CONTAMINANTS AND YIELD OF DIFFERENT STRAINS OF MAPLE OYSTER MUSHROOM (*Pleurotus cystidiosus*) IN SELECTED SUBSTRATES

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This is to certify that the thesis entitled, '**CONTAMINANTS AND YIELD OF DIFFERENT STRAINS Of MAPLE OYSTER MUSHROOM (*Pleurotus cystidiosus*) IN SELECTED SUBSTRATES**' submitted to **the Department of Plant Pathology**, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207 in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY**, embodies the results of a laboratory research work carried out by Registration No. **18-09163,** under my direct supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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CONTAMINANTS AND YIELD OF DIFFERENT STRAINS Of MAPLE OYSTER MUSHROOM (Pleurotus cystidiosus) IN SELECTED SUBSTRATES

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ABSTRACT

The experiment was carried out at the mushroom culture house of Mushroom Development Institute, Saver, Dhaka and central laboratory of SAU during the period from October 2019 to March 2020. The experiment was conducted to compare different strains of maple oyster mushroom on contaminants, growth and yield contributing characters. Seven strains of *Pleurotus cystidiosus*, viz. Pcys-1, Pcys-2, Pcys-3, Pcys-4, Pcys-5, Pcys-6 and Pcys-7 and three different substrates S_1 (Saw dust), S_2 (Rice straw), S_3 (Saw dust + Rice straw) were used in this experiment. The experiment was laid out in Completely Randomized Design (CRD) with four replications. *Trichoderma* sp, *Alternaria* sp and *Aspergillus niger* were identified as contaminants, where green mold was detected as the major one. The highest contamination incidence (100%) was observed in S_1P_6, S_1P_7 and S_3P_2, whereas the lowest incidence (60%) was recorded from S_1P_1. The lowest days (7 days) required for mycelium running on spawn was found in S_1P_1, S_1P_2, S_1P_3, S_1P_4 and S_1P_5. No mycelium running was observed in Pcys-6 and Pcys-7. The highest biological yield (98.50 g), economic yield (95.75 g) and biological efficiency (53.25%) was found from S_2P_1, whereas the lowest biological yield (65.25g) was found from S_3P_4. The highest diameter (7.13 cm) of pileus was recorded from S_3P_1, whereas the lowest diameter (4.13 cm) of pileus was found in S_1P_2. Rice straw showed better performance followed by saw dust and combination of the both rice straw and saw dust during cultivation.

Key words: Pcys, Substrate, Biological yield, Economic yield, Biological Efficiency

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LIST OF SYMBOLS AND ABBREVIATIONS

ABBREVIATION	FULL WORDS
%	Percentage
PDA	Potato Dextrose Agar
PCYS	*Pleurotus cystidiosus*
Sp	Species
et al.	and others (at ell)
Cm	Centimeter
SAU	Sher-e-Bangla Agricultural University
J.	Journal
DAI	Days after Incubation
CRD	Complete Randomized Design
BE	Biological Efficiency
G	Gram
MDI	Mushroom Development Institute
Viz.	Namely
&	And
℃	Degree Celsius
Ml	Millilitre
etc.	Etcetera
L	Liter
MCH	Mushroom Cultre House

CHAPTER I

INTRODUCTION

Pleurotus cystiodiosus, is a common edible mushroom. It is one kind of oyster mushroom. It was first described by k. Millar in 1969 from a maple in Indiana (Miller, 1969; Pollack & Millar 1979). It is also known as *Pleurotus abalonus,* commercially cultivated in Asia and many other parts of the world. It belongs to Basidiomycota fungi and possesses on class *Agaricomycetes,* order *Agaricales,* family *Pleurotaceae*, genus *Pleurotus*.

Pleurotus spp are characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. Pileus of *Pleurotus cystidiosu*s convex to hemispheric or plane, measuring 2-5 cm wide and cream to off-white in colour (Hanelt, 2001; Croan, 2004; Lechner *et al*., 2004; Abdullah *et al*., 2012; Usami *et al*., 2014). Edge of pileus is often irregular and the gills often broad, widely spaced and sometimes become irregular and is thick and relatively short.

The mycelium of *Pleurotus cystidiosus* resembles an oyster strain – white, racing linearly, soon fluffy white and aerial. But it produces darkly pigmented arthroconidia forming a black pigment on the mycelium or basidiomata. As it grows outwards, black droplets form, radiating outwards from the centre as the mycelium matures. These are coremia – stalk-like cells whose tops are fitted with liquid droplets of black spores (Croan, 2004; Bao *et al*., 2004; Lechner *et al*., 2004; Selvakumar *et al*., 2008; Abdullah *et al*., 2012; Usami *et al*., 2014; Stamets, 2011). The genus of oyster mushroom (*Pleurotus*) is represented by many species (Bao *et al*. 2004). *Pleurotus cystidiosus* has 7 strains, they are Pcys-1, Pcys-2, Pcys-3, Pcys-4, Pcys-5, Pcys-6, and Pcys-7. *Pleurotus cystidiosus* strains are distinct from each other biologically, physically, or chemically.

The pileus and stems of *Pleurotus cystidiosus* are edible. In addition to the culinary qualities of fruiting bodies, it has very good nutritional value (proteins, fibre, minerals and vitamins) and it has high amount of health promoting

biologically active substance. It has antitumor, anti-inflammatory, anticleric, antiatherosclerotic, antibacterial, antiviral, antifungal, immunomodulatory and hepato protective properties. It can minimize blood sugar levels and the effect of blood cholesterol. This mushroom is low in calories due to the limited content of lipids (Manzi and Pizzoferrato, 2000; Wasser, 2002; Croan, 2004; Thekkuttuparambil and Kainoor, 2007; Karaman *et al*., 2010; Abdullah *et al*., 2012; Patel *et al*., 2012; Lau *et al*., 2013; Siwulski *et al*., 2014; Usami *et al*., 2014).

Pleurotus cystidiosus can be grown on substrates prepared on a base of straw and various types of agricultural, horticultural, forestry and textile industry waste (Cohen *et al*., 2002; Croan, 2004; Lau *et al*., 2013; Usami *et al*., 2014). It is cultivated mainly in Asia, particularly in China, Thailand and Taiwan (Hanelt, 2001; Stamets, 2011; Usami *et al*., 2014). Earlier documentation reveals that oyster mushrooms can be grown on a variety of waste materials, such as: various types of straw and sawdust, rice straw, paper waste, cotton waste, chopped and corn stover, waste from the production of palm oil, tea leaves, chopped cocoa pods (Croan, 2004; Lau *et al*., 2013; Usami *et al*., 2014).

The most widely used substrates for oyster mushroom cultivation in Asia are rice straw and saw dust (Akhter, 2017; Thomas *et al*., 1998). Using such crop residues as a mushroom substrate would subsequently convert them into a more protein-rich biomass and influence the mushroom yields. Crop residues such as straw are characterized by the predominance of lingo-cellulose with cellulose, hemi-cellulose and lignin as the main components (Yildiz *et al*., 2002; Das and Mukherjee, 2007; Jonathan *et al*., 2012). There are usually some differences in the nutrient content of the mushroom cultivated on different substrates (Mabrouk and Ahwanyi, 2008; Akinyele *et al*., 2011; Kulshreshtha *et al*., 2013). However, this changes in nutritional content never found to affect their edibility.

Mushroom is now-a-days one of the promising concepts for crop diversification in Bangladesh. The climatic condition of Bangladesh is suitable for mushroom cultivation. It requires short time, little capital and easy technique for cultivation.

This is why all types of people like male and female, youth and old even children can easily participate in its cultivation. Its cultivation can transfer as a cottage industry and create a good opportunity for export. Therefore, it can generate huge scope of employment opportunities for unemployed people.

Bangladesh is developing country with large number of population, but the availability of cultivable land is decreasing day by day. In such situation, mushroom production can play an important role because it grows fast and does not require any fertile land. Among various waste materials sawdust and rice straw are most commonly used for mushroom cultivation in Bangladesh now-a-days.

