EFFECT OF SUBSTRATE ENRICHMENT ON CONTAMINATION AND PRODUCTIVITY OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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SHER-E-BANGLA NAGAR, DHAKA-1207, BANGLADESH

June, 2021

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BY

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Reg. No. 14-06294

A Thesis

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 (MS)
IN
PLANT PATHOLOGY

SEMESTER: JANUARY-JUNE, 2019

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CERTIFICATE

This is to certify that thesis entitled ***"EFFECT OF SUBSTRATE ENRICHMENT ON CONTAMINATION AND PRODUCTIVITY OF OYSTER MUSHROOM (Pleurotus ostreatus)"*** *submitted to the* ***Department of Plant Pathology,*** *Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS) in Plant Pathology,*** *embodies the result of a piece of bona fide research work carried out by SHEIKH SAHA ALI, Registration No. 14-06294 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 and style of this thesis have been approved and recommended for submission.

Dated: June, 2021
Place: Dhaka, Bangladesh

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DEDICATED
TO
MY BELOVED
PARENTS

ACKNOWLEDGEMENT

All the praises and gratitude are due to the omniscient, omnipresent and omnipotent Almighty Allah, who has kindly enabled the author to complete her research work and complete this thesis successfully for increasing knowledge and wisdom.

The author really does not have adequate words to express his heartfelt sense of gratification, ever indebtedness and sincere appreciation to his benevolent teacher and research supervisor, **Prof. Dr. Khadija Akhter,** Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, for her constant help, scholastic guidance, planning experiment, valuable suggestions, timely and solitary instructive criticism for successful completion of the research work as well as preparation of this thesis.

It is a great pleasure for the author to express his sincere appreciation, profound sense, respect and immense indebtedness to his respected co-supervisor & Chairman **Prof. Dr. Fatema Begum,** Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, for providing necessary facilities and conductive atmosphere to accomplish the research work.

Special thanks and indebtedness are also due to all the teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for their valuable teaching, sympathetic co-operation and inspiration throughout the period of the study.

The author would like to express his deepest respect and boundless gratitude to all the respected teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 for their sympathetic co-operation and inspiration throughout the course of this study and research work.

The author deeply acknowledges the profound dedication to his beloved Parents and Sisters for their moral support, steadfast encouragement and continuous prayer in all phases of academic pursuit from the beginning to the completion of study successfully.

Finally, the author is deeply indebted to his friends and well-wishers especially Md. Mehedi Hasan, Md. Shafiqul Islam, Tanjila Hasan, Rekhea Bhaumik and Mohammad Kabil khan for their kind help, constant inspiration, co-operation and moral support which can never be forgotten.

Date: June, 2021 The Author

SAU, Dhaka

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ABSTRACT

The experiment was carried out during the period from November 2020 to March 2021 to find out the effect of substrate enrichment on contamination and productivity of oyster mushroom (*Pleurotus ostreatus*). The experiment consists of eight different types of nutrient supplements with one control to achieve the desired objectives. The treatments were T_0 = Control (without supplementation of nutrients); T_1 = 1% Gypsum; T_2 = 10% Extract of sawdust; T_3 = 0.0001% Torus; T_4 = 1% Macboron; T_5 = 10% Extract of rice straw; T_6 = 1% Shakti zinc; T_7 = 0.5% Grow-cell and T_8 = 0.5% American NPKS. The experiment was laid out in single factor Completely Randomized Design (CRD) with three replications. The highest mycelium running rate (0.82 cm/day) was observed when the substrate was enriched with 0.5% Grow-cell. The minimum duration required from incubation to completion of mycelium running (17.33 days), from stimulation to primordial initiation (5.89 days), from primordial initiation to 1st harvest (4.67 days) and the lowest total harvesting period (40.00 days) were found when sawdust was enriched with 0.5% Growcell. Again, the maximum number of primordia (86.33), effective fruiting body per packet (30.00), the highest length of pileus (5.11 cm) and width of pileus (7.45 cm) were found at 0.5% Growcell enriched substrates. Oyster mushroom grown on sawdust substrates that enriched with 0.5% Growcell gave the highest biological yield (174.7 g), economic yield (167.3 g) and biological efficiency (34.93%) but the highest cost benefit ratio (3.26) was calculated from 10 ppm Torus. In the experiment, the highest moisture content (91.30 %) and carbohydrate (45.80 %) content was recorded from control spawn whereas the highest protein (27.16 %), lipid (4.70 %), fiber (25.58 %) and ash (13.70 %) was recorded from 0.5% Growcell enriched packets. During the cultivation period four contaminants namely *Trichoderma harzianum*, *Penecillium* sp, *Rhizopus stolonifer* and *Aspergillus niger* were isolated and identified from contaminated substrates. Percent contamination of fungi gradually increased with the increase of days after stimulation. After 45 days after stimulation (DAT), 0.0001% Torus and 0.5% Growcell enriched substrates were not contaminated. Among the treatments, the performance of 0.5% Growcell was better economically as well as growth and yield performance for the cultivation of oyster mushroom.

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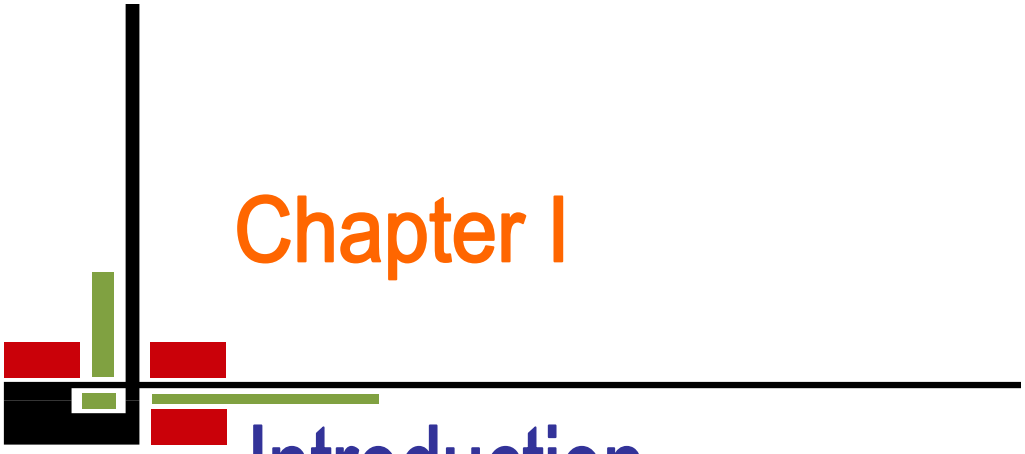
LIST OF ABBREVIATIONS AND ACRONYMS

Abbreviation	Full meaning
%	Percent
@	At the rate
^{0}C	Degree Centigrade
Agril.	Agricultural
BAU	Bangladesh Agricultural University
BBS	Bangladesh Bureau of Statistics
BCR	Benefit Cost Ratio
BE	Biological Efficiency
Cm	Centi-meter
CRD	Completely Randomized Design
CV	Coefficient of variation
d.f.	Degrees of freedom
DAS	Days After Stimulation
DMRT	Duncan's Multiple Range Test
e.g.	For example
et al.	And others
FAO	Food and Agriculture Organization
G	Gram
J.	Journal
Kg	Kilogram
LSD	Least Significant Difference
Mg	Milligram
m^2	Meter Squares
MRR	Mycelium Running Rate
MDI	Mushroom Development Institute
SAU	Sher-e-Bangla Agricultural University



Chapter I

Introduction

CHAPTER I

INTRODUCTION

Over 200 species of mushrooms have long been used as functional foods around the world (Kalac, 2013), but only about 35 species have been commercially cultivated (Aida *et al.,* 2009; Xu *et al.,* 2011). They are a rich source of nutrients, particularly proteins, minerals as well as vitamins B, C and D (Panjikkaran and Mathew, 2013). Mushrooms contain 20–35% of protein (dry weight), are low in lipids and contain all the nine essential amino acids (Kalac, 2009). Mushrooms are delicacy food items praised for their characteristic texture when biting and enjoyable flavor. Mushrooms may have health-promoting benefits due to a multitude of compounds with antifungal activity (Ye *et al.,* 1999), antigenotoxicity (Wang *et al.,* 2005), antioxidation (Roupas *et al.,* 2012), antiproliferative (Zhou *et al.,* 2013), anti-tumorigenic (Kim *et al.,* 2015), anti-hypertensive, anti-nociceptive, immune stimulation (Vaz *et al.,* 2011), hypocholesterolaemic/anti-atherogenic properties (Han *et al.,* 2011), stress-reducing properties and are also good for diabetic patients (Akata *et al.,* 2012). Mushrooms are generally low in saturated fats and high in fiber and protein, and may reduce harmful blood cholesterol and act as an appetite suppressant. (Kim *et al.,* 2011).

The *Pleurotus* spp. of the class basidiomycetes belongs to a group known as ‘‘white rot fungi” (Tsujiyama and Ueno, 2013) as they produce a white mycelium and are generally cultivated on non-composted lignocellulosic substrates (Savoie *et al.,* 2007) in which various kinds of *Pleurotus* are commercially cultivated and have considerable economic value, including *P. ostreatus* (oyster mushroom), *P. eryngii* (king oyster or Cardoncello), *P. pulmonarius* (phenix mushroom), *P. djamor* (pink oyster mushroom), *P. sajor-caju* (indian oyster), *P. cystidiosus* (abalone oyster), *P. citrinopieatus* (golden oyster mushroom) and *P. cornucopiae* (Knop *et al.,* 2015; Zhang *et al.,* 2016). *Pleurotus* species require a short growth time, compared to other mushrooms. It can be

grown in a simple and cheap way, with high yield, wider substrate utilization, sporelessness, wide temperature and chemical tolerance, as well as environmental bioremediation. It is an edible mushroom and also has several biological effects, as it contains important bioactive molecules (Yang *et al.,* 2013). *P. ostreatus* is characterized by high water content and low caloric value (1510 kJ kg^{-1} edible parts), making it suitable for inclusion into calorie-controlled diets (Jaworska and Bernas, 2009). The pileus of *P. ostreatus* are valued not only for their taste but also for their nutritional qualities, especially in vegetarian diets. It is very popular in many countries particularly in Southeast Asia where button mushroom cultivation is difficult under natural condition. This mushroom is easy to grow and its cultivation is a unique enterprise, where unlike other farm activities competition would not be for land but for labor and capital. From the economic evaluation, it may be assumed that the oyster mushroom production would preferably be assured profit.

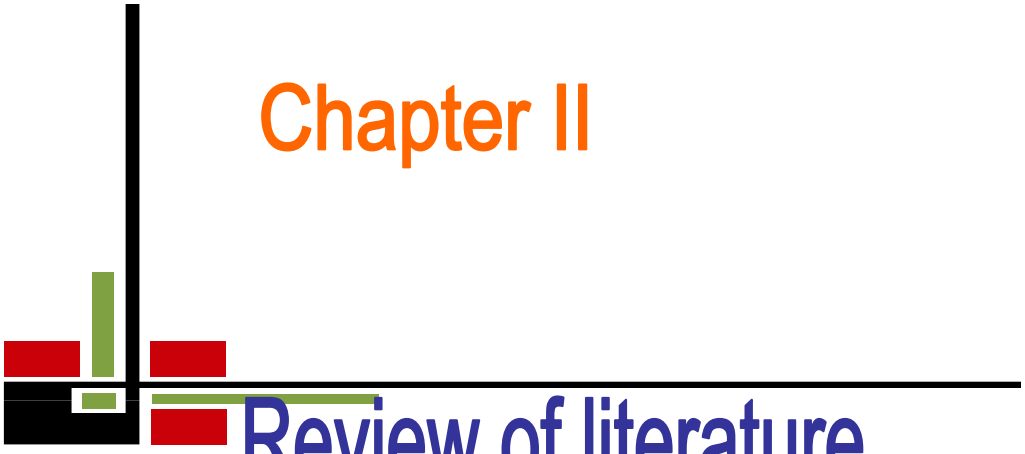
The enormous increase in our population has necessitated more and more food production through alternate resources such as mushroom as the availability of more arable land. In the developed countries, mushrooms have become one of the most important horticultural crops (Biswas, 2001). Mushroom needs labor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

composition, water activity, ratio of carbon to nitrogen, nutrients, surfactant, pH, moisture, sources of nitrogen, particle size, and amount of inoculum, antimicrobial agents and the presence of interactions between microorganisms are considered as chemical, physical and biological factors that are linked to mushroom production (Mangat *et al.,* 2008). Generally, lignocellulosic materials are low in mineral content, and they require additives to provide them with different minerals, and thus, enhance mushroom production (Alananbeh *et al.*, 2014). Minerals such as nitrogen, phosphorus, magnesium, sulfur, boron, calcium, iron, potassium, copper, zinc, manganese and cobalt, as well as vitamins, are used in culture media. There are several parameters that affect the enzyme production; however, its nitrogen source is a major factor (Singh *et al.,* 2008). Nitrogen is important in protein, nucleic acid, purine, pyrimidine and polysaccharide synthesis (Abdullah *et al.,* 2015) constituents of the cell wall of many fungi, Supplementation with nitrogen can increase crop productivity. Besides this, sulfur ions, phosphorus, potassium and magnesium stimulate the development of *Pleurotus* spp. The micronutrients such as calcium, zinc, manganese, iron, copper and molybdenum cations are trace elements that may be supplement the substrate for these mushrooms (Bhatti, 2007).

Considering the above facts, the experiment has been undertaken with the following objectives:

- To determine the incidence and severity of oyster mushroom contaminants after using of different nutrients with substrates
- To evaluate the effect of different nutrient supplements on growth, yield and cost benefit ratio of oyster mushrooms
- To know the proximate composition of oyster mushroom under different nutrient supplements

Chapter II



Review of literature

CHAPTER II

REVIEW OF LITERATURE

The purpose of this chapter was to review the literatures having relevance to the present study. The review of literature of the past studies and opinions of the researchers pertinent to the present experiment were collected through reviewing of journals, thesis, reports and other form of publications. The information was compiled and presented below:

2.1. What are mushrooms?

Mushrooms are very special in the scientific classification. They are neither plants nor animals, but are still organisms because they perform all the life processes of other organisms. They belong to their special group called the fungi which are microscopic. Notwithstanding, there are other members in the group that are macroscopic and mushrooms can be described as one of the macroscopic groups. What then is a mushroom? The word 'mushroom' has many meanings in different parts of the world. Many researchers have tried to explain or more still define the word 'mushroom'. According to Ganopedia (2011), a mushroom is a fungus that has a stem, a cap and gills or pores on the underside of the cap. Cho and Kang (2004) defined mushroom as "a macrofungus with a distinctive fruiting body which can be either epigeous (growing on or close to the ground) or hypogeous (growing underground)". The word mushroom refers only to the fruit and must be large enough to be seen with the naked eye and to be picked by hand (Cho and Kang, 2004). The word mushroom actually refers to the fungi that is seen with the naked eyes and that is picked by other organisms and sometimes used as food. In a broad sense "Mushroom is a macrofungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough

to be seen with naked eye and to be picked by hand" (Chang and Mills, 1992). Mushrooms are not only basidiomycetes, they can also be ascomycetes, grow underground, have a non-fleshy texture and could be inedible (Chang, 2007). All the poisonous and the non-poisonous fungi that can be seen with the naked eye and can be picked with the hand are described as mushrooms. The various types and shapes of mushrooms that can be picked from the wild include the most common type of umbrella shape with a pileus (cap) and a stipe (stem) i.e., *Lentinula edodes* (Chang, 2007). There are other species that have different shapes such as volva (cup) in *Volvariella volvacea* or an annulus (ring) in *Agarius campestris* and some even, like the human ear such as *Plearotus ostreatus* (Chang, 2007). The life cycle of a mushroom may be traced from - a spore which under favorable conditions germinates to form a mass of branched hyphae of mycelia which colonize a substrate (Dike *et al.,* 2011). Mushrooms go through two stages, the vegetative stage and the reproductive phase. The vegetative stage ceases when the hyphae fully colonize its substrate. The reproductive phase starts when the hyphae develop primordia. The mushroom is a fruit that results from fully matured primordia of the fungi (Dike *et al.*, 2011).

