EFFECT OF DIFFERENT TYPES AND AMOUNT OF SUBSTRATES ON CONTAMINATION GROWTH AND YIELD OF PINK OYSTER MUSHROOM *(Pleurotus djamor)*

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JUNE, 2020

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REGISTRATION NO. 18-09049

A Thesis

Submitted to the Faculty of Agriculture

Sher-e-Bangla Agricultural University, Dhaka

In partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE (MS)

IN

PLANT PATHOLOGY

SEMESTER: JAN-JUNE, 2018

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CERTIFICATE

*This is to certify that the thesis entitled '**Effect of different substrates and spawn packet sizes on severity of contamination, growth and yield of pink oyster mushroom (Pleurotus djamor)**' submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in PLANT PATHOLOGY**, embodies the results of a piece of bona fide research work carried out by **SURAYA PERVIN**, Registration No. **18-09049** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated: 20/12/2020
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DEDICATED TO

MY

BELOVED PARENTS, MY

FAMILY & SIBLINGS

ACKNOWLEDGEMENTS

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

The author sincerely desires to express her deepest sense of gratitude and respect to her research Supervisor, ***Dr. Khadija Akhter****, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for her scholastic guidance, untiring effort, valuable suggestions, co-operation, constructive criticisms and for providing necessary facilities and conductive atmosphere to accomplish the research work and the preparation of the manuscript of this thesis.*

The author expresses heartfelt gratitude and indebtedness to her Co-supervisor, **Dr. F. M. Aminuzzaman** *Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his cooperation, criticisms on the manuscript and helpful suggestions for the successful completion of the research work.*

The author wishes to extend her special thanks to Ferdaous Jahan Urmi, Anannya Biswas Soma, for their great support and assistance during experimentation. Special thanks to all other friends for their support and encouragement to complete this study.

The author is deeply indebted to her respectful father, mother, sister and Mokhlesur Rahman Mukul for their moral support, encouragement and unquantifiable love with cordial understanding.

Finally the author also extends her thanks to all the staff of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for their help and co-operation during the research work.

The Author

Effect of different types and amount of substrates on contamination growth and yield of pink oyster mushroom (*Pleurotus djamor*)

BY

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ABSTRACT

An experiment was carried out to find out the effect of different substrates and spawn packet sizes on the growth and yield of pink oyster mushroom (*Pleurotus djamor*) and to estimate the rate of contamination of spawn packets. Among the selected 5 substrates namely rice straw, mustard straw, saw dust, sugarcane bagasse and waste paper, the minimum days (15.43 days) for mycelium running was recorded in mustard straw whereas the maximum days (18.93 days) required for mycelium running was recorded in waste paper. Number of effective fruiting bodies was maximum at rice straw (21.43) and weight of individual fruiting body was highest at waste paper (4.88g). The highest biological yield (83.36g) and biological efficiency (76.76%) was recorded in rice straw, whereas the lowest biological yield (49.14g) and biological efficiency (43.95%) was recorded in waste paper substrate. In case of 250g and 500g spawn packet size, days required for mycelium running, days required for primordia formation and total harvest days was minimum in 250g substrate packet. The number of effective fruiting body, weight of individual fruiting body was maximum in 500g spawn packet. Maximum biological yield (79.44g) was recorded in 500g spawn packet whereas the maximum biological efficiency (65.90%) was recorded in 250g spawn packet. Mustard straw at 250g substrate/packet required minimum time (14.43 days) for mycelium running and primordial formation (5.43 days) whereas waste paper at 500g substrate/packet required maximum time for mycelium running (19.43 days) which was statistically similar with sugarcane bagasse at 500g substrate/packet (19.29 days). Total harvesting day was highest (49.57 days) both in waste paper and saw dust @ 500g substrate/packet. Rice straw at 500g spawn packet gave maximum biological yield (103.14g). whereas rice straw at 250g spawn packet gave maximum biological efficiency (84.76%) followed by rice straw at 500g spawn packet (68.76%) and saw dust 250g spawn packet (68.75%). Total 3 fungi namely *Trichoderma harzianum, penicillium sp. and Aspergillus niger* were isolated and identified. Percent contamination of fungi gradually increased from 1st harvesting to 3rd harvesting stage. Maximum severity of contamination 10.71% was observed in sugarcane bagasse at 500g spawn packet during 3rd harvest.

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LIST OF SYMBOL AND ABBREVIATIONS

ABBREVIATION	FULL WORDS
%	Percentage
PO	*Pleurotus ostreatus*
PDA	Potato Dextrose Agar
DAE	Department of Agricultural Extension
CV	Co efficient of variation
Sp	Species
Temp.	Temperature
e.g.	Exempli gratia (by way of example)
et al.	and others (at ell)
FAO	Food and Agricultural organization
Cm	Centimeter
SAU	Sher-e-Bangla Agricultural University
J.	Journal
@	At the rate of
ml	Milliliter
CRD	Complete Randomized Design
BE	Biological Efficiency
LSD	Least significant difference
df.	Degrees of freedom
g	Gram
ANOVA	Analysis of variances
Kg	Kilogram
MDI	Mushroom Development Institute
hr	Hour

CHAPTER I

INTRODUCTION

Mushroom, a macro fungus with a distinctive fruiting body, belonging to Basidiomycotina, is a unique biota which assembles it's food by secreting degrading enzymes. It decomposes the complex organic materials on which it grows (the substrate) to generate simpler compounds for its nutrition (Chang and Miles 1992). It comprises a large heterogeneous group having various shapes, sizes, appearance and edibility. *Pleurotus* mushrooms, commonly known as oyster mushrooms, grow in the wild in tropical, subtropical and temperate regions and are easily cultivated (Akindahunsi and Oyetayo, 2006). There are about 41,000 different types of mushrooms identified (Manoharachary *et al*., 2005). Among them which have no toxic effect are to be considered as edible mushroom. Out of 2000 species of prime edible mushrooms about 80 have been grown experimentally, 20 cultivated commercially and around 5 are produced on industrial scale throughout the world (Chang and Miles, 1988). The edible mushrooms are grown in controlled, clean environment where there is no infection of other fungi or germs.

Oyster mushrooms (*Pleurotus* spp) are characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. Oyster mushroom can be grown on various substrates including paddy straw, maize stalks, saw dust, vegetable plant residues, bagasse etc. (Hassan *et al*., 2011) and this has been reported to influence its growth, yield and composition (Iqbal *et al*., 2005; Kimenju *et al*., 2009; Khare *et al*., 2010). The most widely used substrates for oyster mushroom cultivation in Asia are rice straw and saw dust (Akhter, 2017; Thomas *et al*., 1998). Using such crop residue as a mushroom substrate would subsequently convert them into a more protein-rich biomass and influence the mushroom yields. Crop residues such as straw are characterized by the predominance of lingo-cellulose with cellulose, hemi-cellulose and lignin as the main components (Yildiz *et al*., 2002; Das and Mukherjee, 2007; Jonathan *et*

al., 2012). There are usually some differences in the nutrient content of the mushroom cultivated on different substrates (Mabrouk and Ahwanyi, 2008; Akinyele *et al*., 2011; Kulshreshtha *et al*., 2013b). However, this changes in nutritional content never found to affect their edibility.

Mushrooms are recognized as the alternate source of good quality protein and arc capable of producing the highest quantity of protein per unit area within a short time from the worthless agro-wastes (Chanda and Sharma, 1995). They are good source of protein, vitamins and minerals. Kovfeen (2004) stated that, the fresh mushroom contains about 85-90% moisture, 3% protein, 4% carbohydrates, 0.3-0.4% fats and 1% minerals and vitamins. It is also a source of Niacin (0.3 g) and Riboflavin (0.4 mg). Mushroom protein is intermediate between that of animal and vegetable and the amount of niacin, pantothenic acid and biotin are of appreciable level. The detrimental cholesterol is absent in mushroom but necessary ergosterol is usually present (Chanda and sharma, 1995). The popularity of oyster mushroom has been increasing due to its ease of cultivation, high yield potential and high nutritional value (Banik and Nandi, 2004). Mushrooms are now-a-days popularly known as functional foods (Liu *et al*., 2009). Edible mushrooms have been treated as an important tool in modern medicine for their medicinal values (Kovfeen, 2004). Mushroom reduces the diabetic on regular feeding and also reduces the serum cholesterol in human bodies which reduces hypertension (Gregori *et al*., 2007).

Mushroom is now-a-days one of the promising concepts for crop diversification in Bangladesh. The climatic condition of Bangladesh is suitable for mushroom cultivation. It doesn't require any cultivable land. It requires short time, little capital and easy technique for cultivation. This is why all types of people like male and female, youth and old even children can easily participate in its cultivation. It's cultivation can transfer as a cottage industry and create a good opportunity for export. Therefore, it can generate huge scope of employment opportunities for

unemployed people. Among various waste materials sawdust and rice straw are most commonly used for mushroom cultivation in Bangladesh now-a-days. The substrates of oyster mushroom are contaminated by various kinds of mycoflora, most of them act as competitor moulds thereby spawn run is adversely affected either by competition for food material or through production of toxic substances (Vijay and Sohi, 1987). During oyster mushroom cultivation, mushroom growers facing various problems especially competitor moulds, which damage the mushroom beds and reduce yield. Studies on various aspects of fungal contaminants and diseases of *Pleurotus spp.* were undertaken by various workers (Akhter, 2017; Mamoun *et al*., 2000; Castle *et al*., 1998) and they reported *Trichoderma harzianum, Aspergillus* sp.,, *Rhizopus, Penicillium sp., Alternaria sp., Ceratocytis sp., Coprinu sp.* and *Chaetomium sp.* were the major contaminants of *Pleurotus sp.* where green mold was detected as the major one. Spillman (2002) recognized *Trichoderma* as green mould on the production bed of oyster mushroom. *Trichoderma*, *Aspergillus*, *Fusarium* and *penicillium* on oyster mushroom bed were predominant microorganisms (Urmi, 2019). The ability to identify and control of contamination helps to protect mushroom crops and these common molds associated with edible mushrooms can be controlled by several treatments or sterilizations of substrates. Though the weather and climate of Bangladesh is quite suitable for year-round oyster mushroom cultivation but the farmers can't cultivate the mushroom due to lack of proper knowledge of sterilization. Without sterilization of substrates, it is not possible to eradicate the contamination of mushroom spawn. So, research should be conducted to investigate the source and causal organisms of the contamination of substrates and proper sterilization method to grow oyster mushroom.