Substrate is an important item for growing mushroom. It grows on agro wastes like wheat or paddy straw, banana leaves, sugarcane bagasse and leaves, wheat bran, rice husk, sawdust, straw etc. (Sarker *et al*., 2007). Yoshida *et al*. (1993) reported the highest yield of mushroom in substrates (chopped straw or sawdust) mixed with wheat bran, rice bran, and bean curd at the rate of 45%. Sarker *et al*. (2008) achieved higher yield on waste paper and wheat straw using wheat bran and rice bran as supplements.

Despite of several advantages of its cultivation, mushroom cultivation has not picked up to the desired momentum due to the occurrence of contamination by several competitor microflora in the substrate. They reduce the mushroom yield by competing for oxygen, water, space and nutrition. In addition to microflora being competitive, some have been shown to produce metabolites which directly inhibit the growth of mushroom mycelia during spawn run on the substrate.

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

- to investigate and compare the performance of different strains of maple oyster

- to assess the incidence of contaminants and identify the competitive microorganisms
- to determine suitable substrate for cultivation of maple oyster mushroom

CHAPTER II

REVIEW OF LITERATURE

In recent years, the cultivation of mushroom for food or medicine has increased tremendously. The success of mushroom cultivation and its yield depend to large extent on the purity and quality of the spawn used. There are many types of spawn, different types of substrates that are used for the production of the oyster mushroom spawn. During the preparation of oyster spawn it is infected by the pathogens and reduces quality of the spawn packet. 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 becomes contaminated from many sources during production and processing, including the humans harvesting the crop. Bacteria, yeasts and moulds cause most problems. The information on substrate spawn production and isolation and identification the pathogens infecting spawn of oyster mushroom and growth and yield of oyster mushroom presented here.

2.1 Production of spawn of oyster mushroom on different substrates

Jegadeesh *et al*., (2018) found that Cultivation of the oyster mushroom, *Pleurotus* spp., has increased greatly throughout the world and commonly grown on pasteurized agro wastes. It can be cultivated on a wide variety of lignocellulosic organic waste materials. Mushroom cultivation is a simple, low cost and environmentally friendly technology for the utilization of rural and agro industrial residues. The substrate used for the cultivation of one such species is pink oyster mushroom, *Pleurotus djamor* var. *roseus*, which is becoming important as this is an unfamiliar edible mushroom and can be cultivated easily throughout the year.

Different substrates viz. paddy straw, sugarcane bagasse, coir pith, sorghum straw, ragi straw and mixed bed were used for the cultivation of pink oyster

mushroom. Primordium initiation was observed on 17-22nd day after spawning. Maximum yield of *P. djamor* var. *roseus* was obtained using paddy straw.

Akhter, K. (2017) surveyed and recorded that 55.7% mushroom growers used rice straw and 21.6% used saw dust for cultivation of oyster mushroom in rural areas of Bangladesh.

Kumbhar (2012) showed that mycelium of *P. eous* indicated marked preference for cereal grains over pulses and crop residues. Among the cereals, ragi grain was colonized the best only in 6 days, followed by maize, pearl millet, sorghum, wheat and paddy grains.

Shahu *et al*., (2012) conducted an experiment on screening of suitable grains substrates for spawn development 10 of *P. eous* and showed that sorghum (7.33 days), paddy grain (8.66 days) and maize grains (9 days) took significantly less time for spawn development.

Narh *et al*., (2011) showed that combination of sorghum and millet grains in a 3:1 (w/w) ratio showed fastest mycelial growth of *P. ostreatus* (16 days) followed by sorghum only recording a value of 18 days.

Senthilnambi *et al*., (2011) revealed that sorghum grains was the most suitable substrate for early spawn run, which took only 13.7 days for hundred per cent mycelial growth of *Calocybe indica*.

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

of colors. They can be white, yellow, brown, tan and even pink. They have a unique scent that is often described as sweet like anise or licorice (liquorice).

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

Stanely and Waddu (2010) used various substrate s like wheat, yellow maize, guinea corn, millet, red sorghum and white maize for spawn production of two oyster mushroom species viz., *P. tuber-regium* and *P. pulmornious* and they found that white maize showed maximum growth rate whereas wheat showed least mycelial extension for both species.

Pathmashini *et al*., (2008) conducted an experiment to examine the effect of different types of spawn on oyster mushroom *(P. ostreatus*) production using sawdust. Locally available grains of kurakkan (*Eleusine coracana*), maize (broken) (*Z. mays*), sorghum (*S. bicolor*), and paddy (*Oryza sativa*) were used for spawn production. The kurakkan spawn caused an acceleration of spawn running. The fastest spawn running of 21 ± 1 days was seen for kurakkan spawn.

Sangeetha *et al*., (2008) conducted an experiment to evaluate various substrates for spawn production and its effect on sporophore yield of oyster mushroom. The substrates comprised of proso millet, pearl millet, chaffy paddy grain, horse gram, maize, sorghum, barnyard millet, wheat, finger millet, kodo millet and foxtail millet. Sorghum grain was found to be the best medium for spawn running followed by wheat. The number of days required for completion of spawn run was less (10.67 days) in sorghum grain, while it was 12 days in wheat.

Sharma (2003) conducted an experiment to determine the best substrate for production of *Pleurotus djamor* spawn. The cereal grains of jowar (Sorghum bicolor), kutki (*Panicum miliare* [*Panicum sumatrense*]), kodo (*Paspalum scrobiculatum*), maize (*Zea mays*), and wheat (*Triticum aestivum*) were evaluated as spawn substrate and found that shortest period for spawn development (8 days) was obtained with kutki grains indicating its suitability for efficient spawn production.

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

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

2.2 Isolation and identification the contaminants from contaminated spawn packets of oyster mushroom

Urmi, F. J., (2019) carried out an experiment to isolate contaminants from contaminated packets of oyster mushroom and found 5 fungi namely *Sclerotium rolfsii*, *Trichoderma harzianum*, *Fusarium oxysporum*, *penicillium* sp. and 20 *Aspergillus niger*. She also found that, 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.

Akhter, K., (2017) experimented the occurrence of contaminants in mushroom packets and 8 contaminants namely *Trchoderma*, *Rhizopus*, *Aspergillus*, *Penicillium*, *Alternaria*, *Ceratocytis*, *Coprinus*, *Chaetomium* sp. were found to be associated where green mold was detected as the major one.

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*.

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%) *T. koningii* (5.5%) and the majority of the isolates (65.5%) belonged to an unidentified species of *Trichoderma* causing disease in mushrooms.

Wickremasinghe *et al*., (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.

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*.

2.3 Growth and yield of maple oyster mushroom

Howlader *et al*., (2011) observed significant variation in growth, yield and yield contributing characters of *Pleurotus cystidiosus* strains. The highest mycelial growth (0.58 cm/day) was observed in Pcys-4, whereas the lowest mycelial growth (0.22 cm/day) was observed in Pcys-3. The highest biological and economic yield, 196.3 and 189.0 g/packet respectively were obtain from Pcys-1 and the lowest biological and economic yield were observed in Pcys-6. The number of effective fruiting bodies was the highest (37.25) in Pcys-1, while the weight of individual fruiting body was the highest (26.88 g) in Pcys-6.The highest length of stipe (4.95 cm) and thickness of pileus (1.35 cm) were observed in Pcys-1. The strain also showed the highest biological efficiency (BE) among the strains.

Shelly *et al*., (2010) conducted a study to determine the effect of rice straw on the growth and yield of *Pleurotus cystidiosus.* The minimum days required from opening to primordia initiation (4.75) and first harvest (8.00) were recorded in Pcys-1 on 1500g rice straw. The highest number of effective fruiting bodies (103.50), length of the stipe (9.19 cm), diameter of stipe (1.70 cm) and diameter

of pileus (11.63 cm) found in Pcys-1 on 1500g rice straw. The biological yield was increased with increasing amount of rice straw. The highest biological yield 870.30g and lowest biological yield 164.00g was recorded in Pcys-1 on 1500g and 250g rice straw respectively. The highest biological efficiency (BE) (207.40 %) and lowest BE (135.50 %) was observed in Pcys-2 on 250g and 1000g rice straw respectively.