2.2 Classification of mushrooms

Classification is the arrangement of things or organisms into classes according to common features shared by the organism. Mushroom classification is therefore the arrangement of mushrooms based on their common characteristics. Mushrooms can be classified by their tropic pattern as saprophytes, parasites or mycorrhizae (Cho and Kang, 2004). The saprophytes are decomposers growing on organic matters like wood, leaves and straw in nature. They produce enzymes to digest the organic waste outside their body before they absorb them into their body (Austin, 2004). The parasites on the other hand grow, feed and are sheltered on or in a different organism while contributing

nothing to the survival of their host, while mycorrhizas form a symbiotic association of their mycelia with the roots of certain plants (Cho and Kang, 2004).

There are three groups of mushrooms according to their economic importance; these are edible mushrooms, toxic mushrooms and medicinal mushrooms (Ganopedia, 2011). Edible mushrooms are mushrooms that have desirable taste and aroma without poisonous effect and are used extensively in cooking; toxic mushrooms produce toxin, mind altering substances, antibiotics and antiviral substances, therefore, ingestion of toxic mushrooms may cause harmful effects that vary from mild symptoms such as gastric upset to severe life-threatening organ-failure which may result in death (Ganopedia, 2011). Medicinal mushrooms on the other hand have extracts that are possibly used for treatment of diseases. Oyster mushroom belongs to the edible type.

On the other hand, Chang (2007) had earlier reported that mushrooms can be grouped into four main categories, these include "(1) those which are fleshy and edible fall into the edible mushroom category, e.g., *Agaricus bisporus;* (2) mushrooms which are considered to have medicinal applications, are referred to as medicinal mushrooms, e.g., *Ganoderma lucidum;* (3) those which are proven to be, or suspected of being poisonous are named as poisonous mushrooms, e.g., *Amanita phalloides;* and (4) a miscellaneous category which includes a large number of mushrooms whose properties remain less well defined, which may tentatively be grouped together as 'other mushrooms'."

Mushrooms can also be classified according to the substrates they grow on (Dzomeku, 2009). These include cellulolytic mushrooms, lignocellulolytic and termitomyces. The cellulolytic mushrooms grow mainly on cellulose such as straws; examples include *Vovariella volvacea*, *Agaricus bisporus* etc. The lignocellulolytics grow well on both straws and decaying wood such as sawdust; examples include *Pleurotus ostreatus*. The

termitomyces grow mainly on anthills and their life cycles are completed by the help of ants or termites; examples include the Termitomyces family.

2.3 Description and classification of oyster mushroom

Oyster mushroom belongs to the class Agaricomycetes, order Agaricales, family Pleurotaceae or Tricholomataceae, genus *pleurotus* and species *ostreatus*. Scientifically, oyster mushroom is known as *Pleurotus ostreatus* (Kuo, 2011). The Latin word Pleurotus means 'beside the ear' and ostreatus means 'oyster shaped' (Cohen *et al.,* 2002). Oyster mushrooms include many species such as *P. flobellotus P. sojar - caju, P. eryngii, P. osfreafies, P. floride and P. sapidus* etc. (Dike *et al.,* 2011). There are over 70 species of *Pleurotus* for which new species still being discovered (Kong, 2004). All the varieties or species of oyster mushrooms are edible except *P. olearius* and *P. nidoformis* (Agridaksh, 2011).

Mukhopadhyay (2019) reviewed that the fruit bodies of *Pleurotus* are generally referred to as 'oyster mushroom'. It is a lingo-cellulolytic fungus of basidiomycetes and grows naturally in the temperate and tropical forests.

Chowdhury *et al.,* (2011) demonstrated 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 versatile mushrooms. They are easy to cultivate and common all over the world. The latin name *Pleurotus ostreatus* means "sideways oyster", referring to the oyster-like shape of the mushroom. They are found on hardwoods in the spring and fall. The caps usually range between 5 to 25 cm (2 to 10 inches) and are shaped like 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).

Oyster mushroom is an edible, saprophytic and lignocellulolytic type of mushroom. The fruiting bodies of oyster mushroom are usually flat with the cap offset from the stalk, or the stalk hardly present at all (Woller, 2007). *Pleurotus ostreatus* (roughly translating to "beside-the-ear oyster-shaped") predominantly grows on hardwoods such as stumps, logs, and trunks of deciduous trees. It has a pale lilac-grey spore print and a soft fleshy fruiting body that ranges in color from white to grey, brown or even blackish (Woller, 2007). There is some variability among the species due to the wide distribution and reproductive isolation between continents. The caps of *Plearotus ostreatus* are shell shaped, semicircular to elongate. The margins are smooth and sometimes wavy and are whitish to grayish to tan; the texture is velvety, the flesh is thick and white, gills are narrow, the stalk is short, thick and white and the base being hairy (Mdconline, 2013). The spores look narrowly elliptical, smooth and colorless when magnified. On average, the cap width ranges between 2 – 15 cm, stalk length is around 4 cm and stalk width is around 2 cm (Mdconline, 2013). *P. ostreatus* fruits year-round, especially after a good rain, if the weather is mild (Mdconline, 2013).

2.4 The oyster mushroom life cycle

The oyster mushrooms are basidiomycetes that bear their spores externally on basidia. The basidia grow to release the reproductive basidiospores. The oyster bears so many spores. The spores on landing in a favourable environment germinate into haploid mycelia. When the mycelia meet other haploid mycelia, they mate and then undergo plasmogamy. This results in the fusion of their cell membrane to create a dikaryotic cell, one with two genetically different haploid nuclei (Woller, 2007). The oyster mushroom has four mating types to ensure the chance of successful mating. The new

dikaryotic cell multiplies and divides to live as a multi-cellular dikaryotic organism. This is the dominant stage for growing and gathering of nutrients. The dikaryotic mycelia then mature to a mushroom. The mushroom develops dikaryotic basidia within the gills (Woller, 2007). The nuclei in the basidia finally undergo karyogamy, fusion of the nuclei, and at last form a diploid nucleus that quickly undergoes meiosis. Each diploid nucleus yields four haploid nuclei of different mating types that develop into a basidiospore to repeat the cycle (Woller, 2007). The oyster really begins its life with a spore, from the spore the oyster grows in a thread-like, branching formation known as a hypha. The hyphae continue to elongate and branch repeatedly to form a network of vegetative hyphae which is known as mycelia. The spores and the initial hyphae are haploid (contains only one copy of each chromosome) (IMA-Fungus, 2002). A haploid mycelium meeting another haploid mycelium of the same species join together and exchange nuclear materials. The process is called diploidization (IMA-Fungus, 2002). This is normally done before meiosis and spore production – Karyogamy. The two nuclei duplicate themselves, fuse, mix-and-match their genes, divide, and divide again to produce four new sexual nuclei which become the nuclei of the four-spore produced on a "typical basidium‖ (IMA-Fungus, 2002).

Uddin *et al.* (2011) executed an investigation of four species of oyster mushroom: *Pleurotus ostreatus, P. florida, P. sajor-caju* and P. high king cultivated in every season (January to December) in Bangladesh to observe the environmental condition for better production. In all of the selected species of this study, the minimum days required for primordial initiation, and the maximum number of fruiting bodies, biological yield and biological efficiency were found during December to February (14-27 ^{0}C, 70-80% RH). The production was found minimum during the cultivated time August to October.

2.4.1 Mode of feeding of oyster mushroom

Oyster mushrooms are unable to manufacture their own food; they feed by excreting digestive enzymes through the tips of their hyphae (Woller, 2007; Volk, 2001). The hyphae branch to form thick mass mycelia to increase their surface area through which feeding can be maximized. The oyster mushrooms feed by secreting a range of enzymes such as peroxidases, laccases, cellulases, hemicellulases and xylanases (Cohen *et al.*, 2002). This makes the oyster mushroom well adapted on lignin and cellulose containing substrates such as sawdust, rice straw etc. These mushrooms, as a result of their saprophytic and the lignocellulotic ability, are able to grow on lignocellulosic substrates with little composting. This is because oyster prefers the lignin that makes up the secondary cell walls of hard woods from angiosperm trees (Woller, 2007). The oyster mushrooms take their protein by secreting a potent toxin to kill nematodes or roundworms that may be present in the rotten wood (Mdconline, 2013; Woller, 2007). The hypha of the fungi then secretes enzymes to digest these microorganisms, and then the mushroom absorbs the nutrients within them. That is how oyster mushrooms solve their nitrogen problem and defend themselves from a potential predator (Woller, 2007).

2.4.2 Nutritional requirement of oyster mushroom

The ability of a fungus to synthesize enzymes to degrade the substrate is influenced by the strain, substrate composition and nitrogen concentration in the cultivation medium (Elishashvili *et al.*, 2008; Stajic *et al.*, 2006). The substrate on which the mushroom is grown should supply specific nutrients required for oyster cultivation and the main nutritional sources for oyster mushrooms are cellulose, hemicelluloses and lignin (Kang, 2004). Cellulose and hemicelluloses which are the main sources of carbohydrates for oyster mushrooms are often incrusted within lignin, which forms a physical seal around cellulose and hemicelluloses and the proportion of these three

structural components along with nitrogen content of residues affect mycelia growth, mushroom quality and crop yield (Philippoussis and Diamantopoulou, 2011). The strategy of the oyster mushroom is to decompose the lignin in wood so as to gain access to the cellulose and hemicelluloses embedded in the lignin matrix (Philippoussis and Diamantopoulou, 2011). Oyster mushrooms require more carbon and less nitrogen, however most of the substrates must be supplemented with nitrogen source to reach optimal Carbon: Nitrogen (C:N) ratio for the mushroom (Philippoussis and Diamantopoulou, 2011). Different researchers have suggested different C:N ratios for the growth of oyster mushroom. According to Srivastava and Bano (2010) C:N ratio between 30:1 and 117:1 was appropriate for *Plearotus fabellatus.*

Ogbo and Okhuoya (2009) were conducted to the effect of crude oil contamination which is a perennial problem in the Niger Delta of Nigeria on the yield and nutrient status of *Pleurotus tuber-regium* was investigated. In this study, after cultivating *P. tuber-regium* in crude oil contaminated soils to which sawdust, shredded banana leaf blades, NPK fertilizer and poultry litter were added, the yield and nutrient or chemical composition were determined. Crude oil contamination caused significant increase in the size and yield of the mushroom by increasing the pileus and stipe size and also fresh and dry weights showing a fertilizer effect. The addition of sawdust and poultry litter enhanced the fertilizer effect by further significant increases in size and yields of treatments that had them. The addition of NPK and shredded banana leaf blades to crude oil contaminated soils did not enhance the fertilizer effect of crude oil as there was significant decrease in the size and yield of *P. tuber-regium* in treatments that had them. The nutrient chemical composition of the mushroom was also affected by the presence of the crude oil and supplementation with the various materials. There was a reduction in the moisture and carbohydrate content caused by the addition of poultry litter and

sawdust to the contaminated soils and an increase in the ash, fat, protein and fibre content of the mushroom. On the other hand, the addition of NPK and shredded banana leaf blades caused a reduction in the moisture, protein and carbohydrate content and an increase in the fat, moisture, ash and fibre content. The performance of the fungus in crude oil contaminated substrates can be optimized by the addition of sawdust and poultry litter but not shredded banana leaf blades and NPK. This is evident from the fact that sawdust and poultry litter enhanced growth while shredded banana leaf blades reduced growth in crude oil contaminated soil. The improvement in nutrient status of the mushroom indicates that the fertilizer effect of crude oil also affects the general well-being of the fungus.

Bhuyan (2008) conducted an experiment to study the effect of various supplements at different levels with sawdust showed significant effort on mycelium running rate and reduced the required days to complete mycelium running in the spawn packet. The supplementation of sawdust found to be significant in yield and yield contributing characters of oyster mushroom with some extent. The highest biological yield, economic yield, dry yield, biological efficiency (BE) and benefit cost ratio (BCR) of 270.5 g, 266.5 g, 26.34 g, 93.29, 9.57%, respectively was observed in sawdust supplemented with NPK mixed fertilizer (N=0.6%, P=0.3%, K=0.3%).

Wu *et al.*, (2004) recommended C:N ratio of 18:1 to 36:1 to be appropriate for *Pleurotus* tuber-regium, while Rajarathnam and Bano (2005) suggested a C:N ratio around 85:1 as appropriate for *Pleurotus ostreatus* which falls within a C:N ratio ranging from 40:1 to 90:1. Shroomery (2011) on the other hand lowered the optimum range of C:N ratio for *P. ostreatus* to between 40:1 and 60:1.

Oyster mushrooms do not require more nitrogen for their growth. Excess nitrogen may cause stratum degradation when nitrogen is excessively added (Rajarathan and Bano,

2005). The activities of Laccase which is the main enzyme used by oyster mushroom to degrade the lignin content of the substrate is reduced when excess nitrogen is added to the substrate, although excess nitrogen increases mycelia growth (D'Agostini *et al.,* 2011). When working on "Effect of Organic Nitrogen Supplementation in *Pleurotus* species", Upadhyay *et al.* (2002) concluded that substrates supplemented with 1% de-fatted soybean meal performed better than those supplemented with 2.5%, 5%, 7.5% and 10% of the same material or cotton seed cake and that substrates with higher supplementation gave lower yields of the mushroom.

Tagwira *et al.* (1998) noticed that supplementation with 15% pigeon pea on sugarcane bagasse gave an ABE of 97.9%. Wheat straw supplemented with soya bean and alfalfa increased the production of *P. sajor-caju* by 300% but when ammonium nitrate was used, production increased by 50%.

According to Tagwira *et al.* (1998), addition of nitrogen to an alkaline substrate stimulated mycelium formation and production of mushrooms. However, excessive addition of inorganic nitrogen can inhibit the synthesis of the lignin degrading enzyme, thus causing decrease in productivity of the substrate.

Ayodele and Okhuoya (2007) was conducted to three agricultural by-products (oil palm fruit fibre, banana leaves and sawdust) were supplemented with wheat bran, NPK fertilizer and urea at 5 - 20% (w/w) for wheat bran and 0.1, 0.5, 1.0 and 2% (w/w) for NPK and Urea. The supplemented substrates were used to cultivate *Psathyrella atroumbonata*. There was sporophore formation in all the substrates supplemented with NPK except at 1% and 2% in oil palm fruit fibre and 2% in banana leaves. The highest yield was on sawdust supplemented with wheat bran at 5%. Urea as supplement did not support sporophore formation in all the substrates except in sawdust but with low yield at 0.5%.

Kim *et al.* (2002) found 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.

Shalahuddin *et al.*, (2018) carried out an experiment where *Pleurotus ostreatus* was evaluated for their growth and yield performance on different chemical nutrients (NPK), both for spawn production and cultivation with only 10 kg straw, 2g NPK in 10 kg straw, 4g NPK in 10 kg straw and 6g NPK in 10 Kg NPK. Where NPK was kept as 2:1:1. The highest economic yield (267.38 g) was recorded in 4g NPK with 10 kg straw treatment, again the lowest economic yield (208.11 g) was observed in NPK controlled condition. The highest moisture content was found in controlled (88.57%), while the lowest moisture content was recorded in 4g NPK with 10 kg straw treatment (84.12%). The highest biological yield (282.36 g) and economic yield (267.38 g) was attained from 4g NPK with 10 kg straw treatment. The highest benefit cost ratio (5.17) was found from 4g NPK with 10 kg straw treatment. Chemical nutrients (4 g NPK) with 10 kg rice straw performed significantly better on growth and yield of oyster mushroom.

