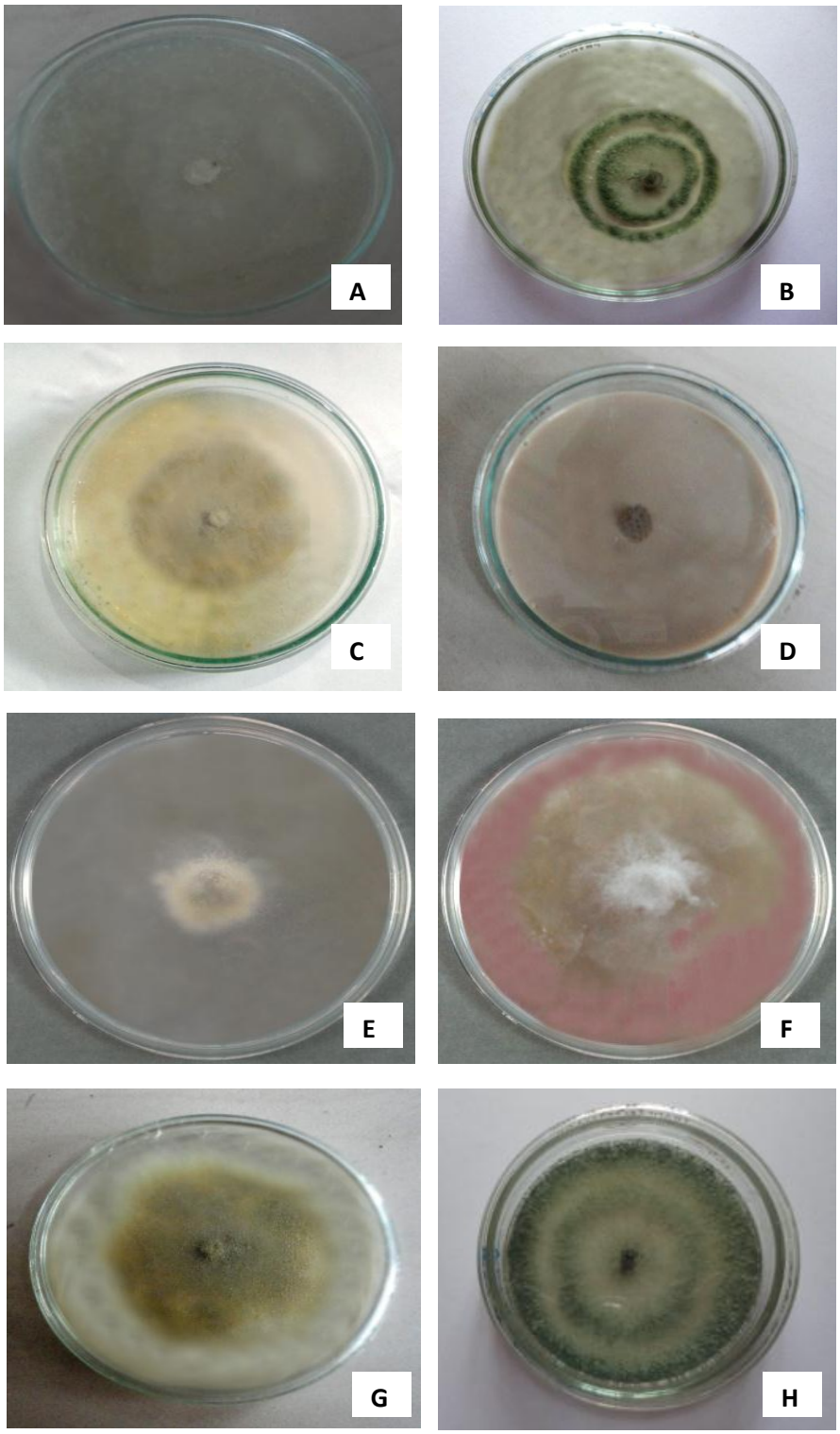


Plate 22. Radial mycelium growth of *Trichoderma* sp. on PDA amended with six chemicals (A-G):
A. Bavistin, **B.** Formalin, **C**. Alkalanized lime, **D.** Hydrogen peroxide, **E**. Chlorox, **F.** Surf x-cel, **G.** Manganese chloride, **H.** Control

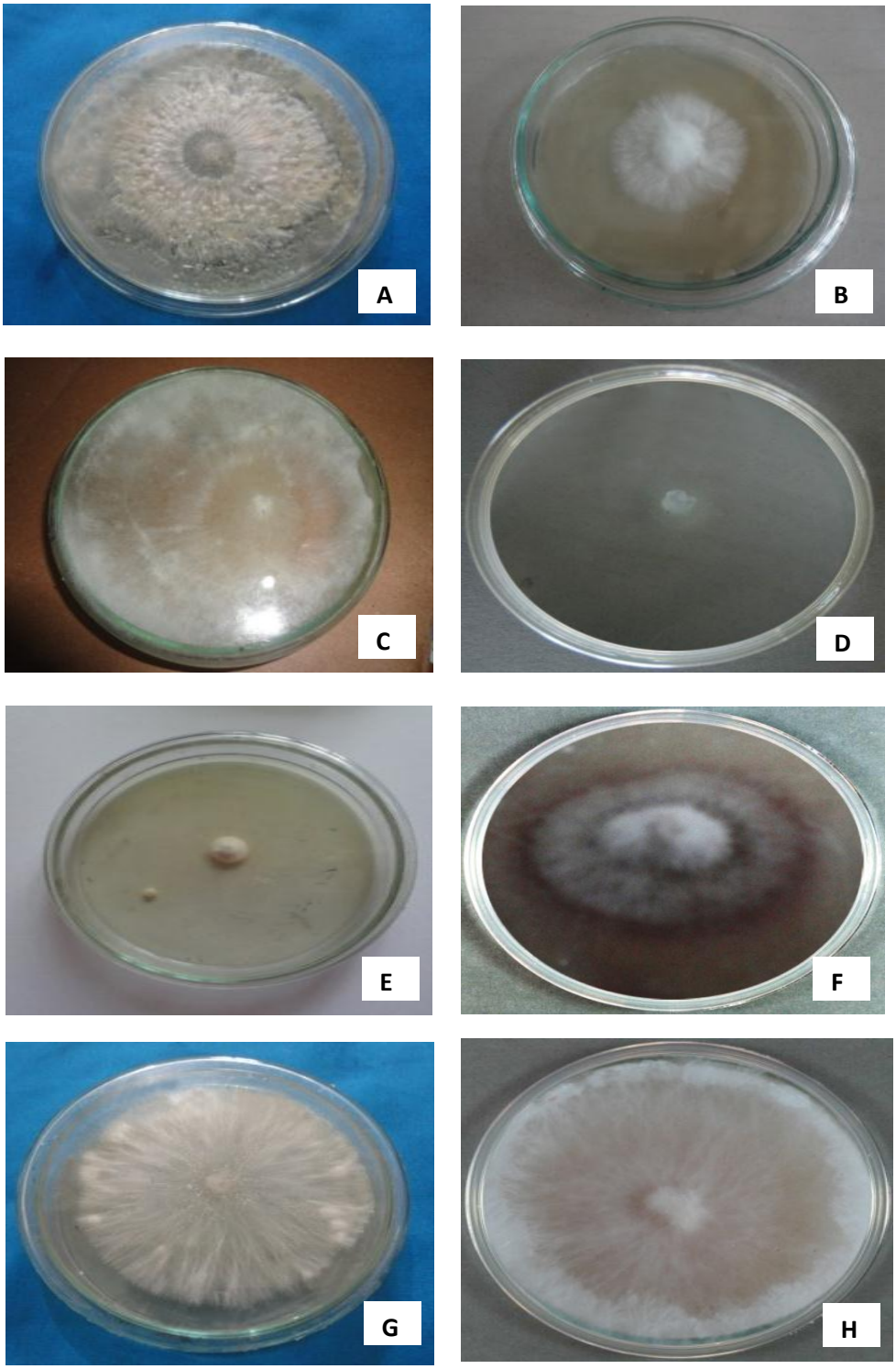


Plate 23. Radial mycelium growth of *Sclerotium* sp. on PDA amended with six chemicals (A-G):
A. Bavistin, **B.** Formalin, **C**. Alkalanized lime, **D.** Hydrogen peroxide, **E**. Chlorox, **F.** Surf x-cel, **G.** Manganese chloride, **H.** Control

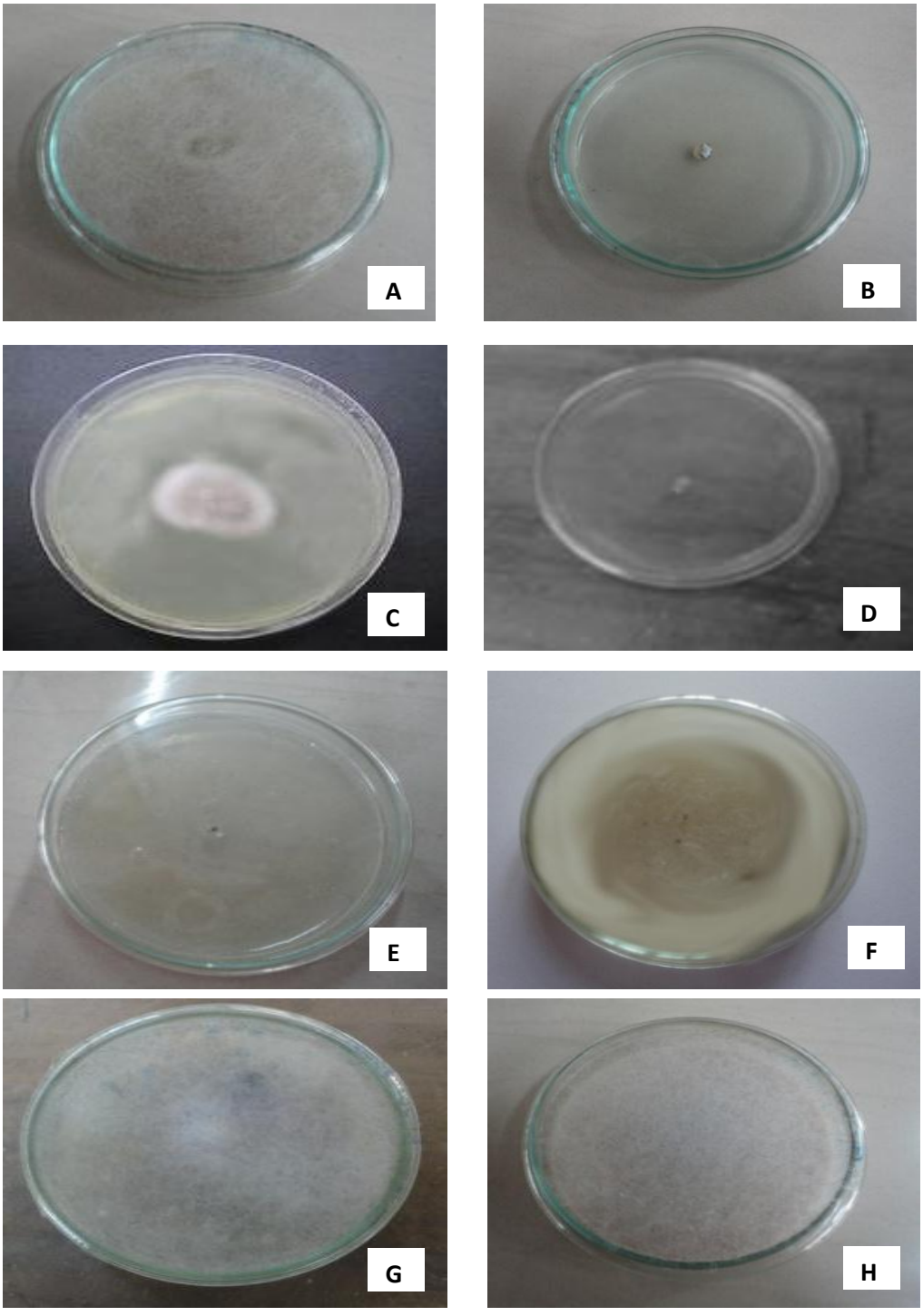


Plate 24. Radial mycelium growth of *Rhizopus* sp. on PDA amended with six chemicals (A-G):
A. Bavistin, **B.** Formalin, **C**. Alkalanized lime, **D.** Hydrogen peroxide, **E**. Chlorox, **F.** Surf x-cel, **G.** Manganese chloride, **H.** Control

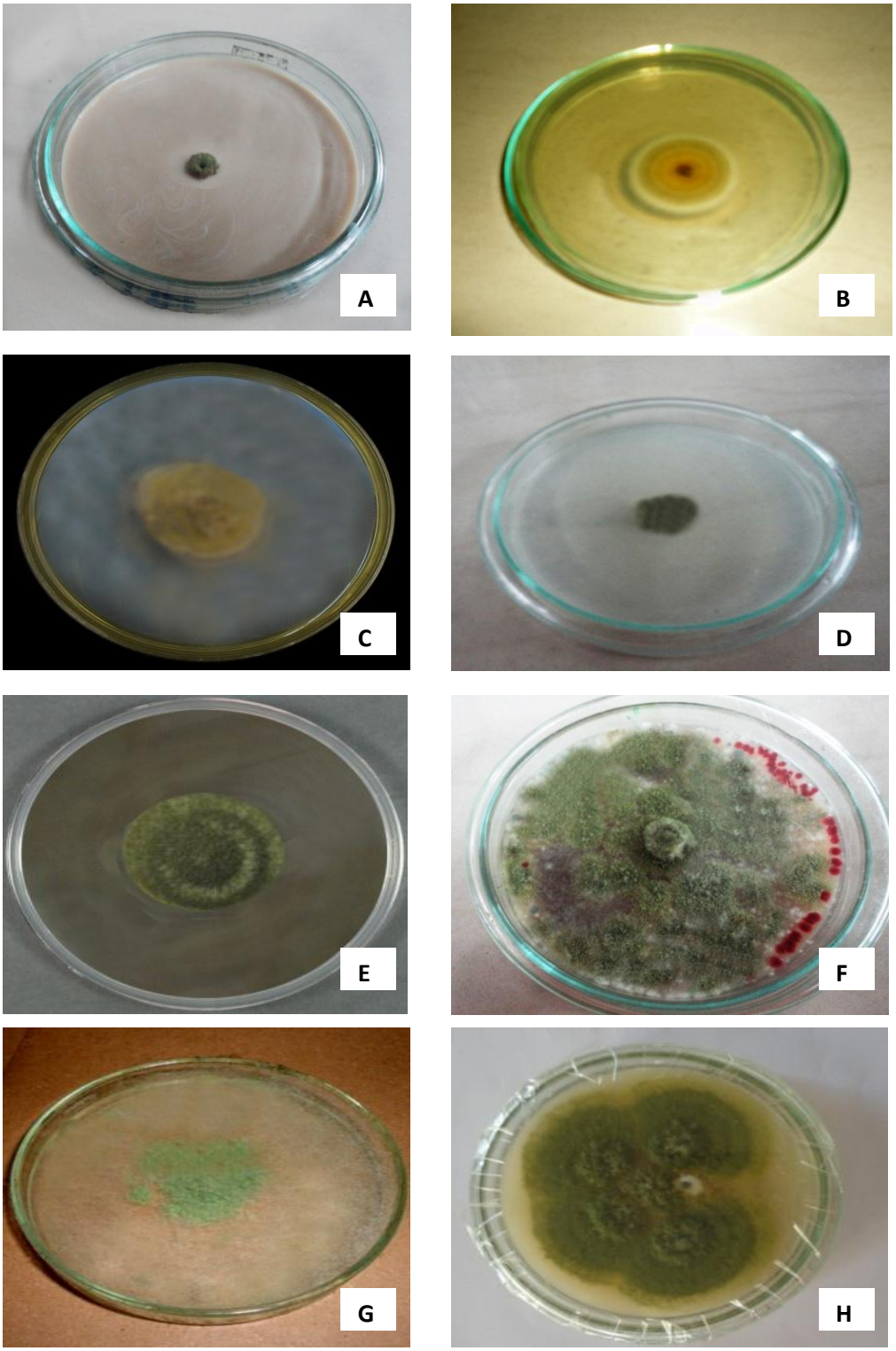


Plate 25. Radial mycelium growth of *Aspergillus flavus* on PDA amended with six chemicals (A-G):
A. Bavistin, **B.** Formalin, **C**. Alkalanized lime, **D.** Hydrogen peroxide, **E**. Chlorox, **F.** Surf x-cel, **G.** Manganese chloride, **H.** Control

4.4.2. *In-vivo* evaluation of chemicals for substrate sterilization against contaminating fungi and production of oyster mushroom

4.4.2.1. Severity of contamination in spawn packet containing chemical sterilized substrate

The contamination with fungi was found under control only at first and second harvest where the severity was 19.0 and 36.0%, respectively. The contamination severity was 10.0 and 6.0% under third harvest when substrate was treated with Alkalanized hydrated lime and Hydrogen peroxide, respectively, and 4.0 and 8.0% under fourth harvest where Chlorox and $MnCl_2$ were used for sterilization, respectively. Treatment of substrate with Surf x-cel and $MnCl_2$ showed respectively 12.6 and 27.0% contamination under fifth harvest. In other cases the spawn packets were free from contamination (Table 25).

Table 25. Effect of chemical treatment of substrate of oyster mushroom on contamination

Treatment	Contamination severity (%)				
	First harvest	Second harvest	Third harvest	Fourth harvest	Fifth harvest
T_1	-	-	-	14.0	-
T_2	-	-	-	8.0	-
T_3	-	-	10.0	-	-
T_4	-	-	6.0	-	-
T_5	-	-	-	4.0	5.0-
T_6	-	-	-	-	12.6
T_7	-	-	-	8.0	27.0
T_0	19.0	36.0	50.0	80.5	100

*T_1= Bavistin (75 ppm) for 24 hr T_2=Formalin (500 ppm) for 24 hr, $T_{3=}$Alkalanized with hydrated lime for 36 hr, T_4=Hydrogen peroxide (3%), T_5=Chlorox, $T_{6-=}$Surf x-cel and $T_{7=}$ Manganese chloride (100 ppm) and T_0 =Control

4.4.2.2. Mycelium running and days required for primordia formation, first harvest and total harvest

Sterilization of substrate with different chemicals significantly increased mycelium run rate over control. The range of mycelium running of oyster mushroom under seven different chemicals was 0.4-1.3 cm/day. Significantly the highest run rate was observed in packets containing substrate sterilized with Hydrogen peroxide followed by Chlorox

and Alkalanized hydrated lime. The minimum mycelium run rate was recorded from surf x-cel. The run rate was only 0.2 cm/day under control (Table 26).

Under control, the days require for completion of mycelium running was 26.20, Except Formalin, all other chemicals significantly decreased days required for completion of mycelium running compared to control. The minimum of 15.4 days for completion of mycelium running was obtained with H_2O_2 followed by Surf x-cel (17.0 days), Bavistin (17.2 days) and $MnCl_2$ (18.6 days). Except control, the maximum days (23.6) required for mycelium running was obtained with Formalin followed Chlorox (22.8 days) and Alkalanized hydrated lime (Table 26).

Maximum of 8.4 days were required for primordia formation under control. All chemicals tested for substrate sterilization decreased days required primordia formation significantly compared to control. The minimum of 4.8 days for primordia formation was obtained with Surfx-cel followed by H_2O_2 (5.0), Bavistin (6.2 days) and Chlorox (6.8 days).Except control, the highest 7.6 days required for primordia formation was obtained with Alkalanized hydrated lime followed by Formalin (7.4 days) and $MnCl_2$ (7.2 days). Under control, days required for first harvest were 5.8, which was statistically similar to $MnCl_2$ (5.6 days), Surf x-cel (5.2 days) and Chlorox (5.8 days). The highest (8.6 days) was recorded from Bavistin followed by Alkalanized hydrated lime (6.6 days) and Hydrogen peroxide (6.0 days). Differences in this parameter among above three chemical were significant (Table 26)

The lowest days (26.6) were required for total harvest after stimulation of oyster mushroom under control, which was statistically similar to Alkalanized hydrated lime (27.6 days) and Bavistin (40.0 days). Sterilization of substrate with other chemicals significantly increased days required for total harvest over control. Hydrogen peroxide, Formalin, Chlorox, $MnCl_2$ and Surf x-cel used for substrate sterilization significantly increased the parameter within the range of 56.6 - 97.4 days. Efficacy of Hydrogen peroxide, Formalin and Chlorox was statistically similar and significantly higher compared to other chemicals except Surf x-cel (Table 26).