Considering the above facts the present investigation was carried out under the following objectives:

(1) To observe the effect of different substrates on yield and contamination of oyster mushroom

(2) To estimate the rate of severity of contamination on the different amount of substrate

(3) To isolate and identify the contaminants from contaminated spawn of oyster mushroom during cultivation

CHAPTER II
REVIEW OF LITERATURE

Like many other crops, mushroom is also attacked by various diseases right from spawn preparation to maturity. A range of fungi, bacteria and viruses are pathogenic to mushrooms. Mushrooms become contaminated from many sources during production and processing, including the humans harvesting the crop. Mushrooms get spoiled quickly if kept at room temperature for long time. Some of the important and informative works and research findings related to the effect of different substrates on mushroom cultivation, isolation and identification the pathogens infecting spawn of mushroom, and estimation of disease incidence and severity so far been done at home and abroad have been reviewed in this chapter under the following headings-

2.1. Oyster mushroom

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

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

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

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

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

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

2.2. Effect of Substrate on mushroom production

Elattar *et al.* (2019) demonstrated growing oyster mushroom using various agricultural wastes, including wheat straw (W.S), rice straw (R.S), saw dust (S.D) and water hyacinth (W.H), either single or mixed with wheat straw (R.S+W.S, S.D+W.S and W.H+W.S) at a ratio of 1:1 (w/w), in order to determine their significance on growth, composition and consumer acceptance. The experiments were conducted during the winter season (September to December and January to April) 2017/2018 at Agricultural Research., elsabahia, Alexandria, Egypt. Results revealed that, rice straw + wheat straw (R.S+W.S) and single rice straw (R.S) produced the highest mushroom yield from the harvesting periods (7600 g and

6650 g respectively). The product grown on a mixture of rice straw and wheat straw had the highest yield score. It showed to be a rich source of protein, minerals and fibers. It could be approved that oyster mushroom developed on blend of rice straw and wheat straw is nutritious as well as a rich source in pharmaceutical-type products.

Akhter, K. (2017) surveyed that, in case of cultivation of oyster mushroom in rural areas of Bangladesh, rice straw (55.7%) and saw dust (21.6%) was mainly used as substrate. And the yield and biological efficiency was quite satisfactory in these substrates.

Jegadeesh *et al.* (2018) found that Cultivation of the oyster mushroom, *Pleurotus spp*., has increased greatly throughout the world and commonly grown on pasteurized agro wastes. It can be cultivated on a wide variety of lignocellulosic substrates, enabling it to play an important role in managing organic wastes whose disposal is problematic. Mushroom cultivation is a simple, low cost and environmentally friendly technology for the utilization of rural and agro-industrial residues. The substrate used for the cultivation of one such species is pink oyster mushroom, *Pleurotus djamor var. roseus*, which is becoming important as this is an unfamiliar edible mushroom and can be cultivated easily throughout the year. In the present study different substrates viz. paddy straw, sugarcane bagasse, coir pith, sorghum straw, ragi straw and mixed bed were used for the cultivation of pink oyster mushroom. The selected substrates were chopped into 5 cm long pieces and soaked in clean tap water for 12 h. The pre-soaked straw was sterilized for 30 min at 15 lb/sq inch pressure. After cooling the substrate, a handful of spawn of *P. djamor var. roseus* was inoculated in perforated polypropylene bags (15 cm x 25 cm) row by row until it covers the whole size of the bag. The inoculated bags were incubated under dark for 12-14 d with the humidity range at 80-90% for mycelial formation. Primordium initiation

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

Onyeka *et al.* (2018) investigated the effect of substrate (medium) on growth, yield and nutritional composition of domestically-grown oyster mushroom (*Pleurotus ostreatus*). Six different substrates namely sawdust only (SDO), sawdust + corn waste + $CaCO_3$ (SDW), sawdust + rice bran + $CaCO_3$ (SDR), sawdust + banana leaves (SBL), sawdust + cassava peel (SDC) and cassava peel only (CPO) were used. The substrates were pasteurized with hot water (90°C for 4 h) before spawns of oyster mushroom were inoculated to them. After inoculation, the substrates were kept in a controlled environment until fruiting took place. The SDC substrate gave the highest number (22) of fruiting body, highest yield (463 g/kg) and best biological efficiency (46.30%). This was followed closely by the harvest from SDR substrate. The differences in the nutrient composition of mushroom from the different substrates were significant at 0.05 % confidence level. Harvest from SDR contained higher vitamins and minerals compared to others. Harvest from CPO substrate had the lowest (20.10%) protein content as well as other nutrients. SDC and SDR substrates are considered good for domestic cultivation of oyster mushroom.

Garuba *et al*, (2017) investigated the influence of agro-wastes as substrates on the nutritional quality of *Pleurotus pulmonarius* and *Pleurotus ostreatus*. Cassava peels, banana leaves and amended sawdust (sawdust mixed with rice bran in ratio 4:1) were used as growth substrates. Proximate and mineral analyses were carried out using DA 7250 NIR Analyzer and Atomic Absorption of Spectrophotometer machine (AA320N). Both species in amended sawdust had the highest stipe length and pileus diameter of the fruiting body. Starch was the most abundant proximate constituent in the two species. The starch was the most

abundant proximate constituent in the two species but highest starch contents were observed in. *P. pulmonarius* grown in cassava peels substrate and *P. ostreatus* raised in banana leaves substrate. Fat appeared to be the lowest proximate constituent in the two species. Potassium was predominant among the minerals in both *P. pulmonarius* and *P. ostreatus* and the highest value (68.204 mg/L) was observed in *P. ostreatus* cultivated in amended sawdust.

Girmay *et al.* (2016) reported mushroom cultivation as an economically viable bio-technology process for conversion of various lingo-cellulosic wastes. Given the lack of technology know-how on the cultivation of mushroom, this study was conducted in Wondo Genet College of Forestry and Natural Resource, with the aim to assess the suitability of selected substrates (agricultural and/or forest wastes) for oyster mushroom cultivation. Accordingly, four substrates (cotton seed, paper waste, wheat straw, and sawdust) were tested for their efficacy in oyster mushroom production. Pure culture of oyster mushroom was obtained from Mycology laboratory, Department of Plant Biology and Biodiversity Management, Addis Ababa University. The pure culture was inoculated on potato dextrose agar for spawn preparation. Then, the spawn containing sorghum was inoculated with the fungal culture for the formation of fruiting bodies on the agricultural wastes. The oyster mushroom cultivation was undertaken under aseptic conditions, and the growth and development of mushroom were monitored daily. Results of the study revealed that oyster mushroom can grow on cotton seed, paper waste, sawdust and wheat straw, with varying growth performances. The highest biological and economic yield, as well as the highest percentage of biological efficiency of oyster mushroom was obtained from cotton seed, while the least was from sawdust. The study recommends cotton seed, followed by paper waste as suitable substrates for the cultivation of oyster mushroom.

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

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

Ashraf *et al*. (2013) conducted an experiment to compare the effect of different agricultural wastes on growth and yield of mushroom production, three species of *Pleurotus* viz. *P*. *sajor-caju* (V1), *P*. *ostreatus* (V2), and *P*. *djmor* (V3) were grown on three different substrates cotton waste (T1), wheat straw (T2) and paddy straw (T3). The fastest spawn running, primordial initiation, harvesting stage, maximum number of fruiting bodies and maximum yield was observed in T1 took minimum number of days T3 showed maximum yield in 1st flush showing no significant differences with treatment T1 whereas T1 took maximum yield in 2nd flush and 3rd flush. *P. djmor* showed the highest percentage of dry matter (17.23%) and moisture content was found high in *P. sajor-caju* (87.37%). *P. ostreatus* and *P. sajor-caju* showed the maximum protein (27.23%) and fiber (26.28%) contents. The ash contents were found maximum *P. sajor-caju* (9.08%).The highest fat and carbohydrate contents were found in *Pleurotus djmor* (3.07%) and *P*. *djmor* (37.69) respectively.

Sonali (2012) conducted an experiment to study the growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis. The development of Oyster mushroom (Grey and pink) production methodologies on agricultural waste like Paddy straw and wheat straw gave very high yield as well as the nutritional content like carbohydrate, protein, ash, calcium, magnesium, crude fibers and lipid were checked.

Siqueira *et al.* (2011) used banana stalks and Bahia grass as basic starting materials for the production of the mushroom *Pleurotus sajor-caju*. Banana stalks were combined with other waste or supplement products (wheat bran, coast-cross hay, bean straw and cotton textile mill) to obtain different nitrogen concentrations. Since Bahia grass is relatively rich in protein, it was combined with other substrates (banana stalk, coast-cross hay and bean straw) to maintain a substrate nitrogen concentration of about 1.5%. Banana stalks and Bahia grass were both more efficient in the production of the mushroom *P. sajor-caju* when utilized

without the addition of other substrates, with biological efficiencies of 74.4% and 74.12%, respectively. When combined with other substrates or grasses, there was a drop in biological efficiency, independent of the concentration of nitrogen.

Fatema *et al.* (2011) found that the best response in the form of pin head appearance and productivity of mushroom came from the bags containing wheat straw only (3.1 kg), followed by the 3:1 combination of wheat straw and water hyacinth (2.6 kg), 1:1 combination of wheat straw and water hyacinth (1.9 kg), 1:3 combinations of Wheat straw and water hyacinth (1.5 kg) and only water hyacinth (0.77 kg), where respectively it took 16, 20, 25, 30 and 40 days for the appearance of pin heads.

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

Kumari and Achal (2008) conducted an experiment to investigate the effect of five different substrates viz. paddy straw, wheat straw, mixture of paddy and wheat straw (in the ratio of (1 : 1), bamboo leaves and lawn grasses on the production of Oyster mushroom (*Pleurotus ostreatus*). Wheat straw and a mixture of paddy and

wheat straw gave the earliest colonization of fungus. The highest yield of *P. ostreatus* was recorded on wheat straw (29.27 g fresh weight/kg substrate), followed by the combination of paddy and wheat straw (27.96 g fresh weight/kg substrate) and dry fruit body (5.93 mg/g) of *P. ostreatus*.