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

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

Namdev *et al*., (2006) conducted a study to determine the effect of different straw substrates on spawn growth and yield of oyster mushroom. The number of days required for spawn run was significantly less (14 days) in case of gram straw, parthenium straw, sugarcane straw and wheat straw, compared with 20 days for sunflower stalk, mustard straw and paddy straw. Yield was very poor on parthenium straw (95 g/500 g dry substrates) and it was highest on paddy straw

(666 g/500 g), followed by wheat straw and mustard straw (427 and 400 g/500 g respectively).

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

Shah *et al*., (2004) carried out an experiment to investigate the performance of oyster mushroom on the following substrates: 50 % sawdust + 50 % wheat 16 straw, 75 % sawdust + 25 % leaves, 50 % wheat straw + 50 % leaves, 100 % sawdust, 100 % wheat straw and 100 % leaves. The temperature was kept at 25ºC for spawn running and 17-20ºC for fruiting body formation. The time for the completion of mycelial growth, appearance of pinheads and maturation of fruiting bodies on different substrates were recorded. The number of fruiting bodies and the biological efficiency of substrates were observed. The results show that spawn running took 2-3 weeks after inoculation, while small pinhead-like structures formed 6-7 days after spawn running. The fruiting bodies appeared 3-6 weeks after pinhead formation and took 27-34 days later after spawn inoculation. Sawdust at 100 % produced the highest yield (646.9 g), biological efficiency (64.69%) and the number of fruiting bodies (22.11). Therefore, sawdust is recommended as the best substrate for oyster mushroom cultivation.

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 fruiting bodies were sun-dried and analyzed for various nutritional parameters. Considerable variation in the composition of fruit bodies grown on different substrates was observed.

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.

Amin (2004) in his experiment reported 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.

Baysal *et al*., (2003) conducted an experiment to spawn running, pin head and fruit body formation and mushroom yield of oyster mushroom (*Pleurotus ostreatus*) on waste paper supplemented with peat, chicken manure and rice husk (90+10; 80 + 20 W:W). The fastest spawn running (mycelia development) (15.8 days), pin head formation (21.4 days) and fruit body formation (25.6 days) and the highest yield (350.2 g) were realized with the substrate composed of 20% rice husk in weight. In general, increasing the ratio of rice husk within the substrate accelerated spawn running, pin head and fruit body formation and resulted increased mushroom yields, while more peat and chicken manure had a negative effect on growing.

CHAPTER III

MATERIALS AND METHOD

The experiment was carried out to study the comparison among different strains of maple oyster mushroom on contaminants, growth and yield contributing characters grown on different substrates like sawdust, rice straw and mixture of saw dust and rice straw and an examination was made to isolate and identify different weed fungi associated with colonized substrate of oyster mushroom. This chapter deals with a brief description on location and design of experiment, 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 Experimental location

The field experiment was conducted at Mushroom Culture House (MCH) of Mushroom Development Institute, Savar, Dhaka and laboratory experiment was done in Plant Pathology laboratory, Sher-e-Bangla Agricultural University, Dhaka for isolating and identifying different microorganisms.

3.2 Duration of the experiment

The experiment was carried out during the period from October 2019 to March 2020

3.3 Spawn production

3.3.1 Collection of materials for spawn production

Saw dust, Rice straw were collected from the local market of Savar. Mother culture of *Pleurotus cystidiosus* strain, neck and 7×10 inch polypropylene bag were collected from Mushroom Development Institute, Savar, Dhaka.

3.3.2 Design and layout of the experiment

The experiment was laid out in completely randomized design (CRD) with four replications. The experiments with three substrates with four replications were conducted to achieve the desired objectives.

3.3.3 Preparation of mother culture

To prepare mother culture of *Pleurotus cystidiosus* strains saw dust was used as media of mother culture. In saw dust media water was added to make the moisture content 60% and CaO was added at the same ratio. Then the media were filled into polypropylene bags (18cm × 25cm) at 250-300 g/bag and their mouths were plugged by inserting absorbing cotton without neck. The packets were autoclaved at 15 Ibs. Pressure and 121℃ temperature for 45 minutes. Then pure culture of different Pcys strais were inoculated to these bags, after inoculation their mouths were plugged by inserting absorbing cotton with neck. All operations were done under sterile condition in a clean bench. The bags were kept in rack at room temperature. After 10 to 15 days the mother culture became white due to complete the mycelium running and then it was ready for spawning of spawn packets.

3.3.4 Preparation of substrates

Sawdust, rice straw and mixture of these two materials were used as substrates. The substrates were prepared by pasteurization method. In case of SD, water was mixed very well and the mixture was poured poured into cribriform nylon bag. In case of rice straw, the straw was chopped to 4-5 cm length and then poured into cribriform nylon bag. The bags were submerged in water for sometimes and then drained out the excess water. After that both the bags containing saw dust and rice straw were kept in a pasteurization chamber at 60-65℃ for one hour. The bags were kept in same place for 18-20 hours to cool down slowly. After 20 hours the prepared sawdust and straw were spread over polythene sheet in open place to reduce moisture 63%. These substrates were ready for spawn packet preparation.

Plate 1. A. Saw dust, B. Rice straw, C. Mixture of saw dust and rice straw D-E. Mushroom mother mix with substrate

3.3.5 Preparation of spawn packets

According to substrates and strains combination prepared substrates and 10% mother culture were mixed thoroughly and filled into 18cm × 25cm polypropylene bags at 500 g/bag. The mouths of the filled polypropylene bags were plugged by inserting absorbing cotton with the help of plastic neck and rubber band.

3.3.6 Incubation of spawn packet

After spawn packets preparation, packets were incubated in a dark room at a temperature ranging from 22-25ºC, 90% relative humidity was maintained till the mycelium running was complete. The spawn packets were placed on iron shelves and observed regularly with 4 days interval to record prevalence of contaminating fungi. When the substrates were fully covered with white mycelium, the rubber band, absorbing cotton plug and plastic neck of the mouth of spawn packets were removed then the mouth of the bags were tightly wrapped with rubber band. Then these spawn packets were transferred to the culture

3.3.7 Cultivation of spawn packets at culture house

Polythene bags were cut in "D" at two ends with a blade and opened by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a blade to remove 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 70-80% by spraying water 3 times a day and the temperature about 21-27°C through air cooler.

3.3.8 Harvesting of produced mushrooms

Mushroom became matured 5 or 6 days after pin head formation depending on substrates and strains combination. Matured fruiting bodies were harvested by twisting to uproot from the base.

Plate 2. A. Incubation of mushroom mother, (B,C and D). Incubation of spawn packets of different substrates, E. Spawn packets transfer to the culture house

3.4 Data collection

Data were taken on the following parameters

3.4.1 Days required for completing mycelium running

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

3.4.2 Days required for the pinhead formation

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

3.4.3 Days required to pinhead formation to 1st harvest

Days required from pinhead formation to first harvest was recorded.

3.4.4 Days required for total harvest

Days required for total harvest was recorded.

3.4.5 Data on yield contributing parameters

Number of fruiting body/packet, effective number of fruiting body/packet were counted.

3.4.6 Dimension of fruiting body (Stalk and Pileus)

Length and width of stalk and diameter and thickness pileus were measured using a measurement scale

3.4.7 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.

Plate. 3 A-B. Pinhead formation, C-D. Fruiting body of different strains on different substrates, E. Matured fruiting body, F. Measurement of whole cluster of fruiting body

3.4.8. Economical yield (g)

Economical yield per 500 g packet was measured by weighing the whole cluster of fruiting body by removing the lower hard and dirty portion.