Table 26. Effect of substrate sterilization with chemicals on mycelium growth, days required for mycelium running, primordia formation and harvest

Treatment	Mycelium run rate (cm/day)	Days required for			
		Mycelium running	Primordia formation	First harvest	Total Harvest
T_1	0.5 e*	17.2 cd	6.2 d	8.6 a	40.0d
T_2	0.5 e	23.6 ab	7.4 bc	5.0 e	96.8 a
T_3	0.8 c	21.8 b	7.6 b	6.6 b	27.6 d
T_4	1.3 a	15.4 d	5.0 e	6.0 c	97.4 a
T_5	1.0 b	22.8 b	6.8 cd	5.8 cd	84.0 ab
T_6	0.4 f	17.0 cd	4.8 e	5.2 de	79.6 b
T_7	0.6 d	18.6 c	7.2 bc	5.6 cde	56.4 c
T_0	0.2 g	26.2 a	8.4 a	5.8 cd	26.6 d

*T_1= Bavistin (75 ppm) for 24 hr T_2=Formalin (500 ppm) for 24 hr, $T_{3=}$Alkalanized with hydrated lime for 36 hr, T_4=Hydrogen peroxide (3%), T_5=Chlorox, $T_{6-=}$Surf x-cel and $T_{7=}$ Manganese chloride (100 ppm) and T_0 =Control

*Means within the same column with a common letter(s) are not significantly different (P=0.05).

4.4.3. Biological yield, economic yield, dry weight and biological efficiency

Sterilization of mushroom substrate with seven chemicals significantly increased biological yield, economic yield, dry weight and biological efficiency of oyster mushroom. All parameters under Hydrogen peroxide and Chlorox were statistically similar but significantly higher compared to other chemicals. So, it was noted that the most effective chemicals were Hydrogen peroxide and Chlorox followed by Alkalanized hydrated lime and $MnCl_2$. The lowest biological and economic yield was observed under Surf x-cel, which was statistically similar to Bavistin and Formalin. The lowest dry weight was recorded from Surf x-cel followed by Formalin and Bavistin. On the other hand, the lowest biological efficiency was found in substrate sterilized with Bavistin followed by Surf x-cel and Formalin (Table 27).

Table 27. Effect of sterilization of substrates with chemicals on different yield contributing parameters of oyster mushroom

Chemicals with dose (ppm)	**Biological yield (g)**	**Economic yield (g)**	**Dry weight (g)**	**Biological efficiency (%)**
	92.2d	87.8 d	24.6 c	18.4e
Bavistin 75 ppm	96.4 d	91.8 d	21.6 cd	19.2d
Formalin 100 ppm	167.8 b	160.0 b	34.0 b	33.5b
Alkalanized lime 286 ppm	219.6 a	211.8 a	41.0 a	43.3 a
H_2O_2 30000ppm	216.8 a	210.8 a	40.6 a	43.2 a
Chlorox 1000ppm	89.6d	84.8 d	16.6 d	18.7 e
Surfx-cel 100ppm	127.4 c	121.8 c	25.6c	25.4 c
$MnCl_2$ 100 ppm	35.8 e	33.4 e	4.2 e	3.78 f
Control				

*Means within the same column with a common letter(s) are not significantly different (P=0.05).

4.4.4. Yield contributing characters of Oyster mushroom

Number of primordia formed in spawn packets containing substrate sterilized with all seven chemicals was significantly increased over control. Significantly the highest number of 89.0 primordia was recorded under H_2O_2. The lowest number of 58.4 primordia was found in substrate sterilized with Alkalanized hydrated lime, which was statistically similar to other chemicals. Significantly the highest mean weight of 8.9 g/fruiting body was obtained with Chlorox followed by H_2O_2 and Bavistin. The lowest mean weight of 2.2 g/fruiting body was recorded from control which was statistically similar to the treatments with Formalin; Alkalanized hydrated lime and Surf x-cel. Sterilization of substrate with Alkalanized hydrated lime, H_2O_2, Formalin and $MnCl_2$ significantly increased weight of per fruiting body over control. Effect of control and treatment with other chemicals on mean weight of fruiting body (g/fruiting body) was not significantly different. The diameter of pileus was 1.5 cm under control which was statistically similar to those of recorded under Surf x-cel and Formalin. Significantly the highest weight of 7.0 g/fruiting body was obtained with Chlorox followed by Hydrogen peroxide and Manganese chloride (Table 28)

Table 28. Effect of sterilization of substrates with chemicals yield attributes of oyster mushroom (*Pleurotus ostreatus*)

Chemicals with dose (ppm)	Number of Primordia	Mean Weight (g/ fruiting body)	Number of fruiting body	Diameter of pileus(cm)
	64.0 b	4.1 b	23.4 cd	3.0 c
Bavistin 75 ppm	67.8 b	2.5 c	31.8 b	1.8 e
Formalin 100 ppm	70.4 b	2.7 c	47.0 a	2.3 d
Alkalanized lime 286 ppm	89.6 a	8.9 b	49.0 a	7.0 a
H_2O_2 30000ppm	67.2 b	4.4 a	26.4 bcd	3.9 b
Chlorox 1000ppm	58.4 b	2.7 c	26.8 bcd	1.5 e
Surfx-cel 100ppm	59.0 b	4.1 b	30.6 bc	3.3 c
$MnCl_2$ 100 ppm	36.2 c	2.2 c	19.4 d	1.6 e
Control				

*Means within the same column with a common letter(s) are not significantly different (P=0.05).

4.4.5. Discussion

Findings of the present *in-vitro* experiment reveal that except $MnCl_2$, other chemicals tested in the experiment enhance mycelium growth of the mushroom fungus (*P. ostreatus*) or the chemicals are not toxic to it. Formalin, Alkalanized hydrated lime, Hydrogen peroxide and Chlorox are effective to control contaminating fungi of oyster mushroom without affecting the growth of *P. ostreatus*. However, the efficacy of the chemicals to sterilize substrates for growing mushroom needs to be tested in vivo under mushroom house conditions before final recommendation for mushroom cultivation.

The findings about the inhibition of fungal growth *in vitro* condition are more or less similar to the findings of many other researchers. Doshi and Singh (1985) and Sharma and Jandaik (1983) obtained complete inhibition of most of competitor moulds of oyster mushroom with the application of 50 ppm benomyl + 100 ppm Thiram. Jain and Vyas (2002) and Biswas (2014) reported that Carbendazim (37.5 ppm) + Formalin 500 ppm is effective against the competitor moulds *in vitro***.** Parvez *et al.*, (2010) reported that growth inhibition of radial colony diameter of *T. harzianum* (96.80%), *A. niger* (94.70%), *R. stolonifer* (94.00%) was achieved at 500ppm of Formalin while the growth inhibition was 85.80% in *Penicillium* sp. Upadhyay e*t al.*, (1987) and Vijay and Sohi (1987)

evaluated different concentration of Carbendazim (Bavistin) and it's combination with formaldehyde (formalin) against the major contaminants of *P. sajor caju, P. flabellatus* and *P. citrinipileatus* found complete inhibition of contaminating fungi under *in vitro* and *in vivo* conditions. Kumar (2011) reported that maximum radial growth was observed at 6th days in ferrous sulphate and copper sulphate supplemented medium of *P. sajor caju, P. florida, P. flabellatus, P. fossulatus* and *P. sapidus*. Minimum time was observed for spawn run in *P. sajor caju, P. fossulatus* and *P. sapidus* supplemented with ferrous sulphate followed by magnesium sulphate, respectively. Prakash *et al.,* 2012; Zaidi and Dahiya, 2015 reported similar finding. Singh *et al*., (2015) and Biswas **(**2014) tested Manganese (Mn) and neem (*Azadirachta indica*) against competitor fungi and recorded maximum inhibitory effect against *Aspergillus* spp., *Trichoderma* spp., *Coprinus* spp. and *Penicillium* spp. and less effective against *Sclerotium rolfsii in* vitro.

Results of the present *in vivo* test indicate that contaminations of spawn packet, days required for completion of mycelium running, primordia formation and first harvest were reduced appreciably over control when substrate was sterilized with chemicals. Sterilization of substrate with chemicals increased the duration of harvesting and amount of yield. Almost similar results have been reported by many other workers. Biswas (2014) noticed that the substrate treated with chemicals (Bavistin 75 ppm + formalin 500 ppm) has taken 14 days for completing the spawn run and contaminating fungi did not attacked mushroom fungi. Pervez *et al.,* (2010) reported that in spawn packets having substrate treated with 3% H_2O_2 required 18.6 days for primordia formation and 3.3 days for development of fruiting bodies and 21.65 days for harvesting of fruiting bodies. Kumar *et al*.,(2011)reported that days required for primordia formation were16.3 days in *P. sajorcaju,*16.6 days in *P. fossulatus* and 15.0 days in *P. sapidus.* Alameda and Mignucci (1998) reported that yield and quality of oyster mushrooms (*P. ostreatus*) are lost due to the associated molds. Biswas (2014) noticed that, the substrate treated with chemicals (bavistin 75 ppm + formalin 500 ppm) has taken 14 days for completing the spawn run and no moulds attacked spawn packet.

Hasan *et. al.,*(2010) obtained the minimum duration (6.67 days) of mushroom production found in substrate (banana leaf mid ribs + 10% cow dung) treated with 1% lime, the

highest yield (119g) was obtained from the 1% lime treatment of substrate (rice straw + 10% poultry litter) and the highest number of effective fruiting bodies(9.72) growing on substrate (banana leaf mid ribs) treated with lime.

Biswas (2014) reported 106 % biological efficiency, 530 g yield/ 500g packets and 7.3 g average weight of fruiting body were obtained from the substrate treated with chemicals (bavistin 75 ppm + formalin 500 ppm).

Zehad *et al*., (2012) reported that significantly the highest number of fruiting bodies per packet (81.66), diameter of pileus (4.67cm), highest yield (680.50g) per spawn packet were recorded in 3% H_2O_2 treated spawn packet.

Pervez *et al.*,(2012) reported that the highest occurrence of contamination was found in 1% H_2O_2 treated spawn packets and the lowest occurrence was found in 3% H_2O_2 concentration packets. Wayne (2001) found and reported that the peroxide radical is a treated substrate and by adding hydrogen peroxide to mushroom substrates, it becomes possible to perform all phases of traditional mushroom cultivation successfully from isolation to fruiting in non-sterile environments with unfiltered air.

Experiment 4.5.Effect of steam pasteurization of substrate on contamination and production of oyster mushroom

4.5.1. Incidence of contamination in spawn packets during incubation

Under control, contamination of spawn packets having substrate inoculated with contaminating fungi was very severe. The contamination of weed fungi in spawn packet having substrate sterilized with steam was also recorded. Severity of contamination varied with period of steam pasteurization (Plate 26).

During incubation, incidence of contamination in spawn packet was 42.6% under control. Steam pasteurization of substrate significantly reduced the contamination over control. Significantly the lowest contamination of 2.8% was recorded from substrate sterilized by autoclaving followed by steam pasteurization for 2, 5 and 4 hours. The least effective duration of contamination of substrate was 1 hour steaming (Table 29).

4.5.2. Incidence of contamination of spawn packets during cultivation

The highest contamination of 90.0% was found under control. The contamination was reduced to 3.3-23.1% due to sterilized with steam for 2-6 hours and autoclaving. The minimum contamination of 3.3% was recorded from autoclaving and steaming for 2 and 3 hours. Steam pasteurization of substrate for 5 and 6 hours reduced the contamination to 6.6 and 9.9%, respectively (Table 29).

4.5.3. Severity of contamination of spawn

Severity of contamination was 40.0, 52.2 and 90.0% under control in first, second and third flushes, respectively. Due to pasteurization of substrate with steam for 2-6 hours and autoclave reduced spawn packet contamination to 0.0% in first flush, 0.0-10.0% in second flush and 10.0-26.0% in third flush (Table 29 and Figure 33).

Plate 26. Contaminated spawns during incubation [A.*Rhizopus* contaminated, B. *Aspergillus niger* contaminated]; contaminated spawns during cultivation [C-contamination by *Trichoderma*, D-left. *Trichoderma* contaminated, right –*Aspergillus* contaminated]

Table 29. Incidence and severity of contamination of spawn packet containing substrate sterilized with steam for 2-6 hours

Duration of steam pasteurization (hour)	Incidence of contamination (%)		Contamination severity (%)		
	During incubation	During cultivation	First flush	Second flush	Third flush
2	21.1 b*	23.1 b*	20.0**	30.0**	42.0**
3	7.1 f	3.3 e	0.0	0.0	10.0
4	14.2 d	3.3 e	0.0	0.0	16.0
5	9.9 e	6.6 d	5.0	10.0	22.2
6	17.0 c	9.9 c	0.0	0.0	26.0
Autoclaving	2.8g	3.3 e	0.0	10.0	15.0
Control	42.6 a	67.9 a	40.0	52.2	90.0

*Means within the same column having a common letter(s) do not differ significantly (P=0.05).
**Statistical analysis was not performed.

4.5.4. Yellowing of fruiting bodies

Yellowing and bacterial oozing on the surface of the pileus of fruiting bodies of mushrooms was observed. Blotch occurred due to higher moisture content of mushroom caps. Firstly, bacteria was found on NA medium from infected portion of mushroom then it formed colony on Kings'B agar (KB) medium which is *Pseudomonas* specific and alsoin Gram's staining method. Rod shaped bacteria were observed. The symptoms of bacterial blotch of fruiting body were recorded (Plate 27 A-G).

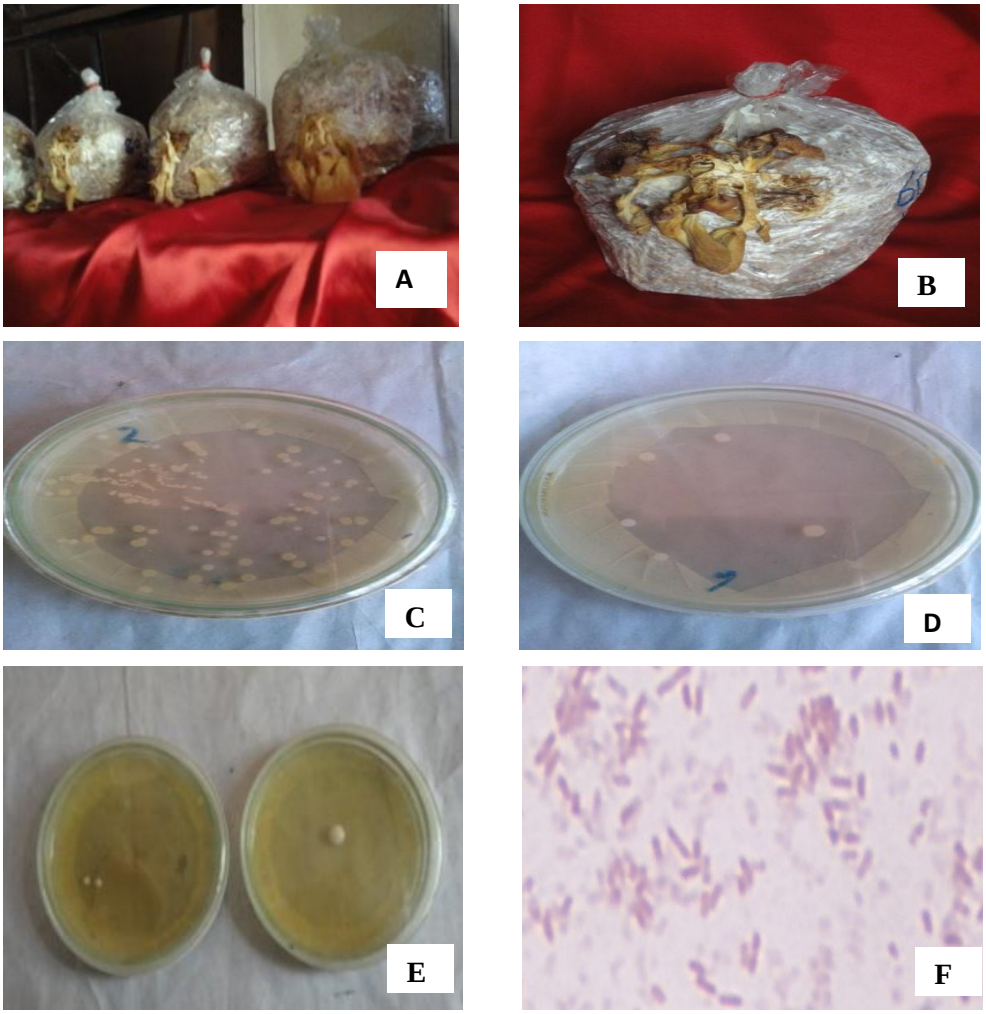


Plate 27. A-B infected fruiting bodies, C-D. Pure culture of *Pseudomonas* sp. on Nutrient Agar medium, E. Pure culture grown on KB medium, F. Microscopic view of *pseudomonas* sp. after gram staining

4.5.5. Mycelium run rate

Under control mycelium run rate was 0.36 cm/day. Pasteurization of substrate with steam for 2-6 hours and by autoclaving significantly increased mycelium run rate to 0.5-1.0 cm/day. The increase was significant compared to control. The highest run rate was achieved with steam pasteurization for 3 hours followed by autoclaving and steam for 4 hours. Effect of steam pasteurization for 2, 5 and 6 hours on mycelium run rate was statistically similar but significantly lower compared other treatments (Table 30)

4.5.6. Days required for completion of mycelium running

In non-sterilized substrate (control) days required for completion of mycelium running were 28.6, which were reduced to 6.6 to 22.8 days due to pasteurization of substrate. The lowest days required for completion of mycelium running was observed in case of autoclaving followed by steam pasteurization for 4, 5 and 3 hours, where the days for completion of mycelium running ranged 11.6-17.0 (Table 30).

4.5.7. Days required for primordia formation

Days required from stimulation to primordia formation was 10.0. Pasteurization of substrate significantly reduced day to primordia formation over control within the range of 3.20 to 9.0 under different treatments. The lowest days to primordia formation was found when substrate was sterilized with steam for 3 hours followed by autoclaving, steam pasteurization for 4, 5 and 2 hours. Pasteurization of substrate with steam for 6 hours reduced days for primordia formation to only 9.0. It might be due to destruction of beneficial moulds or micronutrient in substrate (Table 30).

4.5.8. Days required for primordia formation to first harvest

Under control days required for first harvest after primordia formation were 12.4, which was significantly reduced over control to 4.5-8.4. The maximum reduction was obtained with steam pasteurization for 4 hours followed by 5 and 3 hours. The most ineffective treatment steam pasteurization for 2 hours followed by autoclaving (Table 30).

4.5.9. Days required for total harvest

Days required for total harvest significantly increased due to pasteurization of substrate with steam for 2-6 hours and autoclaving compared to control. The most effective treatment to increase days for total harvest was autoclaving followed by steam pasteurization for 3, 6 and 4 hours. The least effective treatment to enhance duration of total harvest was steam for 2 hours followed by 5 hours (Table 30).

Table 30. Effect of pasteurization of rice straw by steam on the growth rate and colonization of mycelium in spawn packet

Duration of steam pasteurization (hour)	Mycelium growth rate (cm/day)	Days required for			
		Mycelium running	Primordia formation	Primordia to fist harvest	Total harvest
2	0.49 c	19.20 c	6.0 c	8.40 b	33.0 f
3	1.03 a	17.00 d	3.20e	6.80 d	46.0 b
4	0.86 b	11.60 e	4.8 d	4.51 e	43.0 d
5	0.58 c	16.60 d	5.6 c	5.80 e	37.0 e
6	0.56 c	22.80 b	9.0 b	7.00 cd	44.0 c
Autoclaving	0.96 ab	6.60 f	5.8 c	7.20 c	60.0 a
Control	0.36 d	28.60 a	10.0 a	12.40 a	25.0 g

*Means within the same column having a common letter(s) do not differ significantly (P=0.05).

4.5.10. Yield contributing characters of oyster mushroom (*Pleurotus ostreatus*)

In this experiment influence of steam pasteurization with different time duration on yield and yield attributes was studied and these were presented below.

4.5.10.1. Number of primordia and number of fruiting bodies of oyster mushroom (*Pleurotus ostreatus*)

In case of number of primordia statistically significant variation was observed (Table 31). The highest number of primordia (113.8) produced in three (3) hours pasteurized packets (T_2) which followed by four hours pasteurized packets (109.0) and autoclaved spawn packets (104.2). The lowest number of primordia (23.60) recorded from untreated packets (control).

