EFFECT OF DIFFERENT AMOUNTS AND COMBINATIONS OF SUBSTRATES ON YIELD AND CONTAMINATION OF SPAWN PACKET IN OYSTER MUSHROOM CULTIVATION (*Pleurotus ostreatus*)

AYSHA SIDDEQUA URME

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

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BY

AYSHA SIDDEQUA URME

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Approved by:

.....	.....
Dr. Khadija Akhter	**Dr. Nazneen Sultana**
Professor	Professor
Department of Plant Pathology	Department of Plant Pathology
Sher-e-Bangla Agricultural University	Sher-e-Bangla Agricultural University
Supervisor	**Co-Supervisor**

.....
Prof. Abu Noman Faruq Ahmmed

Chairman

Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University

Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

PABX: +88029144270-9
Fax: +88029112649
Web site: www.sau.edu.bd

CERTIFICATE

This is to certify that the thesis entitled, **"EFFECT OF DIFFERENT AMOUNTS AND COMBINATIONS OF SUBSTRATES ON YIELD AND CONTAMINATION OF SPAWN PACKET IN OYSTER MUSHROOM CULTIVATION (*Pleurotus ostreatus*)"** *submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirement for the degree of* **MASTER OF SCIENCE IN PLANT PATHOLOGY** *embodies the results of a piece of bona fide research work carried out by* ***AYSHA SIDDEQUA URME,*** *bearing Registration No* ***19-10212*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma, elsewhere in the country or abroad.*

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

Dated:

Place: Dhaka, Bangladesh

Dr. Khadija Akhter
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka- 1207
Supervisor

DEDICATED TO

MY

BELOVED PARENTS, MY

FAMILY & SIBLINGS

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ABSTRACT

The experiment was conducted during the period from November 2020 to March 2021 to study the effect of different amounts and combinations of substrates on the productivity, contamination and cost benefit ratio of oyster mushroom (*Pleurotus ostreatus*). The experiment was conducted with fifteen (15) different type treatments which were controlled amount of waste paper alone and combinations of waste paper and sawdust with three replications to achieve the desire objectives. The experiment was laid out in single factor Completely Randomized Design (CRD).Highest mycelium running rate (0.97 cm/day) was recorded in T_2 =500 g waste paper. The lowest harvesting period (39.33 days) was recorded in T_1 =250 g waste paper and T_2 =500g waste paper. Maximum average number of primordia and fruiting body per packet (59.33) was observed from T_{10} =1500 g(75%Waste paper + 25% Sawdust) but the highest number of effective fruiting body per packet (18.67) was recorded from T_{15} =1500g (25% Waste paper + 75% Sawdust). T_{15} =1500g (25% Waste paper + 75% Sawdust) provided with the highest biological yield (165.33 g), economic yield (162 g), dry yield (11.44 g) and net returns (34.6 TK) but the highest of biological efficiency (44.93%) was observed when 75% of waste paper was added to 25% of sawdust to prepare 250g substrate. *Trichoderma sp.*, *Penecillium sp.*, *Rhizopus stolonifer* and *Aspergillus niger* were isolated and identified from contaminated substrates. Contamination severity was highest in T_{15} =1500g (25%Waste paper + 75%Sawdust) and it increased with the increase of days after incubation. Among all the treatments, T_{15} =1500 g (25%Waste paper + 75%Sawdust) was better economically as well as showed the best yield performance for the cultivation of oyster mushroom.

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LIST OF ABBREVIATIONS AND ACRONYMS

Abbreviation	Full meaning
%	Percent
@	At The Rate
0C	Degree Centigrade
Agril.	Agricultural
BCR	Benefit Cost Ratio
BE	Biological Efficiency
Cm	Centi-Meter
CO_2	Carbon Dioxide
CRD	Completely Randomized Design
CV	Coefficient Of Variation
d.f.	Degrees Of Freedom
DAI	Days After Incubation
e.g.	For Example
et al.	And Others
Etc.	Et Cetera (And Other Similar Things)
G	Gram
h	Hour
J.	Journal
Kg	Kilogram
LSD	Least Significant Difference
MCH	Mushroom Culture House
MDI	Mushroom Development Institute
MRR	Mycelium Running Rate
N	Nitrogen
RH	Relative Humidity
SAU	Sher-E-Bangla Agricultural University
Sp.	Species
Viz.	Namely

CHAPTER I

INTRODUCTION

People all over the world are now facing different crises. Even the pandemic situation isn't over yet. High population rate is a problem for the overall development process and death of millions of people all over the world, continuous lockdown, restrictions at different affairs due to covid-19 have made the situation worse. Thousands of people have lost their way of earning. Economic crisis has become acute. These crises ultimately can cause food crisis which will be the most difficult mess to be faced. Hunger of millions of people can lead to an unsteady condition. To avoid this situation we cannot help but increase food productivity and think about other food alternatives. Regarding this situation, mushroom can be of a little help.

Mushroom is the fleshy reproductive structure produced by different kind of fungi under Ascomycotina or Basidiomycotina sub-division. In other words, it can be said that mushroom is the umbrella shaped fruit of fungi which provides a wide range of nutrients. It has a great economic and medicinal importance.

Mushroom is becoming more popular due to their nutritional value, high protein and low fat contents. The mushroom protein contains all the nine essential amino acids required by human and mushrooms are a relatively good source of the other nutrients like phosphorus, iron and vitamins, including thiamine, riboflavin, ascorbic acid, ergosterol and niacin (Kumar, 2015).

According to Zhang *et al*. (2016), mushroom could be developed into a functional food or medicines for prevention and treatment of several chronic diseases, such as cancer, cardiovascular diseases, diabetes mellitus and neurodegenerative diseases. Sevindik (2021) stated that mushroom can be used in the fight against covid-19. Dengue virus is another health issue in our country and mushroom is also combative against Dengue virus. *Pleurotus sp*. Contains hot aqueous, ethanol, hexane, ethyl acetate and aqueous extracts which can fight against Dengue virus (Sevinkdik, 2021).

Mushroom cultivation rate is increasing day by day in Bangladesh. Cultivators are cultivating different types of mushrooms including Oyster mushroom, Shiitake mushroom, Button mushroom, Straw mushroom, Reishi mushroom, Milky white mushroom etc.. Among all of them, Oyster mushroom is most common and easy to cultivate. Kamal *et al.* (2009) reported that Oyster mushroom is cultivated and consumed by 97%.

In Bangladesh, demand for mushroom is getting higher day by day as people are becoming more health conscious. Dried mushroom powder has become popular among the diabetes patients. People are also liking the taste of mushroom and many mushroom based dishes are being served at restaurants.

Beside the nutritional and medicinal value mushrooms also have economic value. It can be he light of hope to many jobless people. The profitability of mushroom cultivation was found comparatively higher than that of rice and wheat, the most popular cash earning crop in Bangladesh (Imtiaj and Rahman, 2008).

Mushroom production rate is increasing due to the increase in domestic market and its export potentiality. According to Ferdousi *et al.* (2019), in Bangladesh 4000 MT mushrooms were produced during 2018-19. Ferdousi *et al.* (2019) also reported that Mushroom production is easy work because it requires only a little technical efficiency and it is a highly profitable agribusiness as evident for its lucrative benefit cost ratio (BCR 1.55-4.25).

Mostly educated youth and rural women are being interested in mushroom production. Although mushroom production increased there are some problems confronting by the mushroom growers during cultivation and marketing including lack of cultivation house, unavailability of good spawn, capital shortage facility etc. (Ferdousi *et al*., 2019). These problems can affect profitability and discourage mushroom cultivators. Khan *et al.* (2018) indicates that per year gross returns in mushroom production were significantly influenced by the level of education, use of human labor and number of spawn (seed) packets of the farmers. Mushroom is an organic vegetable and the production of

mushroom is eco-friendly and profitable agribusiness but labor intensive (Chandha and Sharma, 1995).

Among all the factors affecting mushroom production, the amount of substrate used for packet preparation is one. The common method of Oyster mushroom cultivation is bag culture. For optimum mushroom production, it is necessary to learn about optimum amount of substrate needed for a mushroom bag. Different amounts and combinations of substrates can cause variation in mushroom yield and contamination. Zireva *et al.* (2007) stated that mushroom yield increased with an increased substrate quantity up to 6 kg and thereafter remained constant. The amount of substrate required to optimize production has not been established yet. Use of little amount of substrate per packet can reduce the yield. On the other hand, use of excess amount of substrate per packet can increase the production cost. Use of excessive amount of substrate can also be responsible for contamination which may cause a great loss in profit. After considering the above matters the present supervision was undertaken with following objectives:

(i) To determine the optimum amount and combination of substrates required for cultivation to get maximum yield

(ii) To detect the contaminants from the infested substrates

(iii) To assess the impact of amount and combination of substrates for minimum contamination

(iv) To determine the cost benefit ratio of production cost of Oyster mushroom

CHAPTER II

REVIEW OF LITERATURE

2.1. About mushrooms

Many people all around the earth takes mushroom considering it as a vegetable although this is not the case. All vegetables belongs to plant sources. One of the main characteristics of plants is photosynthesis which requires chlorophyll to perform. But mushrooms are lack of chlorophyll and can't photosynthesize to produce energy. Although they are neither plants nor animals, mushrooms are still organisms as they perform all the life processes like other organisms. For such astonishing character, they are classified into special group called fungi. The organisms of the fungal lineage include mushrooms, rusts, smuts, puffballs, truffles, morels and yeasts, as well as many less well known organisms (Blackwell *et al*., 2011). According to James *et al*. (2006) more than 700, 00 species of fungi have been described however, some estimates of total number suggests that 1.5 million species may exist. Most of the members of the group are microscopic but mushrooms can be described as macroscopic. Carris *et al*. (2012) stated fungus as eukaryote that digests food externally and absorbs nutrients directly through its cell walls.

2.2. Mushroom classification

Mushrooms are classified based on their common characteristics. Mushrooms can be classified by their tropical pattern as saprophytes, parasites or mycorrhizae (Cho and Kang, 2004). The saprophytes grow on organic matter like wood, leaves and straw in nature and acts as decomposer. 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 mushroom that have desired 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-threating organ-failure which may result in death (Ganopedia, 2011).

Mushrooms can also be classified according to the substrates they grow on (Dzomeku, 2009; Oei, 1991). They can be classified as cellulytic mushrooms, lignocellulytic and termitomyces. The cellulytic mushrooms mainly grow on cellulose containing substrates as straws. The lignocellulytics can grow on both straws and decaying wood. The termitomyces grow mainly on anthills and their life cycles are completed by the help of ants or termites.

2.3. Oyster mushrooms

Oyster mushroom, one of the edible mushrooms of genus *Pleurotus* belongs to Basidiomycetes class under Pleurotaceae family. All the varieties or species of Oyster mushrooms are edible except *Pleurotus olearius* and *Pleurotus nidoformis* (Agridaksh, 2011). As it has a shell-like spatulate pileus it is known as Oyster mushroom. Number of species have been cultivated as a result of which Oyster mushrooms of all colours like white, grey, yellow, pink etc. can be gotten.

The Oyster mushrooms are a common saprophytic fungi. Although it is commonly growing on dying trees, it behaves as a facultative parasite of the earliest opportunity (Stamets, 2000).

Oyster mushrooms can be grown on both straws and decaying wood and classified as lignocellulytic mushroom.

Oyster mushrooms grow extremely fast and aggressively. They require very little in terms of fruiting strategy. They display distinct morphological characteristics

when a fruiting condition is not to their liking. They fruit in clusters making it easy to harvest. It tends to be fragile which can create some difficulties in packing and selling.

2.4. Nutritional and medicinal value of oyster mushroom

Mushrooms are considered as a very popular food element. As Oyster mushrooms contain carbohydrate, protein, fats, minerals and water i.e. all the elements of a balanced diet. We can consider it as a model food element. The amount of nutrient contents can vary species to species or variety to variety. It can be an enjoyable source of protein for vegans. Mushrooms are easy to digest. Based on fresh weight mushrooms may be inferior to dairy products and meats but they are superior to all kinds of vegetables and fruits (Aremu *et al*., 2009). Edible mushrooms are regarded as the meat of the vegetables world can give a variety of delicious meals with different dishes and can also use for adding flavors (Hass and patty, 2009).

Oyster mushroom is ideal for people suffering from heart disease and hypertension due to its high potassium to sodium ratio (Sharma *et al*., 2013). And it has no cholesterol control since it is easily digestible (Oei, 2005). It can also cure anemia because of the folic acid present in Oyster mushroom (Randive, 2012). As mushroom is an organic source and edible mushrooms are free from toxicity it may have no or less harmful reaction after consumption.

2.5. Mode of feeding of Oyster mushroom

Oyster mushrooms are lack of chlorophyll and can't produce own food. They are saprophytic fungus that depends on decaying material for nutrients. They intake nutrients through its mycelia. To increase surface area the hyphae branches form thick, mass mycelia through which feeding can be maximized. The Oyster mushrooms feed by secreting a range of enzymes such as peroxidases, lactases, cellulases, hemicellulases and xylanases (Cohen *et al*., 2002). The enzymes helps to break different compounds and make them available for mushroom's intake which makes the Oyster mushroom well adapted on a wide range of substrates

such as sawdust, rice straw, paper waste etc.The Oyster mushrooms take their protein by secreting a potent toxin to kill nematodes or round worms that may be present in the rotten wood (Mdconline, 2013; Woller, 2007). After killing nematodes or roundworms the mushrooms secret enzyme to digest them and absorbs the nutrients within them.

2.6. Oyster mushrooms in Bangladesh

Oyster mushrooms are adapted to a wide range of environmental conditions. In Bangladesh, Oyster mushrooms are most popular and four different species of this mushroom like *Pleurotus ostreatus*, *Pleurotus florida*, *Pleurotus sajor-caju* and *Pleurotus high king* are commercially cultivated all over the year by using sawdust and/or rice straw as main substrate (Amin *et al*., 2007).But these varieties don't give same yield in every season. Uddin *et al*. (2011) found that the production was minimum during the cultivation time August to October and cultivation of selected *Pleurotus sp*. was suggested in winter.