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

Bhatti *et. al*. (2007) carried out experiment on the growth, development and yield of oyster mushroom, as affected by different spawn rates. The oyster mushroom, *Pleurotus ostreatus* (Jacq. ex. Fr. Kummer) was cultivated on wheat straw in polythene bags (containing 500 g wheat straw on dry weight basis per bag) using sorghum grain spawn at different rates. The spawning was done followed by boiling of substrate and sterilization of bags. The minimum period of 4.66 days after pinhead formation for maturation of fruiting bodies was recorded by using 60, 70, 80, 90 and 100 g spawn rate. The pinheads first appeared 32.33 days after spawning, the maximum number of fruiting bodies per bunch (7.30), the maximum flushes (4.00), the maximum yield on fresh weight basis (45.4%) as well as on dry weight basis (4.63%) was also obtained by using 70 g spawn rate per kg on substrate dry weight basis. It was concluded that spawning at 70 g per kg on substrate dry weight basis found to be the best dose for spawning.

Sarker *et al*. (2007 a) revealed the performance of different cheap agricultural household by products, grasses and weeds as substrate available in Bangladesh. Mycelium growth rate and time required to complete mycelium running in spawn packet varied significantly in different substrates. The minimum duration to complete mycelium running was 17.75 days in waste paper, which differed significantly from all other substrates. The minimum duration required from stimulation to first harvest was observed in sugarcane bagasse (6.75 days), which was statistically identical to that in waste paper, wheat straw and sawdust (7.00 days). The highest number of fruiting body per packet (183.25) was recorded on waste paper, which was significantly higher as compared to all other substrates. The lowest number of fruiting body (19.25) was observed in water hyacinth. The highest economic yield (225.43 g/packet) was estimated from the waste paper followed by wheat straw (215.72 g/packet).The economic yield on sugarcane bagasse was 191.98g/packet, which was statistically identical to that grown on rice straw (183.28 g/packet), kash (182.93 g/packet) and ulu (175.15g /packet).The economic yield on sawdust was 160.40g/packet, which was statistically identical to that on ulu. The lowest economic yield was observed in water hyacinth (33.59g/packet). No fruiting body and economic yield were obtained from para and napier grasses.

Namdev *et al.* (2006) conducted a study to determine the effect of different straw substrates on spawn growth and yield of oyster mushroom. The number of days required for spawn running was significantly less (14 days) in case of gram straw, sugarcane straw and wheat straw, compared with 20 days for sunflower stalk, mustard straw and paddy straw. Yield was very poor on parthenium straw (95 g/500 g dry substrates) and it was highest on paddy straw (666 g/500 g), followed by wheat straw and mustard straw (427 and 400 g/500 g respectively).

Ramjan (2006) found that mustard straw performed best as a substrate for the production of fruiting bodies of oyster mushroom.

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

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

Iqbal *et al.* (2005) carried out an experiment to find out the growth and yield performance of oyster mushroom, *Pleurotus ostreatus* (local & exotic strains) and *P. sajar caju* on different substrates. Results regarding the time required for completion of spawn running, formation of pin-heads and maturation of fruiting bodies on different substrates showed that in all the three cases, they appeared earlier on sugarcane bagasse followed by cotton waste and the maximum number of flushes were obtained from wheat straw and banana leaves. Furthermore, it was found out that the minimum flush to flush interval was obtained on millet followed by wheat straw and sugarcane leaves and the maximum yield percentage on fresh and dry weight basis was obtained from banana leaves followed by paddy and wheat straw.

Amin (2004) revealed that the highest number of primordia of oyster mushroom was found in sterilized paddy straw at first flush; whereas the lowest was obtained with saw dust.

Banik and Nandi (2004) conducted an experiment on oyster mushroom for its ease of cultivation, high yield potential as well as its high nutritional value. Laboratory experimentation followed by farm trial with a typical oyster mushroom *Pleurotus sajor- caju* revealed that the yield potential, protein and mineral nutrient contents of *Pleurotus sajor caju* mushroom in Indian subcontinent can be increased significantly when grown on a lignocellulosic crop residue - rice straw supplemented with biogas residual slurry manure in 1:1 ratio as substrate.

Maniruzzaman (2004) utilized wheat, maize, rice and sawdust for the production of spawn in oyster mushroom and found that substrate rice was the best for spawn production of oyster mushroom.

Shah *et al.* (2004) investigated the performance of Oyster mushroom on the following substrates: 50 % sawdust + 50 % wheat straw, 75 % sawdust + 25 % leaves, 50 % wheat straw + 50 % leaves, 100 % sawdust, 100 % wheat straw and 100 % leaves. The results showed that spawn running took 2-3 weeks after inoculation, while small pinhead-like structures formed 6-7 days after spawn running. The fruiting bodies appeared 3-6 weeks after pinhead formation and took 27-34 days later after spawn inoculation. Sawdust at 100 % produced the highest yield (646.9 g), biological efficiency (64.69 %) and the number of fruiting bodies (22.11). Therefore, sawdust is recommended as the best substrate for Oyster mushroom cultivation.

Obodai *et al.* (2003) carried out an experiment on eight lingo-cellulosic by-products as substrate, for cultivation of the oyster mushroom. The yields of mushroom on different Substrates were 183.1, 151.8, 111.5, 87.5, 49.5, 23.3, 13.0 and 0.0 g for composted Sawdust of *Triplochiton scleroxylon*, Rice straw, Banana leaves, Maize stalk, Corn husk, Rice husk, Fresh Sawdust and Elephant grass

respectively. The biological efficiency (BE) followed the same pattern and ranged from 61.0%, for composted Sawdust to 50.0% for elephant grass. The Yield of mushroom was positively correlated to cellulose (r2 = 0.6). Lignin (r2 =0.7) and fiber (r2 = 0.7) contents of the substrates. Based on the yield and BE of the substrates tested, rice straw appeared to be the best alternate substrate for growing oyster mushroom.

Dhoke *et al.* (2001) revealed the effect of different agro-wastes on cropping period and yield of *Pleurotus sajor-caju.* Various plant materials, i.e. soybean, paddy, cotton, wheat and jowar (*Sorghum bicolor*) were used. Cropping period on different substrates was recorded for first, second and third picking. The cropping period for third picking varied from 42.25 to 43.50 days in different substrates. The days required for first picking indicated that soybean straw took 22.00 days to produce first crop of harvestable mushroom while a minimum of 21.25 days were required for paddy and wheat straw. For second picking, jowar and cotton waste took the maximum days of 32.75 days while soybean took the minimum of 31.50 days. The final and third picking was completed in 43.50 days in case of soybean straw which was statistically higher compared to paddy and wheat straw (42.25) and cotton and jowar straw (42.75). The highest yield of 993.00 g/kg was obtained from cotton, followed by soybean straw (935.25 g/kg) and paddy straw (816.0 g/kg). The lowest yield of 445.50 g/kg was recorded in jowar straw.

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

Patil and Jadhav (1999) found that *Pleurotus sajor-caju* was cultivated on cotton, wheat, paddy, sorghum and soybean straws. Cotton stalks + leaves was the best substrate for production (yield of 1039 g/kg dry straw), followed by soybean straw (1019 g/kg). Paddy and wheat straw yielded 650 and 701g/kg. The lowest yield

(475 g/kg) was obtained on sorghum straw. Pileus size and stipe length of *P. sajor-caju* were greatest on sorghum straw.

Chowdhury *et al.* (1998) conducted a study to examine the effects of adding rice husks, soybean meal, pea meal, wheat bran, poultry manure or neem cake (each at 2 or 5%) to rice straw for growing oyster mushrooms (*P. sajor-caju*). Adding 5% soybean or pea meal gave the highest yield of 630 g/kg dry straw.

Zhang-Ruihong *et al.* (1998) cultivated oyster mushroom (*P. sajor-caju*) on rice and wheat straw without nutrient supplementation. The protein content of mushrooms was recorded 27.2% on an average. The dry matter loss of the substrate after mushroom growth varied from 30.1 to 44.3%. Yields were higher from substrates which had been ground-up to 2.5 cm lengths; further size reductions lowered yields. Mushroom cultivation is a highly efficient method for disposing of agricultural residues as well as producing nutritious human food.

According to Biswas *et al.* (1997) methods including spawning percentage, combinations of paddy straw, wheat straw and supplements, to improve the biological efficiency (BE) of *P. florida* were investigated in Madhya Pradesh, India. Increasing spawning rates reduced the time required for spawn runs. The highest BEs (66.8-101.25%) was observed after the use of the highest spawning percentages. A 1:1 mixture of paddy straw wheat straw promoted a high BE (106.5%); supplementation of this substrate with 5% rice flour also promoted BE (125.75%).

Jadhav *et al.* (1996) found that oyster mushroom (*Pleurotus sajor-caju*) was cultivated on wheat straw, paddy straw, and leaves of maize or cotton, jowar, soyabean straw, groundnut creepers plus wheat straw (1:1), soybean straw plus groundnut creepers (1:1), or groundnut creepers alone. Cotton stalks and leaves gave the best results with respect to sporophore number, weight of sporophore (5.12 g) and total yield (914 g/kg of dry straw). Yields obtained on other substrates

were: 796 g on paddy straw; 557 g on soyabean straw; and 508 g on soyabean + wheat straw. The lowest yield was recorded on groundnut creeper (258 g).

Mathew *et al.* (1996) carried out an experiment where *Pleurotus sajor-caju*, *Pleurotus* citrinopileatus, *Pleurotus florida*, *Pleurotus platypus* and *Pleurotus ostreatus* were evaluated for their yield performance on various substrates, both for spawn production and cultivation, in the plains and in the high ranges of Kerala in studies conducted in the summer and rainy seasons. Sorghum, wheat and paddy grains were equally good for spawn production. *Pleurotus sajor-caju* and *Pleurotus florida* were the most suitable species for cultivation in both the plains and the high ranges. These 3 species were successfully cultivated on paddy straw, *Eliocharis plantogena* [*Eleocharis plantaginea*] and rubber wood [Hevea] sawdust, although for commercial cultivation of *Pleurotus sajor-caju*, rubber wood sawdust was not rated as an ideal medium.