The highest number of fruiting bodies (49.40) per packet was harvested from five hours steam pasteurized packets which was statistically significant from other treatment. The lowest number of fruit bodies (20.40) were produced in unpasteurized packets which was followed by T_1 (28.20).

4.5.10.2. Diameter of pileus and length of stipe

The highest diameter (8.16 cm) of pileus was observed from 3 hours steam pasteurized packets (T_2) which was followed by T_1 (8.00cm) and autoclaved packets (7.08cm). The lowest diameter (5.14cm) was recorded in control (Table 30). Sarker *et al*., (2007) observed that diameter of pileus ranged from 4.00 cm to 5.50 cm. The length of stipe differed significantly varied from 1.82 to 4.24 cm. The lowest average length (1.82 cm) was recorded in autoclaved treatment and highest in control treatment. Onyango *et al*., (2011) reported that large sized fruit bodies were considered to be of good quality and rated highly in mushroom production.

Table 31. Effect of pasteurization of substrates through steam on yield attributes oyster mushroom (*Pleurotus ostreatus*)

Treatments	Number of primordia	Number of effective fruiting body	Average length of stipe(cm)	Average diameter of pileus (cm)
T_1	88.60 d	28.20 f	2.36 f	8.00 b
T_2	113.8 a	48.40 b	2.64 e	8.16 a
T_3	109.0 b	36.20 e	3.04 d	6.86 d
T_4	84.40 e	49.40 a	3.28 c	5.88 e
T_5	82.60 f	41.20 c	3.86 b	5.62 f
T_6	104.2 c	36.80 d	1.82 g	7.08 c
T_0	32.60 g	20.40 g	4.24 a	5.14 g
$LSD_{(0.05)}$	**1.028**	**0.363**	**0.163**	**0.094**
CV (%)	**0.90**	**0.75**	**4.12**	**1.08**

Means within the columns and rows, under a parameter having a common letter differ significantly.T_1=Steam pasteurization for two hours, T_2=Steam pasteurization for three hours, T_3= Steam pasteurization for four hours T_4=Steam pasteurization for five hours, T_5=Steam pasteurization for six hours, T_6 = Autoclave sterilization and T_0= Unpasteurized (control)

4.5.11. Fresh yield of oyster mushroom in different flushes grown in substrate sterilized with steam for two to six hours and autoclaving

In substrate without pasteurization (control) or sterilized with steam for 2 hours, mushroom fruiting bodies were not developed. The lowest yield of mushroom was recorded in first, second and third flushes under control and under treatment with steam for 2 hours. Pasteurization of substrate with steam for 3-6 hours or by autoclaving increased fresh yield of mushroom compared to control. Under all treatments, yield of mushroom was maximal in first harvest followed by second and third harvest. In fourth and fifth harvest no mushroom was developed under control (T_0) and T_1. The most effective treatment to produce mushroom was T_2, T_6, T_3, T_4 and T_5 (Figure 35).

4.5.12. Total biological yield

Under control the biological yield was only 53.0 g/packet. Pasteurization of substrate with steam for 2-6 hours and autoclave significantly increased the total biological yield within the range of 123.6 – 311.3 g/packet compared to control. The highest biological yield was obtained with steam pasteurization for 3 hours and the lowest biological yield was recorded from the treatment steaming for 2 hours. The best treatment was steaming for 3 hours followed by autoclaving, steaming for 4 and 5 hours. The least effective treatment was pasteurization of substrate with steam for 2 hours followed by 6 hours (Table 32)

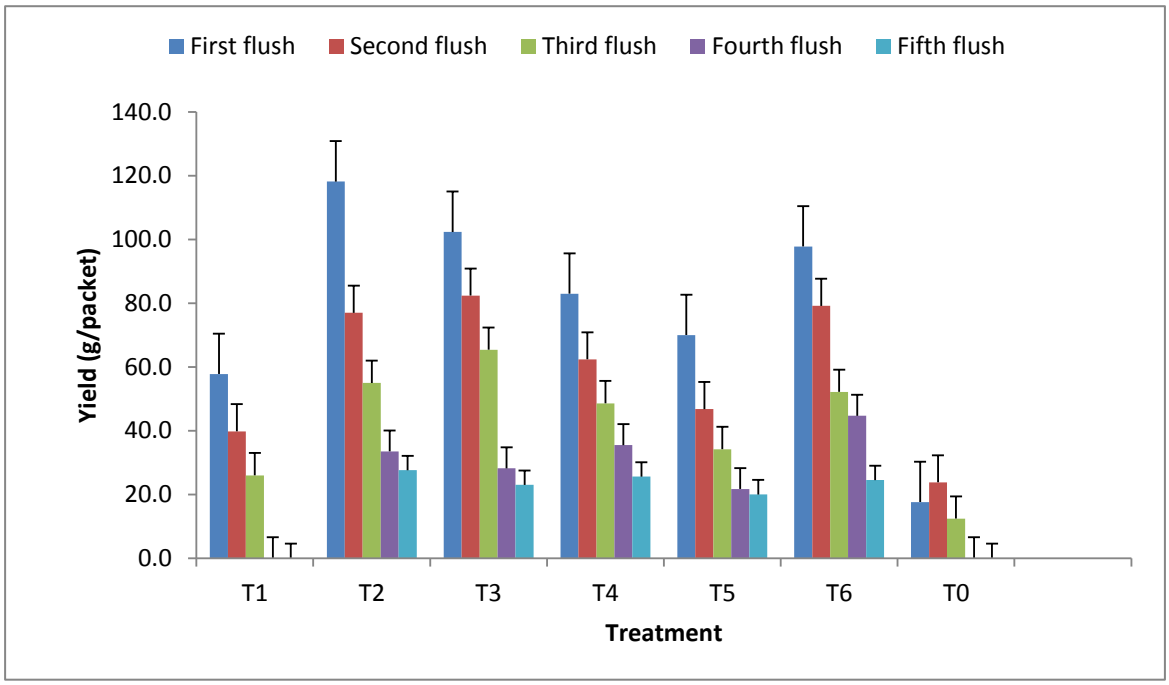


Figure 33. Fresh yield of oyster mushroom in different flushes grown in substrate sterilized with steam for two to six hours

[T_1- Steam pasteurization for 2 hr, T_2- Steam for 3, $T_{3=}$ Steam for 4hrs, T_4= Steam pasteurization for 5hrs, T_5=Steam pasteurization for 6hrs, T_6 =Autoclaving and T_0 –Control]

4.5.13. Economic yield (g)

Economic yield of mushroom was 50 g under control. The economic yield was significantly increased within the range of 120.6 – 304.4 g/packet due to substrate pasteurization by steaming and autoclaving. The highest yield was recorded when substrate was sterilized with autoclave followed by steam for 3, 4 and 5 hours. The lowest economic yield was found when the substrate was sterilized with steam for 2 hours followed by 6 hours (Table 32).

4.5.14. Dry yield

The minimum dry weight of 7.2 g/packet was recorded from control where unsterilized substrate was used. In packets having substrates sterilized with an autoclave or steam for 2-6 hours produced dry yield of 35.0 to 54.1 g/packet. The increase was significant compared to control. The highest dry yield was obtained with autoclaving which were statistically identical to steaming for steaming for 3 hours, which were followed by steam pasteurization for 4, 4 and 5 hours (Table 32).

4.5.15. Biological efficiency

The bio-efficiency was calculated to determine how the mushrooms utilized nutrients present in the substrates efficiently. There were significant effects of substrate pasteurization on the average yield of oyster mushroom and biological efficiency. The highest biological efficiency was obtained with the 3 hours pasteurization of substrate packets which was followed by autoclaving. The lowest biological efficiency was observed in non-treated packet, which was followed by two hours steaming of substrate. From 4 and 5 hours steaming substrates, biological efficiency computed was 58.28 and 54.14%, respectively which were statistically identical with each other. There were considerable different in biological efficiency among the treatments with steaming and autoclaving of substrates (Table 32).

Table 32. Effect of pasteurization of substrates by steam with different time duration on yield and yield attributes

Duration of steam pasteurization (hour)	Biological yield (g/packet)	Economic yield (g/packet)	Dry yield (g/packet)	Biological efficiency(%)
2	123.6 e	120.6 f	28.68 e	24.7 f
3	311.3 a	290.1 b	54.04 a	66.0 a
4	301.4 a	284.2 c	41.74 c	58.3 c
5	255.1 c	264.7 d	44.59 b	54.1 d
6	192.7 d	200.5 e	35.03 d	41.6 e
Autoclaving	294.7 b	304.4 a	54.12 a	62.9 b
Control	53.0 f	50.00 g	7.24 f	10.8 g

*Means within the same column having a common letter(s) do not differ significantly (P=0.05).

4.5.16. Relationship between economic yield and number of primordia, weight of individual fruiting body and biological efficiency

The economic yield of mushroom was correlated positively with primordia, weight of fruiting body and the biological efficiency. The value of correlation (r=0.8633) was linear and could be expressed by the regression equation by y=3.85x-35.61(Figure 34 A). The relationship of between weight of fruiting body and economic yield are shown in Figure 34B. The relationship was also linear, when weight of fruiting body increased gradually; the rate of economic yield was also increased. The relationship might be expressed by the equation y=25.74x-13.87 (r=0.801507). Strong linear correlation (r =1.0) was also observed between economic yield and biological efficiency, where the equation was y=4.650x +4.847, stated that the biological efficiency increased gradually at the rate of 4.650% (Figure 34C).

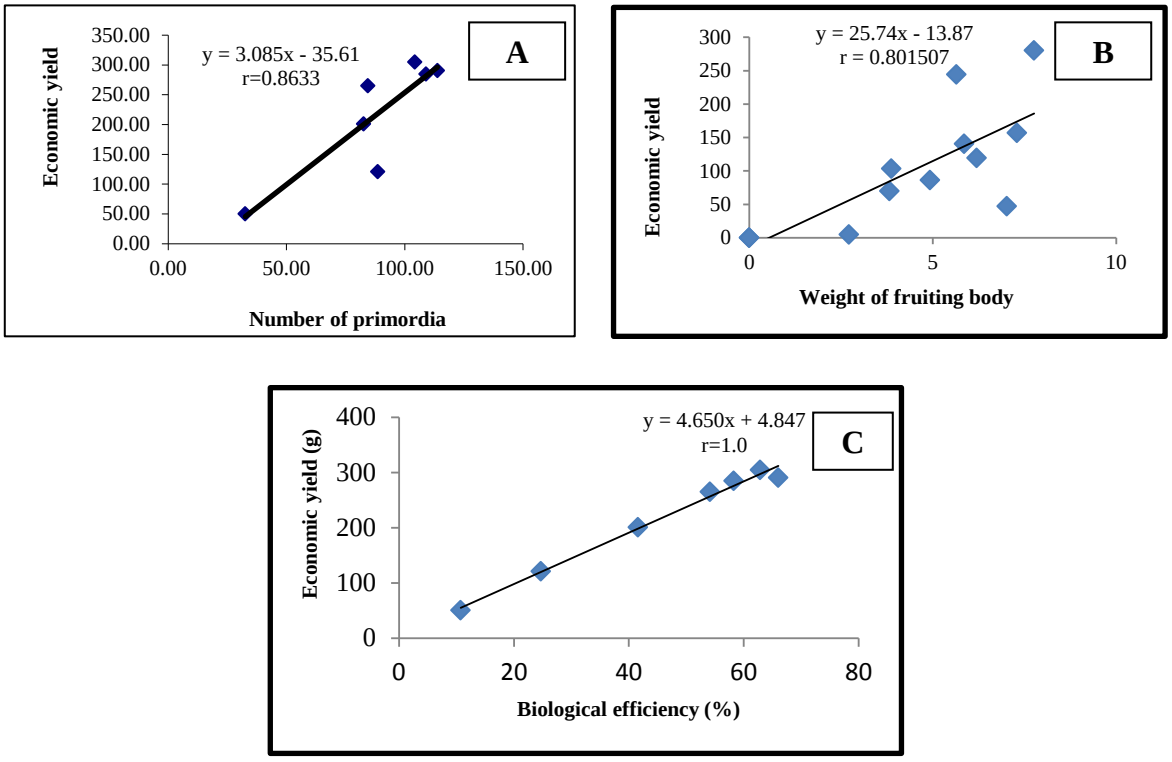


Figure 34. A. Relationship between number of primordia and economic yield, B. between economic yield and weight of fruiting body and C. between economic yield and biological efficiency

4.5.17. Discussion

Results of the present experiment show that pasteurization of contaminated substrate prepared from rice straw reduce contamination of spawn packets, days required for mycelium growth, primordia formation, first harvest and total harvest successfully. On the other hand, mushroom yield and yield contributing characters such as mycelium running, first harvest, total harvest, biological yield, economic yield and biological efficiency increase due to pasteurization with autoclave and steam pasteurization for 2-5 hour. The most effective treatment for substrate pasteurization to remove contamination and to have satisfactory yield of oyster mushroom is seemed to be steam for 3 and 4 hours which are almost similar to autoclaving. Not only fungi, bacteria are also potential contaminant of mushroom spawn packet and bacteria attack fruiting bodies causing bacterial diseases. The contamination of the hot water pasteurized substrate may have occurred probably due to inadequate temperature and exposure times used during pasteurization.

In the present study incidence of bacterial contamination was found, which may be due to stagnant of water drop on the fruiting body or high temperature during cultivation condition. Jandaik and Guleria (1999) reported that *Trichoderma* is often observed in the early stages of the process, especially during spawning run period, but also during cropping period and causes huge losses in mushrooms crops. Oseni *et al.*, (2012) exhibited, the least contamination (3 bags) observed the autoclaved sugarcane bagasse, which was not significantly different from autoclaved horse manure compost (4 bags) and sugarcane bagasse pasteurized for 3 h (7 bags) out of 40 bags. Kumar (2015) observed up to 98.66% reduction in fungal as well as bacterial contamination of spawn with three autoclavings where as it was up to 54.00% in case of fungal and 23.33% in case of bacterial contamination after one autoclaving treatment.

Contamination may be occurred due to inadequate pasteurization in case of non-pasteurized or pasteurization of rice straw for one or two hours. Most of the cases days required for development of yield contributing characters were lower compared to control and some other treatment. The findings of the present study were more or less similar to Kurtzman (2010), who reported several causes of mushroom substrate contamination that was insufficient pasteurization could be attributed to the contamination of mushroom.

Ali *et al*., (2007) reported that the duration for the completion of mycelial growth is affected by treatments of substrates, steam pasteurized cotton waste required 11 days was for spawn-running. Oseni *et al*., (2012) also reported full colonization of mushroom mycelium was completed at 42 days in the autoclaved horse manure compost and 36 days in autoclaved sugar cane bagasse after inoculation of mother culture. The findings concurred with the study of Moonmoon *et al*., (2013) and Mejía and Albertó (2013), where Moonmoon *et al*., (2013) reported the minimum days required from opening to harvesting was 2.67 in Po_2 by autoclaved packets. Mejía and Albertó (2013) observed 8 days required for first harvesting of mushroom from autoclaved wheat straw.

The findings of the present experiment about yield and yield attributes more or less matches with the results of the following researchers. Mejía and Albertó (2013) obtained 282.63g yield from autoclaved (at 120 ˚C for 2 hour 1.2 psi) wheat straw, while Oseni *et. al.*, (2012) found 410.39 g average yield in three flushes per 500 g of autoclaved (121°C for 4 h)sugarcane bagasse, it may be differed due to high content of polysaccharides. Oseni *et al*., (2012) found average the pileus diameter was 4.78 cm and stipe length of fruiting body was 5.48 cm from autoclaved (at 121°C for 4 h) sugarcane bagasse.

Mejía and Albertó (2013) obtained the number of fruiting bodies 49.63 and biological efficiency 94.91% from autoclaved (121^0C, 1.2 PSI for 2 hours) wheat straw. While, Oseni *et al.*, (2012) observed 82.10% and 41.85% BE from autoclaved sugarcane bagasse and autoclaved horse manure compost, respectively. This variation might be due to different substrates of mushroom.

The variations observed in yield may, therefore be attributed the rate of degradation of the cellulose content of substrates by the mushroom enzymes as a result of the different durations of steam pasteurization. From the above study we found that, three hours for steam pasteurization of substrate performed best followed by autoclave for the pretreatment of oyster substrates, it was due to utilization of cellulose and hemicelluloses properly during fruiting stages.

Experiment 4.6. Effect of sterilization of substrate with hot water on contamination, yield and yield contributing characters of oyster mushroom

Results of the experiment with the effect of substrate sterilization with hot water at 60^0C, 80^0C and 100^0C temperature for the duration of 1hr, 2 hrs and 3 hrs durations on microbial contamination of oyster mushroom are presented in following different sections below:

4.6.1. Incidence of contamination in spawn packets during incubation

During incubation period, substrates sterilized with hot water at 60^0C for 1 hour was colonized with contaminant fungi. Mycelium of *Pleurotus ostreatus* failed to colonize in heavily contaminated spawn packet. During incubation and cultivation period of spawn packet, the contaminants *Trichoderma* spp., *Rhizopus* sp., *Coprinus* spp., *Apergillus* spp. and *Penicillium* spp. were observed. *Rhizopus sp, Aspergillus* sp formed a black color spores on the surface of spawn packets of oyster mushroom. The heavily contaminated spawn packets were discarded (Plate 28 A and B). The dominant contaminant was *Trichoderma*, which initially produced a dense white mycelium. The mycelium mat gradually turned green in color due to heavy sporulation, which is a characteristic symptom of green mold disease (Plate 28 C and D).

White dense layer of mycelium were observed on the surface substrates of the packets, where fruiting bodies were not produced i.e. stroma (Plate 28 A), that was also described under experiment 4.1. (4.1.24.5.).With the progress of incubation period, infestation of whole packet with *Trichoderma* spp became green and were covered with green moulds. During cultivation period severely infested spawn packet became decomposed and primordia were not developed (Plate 28 B-D). The emerging fruiting bodies in the affected portion of the substrate were badly spotted, brownish in color and showed reduced growth and yield (Plate 28 E and F).

4.6.1.1. Contamination severity

The effect of hot water sterilization, maximum contamination (60.7%) was found at 60^0C and minimum at 80^0C. Considering the duration of hot water sterilization, maximum contamination (42.77%) was observed in case of one hour treated packets.

The contamination severity varied 0-100% considering the interaction effect of temperature and duration of hot water sterilization (Figure 35 and Figure 36).

The severity of contamination was 0.5-3.8% at 80ºC under all three flushes, 0.5-2.4% at 100ºC under first flush and second flushes, and 6.6-16.1% under first and second flushes at 60ºC. The least effective treatment was 60^0 and 100ºC temperature in third flush. Sterilization of substrate with 60 and 80ºC, hot water was noted as the promising treatments. Considering efficacy of hot water sterilization 80ºC may be noted as the best treatment (Figure 35)

In case of efficacy of sterilization of mushroom substrate with what water for 1, 2 and 3 hours reduced contamination of spawn packets considerably and increase yield appreciably. At every flush, the lowest contamination was recorded at first flush followed by second and third flush. However, 2 hour sterilization was observed as a good treatment under first and second flushes. The least effective treatment was hot water at 60ºC for 1 hour sterilization (Figure 36).

Plate 28. A.*Rhizopus* contaminated packet, during incubation period; B Abounded contaminated spawn C-D: *Trichoderma* contaminated packet showing different successive stage: E-F. Infected fruiting bodies

4.6.1.2. Interaction effect of level of temperature and duration of sterilization of substrate on contamination

Contamination of spawn packets of oyster mushroom was absent in all three flushes under the treatment combinations of 2hr hot water sterilization at 80ºC, in first and second flushes under 3hrx60ºC and 3hrx100ºC and in first flush only under 2hrx100ºC and 3hrx80ºC.

In first flush, severity of contamination was 6.6% under 1hrx60^{0}C, 1hrx80^{0}C, 1hrx100^{0}C and 2hrx60^{0}C and disappeared thereafter. In second flush, severity of contamination was 17.8, 15.8, 15.8 and 6.4% under 1hrx60^{0}C, 1hrx80^{0}C, 1hrx100^{0}C and 2hrx60^{0}C, respectively. Under other two treatment combinations, 2hrx100^{0}C and 3hrx80^{0}C severity was 2.4%. Under other treatment combinations contamination disappeared. In third flush, the highest contamination was found under 1hrx60^{0}C followed by 1hrx80^{0}C, 1hrx100^{0}C and 2hrx60^{0}C. Under those treatment combinations, the severity was 91.7, 61.1, 35.2% and 6.6%, respectively. The contamination reappeared under 2hrx100^{0}C and increased gradually up to 3hrx100^{0}C showing 6.6, 16.0, 18.7 and 38.2%, respectively (Figure 37).