People of Bangladesh are getting more interested in mushroom production day by day. Among the cultivators, 34% cultivated mushrooms on account of its nutritional and medicinal values, 27% to reduce unemployment, 14% for growing mushrooms as more profitable vegetable, 14% to pass leisure time, 10% due to hobby and 1% for other reasons. (Kamal *et al*., 2009).

2.7. Environmental requirements for Oyster mushroom cultivation

There are several factors that affect the growth of Oyster mushroom such as relative humidity (RH), P^H of the substrate, water activity, luminosity and temperature (Belletini *et al*., 2016). Oyster mushrooms are adapted to a wide range of environmental conditions compared to other mushrooms. It requires high relative humidity for optimal growth in fruiting former (80-90%) according to Crosby (2016) with temperature range about 15-30ºC (Crosby, 2016). The Oyster mushrooms should be cultivated in controlled condition for optimal growth (Uddin *et al*., 2010).

The optimal temperature for growth of the mycelium are around 25-28ºC and the range of P^H is about 5.5 to 6.5. The tolerance of mycelia for CO_2 is rather strong. The mycelia of *Pleurotus spp*. can still grow flourishingly at the CO_2 concentration of 15-20%. Only when the concentration of CO_2 raised to 30% does the growth of mycelia rapidly decrease (Chang and Miles, 1989).

The optimal temperatures for the development of fruiting bodies can range from 10 to 18ºC (Chang and Miles, 1989). The color of the caps is closely related to intensity of light and it can be pale if the light intensity is low.

2.8. Life cycle Oyster mushroom

As Oyster mushrooms are Basidiomycetes they undergo sexual reproduction. The gills of the Oyster mushroom contain a macroscopic structure known as basidia. Basidia bear basidiospores that are released into air currents at maturity. Germination of basidiospore in a suitable substrate gives rise to a monokaryotic mycelium containing genetically identical nuclei (n) and capable of indefinite independent growth. When two compatible monokaryotic mycelia are in close contact, they are able to establish a fertile dikaryon (n + n), having clamp connections and binucleate in each hyphal compartment, contains two genetically different nuclei (one from each monokaryon) throughout the mycelium (Martínez-Carrera, 1999; Adebayo *et al*., 2013).

When the dikaryotic mycelium meets appropriate environmental condition, it will differentiate into fruit bodies having basidia. In these club-shaped binucleate cells, which are formed in the lamellae of each fruit body, karyogamy and meiosis take place. The four resulting haploid nuclei move to the steringmata on the basidium, too form four new basidiospores. When the fruit bodies are mature, basidiospores are discharged, starting the sexual life cycle again.

2.9. Effect of substrate on mushroom production

Most organic matters containing cellulose, hemicellulose and lignin can be used as mushroom substrate i.e. rice and wheat straw, cottonseed hulls, corncob, paddy straw sugarcane baggase, sawdust, waste paper, and leaves (Sharma *et al*., 2013). However, an ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth (Khare *et al.*, 2010).

Tisdale *et al.* (2006), conducted an experiment in Hawaii on different wood substrates. Wood of *Falcataria moluccana, Casuarina equisetifolia, Eucalyptus grandis, Psidium cattleinum* and *Trema orientalis* were used and all proved to be suitable substrates for Oyster mushroom cultivation. Economic yield and biological efficiencies achieved by the N-fixing trees (*Falcataria moluccana, Casuarina equisetifolia* and *Trema orientalis*) were greater than those of *Eucalyptus grandis* and *Psidium cattleinum* which do not fix N. The result also shows that fruit trees such as *Psidium cattleinum* are poor substrate for edible mushroom production.

Rashid *et al*. (2016) analyzed the effect of different sawdust substrates namely *Ficus carica* (Fig tree), *Albizia saman* (Rain tree), *Swietenia mahagoni* (Mahogany tree), *Leucaena leucocephala* (*Ipil ipil* tree), *Eucalyptus oblique* (India gum tree) and mixture of all five tree sawdust, supplemented with 30% wheat bran and 1% lime, on the growth and yield performance of Oyster mushroom. Mahogany tree sawdust substrate provided with the highest average number of effective fruiting body. The highest average weight of individual fruiting body, highest amount of biological yield, economic yield and dry yield were found from Rain tree sawdust substrate.

Girmay *et al*. (2016) carried out an experiment to assess the suitability of selected substrates for Oyster mushroom cultivation. Accordingly, four substrates (cotton seed, paper waste, wheat straw and saw dust) were tested for Oyster mushroom production. Results of the study revealed that Oyster mushroom can grow on cotton seed, paper waste, sawdust and wheat straw with varying growth

performance. The highest biological and economic yield, as well as the highest percentage of biological efficiency of Oyster mushroom was obtained from cotton seed where least from sawdust. The study recommended cotton seed, followed by paper waste as suitable substrate for the cultivation of Oyster mushroom.

Dey *et al.* (2008) made the experiment where Oyster mushroom was cultivated on different substrates viz. paddy straw, sugarcane bagasse and mustard straw using cylindrical block system to find out suitable substrate. Different substrates significantly affected the number of primordia and fruiting bodies and the amount of fresh weight was obtained with sugarcane bagasse in all flushes whereas, the lowest with mustard straw.

Naeem *et al*. (2014) cultivated three *pleurotus* species viz. *Pleurotus nebrodensis, Pleurotus ostreatus* and *Pleurotus eryngii* on cotton waste, sawdust, paddy straw and mixture of these substrates (cotton waste + sawdust, cotton waste + paddy straw, sawdust + paddy straw, sawdust + cotton waste + paddy straw). Amongst the three fungi, *Pleurotus ostreatus* showed the best growth and productivity. Sawdust gave the best result in spawn running, time interval between primordial initiation to harvesting stage and in number of flushes. Combination of sawdust, paddy straw and cotton waste gave the best results in emergence of primordia, fruiting bodies weight, moisture percentage, biological efficiency and total yield.

Keneni and Wondimu (2016) carried out this study in order to evaluate the usability of maize stem along with different proportion of cotton seed waste. From all the different treatments tested, those composed the maize stem : cotton seed waste in the ratio of (60:40 and 30:70) showed fastest mycelium run, 3.6 and 7.2cm respectively, on 7^{th} and 14^{th} days of incubation. Maize stem: cotton seed waste (60:40 and 80:20) took shortest time from incubation to 1^{st} flush 30days while maize stem : cotton seed waste (90:10 and 80:20) showed shortest pinning to maturation through throughout the flushes. Maize stem: cotton seed waste (30:70) observed to have higher number of aborts 105, higher number of

fruiting bodies 125 and maize stem: cotton seed waste (30:70) showed larger pileus diameter 9.2cm, higher fresh weight of matures 795g and highest biological efficiency 159%. Shah *et al.* (2004) carried out the research experiment to investigate the cultivation of mushrooms on different substrates as wheat straw, leaves, sawdust. According to this experiment, sawdust produced highest yield, biological efficiency and number of fruiting bodies and was recommended as one of the best substrates for mushroom cultivation.

An experiment was conducted in the mushroom cultivation laboratory, horticulture center, Jessore by Mondal *et al*. (2010) to evaluate the better performance of Oyster mushroom in different substrate compositions as well as to find out the better substrate for mushroom cultivation. Banana leaves and rice straw (1:1) provided with highest mycelium running rate but the lowest in control. Completion of mycelium running time was lowest in banana leaves and rice straw (1:3 and 3:1). Maximum pileus thickness was measured from rice straw but the number of total primordia and effective primordia were found in control. Highest biological yield and economic yield (164.4g and 151.1g) was obtained from rice straw which was much higher than control.

2.10. Effect of amount of substrate

Optimum amount of substrate should be used to manage cost benefit ratio.

Zireva *et al*. (2007) conducted an experiment in Harare (Zimbabwe) to investigate if substrate amount and shelf position had any effect on yield of Oyster mushrooms using plastic tray culture. Mushroom yield increased with an increased substrate quantity of up to 6kg and thereafter remained constant. Shelf position did not show any significant effect on mushroom yield and there was no interaction between substrate quantity and shelf position on yield. Biological efficiency decreased with an increase in substrate quantity per tray. It was concluded that 6kg of substrate per tray (50×35 and 20 cm depth) would result in optimal yields.

2.11. Oyster mushroom and contamination

Like any other agricultural crops, Oyster mushrooms are not free from diseases. Contamination of Oyster mushroom can cause failure in mushroom cultivation.

Since many microbes contaminate mushrooms from substrate preparation to fruiting growing completely disease-free mushrooms is a daunting task. Contaminants like virus, bacteria, fungus, insects, nematodes etc. annually cause huge losses in the mushroom industry, among which fungal contaminants being the major and widespread problem (Fletcher and Gaze, 2008).The fungal contaminants impede the development of mushrooms through competition with them for space and nutrients and could also produce metabolites harmful to the mushroom mycelium (Mumpuni *et al*., 1998*). 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 basidiomes ultimately leading to reduced production and sometimes complete failure of the crop (Gea *et al*., 2021).

Biswas (2014) did a survey that revealed the occurrence of seven contaminants in mushroom beds and out of which *Trichoderma harzianum, Penicillium notatum, sclerotium rolfsii,* and *coprinus spp.* were found to be most dominant fungal contaminants and occurred highly during June and July (28.4% and 35.8%) while the incidence of contaminants were minimum during January (2.87%) and maximum during the month of June (32.8%).

Sauda *et al.* (2015) showed that air–borne fungus were potentially cause failure of Oyster mushroom cultivation with the highest prevalence was *Fusarium spp*. (25.6%), while the highest inhibition ability was *Mucor spp.* (94.7±8.5%). The

most dominant of baglog–borne fungal with its prevalence was *Trichoderma spp*. (35.71%).

Singh *et al.* (2006) recognized *T. harzianum* as the most important species of *Trichoderm*a capable of causing green mold 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).

Rajarathnam *et al*. (1992) reported *Sclerotium rolfsii*. As a serious substrate-borne fungal contaminant found during commercial cultivation of *Pleurotus flabellatus* on unfermented rice straw coming from rice fields. Depending on the degree of contamination, the loss due to *S. rolfsii* in mushroom yield ranges from 80% to 96%), several folds higher than that of the mushroom (Rajarathnam and Zakia, 1987).

2.12. Effect of different substrate pre-treatments

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

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

According to Quimio *et al*. (1990), substrate sterilization is not ideal since both beneficial and harmful organisms in the substrate are killed, while Miroslawa (1991) recommended maintaining the substrate for 24 h at 70°C.

Similarly, sterilization of substrates is not an easy job for the cultivation of mushroom and the right sterilization time and temperature depend on the possible pathogens in a given substrate material (Kwon and Sik Kim, 2004).

Oseni *et al*. (2012) carried out an experiment to devise an easy handy substrate pre-treatment procedure in Oyster mushroom cultivation and according to the

study, although, autoclaving was the best method for substrate pre-treatment, however hot water pasteurization at 60°C for 3 h of sugarcane bagasse proved to be a viable and promising method of substrate pre-treatment, which can be adopted to produce a good yield of Oyster mushroom especially in rural areas, where autoclave sterilization is not feasible.

Shrestha *et al.* (2021) recorded that performance of Oyster mushrooms was best under steam sterilization as it took the shortest time for pinhead formation (34.30 days), fruiting body formation (43.60 days), cropping duration (89.30 days), and produced the highest mushroom yield (1401.9 g per 4 kg bag), and consequently, the highest biological efficiency (101.38%). Average pileus diameter and stipe length were statistically indifferent among the treatments suggesting that the sterilization methods don not affect morphological characters of Oyster mushrooms but yield.

CHAPTER III

MATERIALS AND METHODS

3.1. Location of the experiment

The cultivation of mushrooms was carried out at 'Mushroom Culture House (MCH)' of Sher-E-Bangla Agricultural University, Dhaka and the laboratory work was done at 'Plant Pathology Laboratory' of the same University. Meteorological data during the experimental period was collected from the Bangladesh Meteorological Department, Agargaon, Dhaka and presented in Appendix I.

3.2. Duration of the experiment

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

3.3. Experimental materials

Mother culture was collected from 'Mushroom Development Institute (MDI)', Savar, Dhaka. Variety named 'HK-51' was used for this experiment.

3.4. Varietal characteristics

Oyster mushrooms have a light to dark whitish colored cap depending upon the strain and growing condition. The variety used in this experiment showed gray headed primordia but became lighter with maturation. Mushroom cap was somewhat grayish but is stipe was white after maturation. Stipe was prominent and strong. It was one of the largest sized Oyster mushrooms. Oyster mushrooms show a rapid mycelial growth and high saprophytic colonization activity on cellulosic substrates. It requires temperature below 32ºC and a humid atmosphere. With increased atmospheric temperature the production of Oyster mushroom decreases.

