MICROBIAL CONTAMINATION IN OYSTER MUSHROOM (*Pleurotus ostreatus*) AND THEIR MANAGEMENT USING DIFFERENT CHEMICALS

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CERTIFICATE

*This is to certify that the thesis entitled '**Microbial Contamination in Oyster Mushroom (Pleurotus ostreatus) and their Management Using Different Chemicals**' submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in PLANT PATHOLOGY**, embodies the results of a piece of bona fide research work carried out by **FERDAOUS JAHAN URMI**, Registration No. **17-08237** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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DEDICATED TO

MY

BELOVED PARENTS, MY

FAMILY & SIBLINGS

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The Author

Microbial contamination in oyster mushroom (*Pleurotus ostreatus*) and their management using different chemicals

BY

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ABSTRACT

An experiment was carried out to find out the effect of different chemicals treated rice straw on the growth and yield of oyster mushroom (*Pleurotus ostreatus*) and to isolate contaminants associated with *Pleurotus ostreatus.* Total 5 fungi namely *Sclerotium rolfsii*, *Trichoderma harzianum*, *Fusarium oxysporum, penicillium sp. and Aspergillus niger* were isolated and identified. Three different concentrations namely 10000, 20000, 30000 ppm of hydrogen peroxide, 100 ppm of mancozeb, 100 ppm of mancozeb+metalaxyl, 100 ppm of household bleaching powder and 10000 ppm wood ash were used to manage the contaminated fungi. Among the tested chemicals, higher inhibition of radial mycelial growth was observed with 30000 ppm hydrogen peroxide followed by Mancozeb+Metalaxyl (100 ppm). The maximum days (22.20 days) required for mycelium running was recorded from control whereas the minimum days (16.80 days) found under 30000 ppm hydrogen peroxide. The maximum days (6.60 days) required for primodia initiation to 1st harvest was observed from control whereas lowest days (3.80 days) was observed under 30000 ppm hydrogen peroxide. 30000 ppm hydrogen peroxide showed significantly best performance with the total number of fruiting body and yield per spawn packets. The highest length (5.17 cm) of stipe was recorded from 30000 ppm hydrogen peroxide. Number of fruiting body/packet of oyster mushroom showed statistically significant differences due to use different chemicals for substrate sterilization. The maximum weight (4.54 g) of individual fruiting body was recorded from 30000 ppm hydrogen peroxide which was closely (4.06 g) followed by 20000 ppm hydrogen peroxide and the minimum weight (2.36 g) was found in control. Inhibitory effect against the growth competitor moulds ranged between 72.41-93.46%, maximum yield (202.38 g) and maximum biological efficiency (40.48%) obtained from 30000 ppm Hydrogen peroxide treated packets. Percent contamination of fungi gradually increased from 1st stage to 3rd flash stage. Maximum severity of contamination 68% was observed in control and 36% in ash treated substrate at 3rd harvest. In other cases the spawn packets were free from contamination when the substrate was treated with 30,000 ppm hydrogen peroxide and 100 ppm mancozeb+metalaxyl.

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LIST OF SYMBOL AND ABBREVIATIONS

ABBREVIATION	FULL WORDS
%	Percentage
PO	*Pleurotus ostreatus*
PDA	Potato Dextrose Agar
POP	Pink oyster
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
@	At the rate of
ml	Milliliter
CRD	Complete Randomized Design
BE	Biological Efficiency
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

CHAPTER I

INTRODUCTION

Mushrooms are large reproductive structure of edible fungi belonging to Basidiomycotina. They are non-green fungal plants arising seasonally all over the world in various habitats varying from sandy plains to thick forests or green meadows to roadside pathways. They construct a large heterogeneous group having various shapes, sizes, colors, appearance and edibility (Chang and Miles, 1988). There are about 41,000 different types of mushrooms are identified (Manoharachary *et al*., 2005). Among them which are fully edible and have no toxic effect are to be considered as edible mushroom. Out of 2000 species of prime edible mushrooms about 80 have been grown experimentally, 20 cultivated commercially and 4-5 produced on industrial scale throughout the world (Chang and Miles, 1988). The edible mushrooms are grown in controlled, clean environment where there is no infection of other fungi or unhealthy germs.

Mushroom in Bangladesh is a relatively new enterprise and gradually being incorporated into the existing food habit of the people in our country. Even though mushroom industry is gradually enlarging in Bangladesh but the movement is relatively slow because of inadequate scientific support and lack of training programs. On the other hand, crop diversification and changes food habit is an urgent need to build up our national health. Mushroom cultivation does not compete with other crops, can be grown in homestead with the active participation of family members. The agricultural wastes can be used as substrates. Thus it can be produced in large quantity within a short time. It provides more protein per unit area and can serves as income generating source in unemployed sector.

A number of local edible mushrooms are being produced in hilly areas of our country from ancient time. The Department of Agricultural Extension (DAE) has started mushroom production since 1979 with technical assistant of Japan overseas corporation volunteers (JOCV). In early 1980s commercial mushroom cultivation was initiated by Bangladesh Agricultural Research Council and then a mushroom culture centre at Savar.

From the various kinds, oyster mushroom scientifically known as *Pleurotus ostreatus is* most suited to the climatic condition of Bangladesh. The common name "oyster mushroom" comes from the white shell-like appearance of the fruiting body. The oyster mushrooms have three

distinct parts- a fleshy shell or spatula shaped cap (pileus), a short or long lateral or central stalk called stipe and long ridges and furrows underneath the pileus called gills or lamellae.

The popularity of oyster mushroom has been increasing due to its ease of cultivation, high yield potential and high nutritional value (Banik and Nandi, 2004). Mushrooms are now-a-days popularly known as functional foods (Liu *et al*., 2009). Edible mushrooms have been treated as important tool in modern medicine for their medicinal values (Kovfeen, 2004). Mushroom reduces the diabetic on regular feeding. It also reduces the serum cholesterol in human bodies which reduces hypertension (Gregori *et al*., 2007). Kovfeen (2004) reported that the fresh mushroom contains about 85-90% moisture, 3% protein, 4% carbohydrates, 0.3-0.4% fats and 1% minerals and vitamins. It is the source of Niacin (0.3 g) and Riboflavin (0.4 mg). Mushroom is also a good source of trypsin enzyme. It is rich of low in calories, riches in vegetable proteins, iron, copper, calcium, potassium, zinc, chitin fibre and also contains vitamin D and folic acid (Alam *et al*., 2007, Banik and Nandi, 2004). It inhibits the growth of tumor and cancer (Mori *et al.,* 1986). *Pleurotus* species are very much effective in reducing the harmful body plasma lipids (Alam *et al*., 2007).

Oyster mushroom can grow on sawdust, rice and wheat straw and other agro-waste. Sarker *et al*., (2007a) noticed a remarkable variation in nutritional content of oyster mushroom in different substrates. Amin (2004) reported that the highest mycelium growth would be achieved in using wheat substrate and the highest yield in using sterilized rice straw. A huge amount of rice straw is produced in Bangladesh annually. If we use a small part of this for oyster mushroom production, then we can produce notable amount of mushroom.

During oyster mushroom cultivation, mushroom growers facing various problems especially competitor moulds damage the mushroom beds and reduce yield. Studies on various aspects of fungal contaminants and diseases of *Pleurotus ostreatus* were undertaken by various workers (Castle *et al*., 1998; Mamoun *et al*., 2000) and they reported *Trichoderma harzianum*, *Aspergillus* sp., *Penicillium* sp., *Monilia sitophila*, *Stemonitis* sp. and *Coprinus* sp. were the major contaminants of *Pleurotus* sp. Different saprophytic and plant pathogenic fungi occurring in the substrate and competing with mushroom mycelium for space and nutrition are *Aspergillus niger*, *A. flavus*, *Alternaria alternata*, *Drechslera bicolor*, *Fusarium moniliforme*, *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Rhizpus stolonifer*, *Sclerotium rolfsii*, *Trichoderma viride* (Sharma *et al*., 2007). Spillman (2002) recognized *Trichoderma* as green

mould on the production bed of oyster mushroom. *Trichoderma*, *Aspergillus* and *Rhizopus* on oyster mushroom bed were predominant microorganisms.

Considering the above discussions, an experiment was conducted to develop a suitable management practice against the competitor moulds of substrate of *Pleurotus ostreatus* using different chemicals. To overcome the dissonance among the effect of chemicals and their concentrations, suitable chemicals at optimum concentration is needed to control different contaminants associated with oyster mushroom to increase the growth and yield. With this background and situation the present study was conducted for fulfilling the following objectives:

- To isolate and identify the organisms from contaminated spawn of oyster mushroom
- To estimate the severity of spawn contamination in oyster mushroom cultivation
- To evaluate the efficacy of different chemicals under *in- vitro* and *in-vivo* condition for management of the contaminants of spawn of oyster mushroom

CHAPTER II

REVIEW OF LITERATURE

Among the several constraints responsible for low productivity of mushroom, the diseases are one of them, causing serious losses by reducing the yield in terms of quality and/or quantity. Like other crops, mushroom is also attacked by many diseases right from spawn preparation to maturity. A range of fungi, bacteria and viruses are pathogenic to mushrooms. Mushrooms become contaminated from many sources during production and processing, including the humans harvesting the crop. Bacteria, yeasts and moulds cause most problems. Mushrooms have soft flesh which is easily bruised, helping bacteria such as Pseudomonas species to spread. These cause brown blotches on the caps. Other microbes induce shape distortions. Mushrooms get spoiled quickly if kept at room temperature for long. For successful management of the spawn infecting pathogens, chemicals and botanicals are used. But research works related to successful management of the spawn infecting pathogens are limited in Bangladesh as well as world context. However, some of the important and informative works and research findings related to the isolation and identification the pathogens infecting spawn of mushroom, estimation of disease incidence and severity and successful management of the spawn infecting pathogens so far been done at home and abroad have been reviewed in this chapter under the following headings-

2.1. Oyster mushroom

According to Chowdhury *et al.,* (2011) 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 on 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 5 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 0C, 70-80% RH). The production was found minimum during the cultivated time August to October.

Kim *et al*., (2002) and Rosado *et al*., (2003) reported 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.

Gupta (1989) observed 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.

2.2. Effect of substrate on mushroom production

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.

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 7 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.

Siqueira *et al.,* (2011) used 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.

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*., (2007) conducted 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 8 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) noticed 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.

According to Namdev *et al.,* (2006) 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 observed 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 an experiment 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.*

Iqbal *et al.,* (2005) demonstrated 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.

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 (r2 = 0.6). Lignin (r2 =0.7) and fiber (r2 = 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.

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.

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.

2.3 Prevalence of contamination in mushroom

Kumar and Sarathi (2017) carried out a survey ten home scale mushroom farms of Thanjavur its nearby areas, Tamilnadu, India and survey revealed that the occurrence of eight contaminants in mushroom beds and out of which *Trichoderma viride*, *Aspergillus niger*, *Coprinus* sp. were found to be the dominant fungal contaminants and occurrence was high during may to July (23.5-26.7%) causing maximum loss to mushroom yield. The incidence of contaminants were minimum during December and January (3.60%) and maximum during the month of May (26.5%). A good harvest of mushroom (107% Biological Efficiency) was obtained during the month of October. A range of average maximum temperature (23.5-34.6^{0}C), minimum temperature (13.4-24.2^{0}C) was found most appropriate for the cultivation of oyster mushroom in this region.

Shamoli *et al.,* (2016) conducted an experiment to find out the fungal competitors and symptom studies in damaged Oyster Mushroom spawn packets at National Mushroom Development and Extension Center, Savar, Dhaka, Bangladesh. A total of nine fungal competitors of oyster mushroom were isolated and identified namely- *Trichoderma harzianum Rifai*, *T. viride* Pers. (Green strain), *T. viride* Pers. (Yellow strain), *T. koningii* Oudem, *Mucor hiemalis* Wehmer, *Papulaspora byssina* Hotson, *Neurospora* sp. Shear and

B.O. Dodge., *Aspergillus flavus* Link., and *Botryodiplodia theobromae* Pat. on the basis of microscopic, morphological and cultural characteristics. To produce oyster mushroom in an eco-friendly manner and to find out their antifungal potency, 23 plant species belonging to 19 families were screened out against isolated nine fungal competitors of oyster mushroom. Among 23 extracts, the maximum (44%) mycelial inhibition of *T. harzianum* was found due to *Aegle marmelos* whereas *Eclipta alba* showed the highest mycelial inhibition (62%) of *T. viride* (Green strain); in case of *T. viride* (Yellow strain), *Cassia tora* exhibited the highest mycelial inhibition (39%); *Diospyros cordifolia* showed the maximum mycelial inhibition (48%) of *T. koningii*; *Curcuma longa* (rhizome) gave the maximum mycelial inhibition (90%) of *Neurospora* sp.

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.

Kim *et al*., (2013) showed that fungal pathogens caused severe damage to the commercial production of *P. eryngii*. Four strains of pathogenic fungi, including *T. koningiopsis* DC3, *Phomopsis* sp. MP4, *Mucor circinelloides* MP5, and *Cladosporium bruhnei* MP6, were isolated from the bottle culture of infected *P. eryngii*.

Shah *et al*., (2011) examined green mold infecting substrate in poly bag and spawn bottles of *P. sajor-caju* and found that the fungus causing green mold was identified as *T. harzianum*.

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*).

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.

Young *et al*., (2010) studied that *Agrocybe aegerita* can be cultivated throughout the year using culture bottles but it was more susceptible to contamination than other mushrooms. They isolated 22 pathogens from the fruiting bodies and compost of *A. aegerita* and 7 isolates from *P. ostreatus* and found that among the 29 isolates, 26 were identified as *Trichoderma* sp. and the remaining three were *Aspergillus* spp., *Mucor* sp., and *Penicillium* sp.

Mazumder *et al*., (2005) observed month-wise variation in spawn contaminations caused by various fungal and bacterial contaminants and isolated and identified eight fungal and one bacterial contaminants from naturally contaminated spawn of oyster mushroom. They were *A. flavus* var. columneris, *A. niger*, *Alternaria alternata*, *Penicillium janthinellum*, *Penicillium* sp., *Rhizopus stotonifer*, *T. harzianum*, *T. viride* and *Bacillus brevis*.

Yu (2002) examined the cultural and morphological characteristics of more than one hundred *Trichoderma* strains isolated from oyster mushroom substrate causing green mold disease and resulted that *T. viride* (13.6%), *T. harzianum* (8.2%) and *T. koningii* (5.5%) and the majority of the isolates (65.5%) belonged to an unidentified species of *Trichoderma* causing disease in mushrooms.

Thakur *et al*., (2001) stated that frequency and total number of mycoflora associated with paddy straw substrate during *pleurotus florida* cultivation was three fold higher on untreated straw substrate as compared to chemically treated paddy straw substrate. A sum of 12 fungal species belonging to 7 genera were associated with untreated paddy straw compared to only 8 fungal species belonging to 5 genera with treated paddy straw substrate. Among the isolated fungi, *Aspergillus flavus*, *Rhizopus* sp, *A. niger* and *Trichoderma* sp. were most predominant with untreated and treated straw substrate.

Wickremasinghe (1999) showed that frequency of contaminants, A. fumigates and T. harzianum occurrence was 100% irrespective of the stage of processing of straw and oyster compost.

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%.

Castle *et al*., (1998) found that *Trichoderma* species are common contaminants of spawn, compost, and wood. They analyzed 160 isolates of *Trichoderma* from mushroom farms based on morphological, cultural, and molecular characteristics and it was identified as a strain of *T. harzianum*.

Liao (1993) studied that microorganisms caused contamination in the process of cultivation and their effect on the production of shiitake and found that species of *Trichoderma*, *Penicillium*, *Neurospora*, and *Aspergillus* were the most frequently isolated fungi from the saw dust spawn, while *Trichoderma*, *Bacillus* and *Aspergillus* were often isolated in the wheat grain spawn.