Singh *et al.* (1995) revealed that the *Pleurotus florida* was cultivated on wheat straw, paddy straw and sugarcane trash (dried leaves) used either separately or in 1:1 ratio, yield and biological efficiency were the highest in paddy straw.

Patil (1989) cultivated *P. sajor-caju* on six different substrates, i.e. wheat straw, bajra (*Pennisetunz americana*), maize straw, paddy straw, jower and cotton stick and found that all the substrates could be used for commercial cultivation of the oyster mushroom.

Chang and Miles (1988) revealed that substrate is an important item for growing mushroom. It is a kind of media which supports the growth, development and fruiting of mushroom.

2.3. Contamination of spawn

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

Aspergillus niger. She also found that, Percent contamination of fungi gradually increased from 1st stage to 3rd flash stage. Maximum severity of contamination 68% was observed in control and 36% in ash treated substrate at 3rd harvest.

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

Kumar *et al*. (2017) conducted a survey on ten home scale mushroom farms of Thanjavur its nearby areas, Tamilnadu, India and survey revealed that the occurrence of eight contaminants in mushroom beds and out of which *Trichoderma viride*, *Aspergillus niger*, *Coprinus* sp. were found to be the dominant fungal contaminants and occurrence was high during may to July (23.5-26.7%) causing maximum loss to mushroom yield. The incidence of contaminants were minimum during December and January (3.60%) and maximum during the month of May (26.5%). A good harvest of mushroom (107% Biological Efficiency) was obtained during the month of October. A range of average maximum temperature (23.5-34.60C), minimum temperature (13.4-24.20C) was found most appropriate for the cultivation of oyster mushroom in this region.

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

Biswas (2014) experimented the occurrence of seven contaminants in mushroom beds and out of which *Trichoderma harzianum, Penicillium notatum, Sclerotium rolfsii* and *Coprinus* spp. were found to be most dominant fungal contaminants and occurrence was high during June and July (28.4 & 35.8 %) causing maximum loss to mushroom yield.

According to Kim *et al*. (2002) fungal pathogens caused severe damage to the commercial production of *P. eryngii*. Four strains of pathogenic fungi, including *T. koningiopsis* DC3, *Phomopsis* sp. MP4, *Mucor circinelloides* MP5, and *Cladosporium bruhnei* MP6, were isolated from the bottle culture of infected *P. eryngii*.

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

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

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

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

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

According to Wickremasinghe (1999) the frequency of contaminants and *T. harzianum* occurrence was 100% irrespective of the stage of processing of straw and oyster compost.

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

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

2.4. Sterilization of substrate

Gowda and Manvi (2019) found that agro residues have effectively been utilized as a substrate in mushroom cultivation. The substrates used in cultivating edible mushroom requires varying degree of pre-treatments in order to promote the growth of mushroom mycelium by excluding other competitor microorganisms. Sterilization using hot water, steam or chemical methods is done to disinfect the substrate. However, sterilization is not an ideal disinfection method as it kills both beneficial and harmful organisms in the substrate. Pasteurization of the substrate seems to be the better alternative which permits re-growth of beneficial organisms

during the cooling period. This paper presents an outline on the utilization of agro residues as substrate in mushroom cultivation. Various methods of chemical, non-chemical sterilization and pasteurisation of substrate have been discussed. It also highlights that more focus is needed to promote mushroom growers to adopt the pasteurization technique than sterilization by developing simple and low cost pasteurization equipments for rural and small scale mushrooms growers.

Akhter, K., (2017) observed that pasteurization of small bags (500g) for 3 hours were found most ideal for less contamination for oyster mushroom. Efficiency of steam pasteurization and autoclaving were compared at different time duration to control substrate contamination and the result showed that highest growth rate, better yield performance and the highest biological efficiency (66.01%) with minimum contamination were observed while packets steam pasteurized for three hours. Again, performance parameters such as, economic yield and BE was found while packet treated with 80◦C for 2 hours. As such pasteurization of rice straw through steam in steel drum for 3 hours in lieu of other treatments namely hot water treatment, chemical treatment or autoclaved treatment would be a promising technique for substrate pre-treatment that can be adopted to produce a good yield of oyster mushroom in most rural areas, where autoclave sterilization may not be feasible.

Oseni *et al.* (2012) experimented on substrate pre-treatments by autoclaving at 121°C, hot water dipping (pasteurization) in steel drum at 60°C for 2 h and hot water dipping (pasteurization) in steel drum at 60°C for 3 h. The percent contamination was significantly higher in horse manure compost (70%) compared to sugarcane bagasse (12.5%). The oyster mushroom took significantly less time to colonize the autoclaved sugarcane bagasse (36 days) compared to sugar cane bagasse pasteurized for 2 h (64 days). Autoclaved horse compost manure was fully colonized in 42 days, while those pasteurized with hot water at 2 and 3 h failed to colonize due to heavy contamination by *Trichoderma harzianum* presumably due

to insufficient sterilization. Despite the s hottest days to full colonization, there was no significant difference in the yield (410.4 g) and bio-efficiency (82.10%) of autoclaved sugarcane bagasse compared to the yield (301.1 g) and bio-efficiency (60.22%) of sugarcane bagasse pasteurized in hot water for 3 h.

Khan *et al.* (2011) carried out an experiment to investigate different sterilization methods viz., Lab autoclave, Country style autoclave (2hr), Country style autoclave (1hr), Hot water treatment (1/2hr) and Ordinary water (1/2 hr). Oyster mushroom was cultivated on saw dust, wheat straw, and rice husk with different treatments which included, wheat straw 50 %+saw dust 50%, saw dust 100 %, wheat straw 50% + rice husk 50% and rice husk 100%. Among the sterilization methods, the significantly effective method was lab autoclave followed by others. It was observed that the *Pleurotus ostreatus* (P-19) gave the maximum yield in the first flush followed by second, third and fourth flush and lab autoclave was recommended one of the best method for the yield improvement of *Pleurotus spp.*

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

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

Moda *et al*. (2005) conducted a study of cultivation of *P. sajor caju* whether traditional composting and pasteurization processes could be replaced by washed and supplemented (mineral or organic) sugarcane bagasse. In one experiment, fresh sugarcane bagasse was immersed in hot water at 80°C for two hours (control). In another experiment, fresh sugarcane bagasse was washed in fresh water (control), and supplemented with corn grits (organic), or supplemented with nutrient solution (mineral). In the first experiment, the washed bagasse presented a

average biological efficiency (ABE) of 19.16% with 44% contamination, and the pasteurized bagasse presented a ABE of 13.86% with 70% contamination. In the second experiment, corn grits presented the poorest performance, with a ABE of 15.66% and 60% contamination, while supplementation with the nutrient solution presented a ABE of 30.03%, whereas the control of 26.62%.

2.5. Meteorological factors

Biswas (2016) surveyed and found that Microbial contamination of oyster mushroom bed is one of the major hindrance in increased yield of *Pleurotus* spp. under the agro-ecological condition of undulating red and lateritic belt of West Bengal, which was maximum during the rainy season.

Uddin *et al*. (2011) investigated the production of four species of oyster mushroom: *Pleurotus ostreatus*, *P. florida*, *P. sajor-caju* and *P. high king* cultivated in every season (January to December) in Bangladesh. The temperature (in ◦C) and relative humidity (% RH) of culture house of each month and parameters of mushroom production were recorded. In all of the selected species of this study, the minimum days required for primordial initiation. The maximum number of fruiting bodies, biological yield and biological efficiency were found during December to February (14-27 ◦C, 70-80% RH). The production was found minimum during the cultivated time August to October. They suggested cultivation of selected *Pleurotus spp*. in winter (temperature zone 14-27 ◦C with relative humidity 70-80%) for better production and biological efficiency.

Sher *et al*. (2010) conducted a study with the objective to examine the suitability of Oyster mushroom cultivation and to compare the growth and yield of Oyster mushroom in two different areas (Peshawar and Swat, North-West region of Pakistan) with different ecological conditions. Stalk height, stalk diameter, cap size and fresh weight of mushrooms were found higher in Peshawar region as compared to those growing in Swat region. On the other side, the spawn running

time, formation of fruiting bodies and the number of productions were higher in Swat region as compared to the mushroom under study in Peshawar region. Mild winter temperatures at Peshawar region and low summer temperatures in Swat, which were found most suitable for growth and yield of *Pleurotus ostreatus*.

Jaivel *et al*. (2010) found that *Trichoderma, Aspergillus* and *Rhizopus* on oyster mushroom bed were predominant microorganisms and especially occurrence of these was severe in summer and spring seasons than autumn and winter. Microbial contamination of oyster mushroom bed is one of the major hindrance in increased yield of *Pleurotus spp*. under the agro-ecological condition of undulating red and lateritic belt of West Bengal, which was maximum during the rainy season.

CHAPTER III

MATERIALS AND METHODS

The experiment was carried out to find out the growth and yield of pink oyster mushroom (*Pleurotus djamor*) grown on different substrates like paddy straw, sawdust, waste paper, mustard straw, sugarcane baggase and an examination was made to isolate and identify different weed fungi associated with colonized substrate of oyster mushroom. This chapter deals with a brief description on location and design of experiment, preparation of substrates, preparation of packets, cultivation of spawn packets, collection of produced mushrooms, data recording and their analysis under the following headings and sub-headings.

3.1 Experiment site

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

3.2 Duration of the experiment

The experiment was carried out during the period from February to May, 2019

3.3 Spawn production

3.3.1 Collection of materials for spawn production

Rice straw, mustard straw and sugarcane bagasse were collected from the farm of Sher-e-Bangla Agricultural University. Waste paper and sugarcane bagasse were collected from local market and mother culture of *Pleurotus djamor*, neck and 7×10 inch polypropylene bag @ 500g and 250g were collected from Mushroom Development Institute, Savar, Dhaka.

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

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

3.3.3 Design and layout of the experiment

The experiment was laid out in a 5x2 factorial experiment in completely randomized design (CRD), with 7 replications. The first factor was substrate with 5 levels (rice straw, saw dust, waste paper, sugarcane bagasse and mustard straw), the second factor was amount of substrate packet with 2 levels (250g and 500g).