3.4.9 Biological efficiency

The biological efficiency was analyzed to determine the suitability of the tested substrates. It depends on the amount of dry substrate used in this experiment Biological efficiency was determined by the following formula:

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

3.4.10 Percent contamination incidence

Percent disease incidence was calculated by using following formulae

$$\text{Percent disease incidence} = \frac{\text{No. of contaminated spawn packet}}{\text{Total. No of Spawn packet}} \text{ X } 100$$

3.5 Analysis of data

The data was recorded for each character from the experiment was analyzed statistically using Statistix 10 computer program.

3.6 PDA media preparation, isolation, purification and identification of contaminants

3.6.1 Composition and preparation of PDA media

Ingredients	Amount (Per liter)
Potato slices:	200 gm
Dextrose:	20 gm
Agar:	20gm
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 45 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% ethyl 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.

3.6.2 Isolation and purification of contaminants from contaminated spawn

Some infected spawn packets were randomly selected for identification of contaminants. The fungi were then sub-cultured on fresh PDA medium for identification. Mycelium was taken from infected spawn material which was placed on Potato Dextrose Agar (PDA) using a sterilized inoculating needle. The plates were incubated at room temperature (27 ± 5 ºC) till fungal growth was visible.

3.6.3 Identification of contaminants

Identification of the contaminants 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).

CHAPTER IV

RESULTS

The experiment was conducted to compare among different strains of Maple Oyster mushroom *(Pleurotus cystidiosus*) on contaminants, growth and yield contributing characters. Data of the different parameters analyzed statistically and the results have been presented in the Tables and Figures. The results of the present study have been presented and discussed in this chapter under the following headings.

4.1 Identified contaminants microflora from contaminated spawn packets

Different types of fungal contaminants were found in contaminated spawn (Plate 4. A-H). Some were green in colour, some were blackish and in some packets were incomplete colonization or lack of complete mycelium running. *Trichoderma* sp. and *Aspergillus niger* and *Alternaria sp*. were detected as contaminants and another fungus contaminants was observed but not identified from these contaminated spawn packets. Among these contaminants, *Trichoderma* was identified from most of the contaminated packets.

Plate 4. Different contaminated spawn packets (A-H)

4.1.1 *Trichoderma* sp

Green color growth of mycelium was observed in spawn packet due to heavy sporulation of causal agent. Conidia were one-celled, ellipsoidal. They were typically green with smooth surface. The conidiophores were branched. Lateral side branches produced from main branches. Normally, the branches formed at or near 90° with respect to the main branch. Paired branches had a pyramidal structure. The typical conidiophore terminated with one or a few phialides that usually arose directly from the axis near the tip (Plate 5. A-B).

4.1.2 *Aspergillus niger*

Aspergillus was found from contaminated the spawn packets. *Aspergillus niger* produced black colored spores so it was called black mold. Pure culture of *Aspergillus niger* was prepared from contaminated spawn and the shape was similar to an onion flower stalk and their spores had inside the globose head on these outer surface. Fungal colonies initially 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. The cultural and morphological characters of vegetative and reproductive structures of the fungal isolate indicated its close identity with *Aspergillus niger* (Plate 5. C-D).

4.1.3. *Alternaria* sp.

Conidia of *Alternaria* were ovoid to obclavet, darkly pigmented, muriform smooth or roughened (Plate 5. E-F).

4.1.4 Unknown fungus

An unknown fungus (Plate 5. G-H) was isolated from some infected spawn packets but not identified.

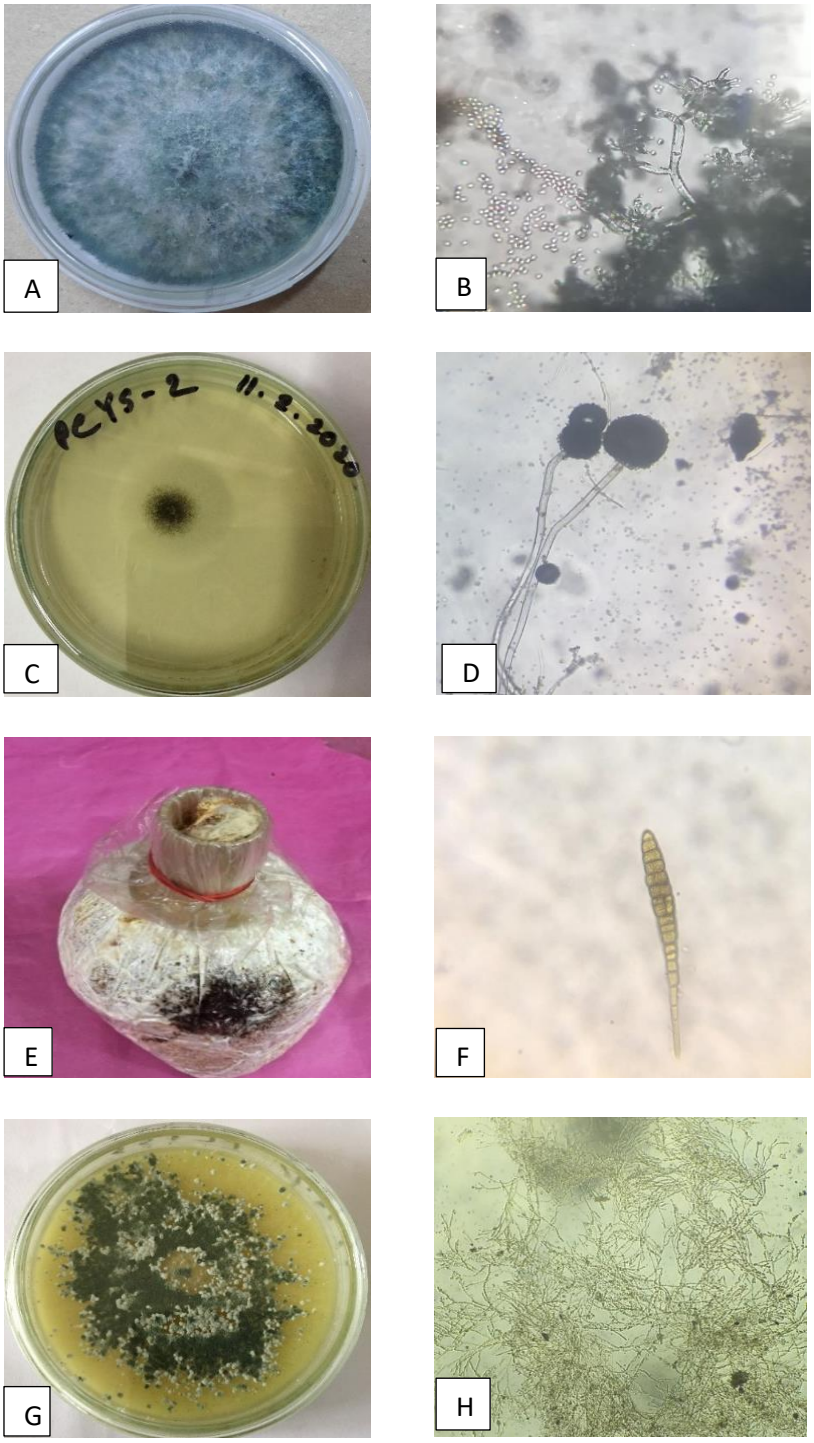

Plate 5. Identified contaminants mycoflora; A. Pure culture of *Trichoderma* sp B. Microscopic structure of *Trichoderma* sp.; C. Pure Culture of *Aspergillus niger*, D. Microscopic Structure of *Aspergillus niger* ; E. Alternaria contaminated spawn packet, F. Microscopic structure of *Alternaria* sp.; G. Pure culture of unknown fungus, H. Miroscopic structure of unknown fungus

4.2 Incidence of contaminants on different strains of Pcys in spawn packets

Statistically significant variation was found in case of contamination incidence in different strains of Maple oyster mushroom in different substrates and it was varied from 60-100%. The highest contaminant incidence (100%) was recorded from S_1P_6, S_1P_7 and S_3P_2. Whereas the lowest incidence (60%) was observed in S_1P_1 followed by S_1P_2 (66%), S_1P_4 (74%) and the similar contamination (85%) was found from S_1P_3 and S_1P_5.