2.4 Management the contaminated organisms

Kumar and Sarathi (2017) carried out a survey ten home scale mushroom farms of Thanjavur its nearby areas, Tamilnadu, India and survey revealed that the occurrence of eight contaminants in mushroom beds and out of which *Trichoderma viride*, *Aspergillus niger*, *Coprinus* sp. were found to be the dominant fungal contaminants. Among the chemicals tested for management of competitors moulds *Carbendazim* 50% WP + formalin 0.01g+0.15 ml/100 ml showed its supremacy and exhibited maximum inhibitory effect (44.1 to 61.6%) against *Aspergillus* sp., *Trichoderma* sp., *Coprinus* sp. and *Penicillium* sp. and was found to be less effective against *Sclerotium rolfsii* in vitro.

Shamoli *et al.,* (2016) conducted an experiment to find out the fungal competitors and symptom studies in damaged Oyster Mushroom spawn packets at National Mushroom Development and Extension Center, Savar, Dhaka, Bangladesh. A total of nine fungal competitors of oyster mushroom were isolated and identified namely- *Trichoderma harzianum Rifai*, *T. viride* Pers. (Green strain), *T. viride* Pers. (Yellow strain), *T. koningii* Oudem, *Mucor hiemalis* Wehmer, *Papulaspora byssina* Hotson, *Neurospora* sp. Shear and B.O. Dodge., *Aspergillus flavus* Link., and *Botryodiplodia theobromae* Pat. on the basis of

microscopic, morphological and cultural characteristics. To produce oyster mushroom in an eco-friendly manner and to find out their antifungal potency, 23 plant species belonging to 19 families were screened out against isolated nine fungal competitors of oyster mushroom. There were no significant effects found to control of *P. byssina*, *B. theobromae*, *M. hiemalis* and *A. flavus* due to 23 different types of botanicals tested. *Trichoderma harzianum*, *T. viride* (Green strain), *T. viride* (Yellow strain), *T. koningii*, *A. flavus*, *Neurospora* sp. and *P. byssina* was successfully inhibited by 30, 50 and 70 ppm of fungicide-Bavistin 50 WP but *B. theobromae* and *M. hiemalis* were not affected by Bavistin at mentioned concentration.

Oyelakin *et al*., (2014) examined inhibitory effect of the pesticides against the growth of the pathogenic fungi associated with cultures of the *P. tuber-regium*. *A. niger*, *A. flavus*, *Trichoderma* sp. and *Mucor* sp. were isolated from the mycelial culture plates of *P. tuber-regium* and found that mycelial growth of *A. niger* was effectively inhibited at 50, 100, 150, 200 and 250 ppm of copper sulphate, benlate, brestan and kocide, while the mycelial growth of *A. flavus* was effectively inhibited at 50, 100, 150, 200 and 250 ppm of benlate and brestan.

Paswan and Verma (2014) prepared method of management of fungal and bacterial contaminants affecting commonly grown mushroom viz. *Pleurotus* spp., *Hypsizygus ulmarius* and *Volvariella volvacea*. Due to development of fungicide resistant strains of *Trichoderma*, a neem based formulation viz. Mahaneem containing 0.15% *Azadirachtin* was evaluated and found effective.

Saxena *et al*., (2014) studied the in vitro effect of some commonly used fungicides, insecticides and herbicides on the mycelial growth of *T. harzianum* PBT 23. Benomyl, thiophanate methyl, bayleton and ipridione were found incompatible with the test organism even at 25 µg/ml. While mancozeb up to 250 µg /ml did not adversely affect the growth of *T. harzianum*.

Gangwar (2013) studied compatibility of fungal bioagent for bacterial leaf blight of rice with chemical pesticides, commonly used in rice cultivation and showed that mancozeb exhibited compatibility only on lower concentrations (500 and 250 ppm). Whereas, at higher concentration (1000 and 2000 ppm) gave cent per cent inhibition of *T. harzianum*.

Shah *et al*., (2013) examined in vitro efficacy of five fungicides carbendazim, bitertanol, hexaconazole, captan, and mancozeb against *Trichoderma* spp. and revealed that the

maximum average inhibition of *T. harzianum* was recorded in carbendazim (90.8%), followed by bitertanol (40.0%), captan (36.6%) and hexaconazole (16.1%). The least inhibition (11.7%) of *T. harzianum* was exhibited by mancozeb. It was further observed that carbendazim exhibited the least inhibition (24.9%) of *P. sajor- caju*, followed by captan (45.5%), bitertanol (63.0%) and hexaconazole (74.5%). The maximum inhibition (87.4%) of *P. sajor-caju* was exhibited by mancozeb.

Onyeani (2012) found the association of fungal species with the storage of four staple food crops and its management by using some indigenous plant extracts under in vitro conditions. *A. flavus*, *A. niger*, *Penicillium expansum* and *Rhizopus stolonifer* were found associated with the spoilage of horticultural crops in storage. The results of the investigation revealed that, *Acalypha ciliata*, *Aloe vera*, *A. indica* and *Vernonia amygdalina* were effective in the inhibition of *A. flavus* and *P. expansum*. *Annona squamosa* effectively inhibited the mycelial growth of *A. niger* and *R. stolonifer*. Aloe vera extract was equally effective in the reduction of mycelial growth of *A. niger*.

Pervez *et al*., (2012) revealed that botanicals *Allium cepa* (34.85%), *A. sativum* (28.95%), *Curcuma longa* (24.1%), *Aloe vera* (24.7%), *A. indica* (47.75%), *Lantana camara* (51.25%), *O. sanctum* (17.2%) and *D. stramonium* (19.7%) showed significant reduction of the pathogen *Trichoderma* and minimum inhibition of the mushroom under in vitro conditions.

Verma and Ratnoo (2012) tested the different fungicides against green mould pathogen (*T. harzianum*). Thiophanate methyl (75 ppm) was found the best with less affecting the growth of *A. bisporus* in in vitro condition. Further, combination of thiophanate-methyl + copper-oxychloride each at 50 ppm was found best for inhibiting the growth of *T. harzianum*, followed by carbendazim 50 WP+copperoxychloride (each at 100 ppm). They also observed that Garlic extract (10 g/100 ml) gave best results in inhibiting the growth of *T. harzianum* followed by Garlic extract (7.5 g/100 ml) and *Azadiractin* (0.75 ml/100 ml), and both the treatments did not inhibit the growth of *A. bisporus*.

Shah *et al*., (2011) revealed that in oyster mushroom the pathogen *T. harzianum* can be effectively controlled by botanicals *A. indica* (34.1%), *Allium sativum* (28.4%), *Artemesia indica* (21.8%), *Urtica dioeca* (22.2%), *Lycopersicon esculentum* (17.4%), *D. stramonium* (17.6%), *Mentha spicata* (20.8%), and *Juglans regia* (51.9%) with minimum inhibition of the mushroom under in vitro conditions.

Wani and Nisha (2011) studied the effect of different concentrations of systemic and non-systemic fungicides evaluated for their effect on the inhibition of the mycelial growth, lesion diameter and disease severity of black mold (*A. niger*) rot of onion and showed that among systemic fungicides carbendazim at highest concentration (1000 ppm) brought about highest reduction in colony diameter (0 mm) followed by hexaconozole, (17mm), bitertanol (24.8mm) and myclobutanil (27mm). Whereas, among non-systemic fungicides the mancozeb was found highly effective in reducing the colony diameter (0mm) of A. niger followed by Zineb (6.00mm) and Captan (15mm), respectively over control.

Sheth and Patil (2010) revealed that carbendazim, benomyl and carbendazim (12%) + mancozeb (63%) (Sixer 75% WP) at both the concentrations (500 & 1000 ppm) completely inhibited the mycelial growth of *A. niger* infecting citrus fruits. The severity of *Aspergillus* fruit rot was significantly lowest in fruits treated with benomyl at 1000 ppm concentration both in pre-inoculation (6.75%) and postinoculation (8.25%) treatments.

Bagwan (2010) evaluated different phytoextracts against *Trichoderma*. Among the botanicals tested, 10% fresh leaf extract of karanj leaves (*Pongamea pinnata*) and cumin leaves inhibited 32.19% and 27.15% growth of *Trichoderma*, respectively as compared to control.

Sheth and Patil (2010) showed that leaf extracts (10%) of tulsi (*Ocimum sanctum* L.), datura (*Datura stramonium* L) and ginger (*Zingiber officinale* Rose) rhizome extract gave complete inhibition of *Aspergillus* fruit rot of lime (*Citrus aurantifolia*) under in vitro and in vivo conditions.

Pervez *et al*. (2009) conducted a laboratory trial with formalin, bavistin and combination of formalin and bavistin at three different concentrations against identified associated mycoflora of oyster mushroom substrates and showed that combination of formalin and bavistin at the highest concentration (500+75 ppm) was found to be the best in inhibiting the radial colony growth of all the identified fungi. At the lowest dose of bavistin (25 ppm) the highest inhibition of radial growth of R. stolonifer (84.00%) was observed followed by *T. harzianum* (70.66%).

Khan and Shahzad (2007) examined *Trichoderma* species viz., *T. harzianum*, *T. pseudokoningii*, *T. longibrachiatum*, *T. viride* for tolerance to fungicides. They were evaluated different fungicides viz., benomyl, Topsin- M, carbendazim and cuprocaffro at different concentrations i.e., 0, 1, 10, 100, 1000 and 10,000 ppm and found that Topsin-M and carbendazim were the most effective fungicides that inhibited the growth of Trichoderma

species even at low concentration. Topsin-M completely suppressed the growth of *T. harzianum* at 10ppm.

Narzari *et al*., (2007) showed that complete inhibition of *T. harzianum* was obtained by 0.4% extract of garlic while 0.2% extract of consumable and non-consumable garlic inhibited the green mould by 42.2 and 12.2 per cent in oyster mushroom.

Alawiye and Tosin (2006) *Azadirachta indica* and *Carica papaya* effectively reduced the mycelial growth of *A. niger* as compared to control. *A. indica* and *C. papaya* significantly inhibited the growth of *A. niger* and *A. flavus* and among them *A. indica* had higher inhibitory effect on *A. flavus* than *C. papaya*.

Raju and Naik (2006) revealed that significantly higher inhibition of A. niger (100%) in storage diseases of onion was observed in SAFF, benomyl and carbendazim which were at par with each other at 0.1 and 0.15% concentrations and were significantly superior over other treatments. The least inhibition of growth was recorded by thiophanate methyl (0%) at 0.05 per cent concentration.

Kumar *et al*., (2005) who reported that NSKE and nimbicidin effectively inhibited the mycelial growth of *A. flavus* on chilli fruits.

El-Katatny *et al*., (2004) showed that on PDA-benomyl plate's growth of *T. harzianum* was inhibited by 20 and 30 per cent at benomyl 1 and 2 µg/ml, respectively, and it was completely inhibited at 5 µg/ml.

Sangoyami (2004) reported that aqueous extract of garlic effectively inhibited mycelial growth, conidia, pycnidia and sclerotial production of *Botryodiplodia theobromae*, *A. niger*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Nattrassia mangifera* fungal pathogens of yam in storage.

Kumar *et al*., (2002) found that penicillin was most effective against Bacillus sp. In non-autoclaved conditions. Tetracycline controlled spawn rotting at a minimum concentration (400 ppm) followed by Chloramphenicol (500ppm). These antibiotics had no adverse effect on mycelial growth of mushroom.

Khattabi *et al*., (2001) studied the effect of fungicides on mycelial growth of isolates of *T. harzianum* and showed that the mycelial growth of all *T. harzianum* isolates was reduced by benomyl and oxyquinoleine, where the highest fungicide concentration caused the greatest

reduction in mycelial growth. At the highest concentrations, mycelial growth was completely inhibited. With benomyl, at the lowest concentration (5µg/ml) the percentage of inihibition was higher than 50% for all isolates of *T. harzianum*.

Kumar and Dubey (2001) showed that benomyl, carbendazim and thiophanate methyl inhibited cent per cent mycelial growth of *T. harzianum* at both the concentrations (0.025% and 0.05%).

Singh and Sharma (2001) revealed that thiophanatemethyl (10 µg/ ml) and mancozeb at (500 µg/ ml) inhibited the mycelial growth of *Mycogone perniciosa*, incident of wet bubble disease in white button mushroom under in vitro condition.

Mohammadi *et al*., (2000) studied the effects of different fungicides on cobweb disease of white button mushroom and found that benomyl, carbendazim, thiophanate methyl, zineb and mancozeb @ 200, 200, 250, 500 and 500 ppm, respectively, showed good fungal growth inhibition.

Viji *et al*., (1997) observed that the benzimidazole group of fungicides, carbendazim and benomyl were toxic to the antagonists *Gliocladium virens*, *Trichoderma longibrachiatum* and *T. harzianum*.

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CHAPTER III

MATERIALS AND METHODS

The experiment was carried out to find out the growth and yield of oyster mushroom (*Pleurotus ostreatus*) grown on paddy straw with different chemical treatments and an examination was made to isolate and identify different microorganisms associated with substrate colonized oyster mushroom and their control with different chemicals *in vitro*. This chapter deals with a brief description on location and design of experiment, treatments, preparation of substrates, preparation of packets, cultivation of spawn packets, collection of produced mushrooms, data recording and their analysis under the following headings and sub-headings.

3.1 Experiment site

The field experiment was conducted at Mushroom Culture House (MCH) of Sher-e-Bangla Agricultural University, Dhaka and in *vitro* experiment was done in Plant Pathology laboratory, Sher-e-Bangla Agricultural University, Dhaka for evaluating the efficacy of different chemicals.

3.2 Duration of the experiment

The experiment was carried out during the period from April to October, 2019

3.3 Collection of contaminated spawn packet

Contaminated spawn packets were collected from National Mushroom Development Institute (MDI), Savar and collected samples were preserved in the laboratory at $4^{o}C$. Isolation of contaminating microorganisms causing spoilage of spawn packets and fruiting bodies were done by the following appropriate methodology (Dhingara and Sinclair, 1995).

3.3.1 Composition and preparation of agar media

Ingredients	Amount (Per liter)
Potato slices :	200 gm
Dextrose :	20 gm
Agar :	20 gm
Water :	1 L

The glassware's *viz.,* petri plates, test tubes, conical flasks, measuring cylinders, glass rods were sterilized in electrical hot air oven at 160 ºC for an hour. 200 gm sliced, peeled potatoes were boiled in 1 liter distilled water to make potato infusion for 30 min. Potato infusion was filtering through sieve and dextrose, agar and water (if needed to fill 1 L) was mixed and boiled to dissolve. The mixture was sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. After autoclaving the media the conical flask are then taken into the laminar airflow chamber in order to avoid contamination. The laminar airflow chamber must be wiped thoroughly with cotton cloth dipped in 70% alcohol. So prepared agar media is then poured into the sterile petri plates at equal volumes. After the agar is poured into the sterile petri plates, it is allowed to cool down.

3.3.2 Isolation and purification of competitor moulds from collected spawn

10g samples were taken from the contaminated packets and mixed with 100 ml sterile distilled water. A series of dilutions were made by taking 1 ml from the stock solution to add with 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 (Dhingra and Sinclair 1985). From the each of the substrate dilutions 0.5 ml volumes were pipetted on PDA media and incubated at 27ºC (±2)ºC for 3-4 days. The pathogen grown as the mixed colony then individual culture plates of substrate samples were isolated. To prepare pure culture sufficient number of sub culturing were done by hyphal tip technique (Hyakumachi, 1994). All the pure cultures were kept in refrigerator at 4ºC for preservation.