3.5. Treatments of the experiment

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

T_1 = 250 g waste paper

T_2 = 500 g waste paper

$T_{3:}$ = 750 g Waste paper

T_4 = 1000 g Waste paper

T_5 = 1500 g Waste paper

T_6 = 250 g (75% Waste paper + 25% Sawdust)

T_7 = 500 g (75% Waste paper + 25% Sawdust)

T_8 = 750 g (75% Waste paper + 25% Sawdust)

T_9 = 1000 g (75% Waste paper + 25% Sawdust)

T_{10} = 1500 g (75% Waste paper + 25% Sawdust)

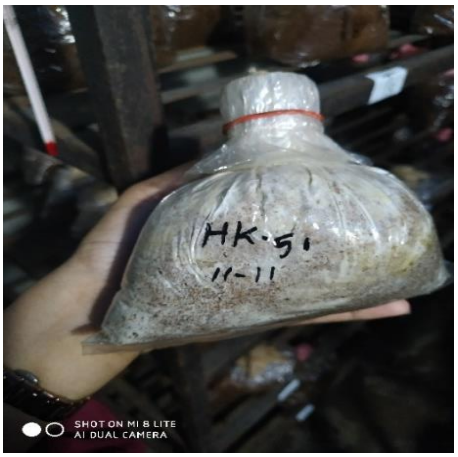
T_{11} = 250 g (25% Waste paper + 75% Sawdust)

T_{12} = 500 g (25% Waste paper + 75% Sawdust)

T_{13} = 750 g (25% Waste paper + 75% Sawdust)

T_{14} = 1000 g (25% Waste paper + 75% Sawdust)

T_{15} = 1500 g (25% Waste paper + 75% Sawdust)



A. "HK-51" variety mother spawn

B. Waste paper

C. 75% Waste paper + 25% Sawdust

D. 25% Waste paper + 75% Sawdust

Plate 1. Substrate preparation**; A.** "HK-51" variety mother spawn**, B-C.** Different substrates for spawn packet preparation

3.6. Design and layout of the experiment

Fifteen treatments with three replications were arranged in single factor Completely Randomized Design (CRD).

3.7. Preparation of substrates and spawn packet

As the treatments of the experiment were related to spawn packet size, packets preparation was done with much caution. Two types of substrates (waste paper and sawdust) were used at different percentage (100% waste paper, 75% waste paper + 25% sawdust, 25% waste paper + 75% sawdust). Waste papers were cut into small pieces and soaked in water overnight. Then water was drained off and paper pieces were squeezed very well to remove excess water. After that waste paper was mixed with calcium carbonate (1 gram per packet) thoroughly by hand. Moisture was adjusted to 65% moisture content measuring through moisture meter. Then the waste paper substrate was packaged in polypropylene bags of different sizes. During packaging weight was measured through weight machine to maintain proper treatments.

Water soaked waste paper in 75% amount and 25% sawdust were mixed to prepare another substrate. Some calcium carbonate was also added moisture content was kept at 65%. Then the substrate was packaged in polypropylene bags.

The same procedure was followed for 25% waste paper + 75% sawdust packet preparation.

Plastic necks were used to the filled polypropylene bags to be finally completed and plugged the neck with cotton, cover the brown paper and bound with rubber bands tightly.

3.8. Sterilization and inoculation

The packets were sterilized by autoclave. These were kept in autoclave for an hour at 15 PSI. Afterword these were kept in a sterilized place for cooling. After cooling, the sterilized spawn packets were inoculated with 5g of mother spawn. This inoculation process was done in laminar air flow cabinet carefully. The inoculated packets were incubated at 20-22ºC temperature.

A. Prepared spawn packets

B. Spawn packets taken for autoclave

C. Spawn packets kept for cooling after autoclave

D. Incubation of spawn packets

Plate 2. Steps of mushroom cultivation; **A.** Prepared spawn packets**, B.** Spawn packets taken for autoclave, **C.** Spawn packets kept for cooling after autoclave**, D.**Incubation of spawn packets

3.9. Mycelium running in spawn packets

During incubation period mycelium colonization starts. The rubber band, brown paper, cotton plug and plastic neck settled with spawn packets were removed when mycelium running was started. Then the opening of the packets was bound with rubber band tightly and the packets were transferred to the culture house.

3.10. Completion of mycelium running and cutting of spawn packets

Spawn packets were observed if the mycelium colonization were completed. When the packets became White with mushroom mycelium, 2 'D' shaped cut were made at one side of each packet and scraped slightly. Cut packets were soaked in clean water for 10 to 15 minutes. Excess water was removed by keeping the soaked spawn packets in inverted position in the shelf.

3.11. Cultivation of spawn packets

After removal of excess water, the packets of each treatment were arranged on the shelf of culture room covered with newspaper. Sprayed water 3 times a day to keep the moisture of culture room at 80-85% relative humidity. The light was around 300-500 lux and temperature was kept at 22-25º C. Ventilation of culture room was maintained uniformly.

3.12. Harvesting

Mushrooms should be harvested before the caps were completely flatten out. So, the mature fruiting bodies were harvested by gently twisting the fruiting body before water is sprayed. After first harvesting. Another two 'D' cuts were made at the other side of bags and the bags were soaked in clean water for 10-15 minutes. Removing excess water, bags were kept in growing shelf so that other mycelium can produce more fruiting bodies which can then be again harvested. Water was sprayed regularly to maintain enough moisture.

A. 'D' cut at spawn packet after colonization **B.** Spawn packets soaked in water

C. Spawn packets kept in inverted position to remove excess water

Plate 3. Stimulation of spawn packets; **A.** 'D' cut at spawn packet after colonization, **B.** Spawn packets soaked in water, **C.** Spawn packets kept in inverted position to remove excess water

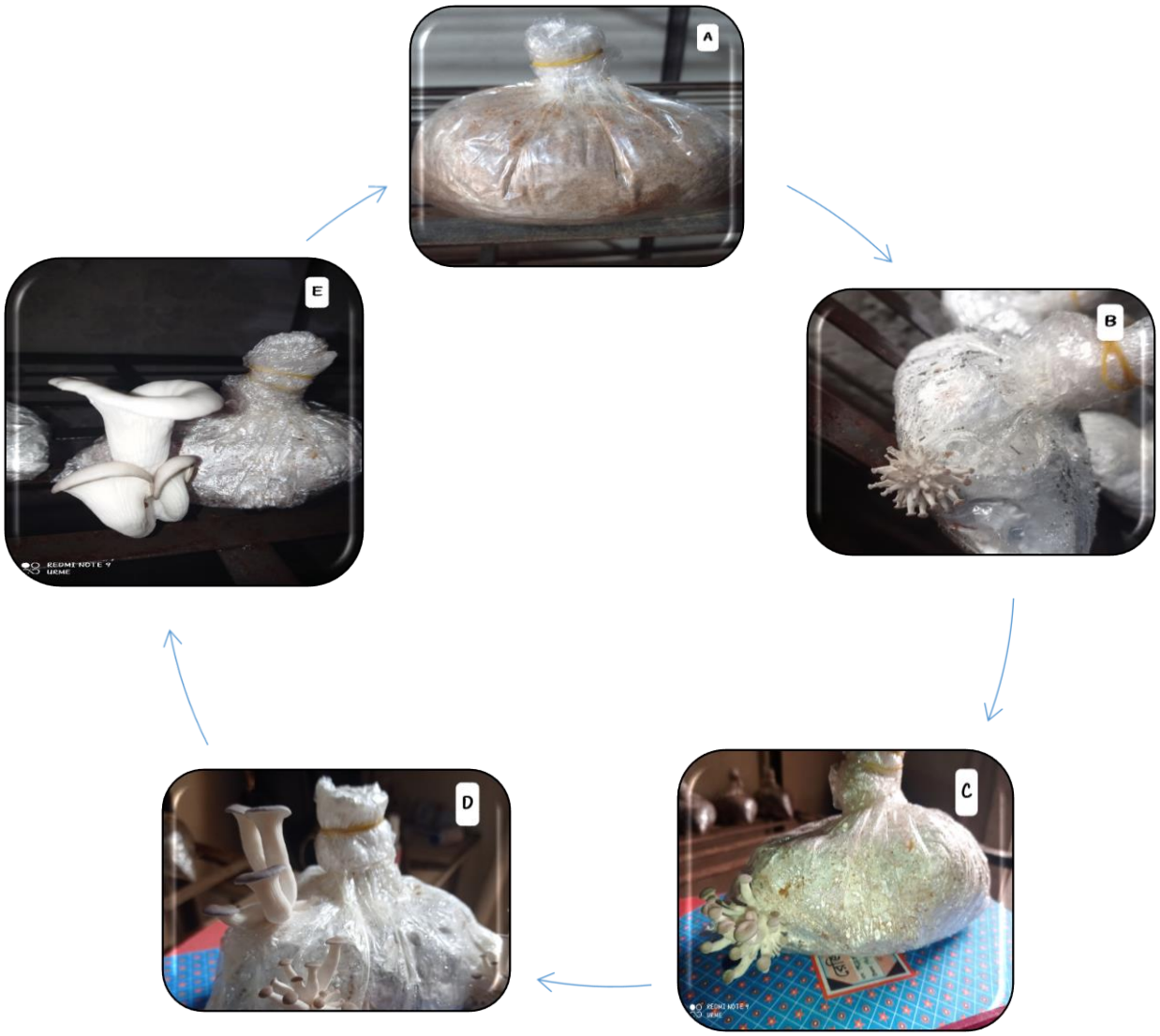


Plate 4. Mushroom growing stages; **A.** Completion of mycelium running, **B**.Primordia initiation, **C-D.** Maturation of primordia, **E.** Matured fruiting body

3.13. Data collection

Data on the following parameter were collected using a standard procedure with some modification where needed:

i. Mycelium running rate in spawn packet (cm)
ii. Days required for completion of mycelium running
iii. Days required for primordia initiation
iv. Days required for first harvest
v. Days required for final harvest
vi. Average number of primordia/packet
vii. Average number of fruiting body/packet
viii. Average number of effective fruiting body/packet
ix. Fresh weight of individual fruiting body (gm)
x. Dimension of fruiting body (cm)
xi. Biological yield (g)
xii. Economic Yield (g)
xiii. Dry yield (g)
xiv. Biological efficiency (%)
xv. Severity of contamination (%) on spawn packets

3.14. Procedure of recording data

3.14.1. Mycelium running rate (MRR) (cm)

Mycelium running rate (MRR) of each treatment was estimated after the mycelium colony crossed the shoulder of packets. The straight length was estimated at different places of packet using the following equation (Sarker, 2004)

$$\text{MRR} = \frac{\text{Average length (L)}}{\text{Number of days (N)}} \text{ cm/Days}$$

3.14.2. Days required for completion of mycelium running

Total days from mother inoculation to completion of mycelium running was recorded.

3.14.3. Days required for primordia initiation

Total days from stimulation to primordia initiation was recorded.

3.14.4. Days required for first harvest

Total days from incubation to first harvest was recorded. Total days required for 1st harvest from primordia initiation was also recorded.

3.14.5. Days required for final harvest

Days required from primordia initiation to final harvest was recorded.

3.14.6. Average number of primordia/packet

Number of primordia in every packet was counted and an average was recorded. Dried primordia were eliminated but those were tiny were included.

3.14.7. Average number of fruiting body/packet

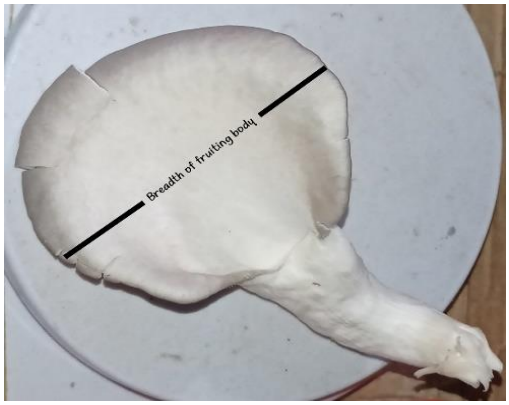
Number of well-developed fruiting bodies in every packet was counted and an average was recorded.

3.14.8. Average number of effective fruiting body/packet

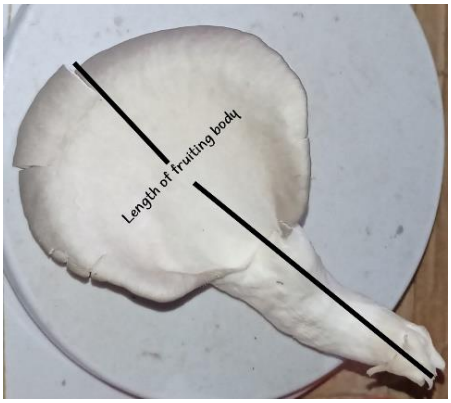
Number of effective fruiting bodies in every packet was counted and an average was recorded.

3.14.9. Fresh weight of individual fruiting body

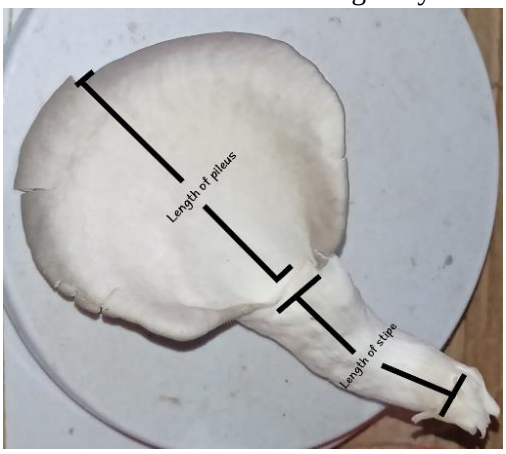
Fresh weight of individual fruiting body determined dividing total weight of fruiting body per packet by total number of fruiting body per packet.