Mushroom spawn packets were free from contamination when hot water sterilization of mushroom substrate was done at 80ºC for 2 hour in three flushes, at 60^0 and 100ºC for 3 hour in first and second flushes and 100ºC for 2 hour in first flush. Contamination of substrate in third flush was 100% under control followed by 60^{0}Cx1hr, 80^{0}Cx3hr, 60^{0}Cx1hr and 100^{0}Cx2hr. In other treatment combinations, contamination was 0.0-16.0%. In general, the most effective treatments were 80^{0}C for 1, 2 and 3 hour and 100^{0}C for 3 hours sterilization (Figure 37).The findings of the experiment reveal that the best treatment combinations are 2hrx60^{0}C, 2hrx80^{0}C and 2hrx100^{0}C for hot water sterilized of substrate for reduction of contamination severity in spawn packet of oyster mushroom.

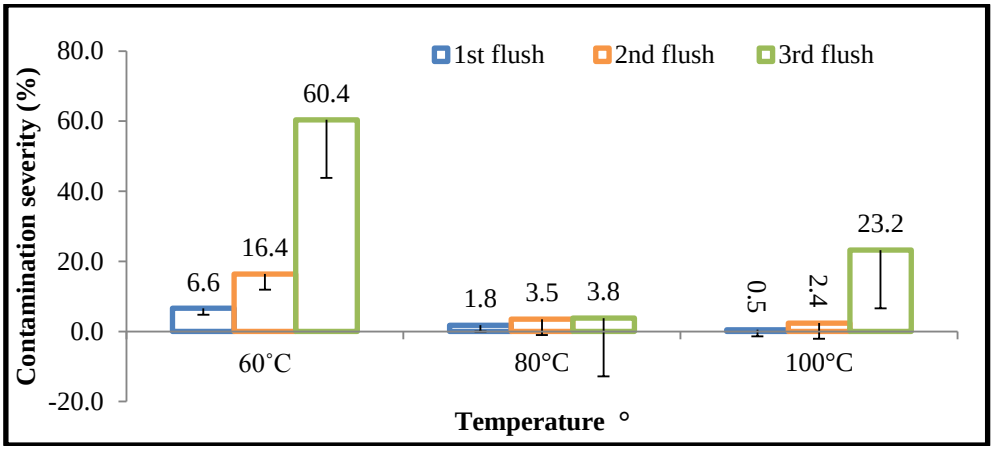


Figure 35. Effectiveness of degree of temperature for sterilization of substrate with hot water against contaminating fungi in spawn packet of oyster mushroom

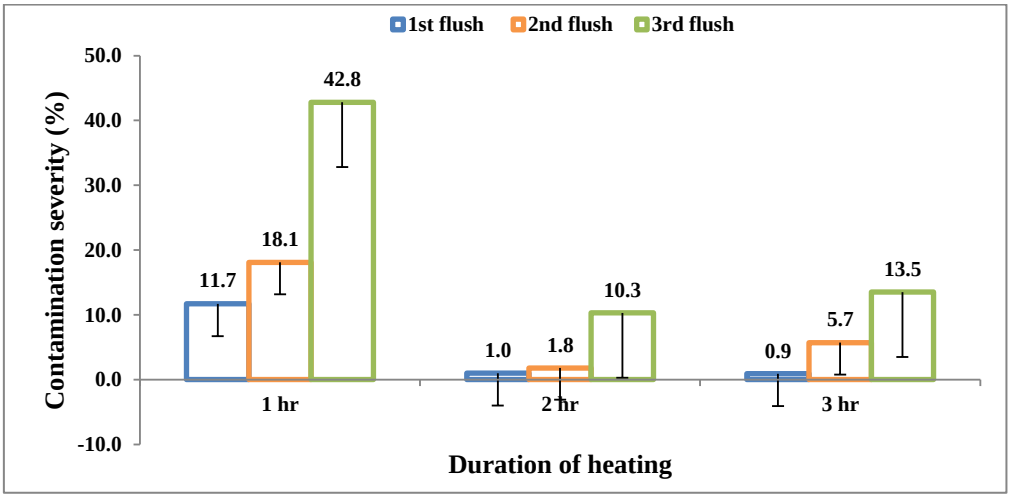


Figure 36. Effectiveness of duration of sterilization of substrate with hot water against contaminating fungi of spawn packet of oyster mushroom

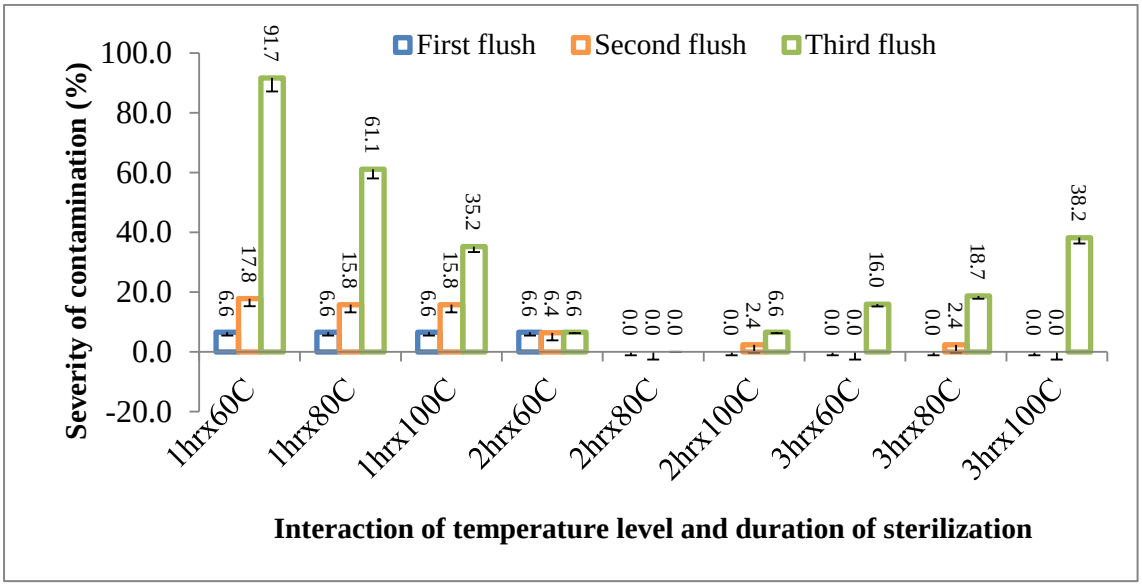


Figure 37. Effect of treatment combinations between level of temperature and duration of sterilization on contamination of spawn packets of oyster mushroom

4.6.2. Effect of substrate hot water sterilization of substrate on mycelium growth

Sterilization of substrate with hot water sterilization at different levels of temperature and duration of sterilization influenced the mycelium growth of *P. ostreatus* in spawn packet. The parameters described hereafter are related to the yield of mushroom:

4.6.2.1. Mycelium run rate in substrate sterilized at 60, 80 and 100°C for 1, 2 and 3 hours

Mycelium run rate in spawn packet was 0.56, 0.43 and 0.38 cm/day when substrates were sterilized with hot water at 80, 60 and 100ºC, respectively. The highest run rate was obtained with temperature 80^0C followed by 60^0C and 100^0C (Table 33). The mycelium run rate per day was 0.38, 0.34 and 0.36 cm when substrate was sterilized for 1, 2 and 3 hours. Their differences were not significant (Table 34). Mycelium growth rate in spawn packet significantly differed due to interaction of different time and temperature. The highest growth rate (0.67cm) was observed in packets treated at 80^0C for 2 hrs and the lowest running rate (0.32 cm) of mycelium was observed at 60^0C for 2 hrs (Table 35).

4.6.2.2. Days required for completion of mycelium running

The parameter was 23.5, 16.1 and 16.8 on substrate sterilized with hot water for 1, 2 and 3 hours. Effect of 2 and 3 hours was statistically similar but significantly lower compared to 1 hour (Table 33). Days required for completion of mycelium running was 21.5, 18.5 and 10.0 in spawn packets containing rice straw as substrate sterilized with hot water for 1, 2 and 3 hours. Their differences were significant (Table 34).

In case of interaction effect of temperature and time of hot water treatment on mycelium growth rate and of oyster mushroom had effects on the duration of full colonization of mycelium and it ranged from 14.4 to 28.2 days. The minimum duration to complete mycelium colonization was recorded from hot water treated bags at 80^0C for 1 hour and maximum days required to complete mycelium running was observed at 100^0C for 3 hours treated packets and at 100^0C for 1 hours treated packets , which were statistically identical. On the other hand, in case of duration of mycelium colonization, statistically similar result was found 22.2, 21.4 and 21.0 days at 60^0C for 1 hour, 60^0C for 2 hours, 60^0C for 3 hours treated spawn (Table 35).

Table 33. Effect of hot water sterilization of substrate at different temperature level on mycelium run rate and completion of mycelium running of oyster mushroom in spawn packet

Temperature for sterilization (ºC)	Mycelium run rate (cm/day)	Days required for completion of mycelium running
60	0.43 b	23.5 a
80	0.56 a	16.1 b
100	0.38 c	16.8 b

- Means with the same column with common letter(s) do not differ significantly.

Table 34. Effect of hot water sterilization of mushroom substrate for different duration on mycelium run rate and days required for completion of mycelium running of oyster mushroom in spawn packet

Duration of sterilization (hour)	Mycelium run rate(cm/day)	Days required for completion of mycelium running
1	0.38 a	21.5 a
2	0.34 a	18.5 b
3	0.36a	10.0 c

- Means with the same column with common letter(s) do not differ significantly.

Table 35. Interaction effect of hot water sterilization of substrate at different levels of temperature and duration on mycelium growth rate and days required for completion of mycelium running of oyster mushroom in spawn packet

Temperature level vs. duration (hour) sterilization	Mycelium growth rate (cm)	Days for full colonization of mycelium
60ºC x1hr	0.40 de	22.2bc
60ºCx2hr	0.32 g	21.4 c
60ºCx3hr	0.57 b	21.0 cd
80ºC x1hr	0.51 c	23.0 b
80ºCx2hr	0.67 a	14.4 f
80ºCx3hr	0.49 c	18.2 e
100ºC x1hr	0.42 d	27.4 a
100ºCx2hr	0.35 fg	20.0 d
100ºCx3hr	0.37 ef	28.2a

*Means with the same column with common letter(s) do not differ significantly

4.6.3. Effect of temperature levels for substrate sterilization on days required for primordia formation, first harvest and total harvest

When mushroom substrate (rice straw) was sterilized with hot water at 60^0, 80^0 and 100ºC, days required for primordia formation were 5.3, 5.4 and 4.1, for first harvest 5.7, 4.8 and 3.7 and for total harvest were 35.0, 31.3 and 38.3, respectively. The best temperature level t474o reduce different parameters was not same. The trends of increasing or decreasing depending on variations of parameters (Table 36).

4.6.4. Effect of duration of hot water sterilization of substrate on primordia formation, first harvest and total harvest

Sterilization of substrate with hot water for 1, 2 and 3 hours required respectively 4.0, 5.1 and 7.5 days for primordia formation; 3.7, 4.9 and 7.1 days for first harvest and 28.0, 25.3 and 30.3 days. Effects of each of the treatments on three parameters were significantly different. The best duration was 1 hour followed by 2 and 3 hours for sterilization of substrate in respect of primordia formation and first harvest. Effect of three durations on total harvest did not follow any logical trend (Table 37).

4.6.5. Interaction effect of temperature and duration of hot water sterilization of substrate on days required for primordia initiation, first harvest and total harvest

The duration required from stimulation to primordial formation and primordial initiation to first harvest ranged 3.6-8.4 days and 4.0-7.3, respectively. The minimum time required from stimulation to primordial initiation and primordial initiation to first crop harvest was observed from spawn packets sterilized at 80^0C for 2 hours followed by 80^0C for 3 hours. To require the highest days to primordia formation was 100^0Cx3hr followed by 100^0Cx2hr. The interaction effect of temperature level and duration for hot water sterilization of substrate required 26.0 to 46.0days to yield total harvest. The minimum duration was required for total harvest under 60^0Cx2hr followed by 80^0Cx1hr and 80^0Cx2hr. While the maximum days was observed under 80^0Cx2hr followed by 100^0Cx2hr, 60^0Cx3hr and 100^0Cx1hr (Table 38).

Table 36. Effect of temperature of hot water at 60, 80 and 100ºC for sterilization of substrate on days required for primordia formation, first harvest and total harvest

Temperature (ºC)	Days required for		
	Primordia formation	First harvesting	Total harvesting
60	5.3 a	5.7 a	35.0 b
80	5.5 a	4.8 b	31.3 c
100	4.07 b	3.7 c	38.3 a

*Means with the same column with common letter(s) do not differ significantly

Table 37. Effect of hot water sterilization of mushroom substrate for 1, 2 and 3 hours on days required for primordia formation, first harvest and total harvest

Sterilization duration (hour)	Days required for		
	Primordia formation	Primordia initiation to first harvesting	Total harvest
1	7.5 a	7.1 a	30.3 a
2	5.1 b	4.9 b	25.3 c
3	4.0 c	3.7 c	28.0 b

*Means with the same column with common letter(s) do not differ significantly

Table 38. Interaction effect of mushroom substrate sterilization with hot water at 60, 80 and 100ºC for 1, 2 and 3 hours on days required for primordia formation, first harvest and total harvest of oyster mushroom *(Pleurotus ostreatus)*

Temperature level x duration (hour)	Days required for		
	Primordia formation	First harvesting	Total harvesting
60ºC x1hr	5.4 d	7.3 a	33.0 cd
60ºCx2hr	6.8 bc	6.2 b	26.0 e
60ºCx3hr	6.4 bc	6.0 bc	31.0 d
80ºC x1hr	6.0 cd	4.4 d	31.0 d
80ºCx2hr	3.6 e	3.6 e	46.0 a
80ºCx3hr	4.0 e	4.0 de	32.0 cd
100ºC x1hr	6.2 bc	5.4 c	37.0 b
100ºCx2hr	7.0	7.2 a	44.0 a
100ºCx3hr	8.40 a	7.2 a	34.0 c

*Means with the same column with common letter (s) do not differ significantly

4.6.6. Effect of hot water sterilization of substrate at different levels of temperature on biological yield

4.6.6.1. Effect of temperature level on biological yield

The maximum biological yield was harvested in first flush followed by second, third and fourth flushes at all three levels of temperature. Sterilization of substrate with hot water produced significantly the highest biological yield of 90.1, 57.7, 34.8, 21.0 and 84.2 g/spawn packet in first, second, third, fourth flushes and total, respectively at 80ºC. The second highest biological yield was recorded from first flush, second flush and total at 100ºC but in third and fourth flushes at 60ºC. The minimum biological yield was obtained at 60ºC in first flush, second flush and total, and in third and fourth

flush at 100ºC. Finding of the present experiment reveal that the most effective temperature level was 80ºC for biological yield of oyster mushroom (Table 39).

4.6.6.2. Effect of duration of hot water sterilization of substrate on biological yield

Hot water sterilization of substrate of an oyster mushroom fungus gave 51.3, 32.0, 14.6 and 10.8 g/packet biological yield in first, second, third and fourth flushes, respectively with an exception where highest yield of 53.1 g/packet was found under 3 hours, which was statistically similar to 2 hours. Except third flush, the second highest biological yield was obtained in first, second and fourth flushes when hot water sterilization was done for 1 hour. In case of total, highest production was achieved with hot water sterilization for 2 hours followed by 3 hours and 1 hour. The yield was 95.7, 111.2 and 117.4 g/packet under 1, 2 and 3 hours of sterilization. Their differences were significant (Table 40).

4.6.6.3. Interaction effect of level of temperature and duration of hot water sterilization on biological yield of a mushroom

Fruiting bodies of mushroom was not produced under the treatment combinations100ºCx3hr in second and third flushes, 60ºCx2hr and 100ºCx1hr in third and fourth flushes. The biological yield ranged 34.8-109.4, 0.0-68.4, 0.0-44.6 and 0.0-31.2 g/packet in first, second, third and fourth flushes, respectively. The highest total yield was obtained from treatment combination 80ºCx2hr followed by 80ºCx3hr, 100ºCx2hr and 80ºCx1hr. So, those treatment combinations may be considered as considerably effective to increase biological yield of oyster mushroom with substrate sterilization with hot water (Table 41).

Table 39. Effect of temperature level of hot water for sterilization of substrate on biological yield of oyster mushroom

Level of temperature (ºC)	Biological Yield(g/packet)				
	1st flush	2nd flush	3rd flush	4th flush	Total
60	45.6 c	22.0 c	7.7 b	8.8 b	84.2 c
80	90.1 a	57.7 a	34.8 a	21.0 a	203.6 a
100	59.9 b	29.3 b	6.1 c	5.4 c	112.5 b

*Means with the same column with ma common letter(s) do not differ significantly

Table 40. Effect of hot water sterilization of mushroom substrate for three durations on biological yield of oyster mushroom

Duration of sterilization (Hour)	Biological yield(g/packet)				
	1st flush	2nd flush	3rd flush	4th flush	Total
1	43.9 b	25.9 b	8.2 c	10.2 b	88.2 c
2	51.3 a	32.0 a	14.6 a	10.8 a	108.7a
3	53.1 a	23.9 c	13.7 b	5.4 c	96.1b

*Means with the same column with ma common letter(s) do not differ significantly

Table 41. Interaction effect of temperature levels and duration of hot water sterilization of substrate on biological yield of oyster mushroom

Temperature level vs duration (hour)	Biological yield (g/packet) in different flushes				
	First	Second	Third	Fourth	Total
60ºC x1hr	40.8 e	21.0 g	13.0 e	18.0 c	92.8g
60ºCx2hr	34.8 f	14.4 h	0.0 g	0.0 g	49.2h
60ºCx3hr	61.2 d	30.6 f	10.2 f	8.5 e	110.5f
80ºC x1hr	59.4 d	39.8 e	19.8 c	22.7 b	141.7d
80ºCx2hr	101.6 b	68.4 a	40.0 b	31.2 a	241.2a
80ºCx3hr	109.4 a	64.8 b	44.6 a	9.0 e	227.8b
100ºC x1hr	69.4 c	42.8 d	0.0 g	0.0 g	112.2e
100ºCx2hr	68.8 c	45.2 c	18.2 d	12.1 d	144.5c
100ºCx3hr	41.6	0.0 i	0.0 g	3.99 f	45.6i

*Means with the same column with common letter(s) do not differ significantly.

4.6.7. Effect of hot water sterilization of substrate on dry yield, economic yield and biological efficiency

4.6.7.1. Effect of temperature level for hot water sterilization on dry yield, economic yield and biological efficiency

Hot water sterilization of substrate at 60, 80 and 100ºC produced 11.4, 26.6 and 16.7 g/packet of dry yield, 79.0, 227.1 and 109.8 g/packet of economic yield, and 16.8, 46.8 and 22.5% economic efficiency, respectively. The maximum of all three parameters were found at 80ºC followed by 100ºC and 60ºC. Differences in each parameter at three temperature levels were significant (Table 42).

4.6.7.2. Effect of duration of hot water sterilization of substrates on dry yield, economic yield and biological efficiency

The dry yield of oyster mushroom was 14.4, 14.1 and 14.9 g/packet when substrate was sterilized with hot water for 1, 2,and 3 hours, respectively. Effect of three durations for sterilization on economic yield was statistically similar. The economic yield was 91.8, 107.9 and 113.4 g/packet; and economic efficiency was 19.1, 22.2 and 23.5% due to hot water sterilization of substrate for 1, 2 and 3 hours, respectively. Economic yield and biological efficiency due to sterilization of substrate for 2 and 3 hours were statistically similar but significantly higher compared to sterilization for 1 hour sterilization (Table 43).