3.3.3 Identification of pathogens

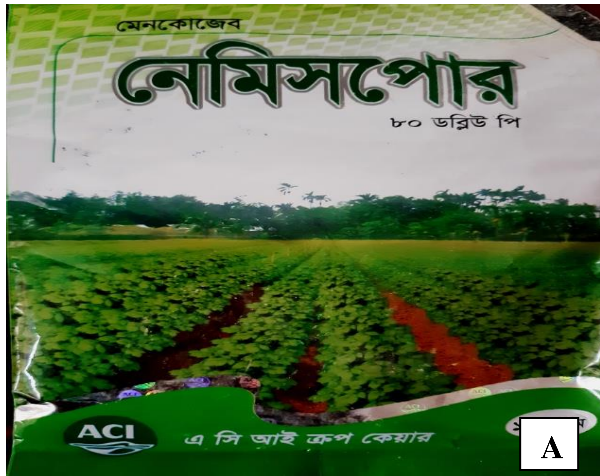
Identification of the pathogens was carried out by studying the cultural and morphological characters of the pathogen. The morphological characters were examined under low (10X) and higher (40X) power magnification from 10 days old culture of pathogens and were confirmed with those given in literature. The microphotograph of pathogens was also taken using microscope. The morphological characteristics of individual fungus were recorded and compared with appropriate key book like CMI description of fungi to identify each fungus (Barnett, 1972).

3.4 Management of the spawn infecting microorganisms by chemicals

Three different concentrations namely 10000, 20000, 30000 ppm of Hydrogen peroxide, 100 ppm of Mancozeb, 100 ppm of Mancozeb+Metalaxyl, 100 ppm of household bleaching powder were selected to evaluate their effect on radial growth of selected fungi. Details of the chemicals are given in the Table 1.

Table 1. List of chemicals, their active ingredients, concentration and dose evaluated under *in-vitro* and *in-vivo* conditions against five fungal isolates causing contamination of spawn

Treatments	Trade name with active ingredient (%)	Concentrations (ppm)	Dose
T_1	Hydrogen Peroxide (30% H_2O_2)	10,000	10ml/ lt. water
T_2		20,000	20ml/ lt. water
T_3		30,000	30ml/ lt.water
T_4	Nemispore 80 WP Mancozeb (80%)	100	100 mg/lt. water
T_5	Nuben 72WP Metalaxyl 8%+ Mancozeb 64%	100	100 mg/lt. water
T_6	Rok (Bleach)	100	1/2 teaspoon/ lt. water
T_7	Wood ash	10,000	10g/lt. water
T_0 (control)	In normal water	-	-



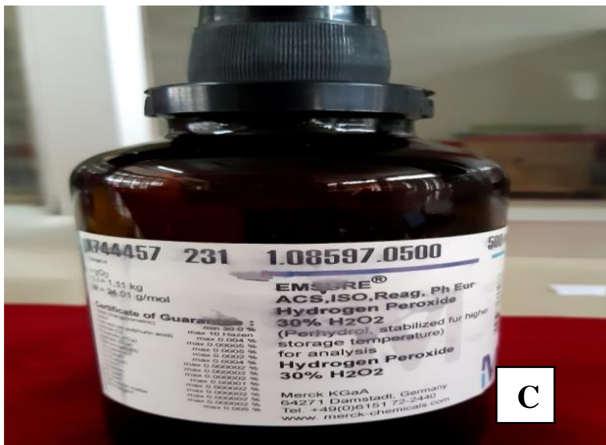


Plate 1. Chemicals used for the management of contaminated microorganisms

Legends:

A. Mancozeb (80% WP)

B. Metalaxyl 8%+ Mancozeb 64% (72WP)

C. Hydrogen Peroxide

D. Bleaching powder

E. Wood ash

3.4.1 *In vitro* evaluation of chemicals against associated contaminants from substrate of *Pleurotus ostreatus*

The Poison food technique was adopted in the experiment. The principle involved in this technique is to supplement the nutrient medium with a toxic chemical and then allowing a test fungus to grow on the medium and evaluate the effect of such chemicals by measuring the growth of the fungus. Required amount of chemicals were added to test fungicide with the help of a sterilized micropipette. The chemically amended medium was poured in sterilized petridishes and allowed to set. With the help of a sterile block cutter, dishes of 0.5 cm diameter were cut from actively growing fungal culture and transferred aseptically in the center of petridish containing the test medium. Three replications of each concentration for each chemical including control (without chemical) were maintained. The inoculated Petri dishes were incubated at 25°C (±2) °C and the radial growth of the fungal colony in each Petri plate was measured after 3rd and 5th days of inoculation and percent inhibition of radial colony growth was calculated by formula given by Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

Where;

I = Percent inhibition (%)

C = Fungal growth in control plate (mm)

T = Fungal growth in treatments (mm)

3.5 Spawn production

3.5.1 Collection of materials for spawn production

Rice straw was collected from the farm of Sher-e-Bangla Agricultural University and mother culture of *Pleurotus ostreatus* (PO_{10}), neck and 7×11 inch polypropylene bag @ 500g was collected from National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka.

3.5.2 Varietal characteristics of oyster mushroom (*Pleurotus ostreatus*)

Oyster mushrooms (*Pleurotus ostreatus*) are characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. Their fruiting bodies are shell or spatula shaped with grey color. If the temperature increases above 32°C, its production markedly decreases.

3.5.3 Design and layout of the experiment

The experiment was laid out in a single factor Completely Randomized Design (CRD) and considered eight treatments with five replications (one packet under each replication).

3.5.4 Preparation of the substrate

The trial was started on 2nd April, 2019. In the first step rice straw were chopped to 2-3 cm size was stored under a covered shade. Chopped rice straw was dipped into the different chemicals to sterilize for 12 hours at given concentrations.

3.5.5 Preparation and inoculation of spawn packets

Therefore the straw was taken off from treated chemicals and left on a perforated sieve for removing the excess water for few hours. Then CaO were added with rice straw substrate @ 1% on dry weight basis. The measured materials were taken in a plastic bowl and mixed thoroughly by hand and moisture was increased by adding water. Then substrates were filled into 7×11 inch polypropylene bag @ 500 g with 50 g mother spawn into three layers in each bag. The filled polypropylene bags were prepared by using plastic neck and plugged the neck with cotton and covered with brown paper placing rubber band to hold it tightly in place. Five spawn packets were prepared for each treatment.

Figure 1. Mixing of CaO with rice straw

Figure 2. Inoculation of spawn packets

3.5.6 Incubation of spawn packets

After preparation of spawn packets, filled packets were incubated in a dark room at a temperature ranging between 22-25°C, where 90% relative humidity was maintained till the mycelium run was complete. When the straw is fully covered with white mycelium, the rubber band, brown paper, cotton plug and plastic neck of the mouth of spawn packet were removed then the bag was cut open and the mouth was wrapped tightly with rubber band. Then these spawn packets were transferred to the culture house.

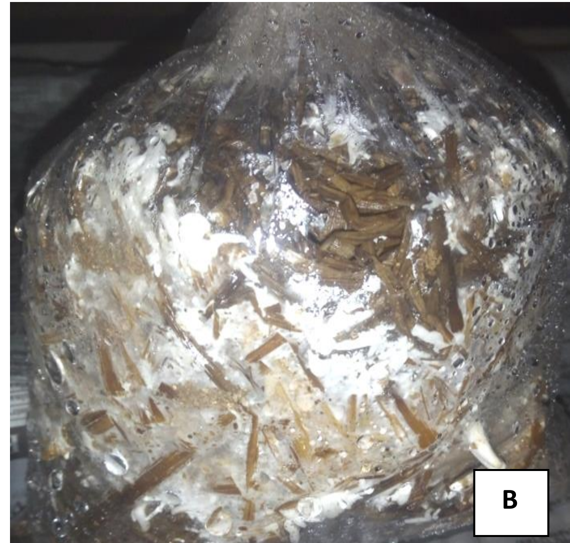
Figure 3. Incubation of spawn packets

3.5.7 Cultivation of spawn packets

Plastic bag were cut in "D" at tow ends with a blade and opened by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a tea spoon for removing the thin whitish mycelial layer. The packet of each type was placed separately side by side on the iron shelves of culture house. The moisture of the culture house was maintained by spraying water 3 times a day. The temperature of culture house was maintained 22°C to 25°C.

3.5.8 Harvesting of produced mushrooms

The matured fruiting body was identified by curial margin of the cap, as described by Amin (2002). Mushrooms were harvested by twisting to uproot from the base. After completing the first harvest again the packets were scraped at the place where the 'D' shaped cut had been done and the spawn packets were soaked in water for 5 minutes and inverted to remove excess water for another 5 minutes and water was sprayed regularly. Then again when the primordia appeared after first harvest and second harvest was done and water spraying was continued until the mushrooms were ready to be harvested.



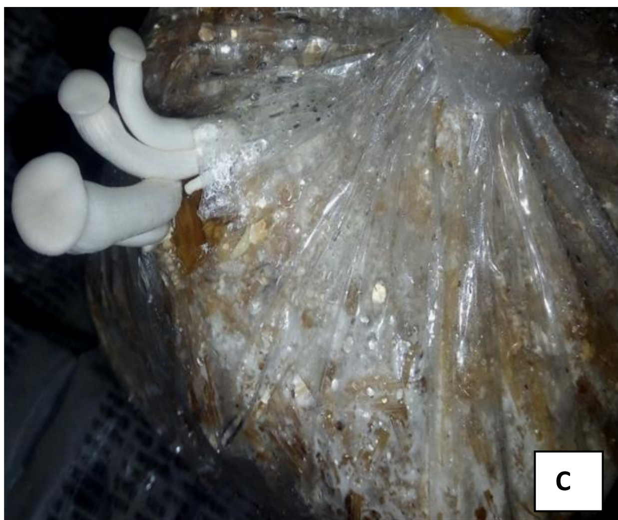


Plate 2. A. Mycelium running in spawn packet, **B**. Mycelium running complete in spawn packet, **C**. Primordia initiation in spawn packet, **D.** Mature fruiting body in spawn packet

3.6 Data collection

Data were taken on the following parameters

3.6.1 Days required for completing mycelium running

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

3.6.2 Days required for the primodia formation

Days required from completion of mycelium to pin head formation were recorded.

3.6.3 Days required to primodia initiation to 1st harvest

Days required from primodia formation to first harvest were recorded.

3.6.4 Days required to final harvest

Days required from primodia formation to final harvest were recorded.

3.6.5 Data on yield contributing parameters

Number of primodia and well-developed fruiting body was recorded. Dry fruiting bodies were discarded. Average weight of individual fruiting body was calculated by dividing the total weight of fruiting body per packet by the total number of fruiting body per packet.

a) Number of primodia per packets
b) Number of fruiting body
c) Number of effective fruiting body
d) Weight of individual fruiting body (g)

3.6.6 Dimension of fruiting body (stipe and pileus)

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

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

3.6.7 Biological yield (g)

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

3.6.8 Economic yield (g)

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

3.6.9 Dry yield (g)

The mushroom was oven dried at 72ºC temperature for 24 hours and weighed again. The dry yield was calculated by using this formula (Sarker, 2004)

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

3.6.10 Biological efficiency

Biological efficiency was determined by the following formula:

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

3.6.11 Percent contamination (%)

Contamination severity was calculated for the test and control beds depended upon the following scale

Grade 0: 0% – Free from infection
Grade 1: >0 – 20% Spawn area coverage by the contaminants
Grade 2: >20 – 40% Spawn area coverage by the contaminants
Grade 3: >40 – 60% Spawn area coverage by the contaminants
Grade 4: >60 – 80% Spawn area coverage by the contaminants
Grade 5: >80 – 100% Spawn area coverage by the contaminants

$$\text{Severity of contamination (\%)} = \frac{\text{Sum of total score}}{\text{Total no.of observation} \times \text{Maximum grade of the scale}} \times 100$$

3.7 Analysis of data

The data was recorded for each character from the experiment was analyzed statistically using Statistix 10 computer program. The mean from all the treatment were calculated and analysis of variance (ANOVA) of characters under study was performed by F variance test.

Plate 3. A-B. Harvested mushroom from spawn packet, C. Measurement of stalk length, D. Measurement of pileus diameter

CHAPTER IV

RESULTS

4.1 Isolation and identification of microbial contamination from spawn

The infected spawn packets were taken for isolation of pathogens infecting substrate. They were isolated on potato dextrose agar. After purification of the fungus as described under materials and methods, morphological characters of the fungus on potato dextrose agar were studied for the purpose of identification and compared with those described in the literature. Based on the morphological characters commonly five pathogens were identified. These are *Sclerotium rolfsii*, *Trichoderma harzianum, Fusarium oxysporum,Penecillium* sp.and *Aspergillus niger.*

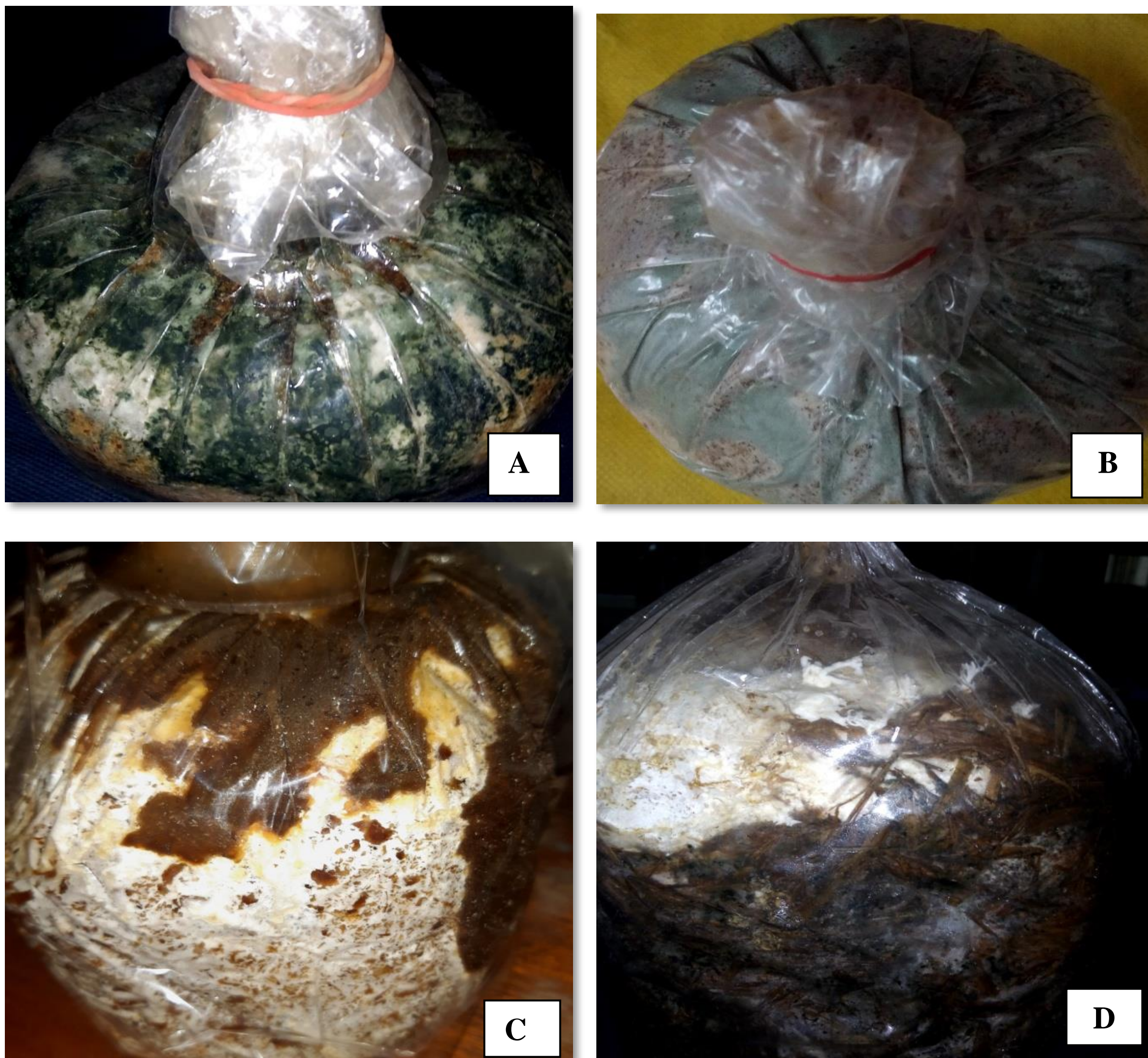


Plate 4. A. *Trichoderma* contaminated spawn packet, B. *Penicillium* contaminated spawn packet, C-D. Different fungi contaminated spawn like *Fusarium, Aspergillus, Sclerotium rolfsii*

4.2 Morphological characterization of isolated fungal contaminants from spawn

4.2.1 *Trichoderma harzianum*

Green colour growth of mycelium was observed in contaminated spawn packet (Plate 5 A) due to heavy sporulation of causal agent. Colonies are usually fast growing and initially whitish in color that later turn into bright green color (plate 6 A-B). *T. harzianum* had occasionally concentric conidiation with whitish yellow conidial area. Conidiophores are branched that cluster into fascicles. Normally branches are formed near 90° with the main branch. The conidiophores terminated with one or few phialides that usually rise from the axis near the tip.