The effort to increase the growth and production of white oyster mushroom (*Pleurotus ostreatus*), it is necessary to add nutrients to sawdust which will be used as a growing medium for white oyster mushroom (Sianturi *et al.*, 2021). One of the nutrients that need to be added is minerals including Ca (calcium) and Magnesium (Mg) contained in dolomite ($CaMg(CO_3)_2$) and macronutrients such as phosphorus contained in TSP fertilizer. The aim of this research to determine the effect of giving TSP fertilizer and dolomite on mushroom growing media (baglog) on the production of white oyster mushrooms. This research was conducted in September 2019 to March 2020 in the village, of, Lau Bakeri village, Kutalimbaru District, Deli Serdang Regency The research used a factorial randomized block design with two treatment factors, namely

TSP fertilizer (control, 1.2%, 2.4%) and dolomite (control, 1%, 2%, 3%. The results showed the application of triple super phosphate fertilizer and dolomite did not significantly affect to all observation parameters of white oyster mushrooms, but the interaction between triple super phosphate fertilizer and dolomite was able to accelerate the age of harvesting, increase the fresh weight of mushrooms in the first and second harvests and the number of mushroom caps at the third harvest.

Maniruzzaman (2004) conducted an experiment to find out the best preparation formulas of horse manure and synthetic compost. Horse manure, wheat straw, gypsum as basic materials and wheat bran, cotton seed meal, sunflower meal, malt sprout, chicken food, molasses, ammonium sulphate, urea as activators were used. The nitrogen content of the starting mixture was brought up 2 in all applications. According to the results, the highest yields with horse manure compost were obtained from the combinations of 1000 kg of horse manure, 50 kg of wheat bran, 3.1 kg of ammonium sulphate, 1.5 kg of urea, 35 kg of gypsum40 kg of chicken food or malt sprout. The highest yields with synthetic compost were obtained from the combinations of 1000 kg of wheat straw, 282 kg of wheat bran, 13 kg of urea, 23.5 kg of ammonium nitrate, 40 kg of molasses, 60 kg of gypsum, 65 kg of cotton seed meal or 100 kg of chicken food.

An experiment was carried out by Nuruddin *et al.* (2010) to investigate the effect of different levels of cowdung (0, 5, 10, 15 and 20%) on yield and proximate composition of *Pleurotus ostreatus*. The highest number of primordial (70.63) and fruiting body (51.92) were observed in rice straw supplemented with 5% level of cowdung. The highest weight of individual fruiting body (4.71g), biological yield (234.24g), economic yield (227.72g), dry yield (22.83g), biological efficiency (140.26%) and benefit cost ratio (5.69) were observed in rice straw supplemented with 10% level of cowdung.

Kulsum *et al.* (2009) conducted an experiment to determine the effect of five different levels of cowdung (0%, 5%, 10%, 15% and 20%) as supplement with sawdust on the performance of oyster mushroom. All the treatments performed better over control. The mycelium running rate in spawn packet and the highest number of primordia/packets were found to be differed due to different levels of supplements used. The highest weight of individual fruiting body was observed in sawdust supplemented with cowdung @ 10% (3.69 g). The supplementation of sawdust with cowdung had remarkable effect on biological yield, economic yield, the dry yield, biological efficiency and cost benefit ratio. The highest biological yield (217.7 g), economic yield (213g), dry yield (21.27g) biological efficiency (75.06%) and cost benefit ratio (8.41) were observed due to sawdust supplemented with cowdung @ 10%.

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.

2.5 Why oyster mushroom cultivation?

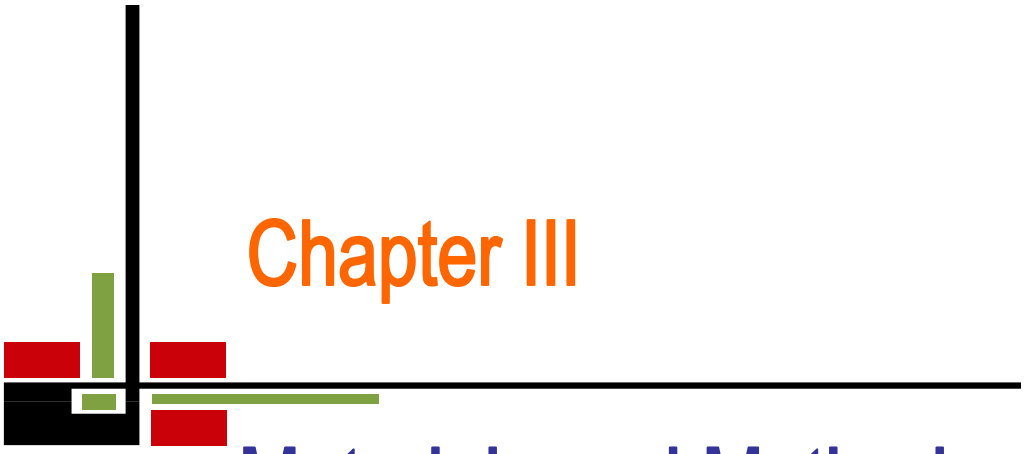
The widespread malnutrition coupled with increasing protein requirement in the country has necessitated the search for new and alternative means to meet the protein requirement of the country and one of the alternative ways is the production of mushroom which is noted for its high protein content (Hasan *et al.,* 2010). Shah *et al.*

(2004) reported that mushrooms contain about 85 – 95% water, 3% protein, 4% carbohydrate, 0.1% fats, 1% minerals and vitamins. Mushrooms also have very strong medicinal properties (Shah *et al.,* 2004). On dry weight basis, mushrooms contain 20 - 40% protein which consists of all the essential amino acids required in human diet (Horn, 2004).

Since mushrooms can grow on a wide variety of agro-waste materials because of their ability to convert these less useful products into a high-quality protein, it naturally opens new job opportunities especially in rural areas where the standard of living is very low (Amuneke *et al.,* 2011). Low-income earners can start this job with very little capital since all these agro wastes can be taken free of charge from the farms.

Shen (2002) reported that the commercial production of oyster mushroom has increased 25-fold worldwide since 1981 and is responsible for the peak production. Constant demand of the mushroom has led to increase in the price (Roach, 2006). This indicates how the usage of this mushroom increases' day by day for its significant role in human health, nutrition and disease; making it the second most cultivated mushroom worldwide (Uddin *et al.,* 2011). Increase in consumer demand for oyster mushroom has continued its rapid space over the last six years and the world demand of the mushroom has remained steady, with about 900,000t annually (Royse *et al.,* 2004). Forty-five percent of the world's supply is consumed un-processed and the rest dried (Roach, 2006). Europe remains the most major consumer of mushrooms (Roach, 2006).

The whole production process of oyster mushroom takes about 42 days and this is less than the life cycle of most arable crops (Horn, 2004). It is therefore an early income generating business that can give rapid income to the family.



Chapter III

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during the period from November 2020 to March 2021 to study the effect of supplementation of nutrients with substrates on the productivity, contamination, nutrient composition and cost benefit ratio of oyster mushroom (*Pleurotus ostreatus*). The chapter includes a brief description of the location of experiment, materials used for the experiment, design of the experiment, preparation of substrates, preparation of packets, cultivation of spawn packet, collection of produced mushrooms, proximate analysis of the mushrooms, data collection and data analysis procedure which are presented below under the following headings-

3.1. Experimental site

The field experiment was conducted at 'Mushroom Culture House (MCH)' of Sher-e-Bangla Agricultural University, Dhaka. On the other hand, the laboratory experiment was done in 'Plant Pathology laboratory', of Sher-e-Bangla Agricultural University, Dhaka and 'Biochemistry laboratory' of Mushroom Development Institute (MDI), Savar, Dhaka. Details of the meteorological data during the period of the experiment was collected from the Bangladesh Meteorological Department, Agargaon, Dhaka and presented in Appendix I.

3.2 Duration of the experiment

The experiment was carried out during the period from November, 2020 to March, 2021.

3.3 Experimental materials

Mother culture of oyster mushroom was collected from Mushroom Development Institute (MDI), Savar, Dhaka.

3.4 Varietal characteristics of Oyster Mushroom

Oyster mushroom is *Pleurotus ostreatus* that has a light to dark whitish colored cap depending upon the strain and growing conditions. Primordia and young mushrooms are light white but become less intensely colored as the mushroom matures. Oyster mushroom is 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 white color. If the temperature increases above 32^0C, its production markedly decreases.

3.5 Treatments of the experiment

Nine different treatments were used with three replications to achieve the desired objectives. The treatments were as follows:

Treatments	Ingredient	Dose
T_0 = Control	Without supplementation of nutrients	-
T_1 = Gypsum	Ca, S	1%
T_2 = Extract of sawdust	Sawdust	10%
T_3 = Torus	Gibberellic Acid	0.0001%
T_4 = Macboron	Boron	1%
T_5 = Extract of rice straw	Rice straw	10%
T_6 = Shakti zinc	Zn, S	1%
T_7 = Grow-cell	N, S, Fe, Mn, Zn, B, Cu	0.5%
T_8 = American NPKS	N, P, K, S	0.5%

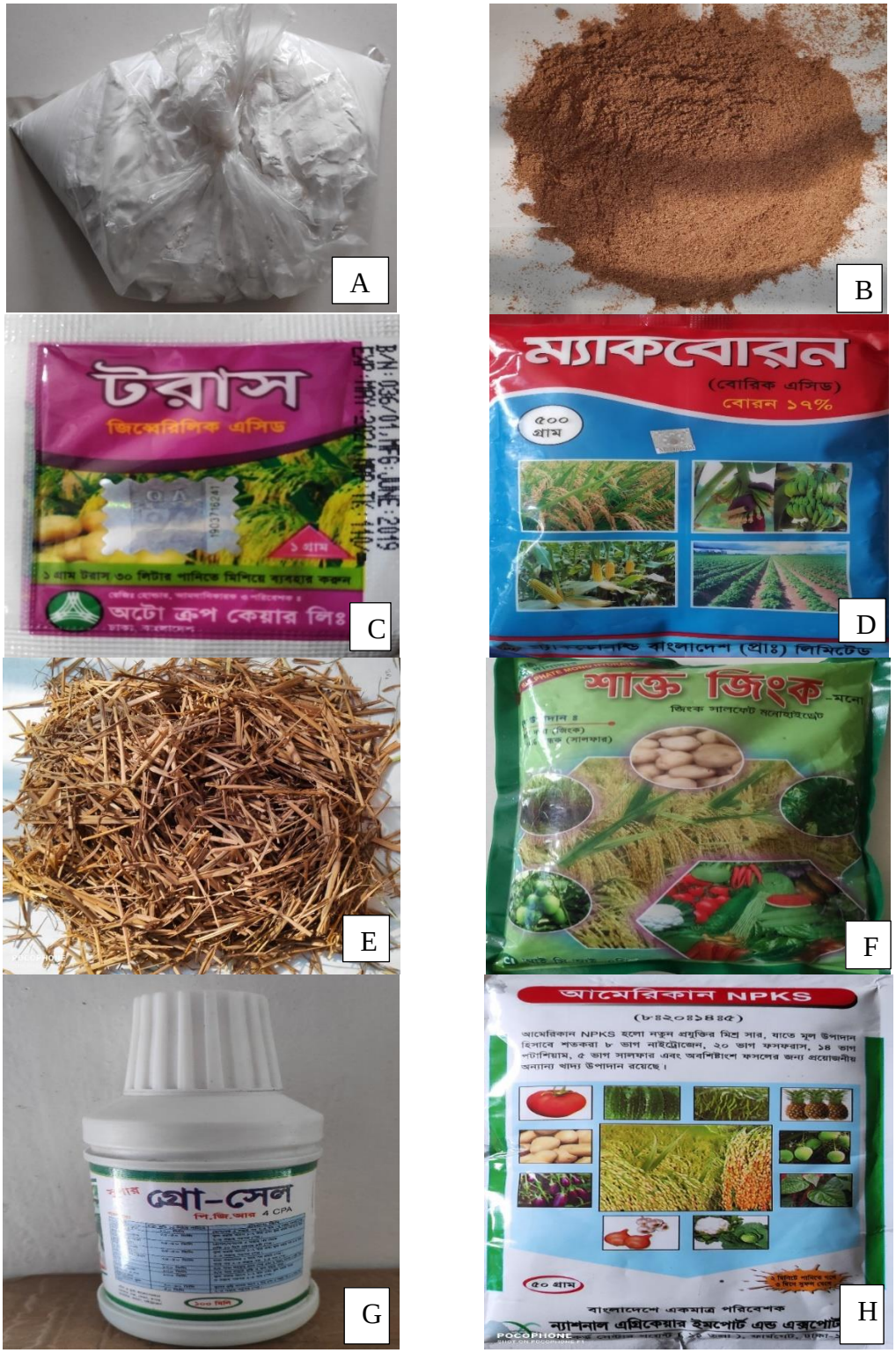


Plate 1. Materials used for different treatments **A.** Gypsum, **B.** Sawdust, **C.** Torus, **D.** Macboron, **E.** Rice straw, **F.** Shakti zinc, **G.** Grow-cell and **H.** American NPKS

3.6 Design and layout of the experiment

The experiment was laid out in single factor Completely Randomized Design (CRD). The experiment included nine treatments with three replications.

3.7 Preparation of spawn packet

Spawn packets were prepared by using sawdust and $CaCO_3$ (1g per packet). The measured materials (300 g) were taken in a plastic bowl and mixed thoroughly by hand and moisture was increased by adding nutrient solution (200 ml) according to the treatments. Moisture was measured by using the moisture meter and adjusted to the moisture content at 65%. The mixed substrates were filled into 7×11 inch polypropylene bag @ 500 g. The filled polypropylene bags were finally completed using plastic neck and plugged the neck with cotton and covered with brown paper and rubber band to hold it tightly in place.

3.8 Sterilization, inoculation and mycelium running in spawn packets

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

Plate 2. A. Mother of *Pleurotus ostreatus*, **B.** Mixing of lime with substrate, **C.** Preparation of substrate packets, **D-E.** Sterilization of substrate packets by autoclave machine, **F.** Incubation of spawn packets

3.9 Preparation of nutrient solution

For the preparation of Gypsum, Macboron and Shakti zinc solution, in each case 10 g Gypsum powder, 10 g Macboron and 10 g Shakti zinc powder were poured into 1 liter normal water individually and mixed properly. Similarly, 5 ml Grow-cell poured into 1 liter normal water as well as 5g American NPKS were poured into 1 liter normal water to prepare 0.5% Grow-cell and 0.5% American NPKS solution. On the other hand, for preparation of 10% Sawdust extract and 10% Rice straw extract, 100 g sawdust and 100 g rice straw soaked into 1 liter normal water for overnight separately. After that, the mixture was filtered to separate the 10% sawdust extraction and 10% rice straw extraction from sawdust and rice straw, respectively. For the preparation of 0.0001% Torus solution, 0.001g Torus powder was added in 1 liter normal water and mixed properly.

3.10 Application of the nutrients

All the treatments were applied to spawn packets at three different stages. The stages are-

1. during spawn packet preparation
2. after completion of mycelium running
3. after first harvest

The nutrient solutions were applied following two methods viz. spraying and immersion. The spraying method was used during spawn packet preparation stage. In this method, the nutrient solution was sprayed with the substrate @ 200 ml per packet during packet preparation to maintain the moisture level of the spawn packet. The immersion method was used after completion of mycelium running when opening/cutting the packet and after the first harvesting of fruiting bodies. In this

method, the spawn packets were immersed for soaking in nutrients solution for 20 minutes. The packets under control were soaked in plain water for same period.

3.11 Cultivation of spawn packet

Two ends, opposite to each other of the upper position of plastic bag were cut in "D" shape with a blade and opened by removing the plastic sheet after which the opened surface of the substrate was scraped slightly with a teaspoon for removing the thin whitish mycelial layer. Then the spawn packets were soaked in nutrient solution according to the treatments for 15 minutes and invested to remove excess water for another 15 minutes. The packets of each type were placed separately on the floor of culture room and covered with newspaper. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3 times a day. The light around 300-500 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22°C to 25°C. The first primordia appeared 2-4 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

3.12 Harvesting of produced mushrooms

The matured fruiting body was identified by curial margin of the cap, as described by Amin (2004). 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 nutrient solution according to the treatments 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 then second harvest was done and water spraying was continued until the mushrooms were ready to be harvested.

3.13 Data collection

3.13.1 Mycelium Running Rate (MRR) in spawn packet

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

Mycelium Running Rate (MRR) = $\frac{L}{N}$ cm/day

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

N = Number of days

3.13.2 Days required from incubation to mycelium initiation

Days required from incubation to mycelium initiation was recorded.