3.3.4 Preparation of the substrate and spawn packets

The trial was started on 15 February, 2019. At first, straw, mustard straw, sugarcane bagasse and waste paper were chopped to 2-3 cm size was stored under a covered shade and dipped in water for overnight before substrate preparation. Then the substrates hanged within a net bag for 60 minutes to drain water and then placed on cemented floor to remove excess water to make the moisture content 60%and CaO was added at the rate of 0.2% of the total substrate. In case of sawdust, water was added to make the moisture content 60% and CaO was added at the same rate of other substrates. Then the substrates were filled into 500g and 250g polypropylene bag. The packets were sterilized by autoclaving at 15 lbs. pressure and 121°C temperature for 45 minutes. Then inoculation of mother culture was done @ 50 g mother spawn per packet layer by layer except sawdust. In case of saw dust each packet was inoculated with the mother culture at the rate of two tea spoonfulls. The filled polypropylene bags were prepared by using plastic neck and plugged the neck with cotton and covered with brown paper placing rubber band to hold it tightly in place for incubation.

3.3.5 Incubation of spawn packets

After preparation of spawn packets, filled packets were incubated in a dark room at a temperature ranging between 22-25°C, where 90% relative humidity was maintained till the mycelium colonization was complete. The spawn packets were placed on iron shelves and observed regularly with 10 days interval to record prevalence of contaminating fungi. Data on severity of contamination were recorded and expressed in percentage computed based on total number of packets checked the number of contaminated packets. When the substrates are fully covered with white mycelium, the rubber band, brown paper, cotton plug and plastic neck of the mouth of spawn packets were removed then the bag was cut open and the mouth was wrapped tightly with rubber band. Then these spawn packets were transferred to the culture house.

Plate 1. **A .** Chopped sugarcane bagasse**, B**. Sawdust, **C.** Mustard straw**, D**. Rice straw, **E**. Waste paper

Plate 2. **A**. Mixing of CaO with substrate **B**. Mother of *pleurotus djamor*, **C-D**. Incubation of spawn packets

3.3.6 Cultivation of spawn packets at culture house

Polythene bags were cut in "D" at two ends with a blade and opened by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a blade to remove the thin whitish mycelial layer. The packet of each type was placed separately side by side on the iron shelves of culture house. The moisture of the culture house was maintained 70-80% by spraying water 3 times a day and the temperature about 21-27◦C through air cooler.

3.3.7 Harvesting of produced mushrooms

The matured fruiting body was identified by curial margin of the cap, as described by Amin (2004). Mushrooms were harvested by twisting to uproot from the base. After completing the first harvest again the packets were scraped at the place where the ‘D’ shaped cut had been done and the spawn packets were soaked in water for 5 minutes and inverted to remove excess water for another 5 minutes and water was sprayed regularly. Then again when the primordia appeared after first harvest then second harvest was done and water spraying was continued until the mushrooms were ready to be harvested.

3.4 Data collection

Data were taken on the following parameters

3.4.1 Days required for completing mycelium running

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

3.4.2 Days required for the primordia formation

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

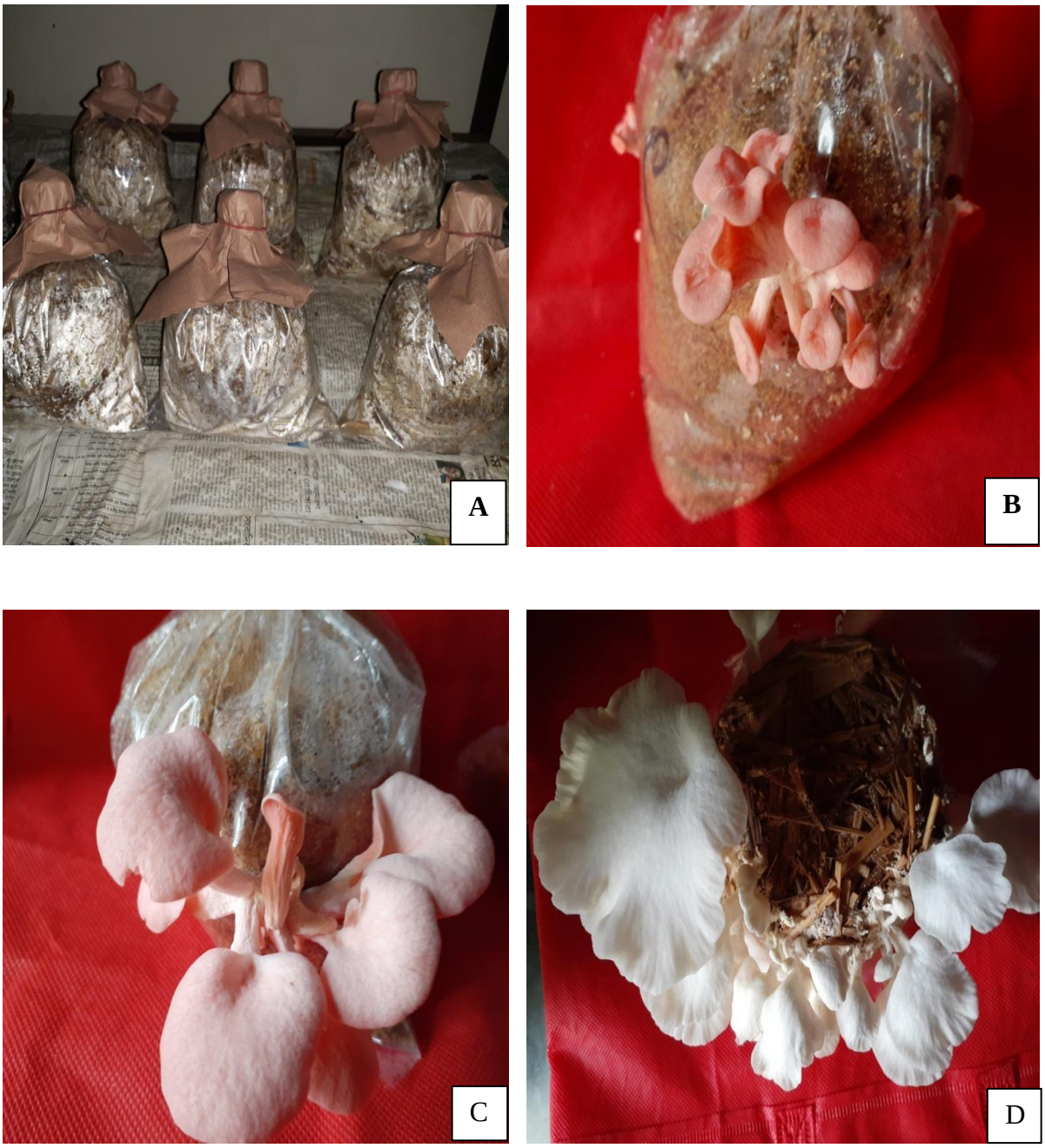

Plate 3. **A**. Mycelium running in spawn packet, **B**. Primordia initiation in spawn packet, **C-D.** Mature fruiting body in spawn packet

3.4.3 Days required to primodia initiation to 1st harvest

Days required from primodia formation to first harvest was recorded.

3.4.4 Days required to final harvest

Days required from primordia formation to final harvest was recorded.

3.4.5 Data on yield contributing parameters

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

a) Number of primodia per packets

b) Number of fruiting bodies

c) Number of effective fruiting bodies

d) Weight of individual fruiting body (g)

3.4.6 Dimension of fruiting body (stipe and pileus)

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

3.4.7 Biological yield (g)

Biological yield per 500 g packet and per 250g packet was measured by weighing the whole cluster of fruiting body without removing the lower hard and dirty portion.

3.4.8 Economic yield (g)

Economic yield per 500 g packet and per 250 g packet was recorded by weighing all the fruiting bodies in a packet after removing the lower hard and dirty portion.

3.4.9 Dry yield (g)

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

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

3.4.10 Biological efficiency

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

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

3.4.11 Severity of contamination (%) on spawn packets

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

Grade 0: 0% – Free from infection

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

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

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

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

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

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

3.5 Analysis of data

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

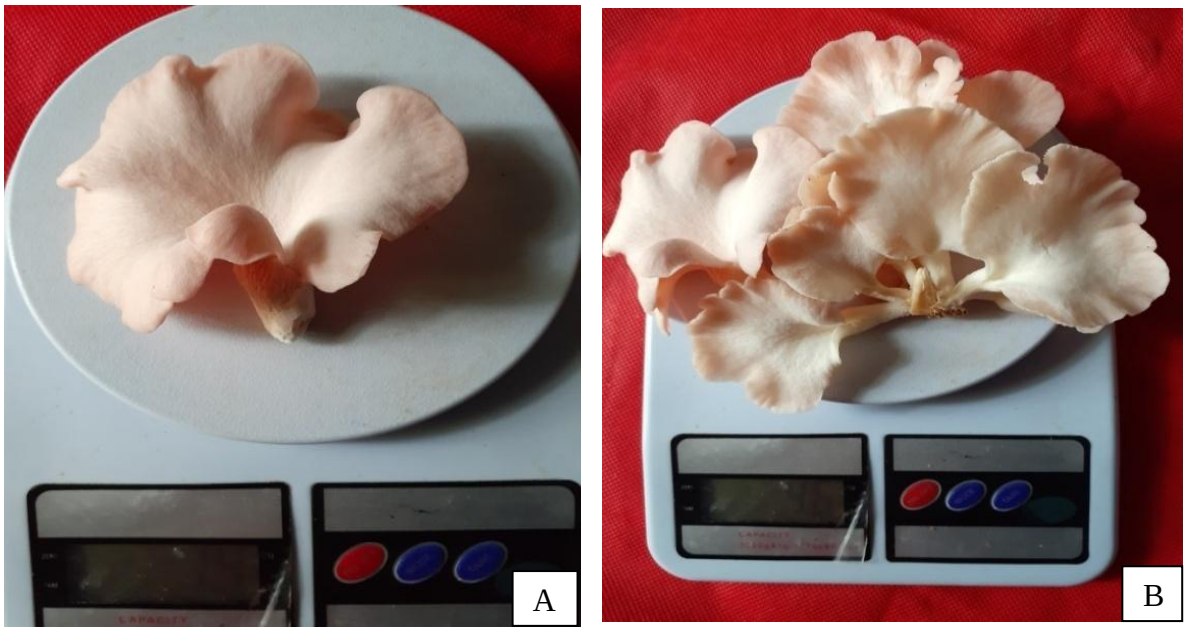

Plate 4. A. Measurement of weight of individual fruiting body, B. Measurement of whole cluster of fruiting body

3.6 Collection of contaminated spawn packet

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

3.6.1 Composition and preparation of PDA media

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

The glassware's *viz.,* petri plates, test tubes, conical flasks, measuring cylinders, glass rods were sterilized in electrical hot air oven at 160 ºC for an hour. 200 gm sliced, peeled potatoes were boiled in 1 liter distilled water to make potato infusion for 30 min. Potato infusion was filtering through sieve and dextrose, agar and water (if needed to fill 1 L) was mixed and boiled to dissolve. The mixture was sterilized by autoclaving at 15 lbs. pressure (121°C) for 45 minutes. After autoclaving the media the conical flask are then taken into the laminar airflow chamber in order to avoid contamination. The laminar airflow chamber must be wiped thoroughly with cotton cloth dipped in 70% ethyl alcohol. So prepared agar media is then poured into the sterile petri plates at equal volumes. After the agar is poured into the sterile petri plates, it is allowed to cool down.