4.2.2 *Fusarium oxysporum*

Colonies color initially white but later turns into pink color in the mycelium on medium (plate-6 C-D). Conidiophores variable, simple or branched single or grouped into sporodochia. Conidia are of two kinds: macroconidia and microconidia, macroconidia are several celled, fusiform to sickle shaped, several celled, slightly curved or bent at the pointed ends and microconidia are 1 or 2 celled smaller than macroconidia, pyriform, fusiform to ovoid, straight or curved and borne singly or in chains.

4.2.3 *Sclerotium rolfsii*

Sclerotium rolfsii produces abundant white mycelium on infected substrate and culture (plate 5 C-D). In culture, mycelium appeared smooth at first but some culture may develop aerial mycelia that cover all the part of the culture after a few days (plate 6 E-F). Aerial mycelium hyphae diminished with age and mature sclerotia had two tissue layer, the rind and medulla. Cells are hyaline with thin cell walls. In agar plate culture, sclerotia are not formed until the mycelium covers the plate. *In vitro or in vivo* sclerotia begin as small tuft of white mycelium that formed spherical sclerotia. Sclerotia darken as they mature, becoming tan to dark brown in color.

4.2.4 *Aspergillus niger*

Aspergillus niger was found in the contaminated the spawn packets in the growing house (plate 5 C-D). It produced black colored spores so it was called black mold (plate 7 A-B). Initially fungal colonies were whitish which quickly became quite black. The hyphae were hyaline and septate. The conidia produced were globose, single celled, pale to dark brown on

maturity. The conidiophores were erect, unbranched, straight, hyaline to light brown, long aseptate and darker near vesicle. The vesicle was globose, thick walled and brown to black.

4.2.5 *Penecillium* sp.

Initially, *Penicillium* appeared as a white colored powder on the substrates of oyster mushroom and later turned into green as timed passed, it is called blue green mold (plate 5 B). Pure culture of *Penicillium* was prepared on PDA from collected contaminated spawn (Plate7 C-D). Conidiophores are hyaline, smooth or rough walled arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides. Conidia hyaline or brightly colored in mass, chain of single celled conidia are produced in basipetal succession from a specialized conidiogenous cell called a phialide.

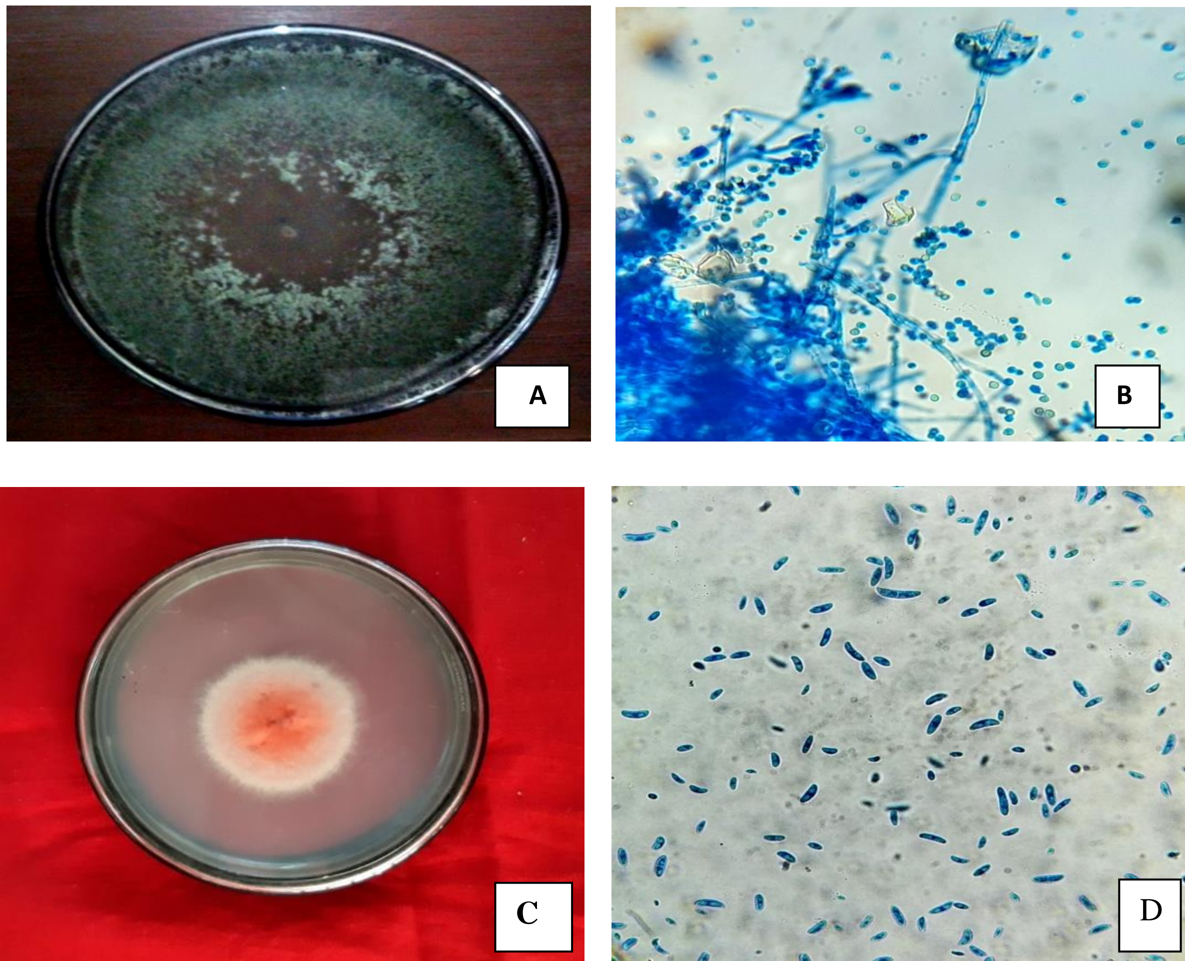
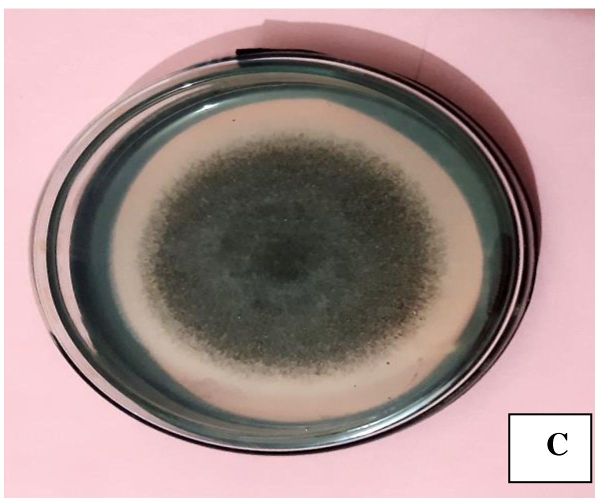
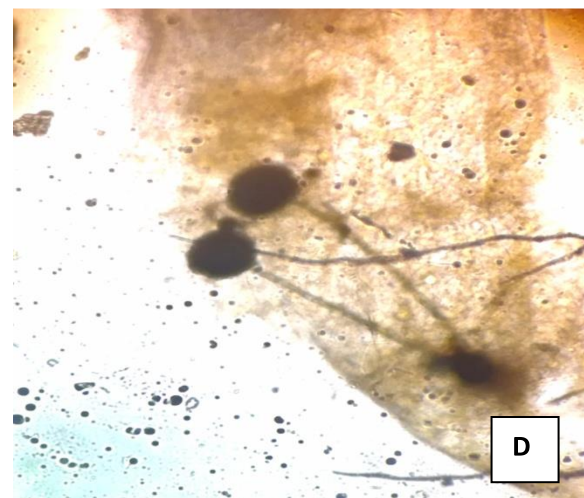
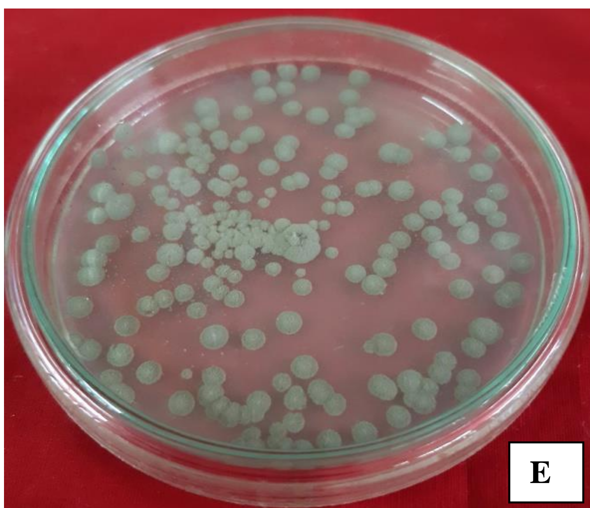


Plate 5. **A.** Pure culture of *Trichoderma* **B.** Pathogenic structure of *Trichoderma*; **C.** Pure culture of *Fusarium* **D.** Pathogenic structure of *Fusarium*.







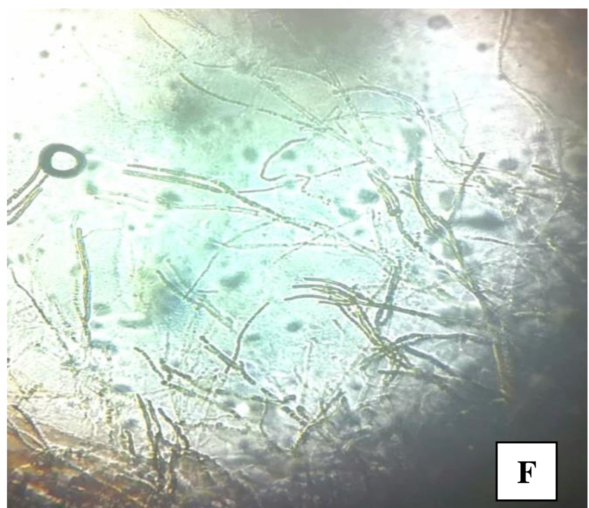


Plate 6. A. Pure Culture of *Sclerotium rolfsii* **B.** Sclerotia of *Sclerotium rolfsii* **C.** Pure Culture of *Aspergillus niger* **D**. Microscopic Structure of *Aspergillus niger*; **E.** Pure culture of *Penicillium* **F.** Pathogenic structure of *Penicillium* isolated from contaminated spawn

4.3 *In-vitro* evaluation of different chemicals against mushroom substrate contaminating fungi

4.3.1 Effect of different chemicals on radial mycelial growth and percent growth inhibition of *Trichoderma harzianum* under *in vitro* condition

The observations regarding mycelial radial growth and percent growth inhibition are presented in Table 6, figure 5 and plate 8. The radial growth of *Trichoderma harzianum* was 73.67 mm at 3 days and 90 mm at 5 days 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 highest mycelial growth (25.90 mm) was observed with 10000 ppm hydrogen peroxide at 3 days which was increased to 88.40 mm at 5 days and that is statistically similar with bleaching powder (24.43 mm at 3 days & 87.00 mm at 5 days) and control. All the chemicales more or less suppressed the growth of *Trichoderma harzianum,* among six chemicals were tested the highest inhibition (72.41%) was obtained with 30000 ppm hydrogen peroxide. The lowest inhibitions (1.78% and 3.33%) were observed with 10000 ppm hydrogen peroxide and bleaching powder which were statistically similar.

Table 2. Radial mycelial growth (mm) and percent growth inhibition (%) of *Trichoderma harzianum* against different chemicals under *in vitro* condition

Treatments	Mycelial growth (mm)		Percent inhibition at 5 days (%)
	3 days	5 days	
T_1	25.90b	88.40a	1.78e
T_2	17.50c	61.90c	31.21c
T_3	10.63e	24.83e	72.41a
T_4	15.43d	68.67b	23.69d
T_5	14.83d	42.23d	53.07b
T_6	24.43b	87.00a	3.33e
T_0	73.67a	90.00a	0.00e
LSD(0.05)	1.48	3.17	3.52
CV (%)	3.25	2.74	7.60

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

Legends

T_1: 10000 ppm Hydrogen Peroxide T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide T_4: Mancozeb (100 ppm)

T_5: Metalaxyl + Mancozeb (100 ppm) T_6: Bleaching powder (100 ppm)

T_0: Control

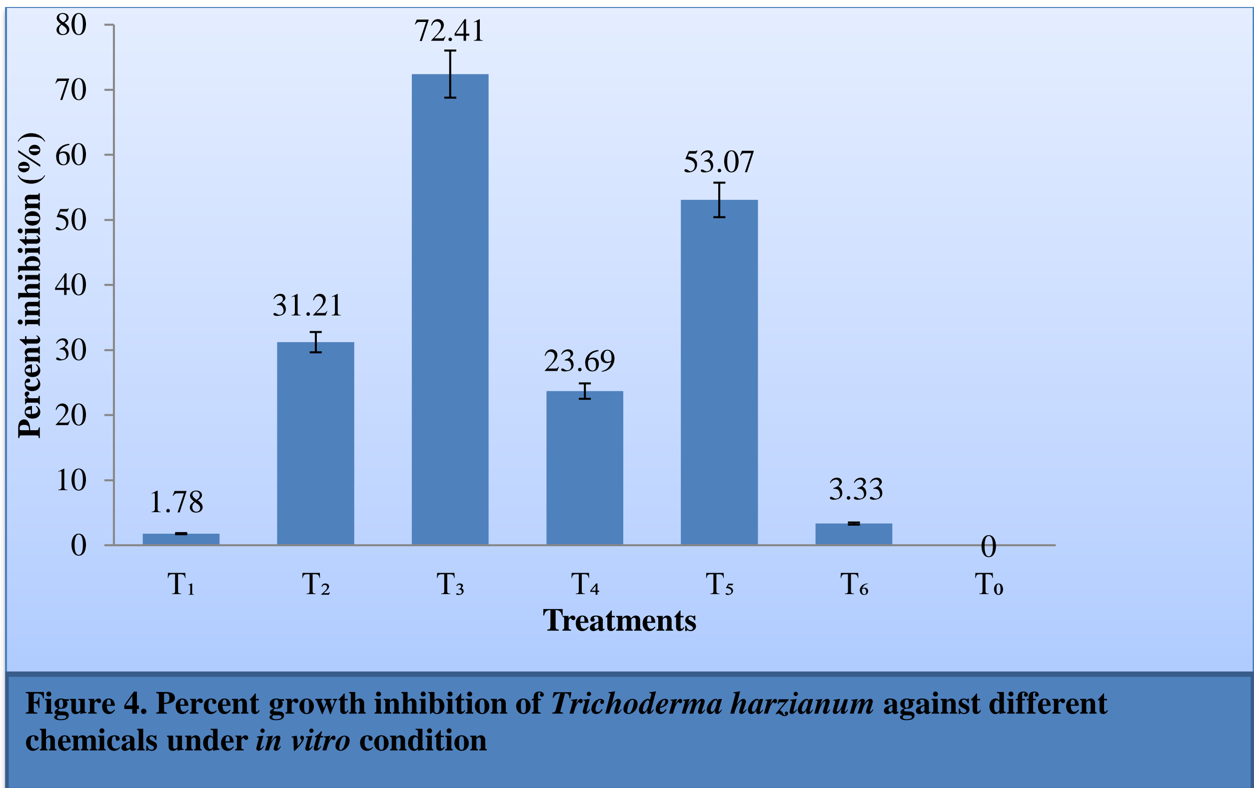


Figure 4. Percent growth inhibition of *Trichoderma harzianum* against different chemicals under *in vitro* condition