4.6.7.3. Interaction effect of temperature and duration of hot water sterilization of substrate on dry yield, economic yield and biological efficiency

Interaction effect of temperature level and duration for hot water sterilization of substrate on three yield related characters was significant. Dry yield ranged 8.3 – 34.9 g/packet under nine different treatment combinations. The maximum dry yield was obtained with 80ºCx3hr followed by 80ºCx2hr and 100ºCx 2hr. Their efficacy was significantly different. The economic yield varied 5.0 to 280.0 g/packet under different treatment combinations. Significantly the highest economic yield was recorded from 80ºC x3hr followed by 80ºCx2hr and 80ºCx1hr. Differences in economic yield under treatment combinations were significant. Biological efficiency ranged 14.4-57.4% under nine different treatment combinations. The highest biological efficiency was recorded from 80ºCx3hr and the lowest biological efficiency was recorded from 100ºCx3hr. Differences in biological efficiency under three treatment combination was significant (Table 44).

Table 42. Effect of temperature level for hot water sterilization of substrate on dry yield, economic yield and biological efficiency of oyster mushroom

Temperature level (ºC) for hot water sterilization	Dry yield (g)	Economic yield (g)	Biological efficiency (%)
60	11.4 c	79.0 c	16.8 c
80	**26.6 a**	**227.1 a**	**46.8 a**
100	16.7 b	109.8 b	22.5 b

*Means with the same column with common letter(s) do not differ significantly

Table 43. Effect of duration of hot water sterilization of mushroom substrate on dry yield, economic yield and biological efficiency of oyster mushroom

Duration of hot water sterilization (hour)	Dry yield(g)	Economic yield	Biological efficiency
1	14.1 a	91.8 b	19.1 b
2	14.1 a	107.9 a	22.2 a
3	14.9 a	113.4 a	23.5 a

*Means with the same column with common letter(s) do not differ significantly

Table 44. Interaction effect of temperature level and duration for hot water sterilization of substrate on dry yield, economic yield, biological efficiency of oyster mushroom

Temperature level and duration (hour)	Dry yield (g)	Economic yield (g)	Biological Efficiency (%)
60ºC x1hr	11.3 g	86.2 f	18.6 e
60ºCx2hr	8.1 i	47.2 g	9.8 f
60ºCx3hr	14.8 f	103.5 e	22.1 d
80ºC x1hr	19.1 d	137.0 c	28.3c
80ºCx2hr	35.7 b	235.2 a	48.2 a
80ºCx3hr	30.9 a	222.2 b	45.6 b
100ºC x1hr	17.5 e	109.2 d	22.4 d
100ºCx2hr	22.6 c	140.3 c	28.9 c
100ºCx3hr	9.9 h	40.0 h	9.1 f

*Means with the same column with common letter(s) do not differ significantly

4.6.7.4. Relationship between economic yield and biological efficiency

The economic yield of mushroom was correlated positively with biological efficiency. The value of correlation (r=0.99983) was strong and linear relationship was obtained (Figure 38). The relationship could be expressed by the regression equation by y=4.8477y+0.003, that indicated that the economic yield per packet increased with the increase of biological efficiency.

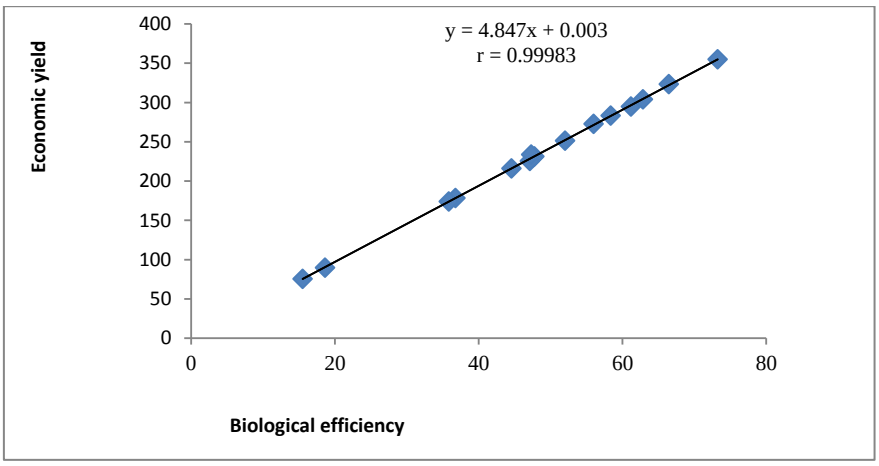


Figure 38. Relationship between economic yield and biological efficiency.

4.6.8. Discussion

Results of the present experiment clearly show that 80^0Cx2hr was better treatment for shorter duration for primordia formation and first harvest compared to others. The hot water temperature level 80^0C and sterilization for 2 hours was also effective treatment to increase mushroom yield. So, the most effective treatment combinations are 80ºCx2hr followed by 80ºCx3hrs. It reveals that at 60^0C temperature level longer time sterilization, on the other hand high temperature for shorter time sterilization may be effective. However, 100^0C for 3 hours is detrimental to mushroom and 60^0C for 1 hour is ineffective to have better mushroom yield.

According to Balasubramanya and Kathe (1996), the microorganism species that competed with *Pleurotus* sp. after pasteurization with hot water (80°C for 2 h) were the fungi *Penicillium* sp. and *Trichoderma* sp., probably due to the partial breakdown of cellulose and hemicelluloses, thus making them available to competitors. The contamination of the hot water pasteurized substrate may have occurred probably due to inadequate temperature and time used during pasteurization, since the literature is quite variable with reference to these characteristics. Maximum contaminants were detected from hot water treatment at 60^0C spawn and untreated packets, whereas

minimum prevalence was recorded from hot water treated packets at 80^0C for 3 hours. The result is more or less similar with Mahjabin *et al*., (2011) who reported that 13.00 days is required to complete mycelium running and 4.75 days required from packet opening to first harvest for *Pleurotus djamor* in hot water treated rice straw. The results contradicted with the findings of Yang *et al*., (2013) who stated that, the non-sterilized substrate exhibited significantly higher mycelial growth rate compared to the sterilized substrate.

Result contradicted with the findings of Oseni *et al.,* (2012) who reported the oyster mushroom took time to colonize sugarcane bagasse (64 days) hot water treatment at 60^0C for 2 hours, while horse compost manure those treated with hot water at 60^0C for 2 and 3 hours failed to colonize due to heavy contamination by *Trichoderma harzianum* presumably due to insufficient sterilization. Oseni *et al.,* (2012) was obtained 118.9 g and 301.1g yield from sugarcane bagasse pasteurized at 60^0C for 2 hand 3 hours, respectively. Mejía and Albertó, (2013) reported that when the distribution of the crop along flushes was observed the first flush was the most important with 76.7–80.82% while, the second flush varied from 5.4 to 14.9% using different strain and the substrate and produced 228.20g yield from wheat straw immersion in hot water at 80^0C for 90 minutes. Oseni *et al.,* (2012) observed sugarcane bagasse pasteurized at 60 ^{0}C for 3 hour and 2 hour 60.22% and 23.78% biological efficiency, respectively. Jaramillo and Albertó (2013) recorded 75.83% BE from wheat straw immersion in 80^0C for 90 minutes hot water.

Ali (2004) reported that steam pasteurization produced the best results followed by hot-water, formalin treatment and control (without pasteurization) treatments. In steam pasteurization, *Pleurotus florida* produced maximum total weight of fruiting bodies. Similar findings were reported by Mejía and Albertó (2013). They proved that immersion in hot water treatment of substrate reduces yields at least 20% when compared to other straw treatments, as steam, chemical or untreated wheat straw. Compounds which are hydro-soluble are lost during wheat straw immersion in hot water. The loss of these nutrients would be the cause of yield decrease. The lowest BE was obtained with immersion in hot water treatment (75.83%).

Houdeau *et al*., (1991) considered that the immersion of substrate in water can have different consequences according to the type of raw material. They pointed out that there is a "nutrients washing" effect that can be negative when old raw material is used, but useful in new raw material because there is a decrease of soluble sugars that can prevent the development of antagonistic microorganisms.

CHAPTER V

SUMMARY AND CONCLUSION

Microbial contamination of oyster mushroom bed is one of the major hindrances in increased yield of *Pleurotus* spp. The substrates for cultivating edible mushrooms (e.g. *Pleurotus ostreatus)* require pre-treatment to promote growth of the mushroom mycelium to the exclusion of other microorganisms.

A survey was conducted on selected 59 Upazillas of 22 districts and total 110 mushroom growers through questionnaire. From the investigation, it was observed 73% mushroom growers cultivated oyster mushroom and Reishi, Milky white, Straw, Button mushroom were cultivated by the rest farmers. Among the strains of Oyster mushroom, the Po_2 was cultivated above 51.2% in Summer and more than 30.5% mushroom growers cultivated Po_2 in Winter. As substrate 55.7% people used rice straw, 21.6% people saw dust, above 52.7% farmers pasteurized substrate (straw) by hot water for 1 hr, 30% spoilage of mother spawn reported by 18.9%, 40% by 40% farmers, 30% wastages occurred in summer reported by 46.3% mushroom farmers and less than 20% in winter. About 65.6% farmers were prepared and cultivated spawn by own, 21.6% bought commercial packets from different centers and 78% mushroom was marketed as fresh rest as dry or powdered form. About 67% did not know the name of contaminants, 24% farmer mentioned only the name *Trichoderma* and 7% mentioned *Aspergillus, Penicillium* and *Trichoderma* as contaminants of mushroom cultivation. During survey, curling, brownish, discolorization, small sized fruiting bodies, less number of fruiting bodies were described by interviewers of different mushroom farms. The surface of the spawn showed different abnormal colours due to formation of spores of contaminants, such as 35.7% noticed black or green, 17.9% noticed for green colorization. Only seed contamination was reported by 16% farmers, both seed crisis and the contamination of spawn were reported by 58% growers as the main problems of cultivation of mushroom.

Contaminated packets were collected to investigate the associated fungi and *Trichoderma, Rhizopus, Aspergillus niger, Aspergillus flavus, Penicillium sp, Alternaria, Ceratocytis, Coprinus* and *Chaetomium* were identified as contaminants.

Different substrates, such as rice straw, waste paper, mango saw dust and combination of rice straw, paper and saw dust were used to observe the contamination level considering size of packets and the duration of traditional steam pasteurization without inoculation mushroom seed. It was revealed that, waste paper was not contaminated.Contamination rate was higher in mixed substrate packets, steam pasteurization of substrates for three (3) hours (3) were effective to minimize the contamination. Large size substrate(1000g) were contaminated highly comparatively with small size bags (500g).

Enrichment of substrates with micronutrients combination (Agrovit plus) with different concentration (1000-5000 ppm), 10000 ppm Boron, 10 ppm $Mncl_2$ was studied. Better economic yield was found from 3000 ppm Agrovit plus enriched packets and also contamination rate was lower, the duration of mushroom harvesting was increased, the rate of carbohydrate, fiber, protein, moisture and mineral contents were also increased.

The chemical treatments of substrates with Bavistin (75 ppm), Formalin (500 ppm), 52.2% sodium hypochlorite containing Chlorox (1000 ppm), Alkalanized lime (1000 ppm), Surf excel (100 ppm), $Mncl_2$ (100 ppm) and Hydrogen Per oxide (30000 ppm) compounds showed good antifungal activity. Among these Bavistin, Formalin, Chlorox and Hydrogen Per oxide showed maximum inhibitory effect against the growth competitor moulds. Maximum yield (219.00 g) and maximum biological efficiency (43.36%) obtained from Hydrogen peroxide treated packets.

In case of steam pasteurization, five time duration, autoclave and non-sterilized rice straw was used to evaluate the pasteurization time for better yield and less contamination. Minimum contamination incidence (3.3%) was observed in three hours pasteurized, four hours pasteurized and autoclaved rice straw packets and the lowest contamination severity (10%) was observed autoclaved packets. The better biological efficiency (66.0%) and total yield was 311.3 g / packet was obtained from three (3) hours pasteurized packets.

The effect of hot water treatment (specially at 60^0 for 1, 2 or 3 hours) of rice straw on contamination was higher than the steam pasteurized spawn packets for three hours. *Aspergillus flavus. Rhizopus* sp, *Aspergillus niger, Trichoderma harzianum, Coprinus* sp. were isolated and identified as contaminants from contaminated spawn packets. Maximum economic yield (235.20g) and biological efficiency (48.2%) was found from the packets treated at 80^0 for 2 hours.

From the present research work it may be concluded that,

Pretreatments of lignocellulose substrates influence on the growth and yield of fruiting bodies of mushroom during cultivation of mushroom. In case of hot water treatment, 80°C for 2 hours is recommended or pasteurization of substrate to avoid contaminations before spawning for the cultivation of oyster mushroom.

Although, autoclaving was the best method for substrate pre-treatment, however steam pasteurization for three (3) hours proved to be a viable and promising method of substrate pre-treatment, which can be adopted to produce a good yield of oyster mushroom especially in rural areas, where autoclave sterilization is not feasible.

Considering yield contributing characters, yield, biological efficiency and nutritional composition it can be concluded that rice straw supplemented with 3000ppm Agrovit Plus is promising treatments for locally grown popular oyster mushroom (*Pleurotus ostreatus)* in Bangladesh.

Hydrogen peroxide (30000 ppm) or Chlorox (1000 ppm) can be used for management of mushroom contaminants which showed the effective antifungal activity against associated fungi as well as good yield of oyster mushroom. Although the chemical treatment was easier but these treatments do not control all possible contaminants (as some fungi or bacteria) ecofriendly. Since mushrooms are considered as an organic product, the use of pesticides and chemicals is unpopular and may be hazardous for environment.

CHAPTER VI

REFERENCES

Adhikari, H.S. and Jha, S.K. (2017) Postharvest Microbial Contamination in Oyster Mushroom and their Management using Plant Essential Oils. *Bio Bulletin* **3**(1): 104-108.

Ahmed, M., Abdullah, N., Ahmed, K.U. and M. H. M. B. Bhuyan, B. (2013).Yield and nutritional composition of oyster mushroom strains newly introduced in Bangladesh *Pesq. agropec. bras.* 48: (2):197-202.

Ahmed. S. (1998). Performance of different substrates on the growth and yield of Oyster mushroom (*Pleurotus sajor-caju* (Fr.) Sing). M.S. thesis, Institute of Postgraduate Studies in Agriculture. Salna. Gazipur.

Akindahunsi, A.A. and Oyetayo, F. L. (2006). Nutrient and antinutrient distribution of edible mushroom, *Pleurotus tuber - regium* (Fries). LWT Food Sci. Tech. **39**: 548–53.

Akinyele, B.J., Olaniyi, O.O., and Arotupin, D.J. (2011). Bioconversion of selected agricultural wastes and associated enzymes by *Volvariella volvacea*: An edible mushroom. *Res. J. Microbiol.* **6**:63–70.

Alam, N., Amin, S.M.R. and Sarker, N.C. (2007). Efficacy of five different growth regulators on the yield and yield attributes of *Pleurotus ostreatus* (Jacq. Ex.Fr) Kummer . *Bangladesh J. Mushroom.* **1**(1):51-55.

Alameda, M. and Mignucci, J. (1998). *Burkholderia cepacia* causal agent of bacterial blotch of oyster mushroom. *J. Agric. Univ. Puerto-Rico.* **82**: (1-2): 109-110.

Alemu, F. (2014). Cultivation of oyster mushroom (*Pleurotus ostreatus*) on teff straw (by-product of agriculture) at Dilla University, Ethiopia Glo. *Adv. Res. J. Med. Sci*. **3**(8): 205-215.

Ali, M.A., Hussain, S., Nawaz, R., Ahsan, A and Siddiq, M. (2004). Effect of pasteurization techniques on mycelial growth of oyster mushroom, *Pleurotus spp*. *J. Agric. Res*. **42**(2): 201-207.

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

Amandeep, K., Atri, N.S. and Munruchi, K. (2014). Taxonomic study on coprophilous species of Coprinopsis (Psathyrellaceae, Agaricales) from Punjab, India. *Mycosphere.* **5** (1): 1–25.

Amin, M.A. (2004). Studies on mycelium, spawn and production of certain edible mushrooms. M.S. Thesis, Department of Biotechnology, BAU, Mymensingh.

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

Amin, S. M. R., Sarker, N. C., Alam, N., Hossain, K. and Uddin, M. N. (2008). Influence of different amount of rice straw per packet and rate of inocula on the growth and yield of oyster mushroom (*Pleurotus ostreatus*). *Bangladesh J. Mushroom.* **2**(1): 15-20.

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

Amin, S.M.R., Sarker, N. C., Moonmoon, M., Khandaker, J. and Rahman, M. (2007b). Officer's Training Manual. (National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh 2007). pp. 7-17.

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

Anonymous, (2008). *Model on Oyster Mushroom Cultivation.* http://planning. up.nic.in. /innovations /inno3 / ph /oyster.htm. 8/04/2011 approaches. *Mushroom Research.* **19**(2):62-67.

Ashraf, J., Ali, M.A., Ahmad, W., Ayyub, C.M. and Shafi, J. (2013). Effect of different substrate supplements on oyster mushroom (*Pleurotus spp.*). *Prodn Food Sci. and Tech.* **1** (3):44-51.

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

Balasubramanya, R. H. and Kathe, A. A. (1996). An inexpensive pretreatment of cellulosic materials for growing edible oyster Mushrooms. *Biol. Resour. Technol*. **57**:303-305.

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

Barnett, H.L. and Hunter, B.B. (1972). Illustrated genera of imperfect fungi. 3rd edition, Burgess Publishing Co., 273 pp.

Barros, L., Cruz, T., Baptista, P., Estevinho, L. M., and Ferreira, I. C. F. R. (2008). Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food and Chemical Toxicology.* **46**: 2742-2747.

Baysal, E., Peker, H., Yalinkiliç, M. K. and Temiz, A. (2003). Cultivation of oyster mushroom on waste paper with some added supplementary materials. *Bioresour. Technol.* **89**: 95–97.

Bernardi, E., Donini, L.P. and Minotto, E. (2007). Cultivation of three *Pleurotus* (Jacq.: Fr.) P. Kummer species on pasteurized elephant grass (*Pennisetum purpureum*) substrate. *International Journal of Medicinal Mushrooms.* **9**: 373–378.

Beluham, S. and Ranogajec, A. (2011). Chemical composition and non-volatile components of Croatian wild edible mushrooms. *Food Chemistry*. **2**(3): 1076-1082.

Bernaś, E., Jaworska, G., and Lisiewska, Z. (2006). Edible Mushrooms as a Source of Valuable Nutritive Constituents. *Acta Scientiarum Polonorum – Technologia Alimentaria.* **1**:5-20.

Bhatti, M.I., Jiskani M.M., Wagan, K. H., Pathanand, M.A. and Magsi, M.R. (2007). Growth, development and yield of oyster mushroom, *Pleurotus ostreatus* (jacq. ex. fr.) kummer as affected by different spawn rates. *Pak. J. Bot*. **39**(7): 2685-2692.

Biserka, B. L. (1972). Dealing with microbiological trouble makers in commercial spawn production of *Agaricus bisporus L. Mushroom Science.* **8**:305-314.

Biswas, S., Hoque, M. S. and Ahmed, K.U. (2009). Effect of a Mineral Supplement on Growth, Yield and Nutritional Status of Oyster Mushroom (*Pleurotus ostreatus*) *Bangladesh J. Mushroom*. **3**(2): 51-58.

Biswas M.K. (2014). Microbial contaminants in oyster mushroom (*Pleurotus ostreatus*) cultivation their management and role of meteorological factors. *Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8).*

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

Biswas, P, Ahmad, A., Ahmed, K.U., Md. Abir Hossain, M.A., Hamja, M.A. and Rabin, M.H. (2016). Yield of Oyster Mushroom (*Pleurotus ostreatus*) Influenced by Different Sawdust Substrate. *Int. J. of Sci. and Res Pub.* **6** (Appendix XVIII): 291-296.

Chang, S. T., Lau, O. W. and Chowdhury, K. Y. (1981). The cultivation and nutritional value of *Pleurotus sajor cuju*. *Eur. J. Appl. Microbiol. Biotechnol.* **12**(1): 58-62.

Chang, S.T. and Miles, P.G. (1989). Edible Mushrooms and Their Cultivation. CRC Press, Inc., Florida, 345 p.

Chang, S. T. (2009). Training Manual on Mushroom Cultivation Technology, United Natons-Asian and Pacific Centre for Agricultural Engineering and Machinery (UN-APCAEM), Beijing, China. Pp. 44-45.

Chen, A.W. and Moy, M. (2004). Mushroom cultivation: building mold contamination. At:<http://www.mushworld.com> Accessed on 28.

Choi, I.Y, Hong, S.B, and Yadav, M.C. (2003). Molecular and morphological characterization of green mold, *Trichoderma* spp. isolated from oyster mushrooms. *Mycobiology*. **31**:74–80.