3.6.2 Isolation and purification of competitor moulds from collected spawn

10g of substrate samples were taken from the contaminated packets and mixed with 100 ml sterile distilled water. A series of dilutions were made by taking 1 ml from the stock solution to add with 9 ml sterile water and shaken thoroughly to

obtain $10^{-}1$ dilution. Similarly $10^{-}2$, $10^{-}3$ and $10^{-}4$ dilutions of the substrate suspension were prepared (Dhingra and Sinclair 1995). From the each of the substrate dilutions 0.5 ml volumes were pipetted on PDA media and incubated at 27°C (±2)°C for 3-4 days. The pathogen grown as the mixed colony then individual culture plates of substrate samples were isolated. To prepare pure culture sufficient number of sub culturing were done by hyphal tip technique (Hyakumachi, 1994). All the pure cultures were kept in refrigerator at 4°C for preservation.

3.6.3 Identification of pathogens

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

CH APTER IV

RESULTS

4.1 Effect of different substrates and amount of substrate on growth and yield contributing characters of oyster mushroom

4.1.1. Main effect of different substrate on growth and yield contributing characters of oyster mushroom

4.1.1.1 Days required for mycelium running

Days required for mycelium running of oyster mushroom varied significantly due to different substrates (Table 1). The highest days (18.93 days) required for mycelium running was recorded from waste paper which was statistically similar (18.86 days) to sugarcane bagasse and closely followed by saw dust (17.93 days)and rice straw (16.29 days), whereas the lowest time (15.43 days) was observed in mustard straw.

4.1.1.2 Days required for primordia formation

Statistically significant variation was recorded in terms of days required for primodia formation of oyster mushroom due to different substrates (Table 1). The highest days (6.86 days) required for primodia formation was recorded from sugarcane bagasse which was closely followed by waste paper (6.29 days), while the lowest time (5.86 days) was found in mustard straw which was statistically similar to rice straw (6.00 days) and saw dust (6.07 days).

4.1.1.3 Days required from primordia initiation to 1st harvest

Different substrates showed statistically significant differences in terms of days required for primodia initiation to 1st harvest of oyster mushroom (Table 1). The highest days (4.79 days) required for primodia initiation to 1st harvest was observed from saw dust which was statistically similar to rice straw (4.64 days)

and sugarcane bagasse (4.57days) and closely followed by waste paper (4.50 days). On the other hand the lowest time (4.21 days) was recorded in mustard straw.

4.1.1.4 Total harvesting period

Statistically significant variation was recorded in terms of days required for final harvest of oyster mushroom due to different substrates (Table 1). The highest total harvesting period (49.07 days) was recorded in saw dust which was statistically similar with waste paper (48.71 days) and sugarcane bagasse (47.86 days), whereas the lowest in mustard straw (41.21days).

Table 1. Effect of different substrates on days required for mycelium running, primordial formation, first and final harvest of oyster mushroom

Substrate	Days required for mycelium running	Days required for primordia formation	Days required from primordia initiation to 1st harvest	Total harvesting period
Rice straw	16.29 c	6.00 bc	4.64 a	42.71 b
Saw dust	17.93 b	6.07 bc	4.79 a	49.07 a
Mustard straw	15.43 d	5.86 c	4.21 b	41.21 b
Sugarcane bagasse	18.86 a	6.86 a	4.57 ab	47.86 a
Waste paper	18.93 a	6.29 b	4.50 ab	48.71 a
$LSD_{(0.05)}$	0.424	0.383	0.3689	1.844
CV (%)	3.21	8.16	10.74	5.31

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

4.1.1.5 Number of primordia/packet

Statistically significant variation was recorded due to different substrates in terms of number of primodia/packet of pink oyster mushroom (Table 2). The maximum number of primodia/packet (39.86) was observed from mustard straw which was statistically similar with rice straw (38.07). in case of sawdust and sugarcane bagasse, the number of primordial was also statistically similar. On the other hand, the minimum number (24.50) was found in waste paper.

4.1.1.6 Number of fruiting body/packet

Number of fruiting body/packet of pink oyster mushroom showed statistically significant differences due to use of different substrates was ranged from 15.21 to 32.29 (Table 2). The maximum numbe of fruiting body/packet was found from mustard straw spawn followed by rice straw and saw dust, whereas the minimum number was recorded in waste paper which was statistically similar to sugarcane bagasse.

4.1.1.7 Number of effective fruiting body/packet

The maximum number of effective fruiting body/packet (21.43) was observed from rice straw which was statistically similar to mustard straw (20.79) and followed by saw dust and sugarcane bagasse, while the minimum number (10.57) was recorded in waste paper (Table 2).

4.1.1.8 Weight of individual fruiting body

The effect of different substrates on weight of individual fruiting body was statistically different and was ranged from 3.29g to 4.88g (Table 2). The maximum weight of individual fruiting body was recorded from waste paper followed by sugarcane bagasse and the minimum weight was found in mustard straw, which was statistically similar with saw dust spawn.

Table 2. Effect of different substrates on primordia and fruiting body of pink oyster mushroom

Substrate	Number of primordia/ packet	Number of fruiting bodies/packet	Number of effective fruiting bodies	Weight of individual fruiting body (g)
Rice straw	38.07 a	29.14 b	21.43 a	3.75 c
Saw dust	30.43 b	24.43 c	18.29 b	3.36 d
Mustard straw	39.86 a	32.29 a	20.79 a	3.29 d
Sugarcane baggase	28.57 b	20.29 d	15.21 c	4.07 b
Waste paper	24.50 c	15.21 e	10.57 d	4.88 a
$LSD_{(0.05)}$	1.995	1.567	1.084	0.197
CV (%)	8.17	8.54	8.31	6.80

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

4.1.1.9. Length of stipe

Length of stipe of pink oyster mushroom varied significantly due to different substrates (Table 3) and it was ranged from 0.50 to 1.00 cm. The maximum length (1.00 cm) of stipe was recorded in both rice straw and saw dust which were followed by mustard straw and waste paper. On the other hand, the minimum length (0.50 cm) of stipe was found in sugarcane bagasse.

4.1.1.10. Length of pileus

Length of pileus of pink oyster mushroom showed statistically significant differences due to different substrates (Table 3). The maximum length (5.46 cm) of pileus was found in rice straw which was statistically similar with mustard straw followed by sugarcane bagasse (5.00 cm) and waste paper (4.75 cm), while the minimum length (3.75 cm) of pileus was observed in saw dust.

4.1.1.11 Width of pileus

Different substrates showed statistically significant variation in terms of width of pileus of pink oyster mushroom and was ranged from 5.18 to 6.11cm (Table 3). The maximum width of pileus was recorded from both waste paper and sugarcane bagasse followed by rice straw and mustard straw, whereas the minimum width was found in saw dust.

Table 3. Effect of different substrates on dimension of fruiting body of pink oyster mushroom

Substrate	Stipe length (cm)	Pileus Length (cm)	Width of pileus (cm)
Rice straw	1.00 a	5.46 a	5.64 b
Saw dust	1.00 a	3.75 d	5.18 c
Mustard straw	0.79 b	5.46 a	5.46 bc
Sugarcane baggase	0.50 c	5.00 b	6.11 a
Waste paper	0.75 b	4.75 c	6.11 a
$LSD_{(0.05)}$	0.045	0.063	0.325
CV (%)	7.40	1.73	7.54

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

4.1.1.12 Biological yield (g)

Statistically significant variation was recorded in terms of biological yield of pink oyster mushroom (Table 4). The highest of biological yield (83.36 g) was found from rice straw which was followed by sugarcane bagasse (61.07 g), mustard straw (59.04 g), saw dust (59.04 g), while the lowest biological yield (49.14 g) was recorded in waste paper.

4.1.1.13 Economical yield (g)

Significant variation was found in economic yield of pink oyster mushroom on different substrates (Table 4). The highest of economical yield (70.00 g) was recorded from rice straw followed by mustard straw (54.71 g), whereas the lowest economical yield (42.79 g) was found in waste paper.

4.1.1.14 Dry yield (g)

Maximum dry yield (20.90 g) was found from rice straw which was followed by mustard straw (15.20 g) and the minimum dry yield (10.69 g) was observed in waste paper. The other substrates differed statistically from each other.

4.1.1.15 Biological efficiency

Remarkable differences were observed in biological efficiency and it was ranged from 43.95% to 76.76%. The highest of biological efficiency (76.76%) was observed from rice straw which was followed by mustard straw (62.95%), while the lowest (43.95%) biological efficiency was recorded in waste paper. So, it was noted that the most effective substrates were rice straw followed by mustard straw, sugarcane bagasse and saw dust.