3.13.3 Days required from incubation to completion of mycelium running

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

3.13.4 Days Required from Stimulation to Primordia Initiation (days)

Days Required from Stimulation to Primordia Initiation (days) was recorded.

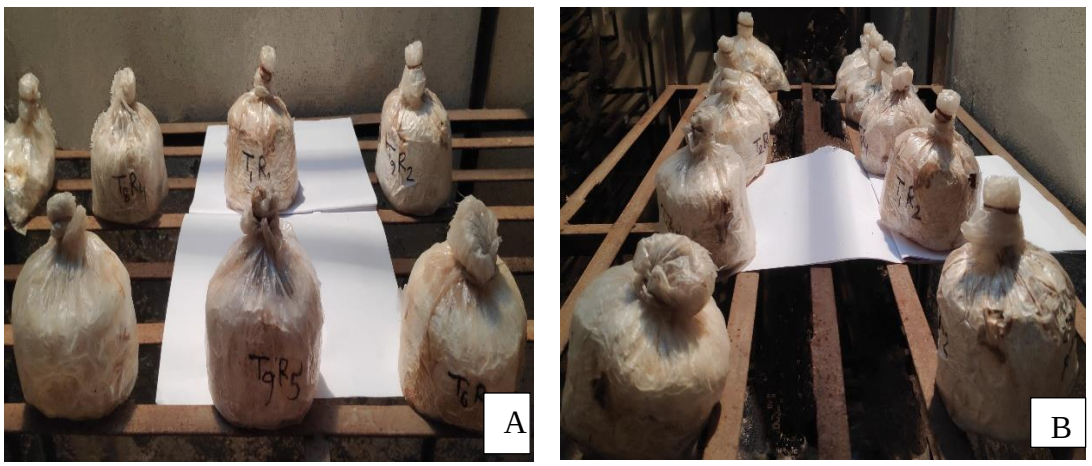


Plate 3. A-B. Mycelium running in spawn

3.13.5 Days Required from Primordia Initiation to 1st Harvest (days)

Days required from primodia formation to first harvest was recorded.

3.13.6 Total harvesting period (days)

Days required from primordia formation to final harvest was recorded.

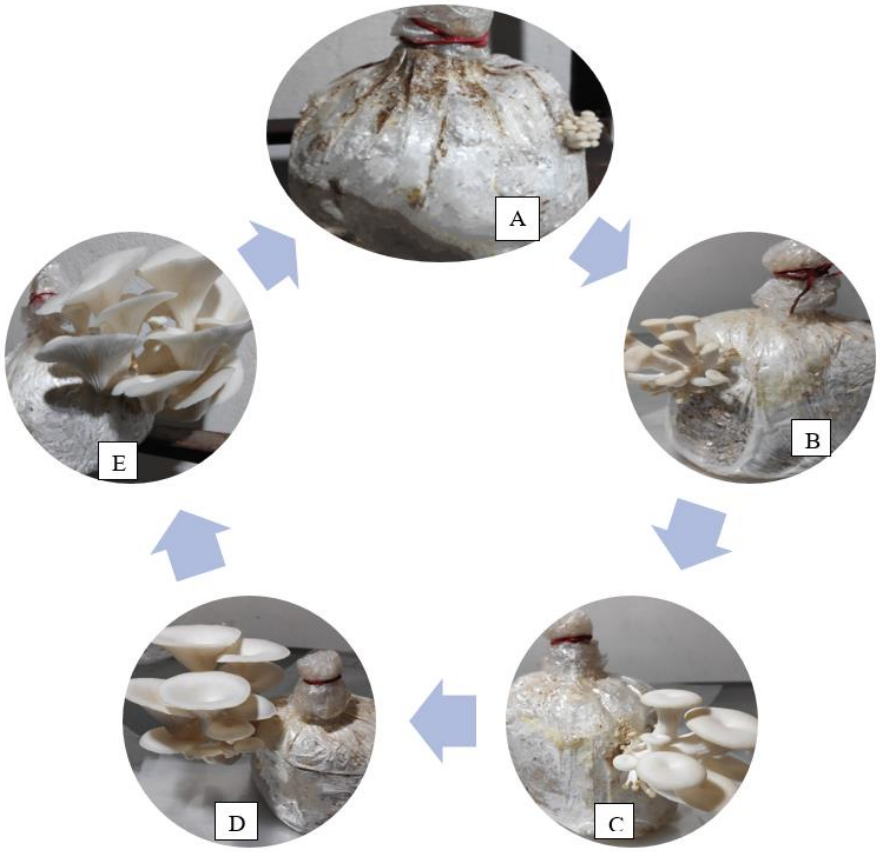


Plate 4. A-E. Steps of primordia initiation to mature fruiting body in spawn packets

3.13.7 Data on yield contributing parameters

Number of primordia and well-developed fruiting bodies was recorded. Dry, undesired fruiting bodies were discarded. Data on the following parameters were also recorded:

a) Number of primordia per packet

b) Number of fruiting bodies per packet

c) Number of effective fruiting bodies per packet

3.13.8 Dimension of fruiting body (stipe and pileus)

Length of stipes and pileus of three randomly selected fruiting bodies from every treatment was measured using a measurement scale. The width of pileus was also measured by measurement scale.

3.13.9 Biological yield (g)

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

3.13.10 Economic yield (g)

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

3.13.11 Dry yield (g)

About 50 g of randomly selected mushroom sample was taken in a paper envelope and was weighed correctly. The mushroom was oven-dried at 72^0C temperature for 24 hours and weighed again. The weight of the blank envelope was subtracted from both the initial weight. The dry yield was calculated using the following formula (Sarker, 2004):

$$\text{Dry yield (g)} = \frac{\text{Economic yield x Oven dry weight of sample (g)}}{\text{Fresh weight of sample (g)}}$$

Figure 1. Measurement of the weight of fruiting body

3.13.12 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:

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

3.13.13 Severity of contamination (%) on spawn packets

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

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

3.13.14 Benefit cost ratio

The benefit cost ratio for different low-cost substrates were computed based on present market price of mushroom and cost of different inputs in the markets (Sarker, 2004).

3.14 Proximate analysis of the mushroom

3.14.1 Determination of moisture

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

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

3.14.2 Determination of protein

The Protein contents of the fruiting bodies or the mushrooms were determined by the standard Biuret method. The Biuret method is based on the complexation of Cu^{2+} to functional groups in the protein's peptide bond. The determination of protein concentration is an essential technique in all aspects of protein studies and proteomics.

Principle

The –CO-NH- bond (peptide) in polypeptide chain reacts with copper sulphate in an alkaline medium to give a purple color which can be measured at 540 nm.

Reagents

1. Biuret Reagent: Dissolve 3 g of copper sulphate ($CuSO_4.5H_2O$) and 9 g of sodium potassium tartarate in 500 ml of 0.2 mol/liter sodium hydroxide; add 5 g of potassium iodide and make up to 1 liter with 0.2 mol/liter sodium hydroxide.

2. Protein Standard: 5 mg BSA/ml.

Procedure

Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard into the series of labeled test tubes and pipette out 1 ml of the given sample in another test tube.

Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank. Then add 3 ml of Biuret reagent to all the test tubes including the test tubes labeled 'blank' and 'unknown'. After that, mix the contents of the tubes by vortexing / shaking the tubes and warm at 37 ºC for 10 min. Then cool the contents to room temperature and record the absorbance at 540 nm against blank. Then plot the standard curve by taking concentration of protein along X-axis and absorbance at 540 nm along Y-axis. Finally, from this standard curve calculate the concentration of protein in the given sample.

By substituting the values in the equation, unknown concentration of the protein can be calculated.

Concentration (mg/ml) = (Absorbance-Intercept)/Slope

3.14.3 Determination of lipid

Lipid was estimated as crude ether extraction of the dry materials. The dried sample (about 5.0 g) was weighed into a conical flask and plugged with fat free cotton. The flask was then placed in an electric shaker and extracted with anhydrous ether for about 16 hours. The ether extract was filtered into another weighed conical flask. The flask containing the original ether extract was washed 4 to 5 times with small quantities of ether and the washings were also transferred to the filter paper. The ether in the conical flask was then removed by evaporation, and the flask with the residual was dried in an oven at 800C to 1000C, cooled in a dessicator and weighed. The result was expressed as follows:

Lipid contents (g) per 100 g of dried sample =

$$\frac{\text{Weight of ether extract x Percentage of dried sample}}{\text{Weight of dried sample taken}}$$

3.14.4 Total carbohydrate estimation

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

% Carbohydrate = 100 – [(moisture + Fat + Protein + Ash + Crude fiber)]

3.14.5 Determination of crude fiber

Ten grams of moisture and fat-free sample was taken in a beaker and 200 ml of boiling 0.255 N H2SO4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a Moslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker and 200 ml of boiling 0.313 N NaOH was added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a Moslin cloth and the residue was washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100°C and weighed (We) in an electric balance (*KEY: JY-2003; China*). The crucible was heated in a muffle furnace (*Nebertherm: Mod-L9/11/c6; Germany*) at 600°C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber (Raghuramulu *et al.*, 2003).

Therefore,

Crude fiber (g/100 g sample) = [100 – (moisture + fat)] x (We – Wa)/Wt of sample

3.14.6 Determination of total ash

One gram of the sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5-6 hours at 600°C. It was then cooled in a desiccator and weighed. To ensure completion of ashing,

the crucible was then heated in the muffle furnace for 1h, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or grayish white in color. Then total ash was calculated as following equation (Raghuramulu *et al.*, 2003):

$$\text{Ash content (g/100 g sample)} = \frac{\text{Weight of ash x 100}}{\text{Weight of sample taken (g)}}$$

3.15 Analysis of data

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

3.16 Collection of contaminated spawn packet

Contaminated spawn packets were collected from Mushroom Culture House (MCH) of Sher-e-Bangla Agricultural University. Isolation of contaminating microorganisms causing spoilage of spawn packets and fruiting bodies were done by following appropriate methodology (Dhingara and Sinclair, 1995).

3.16.1 Composition and preparation of agar media

Ingredients	Amount (per liter)
Potato	200 g
Dextrose	20 g
Agar	20 g
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.

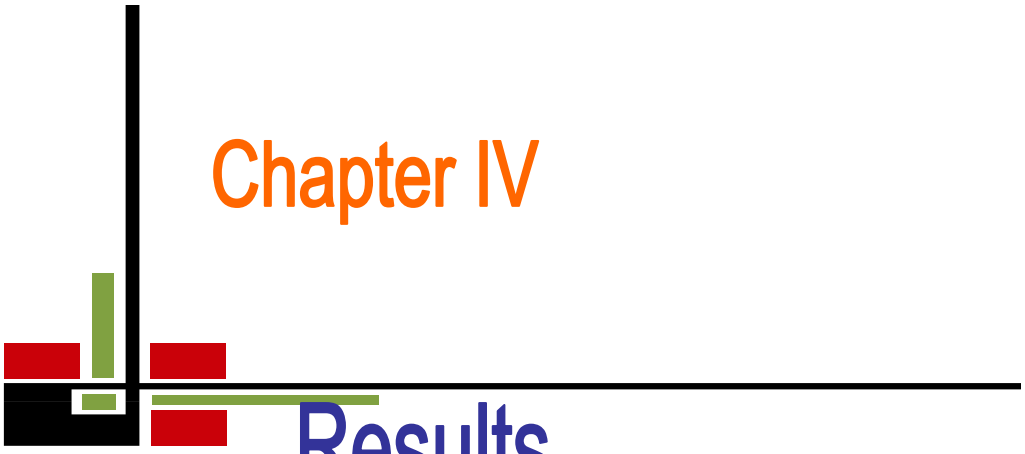
3.15.2 Isolation and purification of competitor moulds from collected spawn

10g of substrate 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 the dilution. 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 subculturing were done by hyphal tip technique (Hyakumachi, 1994). All the pure cultures were kept in refrigerator at 4ºC for preservation.

Plate 5. A-B. Boiling of sliced, peeled potatoes (60 g), **C.** Measurement of the weight of Dextrose and Agar (6 g each), **D.** PDA solution (300 ml), **E.** Sterilization of glassware in the oven, **F.** Prepared PDA solution poured into sterile Petri dish

3.15.3 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 the literature. The microphotograph of pathogens was also taken using a microscope. The morphological characteristics of individual fungi were recorded and compared with appropriate key books like CMI descriptions of fungi to identify each fungus (Barnett and Hunker, 1972).



Chapter IV

Results

CHAPTER IV

RESULTS

4.1 Effect of different nutrient supplements on contamination severity

All the spawn packets under different treatments were free from contamination during incubation period (Table 1). But 15 days after stimulation (DAS), only the spawn packets similar contamination was recorded under T_0 (without supplementation of nutrients) and T_2 (10% extract of saw dust) and that was 6.67%. At 30 DAS, identical contamination severity (6.67%) was recorded from T_1 and T_5. Similarly, 13.33% severity was recorded in case of T_5 and T_8. At 45 DAS, the highest contamination severity (46.67%) was recorded from control (T_o). Similarly, 26.68% contamination was observed at T_4 and T_8 treatment.

Table 1: Effect of different nutrient supplementation on the severity of contamination at different days after stimulation (DAS)

Treatments	Incubation period (%)	Cultivation period (%)		
		15 DAS	30 DAS	45 DAS
T_0	0	6.67	26.68	46.67
T_1	0	0	6.67	13.33
T_2	0	6.67	20	40
T_3	0	0	0	0
T_4	0	0	6.67	26.68
T_5	0	0	13.33	33.33
T_6	0	0	0	6.67
T_7	0	0	0	0
T_8	0	0	13.33	26.68

[T_0 = Control (without supplementation of nutrients); T_1 = 1% Gypsum; T_2 = 10% Extract of sawdust; T_3 = 0.0001% Torus; T_4 = 1% Macboron; T_5 = 10% Extract of rice straw; T_6 = 1% Shakti zinc; T_7 = 0.5% Growcell; T_8 = 0.5% American NPKS]

4.2 Identified contaminants from contaminated spawn

Moulds are the biggest competitor to the mushroom grower. Based on the morphological characters commonly four pathogens were identified, these are *Trichoderma harzianum*, *Penecillium* sp. *Rhizopus stolonifer* and *Aspergillus niger*.

Plate 6. A-D. Different contaminated spawn packets

4.2.1 *Rhizopus stolonifer*

Rhizopus stolonifer is common contaminant. What makes *Rhizopus* tricky to deal with is that it grows extremely fast. Similar in appearance to pin mould, *Rhizopus* develops hair-like sporophores with a tiny head (Plate 7 A-B). Sporangia which are bulbous structures that sprout from the vegetative hyphae and hold the haploid spores. *Rhizopus* has a sour odour. Sometimes, the smell of the fungus may also resemble alcohol.

4.2.2 *Aspergillus niger*

Aspergillus niger was found in the contaminated the spawn packets in the growing house. It produced black colored spores so it was called black mold (Plate 7 C-D). 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 and aseptate.

4.2.3 *Trichoderma harzianum*

Green color growth of mycelium was observed in contaminated spawn packet 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 7 E-F). *T. harzianum* had occasionally concentric condition with whitish yellow conidial area. Conidiophores are branched that cluster into fascicles. Normally branches are formed near 90º with the main branch. The conidiophores terminated with one or few phialides that usually rise from the axis near the tip.

4.2.4 *Penicillium* sp.

Initially, *Penicillium* appeared as a white colored powder on the substrates of oyster mushroom and later turned into green as time passed, it is called blue green mold. Pure culture of *Penicillium* was prepared on PDA from collected contaminated spawn (Plate 7 G). 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 (Plate 7 H). 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.