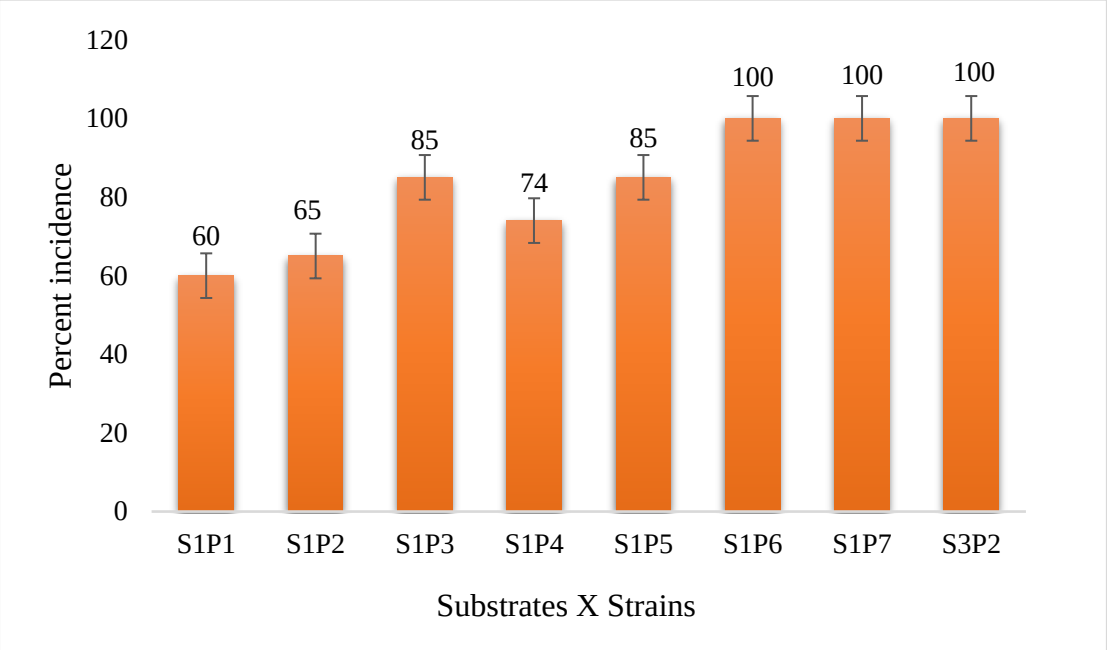

Figure 1. Incidence of contaminants on different strains of Pcys in spawn packets

S= Substrates	**P= Pcys strain**
S_1P_1 = Saw dust + Pcys-1	S_1P_2= Saw dust + Pcys-2
S_1P_3 = Saw dust + Pcys-3	S_1P_4= Saw dust + Pcys-4
S_1P_5 = Saw dust + Pcys-5	S_1P_6= Saw dust + Pcys-6
S_1P_7 = Saw dust + Pcys-7	S_3P_2= Saw dust + Rice straw + Pcys-2

4.3 Mycelial running of different strains of mushroom mother in saw dust

At 9DAI the highest mycelium running (1.96 cm) was recorded from Pcys-5 and Pcys-3 those were closely followed by Pcys-1 (1.89 cm) and Pcys-4 (1.60 cm), whereas no mycelium run in Pcys6 and Pcys-7 (Table 1).

At 12DAI the highest mycelium running (3.68 cm) was recorded from Pcys-2 which was statistically similar (3.25 cm) with Pcys-5 and closely followed by Pcys-1 (2.99 cm) and Pcys-3 (2.99 cm), whereas the lowest mycelium running (0.51 cm) was observed in Pcys-7 and no mycelium run in Pcys-6.

At 16DAI the highest mycelium running (3.67 cm) was recorded from Pcys-2 which was statistically similar to Pcys-1 (3.45 cm) cm and closely followed by Pcys-5 (3.38 cm), Pcys-3 (3.15 cm) and Pcys-4 (3.15 cm), whereas the lowest mycelium running (0.40 cm) was observed in Pcys-6 and Pcys-7 (0.77cm) (Table 1).

Table 1. Mycelial running of different strains of mushroom mother in saw dust at different days after incubation

Pcys strains	Mycelium run rate (cm) at different days after incubation		
	9 DAI	12 DAI	16 DAI
Pcys-1	1.89 a	2.94 ab	3.45 a
Pcys-2	1.23 c	3.68 a	3.68 a
Pcys-3	1.96 a	2.99 ab	3.15 a
Pcys-4	1.60 b	2.78 b	3.15 a
Pcys-5	1.96 a	3.41 a	3.38 a
Pcys-6	0.00 d	0.00 d	0.40 b
Pcys-7	0.00 d	0.51 c	0.78 b
Cv	14.18	15.82	20.32
Lsd (0.05%)	0.12	0.24	0.36

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

4.4 Combined effect of different substrates and strains on days required for complete mycelial initiation, pin head formation, 1st harvesting and total harvesting

There were 7 strains of Pcys. Among them Pcys-6 and Pcys-7 were highly contaminated by pathogens. There was no mycelium running was formed in case of Pcys6, Pcys7 and Pcys-2 in mixed substrates (S_3P_2) due to high contamination by contaminants.

4.4.1 Days required for mycelium running

Days required for mycelium running of oyster mushroom varied significantly due to effect of different substrates and strains (Table 2). The lowest time (7 days) required for mycelium initiation was observed in S_1P_1, S_1P_2, S_1P_3, S_1P_4 and S_1P_5, whereas the highest days (17 days) required for mycelium running was recorded from S_3P_3 which was statistically similar (15 days) to S_2P_5 and closely followed by S_3P_4, S_3P_5 (13 days) and S_2P_4, S_2P_3,S_2P_2, S_2P_1 (12 days), whereas the lowest time (7 days) was observed in S_1P_1, S_1P_2, S_1P_3, S_1P_4 and S_1P_5 respectively.

4.4.2 Days required for pinhead formation

Statistically significant variation was recorded in terms of days required for pinhead formation of oyster mushroom due to different substrates (Table 2). The lowest time (3 days) was found in S_3P_4 which was statistically similar (3 days) to S_3P_5, whereas the highest days (10 days) required for pinhead formation was recorded from S_1P_1 and S_2P_1 which was closely followed by S_1P_2 and S_2P_2 (9 days).

4.4.3 Days required from pin head formation to 1st harvest

Different strains of different substrates showed statistically significant differences in terms of days required for pin head formation to 1st harvest of oyster mushroom (Table 2). The lowest time (3 days) was recorded in S_2P_5 and S_1P_4 which was followed by and S_1P_5 (3.25 days) on the other hand the highest days (12 days) required for pin head formation to 1st harvest was observed from S_3P_4 and S_3P_5 which was closely followed by S_3P_1 (7days).

4.4.4 Days required for total harvesting

Different strains of different substrates showed statistically significant differences in terms of days required for total harvesting period (Table 2). The lowest time was recorded in S_3P_5 (16.50 days) and S_3P_3 (17.00 days) on the other hand the highest days (23.75 days) was observed from S_1P_4 which was closely followed by S_1P_5 and S_1P_1 (22.25days).