T_1: 10000 ppm Hydrogen Peroxide

T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide

T_4: Mancozeb (100 ppm)

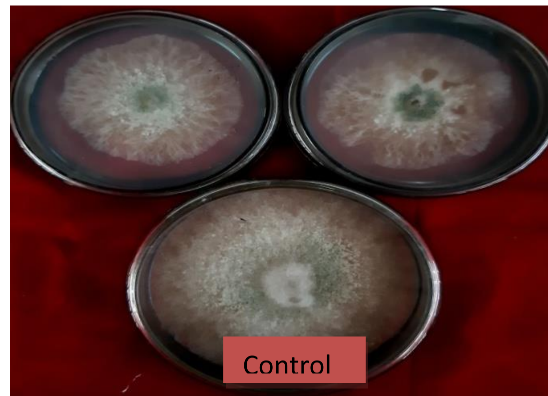
T_5: Metalaxyl + Mancozeb (100 ppm)

T_6: Bleaching powder (100 ppm)

T_0: Control

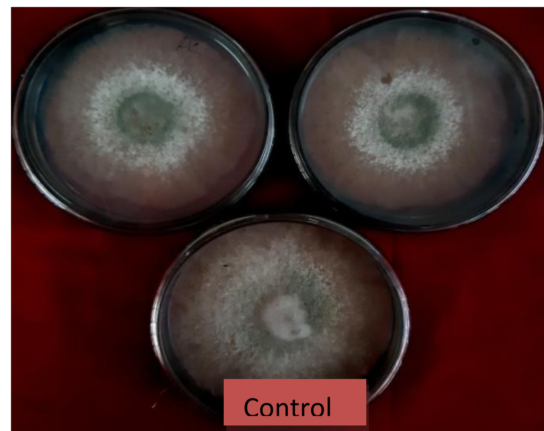
10000 ppm H_2O_2 **20000 ppm H_2O_2**

30000 ppm H_2O_2



100 ppm Mancozeb

100 ppm Mancozeb+Metalaxyl



100 ppm Bleaching powder

Plate 7. In vitro evaluation of chemicals against *Trichoderma harzianum* at 5 days

4.3.2 Effect of different chemicals on radial mycelial growth and percent growth inhibition of *Fusarium oxysporum* under *in vitro* condition

The results of efficacy of different chemicals in the management of *Fusarium oxysporum* have been presented in Table 7, figure 6 and plate 9. The data makes it clear that all the chemicals were more or less effective in inhibiting the growth of *fusarium oxysporum.* The radial mycelium growth of *Fusarium oxysporum* under control condition was 22.50 mm and 56.50 mm at 3 and 5 days, respectively. Maximum radial growth (21.17 mm and 19.83 mm) were observed with 10000 ppm hydrogen peroxide and mancozeb at 5 days, respectively. The lowest radial growth (5.50 mm at 3 days and 5.57mm at 5 days) of *Fusarium oxysporum* was observed under 30000 ppm hydrogen peroxide which was statistically similar with Bleaching powder (6.33 mm at 3 days and 6.66 mm at 5 days). The highest percent inhibition (90.15% and 88.19%) was observed with 30000 ppm hydrogen peroxide and Bleaching powder respectively, both are statistically similar where as the lowest inhibition (62.53%) was obtained under 10000 ppm H_2O_2 followed by Mancozeb (64.90%).

Table 3. Radial mycelial growth (mm) and percent growth inhibition (%) of *Fusarium oxysporum* against different chemicals under *in vitro* condition

Treatments	Mycelial growth (mm)		Percent inhibition at 5 days (%)
	3 days	5 days	
T_1	17.17b	21.17b	62.53e
T_2	14.50c	16.56d	79.68c
T_3	5.50e	5.57f	90.15a
T_4	16.70b	19.83c	64.90d
T_5	13.17d	14.00e	75.22b
T_6	6.33e	6.66f	88.19a
T_0	22.50a	56.50a	0.00f
LSD(0.05)	1.00	1.31	2.26
CV (%)	4.20	3.76	2.00

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

Legends

T_1: 10000 ppm Hydrogen Peroxide T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide T_4: Mancozeb (100 ppm)

T_5: Metalaxyl + Mancozeb (100 ppm) T_6: Bleaching powder (100 ppm)

T_0: Control

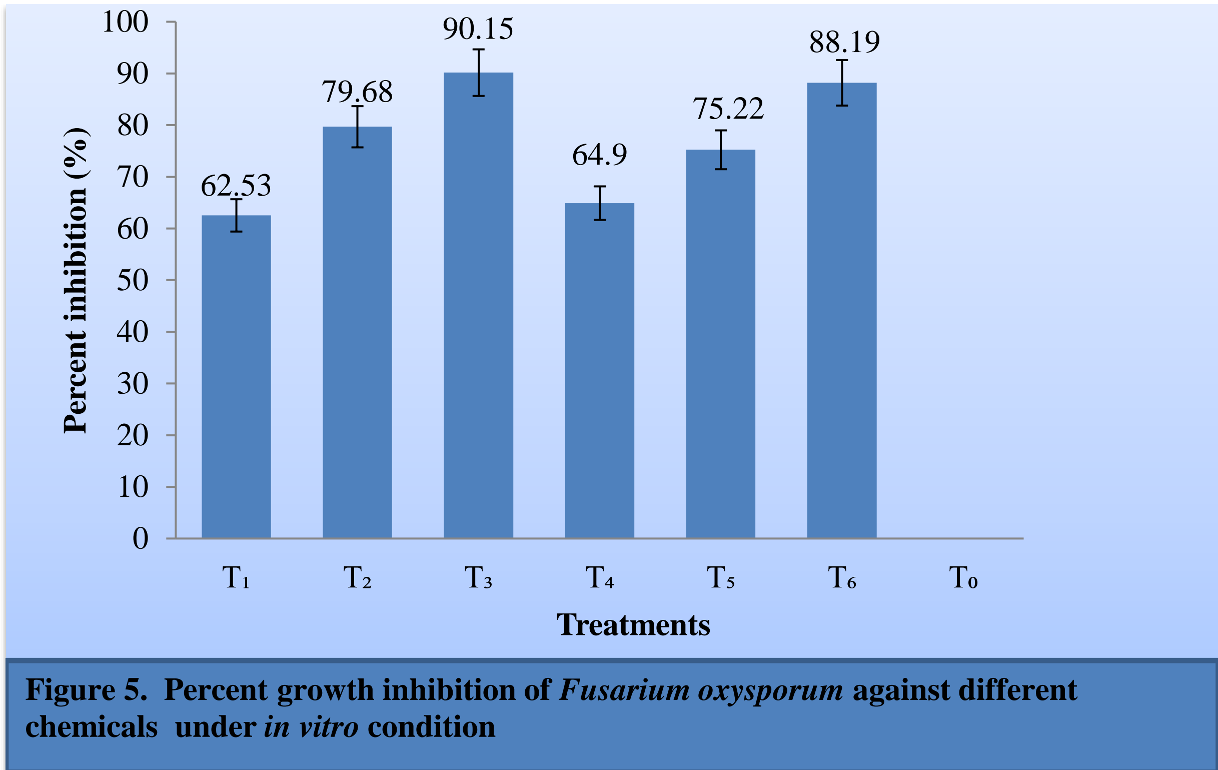


Figure 5. Percent growth inhibition of *Fusarium oxysporum* against different chemicals under *in vitro* condition

Legends

T_1: 10000 ppm Hydrogen Peroxide

T_2: 20000 ppm Hydrogen Peroxide

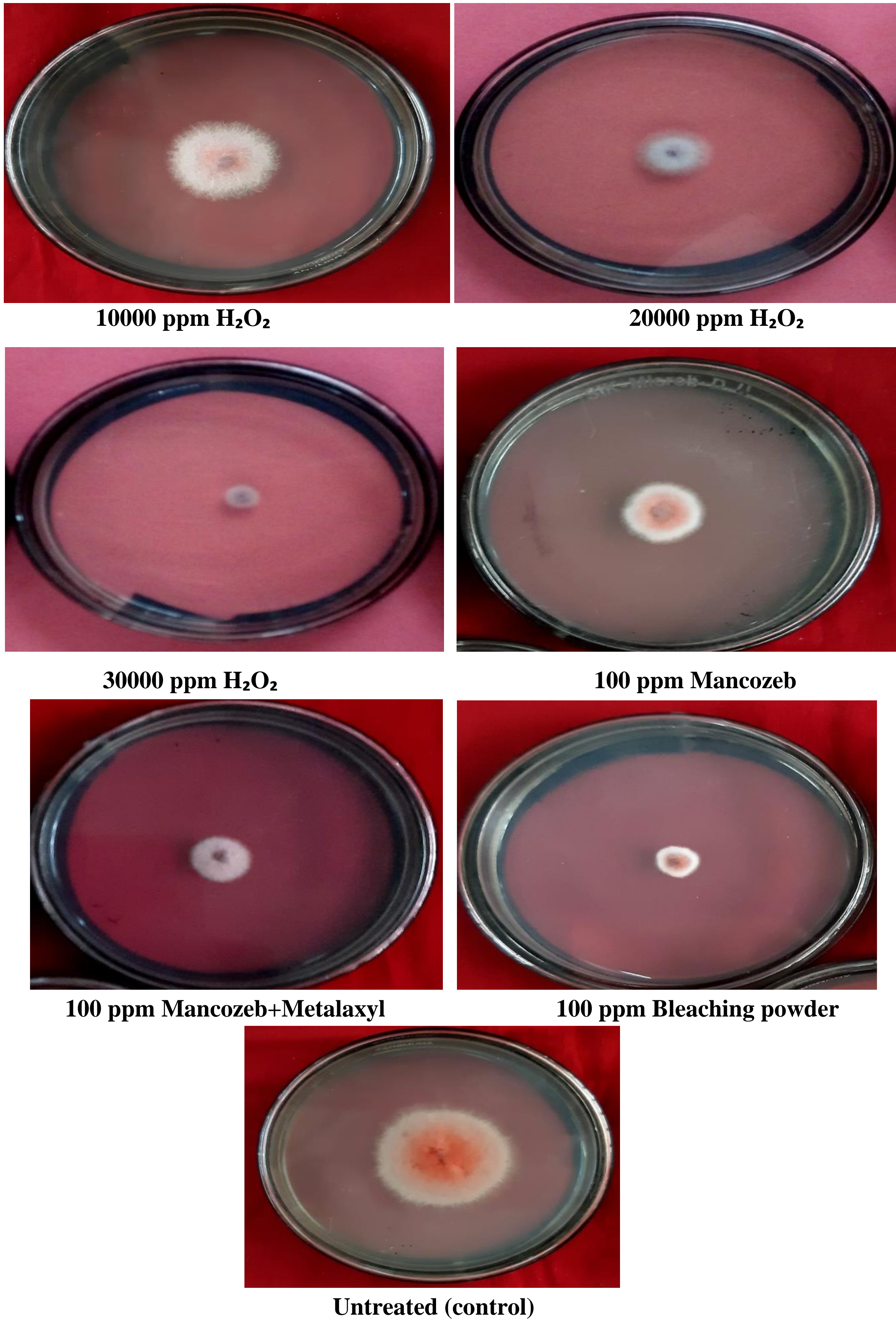
T_3: 30000 ppm Hydrogen Peroxide

T_4: Mancozeb (100 ppm)

T_5: Metalaxyl + Mancozeb (100 ppm)

T_6: Bleaching powder (100 ppm)

T_0: Control



10000 ppm H_2O_2 20000 ppm H_2O_2

30000 ppm H_2O_2 100 ppm Mancozeb

100 ppm Mancozeb+Metalaxyl 100 ppm Bleaching powder

Untreated (control)

Plate 8. In vitro evaluation of chemicals against *Fusarium oxysporum* at 5 days

4.3.3 Effect of different chemicals on mycelial growth and percent growth inhibition of *Sclerotium rolfsii* under *in vitro* condition

The relative efficacies of six different chemicals namely hydrogen peroxide were tested at 10000, 20000, 30000 ppm , mancozeb, mancozeb+metalaxyl and bleaching powder were tested at 100, 100 and 100 ppm concentrations respectively against *Sclerotium rolfsii*. The observations regarding percent growth inhibition are presented in Table 5, figure 4 and depicted in Plate 7. Radial mycelial growth of *Sclerotium rolfsii* under control condition was 77.67 mm and 90 mm at 3 and 5 days, respectively. Maximum radial growth 39.33 mm and 25.50 mm was observed at 3 days under 10000 ppm hydrogen peroxide and 100 ppm bleaching powder respectively. It was increased to 53.33 mm and 46.80 mm respectively at 5 days. The lowest radial growth (5.17mm at 3 days and 5.83mm at 5 days) of *Sclerotium* was observed under 30000 ppm hydrogen peroxide which was statistically similar by Mancozeb+Metalaxyl (5.93mm at 3 days and 6.87mm at 5 days). Therefore it can be concluded that all the fungicides more or less suppressed the growth of *Sclerotium rolfsii*. Among the six chemicals tested 30000 ppm hydrogen peroxide had a maximum inhibitory action (93.47%) against Sclerotium *rolfsii* followed by Mancozeb+Metalaxyl (92.33%). The lowest inhibition (40.73%) was obtained under 10000 ppm hydrogen peroxide followed by Bleach (47.93%).