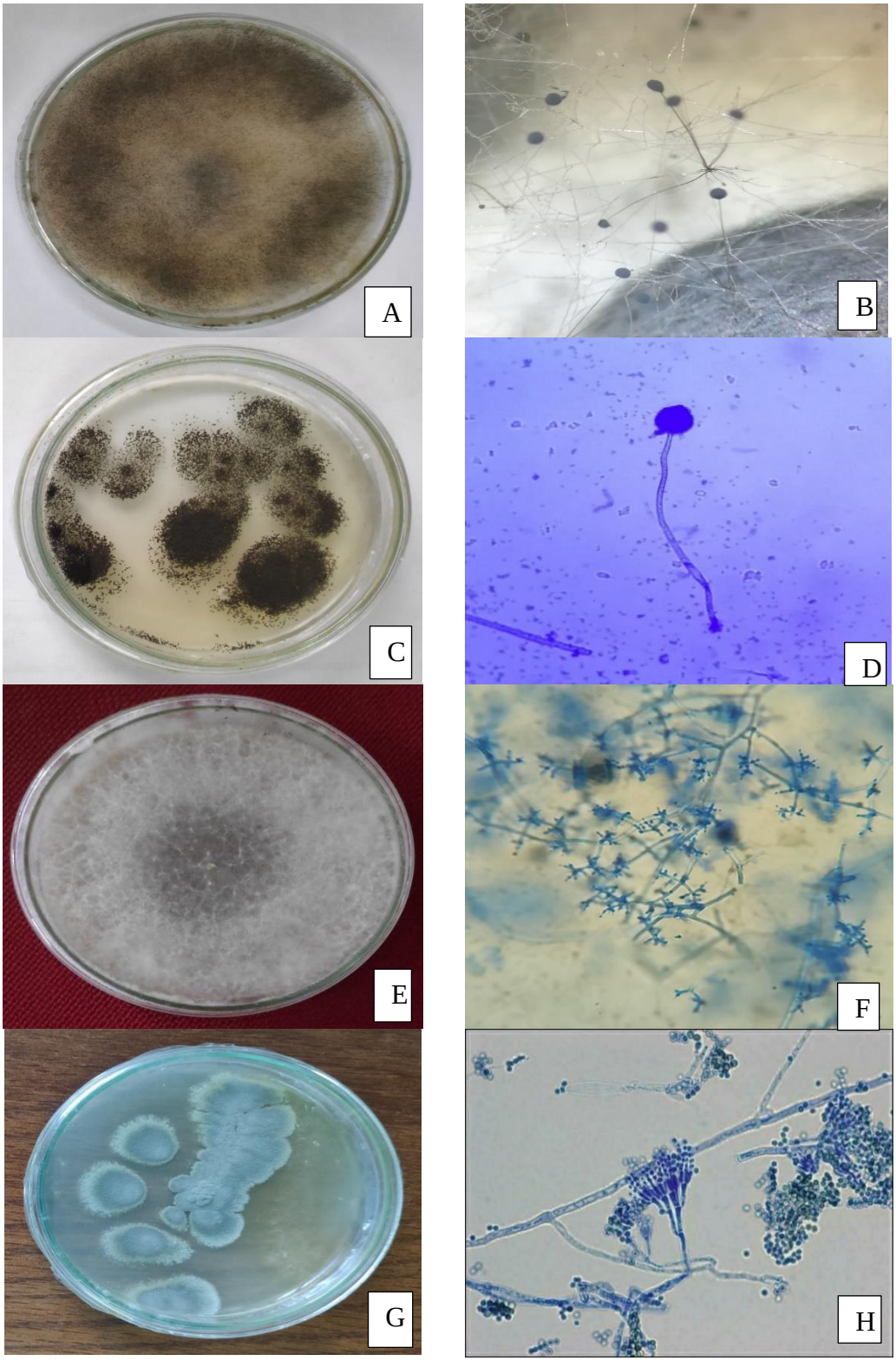


Plate 7. A. Pure culture of *Rhizopus stolonifer*, **B.** Pathogenic structure of *Rhizopus stolonifer*, **C.** Pure culture of *Aspergillus niger*, **D.** Pathogenic structure of *Aspergillus niger*, **E.** Pure culture of *Trichoderma harzianum*, **F.** Pathogenic structure of *Trichoderma harzianum*, **G.** Pure culture of *Penicillium* sp. **H.** Pathogenic structure of *Penicillium* sp.

4.3 Growth and yield contributing characters

4.3.1 Effect different nutrients on mycelium running rate (cm/day)

Mycelium running rate of oyster mushroom (*Pleurotus ostreatus*) showed statistically significant variation due to supplementation of substrates under the present trial (Figure 2 and Appendix II). The highest mycelium running rate (0.82 cm/day) was recorded from T_7 (0.5% Growcell), which was statistically similar with T_3 (0.0001% Torus), while the lowest mycelium running rate (0.60 cm/day) was observed in T_0 (without supplementation of nutrients).

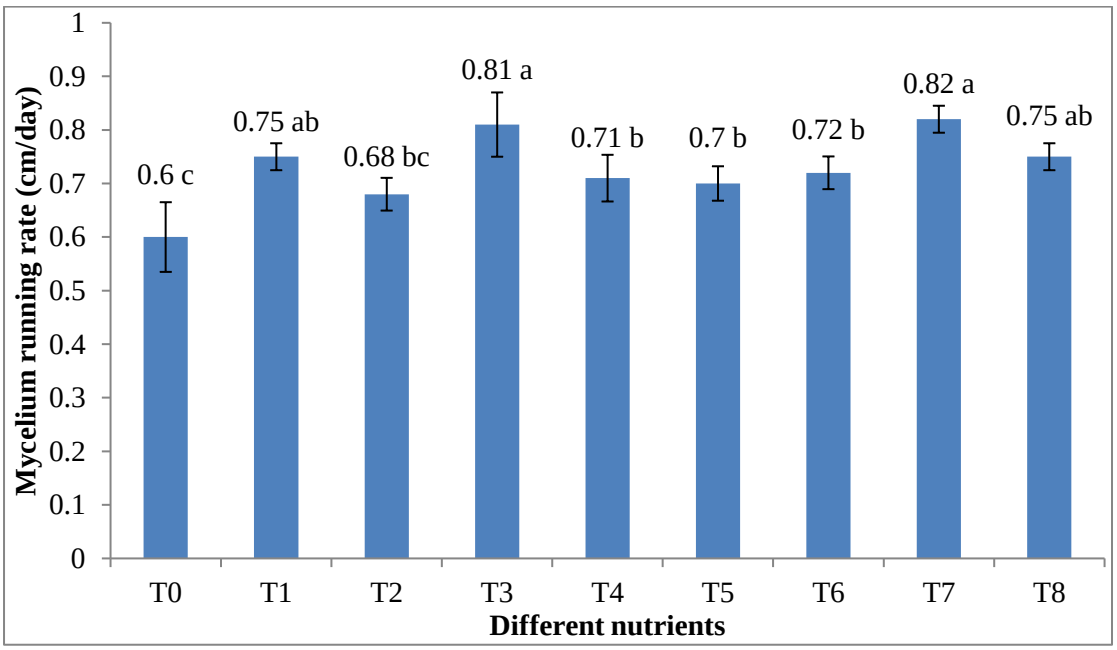


Figure 2. Effect of different nutrients supplement on mycelium running rate of oyster mushroom ($LSD_{0.05}$ = 0.08)

[T_0 = Control (without supplementation of nutrients); T_1 = 1% Gypsum; T_2 = 10% Extract of saw dust; T_3 = 0.0001% Torus; T_4 = 1% Macboron; T_5 = 10% Extract of rice straw; T_6 = 1% Shakti zinc; T_7 = 0.5% Growcell; T_8 = 0.5% American NPKS]

4.3.2 Effect of different nutrients on time required from incubation to mycelium initiation

There was significant variation in terms of time from incubation to mycelium initiation of oyster mushroom (*P. ostreatus*) due to supplementation of different nutrients (Table 2 and Appendix II). The highest time (7.67 days) from incubation to mycelium initiation was found in T_0 (without supplementation of nutrients) followed by T_2 (10% Extract of saw dust), whereas the lowest time (5.00 days) from incubation to mycelium initiation was recorded in T_7 (0.5% Growcell). The duration from incubation to mycelium initiation in T_1, T_3, T_4, T_6 and T_8 was statistically same but significantly different.

4.3.3 Effect of different nutrients on time required from incubation to completion of mycelium running

Days required from incubation to completion of mycelium running of oyster mushroom varied significantly due to supplementation of different nutrients (Table 2 and Appendix II). The highest time (23.33 days) required for mycelium running was recorded in T_0 (without supplementation of nutrients) followed by T_2 (10% Extract of saw dust), whereas the lowest time (17.33 days) required for mycelium running was recorded in T_7 (0.5% Growcell). The duration from incubation to completion of mycelium running in T_1, T_3, T_6 and T_8 was statistically identical but significantly different.

Table 2. Performance of different nutrients on time required from incubation to initiation and completion of mycelium running

Treatments	Time required from incubation	
	to mycelium initiation (days)	to completion of mycelium running (days)
T_0	7.67 a	23.33 a
T_1	5.67 cd	18.33 cd
T_2	7.33 ab	21.67 ab
T_3	5.33 cd	18.00 cd
T_4	6.00 cd	19.67 bcd
T_5	6.33 bc	20.33 bc
T_6	5.67 cd	19.00 cd
T_7	5.00 d	17.33 d
T_8	5.67 cd	18.33 cd
CV (%)	10.02	7.30
$LSD_{(0.05)}$	1.02	2.40

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

[T_0 = Control (without supplementation of nutrients); T_1 = 1% Gypsum; T_2 = 10% Extract of saw dust; T_3 = 0.0001% Torus; T_4 = 1% Macboron; T_5 = 10% Extract of rice straw; T_6 = 1% Shakti zinc; T_7 = 0.5% Growcell; T_8 = 0.5% American NPKS]

4.3.4 Effect of different nutrients on time required from stimulation to primordia initiation (days)

There was significant variation in terms of time from stimulation to primordial initiation of oyster mushroom due to supplementation of different nutrients (Table 3 and Appendix III). The time from stimulation to primordial initiation ranged from 5.89 days to 7.00 days. The highest time from stimulation to primordial initiation (7.00 days) was observed in T_0 (without supplementation of nutrients), whereas the lowest time (5.89 days) from stimulation to primordia initiation was in the treatment T_7 (0.5% Growcell).

The other treatments varied significantly in terms of time from stimulation to primordia initiation.

4.3.5 Effect of different nutrients on time required from primordial initiation to 1st harvest (days)

Data revealed that time from primordial initiation to 1st harvest of oyster mushroom was statistically significant compared to control due to application of different nutrients (Table 3 and Appendix III). The maximum time (6.33 days) from primordia initiation to harvest was observed in T_0 (without supplementation of nutrients), whereas the lowest time (4.67 days) from primordia initiation to harvest was in T_7 (0.5% Growcell).

4.3.6. Total harvesting period (days)

Statistically significant variation was recorded in terms of days for final harvest of oyster mushroom due to supplementation of different nutrients (Table 3 and Appendix III). The highest harvesting period (48.67 days) was recorded in T_0 (without supplementation of nutrients) which was statistically similar with T_2 (48.67 days) and T_5 (47.67 days). On the other hand, the lowest harvesting period (40.00 days) was recorded in T_7 (0.5% Growcell).

Table 3: Performance of different nutrients on time required from stimulation to primordia initiation, primordial initiation to 1st harvest and Total harvesting period

Treatments	Time required from stimulation to primordia initiation (days)	Time required from primordial initiation to 1st harvest (days)	Total harvesting period (days)
T_0	7.00 a	6.33 a	48.67 a
T_1	6.11 b	5.33 abc	46.33 ab
T_2	6.67 ab	6.00 ab	48.67 a
T_3	6.11 b	5.00 bc	42.00 bc
T_4	6.22 ab	5.33 abc	47.33 ab
T_5	6.45 ab	5.67 abc	47.67 a
T_6	6.11 b	5.67 abc	45.67 ab
T_7	5.89 b	4.67 c	40.00 c
T_8	6.11 b	5.00 bc	44.67 abc
CV (%)	7.35	10.60	6.34
$LSD_{(0.05)}$	0.78	0.97	4.86

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

[T_0 = Control (without supplementation of nutrients); T_1 = 1% Gypsum; T_2 = 10% Extract of saw dust; T_3 = 0.0001% Torus; T_4 = 1% Macboron; T_5 = 10% Extract of rice straw; T_6 = 1% Shakti zinc; T_7 = 0.5% Growcell; T_8 = 0.5% American NPKS]

4.3.7 Effect of different nutrients on average number of primordia per packet

Average number of primordia per packet varied from 86.33 to 64.00 significantly due to application of different nutrients supplement with substrate under the present trial (Figure 3 and Appendix IV). The maximum average number of primordia per packet was observed from T_7 (0.5% Growcell) which was statistically similar with T_3 (0.0001% Torus); T_8 (0.5% American NPKS); T_6 (1% Shakti zinc); T_1 (1% Gypsum)

and T_4 (1% Macboron). On the other hand, the minimum number of primordia per packet was found in T_0 (without supplementation of nutrients) which was statistically identical with T_2 (10% Extract of saw dust) and T_5 (10% Extract of rice straw).

4.3.8 Effect of different nutrients on average number of fruiting body per packet

Average number of fruiting body was ranged from 40.67 to 23.33 which had statistically significant differences due to application of different nutrients with saw dust (Figure 3 and Appendix IV). The maximum number of fruiting body per packet was recorded from T_7 (0.5% Grow-cell), whereas the minimum average number of fruiting body per packet was observed in T_0 (without supplementation of nutrients).

4.3.9 Effect of different nutrient on average number of effective fruiting body

Statistically significant variation was recorded due to application of different nutrient supplements in terms of effective fruiting body per packet of oyster mushroom (Figure 3 and Appendix IV). The maximum number of effective fruiting body per packet (30.00) was recorded from T_7 (0.5% Grow-cell) followed by T_3, T_8 and T_6 whereas the minimum average number of effective fruiting body per packet (17.33) was observed in T_0 (without supplementation of nutrients).

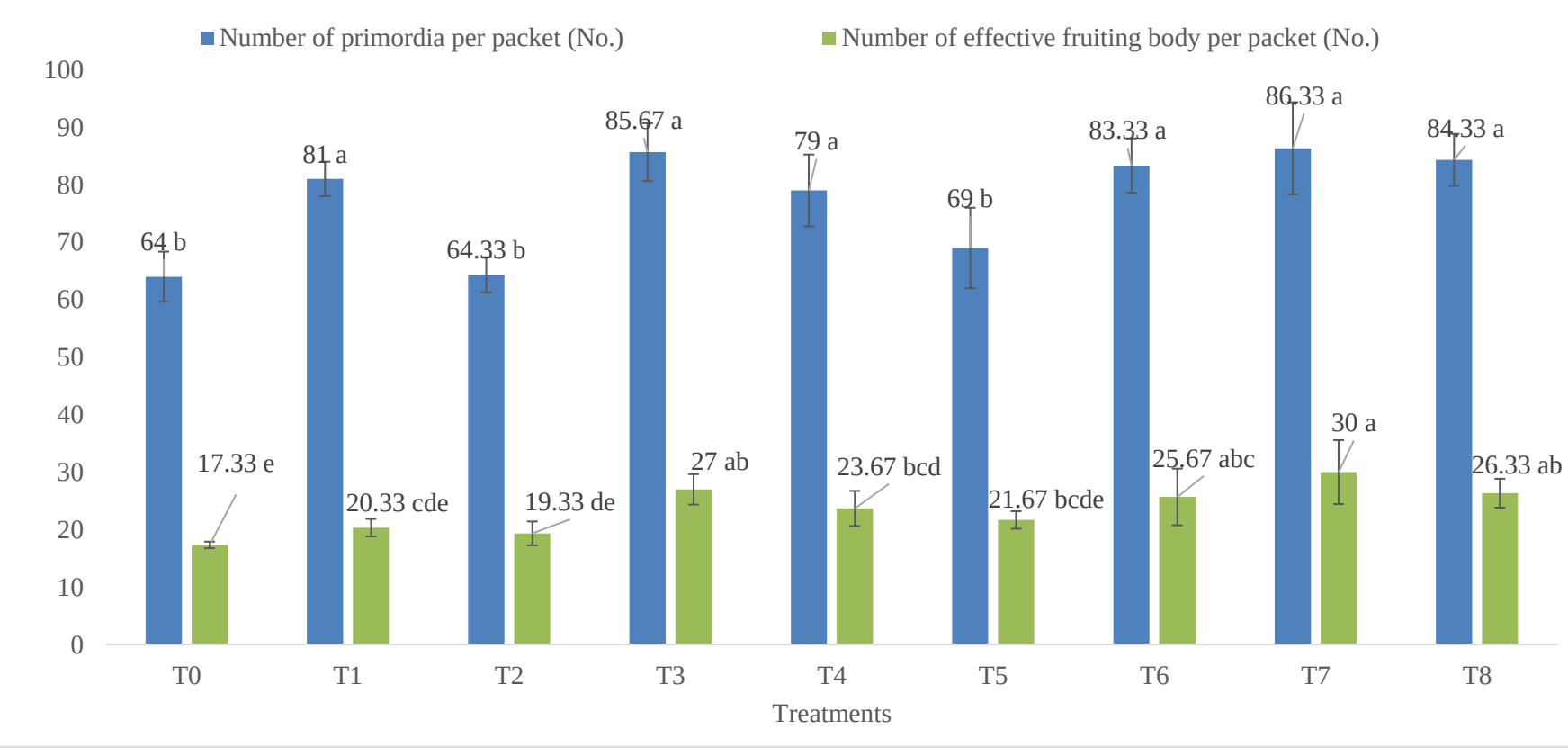


Figure 3. Effect of different nutrients on number of primordia/packet and number of effective fruiting body/packet of oyster mushroom ($LSD_{0.05}$ = 8.98, 9.23 & 5.23).