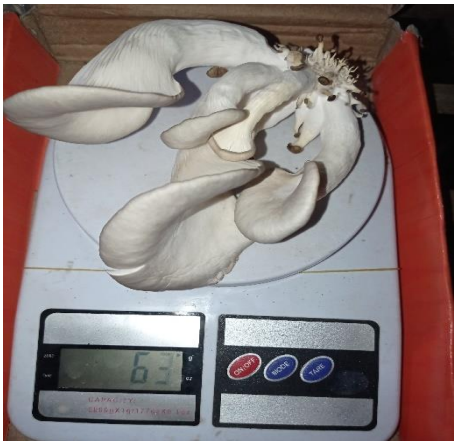
A. Breadth of fruiting body



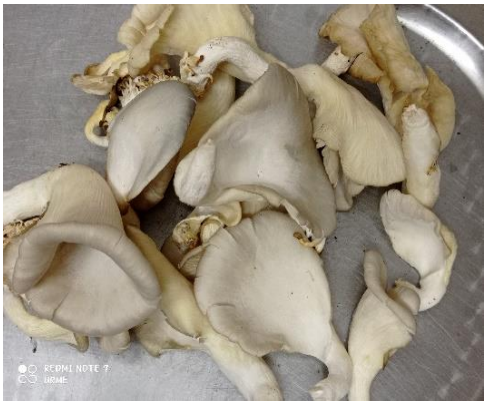
B. Length of fruiting body



C. Length of pileus and stipe



D. Biological yield measurement



E. Harvested mushrooms

F. Contaminated spawn packet

Plate 5. Measurement of harvested mushroom and contamination of spawn packet; **A.** Breadth of fruiting body, **B.** Length of fruiting body, **C.** Length of pileus and stipe, D**.** Biological yield measurement, **E.** Harvested mushrooms, **F.** Contaminated spawn packet

3.14.10. Dimension of fruiting body

Three fruiting bodies were randomly selected and their length and breadth were measured. The measured. Length of pileus, length of stipe was also measured.

a) Length of stipe (cm)

b) Length of pileus (cm)

c) Breadth of fruiting body (cm)

3.14.11. Biological yield (g)

The whole cluster of fruiting body without removing the lower hard and dirty portion was weighed to measure biological yield per packet.

3.14.12. Economic Yield (g)

After removal of lower hard and dirty parts of fruiting bodies, weight was measured to record economic yield per packet.

3.14.13. Dry yield (g)

About 50g of fresh mushrooms from each treatment was taken on brown paper and dried in sun. Then the mushroom was dried in oven for 24 hours at 72ºC to remove remaining moisture and weighed once more. Weight of blank envelop was subtracted from both the weight. The dry yield was determined by using the following equation (Sarker, 2004):

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

3.14.14. Biological efficiency (%)

Following equation was used to determine Biological efficiency (%)

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

3.14.15. Severity of contamination (%) on spawn packets

Contamination severity was calculated for treatments depending upon the following scale:

Grade 0: 0% – Free from infection

Grade 1: >0 – 20% Spawn area coverage by the contaminants

Grade 2: >20 – 40% Spawn area coverage by the contaminants

Grade 3: >40 – 60% Spawn area coverage by the contaminants

Grade 4: >60 – 80% Spawn area coverage by the contaminants

Grade 5: >80 – 100% Spawn area coverage by the contaminants

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

3.14.16. 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).

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 /packet (Tk)

3.15. Preparation and sterilization of PDA media

The following ingredients were used to prepare PDA media and autoclaved:

Ingredients	Amount (per liter)
Potato slices	200g
Dextrose	20g
Agar	20g
Water	1 L

Electric hot air oven was used to sterilize the glass wares viz. measuring cylinder, test tubes, conical flask, glass rods at 160º C for an hour. To prepare potato infusion 200 g peeled, sliced potato was boiled in 1 L distilled water for 30 min. Dextrose and Agar were added to filtered potato infusion and boiled to dissolve. Thus nutrient media was prepared which was sterilized by autoclaving at 15 lbs. pressure and 121ºc temperature for 45 minutes. Sterilized glass wares, nutrients media and other equipment were kept in laminar air flow chamber to avoid contamination. The laminar air flow chamber was wiped with 70% ethyl alcohol. Hot nutrient media was poured in Petridis and was waited to be cooled. When it cooled down, it solidified.

3.16. Isolation and culture of competitor molds from collected spawn

Some contaminated packets where kept isolated after observation and 10 gram of substrate sample was collected from each packet. Collected samples were mixed with 100 ml distilled water separately. A series of dilution were made by adding 1 ml of stock solution to 9 ml of distilled water. From each solution 0.5 ml solution was pipetted on PDA media and incubated at 27 º C ± 2 ºC for 3-4 days. The whole process was done in laminar air flow cabinet very cautiously. Colonies of different colours was seen after incubation period and was isolated. Reculturing process was continued until pure culture was obtained.

3.17. Identification of contaminants

Cultural and morphological characters of pathogens was studied. Slide was prepared from each pure culture and examined under low (10X) and higher (40X) power magnification. Results found from observation were compared with information found from literature. Microphotographs of pathogens were taken. Comparing all information and photograph the contaminating pathogens were identified (Barnett and Hunker, 1972).

3.18. Experimental data analysis technique

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique with the help of a computer package program name Statistix 10 data analysis software and the mean differences were adjusted by Least Significant Difference (LSD) test at 5% level of probability (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS

4.1. Growth contributing characters

4.1.1. Effect of different amounts of substrates on mycelium running rate (cm/day)

Mycelium running rate of oyster mushroom (*Pleurotus ostreatus*) showed statistically significant variation due to different amounts and combinations of substrates under the present trial (Figure 1 and Appendix II). Highest mycelium running rate (0.97 cm/day) was recorded in T_2 = 500g waste paper. The lowest mycelium running rate (0.57 cm/day) was recorded in T_{12} = 500g (25% Waste paper + 75% Sawdust) and T_{14} =1000g (25% Waste paper + 75% Sawdust).

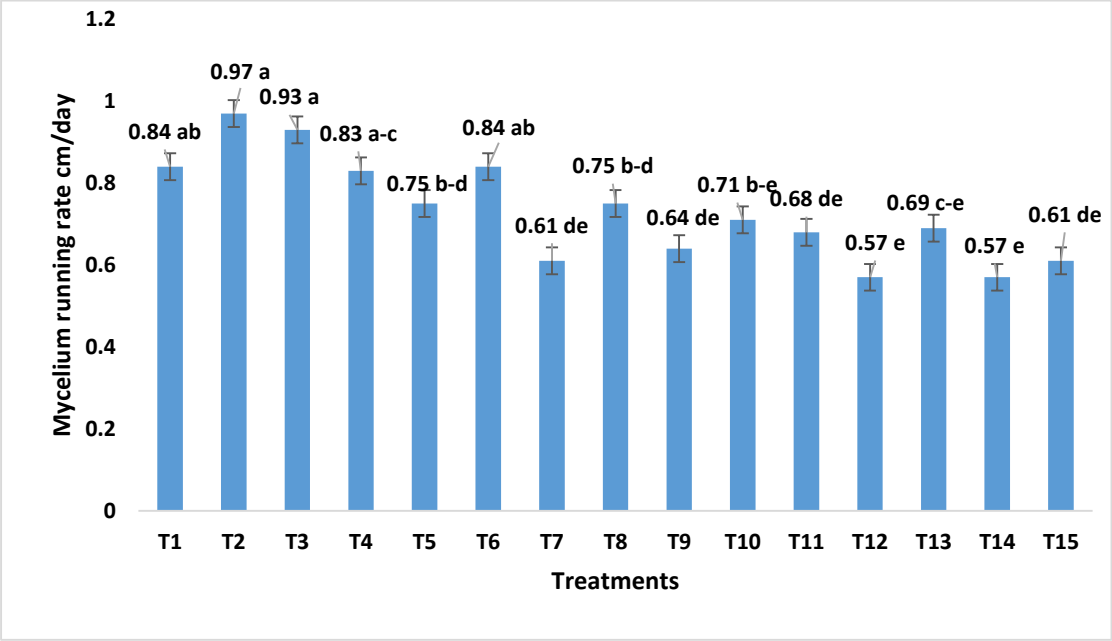


Figure 1. Effect of different amounts and combinations of substrates on mycelium running rate (cm/day)

[T_1 = 250g waste paper, **T_2** =500g waste paper, **T_3** = 750g Waste paper, **T_4** = 1000g Waste paper, **T_5** = 1500g Waste paper, **T_6** = 250g (75% Waste paper + 25% Sawdust), **T_7** = 500g (75% Waste paper + 25% Sawdust), **T_8** = 750g (75% Waste paper + 25% Sawdust), **T_9** = 1000g (75% Waste paper + 25% Sawdust), **T_{10}** = 1500g (75% Waste paper + 25% Sawdust), **T_{11}** = 250g (25% Waste paper + 75% Sawdust), **T_{12}** = 500g (25% Waste paper + 75% Sawdust), **T_{13}** = 750g (25% Waste paper + 75% Sawdust), **T_{14}**= 1000g (25% Waste paper + 75% Sawdust), **T_{15}** = 1500g (25% Waste paper + 75% Sawdust)]

4.1.2. Effect of different amounts and combinations of substrates on time required from inoculation to mycelium initiation

There were significant variation in terms of time from inoculation to mycelium initiation of oyster mushroom (*P. ostreatus*) due to different amounts and combinations of substrates (Table 1 and Appendix II). The highest time (27.00 days) from incubation to mycelium initiation was found in T_{12} = 500g (25% Waste paper + 75% Sawdust) which was statistically identical to $T_{11,}$ $T_{13,}$ T_{14} and T_{15}. The lowest time (10.67 days) required for mycelium running was recorded in T_5 = 1500g Waste paper which was statistically indifferent to $T_{1,}$ T_2, T_3 and T_4 but significantly different. Here $T_{6,}$ T_7; T_8, T_9 and T_{10} also showed statistically similar result.

4.1.3. Effect of different amounts and combinations of substrates on time required from inoculation to completion of mycelium running

Days required from inoculation to completion of mycelium running of oyster mushroom varied significantly due to different amounts and combinations of substrates (Table 1 and Appendix II). The highest time (65.33 days) required for completion of mycelium running was recorded in T_{15} = 1500g (25% Waste paper + 75% Sawdust)followed by which is statistically similar but significantly different whereas, the lowest time (21.67days) required for completion of mycelium running was recorded in T_2 =500g waste paper followed by T_1 = 250g waste paper and T_3 = 750g Waste paper.

Table 1. Performance of different amounts and combinations of substrates on time required from inoculation to initiation and completion of mycelium running

Treatments	Time required from inoculation to	
	mycelium initiation (days)	completion of mycelium running (days)
T_1	12.00 c	22.33 h
T_2	12.00 c	21.67 h
T_3	11.67 c	25.33 gh
T_4	11.67 c	33.67 ef
T_5	10.67 c	40.00 d
T_6	17.67 b	29.67 fg
T_7	17.67 b	37.33 de
T_8	17.33 b	40.33 d
T_9	18.00 b	46.33 c
T_{10}	17.67 b	51.67 b
T_{11}	25.67 a	42.33 cd
T_{12}	27.00 a	52.00 b
T_{13}	24.33 a	52.00 b
T_{14}	25.67 a	64.00 a
T_{15}	24.33 a	65.33 a
CV (%)	10.76	7.62
LSD $_{(0.05)}$	3.27	5.28

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_1 = 250g waste paper, T_2 =500g waste paper, T_3 = 750g Waste paper, T_4 = 1000g Waste paper, T_5 = 1500g Waste paper, T_6 = 250g (75% Waste paper + 25% Sawdust), T_7 = 500g (75% Waste paper + 25% Sawdust), T_8 = 750g (75% Waste paper + 25% Sawdust), T_9 = 1000g (75% Waste paper + 25% Sawdust), T_{10} = 1500g (75% Waste paper + 25% Sawdust), T_{11} = 250g (25% Waste paper + 75% Sawdust), T_{12} = 500g (25% Waste paper + 75% Sawdust), T_{13} = 750g (25% Waste paper + 75% Sawdust), T_{14} = 1000g (25% Waste paper + 75% Sawdust), T_{15} = 1500g (25% Waste paper + 75% Sawdust)]

4.1.4. Effect of different amounts and combinations of substrates on time required from ‘‘D’’ cutting the spawn packet to primordia initiation (days)

There was significant variation in terms of time from ‘‘D’’ cutting the spawn packet to primordial initiation of oyster mushroom due to different amounts and combinations of substrates (Table 2 and Appendix III). The time from stimulation to primordial initiation ranged from 7.67 days to14.00 days. The highest time from stimulation to primordial initiation (14.00 days) was observed in T_4 = 1000g Waste paper followed by T_{15}, T_{14}, T_{11}, T_{12}, T_{13} and T_{10} whereas, the lowest time (7.67 days) from stimulation to primordia initiation was in T_7 = 500g (75% Waste paper + 25% Sawdust), followed by T_1 = 250g waste paper, T_5 = 1500g Waste paper and T_2 =500g waste paper.

4.1.5. Effect of different amounts and combinations of substrates on time required from primordial initiation to 1 st (days) harvest

Data revealed that time from primordial initiation to 1st harvest of oyster mushroom was statistically identical but significantly different (Table 2 and Appendix III). The maximum time (9.33 days) from primordia initiation to harvest was observed in T_6 = 250g (75% Waste paper + 25% Sawdust), whereas, the lowest time (7.33 days) from primordia initiation to harvest was in T_3 = 750g Waste paper and T_4 = 1000g Waste paper

4.1.6.. Effect of different amounts and combinations of substrates on total harvesting period (days)

No significant variation was recorded in terms of days for final harvest of oyster (Table 2 and Appendix III). The highest harvesting period (87 days) was recorded in T_{15} = 1500g (25% Waste paper + 75% Sawdust) followed by T_{14} which is statistically indifferent whereas, the lowest time (39.33 days) was recorded in T_1 = 250g waste paper and T_2 =500g waste paper.