Table 4. Radial mycelial growth (%) and percent growth inhibition (%) of *Sclerotium rolfsii* against different chemicals under *in vitro* condition

Treatments	Mycelial growth (mm)		Percent inhibition at 5 days (%)
	3 days	5 days	
T_1	39.33b	53.33b	40.73e
T_2	24.50d	43.50d	51.64c
T_3	5.17f	5.83f	93.47a
T_4	10.50e	42.77d	52.47c
T_5	5.93f	6.87e	92.33b
T_6	25.50c	46.80c	47.93d
T_0	77.67a	90.00a	0.00f
LSD(0.05)	0.88	0.91	1.01
CV (%)	1.86	1.26	1.07

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

Legends

T_1: 10000 ppm Hydrogen Peroxide T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide T_4: Mancozeb (100 ppm)

T_5: Metalaxyl + Mancozeb (100 ppm) T_6: Bleaching powder (100 ppm)

T_0: Control

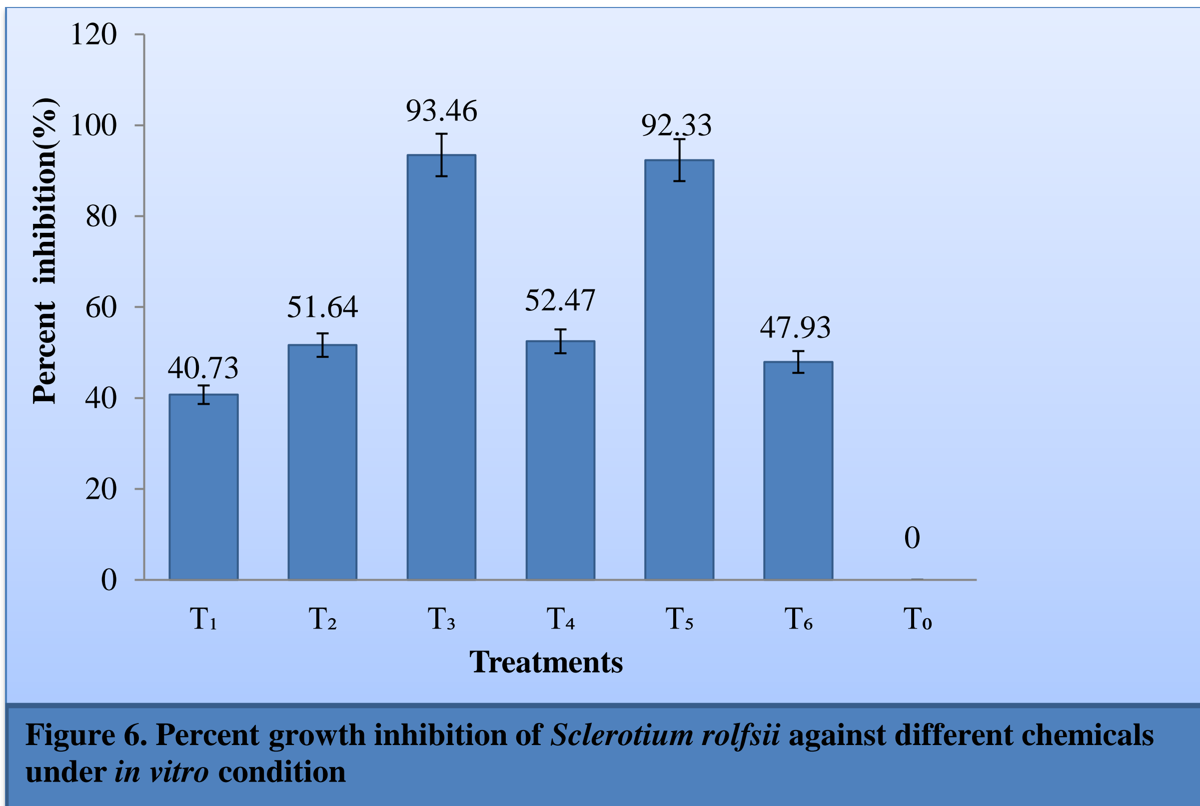


Figure 6. Percent growth inhibition of *Sclerotium rolfsii* against different chemicals under *in vitro* condition

Legends

T_1: 10000 ppm Hydrogen Peroxide

T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide

T_4: Mancozeb (100 ppm)

T_5: Metalaxyl + Mancozeb (100 ppm)

T_6: Bleaching powder (100 ppm)

T_0: Control

10000 ppm H_2O_2

20000 ppm H_2O_2

30000 ppm H_2O_2

100 ppm Mancozeb

100 ppm Mancozeb+Metalaxyl

100 ppm Bleaching powder

Plate 9. In vitro evaluation of chemicals against *Sclerotium rolfsii* at 5 days

4.3.4 Effect of different chemicals against *Aspergillus niger* under *in vitro* condition

Efficacy of different chemicals for the management of *Aspergillus niger* have been presented in Table 9, figure 8 and plate 11. The results reveal significant variation in managing the fungus. Under contro condition the radial growth of *Aspergillus niger* was 49.67mm and 90.00mm at 3 and 5 days respectively. Radial colony diameter under Bleaching powder and Mancozeb was 21.33mm and 21.67mm at 3 days, respectively which was statistically similar. It was increased to 68.00mm and 61.33mm respectively at 5 days under those two chemicals which was highest mycelium growth. The lowest radial growth (7.67mm and 8.33mm at 3 and 5 days respectively) was observed under 30000 ppm H_2O_2. It was found that maximum mycelia inhibition (90.74%) was obtained by 30000 ppm H_2O_2 which was statistically followed by Mancozeb+Metalaxyl (85.55%) where as minimum inhibition (24.44%) was observed by Bleaching powder followed by Mancozeb (31.85%). In the present investigation, 30000 ppm hydrogen peroxide was found most effective.

Table 5. Radial mycelial growth (mm) and percent growth inhibition (%) of *Aspergillus niger* against different chemicals under *in vitro* condition

Treatments	Mycelium growth (mm)		Percent inhibition at 5 days (%)
	3 days	5 days	
T_1	14.33c	43.33d	51.85d
T_2	13.00d	34.67e	61.48c
T_3	7.67f	8.33g	90.74a
T_4	21.67b	61.33c	31.85e
T_5	11.67e	13.00f	85.55b
T_6	21.33b	68.00b	24.44f
T_0	49.67a	90.00a	0.00g
LSD(0.05)	0.99	2.22	2.47
CV (%)	2.85	2.80	2.86

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

Legends

T_1: 10000 ppm Hydrogen Peroxide T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide T_4: Mancozeb (100 ppm)

T_5: Metalaxyl + Mancozeb (100 ppm) T_6: Bleaching powder (100 ppm)

T_0: Control

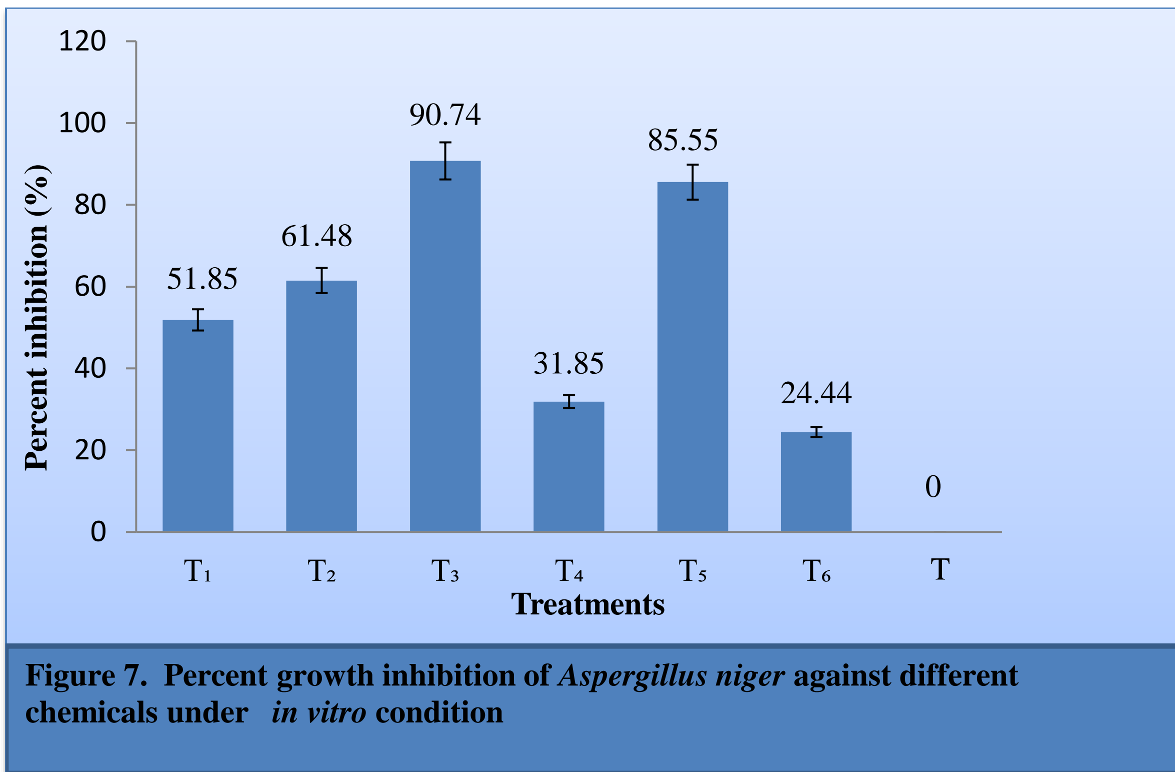


Figure 7. Percent growth inhibition of *Aspergillus niger* against different chemicals under *in vitro* condition

Legends

T_1: 10000 ppm Hydrogen Peroxide

T_2: 20000 ppm Hydrogen Peroxide

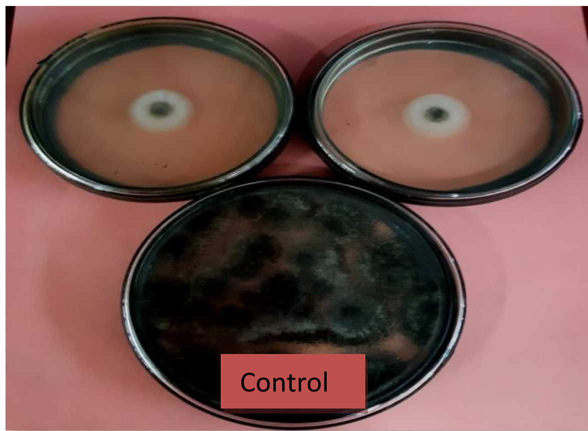
T_3: 30000 ppm Hydrogen Peroxide

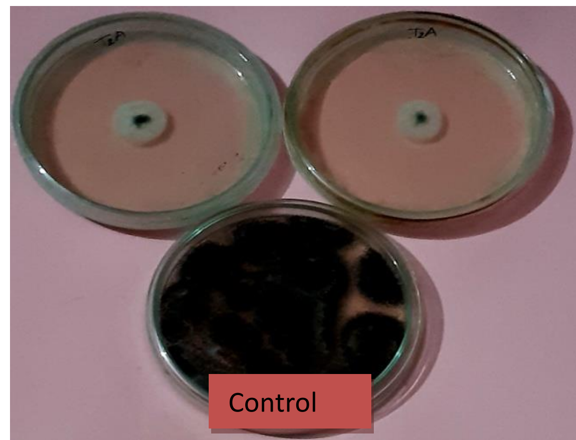
T_4: Mancozeb (100 ppm)

T_5: Metalaxyl + Mancozeb (100 ppm)

T_6: Bleaching powder (100 ppm)

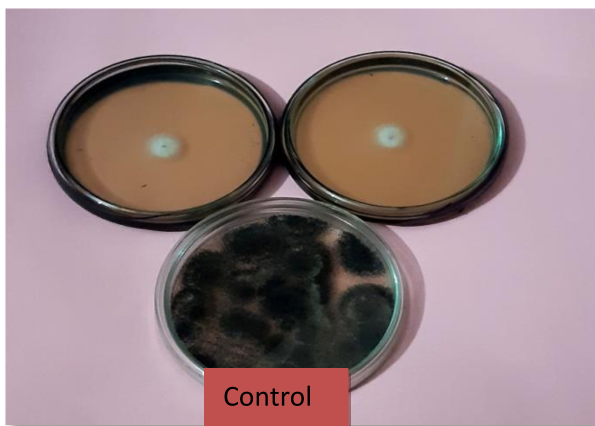
T_0: Control

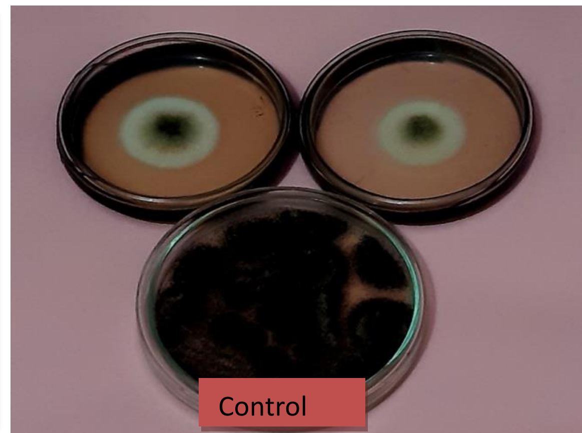




10000 ppm H_2O_2

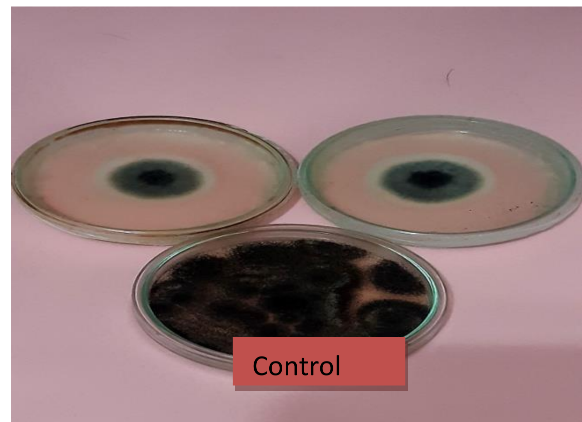
20000 ppm H_2O_2





30000 ppm H_2O_2

100 ppm Mancozeb



100 ppm Mancozeb+Metalaxyl

100 ppm Bleaching powder

Plate 10. In vitro evaluation of chemicals against *Aspergillus niger* at 5 days

4.3.5 Effect of different chemicals on radial mycelial growth and percent growth inhibition of *Penicillium* sp. under *in vitro* condition

Under control condition, the mycelial growth of *Penicillium* sp. covered the full plate of petridish. Radial mycelial growth of *Penecillium* sp. was 73.00 mm at 3 days and 90 mm at 5 days under control. Percent growth inhibition is presented in Table 8, figure 7 and Plate 10. Amended PDA with each chemical inhibited the radial mycelium growth significantly over control. The maximum mycelium growth (11.67 mm at 3 days and 38.33 mm at 5 days) was observed under 10000 ppm H_2O_2. The highest inhibition (92.96%) was obtained with 30000 ppm H_2O_2 followed by Mancozeb (83.88%) where as the lowest inhibition (57.41%) was observed under 10000 ppm hydrogen peroxide.

Table 6. Radial mycelial growth (mm) and percent growth inhibition (%) of *Penicillium* sp. against different chemicals under *in vitro* condition

Treatments	Mycelial growth (mm)		Percent inhibition at 5 days (%)
	3 days	5 days	
T_1	11.67b	38.33b	57.41f
T_2	10.50c	22.33c	75.19e
T_3	6.23f	6.33g	92.96a
T_4	7.17ef	14.50f	83.88b
T_5	7.83de	17.50e	80.56c
T_6	8.67d	18.50d	79.41d
T_0	73.00a	90.00a	0.00g
LSD(0.05)	1.01	0.81	0.91
CV (%)	3.25	1.56	0.78

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

Legends

T_1: 10000 ppm Hydrogen Peroxide T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide T_4: Mancozeb (100 ppm)

T_5: Metalaxyl + Mancozeb (100 ppm) T_6: Bleaching powder (100 ppm) T_0: Control

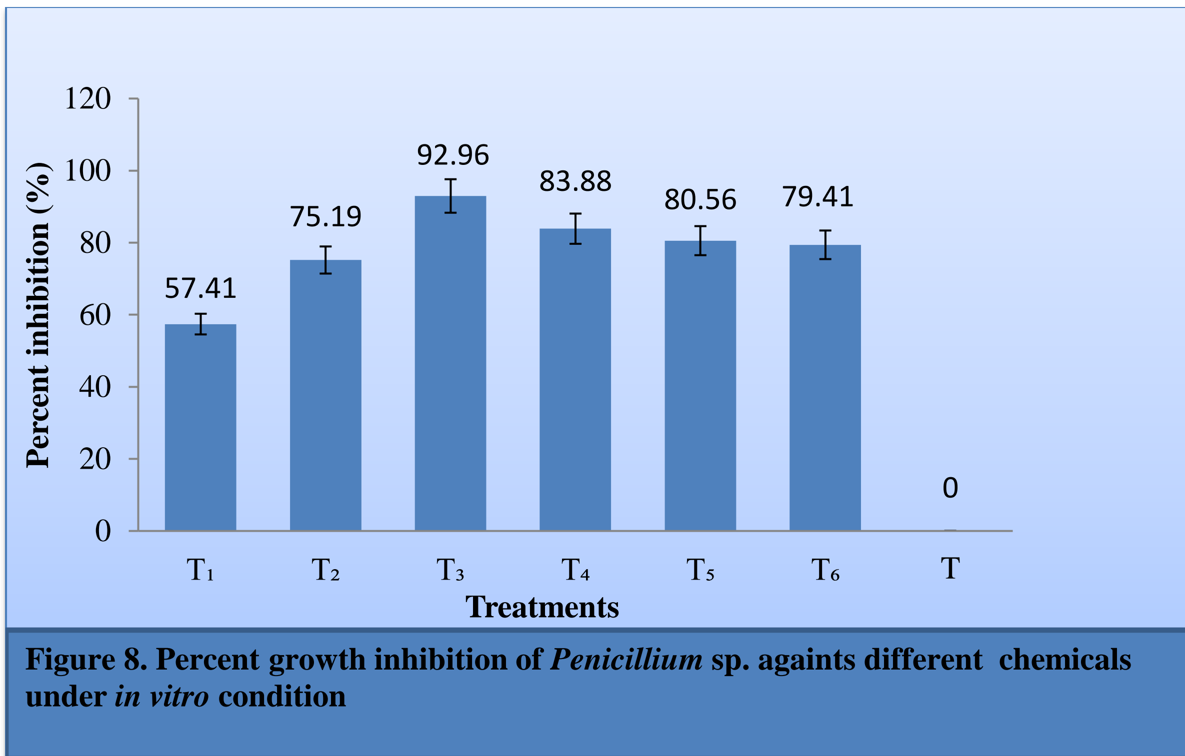


Figure 8. Percent growth inhibition of *Penicillium* sp. againts different chemicals under *in vitro* condition