Table 2. Combined effect of different substrates and strains on days required for complete mycelial initiation, pin head formation, 1st harvesting and total harvesting

Substrates (S) X Strains (P)	Days required for			
	Mycelial initiation	Pinhead formation	1st harvesting	Total harvesting
S_1P_1	7.00 d	10.00 a	6.00 bc	22.25 ab
S_1P_2	7.00 d	9.00 ab	5.00 bcd	20.25 bcd
S_1P_3	7.00 d	6.25 cd	6.00 bc	21.00 abc
S_1P_4	7.00 d	7.50 bcd	3.00 d	23.75 a
S_1P_5	7.00 d	7.50 bcd	3.25 d	22.25 ab
S_2P_1	12.00 c	10.00 a	6.00 bc	19.00 cde
S_2P_2	12.00 c	9.00 ab	5.00 bcd	20.75 bc
S_2P_3	12.00 c	6.00 d	6.00 bc	20.75 bc
S_2P_4	12.00 c	7.00 cd	4.00 cd	18.25 cde
S_2P_5	15.00 ab	8.00 bc	3.00 d	18.50 cde
S_3P_1	11.00 c	7.00 cd	7 b	17.50 de
S_3P_3	17.00 a	6.00 d	6.50 b	17.00 e
S_3P_4	13.00 bc	3.00 e	12.00 a	19.25 cde
S_3P_5	13.00 bc	3.00 e	12.00 a	16.50 e
Cv	19.07	20.77	26.35	10.92
Lsd	1.37	0.98	1.05	1.43

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

S= Substrates **P= Pcys strain**

S_1P_1 = Saw dust + Pcys-1 S_1P_2= Saw dust + Pcys-2

S_1P_3 = Saw dust + Pcys-3 S_1P_4= Saw dust + Pcys-4

S_1P_5 = Saw dust + Pcys-5 S_1P_6= Saw dust + Pcys-6

S_1P_7 = Saw dust + Pcys-7 S_3P_2= Saw dust + Rice straw + Pcys-2

4.5 Interaction effect of different substrates and strains on the yield contributing characters of different strains of maple oyster mushroom

4.5.1 Number of fruiting body/packet

Number of fruiting body/packet of maple oyster mushroom showed statistically significant differences due to combined effect of substrates and strains (Table 3). The highest number (9.75) of fruiting body/packet was found from S_1P_1 which was statistically similar (9.50) to S_1P_5, whereas the lowest number of fruiting bodies (5.00) was recorded in S_3P_3, S_3P_4 and from S_2P_3 (5.50).

4.5.2 Number of effective fruiting body/packet

Statistically significant variation was found in terms of effective fruiting body/packet maple oyster mushroom (Table 3). The maximum number of effective fruiting body/packet (7.75) was observed from S_1P_5 followed by S_1P_1 (6.50) and S_1P_2 (6.00). On the other hand, the minimum number (3.75) was found in S_1P_3 followed by S_3P_4 and S_2P_5 (4.25).

4.5.3 Biological yield (g)

Statistically significant variation was recorded in terms of biological yield of different strains of maple oyster mushroom (Table 3). The highest biological yield (98.50 g) was found from S_2P_1 which was followed by S_2P_2 (94.50 g), S_1P_5 (94.00 g).While the lowest biological yield was recorded in S_3P_4 (65.50), S_3P_3 (67.50 g) and in S_3P_1 (68.75 g) which was followed by S_1P_4 (74.00 g).

4.5.4 Economic yield (g)

Economic yield of maple oyster mushroom showed statistically significant variation due to effect of different strains and substrates (Table 3). The highest economic yield (95.25g) was recorded from S_2P_1 followed by S_2P_2 (92.25 g) and S_1P_5 (92.00 g). Whereas the lowest economic yield (63.00 g) was found in S_3P_4 followed by S_3P_3 (65.75 g).

4.5.6 Biological efficiency (%)

Statistically significant variation was recorded in respect of biological efficiency of maple oyster mushroom due to interaction effect of different strains and substrate (Table 3). The highest biological efficiency (53.24 %) was found from S_2P_1 followed by S_2P_2 (51.08 %) and S_1P_5 (47.00 %).While the lowest biological efficiency was recorded in S_3P_4 (33. 75 %), S_3P_3 (33.75%) and in S_3P_1 (34.30 %).

Table 3. Effect of different strains and saw dust on number of fruiting body and effective number of fruiting body, biological yield and economic yield and biological efficiency

Substrates (S) X Strains (P)	Number of fruiting body	Effective number of fruiting body	Biological yield (g)/packet	Economic yield (%)	Biological efficiency (%)
S_1P_1	9.75 a	6.50 ab	89.00 b	87.25 b	44.50 cd
S_1P_2	8.00 bc	6.00 bc	84.50 bc	82.75 bc	42.25 d
S_1P_3	5.75 de	3.75 e	67.00 f	65.75 e	33.50 f
S_1P_4	8.00 bc	4.75 cde	74.00 e	72.25 d	37.00 e
S_1P_5	9.50 ab	7.75 a	94.00 a	92.25 a	47.00 b
S_2P_1	8.25 abc	6.75 ab	98.50 a	95.75 a	53.24 a
S_2P_2	8.00 bc	6.00 bc	94.50 a	92.25 a	51.08 a
S_2P_3	5.50 e	4.50 de	84.50 bc	83.25 bc	45.67 bc
S_2P_4	7.25 cd	5.50 bcd	84.00 c	83.25 bc	45.40 bc
S_2P_5	6.00 de	4.25 de	79.00 d	77.00 d	42.70 d
S_3P_1	7.25 cd	5.00 cde	68.75 f	66.50 e	34.30 f
S_3P_3	5.00 e	4.25 de	67.50 f	65.75 e	33.75 f
S_3P_4	5.00 e	4.00 e	65.50 f	63.00 e	32.75 f
S_3P_5	9.25 ab	5.50 bcd	87.50 bc	85.25 bc	43.75 cd
Cv	17.52	20.02	4.45	4.63	4.45
Lsd (0.05 %)	0.84	0.70	2.39	2.42	1.23

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

S= Substrates **P= Pcys strain**

S_1P_1 = Saw dust + Pcys-1 S_1P_2= Saw dust + Pcys-2

S_1P_3 = Saw dust + Pcys-3 S_1P_4= Saw dust + Pcys-4

S_1P_5 = Saw dust + Pcys-5 S_1P_6= Saw dust + Pcys-6

S_1P_7 = Saw dust + Pcys-7 S_3P_2= Saw dust + Rice straw + Pcys-2

4.6 Effect of different substrates on the quality of fruiting body of different strains of maple oyster mushroom

4.6.1 Length of stalk (cm)

Statistically significant difference was found in case of length of stalk in maple oyster mushroom and it was ranged from 1.60 cm t0 1.13 cm (Table 4). The highest length of stalk (1.60 cm) was recorded from S_3P_5 and the lowest (1.13 cm) from S_1P_5.

4.6.2 Diameter of pileus (cm)

In case of diameter of pileus in maple oyster mushroom (Table 4), statistically significant variation was observed and it was ranged from 4.25 to 7.13 cm. The highest length of stalk was recorded from S_3P_1 followed by S_2P_3, S_2P_2, and the lowest diameter (4.25 cm) was recorded from S_2P_5.

4.6.3 Thickness of pileus (cm)

Statistically significant differences in terms of thickness of pileus of oyster mushroom was observed and ranged from 0.25 cm to 0. 43cm (Table 4). The maximum thickness of stalk was recorded from S_1P_1 and the minimum found in S_2P_3 (0.33 cm).

Table 4. Effect of different substrates on the quality of fruiting body of different strains of maple oyster mushroom

Substrates (S) X Strains (P)	Length of the stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
S_1P_1	1.45 ab	4.50 de	0.43 a
S_1P_2	1.20 bc	4.13 e	0.35 abc
S_1P_3	1.43 abc	4.88 cde	0.38 abc
S_1P_4	1.20 bc	4.50 de	0.38 abc
S_1P_5	1.13 c	5.00 cde	0.33 abc
S_2P_1	1.18 bc	6.00 abc	0.33 abc
S_2P_2	1.18 bc	5.75 abcd	0.28 bc
S_2P_3	1.30 abc	5.75 abcd	0.25 c
S_2P_4	1.35 abc	6.50 ab	0.30 abc
S_2P_5	1.23 bc	4.25 e	0.28 bc
S_3P_1	1.25 bc	7.13 a	0.38 abc
S_3P_3	1.45 ab	4.75 cde	0.40 ab
S_3P_4	1.43 abc	5.50 bcde	0.33 abc
S_3P_5	1.60 a	5.38 bcde	0.40 ab
Cv	17.51	20.99	30.62
Lsd (0.05 %)	0.15	0.73	0.07

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

S= Substrates **P= Pcys strain**

S_1P_1 = Saw dust + Pcys-1 S_1P_2= Saw dust + Pcys-2

S_1P_3 = Saw dust + Pcys-3 S_1P_4= Saw dust + Pcys-4

S_1P_5 = Saw dust + Pcys-5 S_1P_6= Saw dust + Pcys-6

S_1P_7 = Saw dust + Pcys-7 S_3P_2= Saw dust + Rice straw + Pcys-2

4.7 Correlation and regression study

4.7.1 Functional relationship between economic yield and number of fruiting body.

A positive linear relationship was observed between number of fruiting body and economic yield per packet of saw dust. The equation y = 5.9234x + 31.691 gave a good fit to the data and the value of co-efficient of determination (R^2 = 0.8261) showed that the fitted regression line had a significant regression co-efficient. So, it indicated that economic yield per packet increased as the number of fruiting body increased.