Table 2. Performance of different amounts and combinations of substrates on time required from ''D'' cutting the spawn packet to primordial initiation, primordial initiation to 1st harvest and total harvesting period

Treatments	Time required from ''D'' cutting the spawn packet to primordia initiation (days)	Time required from primordial initiation to 1st harvest (days)	Total harvesting period (days)
T_1	8.67 d-f	*8.33	39.33 i
T_2	8.33 ef	8.67	39.33 i
T_3	10.67 b-f	7.33	43.33 hi
T_4	14.00 a	7.33	55.00 fg
T_5	8.67 d-f	8.00	56.67 ef
T_6	10.00 c-f	9.33	49.00 gh
T_7	7.67 f	8.67	53.67 fg
T_8	10.67 b-f	8.33	59.33 d-f
T_9	10.00 c-f	8.67	65.00 cd
T_{10}	11.00 a-e	7.67	70.33 bc
T_{11}	12.33 a-c	8.00	62.67 de
T_{12}	11.67 a-d	9.00	72.67 b
T_{13}	11.33 a-e	8.67	72.00 bc
T_{14}	12.67 a-c	9.00	85.67 a
T_{15}	13.33 ab	8.33	87.00 a
CV (%)	18.58	20.42	6.94
LSD $_{(0.05)}$	3.33	2.85	7.03

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. *NS= Non-significant

[T_1 = 250g waste paper, **T_2** =500g waste paper, **T_3** = 750g Waste paper, **T_4** = 1000g Waste paper, **T_5** = 1500g Waste paper, **T_6** = 250g (75% Waste paper + 25% Sawdust), **T_7** = 500g (75% Waste paper + 25% Sawdust), **T_8** = 750g (75% Waste paper + 25% Sawdust), **T_9** = 1000g (75% Waste paper + 25% Sawdust), **T_{10}** = 1500g (75% Waste paper + 25% Sawdust), **T_{11}** = 250g (25% Waste paper + 75% Sawdust), **T_{12}** = 500g (25% Waste paper + 75% Sawdust), **T_{13}** = 750g (25% Waste paper + 75% Sawdust), **T_{14}** = 1000g (25% Waste paper + 75% Sawdust), **T_{15}** = 1500g (25% Waste paper + 75% Sawdust)]

4.1.7. Effect of different amounts and combinations of substrates on average number of primordial per packet

Average number of primordia per packet varied from 33.67 to 59.33 significantly due to different amounts and combinations of substrates under the present trial (Figure 2 and Appendix IV). The maximum average number of primordia per packet was observed from T_{10} = 1500g (75% Waste paper + 25% Sawdust), whereas, the minimum number of primordia per packet was found in T_1 = 250g waste paper.

4.1.8. Effect of different amounts and combinations of substrates on average number of fruiting body per packet

Average number of fruiting body was ranged from 10.33 to 22.33 which had statistically significant differences due to different amounts and combinations of substrates (Figure 2 and Appendix IV). The maximum number of fruiting body per packet was recorded from T_{10} = 1500g (75% Waste paper + 25% Sawdust, whereas the minimum average number of fruiting body per packet was observed in T_6 = 250g (75% Waste paper + 25% Sawdust).

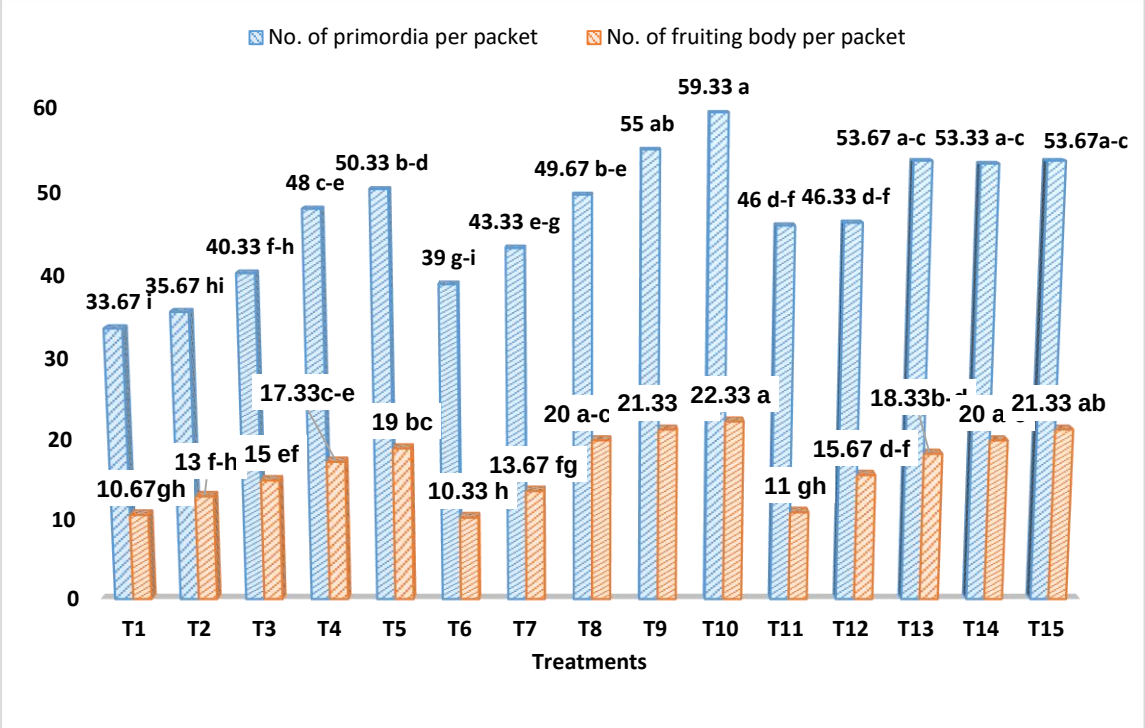


Figure 2. Effect of different amounts and combinations of substrates on average number of primordia and fruiting body per packet ($LSD_{0.05}$ = 6.52, 3.13)

[T_1 = 250g waste paper, **T_2** =500g waste paper, **T_3** = 750g Waste paper, **T_4** = 1000g Waste paper, **T_5** = 1500g Waste paper, **T_6** = 250g (75% Waste paper + 25% Sawdust), **T_7** = 500g (75% Waste paper + 25% Sawdust), **T_8** = 750g (75% Waste paper + 25% Sawdust), **T_9** = 1000g (75% Waste paper + 25% Sawdust), **T_{10}** = 1500g (75% Waste paper + 25% Sawdust), **T_{11}** = 250g (25% Waste paper + 75% Sawdust), **T_{12}** = 500g (25% Waste paper + 75% Sawdust), **T_{13}** = 750g (25% Waste paper + 75% Sawdust), **T_{14}** = 1000g (25% Waste paper + 75% Sawdust), **T_{15}** = 1500g (25% Waste paper + 75% Sawdust)]

4.1.9. Effect of different amounts and combinations of substrates 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 (18.67) was recorded from, T_{15} = 1500g (25% Waste paper + 75% Sawdust) followed by T_{14}. On the other hand, the minimum average number of effective fruiting body per packet (5.00) was observed in T_1 = 250g waste paper followed T_2 =500g waste paper.

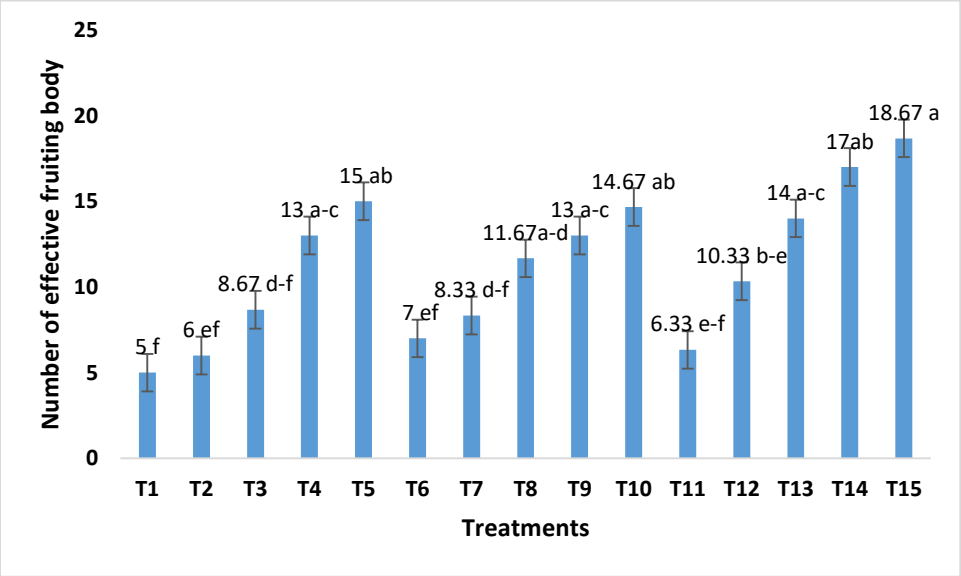


Figure 3. Effect of different amounts and combinations of substrates on average number of effective fruiting body ($LSD_{0.05}$=3.98)

[T_1 = 250g waste paper, **T_2** =500g waste paper, **T_3** = 750g Waste paper, **T_4** = 1000g Waste paper, **T_5** = 1500g Waste paper, **T_6** = 250g (75% Waste paper + 25% Sawdust), **T_7** = 500g (75% Waste paper + 25% Sawdust), **T_8** = 750g (75% Waste paper + 25% Sawdust), **T_9** = 1000g (75% Waste paper + 25% Sawdust), **T_{10}** = 1500g (75% Waste paper + 25% Sawdust), **T_{11}** = 250g (25% Waste paper + 75% Sawdust), **T_{12}** = 500g (25% Waste paper + 75% Sawdust), **T_{13}** = 750g (25% Waste paper + 75% Sawdust), **T_{14}** = 1000g (25% Waste paper + 75% Sawdust), **T_{15}** = 1500g (25% Waste paper + 75% Sawdust)]

4.2. Dimension of fruiting body (cm)

4.2.1. Length of stipe (cm)

Length of stipe of oyster mushroom ranged from 4.43 to 6.77 cm (Table 3 and Appendix V). The highest length of stipe was observed in T_3 = 750g Waste paper whereas, the lowest length of stipe was found in T_{11} = 250g (25% Waste paper + 75% Sawdust). Other treatments showed statistically similar results which were significantly different.

4.2.2. Length of pileus (cm)

Length of pileus of oyster mushroom varied from 5.77 to 8.63 cm significantly due to different amounts and combinations of substrates under present trial (Table 3 and Appendix V). The highest length of pileus was recorded from T_{15} = 1500g (25% Waste paper + 75% Sawdust) whereas, the lowest length of pileus was found in T_1 = 250g waste paper followed by T_2 which is statistically similar but significantly different. Statistically similar result was found in case of all the other treatments which were significantly different.

4.2.3. Breadth of fruiting body (cm)

Significant difference was recorded in terms of breadth of fruiting body of oyster mushroom (Table 3 and Appendix V). The highest breadth of fruiting body (11.40 cm) was observed in T_4 = 1000g Waste paper. On the other hand, the lowest breadth of fruiting body (5.67 cm) was found in T_1 = 250g waste paper.

Table 3. Performance of different amounts and combinations of substrates on dimension of fruiting body

Treatments	Length of stipe (cm)	Length of pileus (cm)	Breadth of fruiting body (cm)
T_1	5.37 ab	5.77 b	5.67 d
T_2	6.20 ab	5.93 b	6.10 cd
T_3	6.77 a	6.23 ab	7.37 b-d
T_4	5.57 ab	8.03 ab	11.40 a
T_5	5.70 ab	6.60 ab	6.60 b-d
T_6	5.80 ab	8.00 ab	7.47 b-d
T_7	6.17 ab	7.57 ab	7.60 b-d
T_8	6.13 ab	6.30 ab	7.47 b-d
T_9	5.73 ab	7.77 ab	9.60 ab
T_{10}	5.90 ab	7.43 ab	9.53 ab
T_{11}	4.43 b	8.10 ab	7.47 b-d
T_{12}	4.87 ab	7.17 ab	9.27 a-c
T_{13}	5.53 ab	7.77 ab	9.27 a-c
T_{14}	6.43 ab	8.13 ab	7.50 b-d
T_{15}	5.63 ab	8.63 a	8.57 a-d
CV (%)	21.66	22.15	25.50
LSD $_{(0.05)}$	2.08	2.69	3.43

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_1** = 250g waste paper, **T_2** =500g waste paper, **T_3** = 750g Waste paper, **T_4** = 1000g Waste paper, **T_5** = 1500g Waste paper, **T_6** = 250g (75% Waste paper + 25% Sawdust), **T_7** = 500g (75% Waste paper + 25% Sawdust), **T_8** = 750g (75% Waste paper + 25% Sawdust), **T_9** = 1000g (75% Waste paper + 25% Sawdust), **T_{10}** = 1500g (75% Waste paper + 25% Sawdust), **T_{11}** = 250g (25% Waste paper + 75% Sawdust), **T_{12}** = 500g (25% Waste paper + 75% Sawdust), **T_{13}** = 750g (25% Waste paper + 75% Sawdust), **T_{14}** = 1000g (25% Waste paper + 75% Sawdust), **T_{15}** = 1500g (25% Waste paper + 75% Sawdust)]

4.3. Yield parameter (g)

4.3.1. Biological yield (g)

Due to use of different amounts and combinations of substrates, biological yield of oyster mushroom showed statistically significant variation (Table 4 and Appendix VI). The highest biological yield (165.33 g) was recorded from T_{15} = 1500g (25% Waste paper + 75% Sawdust) followed by T_{14} = 1000g (25% Waste paper + 75% Sawdust), While the lowest biological yield (102.67 g) was recorded in T_1 = 250g waste paper followed by **T_{11}** = 250g (25% Waste paper + 75% Sawdust).

4.3.2. Economic yield (g)

Statistically significant variation was recorded in terms of economic yield of oyster mushroom due to different amounts and combinations of substrates (Table 4 and Appendix VI). The highest economic yield (162 g) was recorded from T_{15} = 1500g (25% Waste paper + 75% Sawdust) followed by T_{14} = 1000g (25% Waste paper + 75% Sawdust), whereas the lowest economic yield (99.33 g) was found in T_1 = 250g waste paper followed by T_{11} = 250g (25% Waste paper + 75% Sawdust).

4.3.3. Dry yield (g)

Different amounts and combinations of substrates influenced dry yield and statistically significant variation was recorded in this present experiment (Table 4 and Appendix VI). The highest dry yield (11.44 g) was recorded from T_{15} = 1500g (25% Waste paper + 75% Sawdust) followed by T_{14} = 1000g (25% Waste paper + 75% Sawdust), whereas the lowest dry yield (6.95 g) was found in T_1 = 250g waste paper followed by T_{11} = 250g (25% Waste paper + 75% Sawdust).