Legends

T_1: 10000 ppm Hydrogen Peroxide

T_2: 20000 ppm Hydrogen Peroxide

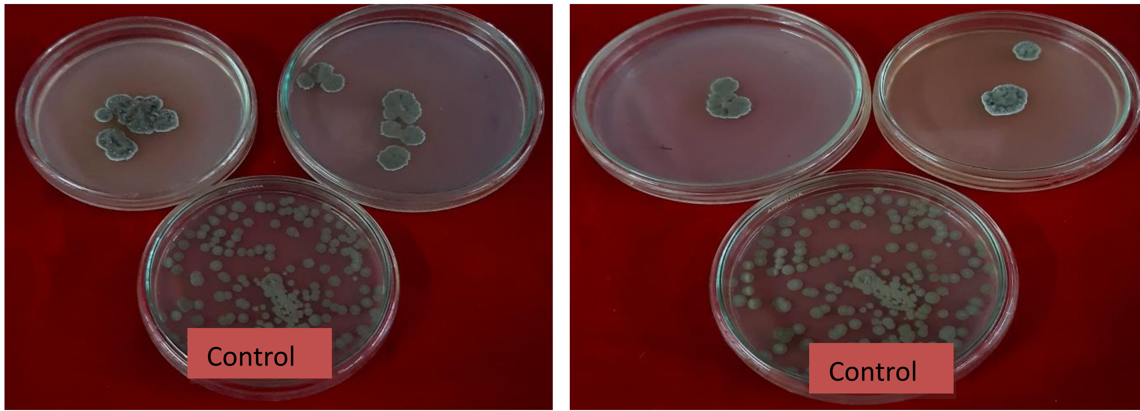
T_3: 30000 ppm Hydrogen Peroxide

T_4: Mancozeb (100 ppm)

T_5: Metalaxyl + Mancozeb (100 ppm)

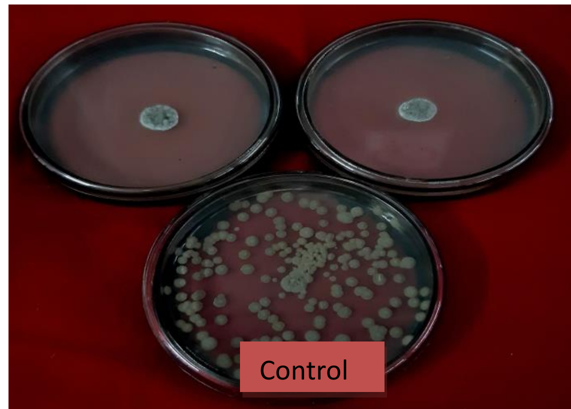
T_6: Bleaching powder (100 ppm)

T_0: Control

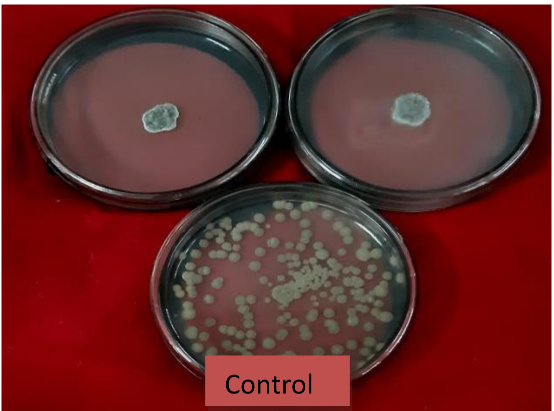


10000 ppm H_2O_2 20000 ppm H_2O_2

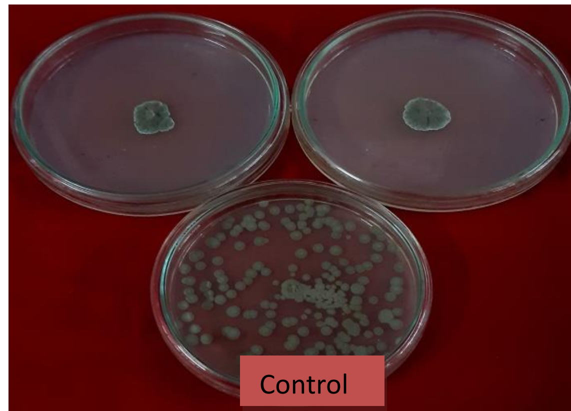
30000 ppm H_2O_2



100 ppm Mancozeb



100 ppm Mancozeb+Metalaxyl



100 ppm Bleaching powder

Plate 11. In vitro evaluation of different chemicals against *Penecillium sp.* at 5 days

4.4 *In-vivo* evaluation of chemicals to sterilize substrate against contaminating fungi and production of oyster mushroom

4.4.1 Severity of contamination in spawn packet containing different chemicals to sterilize substrate

The contamination with fungi was found under control only at first harvest where the severity was 16%. The contamination severity was found with 10000 ppm hydrogen peroxide, ash and control only at 2nd harvest where the severity was 4%, 12% and 28%, respectively. The contamination severity was 8%, 4%, 4%, 8%, 36%, 68% found under third harvest when substrate was treated with 10000 ppm hydrogen peroxide, 20000 ppm hydrogen peroxide, mancozeb , bleaching powder, ash and control, respectively. In other cases (T_3 and T_5) the spawn packets were free from contamination (Table 8).

Table 7. Effect of chemical treatment of substrate of oyster mushroom on contamination severity

Contamination severity (%)			
Treatments	**1st harvest**	**2nd harvest**	**3rd harvest**
T_1	0.0	4.0	8.0
T_2	0.0	0.0	4.0
T_3	0.0	0.0	0.0
T_4	0.0	0.0	4.0
T_5	0.0	0.0	0.0
T_6	0.0	0.0	8.0
T_7	0.0	12.0	36.0
T_0	16.0	28.0	68.0

T_1: 10000 ppm Hydrogen Peroxide　　T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide　　T_4: Mancozeb (100 ppm)

T_5: Metalaxyl + Mancozeb (100 ppm)　　T_6: Bleaching powder (100 ppm)

T_7: Ash (10000 ppm)　　T_0: Control

4.4.2 Effect of substrate sterilization with chemicals on growth and yield contributing characters of oyster mushroom

4.4.2.1 Days required for mycelium running

Days required for mycelium running of oyster mushroom varied significantly due to different substrate sterilization chemicals (Table 9). The highest days (22.20 days) required for mycelium running was recorded from T_0 which was statistically similar (21.20 days) to T_7 and closely followed by T_1 (20.60 days), whereas the lowest time (16.80 days) was found in T_3 which was statistically similar to T_2 (17.00 days).

4.4.2.2 Days required for primordia formation

Statistically significant variation was recorded in terms of days required for primodia formation of oyster mushroom due to different substrate sterilization chemicals (Table 9). The highest days (7.60 days) required for primodia formation was recorded from T_0 which was statistically similar to T_7 (7.20 days) and closely followed by T_6 (6.60 days), while the lowest time (5.20 days) was found in T_3 which was statistically similar to T_4 (5.60 days), T_2 and T_5 (6.00 days).

4.4.2.3 Days required from primordia initiation to 1st harvest

Different substrate sterilization chemicals showed statistically significant differences in terms of days required for primodia initiation to 1st harvest of oyster mushroom (Table 9). The highest days (6.60 days) required for primodia initiation to 1st harvest was observed from T_0 which was statistically similar to T_7 (6.40 days) and T_6 (5.80 days) and closely followed by T_5 (5.40 days). On the other hand the lowest time (3.80 days) was recorded in T_3 which was statistically similar to T_2 (4.20 days) and T_1 (4.60 days).

4.4.2.4 Days required for final harvest

Statistically significant variation was recorded in terms of days required for final harvest of oyster mushroom due to sterilization of substrate with chemicals (Table 9). The highest days (88.00 days) required for final harvest was found from T_3 which was closely followed (85.40 days) by T_2, whereas the lowest time (52.00 days) was observed in T_0.

Table 8. Effect of substrate sterilization with chemicals on days required for mycelium running, primordia formation, first and final harvest of oyster mushroom

Chemicals	Days required for mycelium running	Days required for primordia formation	Days required from primordia initiation to 1st harvest	Days required to final harvest
T_0	22.20 a	7.60 a	6.60 a	52.00 f
T_1	20.60 bc	6.20 cd	4.60 c-e	67.20 c
T_2	17.00 ef	6.00 c-e	4.20 de	85.40 b
T_3	16.80 f	5.20 e	3.80 e	88.00 a
T_4	18.60 d	5.60 de	4.80 cd	64.40 d
T_5	18.40 de	6.00 c-e	5.40 bc	68.60 c
T_6	19.20 cd	6.60 bc	5.80 ab	68.20 c
T_7	21.40 ab	7.20 ab	6.40 a	55.40 e
$LSD_{(0.01)}$	1.410	0.866	0.866	2.569
Level of significance	0.01	0.01	0.01	0.01
CV (%)	4.22	7.94	9.62	2.16

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

Legends

T_1: 10000 ppm Hydrogen Peroxide T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide T_4: Mancozeb (100ppm)

T_5: Metalaxyl + Mancozeb (100ppm) T_6: Bleaching powder (100ppm)

T_7: Ash (10000 ppm) T_0: Control

4.4.2.5 Length of stipe

Length of stipe of oyster mushroom varied significantly due to different substrate sterilization chemicals (Table 10). The highest length (5.17 cm) of stipe was recorded from T_3 which was statistically similar to T_2 (5.08 cm), T_5 (4.84 cm), T_1 (4.78 cm) and T_6 (4.76 cm). On the other hand, the minimum length (4.20 cm) of stipe was found in T_0 which was statistically similar to T_4 (4.52 cm) and T_7 (4.58 cm).

4.4.2.6 Diameter of stipe

Statistically significant variation was recorded due to treatment of substrate with different chemicals for diameter of stipe of oyster mushroom (Table 10). The highest diameter (1.17 cm) of stipe was observed from T_3 which was statistically similar to T_2 (1.14 cm) and closely followed by T_5 (1.09 cm) and T_6 (1.08 cm), whereas the lowest diameter (0.92 cm) of stipe was recorded in T_0.

4.4.2.7 Diameter of pileus

Diameter of pileus showed statistically significant differences due to sterilization of substrate with chemicals in terms of diameter of pileus of oyster mushroom (Table 10). The highest diameter (6.52 cm) of pileus was found from T_3 which was followed by T_5 (5.70 cm) and T_2 (5.36 cm), while the minimum diameter (3.74 cm) of pileus was observed in T_0 which was statistically similar to T_7 (4.30 cm).

4.4.2.8 Thickness of pileus

Different chemicals on substrate for sterilization showed statistically significant variation in terms of thickness of pileus of oyster mushroom (Table 10). The highest thickness (0.84 cm) of pileus was recorded from T_3 which was followed by T_2 (0.79 cm) and T_4 (0.75 cm), whereas the minimum thickness (0.64 cm) of pileus was found in T_0.

Table 9. Effect of substrate sterilization with chemicals on dimension of fruiting body of oyster mushroom

Chemicals	Stipe length (cm)	Diameter of stipe (cm)	Pileus diameter (cm)	Thickness of pileus (cm)
T_1	4.78 a-c	1.01 c	5.07 cd	0.70 d
T_2	5.08 ab	1.14 a	5.36 bc	0.79 b
T_3	5.17 a	1.17 a	6.52 a	0.84 a
T_4	4.52 cd	1.00 c	4.58 de	0.75 bc
T_5	4.84 a-c	1.09 b	5.70 b	0.72 cd
T_6	4.76 a-c	1.08 b	4.68 de	0.69 d
T_7	4.58 b-d	0.98 c	4.30 ef	0.68 d
T_0	4.20 d	0.92 d	3.74 f	0.64 e
$LSD_{(0.01)}$	0.478	0.041	0.564	0.041
Level of significance	0.01	0.01	0.01	0.01
CV (%)	5.82	3.19	6.52	3.93

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

Legends

T_1: 10000 ppm Hydrogen Peroxide

T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide

T_4: Mancozeb (100ppm)

T_5: Metalaxyl + Mancozeb (100ppm)

T_6: Bleaching powder (100ppm)

T_7: Ash (10000 ppm)

T_0: Control

4.4.2.9 Number of primodia/packet

Statistically significant variation was recorded due to treatment of substrate with different chemicals for sterilization in terms of number of primodia/packet of oyster mushroom (Table 11). The maximum number of primodia/packet (76.00) was observed from T_3 which was closely followed by T_2 (76.00), T_5 (75.60) and T_4 (73.40). On the other hand, the minimum number (59.20) was found in T_0 which was statistically similar to T_7 (61.20).

4.4.2.10 Number of fruiting body/packet

Number of fruiting body/packet of oyster mushroom showed statistically significant differences due to use different chemicals for substrate sterilization (Table 11). The maximum number (61.00) of fruiting body/packet was found from T_3 which was closely followed by T_5 (56.00) and T_2 (51.60), whereas the minimum number (43.80) was recorded in T_0 which was statistically similar to T_7 (44.00), T_4 (47.60).

4.4.2.11 Number of effective fruiting body/packet

The maximum number of effective fruiting body/packet (44.60) was observed from T_3 (Table 11) which was closely followed by T_5 (40.80), T_2 (39.60), T_6 (38.80) and T_1 (38.40) and they were statistically similar, while the minimum number (31.90) was recorded in T_0 which was statistically similar to T_7 (32.20)

4.4.2.12 Weight of individual fruiting body

Weight of individual fruiting body of oyster mushroom showed statistically significant differences due to use of different chemicals for substrate sterilization (Table 11). The maximum weight (4.54 g) of individual fruiting body was recorded from T_3 which was closely followed by T_2 (4.06 g) and the minimum weight (2.36 g) was found in T_0.

Table 10. Effect of substrate sterilization with chemicals on primordia and fruiting body of oyster mushroom

Chemicals	Number of primordia/ packet	Number of fruiting body/packet	Number of effective fruiting body	Weight of individual fruiting body (g)
T_1	70.60 c	49.00 c	38.40 bc	2.96 d
T_2	76.00 b	51.60 bc	39.60 bc	4.06 b
T_3	82.20 a	61.00 a	44.60 a	4.54 a
T_4	73.40 bc	50.40 c	37.80 c	3.02 d
T_5	75.60 b	56.00 b	40.80 b	3.46 c
T_6	71.20 c	47.60 cd	38.80 bc	3.24 cd
T_7	61.20 d	44.00 d	32.20 d	2.90 d
T_0	59.20 d	43.80 d	31.90 d	2.36 e
$LSD_{(0.01)}$	3.834	4.525	2.391	0.338
Level of significance	0.01	0.01	0.01	0.01
CV (%)	3.11	5.18	3.63	5.84

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

Legends

T_1: 10000 ppm Hydrogen Peroxide

T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide

T_4: Mancozeb (100ppm)

T_5: Metalaxyl + Mancozeb (100ppm)

T_6: Bleaching powder (100ppm)

T_7: Ash (10000 ppm)

T_0: Control

4.4.2.13 Biological yield (g)

Statistically significant variation was recorded in terms of biological yield of oyster mushroom (Table 12). The highest of biological yield (202.38 g) was found from T_3 which was followed by T_2 (160.38 g), while the lowest biological yield (75.12 g) was recorded in T_0. The rest of the treatments differed significant as compared to control.