[T_0 = Control (without supplementation of nutrients); T_1 = 1% Gypsum; T_2 = 10% Extract of sawdust; T_3 = 0.0001% Torus; T_4 = 1% Macboron; T_5 = 10% Extract of rice straw; T_6 = 1% Shakti zinc; T_7 = 0.5% Grow-cell; T_8 = 0.5% American NPKS]

4.4 Dimension of fruiting body (cm)

4.4.1 Length of pileus

Length of pileus of oyster mushroom varied from 4.03 to 5.11 cm significantly due to application of different level of nutrient supplement under the present trial (Table 4 and Appendix V). The highest length of pileus was recorded from T_7 (0.5% Growcell), whereas the lowest length of pileus was found in T_0 (without supplementation of nutrients). Statistically similar result was found in case of T_1, T_2, T_3, T_4, T_5, T_6 and T_8 which were significantly different.

4.4.2 Diameter of pileus

Significant difference was recorded in terms of width of pileus of oyster mushroom due to supplementation of nutrients (Table 4 and Appendix V). The highest width of pileus (7.45 cm) was observed in T_7 (0.5% Growcell) which was statistically similar with T_3 (0.77 cm). On the other hand, the lowest width of pileus (5.25 cm) was found in T_0 (without supplementation of nutrients).

4.4.3 Length of stipe

Length of stipe of oyster mushroom ranged 1.77 to 3.10 cm significantly due to supplementation of different nutrients (Table 4 and Appendix V). The highest length of stipe was observed in T_7 (0.5% Growcell) whereas, the lowest length of stipe was found in T_0 (without supplementation of nutrients).

Table 4: Effect of different nutrients on the dimension of fruiting body of oyster mushroom

Treatments	Length of pileus (cm)	Diameter of pileus (cm)	Length of stipe (cm)
T_0	4.03 b	5.25 c	1.77 c
T_1	4.69 ab	6.55 ab	2.70 ab
T_2	4.29 ab	5.55 bc	2.27 bc
T_3	4.92 ab	6.78 a	2.97 ab
T_4	4.67 ab	6.52 ab	2.50 abc
T_5	4.48 ab	6.32 abc	2.43 abc
T_6	4.73 ab	6.66 ab	2.77 ab
T_7	5.11 a	7.45 a	3.10 a
T_8	4.90 ab	6.71 ab	2.80 ab
CV (%)	11.40	9.80	16.67
$LSD_{(0.05)}$	0.89	1.06	0.72

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

[T_0 = Control (without supplementation of nutrients); T_1 = 1% Gypsum; T_2 = 10% Extract of saw dust; T_3 = 0.0001% Torus; T_4 = 1% Macboron; T_5 = 10% Extract of rice straw; T_6 = 1% Shakti zinc; T_7 = 0.5% Growcell; T_8 = 0.5% American NPKS]

4.5 Yield parameter (g)

4.5.1 Biological yield (g)

Due to application of different level of nutrient supplements, biological yield of oyster mushroom showed statistically significant variation (Table 5 and Appendix VI). The highest biological yield (174.7 g) was recorded from T_7 (0.5% Growcell) followed by T_3 (169.0 g) and T_8 (165.7 g). While the lowest biological yield (119.3 g) was recorded in T_0 (without supplementation of nutrients) which was statistically similar with T_2 (123.0 g).

4.5.2 Economic yield (g)

Statistically significant variation was recorded in terms of economic yield of oyster mushroom due to application of different level of nutrient supplements (Table 5 and Appendix VI). The highest economic yield (167.3 g) was recorded from T_7 (0.5% Growcell) followed by T_3 (163.7 g) and T_8 (159.0 g), whereas the lowest economic yield (113.7 g) was found in T_0 (without supplementation of nutrients) which was statistically similar with T_2 (117.7 g).

4.5.3 Dry yield (g)

Different supplements influenced dry yield and statistically significant variation was recorded in this present experiment. (Table 5 and Appendix VI). The highest dry yield (17.33 g) was recorded from T_7 (0.5% Growcell) followed by T_3 (17.00 g) and T_8 (16.33 g), whereas the lowest dry yield (11.67 g) was found in T_0 (without supplementation of nutrients) which was statistically similar with T_2 (12.0 g).

Table 5: Effect of different nutrient supplementation on yield parameters of oyster mushroom

Treatments	Biological yield (g)	Economic yield (g)	Dry Yield (g)
T_0	119.3 f	113.7 f	11.67 f
T_1	153.3 cd	148.0 cd	15.33 cd
T_2	123.0 f	117.7 f	12.00 f
T_3	169.0 ab	163.7 ab	17.00 ab
T_4	146.7 d	140.7 d	14.33 de
T_5	134.7 e	129.3 e	13.00 ef
T_6	161.3 bc	155.3 bc	15.67 bcd
T_7	174.7 a	167.3 a	17.33 a
T_8	165.7 ab	159.0 ab	16.33 abc
CV (%)	3.57	3.87	5.54
$LSD_{(0.05)}$	8.97	9.35	1.37

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

[T_0 = Control (without supplementation of nutrients); T_1 = 1% Gypsum; T_2 = 10% Extract of saw dust; T_3 = 0.0001% Torus; T_4 = 1% Macboron; T_5 = 10% Extract of rice straw; T_6 = 1% Shakti zinc; T_7 = 0.5% Growcell; T_8 = 0.5% American NPKS]

4.5.4 Biological efficiency

Remarkable differences were observed in biological efficiency and it was ranged from 23.87% to 34.93%. The highest of biological efficiency (34.93%) was observed when saw dust supplemented with 0.5% Grow-cell, while the lowest (23.87%) biological efficiency was recorded in controlled condition which was statistically similar with T_2 treatment.

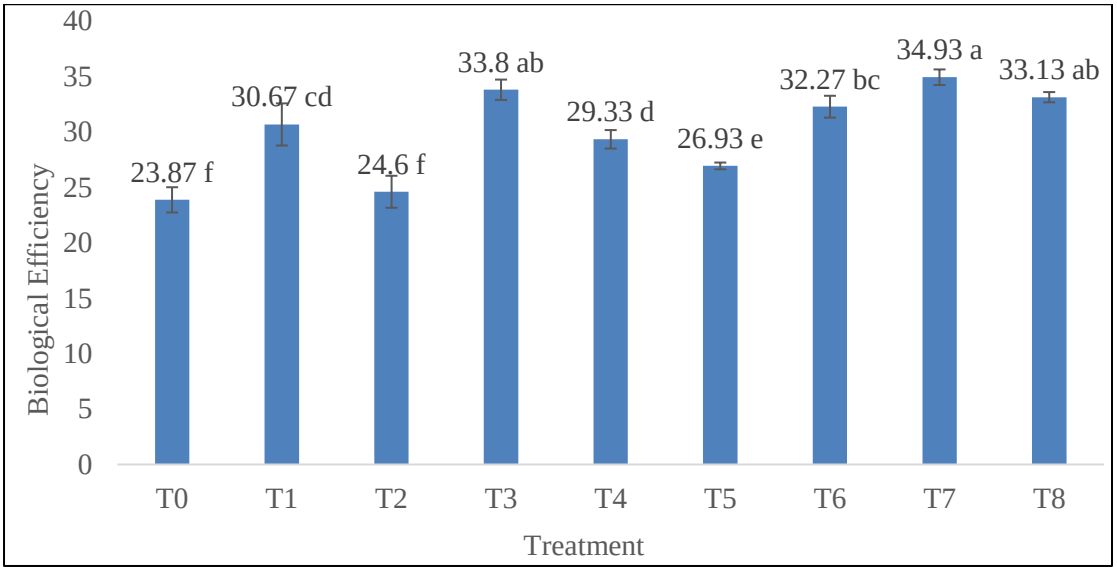


Figure 4. Effect of different nutrients on biological efficiency of oyster mushroom ($LSD_{0.05}$ = 1.80)

[T_0 = Control (without supplementation of nutrients); T_1 = 1% Gypsum; T_2 = 10% Extract of saw dust; T_3 = 0.0001% Torus; T_4 = 1% Macboron; T_5 = 10% Extract of rice straw; T_6 = 1% Shakti zinc; T_7 = 0.5% Growcell; T_8 = 0.5% American NPKS]

4.6 Benefit cost analysis of different nutrient supplements

Cost of the nutrient supplements were analyzed for calculation of the cost benefit ratio to use the best dose of application. For the purpose, cost of total materials and dose of nutrient cost per packet was considered as input and sole price of mushroom fruiting body was considered as output or benefit for this experiment. The total cost after adding nutrients per packet taka 15.00; 15.24; 15.18; 15.06; 16.08; 15.12; 16.02; 18.00 and

16.80 respectively, according to sequence of treatments from T_0 to T_8. The highest net returns was found from T_3 (34.05 Tk) and the lowest net return was recorded in T_0 (19.11 Tk) treatment. The highest benefit cost ratio was found from T_3 (3.26) and the lowest benefit cost ratio was recorded in T_0 (2.27).

Table 6. Benefit cost ratio of different nutrient supplements per packet of mushroom production

Treatments	Cost of total materials per packer (Tk)	Total cost after adding nutrients per packet (Tk)	Economic yield per packet (g)	Economic return per packet (Tk)	Net return per packet (Tk)	Benefit cost ratio
T_0	15.00	15.00	113.7	34.11	19.11	2.27
T_1	15.00	15.24	148.0	44.4	29.16	2.91
T_2	15.00	15.18	117.7	35.31	20.13	2.33
T_3	15.00	15.06	163.7	49.11	34.05	3.26
T_4	15.00	16.08	140.7	42.21	26.13	2.63
T_5	15.00	15.12	129.3	38.79	23.67	2.57
T_6	15.00	16.02	155.3	46.59	30.57	2.91
T_7	15.00	18.00	167.3	50.19	32.19	2.79
T_8	15.00	16.80	159.0	47.7	30.9	2.84

[T_0 = Control (without supplementation of nutrients); T_1 = 1% Gypsum; T_2 = 10% Extract of sawdust; T_3 = 0.0001% Torus; T_4 = 1% Macboron; T_5 = 10% Extract of rice straw; T_6 = 1% Shakti zinc; T_7 = 0.5% Grow-cell; T_8 = 0.5% American NPKS]

Selling price of mushroom per kg 300 Tk.

Economic return/packet (Tk) = Economic yield/packet × Selling price/kg

Net return/packet = Economic return/packet - Cost after adding nutrients/packet

BCR = Economic return/packet (Tk) ÷ Total cost after adding nutrients/packet (Tk)

4.7 Relationship between economic yield and different parameters of oyster mushroom

4.7.1 Relationship between the number of primordia per packet and economic yield of oyster mushroom

A significant and linear positive correlation between economic yield and number of primordia per packet of oyster mushroom when saw dust was supplemented with different nutrients (Figure 5). The relationship between economic yield and number of primordia per packet of oyster mushroom could be expressed by the regression equation, $y = 2.133x – 21.331$ ($R^2 = 0.9687$) where y = economic yield and x = number of primordia per packet. The R^2 value indicated that 96.87% of economic yield of oyster mushroom (*Pleurotus ostreatus*) was attributed to the number of primordia per packet.

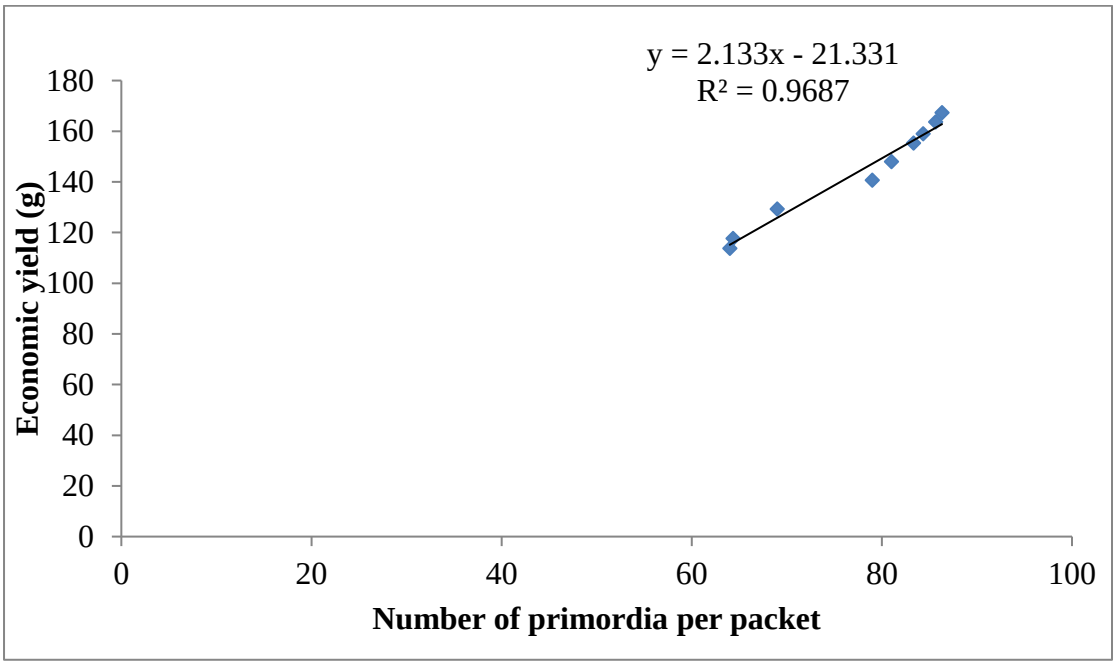


Figure 5. Relationship between the number of primordia per packet and economic yield of oyster mushroom as influenced by supplementation of different nutrient with sawdust

4.7.2 Relationship between the number of effective fruiting body and economic yield of oyster mushroom

A highly significant and linear positive correlation between economic yield and number of effective fruiting body per packet of oyster mushroom when saw dust was supplemented with different nutrients (Figure 6). The relationship between economic yield and number of effective fruiting body per packet of oyster mushroom could be expressed by the regression equation, $y = 4.3911x + 40.747$ ($R^2 = 0.9687$) where y = economic yield and x = number of effective fruiting body per packet. The R^2 value indicated that 83.68% of economic yield of oyster mushroom (*Pleurotus ostreatus*) was attributed to the number of effective fruiting body per packet.