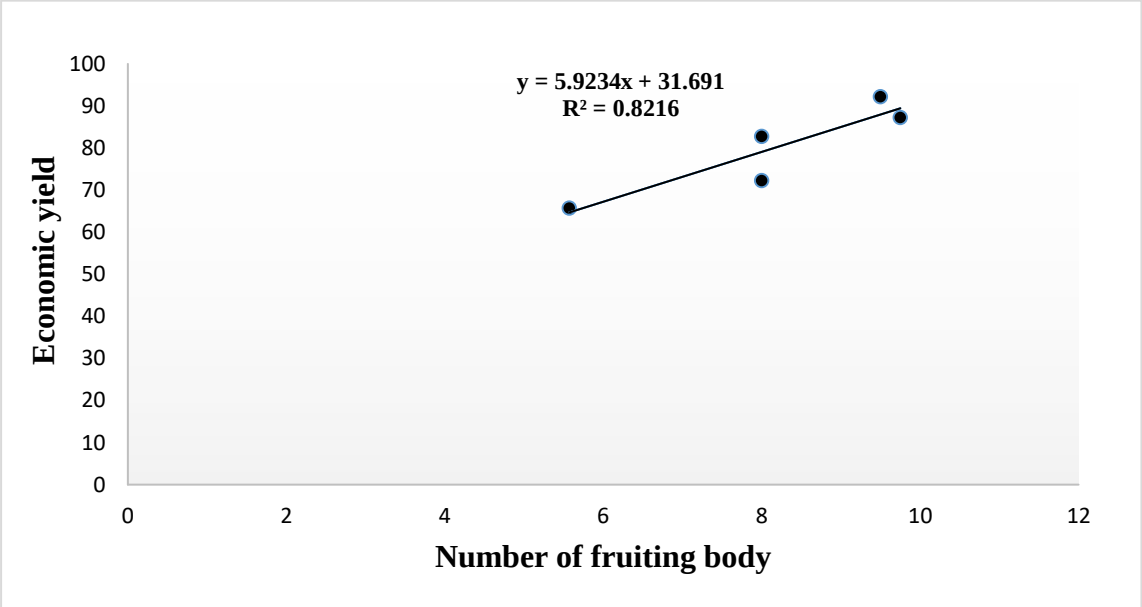

Figure 2. Functional relationship between economic yield and number of fruiting body

4.7.2 Functional relationship between economic yield and biological efficiency at rice straw

A positive linear relationship was observed between economic yield and biological efficiency at rice straw. The equation y = 1.7569x + 2.4415 gave a good fit to the data and the value of co-efficient of determination (R^2 = 0.9975) showed that the fitted regression line had a significant regression co-efficient. So, it indicated that biological efficiency increased with the increased of economic yield.

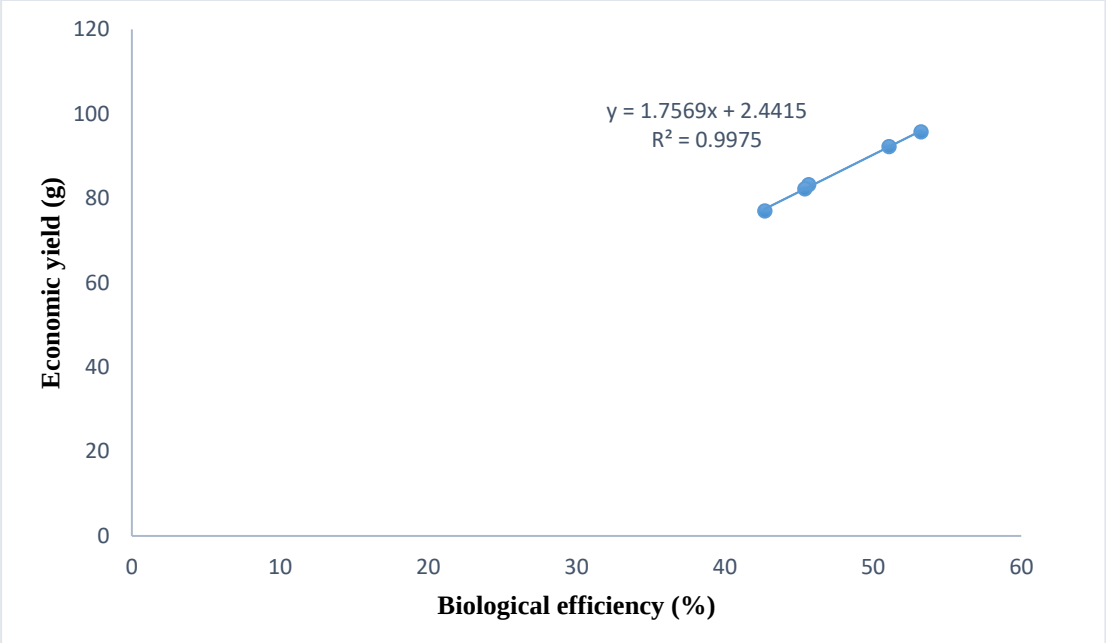

Figure 3. Functional relationship between economic yield and biological efficiency at rice straw

CHAPTER V

DISCUSSIONS

During cultivation period four contaminants namely *Trichoderma* sp. and *Aspergillus niger*, *Alternaria* sp. were isolated and identified and an unknown fungus was isolated but not identified from contaminated spawn packets which was also detected by several scientist previously. Urmi (2019) isolated 5 fungi namely *Sclerotium rolfsii*, *Trichoderma harzianum*, *Fusarium oxysporum*, *penicillium* sp. and *Aspergillus Niger* from contaminated packet of oyster mushroom. Akhter (2017) found that, *Trchoderma*, *Rhizopus*, *Aspergillus*, *Penicillium*, *Alternaria*, *Ceratocytis*, *Coprinus*, *Chaetomium* sp. were associated as contaminants where green mold was detected as the major one. Pervez *et al*., (2010) found that weed microflora namely *Aspergillus* spp, *Penicillium* spp., *Rhizopus stolonifer* and *Trichoderma harzianum* were associated with the substrate of oyster mushroom at different growth stages. According to Mujumdar and Rathaiah (2001), they found *Trichoderma harzianum*, *Aspergillus* spp. and *Penicillium* spp. as the three most dangerous and dominant fungal contaminants during spawn production in oyster mushroom. Choi *et* al., (2010) also isolated and identified *Trichoderma*, *Mucor racemosus* f. *racemosus*, *Aspergillus tubingensis* from *Pleurotus ostreatus* substrates for molecular and morphological characterization. Mejía and Albertó (2013) concluded that the lowest contamination might have occurred due to quality of a substrate.

During cultivation seven strains were evaluated for percent contamination incidence by contaminants. Lowest disease incidence was observed in S_1P_1 showing 60 %, which was followed by S_1P_2 showing 65 % incidence. Whereas highest contamination incidence was recorded from S_1P_6, S_1P_7 and S_3P_2 with 100% incidence respectively. Akhter (2017) showed the incidence of contamination 42.6 % during incubation and 67.9 % during cultivation of oyster mushroom at rice straw substrates, which may be differed due to strain and substrates. Rameshbhai (2014) observed lowest disease incidence of *T. harzianum*, *A. niger* and *A. flavus* sorghum grain spawn showing 6.67%, 6.67

and 0% respectively, whereas highest disease incidence by pathogens was recorded on grain spawn with 36.67%, 36.67% and 33.33% incidence respectively.