Table 4. Effect of different substrates on different yield contributing parameters of pink oyster mushroom

Substrate	Biological yield (g)	Economical yield (g)	Dry yield (g)
Rice straw	83.36 a	70.00 a	20.90 a
Saw dust	59.04 c	52.64 c	13.64 c
Mustard straw	59.04 c	54.71 b	15.20 b
Sugarcane baggase	61.07 c	52.36 c	13.77 c
Waste paper	49.14 d	42.79 e	10.69 e
$LSD_{(0.05)}$	3.366	2.66	0.577
CV (%)	6.91	6.51	5.23

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

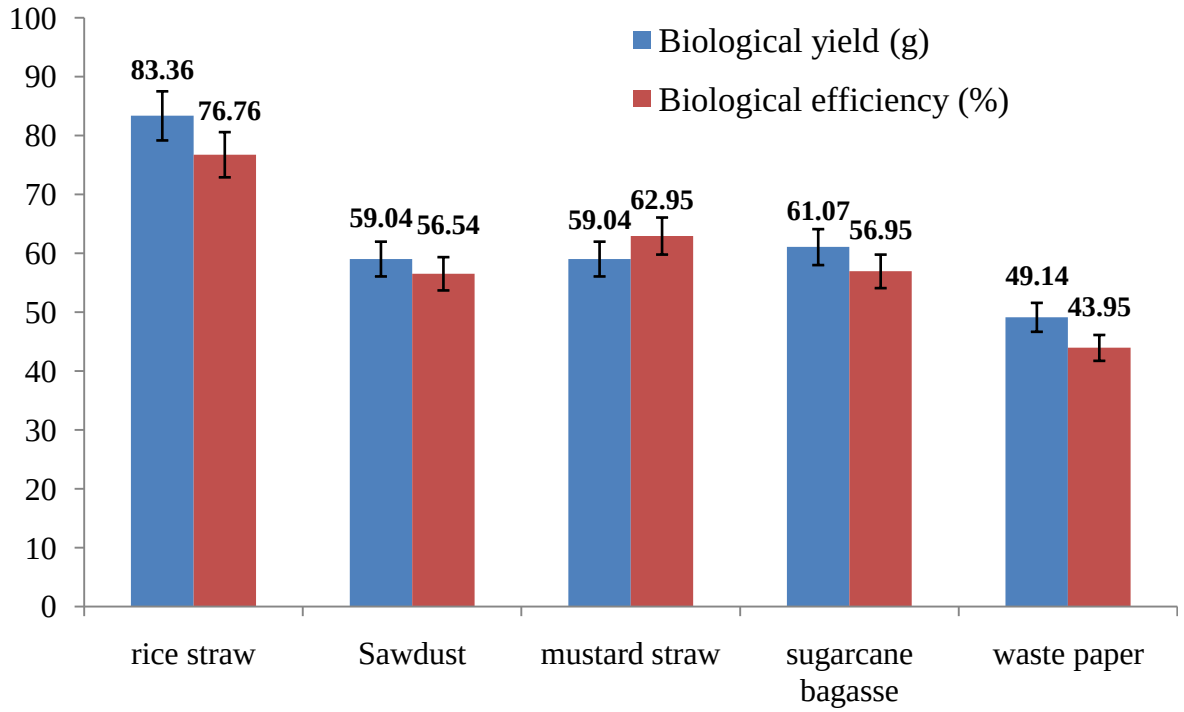

Figure 1. Effect of different substrates on biological yield and biological efficiency

4.1.2. Main effect of different amount of substrate on growth and yield contributing characters of pink oyster mushroom

4.1.2.1 Days required for mycelium running

Days required for mycelium running of pink oyster mushroom varied significantly due to different packet size (Figure 2). The maximum days (18.00 days) required for mycelium running was recorded from 500g substrate/ packet, whereas the minimum time (16.97 days) was found in 250g substrate /packet.

4.1.2.2 Days required for primordia formation

Statistically significant variation was recorded in terms of days required for primodia formation of pink oyster mushroom due to different packet size (Figure 2). The highest days (6.57 days) required for primodia formation was recorded from 500g substrate /packet, while the lowest time (5.86 days) was found in 250g substrate/ packet.

4.1.2.3 Days required from primordia initiation to 1st harvest

Different packet size have significant differences in terms of days required for primodia initiation to 1st harvest of oyster mushroom (Figure 2). The highest days (4.60 days) required for primodia initiation to 1st harvest was observed from 500g substrate /packet. On the other hand the lowest time (4.49 days) was recorded in 250g substrate/packet.

4.1.2.4 Total harvesting period

Statistically significant variation was recorded in terms of total harvesting period of pink oyster mushroom in case of different packet size (Figure 2). The highest days (45.97 days) required for final harvest was found from 500g substrate/ packet, whereas the minimum time (45.86 days) was observed in 250g substrate /packet.

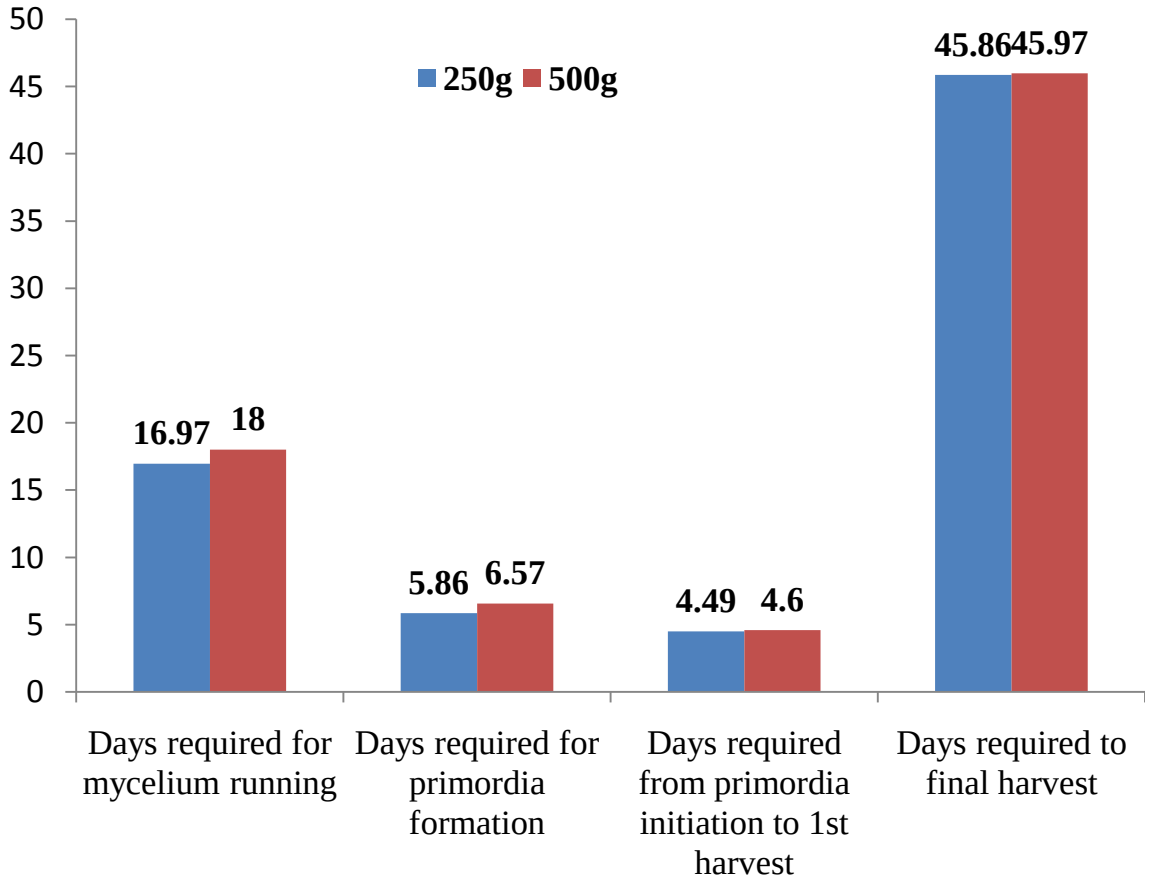

Figure 2. Effect of different Amount of substrate on days required for mycelium running, primordia formation, first and final harvest of oyster mushroom

4.1.2.5 Number of primordia/packet

Statistically significant variation was recorded due to different packet size of substrates in terms of number of primodia/packet of pink oyster mushroom (Figure 3) The maximum number of primodia/packet (37.71) was observed from 500g substrate/ packet. On the other hand, the minimum number (26.86) was found in 250g substrate/ packet.

4.1.2.6 Number of fruiting body/packet

Number of fruiting body/packet of pink oyster mushroom showed statistically significant differences due to use of different packet size of substrates (Figure 3). The maximum number (28.31) of fruiting body/packet was found from 500g substrate/ packet, whereas the minimum number (20.23) was recorded in 250g substrate/ packet.

4.1.2.7 Number of effective fruiting body/packet

The maximum number of effective fruiting body/packet (20.63) was observed from 500g substrate/packet (Figure 3), while the minimum number (13.89) was recorded in 250g substrate /packet.

4.1.2.8 Weight of individual fruiting body

Statistically significant difference was noted in different packet size of substrates where the maximum weight (3.97 g) was recorded from 500g substrate/ packet and the minimum weight (3.69 g) from 250g substrate /packet (Figure 3).

4.1.2.9 Width of pileus

There was no statistical significant variation was found in terms of width of pileus of pink oyster mushroom on different amount of substrates (Figure 3). The width of pileus was statistically identical but insignificantly lower to 500g packets.