Table 4. Effect of different amounts and combinations of substrates on yield parameters of oyster mushrooms

Treatments	Biological yield (g)	Economic yield (g)	Dry yield (g)
T_1	102.67 k	99.33 h	6.95 i
T_2	115.33 h-j	110.67 fg	7.75 gh
T_3	122.33 gh	118.00 ef	8.26 fg
T_4	131.33 fg	126.67 de	8.87 ef
T_5	146.33 cd	141.67 b	9.92 c
T_6	112.33 ij	109.67 fg	7.68 gh
T_7	119.33 hi	114.33 f	8.00 g
T_8	129.67 fg	124.67 de	8.73 ef
T_9	147.67 cd	142.33 b	9.96 c
T_{10}	155.33 bc	152.67 a	10.69 b
T_{11}	109.67 jk	104.67 gh	7.33 hi
T_{12}	136.67 ef	131.00 cd	9.17 de
T_{13}	145.00 de	139.67 bc	9.78 cd
T_{14}	158.67 ab	155.00 a	10.89 ab
T_{15}	165.33 a	162.00 a	11.44 a
CV(%)	4.16	4.46	4.44
$LSD_{(0.05)}$	9.23	9.58	0.67

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_1** = 250g waste paper, **T_2** =500g waste paper, **T_3** = 750g Waste paper, **T_4** = 1000g Waste paper, **T_5** = 1500g Waste paper, **T_6** = 250g (75% Waste paper + 25% Sawdust), **T_7** = 500g (75% Waste paper + 25% Sawdust), **T_8** = 750g (75% Waste paper + 25% Sawdust), **T_9** = 1000g (75% Waste paper + 25% Sawdust), **T_{10}** = 1500g (75% Waste paper + 25% Sawdust), **T_{11}** = 250g (25% Waste paper + 75% Sawdust), **T_{12}** = 500g (25% Waste paper + 75% Sawdust), **T_{13}** = 750g (25% Waste paper + 75% Sawdust), **T_{14}** = 1000g (25% Waste paper + 75% Sawdust), **T_{15}** = 1500g (25% Waste paper + 75% Sawdust)]

4.3.4. Biological efficiency

Remarkable differences were observed in biological efficiency and it was ranged from 10.24% to 44.93%. The highest of biological efficiency (44.93%) was observed in T_6 = 250g (75% Waste paper + 25% Sawdust) followed by T_{11} = 250g (25% Waste paper + 75% Sawdust) while the lowest (10.24%) biological efficiency was recorded in T_5 = 1500g Waste paper followed by T_{10} = 1500g (75% Waste paper + 25% Sawdust).

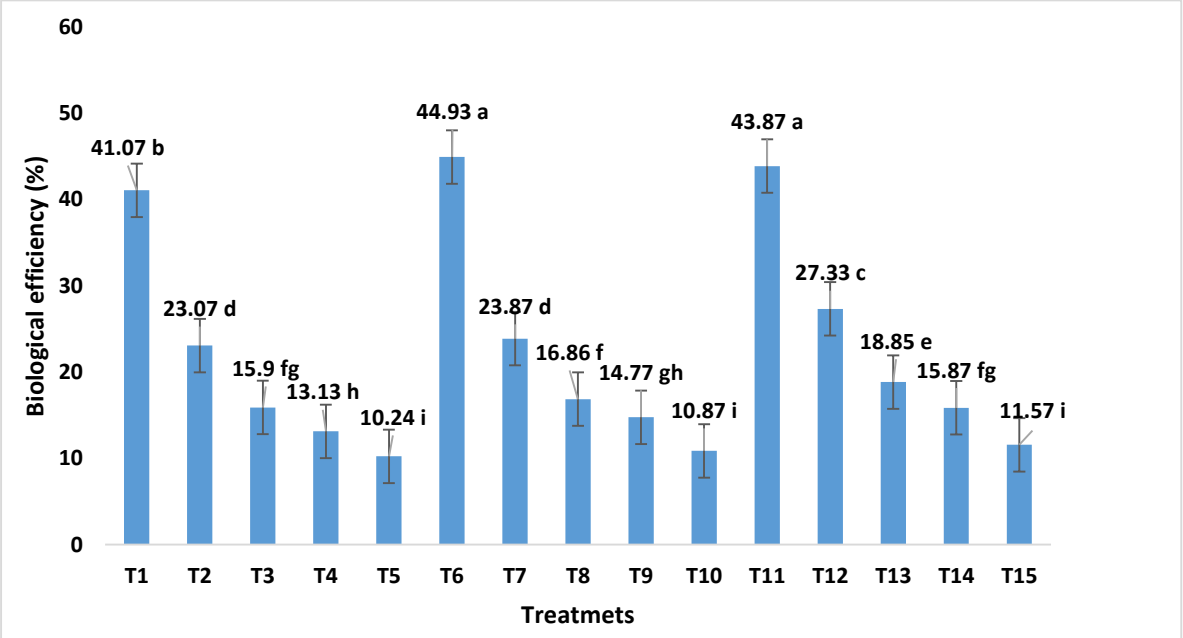


Figure 4. Effect of different amounts and combinations of substrates on biological efficiency (%) of oyster mushrooms ($LSD_{0.052}$ = 2.03)

[**T_1** = 250g waste paper, **T_2** =500g waste paper, **T_3** = 750g Waste paper, **T_4** = 1000g Waste paper, **T_5** = 1500g Waste paper, **T_6** = 250g (75% Waste paper + 25% Sawdust), **T_7** = 500g (75% Waste paper + 25% Sawdust), **T_8** = 750g (75% Waste paper + 25% Sawdust), **T_9** = 1000g (75% Waste paper + 25% Sawdust), **T_{10}** = 1500g (75% Waste paper + 25% Sawdust), = 250g (25% Waste paper + 75% Sawdust), **T_{12}** = 500g (25% Waste paper + 75% Sawdust), **T_{13}** = 750g (25% Waste paper + 75% Sawdust), **T_{14}** = 1000g (25% Waste paper + 75% Sawdust), **T_{15}** = 1500g (25% Waste paper + 75% Sawdust)]

4.4. Benefit cost analysis of different amounts and combinations of substrates

Cost of the every treatment was analyzed for calculation of the cost benefit ratio to determine the optimum amount of substrate. For the purpose, cost of total materials 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 per packet taka 11.5, 13.5, 18, 20, 24.5, 10, 13, 14, 18, 20, 9.5, 11.5, 12.5, 13.5 and 14 respectively, according to sequence of treatments from T_1 to T_{15}. The highest net returns was found in T_{15} (34.6 Tk) followed by T_{14} (33) and the lowest net return was recorded in T_3 (17.40 Tk) treatment. The highest benefit cost ratio (3.47) was found in T_{15} followed by T_{14} (3.44). The lowest benefit cost ratio was recorded in T_5 (1.73).

Table 5. Benefit cost ratio of different amounts and combinations of substrates per packet of mushroom production

Treatments	Total cost per packet (tk)	Economic yield (g)	Economic return per packet (tk)	Net return per packet (tk)	Benefit cost ratio
T_1	11.50	99.33	29.80	18.3	2.59
T_2	13.50	110.67	33.20	19.70	2.45
T_3	18.00	118.00	35.40	17.40	1.97
T_4	20.00	126.67	38.00	18.00	1.90
T_5	24.50	141.67	42.50	18.00	1.73
T_6	10.00	109.67	32.70	22.70	3.27
T_7	13.00	114.33	34.30	21.30	2.64
T_8	14.00	124.67	37.40	23.40	2.67
T_9	18.00	142.33	42.70	24.70	2.37
T_{10}	20.00	152.67	45.80	25.80	2.29
T_{11}	9.50	104.67	31.40	21.90	3.31
T_{12}	11.50	131.00	39.30	27.80	3.42
T_{13}	12.50	139.67	41.90	29.40	3.35
T_{14}	13.50	155.00	46.50	33.00	3.44
T_{15}	14.00	162.00	48.60	34.60	3.47

[**T_1** = 250g waste paper, **T_2** =500g waste paper, **T_3** = 750g Waste paper, **T_4** = 1000g Waste paper, **T_5** = 1500g Waste paper, **T_6** = 250g (75% Waste paper + 25% Sawdust), **T_7** = 500g (75% Waste paper + 25% Sawdust), **T_8** = 750g (75% Waste paper + 25% Sawdust), **T_9** = 1000g (75% Waste paper + 25% Sawdust), **T_{10}** = 1500g (75% Waste paper + 25% Sawdust), = 250g (25% Waste paper + 75% Sawdust), **T_{12}** = 500g (25% Waste paper + 75% Sawdust), **T_{13}** = 750g (25% Waste paper + 75% Sawdust), **T_{14}** = 1000g (25% Waste paper + 75% Sawdust), **T_{15}** = 1500g (25% Waste paper + 75% Sawdust)]

4.5. Relationship between economic yield and different parameters of oyster mushroom

4.5.1. 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 different amounts and combinations of substrates were used (Figure 5). The relationship between economic yield and number of primordia per packet of oyster mushroom could be expressed by the regression equation, $y = 2.2575x + 22.371$ ($R^2 = 0.7596$) where y = economic yield and x = number of primordia per packet. The R^2 value indicated that 75.96% of economic yield of oyster mushroom (*Pleurotus ostreatus*) was attributed to the number of primordia per packet.

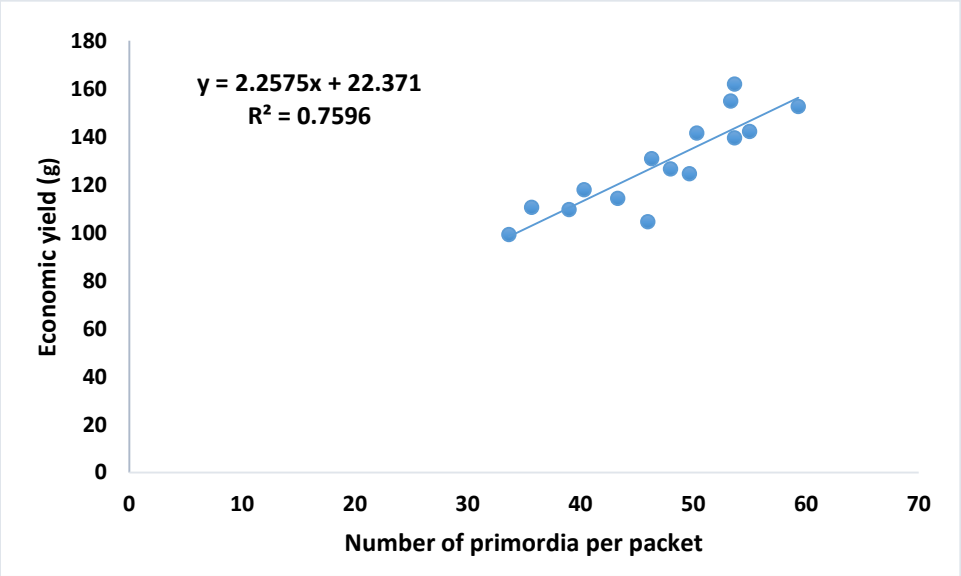


Figure 5. Relationship between the number of primordia per packet and economic yield of oyster mushroom as influenced by different amounts and combinations of substrates

4.5.2. 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 different amounts and combinations of substrates were used (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 = 3.6194x + 86.147 (R^2 = 0.8376) where y = economic yield and x = number of effective fruiting body per packet. The R^2 value indicated that 83.76% of economic yield of oyster mushroom (*Pleurotus ostreatus)* was attributed to the number of effective fruiting body per packet.

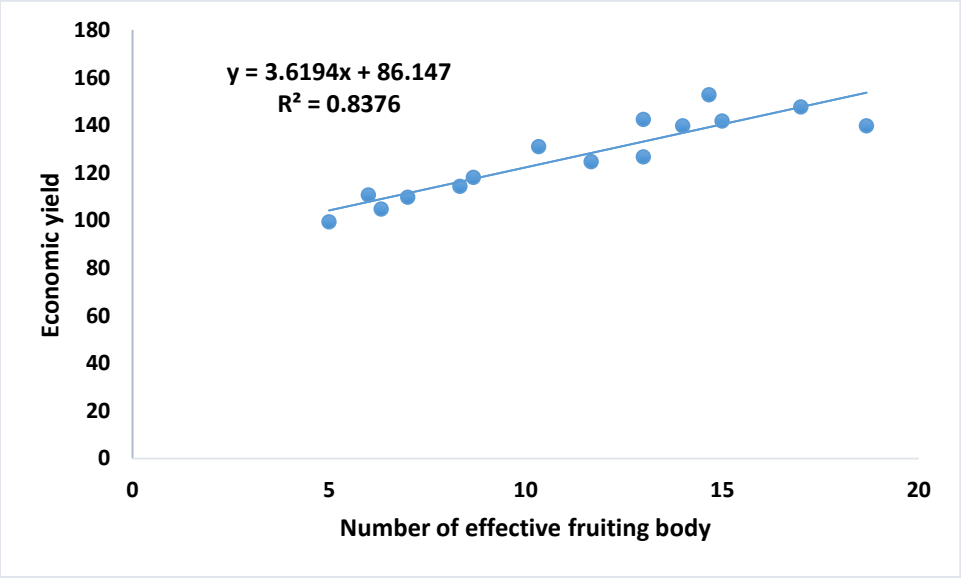


Figure 6. Relationship between the number of effective fruiting bodies per packet and economic yield of Oyster mushroom as influenced by different amounts of substrate

4.5.3. Relationship between biological efficiency and economic yield of oyster mushroom

A highly negative correlation between economic yield and biological efficiency of oyster mushroom when different amounts and combinations of substrates were used (Figure 7). The relationship between economic yield and biological efficiency of oyster mushroom could be expressed by the regression equation, y = -1.2481x + 156.47 (R^2 = 0.5893) where y = economic yield and x = biological efficiency. The R^2 value indicated that 58.93% of economic yield of oyster mushroom (*Pleurotus ostreatus*) was attributed to the biological efficiency.