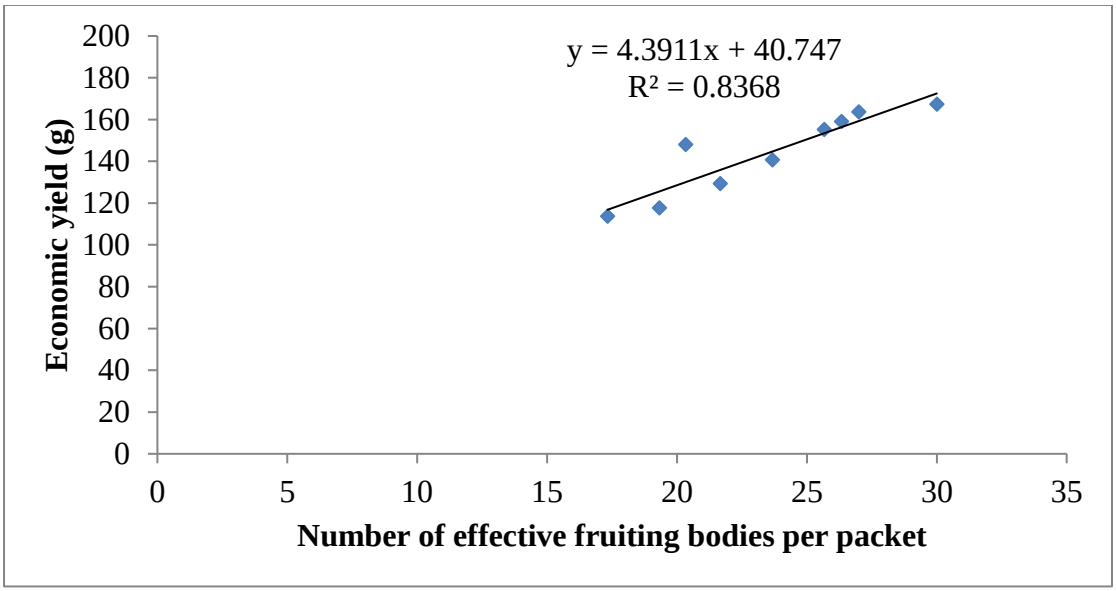


Figure 6. Relationship between the number of effective fruiting bodies per packet and economic yield of oyster mushroom as influenced by supplementation of different nutrient with sawdust

4.7.3 Relationship between biological efficiency and economic yield of oyster mushroom

A highly significant and linear positive correlation between economic yield and biological efficiency of oyster mushroom when saw dust was supplemented with different nutrients (Figure 7). The relationship between economic yield and biological efficiency of oyster mushroom could be expressed by the regression equation, y = 4.8959x – 2.7661 (R^2 = 0.992) where y = economic yield and x = biological efficiency. The R^2 value indicated that 99.92% of economic yield of oyster mushroom (*Pleurotus ostreatus*) was attributed to the biological efficiency.

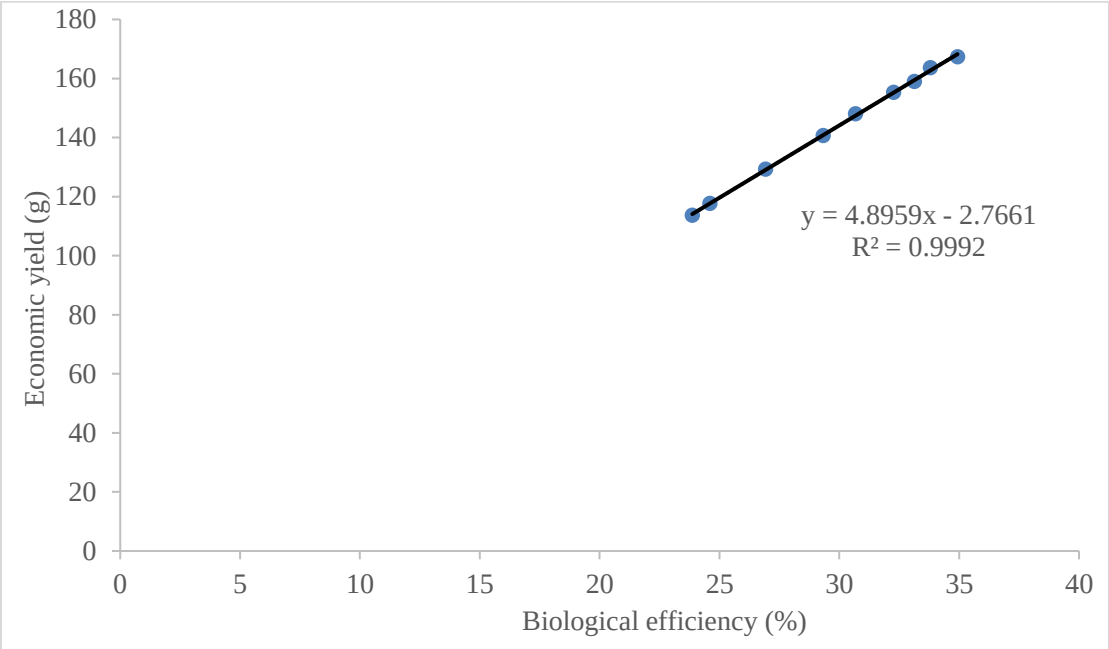


Figure 7. Relationship between biological efficiency and economic yield of oyster mushroom as influenced by supplementation of different nutrient with sawdust

4.8 Effect of different nutrients on proximate composition (%)

4.8.1 Effect of different nutrients on moisture content

Moisture content ranged from 82.8-91.30% which were statistically similar but numerically significant (Table 7 and Appendix VI). The highest moisture content recorded from T_0 (without supplementation of nutrients), while the lowest moisture content was found in T_7 (0.5% Growcell).

4.8.2 Effect of different nutrients on protein content

Protein content of oyster mushroom showed statistically significant variation due to application of different nutrient supplements (Table 7 and Appendix VI). The highest protein content (27.16 %) was recorded from T_7 (0.5% Growcell) which was followed by T_3 (25.50 %) while the lowest protein content (19.94 %) was found in T_0 (without supplementation of nutrients) followed by T_6 (20.60 %).

4.8.3 Effect of different nutrients on lipid content

Statistically significant variation was recorded in terms of lipid content compared to control due to different levels of nutrient supplement (Table 7 and Appendix VI). The highest lipid content was recorded from T_7 (4.70 %) whereas, the lowest percentage of lipid was determined in T_0 (3.20 %).

4.8.4 Effect of different nutrients on carbohydrate content

Statistically significant variation was recorded in case of carbohydrate determination due to application of nutrient supplements (Table 7 and Appendix VI). The highest carbohydrate content was recorded in T_0 (45.80 %) which was statistically identical with T_6 (42.90 %) whereas, the lowest was observed in T_7 (31.28 %).

4.8.5 Effect of different nutrients on crude fiber content

Statistically significant variation was recorded in respect of fiber content due to application of different nutrient supplements to sawdust and it was ranged from 16.28-

25.58% (Table 7 and Appendix VI). The highest percentage of crude fiber was recorded in T_7 and the lowest crude fiber percentage was counted from T_0 which was statistically similar with T_5 (17.46 %).

4.8.6 Effect of different nutrients on ash content

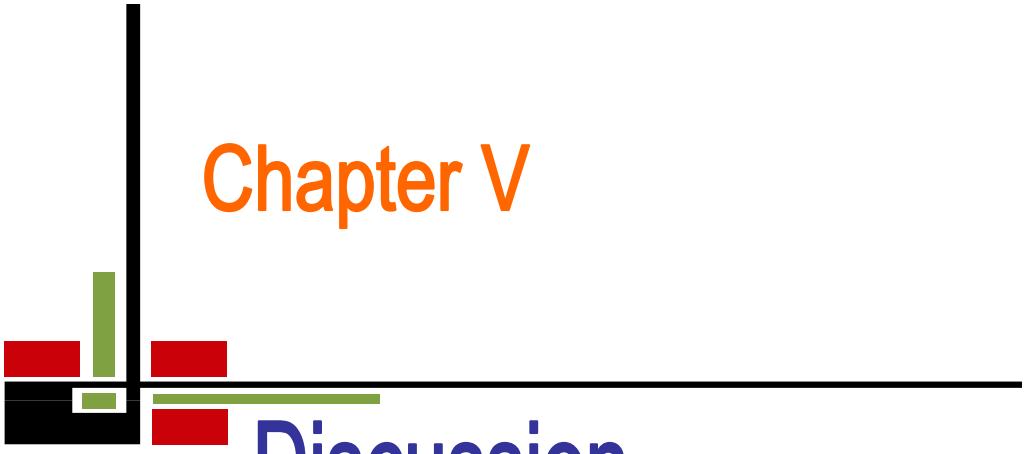
Ash content varied from 8.70-13.70% significantly under the present trial where the highest ash content was found in T_7 and the lowest ash content was recorded in T_0 which was statistically similar with T_6 (9.30 %) (Table 7 and Appendix VI).

Table 7. Effect of different nutrient supplements on proximate composition of oyster mushroom

Treatments	Moisture (%)	Protein (%)	Lipid (%)	Carbohydrate (%)	Crude fiber (%)	Ash (%)
T_0	91.30 a	19.94 f	3.20 d	45.80 a	16.28 e	8.70 d
T_1	87.60 a	23.32 cd	3.94 bc	36.80 d	24.98 ab	11.70 bc
T_2	90.50 a	22.28 cde	4.14 b	40.22 bcd	23.30 bc	11.10 c
T_3	83.00 a	25.50 ab	3.96 bc	42.18 bc	19.68 d	11.90 bc
T_4	89.40 a	23.64 bcd	4.12 b	38.74 cd	21.82 cd	12.70 ab
T_5	89.50 a	24.30 bc	4.24 b	40.94 bc	17.46 e	12.60 ab
T_6	86.30 a	20.60 ef	3.62 c	42.90 ab	20.68 d	9.30 d
T_7	82.80 a	27.16 a	4.70 a	31.28 e	25.58 a	13.70 a
T_8	83.60 a	21.80 def	4.34 ab	40.34 bcd	20.94 d	12.60 ab
CV (%)	6.31	5.36	6.85	6.82	5.89	6.34
$LSD_{(0.05)}$	9.26	1.93	0.38	3.39	2.10	1.24

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

[T_0 = Control (without supplementation of nutrients); T_1 = 1% Gypsum; T_2 = 10% Extract of saw dust; T_3 = 0.0001% Torus; T_4 = 1% Macboron; T_5 = 10% Extract of rice straw; T_6 = 1% Shakti zinc; T_7 = 0.5% Growcell; T_8 = 0.5% American NPKS]



Chapter V

Discussion

CHAPTER V

DISCUSSIONS

The present experiment was conducted to evaluate the effect of enrichment of nutrients with substrates on the productivity, contamination, nutrient composition and cost benefit ratio of oyster mushroom. Eight nutrient supplements namely gypsum, saw dust extraction, torus, macboron, rice straw extraction, shakti zinc, growcell, American NPKS and one control for comparison were used for oyster mushroom cultivation. Enrichment of substrate by nutrients increased the yield, yield attributes, quality and nutrient contents of mushroom over control in the present study. Additional nutrients can be easily used by fungi because the absorption of these molecules is more energetically efficient than synthesizing the molecules, which allow the fungi to obtain more energy for mycelial growth and mushroom formation. The supplements are materials mostly enriched with carbohydrates, proteins, amino acid, macro- and micronutrient (Royse and Sanchez, 2008a; Royse *et al.,* 2008).

In the present study, during cultivation period four contaminants namely *Trichoderma harzianum, Penecillium* sp. *Rhizopus stolonifer* and *Aspergillus niger* were isolated and identified from contaminated substrates. Several researches from all around the world have illustrated the devastating effects of green mould disease in mushroom production caused by *Trichoderma* species like *T. citrinoviride, T. harzianum, T. aggressivum, T. pleuroti, T. viride, T. polysporum, T. longibrachiatum, T. koningii,* and *T. pleuroticola* (Kim *et al.,* 2012; Kumar *et al.,* 2017; Hatvani *et al.,* 2017; Akhter, K., 2017, and Innocenti *et al.,* 2018). Singh *et al.* (2006) recognized *T. harzianum* as the most important species of *Trichoderma* capable of causing green mould disease in many instances and resulting in potential yield losses. *Trichoderma* spp. are very dominant to all fungus and possess high outbreak capacity (Bhandari *et al.,* 2021). *Penicillium*

competes for preoccupancy with green spores and inhibits the formation of fruiting bodies, resulting in the spores spreading out in the middle and top portion of the mushrooms bottles (Choi *et al.,* 2003). *Trichoderma* spp., *Aspergillus niger*, *Coprinus* spp., *Penicillium* spp., *Sclerotium rolfsii, Mycogyone perniciosa, Lecanicillium fungicola, Cladobotryum* spp. are some of the important fungal contaminants of mushrooms that are associated with several economically important diseases like green mould, dry bubble, wet bubble, cobweb, etc. (Biswas and Kuiry, 2013; Fletcher and Gaze, 2008). These contaminants deteriorate quality and damage basidiomycetes ultimately leading to reduced production and sometimes complete failure of the crop (Gea *et al.,* 2021). Oxaley (1985) and Earrana (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. Contamination of spawn and identification of major contaminants has been worked out by Akhter (2017), Mazumder and Rathaiah (2001), Kurtzman (2010) and Kumar (2015) and their results agreed with the result of the present experiment.

The highest mycelium running rate was recorded from 0.5% Growcell (0.82 cm/day) and lowest rate was found from control treatment (0.60 cm/day). The present findings corroborated with the findings of previous workers (Khan *et al.,* 2001 and Kulsum *et al.,* 2009). Khan *et al.* (2001) reported that sawdust amended with different organic supplement like wheat chaff, wheat bran, rice straw, cotton straw, provided suitable condition for spawn running. Kulsum *et al.* (2009) observed that the highest mycelium

running rate was 0.71 cm due to sawdust supplemented with cow dung @ 15% which is more or less similar to the present study.

Among the treatments, the maximum days for incubation to mycelium initiation was required for control treatment (7.67 days) and the minimum days was required for 0.5% Growcell (5.00 days). The result of the present findings was found similar with Sarker (2004) and Amin *et al.* (2007). Sarker (2004) observed that duration from incubation to mycelium initiation of oyster mushroom was significantly lower as compared to control (i.e. no supplement was used). Amin *et al.* (2007) found significant differences on time from incubation to mycelium initiation among the level of supplements used for preparing the substrates. Bhuyan (2008) also found similar effect as found in the present study.

Days required for primordia initiation ranged from 5.89 days to 7.00 days and the minimum time required for primordia initiation was recorded from 0.5% Growcell enriched packets. The result of the present study keeps in with the findings of previous workers (Sarker, 2004; Biswas *et al.*, 2009 and Akhter, 2017). Sarker (2004) observed that duration from primordia initiation to first harvest of oyster mushroom was significantly lower as compared to control where no supplement was used and the duration required for total harvest of oyster mushroom increased with the level of supplement used. In the present study, the time required for total harvest decreased with the levels of supplements increased compared to saw dust alone. Biswas *et al.* (2009) reported that the maximum of 0.45% and 0.35% reduction in days to primordial initiation and time primordia initiation to harvest was obtained with 0.2% wuxul super supplementation (well balanced NPK liquid fertilizer supplemented with a full complement of macro- and micronutrients) which was followed by 0.1 and 0.3%. Akhter (2017) reported that the minimum of 5.90 days was required for primordia

formation at Agrovit Plus at 0 ppm (control) but the maximum 10.40 days was required for the formation of primordia in spawn packet enriched with Boron at 10000 ppm. Minimum 4.67 days was required to harvest from primordia initiation due to use of 0.5% Growcell. The result of the present study keeps in with the findings of several workers. Akhter (2017) also reported that the minimum of 5.10 days was required for first harvest in substrate enriched with Agrovit Plus at 1000 ppm, where the maximum of 11.6 days was required when substrate enriched with $MnCl_2$ at 10. The maximum number of primordia per packet was found from 0.5% Growcell (86.33). The result of the present findings keeps in with the findings of previous scientists (Amin 2004; Pathan *et al.,* 2009 and Shalahuddin, 2014). Pathan *et al.* (2009) reported that 5 g NKP in 10 kg straw was the best in relation to studied characters. Amin (2004) found that the highest number of primordia of oyster mushroom was found by using nutrients with paddy straw but the lowest was found in control. Shalahuddin (2004) in his experiment found that the highest number of primordia (74.40) of oyster mushroom was found by using 4g NPK with 10 kg straw but lowest (63.40) was found in control.