Choi, I.Y., Choi, J.N., Sharma, P.K. and Lee, W.H. (2010). Isolation and Identification of Mushroom Pathogens from *Agrocybe aegerita*. *Mycobiology*. **38**(4): 310–315.

Choudhury, M.B.K., M., Choudhuri, M.S. and Hossain, M.S. (2011). Oyster mushroom (*Pleurotus* spp.): A Friendly and Medicinal Mushroom. *Bangladesh J. Mushroom.* **5** (1):59-81.

Chowdhury, A. K., Panja, B. N. and Laha, S. K. (1998). Organic supplements for better yield of oyster mushroom. *J. Intera cademicia B.C.K.V.*, India., **2**(1-2): 116-117.

Chowdhury, H.H., Rebolj, K., Kreft, M., Zorec, R., Ma•ek, P. and Sepecific, K. (2008). Lysophospholipids prevent binding of a cytolytic protein ostreolysin to cholesterol-enriched membrane domains. Toxicon, in press.

Cohen, R., Persy, L., and Hadar, Y. (2002). Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. *Applied Microbiology and Biotechnology.* **58**: 582–594.

Curvetto, N. R., Figlas, D., Devalis, R., and Delmastro S. (2002). Growth and productivity of different *Pleurotus ostreatus* strains on sunflower seed hulls supplemented with N–NH4 + and/or Mn(II). *Bioresource Technology.* **84**:171–176.

Das, N., and Mukherjee, M. (2007). Cultivation of *Pleurotus ostreatus* on weed plants. Bioresource Technology. **98**: 2723-2726.

Deepalakshmi, K. and Mirunalini, S. (2014). *Pleurotus ostreatus*: an oyster mushroom with nutritional and medicinal properties. *J .Biochem.* **5(2):**718-726.

Dhanda, S., Kakkar, V. K., Garcha, H. S. and Makkar, G. S. (1994). Biological treatment of paddy straw and its evaluation through ruminant feeding. *Indian J. Animal Nutrition*. **11**(2): 73-79.

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

Diana, F., D. Indrea, A.S., Apahidean, M., Apahidean, R. Pop, Z., Moldovan, D., Măniu Ńiu, R., Ganea and Paven, I. (2006). Importance of substrate disinfection on Oyster mushroom (*Pleurotus sp*.) culture. *Not. Bot.Hort. Agrobot. Cluj*. **34:** 1–6.

Dinesh, R. C., Vinay, P., Manasa, P.,Vinothkumar, D. and Ramesh Babu, N. G. (2013). Comparative study of oyster mushroom (*Pleurotus ostreatus)* cultivation by physical and chemical method of Sterilization using two different substrates. *International J. of Scintific & Engineering.*, Dorchester, UK.

Dhingra, O. D. and Sinclair, J. B. (1995). Basic plant pathology methods (second edition). Lewis Publishers, London, 434 p.

Doshi, A. and Singh, R.D. (1985). Control of weed fungi and their effect on yield of oyster mushroom *Pleurotus sajor-caju. Indian J. Mycol. Plant Pathol.* **15:**269-273.

Dundar, A., Acay, H., and Yildiz. A. (2008). Yield performances and nutritional contents of three oyster mushroom species cultivated on wheat stalk, African Journal of Biotechnology. **7**(19): 3497-3501.

Eger, G., Eden, G. and Wissing, E. (1976). *Pleurotus ostreatus*, breeding potential of a new cultivated mushroom. *Theoretical and Applied Genetics*. 47:155-163.

Elhami, B. and Ansari, N. A. (2008). Effect of substrate of spawn production on mycelium growth of oyster mushroom of species. *J. Biol. Sci*. **87(2):** 239-244.

Fatema S., Khan S.S, Khan, M. and Tanveer, A. (2011).Cultivation of *Pleurotus Sajor – Cajo* on Wheat Straw, Water Hyacinth and Their Combinations. *Indian J. of Fundamental and Applied Life Sci.***1**: (3) 56-59.

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

Gupta, G.K., Bajaj, B.S. and Suryanarayana, D. (1975). Studies on the cultivation of paddy straw mushroom(*Volvariella volvacea* and *V. diplasia*) *Indian Phytopath.* **23:** 615-620

Gupta, J.H. (1989). Yield potentiality of oyster mushroom on wheat straw under natural room temperatures, during March-April and September-October at Saharanpur. *Progressive Horticulture***. 21**(1-2): 184.

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

Haque, A. B. M. A. (2004). Use of different substrates and methods on the mycelial growth, spawn production and yield of three mushroom species. M. S. Thesis, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Hasan, M.N., Rahman, M.S., Nigar, S., Bhuiyan, M.Z.A., Ara, N. (2010). Performance of oyster mushroom (*Pleurotus ostreatus*) on different pretreated substrates. *Int. J. Sustain. Crop Prod.* **5**(4): 16-24.

Hassan, S., Mohammad, A.Y. and Kiramat, K. (2011). Cultivation of the oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm.) in two different agroecological zones of Pakistan. *African J. Biotechnol.* **10**: 183–188.

Hatvani L., Antal Z., Manczinger L., Szekeres A., Druzhinina I.S., Kubicek C.P., Nagy A., Nagy E., Vágvölgyi C., Kredics L.(2007). Green mold diseases of *Agaricus* and *Pleurotus* spp. are caused by related but phylogenetically different *Trichoderma sp* species. *Phytopathol*. **97**: 532-537.

Hoa, H. T., Wang, C.L. and Wang, C.H. (2015). The Effects of Different Substrates on the Growth, Yield, and Nutritional Composition of Two Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus). Mycobiology*. **43**(4): 423–434.

Hossain, M.A., Khair, A. and Ahmed, S. (2010). Occurrence of *Coprinus lagopus* (Fr.):A potential weed fungus as a contaminant of mushroom cultivation in Bangladesh. *Bangladesh J. of Mushroom*. **4** (2):67-74.

Houdeau, G., Olivier, J.M., Libmond, S. and Bawadikji, H. (1991). Improvement of *Pleurotus* cultivation. *Mush Sci*. **13**:549–54.

Hunter, J., Schmidt, F., and Jackson, G. (1982). Meta-Analysis: Cumulating research findings across studies. Beverly Hills CA: Sage.

http://www.ppath.cas.psu.edu/MushGrowInfo/Trichoderma%20Green%20Mold.html.

Hyakumachi, M. (1994). Plant-growth-promoting fungi from turfgrass rhizosphere with potential for disease suppression *Soil Microorganism.* **44** (pg. 53 -68).

Ijaz, M. and Khan, S.M. (1992). Biological efficiency of different species/strains of lignicolus fungus *Pleurotus* cultivated on different agro-wastes. *Agril. Res.* Lahore. **30**(8): 423-427.

Iqbal, S.M., Rauf, C.A. and Sheik, M.I. (2005). Yield performance of oyster mushroom on different substrates. *Int. J. Agric. Biol*. **7**: 900–903.

Isik, S. E., Aksu, S., Erkel, I. and Moltay, I. (1995). The effects of some organic nitrogenous substances as activators to the mushroom yield during the preparation of compost. Yalova (Turkey). *Ataturk Central Horticultural Res Inst.* p. 23.

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

Jain, A.K. and Vyas, D. (2002). Yield response of *Pleurotus florida* on wheat straw in combination with other substrates. *Mush. Res*. **11**(1): 19-20.

Jandaik, S., Guleria D.S. (1999). Yield loss in *Agaricus bisporus* due to *Trichoderma sp* infection. *Mushroom Res.* **8**:43-46.

Jaramillo, S. and Albertó, E. (2013) Heat treatment of wheat straw by immersion in hot water decreases mushroom yield in *Pleurotus ostreatus*. *Rev Iberoam Mycol.* **30**(2):125-129.

Jebunnahar, K., Amin, S. M. R., Sarker, N. C., Kamal, S. and Shahin, M. (2007). Performance of bag size and spawning method on yield and yield attributes of *Agaricus bisporus* (Lange) Singer. *Bangladesh J. Mushroom.* **1**(2): 61-66.

Jedinak, A., Dudhgaonkar, S., Jiang, J., Sandusky, G., and Sliva, D. (2010). *Pleurotus ostreatus* inhibits colitis-related colon carcinogenesis in mice. *Int. J. of Mol. Med., 26*:643-650.

Jo, W.S., Rew, Y.H., Kim, S.H., Yun, J.T., and Choi, B.S. (1999).Occurrence of bluish green mold of *Pleurotus eryngii* by *Penicillium corylophilum*. *Korean J. Mycol.* **27**:412–414.

Jonathan, S. G., Okorie, A. N., Babayemi, O. J., Oyelakin, A. O., and Akinfemi, A. (2012). Biodegradation of agricultural wastes (rice straw and sorghum stalk) into substrates of utilizable products using white rot fungus (*Pleurotus florida*). *Nature and Science*. **10**: 131- 137.

Kalac, P. and Svoboda, L. (2000). A review of trace element concentrations in edible mushrooms. *Food Chemistry*. **69**: 273-281.

Khan, N. A., Abbas, M., Rehman, A., Haq, I. and Hanan, A. (2011). Impact of various sterlization methods using different substrates for yield improvement of *Pleurotus spp. Pak. J. Phytopathol.* **23**(1): *20-23.*

Khan, A.S., Sarker, N.C., Howlader, K. R., Kakon, A. J. M., Moonmoon, Hoque, M. M. and Rahman, T. (2012) Effect of Different Amount of Rice Straw on Growth and Yield of *Pleurotus salmoneo-stramineus. Bangladesh J. Mushroom.* **6**(2): 71-75.

Khan, N.A., Ajmal, M., Nicklin, J., Aslam, S. and Ali, M.A. (2013).Nutritional value of *Pleurotus (flabellatus) DJAMOR(R-22)* cultivated on saw dusts of different woods. *Pak. J. Bot.* **45** (3): 1105-1108.

Khare, K.B., Mutuku, J.M., Achwania, O.S. and Otaye, D.O. (2010). Production of two oyster mushrooms, Pleurotus sajor-caju and P. florida on supplemented and un-supplemented substrates. *Bots. J. Agric. Appl. Sci.* **6**: 4-11.

Khatun, K., Mahatab, H., Khanam, P.A., Sayeed M.A. and Khan, K.A. (2007).Oyster mushroom reduced blood glucose and cholesterol in diabetic subjects. **16** (1):94-99.

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

Kim, S. W., Hwang, H. J., Park, J. P., Cho, Y. J., Song, C. H. & Yun, J. W. (2002). Mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media. *Lett. Appl. Microbiol*. **34**: 56–61.

Kimenju, J.W., Odero, G.O.M., Mutitu, E.W., Wachira, P.M., Narla, R.D. and. Muiru W.M. (2009). Suitability of locally available substrates for oyster mushroom (*Pleurotus ostreatus*) cultivation in Kenya. *Asian J. Plant Sci*. **8**: 510–514.

Klingman, A.M. (1950). Hand book of mushroom culture. CRC Publishing co. J. B. Kenneth Square, Pennsylvania, USA.

Kredics, L., García Jimenez, S., Naeimi, D., Czifra1, P., Urbán, L., Manczinger, C. Vágvölgyi, and. Hatvani, L. (2010). A challenge to mushroom growers: the green mould disease of cultivated Champignons. *Applied Microbiology and Microbial Biotechnology.* pp. 295-305.

Komon-Zelazowska, M., Bissett, J., Zafari, D., Hatvani, L., Manczinger, L., Woo, S., Lorito, M., Kredics, L., Kubicek, C.P.and Druzhinina, I.S.(2007).Genetically closely related but phenotypically divergent *Trichoderma sp* species causes green mold diseases in oyster mushroom farms worldwide. *Applied & Environmental Microbiology*. **73**(22):7415-7426.

Kuforiji, O.O. and Fasidi, I.O. (2008). Enzyme activities of *Pleurotus tuber-regium* (Fries) Singer, cultivated on selected agricultural wastes. *Bioresource Technol*. **99**: 4275–4278.

Kulshreshtha, S., Mathur, N., Bhatnagar, P. and Kulshreshtha, S. (2013b). Cultivation of *Pleurotus citrinopileatus* on handmade paper and cardboard industrial wastes. Ind Crop Prod. **41**: 340–346.

Kumar, S. (2015). Evaluation of substrates for quality spawn production of mushrooms. Thesis submitted to Faculty of Postgraduate Studies in partial fulfillment of the requirements for the degree of MS of Science in Agriculture Plant Pathology, pp 1-67.

Kumar, R, k S. hooda, J.C. Bhatt and kumar. R.A. (2011). Influence of chemicals on the growth and yield of five species of oyster mushroom (*Pleurotus spp*.) in North-western Himalayas. *Indian Phytopath.* **64** (2): 178-181.

Kumari, D. and Achal, V. (2008). Effect of different substrates on the production and non-enzymatic antioxidant activity of *Pleurotus ostreatus* (Oyster mushroom). *Life Science Journal*. **5(3):** 73-76.

Kumbhar, C.T. (2012). Effect of spawn substrates on yield of *Pleurotus eous* (Berk.) Sacc. *International Journal of Plant Science.* **7**: 224-229.

Kurtzman, Jr. R., (2010). Pasteurization of mushroom substrate and other solids. *African J. Environ. Sci. Technol.* **4**: 936–941.

Kwon, H. and Kim, B. S. (2004a). Mushroom Growers' Handbook: Shiitake Cultivation, p: 260. Mush world, Korea.

Kwon, H., and Kim, B.S. (2004b). Bag cultivation. In: *Mushroom Growers' Handbook* 1. Seoul: Mush. World. pp 139–152.

Largeteau-Mamoun, M. L., Mata, G. and Savoie, J. M. (2002). Green mold disease: Adaptation of *Trichoderma harzianum* Th2 to mushroom compost. Pp.179-187. In Mushroom Biology and Mushroom Products. Proceedings of The 4th International Conference on Mushroom Biology and Mushroom Products, Cuernavaca, Mexico.

Lee, C.Y., Park, J.E., Kim, B.B, Kim, S.M. and Ro, H.S. (2009). Determination of Mineral Components in the Cultivation Substrates of Edible Mushrooms and Their Uptake into Fruiting Bodies. *Microbiology*. **37**(2): 109–113.

Leong, P.C. (1980). Utilization of cotton waste substrate with temperature treatments for the cultivation of oyster mushroom (*Pleurotus*) in Singapore. *Singapore J. Primary Indus.* **8**(10): 21-27.

López, L.Z., Sánchez, J.E. and Bello, R. (1995). Quality of effluent water from the pasteurizing of coffee pulp. In: Proceedings of the 16th international conference on coffee science.

Loss, E., Royer, A. R., Barreto-Rodrigues, M., and Barana, A. C. (2009). Use of maize wastewater for the cultivation of the *Pleurotus* spp. mushroom and optimization of its biological efficiency. *Journal of Hazardous Materials.* **166:**1522-1525.

Mabrouk, E.M. and Ahwany, M.D. (2008). Production of mannanase by *Bacillus amylolequifaciens* 10A1 cultured on potato peels. *Afr J. Biotechnol.* **7**:1123–1128.

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

Mandeel, Q. A., Al-Laith, A. A. and Mohamed, S. A. (2005). Cultivation of oyster mushrooms (*Pleurotus* spp.) on various lignocellulosic wastes. *World J. Microbiol. Biotechnol.* **21**: 601–607.

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

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

Mazumdar, N. and Rathaiah, Y. (2001).Management of fungal and bacterial contaminations of oyster mushroom spawn. *Mushroom Research.* **10**: 113-115.

Mazumder, N., Rathaiah, Y. and Gogoi, R. (2005). Seasonal variation in microbial contamination of Pleurotus ostreatus spawn. *Indian Phytopathology*. **58**(1): 84-88.

Mbogoh, J.M., Anjichi, V.E.,Rotich, F. And Ahoya, N.K. (2011). Proceedings of first all African Horticultural congress Eds. J. Wesonga and R. Kahane. Acta Hort. 911, ISHS.

Mejía, S.J. and Albertó, E. (2013). Heat treatment of wheat straw by immersion in hot water decreases mushroom yield in *Pleurotus ostreatus. Micol.* **30** : 125-129 .

Mishra, K. K. and Singh, R. P. (2010). Cultural and biochemical variability amongst indigenous *Ganoderma lucidum* isolates from Uttarakhand. *Mushroom Research.* **19 (**2): 74-81.

Moda, E.M. Horii, J. and Spoto, M.H.F. (2005). Edible mushroom *Pleurotus sajor-caju* production on washed and supplemented sugarcane bagasse *Sci. Agric*. **62**(2).

Mona, M.R., Abdou, H. M., Mahmoud, A. E., and Nooman, M. U. (2009). Nutritional analysis and enzyme activities of *Pleurotus ostreatus* cultivated on *Citrus limonium* and *Carica papaya* wastes, *Australian Journal of Basic and Applied Science*. **3**(4): 3352-3360.

Mondal, S. R., Rehana, M. J., Noman, M. S. and Adhikary, S. K. (2010). Comparative study on growth and yield performance of oyster mushroom (*Pleurotus florida*) on different substrates. *J. Bangladesh Agril. Univ.* **8**(2): 213–220.

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

Moonmoon, M., Shelly, N. J., Khan, M. A., Uddin, M. N., Hossain, K., Tania, M., and Ahmed, S. (2011). Effects of different levels of wheat bran, rice bran and maize powder supplementation with saw dust on the production of shiitake mushroom (*Lentinus edodes* (Berk.) Singer). *Saudi Journal of Biological Sciences.* **18:** 323-328.

Moonmoon, M., Kakon, A. J., Hoque, M. M., Sarker H. K., Huda, N., Nahar, K., Kutubuddin, M., Mujib, T.B. and Khan, A. S.(2013). Yield performance of oyster Mushroom variety at Different Location in Bangladesh. *Bangladesh J. Mushroom.* **7**(1): 27-34.

Muhammad, I. and Khan, S.M. (1993). Yield performance of different species strains for Oyster mushroom (*Pleurotus* spp.) on cotton waste. *Pakistan J. Phytopathol*. **5**(12): 53-57.

Munsur, M.A., Miah, A., Rahman, M.H. and Yahia, A.S.M. (2012). Effect of varieties and media on mycelial growth and substrate on spawn production of oyster mushroom. *Bangladesh Research Publications Journal.* **7**(4): 361-366.

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

Nawnze, P. I., Khan, A. U., Ameh, J. B. and Umoh, V. J. (2004). The effect of various grains, culture media, oil type and rate on the stipe lengths and diameters, wet and dry weights and pileus diameters of *Pasthyrella atroumbonata*. ROAN. **3**(1-2)**:** 85-97.

Nayana, J. and Janardhanan, K. K. (2000). Antioxidant and antitumor activtity of Pleurotus florida. *Current Sci*. **79**: 941-943.

Nunes, M. D., José Maria Rodrigues da Luz, Sirlaine Albino Paes, João Júlio Oliveira Ribeiro, Marliane de Cássia Soares da Silva and Maria Catarina Megumi Kasuya. (2012). Nitrogen Supplementation on the Productivity and the Chemical Composition of Oyster Mushroomwww.ccsenet.org/jfr *Journal of Food Research.* **1**(2).

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

Obodai, M., Okine, C. and Vowotor, K.A. (2003). Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products. Food Res. Inst. Accra, Ghana. *J. Industrial Microbio. and Biotech.* **30**(3): 146-149.

Odero, G.M.O. (2009). Substrates evaluation and effects of ph and nutritional supplementation on production of oyster mushroom (*Pleurotus ostreatus*). A research thesis submitted in partial fulfilment for the award of master of science degree in agricultural resource management, university of Nairobi.

Oei, P. (1996). Mushroom cultivation with special emphasis on appropriate techniques for developing countries. CTA, The Netherlands. P. 145.

Olsen, S., Cole, C., Watanabe, F. and Dean, L. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular Nr 939, US Gov. Print. Office, Washington, D.C.

Onyango, B.O., Palapala,V.A., Arama, P.F. Wagai, S.O and Gichimu, B.M. (2011). Suitability of selected supplemented substrates for cultivation of Kenyan native wood ear mushrooms (*Auricularia auricula*). *American J. Food Technol*. **6**: 395–403.

Oseni, T.O., Dlamini, S.O. Earnshaw, D.M. and Masarirambi, M.T. (2012). Effect of substrate pre-treatment methods on oyster mushroom (*Pleurotus ostreatus*) production. *Int. J. Agric. Biol*. **14:** 251–255.