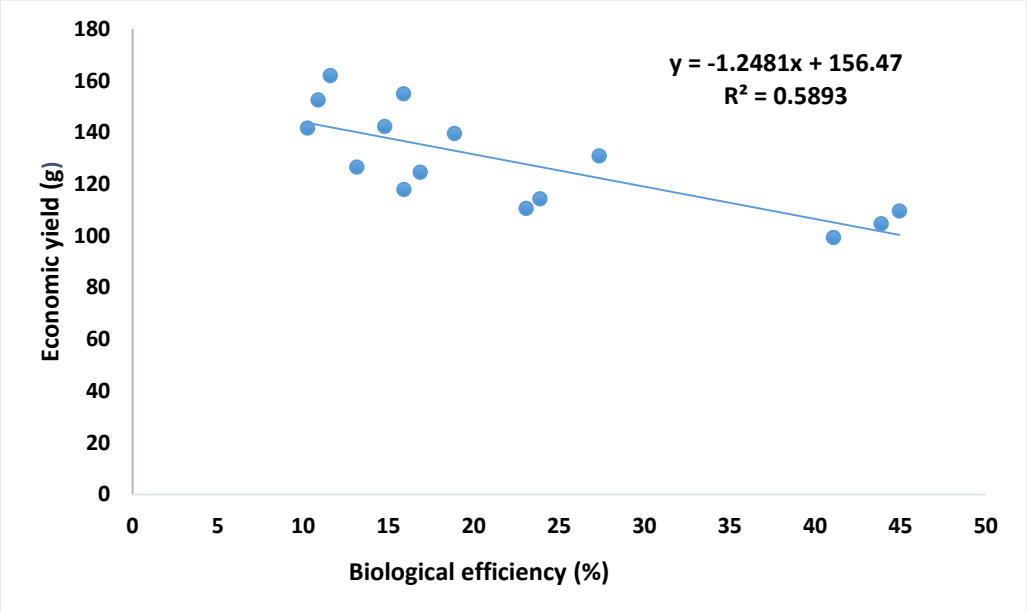


Figure 7. Relationship between biological efficiency and economic yield of oyster mushroom as influenced by different amounts and combinations of substrates

4.6. Effect of different amounts and combinations of substrates on contamination severity

All the spawn packets were kept under observation if there were any contamination. All the packets were free from contamination during incubation but some packets started showing contamination after few days (Table 6). Fifteen (15) days after incubation (DAI), T_{10}, T_{14} and T_{15} showed 3.87%, 4.58% and 3.17% contamination respectively. After thirty (30) DAI, highest contamination severity (17.24%) was in T_{14} and after 45 DAI it was 24.16%. After sixty (60) DAI, T_3, T_5, T_{10}, T_{14} and T_{15} was recorded having contamination. Highest contamination severity (63.76%) was in T_{15} and lowest (29.38%) was in T_5. During total harvesting period T_1, T_2, T_4, T_6, T_7, T_8, T_{11}, T_{12} and T_{13} were completely free from contamination.

Table 6: Effect of different amounts and combinations of substrates on contamination severity of Oyster mushroom

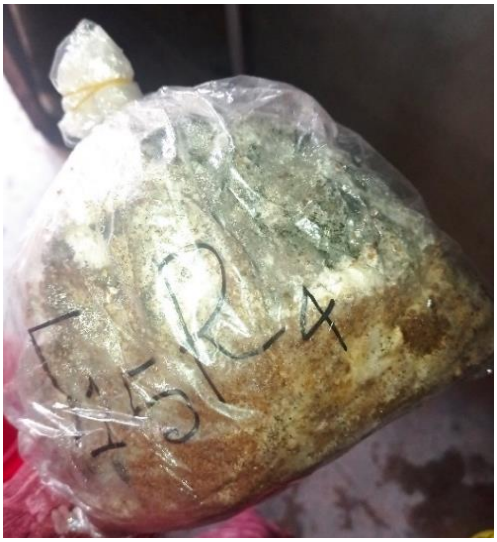
Treatments	Contamination of spawn (%)			
	15 DAI	30 DAI	45 DAI	60 DAI
T_1	0	0	0	0
T_2	0	0	0	0
T_3	0	9.61	21.32	43.86
T_4	0	0	0	0
T_5	0	0	13.36	29.38
T_6	0	0	0	0
T_7	0	0	0	0
T_8	0	0	0	0
T_9	0	0	0	7
T_{10}	3.87	12.83	24.16	47.51
T_{11}	0	0	0	0
T_{12}	0	0	0	0
T_{13}	0	0	0	0
T_{14}	4.58	17.24	26.73	51.47
T_{15}	3.17	11.52	23.81	63.76

DAI= Days after incubation

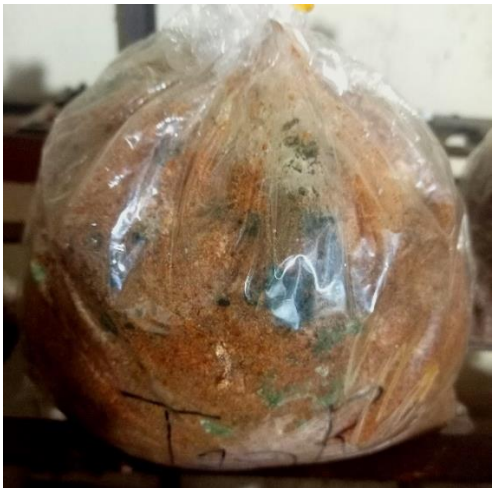
[T_1 = 250g waste paper, **T_2** =500g waste paper, **T_3** = 750g Waste paper, **T_4** = 1000g Waste paper, **T_5** = 1500g Waste paper, **T_6** = 250g (75% Waste paper + 25% Sawdust), **T_7** = 500g (75% Waste paper + 25% Sawdust), **T_8** = 750g (75% Waste paper + 25% Sawdust), **T_9** = 1000g (75% Waste paper + 25% Sawdust), **T_{10}** = 1500g (75% Waste paper + 25% Sawdust), **T_{11}** = 250g (25% Waste paper + 75% Sawdust), **T_{12}** = 500g (25% Waste paper + 75% Sawdust), **T_{13}** = 750g (25% Waste paper + 75% Sawdust), **T_{14}** = 1000g (25% Waste paper + 75% Sawdust), **T_{15}** = 1500g (25% Waste paper + 75% Sawdust) **]**

4.7 Identification of contaminants from contaminated spawn

Molds are the first tool for contaminants identification of contaminated mushroom spawn packets. If green, blue, black or grey patches were spotted in spawn packets and considered as contaminated packets. *Trichoderma sp.*, *Penecillium sp.*, *Rhizopus stolonifer* and *Aspergillus niger* were identified based on their morphological characteristics in spawn packets.



A. Packet contaminated with *Aspergillus sp.* **B.** Packet contaminated with *Penicillium sp.*



C. Packet contaminated with *Trichoderma sp.* **D.** Packet contaminated with *Rhizopus sp.*

Plate 6. Contaminated spawn packets; **A.** Packet contaminated with *Aspergillus sp.* **B.** Packet contaminated with *penicillium sp.* **C.** Packet contaminated with *Trichoderma sp.* **D.** Packet contaminated with *Rhizopus sp.*

4.7.1. *Aspergillus niger*

This fungus produces black mold which is usually fuzzy though initially the colony was whitish in color (plate 7-A). They grow rapidly on culture media. They have a smooth surface. Hyphae were hyaline and septate. Under microscope, smooth and colorless conidiophores and spores were found

(Plate 7-B). A closer look revealed globose and dark brown colored conidial head. The produced conidia was single celled. Stipes having gray color around the apex were seen.

4.7.2. *Penicillium sp.*

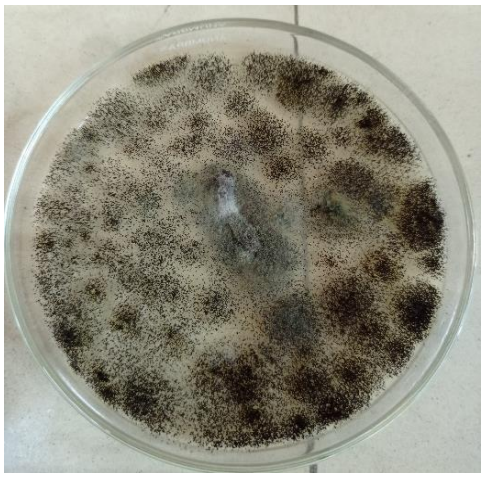
This was characterized by fast growing colonies in shades of green teal with a white outline, mostly consisting of a dense felt of conidiophores (Plate 7-C). Conidiophores are hyaline, smooth or rough walled arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides (Plate 7-D). Conidia are in long dry chains, divergent or in colums, hyaline or brightly colored, single celled produced in basipetal succession from a specialized conidiogenous cell called a phialide. Phialides are usually flask-shaped, consisting of a cylindrical basal part and a distinct neck.

4.7.3. *Trichoderma sp.*

Green molds were observed in spawn packet which was grow into pure culture later. Colonies grew rapidly and matured in 5 days. The colonies were wooly and became compact in time (Plate 8-A).*Trichoderma sp.* 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.

4.7.4. *Rhizopus stolonifer*

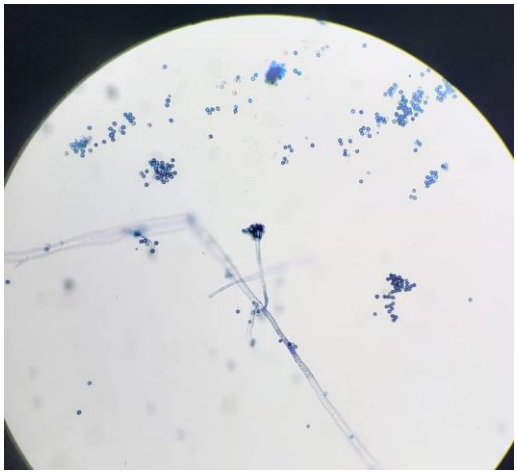
The colony was grayish brown and sort of fuzzy (plate 7). Initially the colony was white. It grows very first and hard to control. Rhizopus develops hair-like sporophores with a tiny head (Plate 8-C). stolon and pigmented rhizoids were found under microscope (Plate 8-D). Sporangia which are bulbous structures that sprout from the vegetative hyphae and hold the haploid spores. Rhizopus has a sour odour. Sometimes, the fungus may also resemble alcohol like smell.



A. Pure culture of *Aspergillus niger*

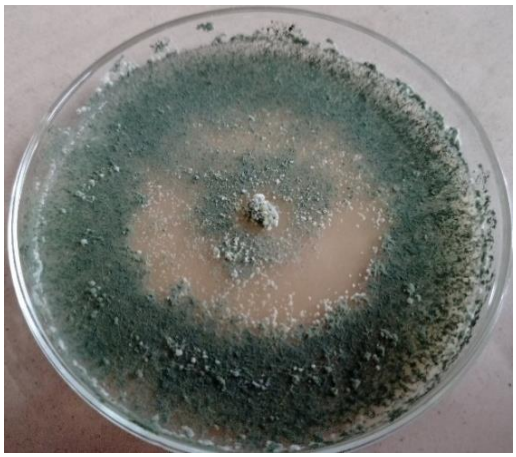
B. Microscopic view of *Aspergillusniger*

C. Pure culture of *Penicillium sp.*

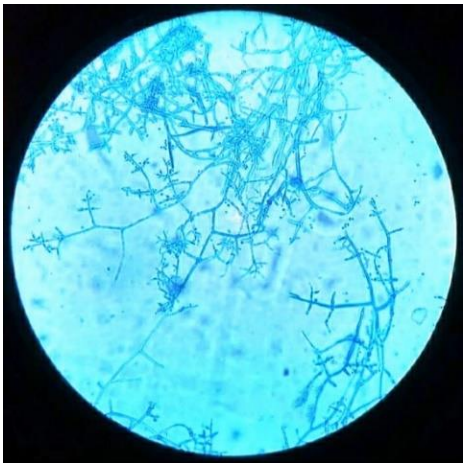


D. Microscopic view of *Penicillium sp.*

Plate 7. Pure culture and microscopic view of contaminants; **A.** Pure culture of *Aspergillus niger*, **B.** Microscopic view of *Aspergillus niger*, **C.**Pure culture of *Penicillium sp*., **D.** Microscopic view of *Penicillium sp.*

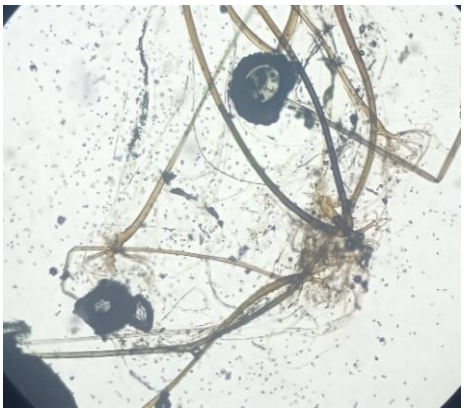


A. Pure culture of *Trichoderma sp.*



B. Microscopic view of *Trichoderma sp.*

C. Pure culture of *Rhizopus stolonifer*



D. Microscopic view of *Rhizopus stolonifer*

Plate 8. Pure culture and microscopic view of contaminants; **A.** Pure culture of *Trichoderma sp.*, **B.** Microscopic view of *Trichoderma sp.*, **C.** Pure culture of *Rhizopus stolonifer*, **D.** Microscopic view of *Rhizopus stolonifer*

CHAPTER V

DISCUSSION

The present study was carried out to evaluate the effect of different amounts and combinations of substrates on the productivity, contamination and cost benefit ratio of oyster mushroom. packets of five different weight (250 g, 500 g, 750 g, 1000 g and 1500 g) were prepared using waste paper supplemented with sawdust at different level (100% waste paper, 75% waste paper supplemented with 25% sawdust and 25% with paper with 75% sawdust) which ultimately resulted fifteen(15) treatments in total. Substrate is the core material of Oyster mushroom cultivation and its amount can matter a lot. Time required from incubation to mycelium initiation was highest (27 days) in T_{12} = 500g (25% waste paper + 75% sawdust) and the lowest (10.67days) in T_5 = 1500g waste paper. Some packets of different sizes showed same result. T_1 = 250 g waste paper and T_2 = 500 g waste paper both took 12 days for mycelium initiation. T_3 = 750g Waste paper and T_4 = 1000 g Waste paper both took 11.67 days. T_{11} = 250 g (25% Waste paper + 75% Sawdust)} and T_{14} = 1000 g (25% Waste paper + 75% Sawdust) both took 25.67 days. Thus, it can be assumed that different amounts of substrates didn't affect days required for mycelium initiation from incubation. The difference can be due to supplementation of waste paper with sawdust at different levels which is similar to the result found by Amin *et al.* (2007). Mycelium running rate ranged from 0.57 to 0.97 cm/day. T_2 = 500 g waste paper was recorder with highest mycelium running rate. T_{12} = 500 g (25% Waste paper + 75% Sawdust), also a packet of 500 g but was recorded with lowest (0.57cm/day) mycelium running rate. Then it can be said that it was not different amounts of substrates which affected mycelium running rate but different combinations. The difference in mycelium running rate can be due to combination of waste paper with saw dust which is similar to the findings of Tesfay *et. al.* (2020) who used waste paper supplemented with corn stalk and wheat bran.