Yoshida *et al.* (1993) reported that the number of fruiting bodies was increased when the different supplements were mixed with substrates. Sarker (2004) found that the number of fruiting body increased with the increase levels of supplement and continued up to a certain range and decline thereafter. In another study, Bhuyan (2008) found similar results. Sarker *et al.* (2011) reported that the highest number of effective fruiting bodies (21.00) in autoclaved N, P and K (2:1:1) and it was the lowest (7.50) in autoclaved N, P and K (1:1:1). More or less similar findings have been recorded in present study where the maximum number of effective fruiting body per packet (30.00) was obtained when treated with 0.5% Growcell. In the experiment, the highest length of pileus (5.11 cm), width of pileus (7.45 cm) and length of stipe (3.10 cm) was recorded

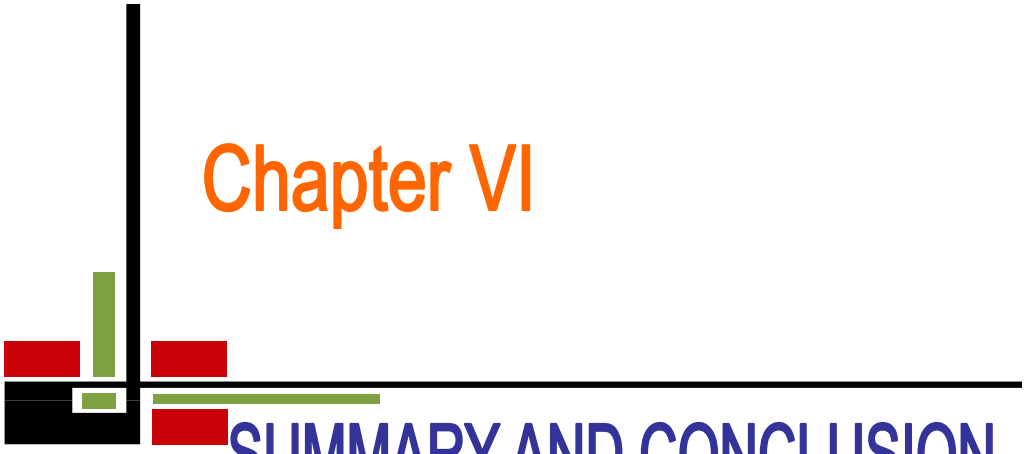
in 0.5% Growcell enriched packets. More or less similar findings have been reported by previous scientists. Sarker *et al.* (2011) reported the length of pileus ranged from 5.66 to 7.44 cm; thickness of pileus ranged from 7.47 to 5.55 cm and length of stalk ranged from 1.80 to 2.57 cm. The highest length and thickness of pileus and stalk was found in NKP (2:1:1). Sarker *et al.* (2008) also observed that number of fruiting bodies of oyster mushroom ranged from 20 to 98.25/ packets on wheat straw supplemented with different levels of wheat and rice bran.

The maximum biological and economic yield was observed in 0.5% Growcell among the eight nutrients. Biswas *et al.*, (2016) also recorded the highest biological yield (368.18g) from mango tree sawdust supplemented with 30% wheat bran of oyster mushroom (*Pleurotus ostreatus*) spawn. Baysal *et al.* (2003) found the highest yield of oyster mushroom (*Pleurotus ostreatus*) where 20% rice husk was mixed with substrate in weight. Appreciable variations in economic yield also observed at different levels of supplements under different substrate-supplement combinations. Payapanon *et al.* (1994) mentioned that maximum economic yield of oyster mushroom was recorded by adding suitable amount of supplements at optimum production cost. Bhuyan (2008) observed that the yield of *Pleurotus ostreatus* responded with the levels of supplements used with sawdust and increased with the level of supplementation and declined thereafter. Sarker *et al.* (2011) recorded the highest economic yield in autoclaved NPK at ratio 2:1:1. The highest dry weight of oyster mushroom (17.33 g) was also recorded from 0.5% Growcell and this result of the present study was also supported by the study of previous researcher Pathan *et al.* (2009) who found that NPK soaking was the best in relation to studied characters. Kulsum *et al.* (2009) recorded the highest dry yield of 21.27 g due to 6 g NPK socking. Baysal *et al.* (2003) found the highest yield (350.2 g) in supplemented the substrate with 20% rice husk in weight of oyster mushroom. In

similar study, Biswas *et al.* (2009) found the highest economic yield (224.3g) and biological efficiency (79.54%) from 0.2% wuxul super supplemented spawn packets followed by 0.1 and 0.3% of the supplement. Substrate products are tuned on the nutritional needs of mushroom and complete the deficits in the compost of essential nutrients like nitrogen, carbohydrates or trace- elements. It gives higher yield (up to 30%) and offers a direct economic benefit for every mushroom grower. Royse and Sanchez (2008b) tested 16 supplements of mushroom compost, including five crystalline amino acids, one amino acid blend, one egg white and four hydrolyzed proteins, Micromax (a micronutrient containing nine minerals) and four commercial supplements to find out their effect on mushroom yield and biological efficiency.

Mushroom can play an important role to meet up the nutritional requirements of the population of Bangladesh (Amin *et al.,* 2007). The nutritional advantages of mushrooms include a low content of calories and a high content of proteins, minerals and dietary fiber (Beluhan and Ranogajec, 2011). Mushrooms are a good source of protein, vitamins and minerals (Khan *et al.,* 2013). The efficiency of mushroom species in producing food protein in the form of biomass or fruiting bodies from different wastes lies in their ability to degrade waste via secretion of a variety of hydrolyzing and oxidizing enzymes (Kuforiji and Fasidi 2008 and Zhu *et al.,* 2013). In the experiment, highest moisture and carbohydrate content was recorded in control. Bhuyan (2008) found no significant differences in moisture content among the mushrooms produced in sawdust supplemented with cow dung. Dundar *et al.,* (2008) found that the carbohydrate content of *P. sajor-caju*, *P. ostreatus* and *P. eryngii* are 37.72, 37.87 and 39.85 (g/100 g dried matter), respectively. Biswas *et al.* (2009) reported that the moisture and dry matter content of oyster mushroom grown under different levels of wuxal super varied from 89.5 to 90.23 % and 9.77 to 10.46%, respectively. Ragunathan

et al. (1996) recorded the carbohydrate content ranged from 40.6 to 46.3%. The highest protein content, lipid content, fiber content and ash content were recorded when the spawn was enriched with 0.5% Growcell. Kulsum *et al.* (2009) found that lipid content was ranged from 3.44 to 5.43% due to sawdust supplemented with different levels of cow dung. Mona *et al.*, (2009) investigated nutritional analysis and enzyme activities of *Pleurotus ostreatus* cultivated on *citrus limonium* and *Carica papaya* wastes and they concluded that fruit bodies containing 26.0-31.5% digestible protein, 20.9-33.0% total soluble carbohydrates and 2.0-5.9% fat (on dry basis). Beluham and Ranogajec (2011) reported mushrooms are a potential source of total carbohydrates in the range of 42.62-66.78 g/100g and protein in the range of 27.95-38.89 g/100g and very low-fat contents 1.34-6.45g/100g depending upon the species of mushroom. Ashraf *et al.* (2013) showed crude protein was ranged 24.83%-27.23%, fiber content showed ranges between 22.03%-26.28, ash contents in three varieties of oyster mushroom were found 6.76-9.08%, the carbohydrate content (36.74%), the total fat content (2.37%), protein (27.23%) and fiber contents (26.28%) was found in *P. ostreatus* depending upon the different substrates. Ashraf *et al.* (2013) observed that the percentage of dry matter and the moisture content varied from 12.63-17.23% and 82.77-87.37% among the interaction of different oyster species and substrates. Zahid *et al.* (2010) studied nutritional status, where, crude protein, total lipids, available carbohydrates, dietary fiber, total carbohydrates, moisture, total solids and ash content in mushrooms were found to be in the ranges of 3.22-4.83, 0.41-1.05, 4.2-6.37, 0.58-1.11, 4.82-7.48, 85.95-90.07, 9.93-14.05 and 0.98-2.3 g/100g of fresh edible portion, respectively on the basis of different species of oyster mushroom.



Chapter VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

The field experiment was conducted at 'Mushroom Culture House (MCH)' of Sher-e-Bangla Agricultural University, Dhaka. On the other hand, the laboratory experiment was done in 'Plant Pathology Laboratory', of Sher-e-Bangla Agricultural University, Dhaka and 'Biochemistry laboratory' of National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka during the period from November 2020 to March 2021 to study the effect of supplementation of nutrients with substrates on the productivity, contamination, nutrient composition and cost benefit ratio of oyster mushroom (*Pleurotus ostreatus*). The experiment consists of nine different type of nutrient supplement with three replications to achieve the desire objectives. The treatments are T_0: Control (without supplementation of nutrients); T_1: 1% Gypsum; T_2: 10% Extract of saw dust; T_3: 0.0001% Torus; T_4: 1% Macboron; T_5: 10% Extract of rice straw; T_6: 1% Shakti zinc; T_7: 0.5% Growcell; T_8: 0.5% American NPKS]. The experiment was laid out in single factor Completely Randomized Design (CRD). The results obtained in the study have been summarized below. The highest mycelium running rate (0.82 cm/day) was recorded when the substrate treated with 0.5% Growcell, while the lowest mycelium running rate (0.60 cm/day) was observed in controlled condition (without supplementation of nutrients). But the maximum time required for primordia initiation to 1st harvest (6.33 days) was found in control treatment. The maximum number of effective fruiting body per packet (30.00) was found from 0.5% Growcell and the minimum number of effective fruiting (17.33) body was found from control treatment. The highest length (5.11 cm) and width (7.45 cm) of pileus was observed in 0.5% Growcell and the minimum length and width of pileus was observed in control

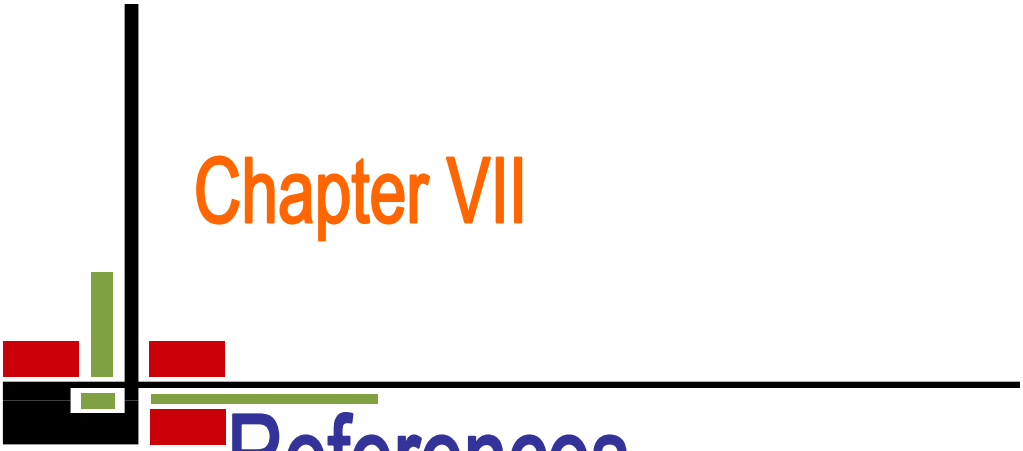
treatments. The highest biological yield (174.7 g) was recorded from 0.5% Growcell. But the highest benefit cost ratio (3.26) was obtained from 10 ppm Torus.

In the experiment, highest moisture content (91.30 %) and highest carbohydrate (45.80 %) content was recorded in control but the highest protein (27.16 %), lipid (4.70 %), fiber (25.58 %) and ash content (13.70 %) was recorded in 0.5% Growcell enriched spawn packets.

Percent contamination of fungi were gradually increased with the increase of days after stimulation. After 45 days after stimulation (DAT), the spawn packets treated with 10 ppm Torus and 0.5% Growcell were not contaminated. But the severity of contamination (46.67 %) was observed in control treatment, 40.00% in saw dust solution treatment and 33.33% in rice straw solution.

Based on the experimental results, it may be concluded that-

- Comparatively less contamination severity was observed in spawn packet when enriched with 0.0001% Torus and 0.5% Growcell during the cultivation of mushroom and four fungi namely *Trichoderma harzianum*, *Penecillium* sp. *Rhizopus stolonifer* and *Aspergillus niger* were isolated and identified.
- Sawdust supplemented with 0.5% Growcell nutrient is suitable for oyster mushroom (*Pleurotus ostreatus*) cultivation in Bangladesh based on growth and yield contributing characters but for getting highest benefit cost ratio from oyster mushroom (*Pleurotus ostreatus*) cultivation, 0.0001% Torus gave the best result.
- Saw dust enriched with 0.5% Growcell nutrient performs best in terms of protein, lipid, fiber and ash content.



Chapter VII

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CHAPTER VII

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Chapter VIII

APPENDICES

CHAPTER VIII

APPENDICES

Appendix I: Temperature and relative humidity of culture house and outside during oyster mushroom cultivation

Duration	Average Temperature (^{0}C) of culture house	Average RH (%) of culture house	Average temperature of outside house (^{0}C)	Average RH (%) of outside of culture house
November	21	85%	28	50%
December	20	80%	25	45%
January	20	75%	25	45%
February	20	75%	27	50%
March	21	85%	28	55%

RH: Relative humidity

Appendix II: ANOVA table for Mycelium Running Rate (cm/day), days required from incubation to mycelium initiation and days required from incubation to completion of mycelium running (days)

Source of variation	Degrees of freedom	Mean Square		
		Mycelium Running Rate	Days Required from Incubation to Mycelium Initiation	Days Required from Incubation to Completion of Mycelium Running
Between	8	0.013	2.398	11.25
Within	18	0.002	0.37	2.037
Total	26			

Appendix III: ANOVA table for time required from stimulation to primordia initiation, primordial initiation to 1st harvest and Total harvesting period

Source of variation	Degrees of freedom	Mean Square		
		Time required from stimulation to primordia initiation	Time required from primordial initiation to 1st harvest	Total harvesting period
Between	8	0.361	0.833	26.917
Within	18	0.214	0.333	8.37
Total	26			

Appendix IV: ANOVA table for number of primordia per packet, number of fruiting body per packet and number of effective fruiting body per packet

Source of variation	Degrees of freedom	Mean Square		
		Number of primordia per packet	Number of fruiting body per packet	Number of effective fruiting body per packet
Between	8	250.417	76.75	51.009
Within	18	28.63	30.259	9.704
Total	26			

Appendix V: ANOVA table for dimension of fruiting body of oyster mushroom

Source of variation	Degrees of freedom	Mean Square		
		Length of pileus	Width of pileus	Length of stipe
Between	8	0.336	1.311	0.489
Within	18	0.281	0.396	0.186
Total	26			

Appendix VI: ANOVA table for yield parameters of oyster mushroom

	Degrees of freedom	Mean Square			
		Biological yield	Economic yield	Dry Yield	Biological efficiency
Between	8	1226.065	1177.593	13.398	49.043
Within	18	28.593	31.037	0.667	1.144
Total	26				

Appendix VII: Fruiting bodies of oyster mushroom in the spawn packets of different nutrient supplements

Appendix VIII: Table of different nutrient prices

Treatments	Purchase quantity (kg/g/ml)	Purchase price (Tk)	Amount of nutrient required to prepare 1 L solution (g/ml)	Number of packets treated by 1 liter solution	Number of application of treatment per packet	Cost of nutrient per packet
T_1	1 kg	40	10 g	5	3	0.24
T_2	100 kg	300	100 g	5	3	0.18
T_3	1 g	100	0.001 g	5	3	0.06
T_4	500 g	90	10 g	5	3	1.08
T_5	100 kg	200	100 g	5	3	0.12
T_6	1 kg	170	10 g	5	3	1.02
T_7	100 ml	100	5 ml	5	3	3.00
T_8	50 g	30	5 g	5	3	1.80