Özçelik, E. and Peksen, A. (2007). Hazelnut husk as a substrate for the cultivation of shiitake mushroom (*Lentinula edodes*). *Bioresource Technology*. **98:** 2652-2658.

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

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

Pegler, D.N. (1977). A Preliminary Agaric flora of East Africa. Kew Bulletin 6, 1–615.

Pervez, Z., Bhuiyan, K. A., Rahman, H. and Islam, S. (2010). Prevalence of Mycoflora Associated with Oyster Mushroom (*Pleurotus ostreatus*) Substrates and Evaluation of Formalin and Bavistin Against them. *Bangladesh J. Mushroom.* **4**(1): 45-50.

Pervez, Z. Alam, M.I., Islam, M. S. and Islam, S.M.A. (2012). Evaluation of H_2O_2 in Controlling Mycoflora Associated With the Substrate of Oyster Mushroom. *Bangladesh Res. Pub. J.* 7(2): 189-193.

Ponmurugan, P., Sekhar, Y. N., and Sreesakthi, T.R., (2007). Effect of various substrates on the growth and quality of mushrooms. *Pakistan J. Biol. Sci.* **10:** 171–173.

Prakash, D., Upadhyay, G., Gupta, C., Pushpangadan, P. and Singh, K.K. (2012). Antioxidant and free radical scavenging activities of some promising wild edible fruit*s International Food Research Journal.* **19** (3): 1109-1116.

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

Quimio, T.H., S.T. Chang and Royse, D.J. (1990). *Technical Guidelines for Mushroom Growing in the Tropics*, p: 154. FAO, *Plant Prod and Protec. paper* No-106. Rome, Italy.

Raghuramulu, N., Madhavan, N.K. and Kalyanasundaram, S. (2003). A Manual of Laboratory Techniques. National Institute of Nutrition. *Indian Council of Medical Research*. pp: 56-58.

Rahman, M. H., Ahmed, K. U., Roy, T. S., Alam, M. R. And Kabir, M. A. (2012 a). Effect of wheat bran supplements with rice straw on the growth and yield of oyster mushroom (*Pleurotus ostreatus*) *Int. J. Sustain. Agril. Tech*. **8**(11): 10-14.

Rahman, M.H., Ahmed, K.U., Roy, T. S., Shelly, N.S. and Rahman, M.S. (2012 b). Effect of wheat bran supplements with rice straw on the proximate composition of oyster mushroom (*Pleurotus ostreatus*). *Bangladesh res. publications j*. **7**(4): 306-311.

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

Reyes, R. G., Encarnacion, A. D. and Abella, E. A. (1994). Utilization or selected agro-industrial wastes for mushroom (*Pleurotus sajor-caju* (Fr.) Singer) production. *CLSU Sc. J*. **14** (1): 9-23.

Roye. (2002). Specialty mushroom and their cultivation. *Hort. Rev*. **19:** 59-97.

Royse, D. (2002) Influence of spawn rate and commercial delayed release nutrient levels on *Pleurotus cornucopiae* (oyster mushroom) yield, size, and time to production *Applied microbiology and biotechnology*. **58**(4): 527–531.

Royse D. J., Sanchez, J. E., Beelman, R. B. and Davidson, J. (2008). Re-supplementing and recasing mushroom (Agaricus busporus) compost for a second crop. *World J. Microbiol. Bidotech*. **24**: 319-325.

Royse, D. J. and Sanchez. J. E. (2008a). Supplementation of first break mushroom compost with hydrolyzed protein, commercial supplements and crystalline amino acids. *World Journal Microbiology Biotechnology***. 24**: 1333-1339.

Royse D. J. and J. E. Sanchez. (2008b). Supplementation of second break mushroom compost with isoleucine leucine, valube, phenylalanine and Soypluse. *World J. Microbiol. Biotech*. **24**:2011-2017.

Samuels, G.J, Dodd, S.L, Gams, W., Castlebury, L.A., Petrini, O. (2002) *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. *Mycologia*. 94(1): 146-170.

Samson, R.A., Hong, S.B., Frisvad, J.C. (2006). Old and new concept of specific differentiation in *Aspergillus*. *Med Mycol*. **44** (Suppl):133–148.

Sanchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Appl. Microbiol. Biotechnol.* **85**: 1321–1337.

Sánchez, J., Moreno, L., René and Rrade-gallegos, R. (2011*)*.Pasteurization of substrate for growing *Pleurotus ostreatus* by self heating *Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7).*

Sarker, N. C., Hossain, M.M., Sultana, N., Karim, A.J.M.S. and Amin S.M.R. (2007). Performance of different substrates on growth and yield of *Pleurotus ostreatus* (Jacq. Ex.Fr) Kummer. *Bangladesh J. Mushroom.* **1**(2): 9-20.

Sarker, N. C., Hossain, M.M., Sultana, N., Mian, I., H., Karim, A. J. M. and Amin, S.M.R. (2008). Effects of wheat bran and rice bran supplements to waste paper and wheat straw substrates on growth and yield of *Pleurotus ostreatus* (Jacq. Ex.Fr) Kummer. *Bangladesh J. Mushroom.* **2**(2): 1-15.

Sarker, N.C. (2004). Oyster mushroom (*Pleurotus ostreatus*) Production Technology Suitable for Bangladesh and its Nutritional and Postharvest Behavior. PhD Thesis. Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur.

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

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

Sarker, N.C., Hossain, M. M., Sultana, N., Mian, I. H., Karim, A. J. M. S. and S. M. R. Amin (2008). *Bangladesh J. Mushroom.* **2**:27.

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

Schaad, N. W. (1988). Laboratory Guide for Identification of plant pathogenic Bacteria. 2nd ed. *American Phytopathological Society*, St. Paul, Minnesota. pp. 185-197.

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

Sharma, A.D. and Jandaik, C.L. (1983). Some preliminary observation on the occurrence of Sibrina rot of cultivated mushrooms in India and its control. *Tai. Mush*. **6** (1&2): 38-42.

Sharma, S.R., Kumar, S. and Sharma, V.P. (2007). Diseases and Competitor Moulds of Mushrooms and their Management. In Technical Bulletin, National Research Centre for Mushroom (*Indian Council of Agricultural Research*). pp 1-86.

Singh, K.L., Kannayan, S. and Ramasamy, K. (1990). *Hand Book of Edible Mushrooms-Cultivation of Pleurotus*. pp: 38–72.

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

Singh *et al.,(*2015).Chemical composition of essential oil from leaves of seeded and seedless Citrus reticulata blanco var. kinnow *Bangladesh Journal of Scientific and Industrial Research.* **49**(3).

Siqueira, F.G., Emerson, T. Martos, T. E., Silva, R. D and Dias, E.S. (2011) Cultivation of *Pleurotus sajor-caju* on banana stalk and Bahia grass based substrates. *Hortic. Bras Brasília* **29** (2).

Siqueira, S.F.G., Maciel, W.P., Martos, E.T., Duarte, G.C. and Miller Silva, R.N.G. (2012). Cultivation of *Pleurotus* mushrooms in substrates obtained by short composting and steam pasteurization. *Afr. J. Biotechnol*. **11:**11630-11635.

Sofi, B., Ahmad, M. and Khan, M. (2014) Effect of different grains and alternate substrates on oyster mushroom (*Pleurotus ostreatus*) production *Microbiology Research.* **8** (14): 1474-1479.

Sonali, D. R. (2012). Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis. *Adv. Appl. Sci. Res*. **3**(4):1938-1949.

Stamets, P. (1993). Cultivation of Morels mushroom. *J. Wild Mushrooming*.

Stanly, H. O. (2010). Effect of substrates of spawn production on mycelial growth of oyster mushroom species. *Agril. and Biol. J. of North America.* **1**(5): 817-820.

Stoller, B.B. (1962). Some practical aspects of mushroom spawn. *Mushroom Science,* **5**:170-184.

Tan, K.K. (1981). Cotton waste is a good substrate for cultivation of (*Pleurotus ostreatus*) the Oyster Mushroom. *Mush. Sci.* **11**(1): 705-710.

Tewari, R. P. (1986). Mushroom cultivation. Extension Bulletin. *Indian Institute of Horticulture Research.* **8:**36.

Thapa, C.D, Seth, P.K and Pal, J. (1979). Occurrence of olive green mould (*Chaetomium globosum*) in mushroom beds and its control. *Indian. J. Mush.* **5:** 9-13.

Tinoco, R., Pickard, M.A. and Vazquez, D. R. (2001). Kinetic differences of purified laceases from six white button mushroom strains. *Lett. Applied Microbiology.* **32**:331- 335.

Uddin, M. N., S. Yesmin, M. A. Khan, Tania, M., Moonmoon, M. and Ahmed, S. (2011). Production of Oyster Mushrooms in Different Seasonal Conditions of Bangladesh. *J. Sci. Res.* **3 (1):** 161-167.

Upamanya, G. K. and Rathaiah, Y. (2000). Effect of fortification of rice straw with rice bran on yield and protein content of oyster mushroom (*Pleurotus cornucopiae*). *Indian J. Hill-Farm***. 13(1-2):** 104-105.

Vetayasuporn, S., Chutichudet, P. and Cho-Ruk, K. (2006). Bagasse as a possible substrate for *Pleurotus oesterotus* (Fr.) Kummen cultivation for the local mushroom farms in the Northeast of Thailand. *Pakistan J. Biol. Sci*. **9**: 2512–2515.

Vijay B and Sohi HS. (1987). Effect of different sterilants and farm wastes on yield of *Pleurotus citrinopileatus. Mush. J. Tropics*.**7**: 67-75.

Williams, J., Clarkson, J. M., Mills, P. R., and Cooper, R. M. (2003). Saprotrophic and mycoparasitic components of aggressiveness of *Trichoderma harzianum* groups toward the commercial mushroom *Agaricus bisporus .Appl. Environ. Microb*. **69**:4192–4199.

Wayne, R. R., (2001). Growing Mushrooms the Easy Way Home Mushroom Cultivation with Hydrogen Peroxide. **3**(1): 11-14

Won, Y. (2000).Oyster Mushroom Disease: Green Mould. At: <http:// www. mushworld. com> Accessed on 1.

Woo S. L., Donzelli, B., Scala, F., Mach, R., Harman, G. E., Kubicek, C. P., del Sorbo, G, and Lorito, M. (1999). Disruption of the *ech42* (endochitinase-encoding) gene affects biocontrol activity in *T. harzianum P1*. *Mol. Plant Microbe* In. **12:** 419–429.

Yamakawa, T. (1992). Laboratory method for soil science and plant nutrition. JICA-IPSA Project Publication. IPSA, Gazipur, Bangladesh. pp. 1-14.

Yang, W.J., Guo, F.L. and Wan, Z.J. **(**2013) .Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. *Saudi J Biol Sci*. **20**(4): 333–338.

Yildiz, S., Yildiz, U.C., Gezer, E. D., and Temiz, A. (2002). Some lignocellulosic wastes used as raw material in cultivation of the Pleurotus ostreatus culture mushroom. Process Biochemistry. **38:**301-306.

Yoshida, N., Takahashi, T., Nagao, T. and Chen, J. (1993). Effect of edible mushroom (*Pleurotus ostreatus*) cultivation on *in vitro* digestibility of wheat straw and sawdust substrate. *J. Japanese Soc. Grassland Sci*. **39** (2): 177-182.

Yu, S. H. (2002). Integrated Control of Oyster Mushroom Green Mould (2). At: <http: // www.mushworld.com> Accessed on 1.

Zahid, M. K., Barua, S. and Haque, S. M. I. (2010). Proximate Composition and Mineral Content of Selected Edible Mushroom Varieties of Bangladesh. *Bangladesh J. Nutr*. **22-23**: 61-68.

Zaidi, S., and Dahiya, P. (2015). In vitro antimicrobial activity, phytochemical analysis and total phenolic content of essential oil from *Mentha spicata* and *Mentha piperita*. Ifrj. **22**(6): 2440-2445.

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

Zhang, R., Li, X. and Fadel, J. G. (2002). Oyster mushroom cultivation with rice and wheat straw. *Bioresource Technology*. **82**(3): 277-284.

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

Zhu, M.J., Du, F., Zhang, G.Q., Wang, H.X. and Ng, T.B. (2013). Purification a laccase exhibiting dye decolorizing ability from an edible mushroom Russula virescens. *Int Biodeterior, Biodegrad.* **82**:33–39.

CHAPTER-VII

APPENDICS

Appendix 1. English version of questionnaire used for collection of data on mushroom contamination related information and analysis.

"Questionnaire for mushroom cultivator to determine the prevalence of contamination of oyster mushroom in Bangladesh." (Please help by answering the following questions by giving code number in the box)

1. **NAME OF THE MUSHROOM FARM/ Center:**
2. **Personal Information of mushroom grower:**

2.1 Name:----------

2.2 Cell No.:----------

2.3 Address of the mushroom farm----------

2.4 Gender: (Code:1 =male, 2= Female) ☐

2.5 Age: (Code:1 =20 or less, 2= 21 to 30, 3= 31 to 40, 4=41 to 50, 5=51 or more) ☐

2.6 Educational Background: (Code: 1=below SSC, 2=SSC, 3=HSC, 4= Graduate, 5= post graduate). ☐

2.7 Status of the service

1. Owner, 2. Worker ☐

3. **Information about mushroom production:**

3.1 In which season mushroom are cultivated?

I. Summer season ii. Winter season

3.2 Which mushrooms are cultivated here?

Code: a) Oyster mushroom b) Reishi mushroom c) Milky white d) Button mushroom e) straw mushroom

3.3. Which varieties of oyster mushroom are cultivated here in Winter season?

a.$Po_{2,}$ d. Pop c.High King e. ws f. PCYS g. PCS

2.3. Which varieties of oyster mushroom are cultivated here in Summer season?

(Code: a.Po2, b. PCYS, c. POP, d. WS, e.HK, f. PCS)

3.0. **Information about preparation of substrate:**

3.3 .Which substrates are used for oyster mushroom production?

(Materials/component of substrate)

(Code:1. Saw dust 2. Wheat straw, 3. Rice straw 4. Waste paper)

4.2 . Which supplements are used with substrates?

(Code:1.Rice bran 2. Wheat bran. 3. Rice husk 4. No)

4.3. Problems during substrate collection:

1.high Cost, 2. Not available 3.No problem, 4. Collection)

4.4. Which procedure is used for substrate sterilization?

(Code: 1.Autoclave 2. Hot water 3.Steam 4. Solar 5.NAMDEC Pasteurization chamber)

4.5. Problems during substrate sterilization:

4.6. Duration of sterilization

(Code: 1.1hr, 2. 2 hr, 3. 3 hr, 4. 4hr)

4.7. How many spawn packets can be prepared once a time?

(Code:1.500-1000 2. 1000-1500 3.2000-2500 4.2500-3000 5.3000-4000 6.4000-5000.)

4.8. Number of shelf for keeping oyster spawn packets:

(Code: 1= 5-10, 2=10-15, 3 =15-20, 4= 20-25, 5=25 or more)

4.9. Number of tak/ shelf:

(Code: 1=4, 2=5, 3=6)

4.10. Number of spawn packets /shelf

(Code:1=100-150, 2=200-250, 3=300-350, 4=400-450, 5.500 or more)

4.11. Spoiled spawn packets in Winter (percentage)

(Code: 1=10, 2=20, 3=30, 4=40, 5=50, 6=50 or more)

4.12.How many spawn packets are spoiled in summer ?

(Code: 1=10%, 2=20%, 3=30% , 4=40%, 5= 50% , 6=60% 7. More %)

5.0. Information about mother spawn

5.1.Media of mother:

(Code: 1.Rice B. Jute stick,C. Wheat, D. Cheena, E.Kaon)

5.2. In which place inoculation is done?

(Code: 1.Clean bench 2.glass box C. Open place)

5.3. Source of mother:

(Code: 1.Mother taken from Centre B. Private Enterpretnar ,3. Self production)

5.4..Amount of mother per spawn packet

(Code: 1.30 g B. 40g ,3. 50 g)

5.5. Size of Spawn packet

(Code: 1.1Kg B. 2 Kg 3. 3Kg)

5.6. Distance of inoculation chamber from sterilization chamber

(Code: 1.Zero distant 2. 1-5 meter D. 6-10 meter E. More than 10 meter)

6. **Information about mushroom yield**

6.1.How many times harvesting can be done in Summer?

(Code: 1=2 times, 2=3times, 3-=4 times, 4=5 times or more)

6.2.How many times harvesting can be done in Winter ?

(Code: 1=2 times, 2=3times, 3-=4 times, 4=5 times or more)

6.3.Total weight of fruiting bodies/ spawn packets.

1. 100-150g 2. 200-250g 3. 300-350g 4. 400-450g

7. Information about mushroom contamination/ Competitor moulds

7.1. Number estimated infected spawn packets from hundred spawn packets.

7.2. Status of infested spawn packets

Colour from the outside: (Code: 1=Black, 2= Yellow, 3= Green, 4.Others)

7.3.Status of infected fruiting bodies

7.4.Twisted 2. Cracked 3. Black 4. Brown 5. Small Size 6. Less Number

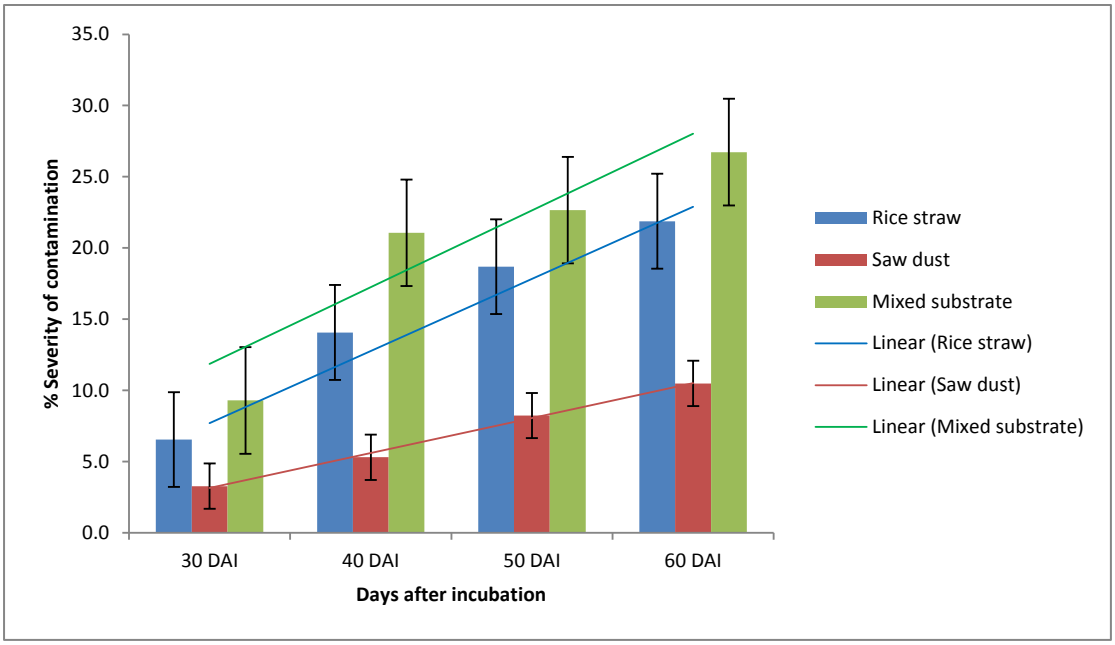
7.5.. Name of competitors:

(Code: 1=*Trichoderma, 2= Aspergillus, 3=Penicillium,* 4=Unknown)