Time required for a ‘‘D’’ cutting the spawn packet to Primordia initiation was the highest (14 days) in T_4 = 1000 g Waste paper and the lowest (7.67 days) in T_7 = 500 g substrate (75% Waste paper + 25% Sawdust). As packet of different sizes showed statistically similar data it can be assumed that there was no effect of different amounts and combinations of substrates on days required for primordia initiation. In case of Primordia initiation to first harvest all the treatment showed statistically same result. Total harvesting period was highest (87 days) in T_{15} = 1500 g (25% Waste paper + 75% Sawdust) and lowest (39.33 days) T_1 = 250g waste paper and T_2 = 500g waste paper. 250g waste paper and 500g waste paper had different amount of substrate but showed indifferent result.

Number of primordia ranged from 33.67 to 59.33. Packets of 1500g was recorded to have higher number of primordia than the packets weighed 250g. It can be assumed that different amounts and combinations of substrates have an effect on number of primordia. Average number of fruiting body ranged from 10.33 to 22.33. Packets of 1500g were recorded to have higher number of fruiting bodies and packets of 250g were recorded with lower number of fruiting bodies. Number of primordia and fruiting body increased with the increase of amount of substrate. The maximum number of effective fruiting body per packet (18.67) was recorded from T_{15} = 1500g (25% Waste paper + 75% Sawdust)} and the minimum average number of effective fruiting body per packet (5.00) was observed in T_1 = 250g waste paper.

Length of a stipe was highest (6.77 cm) in 750g Waste paper and lowest (4.43 cm) in T_{11} = 250g (25% Waste paper+ 75% Sawdust). Other treatments were recorded to have statistically similar stipe length. Highest (8.63 cm) length of pileus was recorded from T_{15} = 1500g (25% Waste paper + 75% Sawdust) and lowest (5.77 cm) was from 250g waste paper. Other treatments have statistically same data. Breadth of fruiting body ranged from 5.67 cm to 11.40 cm. Kivaisi *et al.* (2003) have indicated that the size of the pileus depends on the aeration and amount of light and that may be the case behind the various pileus size. Different amounts and combinations of substrates didn't affect dimension of

fruiting body. The variation can be due to the variation in nutrient factors of multiple substrates which is similar to the finding of Shrestha *et al.* (2021).

Biological yield ranged from 102.67g per packet to 165.33g per packet. Higher biological yields were recorded from packets of 1500g and lower biological yields from packets of 250g. The result was same in the case of economic yield and dry yield. Highest economic yield (162 g per packet) was recorded from T_{15} = 1500 g (25% Waste paper + 75% Sawdust), and lowest (99.33 g per packet) in T_1 = 250g waste paper. Highest dry yield (11.44 g per packet) was from T_{15} = 1500 g (25% Waste paper + 75% Sawdust) and lowest (6.95) from T_1 = 250 g waste paper. Three types of substrate were used for the assessment of most probable accurate result. Yield increased with the increase in amount of substrate and that was true for all the three types of substrate. This finding was similar to the finding of Zireva *et al*. (2007) who used different amounts of substrate from 2 kg to 10kg and yield increased with increase in amount of substrate up to 6 kg substrate. Using lower substrate amount of either 250 g or 500 g resulted in significantly lowered yield compared to substrate amounts of 750 g, 1000 g or 1500 g per packet. Decrease in mushroom yield in lower amount of substrate is most likely attributable to earlier depletion of nutrients with limited amount of substrate as supported by Choi (2003).

Waste paper substrate showed comparatively lower yield than those of supplemented waste paper which is similar to the findings of Tesfay *et al*. (2020).

Biological efficiency ranged from 10.24% to 44.93%. T_6 = 250 g (75% waste paper + 25% sawdust) recorded with highest (44.93%) biological efficiency and T_5 = 1500g waste paper with the lowest (10.24%). T_5 = 1500g Waste paper, T_{10} = 1500 g (75% Waste paper + 25% Sawdust) and T_{15} = 1500g (25% Waste paper + 75% Sawdust) were recorded with statistically similar yield. Biological efficiency (%), the amount of fresh mushroom per unit substrate decreased from 41.07% to 10.24%, 44.93% to 10.87% and 43.87% to 11.57% with the increase in amount of substrate respectively in case of three types of substrate. Lower substrate amount provided with higher BE. Yield increased with the increase in

amount of substrate but that was not much satisfactory. This can be due to substrate utilization by mushroom to produce fruiting bodies and the result is similar to the finding of Zireva *et al*. (2007) who assumed that as substrate quantities increased, substrate utilization decreased, either because space within packet became limiting or other factors, like greater humidity levels within the substrate mass at higher substrate quantities.

Although substrate sterilization was done and other precautionary measures were taken during the experiment four types of contaminants were found namely *Aspergillus niger, Penecillium sp., Trichoderma harzianum* and *Rhizopus stolonifer.* They were isolated from contaminated substrate, pure cultured in PDA media and identified based on their morphology. Occurring in the substrate and competing with mushroom mycelium for space and nutrition are *Aspergillus niger, A. flavus, Alternaria alternata, Drechslera bicolor, Fusarium moniliforme, Mucor sp., Penicillium sp., Rhizopus sp.., Rhizpus stolonifer, Sclerotium rolfsii,, Trichoderma viride* (Sharma et al., 2007; Sharma and Kumar, 2011). There might be an interaction between *Trichoderma sp*. and the mushroom due to the enzymatic action on substrate by mushroom that favors green mold fungal growth which is similar to the findings of Colavolpe *et al*. (2014). *Penicillium* competed 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 just like happened in the experiment carried out by 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 mold, dry bubble, wet bubble, cobweb, etc. (Biswas and Kuiry, 2013; Fletcher and Gaze, 2008). These contaminants deteriorated quality and damaged basidiomycetes ultimately leading to reduced production and sometimes complete failure of the crop like the experiment by Gea *et al*. (2021).

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 during the period from November 2020 to March 2021 to study the effect of different amounts and combinations of substrates on the productivity, contamination, and cost benefit ratio of oyster mushroom (*Pleurotus ostreatus*). The experiment was conducted with fifteen different type treatments which were controlled amount of waste paper alone and mixture of waste paper and sawdust with three replications to achieve the desire objectives. The experiment was laid out in single factor Completely Randomized Design (CRD). The results obtained in the study have been summarized below:

Highest mycelium running rate (0.97 cm/day) was recorded in 500 g waste paper and the lowest mycelium running rate (0.57 cm/day) was recorded in a 500g substrate (25% Waste paper + 75% Sawdust)} packet and a 1000g substrate (25% Waste paper + 75% Sawdust)} packet. The highest time (27.00 days) from incubation to mycelium initiation was found in 500g substrate (25% Waste paper + 75% Sawdust)} and the minimum required time (10.67 days) was recorded in 1500 g Waste paper. The highest time (65.33 days) required for completion of mycelium running was recorded in 1500 g substrate (25% Waste paper + 75% Sawdust)} and the lowest required time (21.67days) was recorded in 500g waste paper. The highest time from ''D'' cutting the spawn packet to primordial initiation (14.00 days) was observed in 1000g Waste paper whereas, the lowest time (7.67 days) from ''D'' cutting the spawn packet to primordia initiation was in 500 g substrate (75% Waste paper + 25% Sawdust). The maximum time (9.33 days) from primordia initiation to harvest was observed in 250 g substrate (75% Waste paper + 25% Sawdust)} whereas, the lowest time (7.33 days) from primordia initiation to harvest was in 750 g Waste paper and 1000 g Waste paper. The highest harvesting period (87 days) was recorded from 1500 g substrate

(25% Waste paper + 75% Sawdust) and the lowest time (39.33 days) was recorded in 250 g waste paper and 500 g waste paper. The maximum average number of primordia per packet (59.33) was observed from 1500 g substrate (75% Waste paper + 25% Sawdust) whereas, the minimum number of primordia per packet (33.67) was found in 250 g waste paper. The maximum number of fruiting body per packet (22.33) was recorded from 1500 g substrate (75% Waste paper + 25% Sawdust), whereas the minimum average number of fruiting body per packet (10.33) was observed in 250g substrate (75% Waste paper + 25% Sawdust). The maximum number of effective fruiting body per packet (18.67) was recorded from 1500 g substrate (25% Waste paper + 75% Sawdust)} and the minimum average number of effective fruiting body per packet (5.00) was observed in 250 g waste paper. The highest length of stipe (6.77 cm) was observed in 750 g Waste paper whereas, the lowest length of stipe (4.43 cm) was found in 250 g substrate (25% Waste paper + 75% Sawdust). The highest length of pileus (8.63 cm) was recorded from 1500 g substrate (25% Waste paper + 75% Sawdust) whereas, the lowest length of pileus (5.77 cm) was found in 250 g waste paper. The highest breadth of fruiting body (11.40 cm) was observed in 1000 g Waste paper. On the other hand, the lowest breadth of fruiting body (5.67 cm) was found in 250 g waste paper. The highest biological yield (165.33 g) was recorded from 1500 g substrate (25% Waste paper + 75% Sawdust) while the lowest biological yield (102.67 g) was recorded in 250 g waste paper. The highest economic yield (162 g) was recorded from 1500g substrate (25% Waste paper + 75% Sawdust) and the lowest economic yield (99.33 g) was found in 250 g waste paper. The highest dry yield (11.44 g) was recorded from 1500g substrate (25% Waste paper + 75% Sawdust), whereas the lowest dry yield (6.95 g) was found in 250 g waste paper. The highest of biological efficiency (44.93%) was observed when 75% of waste paper was added to 25% of sawdust to prepare 250 g substrate, while the lowest (10.24%) biological efficiency was recorded in 1500 g waste paper. The highest net returns (34.6 TK) was found from 1500 g (25% Waste paper + 75% Sawdust) and the lowest net return (17.40 TK) was recorded in 750 g waste paper substrate.

Percent contamination of fungi were gradually increased with the increase of days after incubation. Packets with lower amounts of substrate were free from contamination. After 60 DAI, 1500 g substrate (25% Waste paper + 75% Sawdust) was with highest contamination severity. *Trichoderma sp., Penecillium sp. Rhizopus stolonifer* and *Aspergillus niger* were isolated from contaminated packets, pure cultured in PDA media and identified based on their morphological characteristics.

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

- 1500 g substrate (25% Waste paper + 75% Sawdust) is suitable for oyster mushroom (*Pleurotus ostreatus*) cultivation in Bangladesh based on yield contributing characters.
- During the cultivation of mushroom and four fungi namely *Aspergillus niger, Trichoderma sp., Penecillium sp.*and *Rhizopus stolonifer* were isolated and identified.
- Comparatively less contamination severity was observed in spawn packet with lower amounts of substrate.
- For getting highest benefit cost ratio from oyster mushroom (*Pleurotus ostreatus*) cultivation, 1500 g substrate (25% Waste paper + 75% Sawdust) gave the best result.

CHAPTER VII
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APPENDICES

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

Duration	Average Temperature (^{o}C) of culture house	Average RH (%) of culture house	Average temperature of outside house (^{o}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 (cm/day	Days required from incubation to mycelium initiation	Days required from incubation to completion of mycelium running
Between	14	0.049	103.89	571.39
Within	30	0.008	3.84	10.04
Total	44			

Appendix III: ANOVA table for time required from ‘‘D’’ cutting the spawn packet to primordial initiation, primordial initiation to 1st harvest and total harvesting period

Source of variation	Degrees of freedom	Mean square		
		for time required from ‘‘D’’ cutting the spawn packet to primordia initiation (days)	primordial initiation to 1st harvest (days)	total harvesting period (days)
Between	14	10.68	1.07	681.78
Within	30	3.98	2.91	17.76
Total	44			

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	14	170.041	51.63	54.12
Within	30	15.311	3.53	5.69
Total	44			

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

Source of variation	Degrees of freedom	Mean square		
		Length of stipe (cm	Length of pileus (cm)	Breadth of fruiting body (cm)
Between	14	1.03	2.47	6.99
Within	30	1.55	2.61	4.22
Total	44			

Appendix VI: ANOVA table for yield parameter of Oyster mushrooms

Source of variation	Degrees of freedom	Mean square			
		Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)
Between	14	1129.52	1140.66	5.73	431.40
Within	30	30.64	32.98	0.16	1.48
Total	44				

Appendix VII: Fruiting bodies of oyster mushroom in the spawn packets of different amounts and combinations of substrates