The highest days (17 days) required for mycelium running was recorded from S_3P_3 which was statistically similar (15 days) to S_2P_5, closely followed by S_3P_4, S_3P_5 (13days) and S_2P_4 (12 days). Maximum days (10 days) required for pin head formation was recorded from S_1P_1 which was closely followed by S_1P_2 (9 days), while the lowest time (3 days) was found in S_3P_4 which was statistically similar to S_3P_5 (3 days). More or less similar findings have been reported by previous scientists. Namdev *et al*., (2006) found that the number of days required for spawn run was significantly less than 14 days to 20 days in different straw substrates (gram straw, parthenium straw, sugarcane straw and wheat straw, sunflower stalk, mustard straw and paddy straw). Sarker *et al*., (2007) revealed that, the duration to complete mycelium running was 17.75 days in waste paper. Similar kind of results has been recorded in the present study.

In present study maximum biological yield was found in S_2P_1 among the seven strains and highest number of effective fruiting body was also found in S_3P_3 in the present study, which is more or less similar with reports of previous scientists. Jegadeesh *et al*., (2018) stated that, maximum yield of *P. djamor* var. *roseus* was obtained using paddy straw. Maniruzzaman (2004) utilized wheat, maize, rice and sawdust for the production of mother spawn in oyster mushroom and 75 found that substrate rice was the best for mother spawn production of oyster mushroom. Dhoke *et al*., (2001) also found significant effect of different agro-wastes on yield of oyster mushroom. Hassan *et al*., (2011) said that oyster mushroom can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, bagasse etc. and this has been reported to influence its growth, yield and composition (Iqbal *et al*., 2005; Kimenju *et al*., 2009; Khare *et al*., 2010).

In the present experiment 77-99.77 % economic yield was estimated from the rice straw showed highest economic yield was estimated from Pcys-1 cultivated

in rice straw (S_2P_1). Urmi (2018) recorded, 64.34g economic yield and 75.12g biological yield from rice straw in control which was more or less similar to present study. Similarly Sarker *et al*., (2007) found that highest economic yield was estimated from the waste paper. The economic yield on sugarcane bagasse was statistically identical to that grown on rice straw, which does not support the present study. In the present experiment highest economic yield is estimated from rice straw.

In the present study 33.5-47 %, 42.7-53.24 %, 32.75-43.75 % BE was found in saw dust, rice straw and mixed substrates whereas the highest BE was in S_2P_1. Biological efficiency varies from substrate to substrate. Similar results were found by scientist previously. According to Bernardi *et al*., (2007) the productivity and biological efficiency will vary according to different strains and various kinds of substrates used. This confirms the finding of Mandeel *et al*., (2005) that BE is highly affected by the quality of the spawn of the cultivated mushroom strain.

CHAPTER VI

SUMMARY AND CONCLUSION

To identify the contaminants and yield of different strains of maple oyster mushroom the experiment was carried out at the Mushroom Culture House of Mushroom Development Institute, Savar, Dhaka, and central laboratory of SAU during the period from October 2019 to March 2020. The experiment consisted of seven strains of *Pleurotus cystidiosus*, viz. Pcys-1, Pcys-2, Pcys-3, Pcys-4, Pcys-5, Pcys-6 and Pcys-7 and 3 substrates were used, those were S_1= Saw dust, S_2= Rice straw and S_3= Saw dust + Rice straw. The experiment was laid out in Completely Randomized Design (CRD) with four replications.

For the successful cultivation of mushroom, good quality contaminants free spawn is needed. During preparation of the spawn there are occurrence of some contaminants which infect spawn thereby reducing the quality of the spawn. Among these contaminants, *Trichoderma* sp., *Aspergillus niger*, *Alternaria* sp. sp. were isolated and identified.

Percent contamination incidence were recorded for different strains in different substrates. 100% contamination incidence were recorded from S_1P_6, S_1P_7 and S_2P_3 spawn packets and the lowest for S_1P_1 (60%)

Mycelium running rate of different strains of mushroom mother also evaluated. At 9DAI highest mycelium running was recorded from Pcys-5 and at 12DAI highest mycelium running was observed in Pcys-2, and within 16DAI all the strains complete their mycelium running except Pcys-6 and Pcys-7 due to contamination.

Strains in saw dust substrates took lowest time (7 days) to complete mycelium running, while S_3P_3 took highest time (17 days) to complete mycelium running. S_3P_4 took lowest time (3 days) to pinhead formation and S_1P_1 and S_2P_1 took highest time (10 days). S_2P_5 took lowest time (3days) and S_3P_4 took highest time (10 days) for pin head formation. Lowest time (3 days) was recorded in S_2P_5 and on the other hand the highest days (12 days) required for pin head formation to

1st harvest was observed from S_3P_4 and S_3P_5 which was closely followed by S_3P_1 (7 days). The lowest time for total harvesting was recorded in S_3P_5 (16.50 days) and S_3P_3 (17.00 days) on the other hand the highest days (23.75 days) was observed from S_1P_4 which was closely followed by S_1P_5 and S_1P_1 (22.25 days).

The highest number (9.75) of fruiting body/packet was found from S_1P_1, whereas the lowest number of fruiting bodies (5.00) was recorded in S_3P_3, S_3P_4 and from S_2P_3 (5.50). The maximum number of effective fruiting body/packet (7.75) was observed from S_1P_5, the minimum number (3.75) was found in S_1P_3 followed by S_3P_4 and S_2P_5 (4.25), the highest biological yield (98.50 g) was found from S_2P_1 which was followed by S_2P_2 (94.50 g), S_1P_5 (94.00 g).While the lowest biological yield was recorded in S_3P_4 (65.50), S_3P_3 (67.50 g) and in S_3P_1 (68.75 g) which was followed by S_1P_4 (74.00 g). The highest economic yield (95.25g) was recorded from S_2P_1. Whereas the lowest economic yield (63.00 g) was found in S_3P_4, The highest biological efficiency (53.24 %) was found from S_2P_1 followed by S_2P_2 (51.08 %) and S_1P_5 (47.00 %).While the lowest biological efficiency was recorded in S_3P_4 (33. 75 %), S_3P_3 (33.75%) and in S_3P_1 (34.30 %).

Significant differences were observed in terms of quality of fruiting body of different strains of maple oyster mushroom. The highest length of stalk (1.60 cm) was recorded from S_3P_5 and the lowest (1.13 cm) from S_1P_5. The highest length of stalk was recorded from S_3P_1 followed by S_2P_3, S_2P_2, and the lowest diameter (4.25 cm) was recorded from S_2P_5. The maximum thickness of stalk was recorded from S_1P_1 and the minimum found in S_2P_3 (0.33 cm).

From the present research work it may be concluded that,

• From the contaminated spawn packets *Trichoderma* sp, *Aspergillus niger*, *Alternaria* sp. were isolated and identified. An unknown fungus was isolated but not identified. Among the strains of maple oyster mushroom Pcys-6 and Pcys-7 were slow growing and highly contaminated.

• Considering the yield and yield contributing characters it can be concluded that Pcys-1, Pcys-2, Pcys-3, Pcys-4 and Pcys-5 strain are suitable for cultivation in Bangladesh.

• Comparing the three types of substrates Pcys-1 performed better on rice straw and Pcys-5 on saw dust and mixed substrates. The highest yield was found in Pcys-1 when grown on rice straw.

CHAPTER VII

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CHAPTER VIII

APPENDICES

Appendix. 1 Pasteurization and mixing mushroom mother with substrates (Plate 1. A-B)

Plate. 1. A. Pasteurization of substrates, 2. Mixing of mushroom mother with the substrates

Appendix. 2 Mushroom culture house (Plate C-D)

Plate 2. Inner view of mushroom culture house (C-D)

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