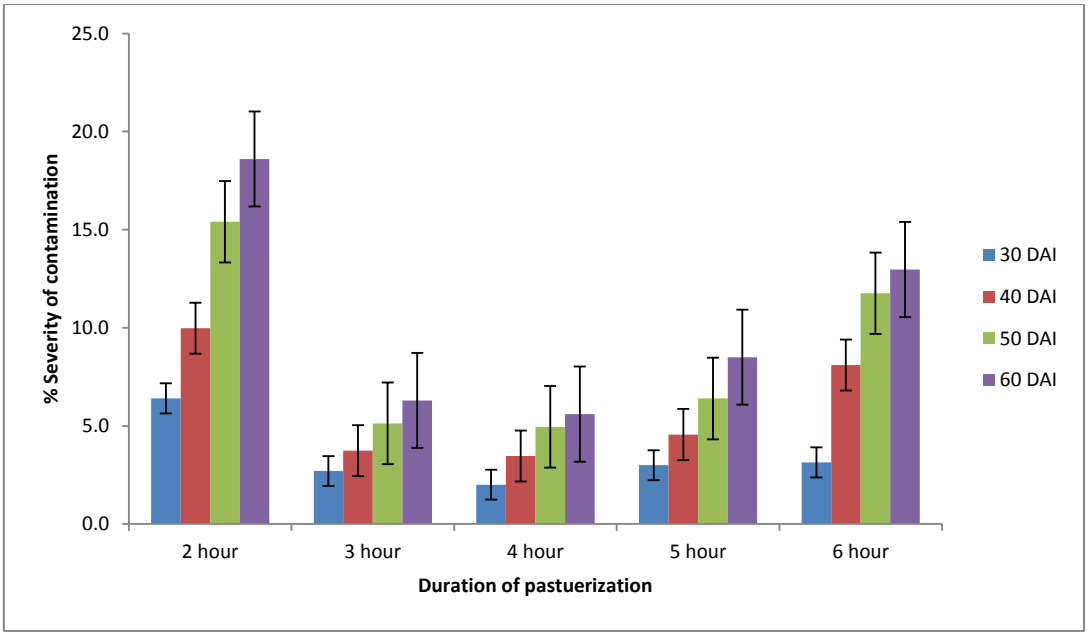
Name of informer: **Signature and date:**

Name of the supervisor: **Signature and date:**

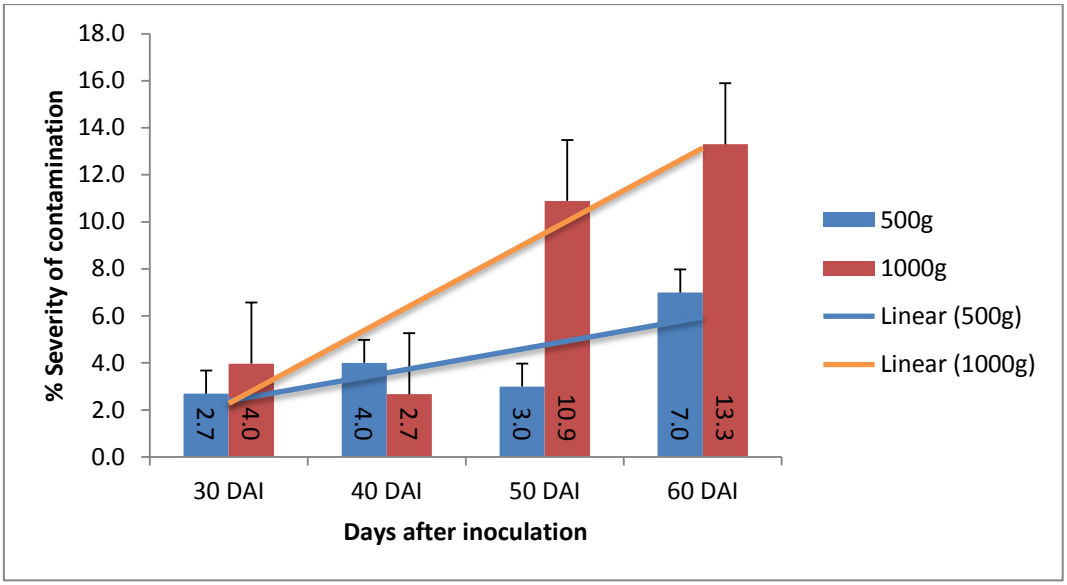
(THANKS FOR CO-OPERATION)



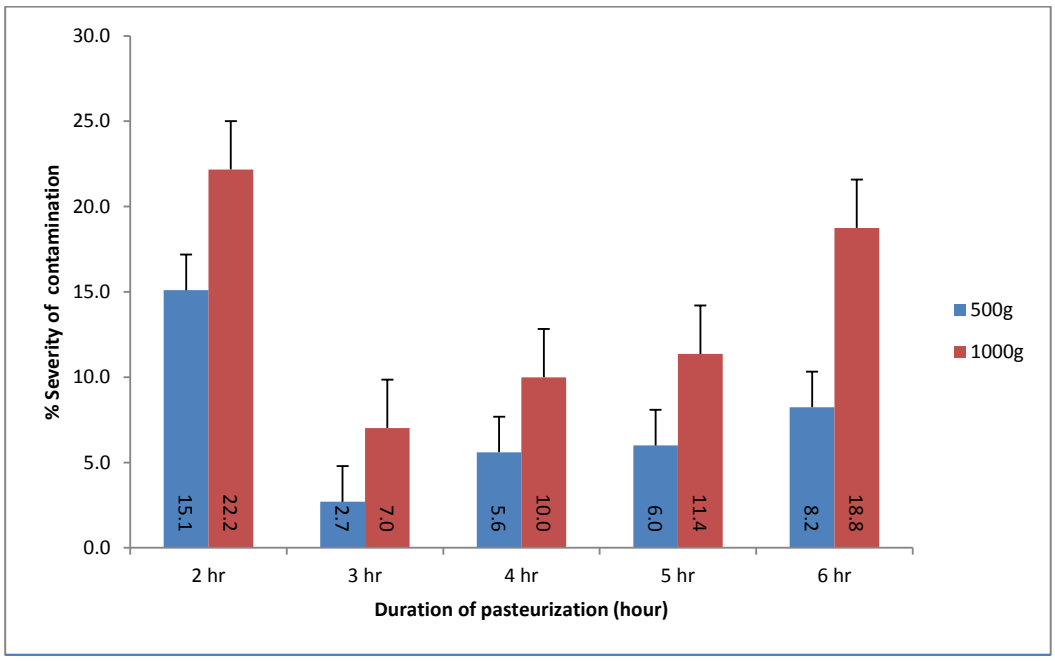
Appendix 2. Severity of contamination in substrate packets prepared with three substrates as recorded at 30-60 days after incubation with 10 days interval



Appendix 3. Effect of duration of pasteurization of substrates with steam on contamination severity of in substrate packets recorded up to 60 days after inoculation with 10 days interval



Appendix 4. Severity of mycoflora contamination in 500g and 1000g substrate packetsrecorded at 30-60 days after incubation with 10 days interval



Appendix 5. Interaction effect of bag size (500 and 1000g substrate) and pasteurization duration on severity of contamination in packet 60 DAI

Appendix 6. Analysis of variance for the percentage of contamination as influenced by concentration and methods of micronutrient enrichment

Source of variations	Degrees of freedom	Mycelium growth rate	Number of primordia	Number of effective fruiting bodies	Total harvesting
Factor A (Treatment)	7	201.327**	23.698**	52.041**	2699.25**
Factor B (Methods)	1	1.513ns	0.313ns	23.112**	1445**
AB (Treat × Methods)	7	4.112**	0.427*	35.684**	90.600**
Error	64	1.444	0.291	1.144	11.494

Appendix 7. Analysis of variance for the yield attributes as influenced by micronutrient enrichment

Source of variations	Degrees of freedom	Dry weight	Economic yield	Dry yield	Biological efficiency
Factor A (Treatment)	7	2869.713**	47384.429**	2772.25**	2021.837**
Factor B (Methods)	1	159.613**	54184.05**	428.83**	2184.05**
AB (Treat × Med)	7	264.727**	8052.964**	118.089**	353.797**
Error	64	6.706	839.538	4.907	36.174

Appendix 8. Analysis of variance for biological yield as influenced by micronutrient enrichment

Analysis of variance	Degrees of freedom	1st flush	2nd flush	3rdflush	4thflush	5thflush	Total
Rep	2	29.338	26.645	16.188	8.080	6.480	103.824
Treat	7	1081.9	649.5	324.4	340.2	340.9	40755.6
Error	14	0.301	0.216	0.088	0.105	0.107	1.477
Non-additivity	1	2.892	2.591	0.685	1.297	1.276	15.903
Residual	13	0.102	0.033	0.042	0.013	0.017	0.368
Total	23	1111.636	676.367	340.717	348.427	347.464	40860.9

Appendix 9. Analysis of variance for dimension of fruiting body as effected by micronutrient enrichment of substrates of oyster mushroom

Source of variations	Degrees of freedom	Number of primordia	Pileus diameter	Stipe Length	Weight of fruiting body	Number of flush
Factor A (Treatment)	7	6071.655**	4.768**	7.488**	6.245**	9.784**
Factor B (Methods)	1	52685.113**	0.112ns	0.006ns	0.198**	0.012ns
AB (Treat × Med)	7	3292.255**	0.723**	0.102**	0.833**	0.013ns
Error	64	43.438	0.081	0.014	0.017	0.077

Appendix 10. Mean square of minerals influenced by micronutrient enrichment

Analysis of variance	Degrees of freedom	K (%)	P (mg/g)	Zn (mg%)	Fe (mg/g)	Cu (mg/g)
Rep	2	1.140	0.130	15.180	37.498	14.770
Treat	7	0.906**	0.420**	6.294**	148.5**	61.84**
Error	14	0.002	0.001	0.002	0.367	0.009
Non-additivity	1	0.001	0.000	0.002	0.224	0.008
Residual	13	0.002	0.001	0.002	0.378	0.009
Total	23	1.145	0.132	15.186	38.467	14.796

Appendix 11. Effect of incidence of contamination of spawn packets as substrate enriched by different chemicals

Treatments	Incubation Period	First Harvest	Second harvest	Third harvest	Fourth harvest	Fifth Harvest	Total (%)
T_1	10%	-	-	-	-	10%	20
T_2	10%	-	-	-	10%		20
T_3	-	-	-	-	-	10%	10
T_4	-	-		-	-		0
T_5	-	-	-	10%		10%	20
T_6	-	-				30%	30
T_7	-	-		10%	-	-	10
T_0	60%	-	30%	-	-	-	90

T_1=0.1 % Agrovit Plus, T_2=0.2 % Agrovit Plus, T_3=0.3 % Agrovit Plus, T_4=0.4 % Agrovit Plus T_5=0.5 % Agrovit Plus, T_6=boron,T_7= Treatment with 10 ppm $Mncl_2$ treated, T_0= untreated (Control).

Appendix 12. Mean square Contamination as influenced by steam pasteurization

Source of variation	Degrees of freedom	Contamination			Dry yield	Economic yield
		1st	2nd	3rd		
Rep	4	1.155	1.242	10.056	6.299	5.212
Treatment	6	1184.53**	1943.93**	3886.44**	855.131**	2791.08**
Error	24	0.302	0.213	0.273	0.281	0.409
Non-additivity	1	6.098	4.726	6.042	5.932	5.742
Residual	23	0.05	0.017	0.023	0.036	0.177

Appendix 13. Contamination severity of spawn packets of oyster mushroom (*Pleurotus ostreatus*) pasteurization of substrate through steam

Treatments	Contamination severity (%)		
	First flush	Second flush	Third flush
T_1	20.00 b	30.00 b	42.00 b
T_2	0.00 d	0.000 d	10.00 g
T_3	0.00 d	0.000 d	16.00 e
T_4	5.00 c	10.00 c	22.20 d
T_5	0.00 d	0.000 d	26.00 c
T_6	0.00 d	10.00 c	15.00 f
T_0	40.00 a	52.20 a	90.34 a
$LSD_{(0.05)}$	**0.717**	**0.602**	**0.682**
CV (%)	**5.92**	**3.16**	**1.65**
Level of significance	**	**	**

Appendix 14. Mean square for duration from mycelium colonization to final harvest as influenced by steam pasteurization

Source of variation	Degrees of freedom	Mycelium run rate	Days required for Colonization	Days to primordia formation	Primordia to 1st harvest	Total harvest day
Rep	4	0.451	18.744	5.626	4.607	20.221
Treatment	6	0.329**	257.39**	28.066**	31.215**	612.381**
Error	24	0.007	0.437	0.113	0.076	0.134
Non-additivity	1	0.137	7.732	2.448	1.378	2.288
Residual	23	0.001	0.12	0.012	0.019	0.04

Appendix 15. Mean square for Dimension of fruiting bodies as influenced by Steam pasteurization

Source of variation	Degrees of freedom	Number primordia	Number of effective fruiting bodies	Length of stipe	Weight of fruiting body	Number of flush
Rep	4	43.291	13.894	3.002	4.794	3.739
Treatment	6	3733.86**	545.562**	3.567**	21.869**	10.714**
Error	24	0.62	0.077	0.016	0.054	0.029
Non-additivity	1	8.912	1.605	0.332	0.952	0.629
Residual	23	0.259	0.011	0.002	0.015	0.003

Appendix 16. Mean square for yield attributes as influenced by steam pasteurization

Source of variation	Degrees of freedom	Diameter of pileus	Biological efficiency	Dry weight	Economic yield
Rep	4	3.763	24.998	20.401	99.297
Treatment	6	6.883**	2178.82**	1596.800**	47683.60**
Error	24	0.005	0.229	0.43	0.885
Non-additivity	1	0.091	3.321	9.004	17.612
Residual	23	0.001	0.094	0.057	0.157

T_1=Steam for two hour, T_2=Steam for three hour, T_3= steam for four hour T_4=Steam for five hour, T_5=steam for six hour, T_6 = Autoclaved sterilization and T_0= control.

Appendix 17. Analysis of variance for the percentage of yield attributes as influenced by hot water treatment

Source of variations	Degrees of freedom	Mycelium growth rate	Number of primordia	Number of effective fruiting bodies	Weight of fruiting body
Factor A (Temp.)	3	0.676**	22404.105**	2894.067**	99.4**
Factor B (Time)	2	0.008ns	329.4**	148.617**	10.175**
AB (Time × Temp)	6	0.059**	1872.755**	374.55**	9.813**
Error	48	**0.003**	**28.211**	**4.272**	**0.237**

Appendix 18. Analysis of variance for the days required for mycelium colonization to final harvesting as effected by hot water treatment

		Days required for			
Source of variations	**Degrees of freedom**	**Mycelium colonization**	**Primordia formation**	**First Harvesting**	**Total harvesting**
Factor A (Temp.)	3	629.706**	25.000**	22.523**	3109.444**
Factor B (Time)	2	331.817**	62.55**	58.414**	125.417**
AB (Time × Temp)	6	455.039**	70.950**	59.769**	396.528**
Error	48	**2.527**	**0.853**	**0.850**	**7.998**

Appendix 9. Analysis of variance for the biological yields influenced by hot water treatment

Source of variations	**Degrees of freedom**	**Biological Yield**			
		1st	**2nd**	**3rd**	**4th**
Factor A (Temp.)	3	20156.772**	8498.195**	3585.794**	1186.396**
Factor B (Time)	2	471.817**	359.450**	237.650**	177.388**
AB (Time × Temp)	6	1808.239**	1473.161**	472.894**	348.477**
Error	48	23.191	6.999	0.746	0.376

Appendix 20. Analysis of variance for the percentage of contamination as influenced by hot water treatments

Source of variations	**Degrees of freedom**	**Contamination (%)**		
		1st flush	**2nd flush**	**3rd flush**
Factor A (Temp.)	3	13.236**	20.184**	93.873**
Factor B (Time)	2	38.962**	43.541**	64.838**
AB (Time × Temp)	6	22.821**	41.492**	43.636**
Error	48	0.010	0.009	0.288

Appendix 21. Analysis of variance for different kinds of yield as influenced by hot water treatment

Source of variations	Degrees of freedom	Biological Efficiency	Economic yield	Dry weight
Factor A (Temp.)	3	5526.622**	131387.095**	1886.283**
Factor B (Time)	2	100.068**	2526.080**	39.017**
AB (Time × Temp)	6	397.882**	9438.731**	189.217**
Error	48	**5.457**	**91.080**	**2.441**

Appendix 22. Analysis of variance for the dimension of fruiting bodies as influenced by interaction effect of temperature and time of hot water treatment

Source of variations	Degrees of freedom	Pileus diameter	Stipe length	Number of flush
Factor A (Temp.)	3	40.272**	20.450**	47.350**
Factor B (Time)	2	1.631**	2.909**	1.350**
AB (Time × Temp)	6	4.324**	2.216**	4.017**
Error	48	**0.061**	**0.059**	**0.051**

Appendix 23. Different stages of mushroom growth

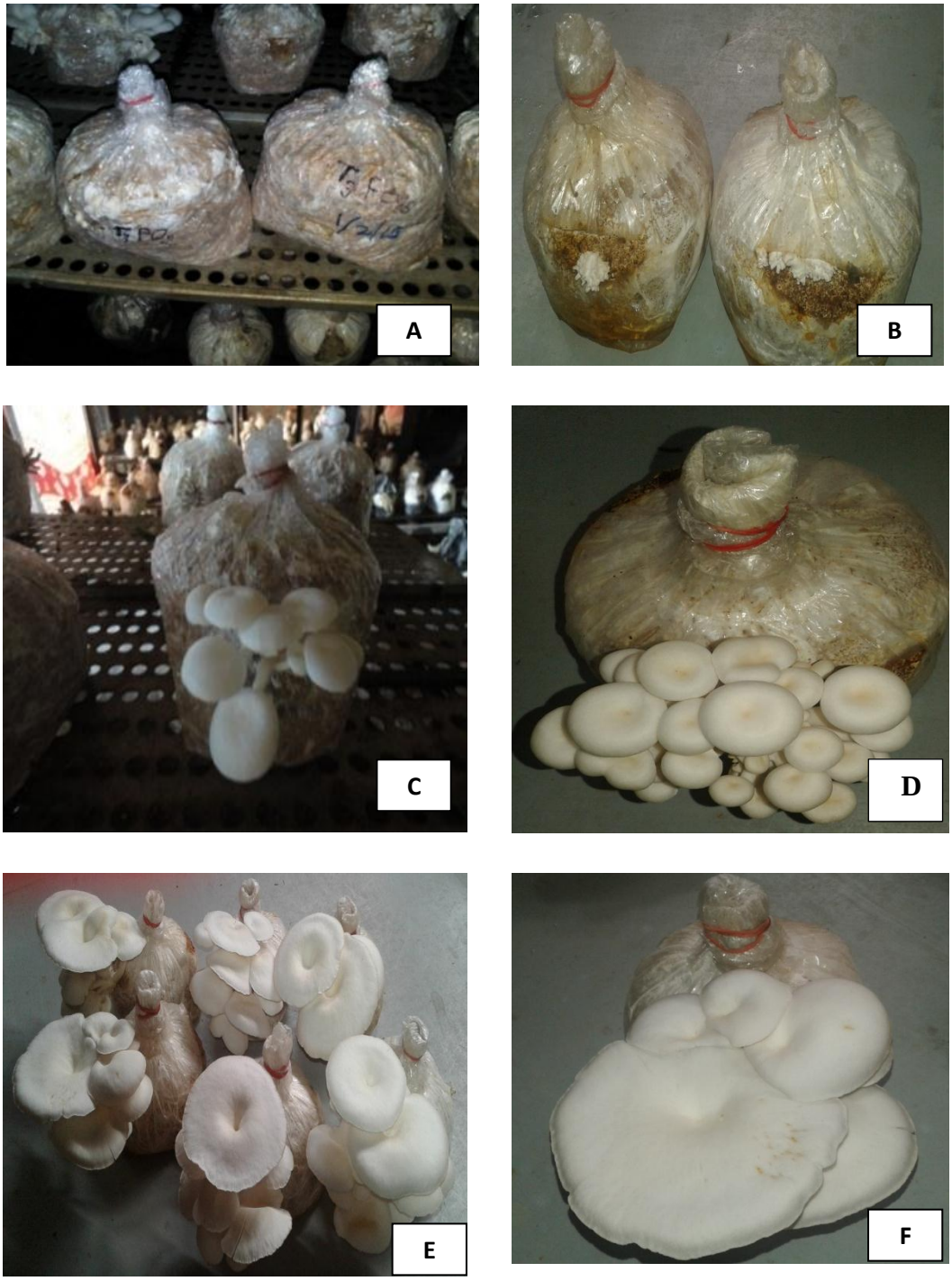


Plate 1 A., Mycelium colonization B. primordia formation; C-D. Spawn packets of *P. ostreatus* with young fruiting bodies; E-F. matured fruiting bodies

Appendix 24. Association of fungi in different substrates during 30-60 days after incubation

Plate 2. Association of fungi in substrates after incubation A. 30 DAI, B. 40 DAI, C. 50 DAI, D. 60 DAI (**DAI**-days after incubation)

Appendix 25. Different experimental view


Figure 3. Cultivation period of oyster mushroom Spawn packets enriched with micronutrients


Figure 4. Cultivation period of oyster mushroom Spawn packets treated by chemicals


Figure 5. Cultivation period of oyster mushroom Spawn packets treated by hot water