4.4.2.14 Economical yield (g)

Economical yield of oyster mushroom showed statistically significant variation due to use of different substrate sterilization chemicals (Table 12). The highest of economical yield (191.32 g) was recorded from T_3 which was followed by T_2 (139.38 g), whereas the lowest economical yield (64.34 g) was found in T_0.

4.4.2.15 Dry yield (g)

Maximum dry yield (41.24 g) was found from T_3 which was followed by T_2 (32.30 g) and the lowest dry yield (15.10 g) was observed in T_0. The other treatments differed statistically compared to control.

4.4.2.16 Biological efficiency

The highest of biological efficiency (40.48%) was observed from T_3 which was followed by T_2 (32.11%), while the lowest (15.02%) biological efficiency was recorded in T_0. So, it was noted that the most effective chemicals were T_3 and T_2 followed by T_5.

Table 11. Effect of substrate sterilization with chemicals on different yield contributing parameters of oyster mushroom

Chemicals	Biological yield (g)	Economical yield (g)	Dry yield (g)	Biological efficiency (%)
T_1	113.52 e	99.24 e	28.24 cd	22.70 e
T_2	160.54 b	139.38 b	32.30 b	32.11 b
T_3	202.38 a	191.32 a	41.24 a	40.48 a
T_4	114.10 e	97.24 e	27.34 d	22.82 e
T_5	140.90 c	125.38 c	30.18 bc	28.18 c
T_6	125.50 d	111.44 d	21.24 e	25.10 d
T_7	93.22 f	71.32 f	18.26 f	18.64 f
T_0	75.12 g	64.34 g	15.10 g	15.02 g
$LSD_{(0.01)}$	8.455	6.156	2.634	1.691
Level of significance	0.01	0.01	0.01	0.01
CV (%)	3.81	3.20	5.69	3.81

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

Legends

T_1: 10000 ppm Hydrogen Peroxide
T_2: 20000 ppm Hydrogen Peroxide

T_3: 30000 ppm Hydrogen Peroxide
T_4: Mancozeb (100ppm)

T_5: Metalaxyl + Mancozeb (100ppm)
T_6: Bleaching powder (100ppm)

T_7: Ash (10000 ppm)
T_0: Control

4.4.3 Functional relationship between economic yield and number of primordia, weight of individual fruiting body and biological efficiency

The economic yield of oyster mushroom was correlated positively with number of fruiting bodies, weight of individual fruiting body and the biological efficiency. The value of correlation ($R^2 = 0.8593$) was linear and could be expressed by the regression equation by y = 6.0009x-191.39 (Figure 9 A). The relationship of between weight of individual fruiting body and economic yield are shown in Figure 9 B. 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 = 52.84x-64.108 (R^2=0.9404). Strong linear correlation (R^2 = 0.9921) was also observed between economic yield and biological efficiency, where the equation was y = 4.7185x- 9.7323, stated that the biological efficiency increased gradually at the rate of 4.728% (Figure 9 C).

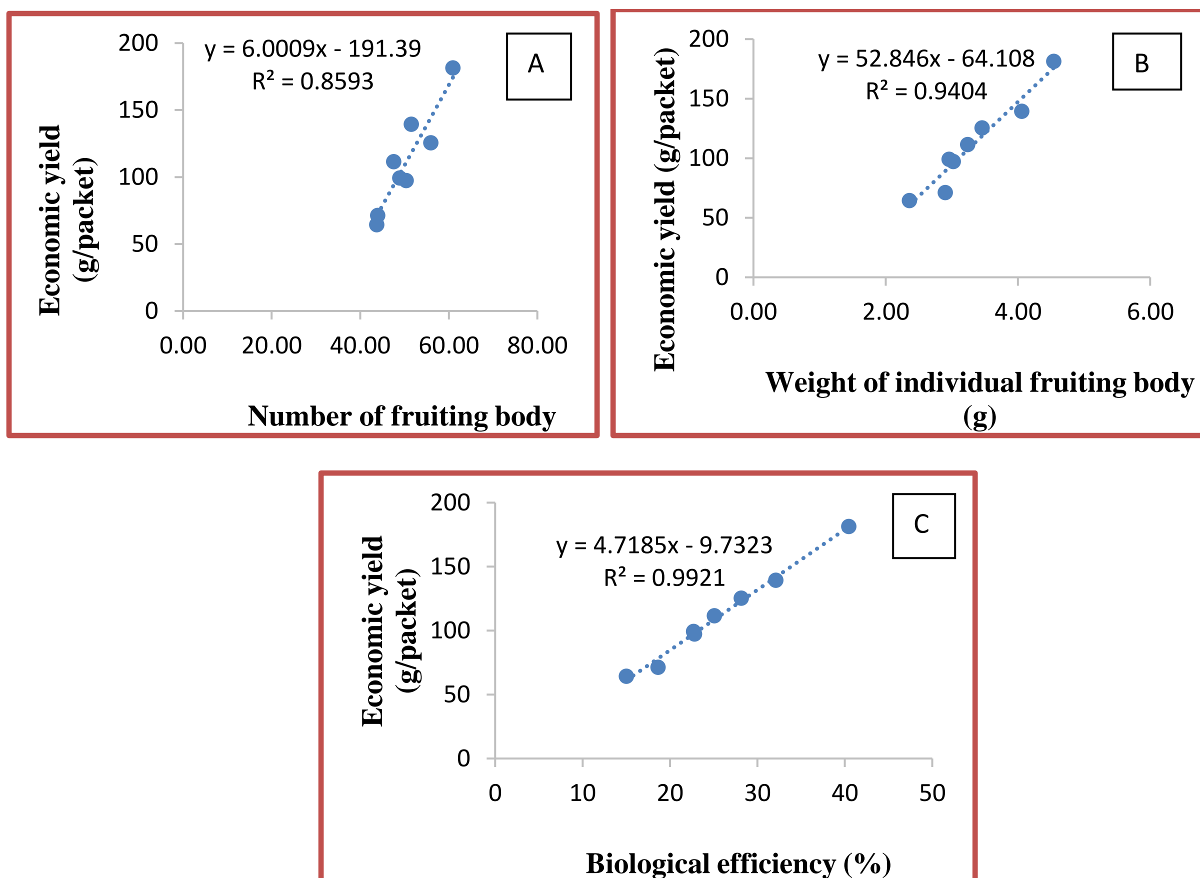


Figure 9. A. Relationship between economic yield and number of fruiting body

B. Relationship between economic yield and number of individual fruiting body

C. Relationship between economic yield and biological efficiency

CHAPTER V

DISCUSSIONS

The present experiment was conducted to evaluate the effect of different chemicals against the contaminants of oyster mushroom. Five contaminants namely *Sclerotium rolfsii*, *Trichoderma harzianum*, *Fusarium oxysporum, penicillium* sp. *and Aspergillus niger* were isolated and identified from contaminated substrates and more or less similar findings have been reported by previous scientists. According to Pervez *et al.,* (2010) 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. Oxaley (1985) and Earanna (1991) reported that the spawn contamination was occupied to be caused by the air-borne microflora present inside the incubation room. During the transfer of the mother spawn into the autoclaved bags, air carrying air-borne microflora might enter into the bags quickly and lead to contamination of spawn during incubation. Similar kind of results has been recorded in the present study. The cultural and morphological characteristics of vegetative and reproductive structures of the fungal colony indicated its close identity with *T. harzianum*. The fungus that isolated and identified as *T. harzianum* as described in the literature (Samuels *et al*., 2002 and Choi *et al*., 2003).

Sharma *et al., (*2007) noticed that one of the most common and destructive disease in mushroom cultivation is the green mould which was mainly caused by different species of *Trichoderma, Penicillium and Aspergillus*. Similar result was observed in the present experiment. 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. Komon-Zelazowska *et al.,* (2007) reported that, *Trichoderma* sp. infections 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) reported 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 more or less similar with 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.

In-vitro experiment revealed that chemicals tested in the experiment enhance mycelium growth of the *P. ostreatus* or the chemicals are not toxic to it. The findings about the inhibition of fungal growth *in vitro* condition are more or less similar to the findings of many other researchers. Hydrogen peroxide (30000 and 20000 ppm) and mancozeb+metalaxyl 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.

This findings is supported by Block (2001) and Rutala and Weber (2008), that hydrogen peroxide is active against a wide range of microorganisms, including bacteria, yeasts, fungi, viruses, and spores depending on the concentration and time of exposure. Hydrogen peroxide is increasingly used in number of medical, food and industrial applications for surface disinfection (Linley *et al*., 2012) its main advantage is its broad spectrum activity, their lack of environment toxicity following their complete degradation. Hydrogen peroxide is particularly interesting for its application in liquid but also vaporized form for antisepsis and for the disinfection of surfaces. In our findings, it has proved to be equally good for surface disinfection of spawn bags.

The present findings of the experiment differed with the findings of Mamza *et al*., (2008) reported that carbendazim and mancozeb showed maximum inhibition when their concentration was raised under *in vitro* condition against *Fusarium pallidoroseum.* This finding is differ from Singh *et al.* (2014), that all employed concentrations of carbendazim and carbendazim (12%) + mancozeb (63%) totally suppressed *A. flavus* and *A. niger*. Effect of all applied concentration of mancozeb, copper oxychloride and thiophanate methyl showed least mycelial inhibition, but higher dose suppressed both fungi. Thapa and Seth (1977) as they reported that carbendazim exhibited minimum efficacy against mushroom and mancozeb showed the maximum inhibition of *Pleorotus ostreatus* mycelium.

Results of the *in vivo* test indicated that contaminations of spawn packet, days required for completion of mycelium running, primordia formation and first harvest were reduced over control when substrate was sterilized with chemicals. Sterilization of substrate with different chemicals increased the duration of harvesting and amount of yield. Bitner (1972) noticed

type of substrate was found to have influence on spawn contamination. Paddy grain based spawn recorded significantly lowest (15.00%) contamination as compared to wheat grain based (30.00%) spawn. The present findings of the experiment differed with the findings of Sarker *et al*., (2011) where they observed that the number of effective fruiting bodies was the highest (21.00) in autoclaved sawdust with pasteurized straw. Almost similar results have been reported by many other researchers. Sarker (2004) observed that duration of primordia initiation of oyster mushroom was significantly lower as compared to control, when water or chemical sterilizing agent was used. Pervez *et al.,* (2010) reported that in spawn packets having substrate treated with 3% H2O2 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.

The finding of the present experiment about yield and yield attributes more or less matches with the results of the following researchers. Akhter (2017) observed that minimum days (15.4) required for completion of mycelium running, minimum days (5.0) for primodia formation, minimum days (6.0) for 1st harvesting in 30000 ppm hydrogen peroxide treated rice straw and biological yield (216.8 g), economic yield (210.8), dry yield (40.6), biological efficiency (43.3%) obtained from 30000 ppm hydrogen peroxide treated rice straw which is more or less similar with the present findings.

Ali *et al*., (2004) reported that steam pasteurization gave maximum mycelia growth which completed in shortest period of time. Formalin treatment behaved poorly as the species took maximum time to complete their mycelial growth and final harvest. Mahjabin *et al*., (2011) reported minimum days (13.25) required for completion of mycelial growth in pasteurized rice straw substrate whereas the highest days (31.75) required for mycelial growth in sugarcane baggase when pasteurized with chemicals. This finding is also supported by Zehad *et al*., (2012) that noticed significantly the highest number of fruiting bodies per packet (81.66), diameter of pileus (4.67cm), highest yield (680.50g) per spawn packet was recorded in 3% H2O2 treated spawn packet. Pervez *et al.,* (2012) observed that the highest occurrence of contamination was found in 1% hydrogen peroxide treated spawn packets and the lowest occurrence was found in 3% hydrogen peroxide 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.

CHAPTER VI

SUMMARY AND CONCLUSION

A series of experiments were conducted to isolate, identify and control the associated microorganisms of oyster mushroom substrate. *Pleurotus ostreatus* growing 40 spawn packets were collected randomly from Mushroom Culture Centre, Savar. A total 5 fungi namely *Sclerotium rolfsii*, *Trichoderma harzianum*, *Fusarium oxysporum, penicillium* sp. *and Aspergillus niger* were isolated and identified. Duration for the completion of mycelium over petridishes was recorded. Among the tested chemicals, higher inhibition of radial mycelial growth was observed with 30000 ppm hydrogen peroxide followed by Mancozeb+Metalaxyl (100 ppm). The result of *in-vitro* evaluation of fungicides revealed that out of six chemicals tested against five fungal contaminants, 30000 ppm hydrogen peroxide was found to be more effective as it showed maximum inhibition against the growth of *Trichoderma harzianum* (72.41%), *Fusarium oxysporum* (90.15%), *Sclerotium rolfsii* (93.46%), *Aspergillus niger* (90.74%) and *Penicillium* (92.96%).

Percent contamination of fungi gradually increased from mother spawn stage to 3rd flash stage. Severity of contamination was observed 36% in ash treated substrate and 68% in control at 3rd harvest .The chemical treatments of substrates with hydrogen peroxide (30000, 20000, 10000 ppm), mancozeb (1000 ppm), mancozeb+metalaxyl (1000 ppm), bleaching powder (100 ppm) and ash (10000 ppm) showed good antifungal activity. Among these chemicals, 30000 ppm hydrogen peroxide showed maximum inhibitory effect against the growth competitor moulds. Maximum biological efficiency (40.48%) obtained from 30000 ppm hydrogen peroxide treated packets.

Time required for completing mycelium running varied in different chemicals. The maximum days (22.20 days) required for mycelium running was recorded from T_0 whereas the minimum days (16.80 days) found under hydrogen peroxide (30000 ppm). The maximum days (6.60 days) required for primodia initiation to 1st harvest was observed from control whereas lowest days (3.80 days) was observed under hydrogen peroxide (30000 ppm). 30000 ppm hydrogen peroxide showed significantly best performance with the total number of fruiting body and yield per spawn packets. The highest length (5.17 cm) of stipe was recorded from 30000 ppm hydrogen peroxide whereas the minimum length (4.20 cm) of stipe was found in control. The highest of biological yield (202.38 g) was found from 30000 ppm

hydrogen peroxide which was followed by 20000 ppm hydrogen peroxide (160.38 g), while the lowest biological yield (75.12 g) was recorded in control.

From the present research work it may be concluded that,

- Among the fungal contaminants total 5 fungi namely *Sclerotium rolfsii, Trichoderma harzianum, Fusarium oxysporum, penicillium* sp. *and Aspergillus niger* were isolated and identified.
- Under *In-vitro* evaluation of chemicals, maximum inhibition was observed by 30000 ppm hydrogen peroxide on *Trichoderma harzianum* (72.41%), *Fusarium oxysporum* (90.15%), *Sclerotium rolfsii* (93.46%), *Aspergillus niger* (90.74%) and *Penicillium* (92.96%) whereas 30000 ppm hydrogen peroxide was found most effective against *Sclerotium rolfsii.*
- Minimum contamination of spawn packets was observed when the substrate was treated with 30000 ppm hydrogen peroxide.
- Hydrogen peroxide (30000 ppm) can be used for management of oyster mushroom contaminants which showed the effective antifungal activity against associated fungi as well as good yield of oyster mushroom.

CHAPTER VII

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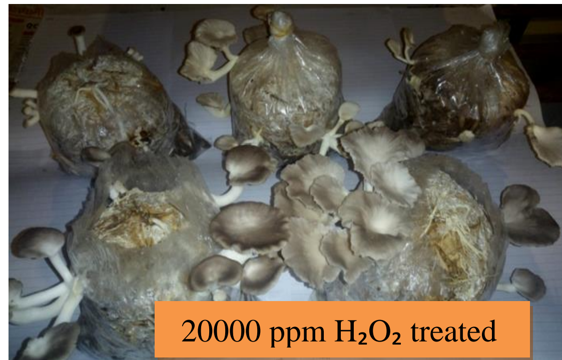
CHAPTER VIII

APPENDICS



Appendix 1. Fruiting bodies of oyster mushroom in the treated spawn packets