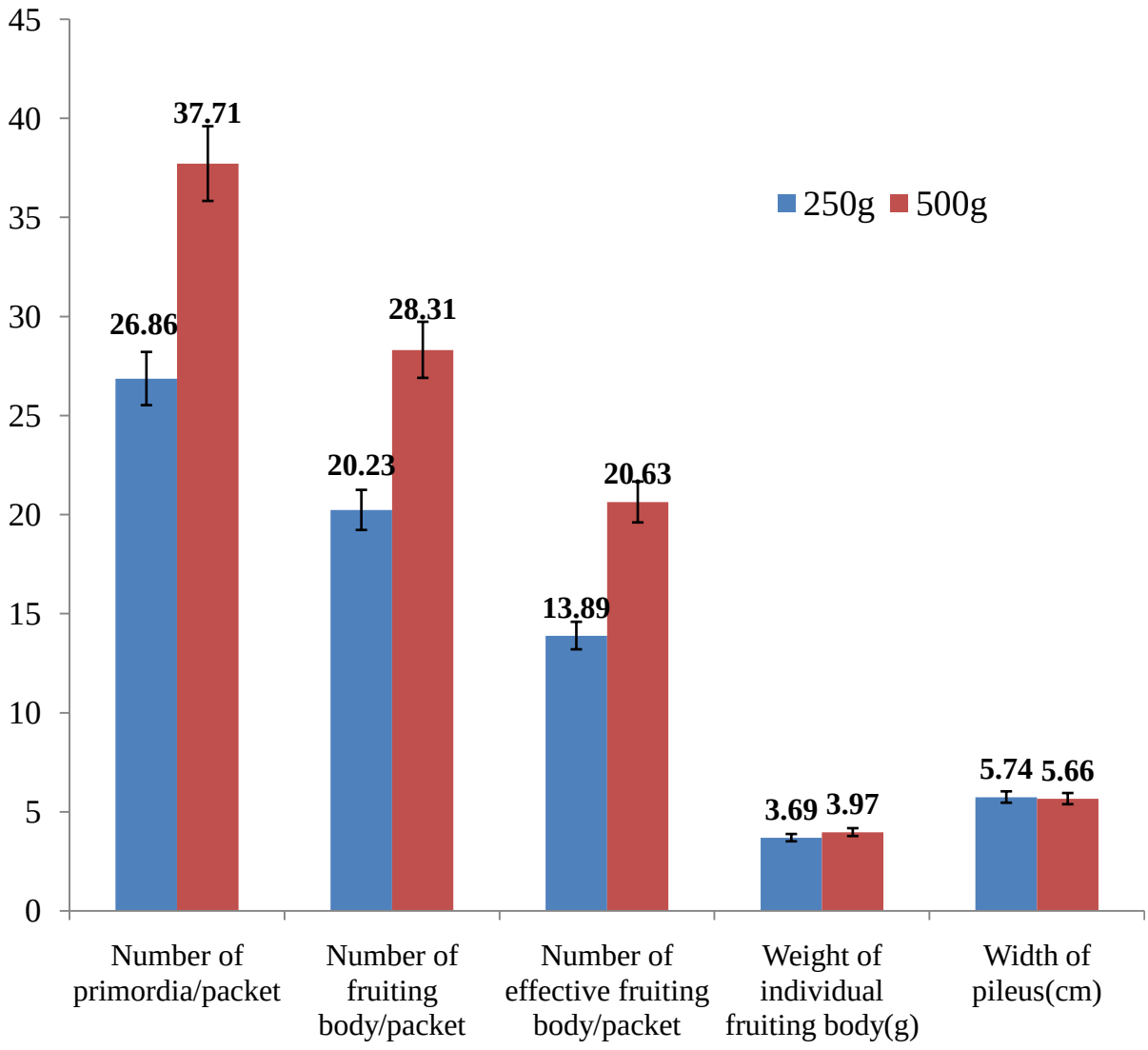

Figure 3. Effect of different Amount of substrate on number of primordia, on number of fruiting body, number of effective fruiting body, weight of individual fruiting body and width of pileus.

4.1.2.10 Biological yield (g)

Statistically significant variation was recorded in terms of biological yield of pink oyster mushroom (Figure 4). The highest of biological yield (79.44 g) was found from 500g substrate packet, while the lowest biological yield (49.43 g) was recorded in 250g substrate packet.

4.1.2.11 Economical yield (g)

Economical yield of pink oyster mushroom showed statistically significant variation due to use of different packet size of substrates (Figure 4). The highest of economical yield (67.57 g) was recorded from 500g substrate packet, whereas the lowest economical yield (40.63 g) was found in 250g substrate packet.

4.1.2.12 Dry yield (g)

Maximum dry yield (17.23 g) was found from 500g substrate packet and the minimum dry yield (11.95 g) was observed in 250g substrate packet.

4.1.2.13 Biological efficiency (%)

The highest of biological efficiency (65.90 %) was observed from 250g substrate packet, while the lowest (52.96%) biological efficiency was recorded in 500g substrate packet.

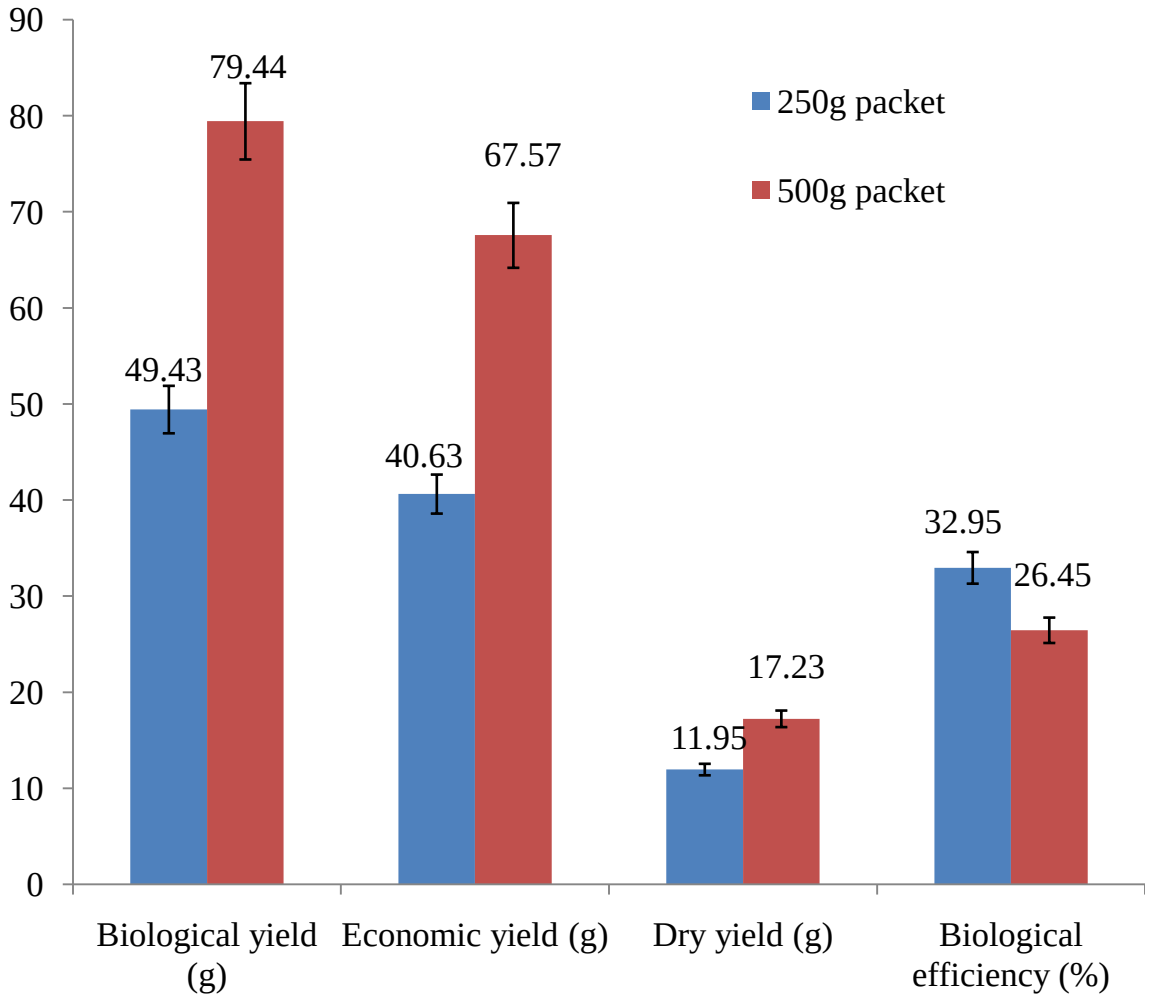

Figure 4. Effect of different Amount of substrate on different yield contributing parameters of pink oyster mushroom

4.1.3. Interaction effect of substrates and amount of substrate on growth and yield contributing characters of pink oyster mushroom

4.1.3.1 Days required for mycelium running

Days required for mycelium running of pink oyster mushroom varied significantly due to interaction effect of substrates and amount of substrate and it was ranged from 14.43 to 19.43 days (Table 5). The minimum time was found in mustard straw at 250g/ packet followed by rice straw at 250g/packet (15.43 days), whereas maximum days required for mycelium running was recorded in waste paper at 500g/packet followed by sugarcane bagasse at 500g/packet (19.29 days), waste paper at 250g/packet (18.43 days) and sugarcane bagasse at 250g/packet (18.43 days).

4.1.3.2 Days required for primordia formation

Statistically significant variation was recorded in terms of days required for primordia formation of pink oyster mushroom due to interaction effect of substrates and amount of substrate (Table 5). The minimum time (5.43 days) was found in mustard straw at 250g/packet, whereas the maximum days (7.29 days) required for primordia formation was recorded from sugarcane bagasse at 500g/packet followed by rice straw at 500g/packet(6.43 days), saw dust at 500g/packet (6.43 days), waste paper at 500g/packet and sugarcane bagasse at 250g/packet(6.43 days).

4.1.3.3 Days required from primordia initiation to 1st harvest

Interaction effect of substrates and amount of substrate have significant differences in terms of days required for primordia initiation to 1st harvest of oyster mushroom (Table 5). The minimum time for primordia initiation to 1st harvest (4.14 days) was found in mustard straw at 250g/packet. Whereas maximum days (4.86 days) was recorded from saw dust at 500g/packet followed

by rice straw at 250g/packet(4.71 days), saw dust at 250g/packet (4.71 days) and sugarcane bagasse at 500g/packet(4.71 days).

4.1.3.4 Total harvesting period

Statistically significant interaction effect of different substrates and amount of substrate was varied from 41 to 49.57 days considering the total harvesting period (Table 5). On the other hand, rice straw at 250g/packet, rice straw at 500g/packet, mustard straw at 250g/packet and mustard straw at 500g/packet were statistically identical but significantly different from each other. The highest harvesting period was recorded from saw dust and waste paper at 500g/packet, which were statistically and significantly identical followed by sugarcane bagasse at 250g/packet, saw dust straw at 250g/packet(48.57 days), waste paper at 250g/packet and sugarcane bagasse at 500g/packet, while the lowest time was found in mustard straw at 250g/packet.

Table 5. Interaction effect of substrate and amount of substrate on days required for mycelium running, primordia formation, first and final harvest of pink oyster mushroom

Substrate x Amount of substrate	Days required for completion of mycelium running	Days required for primordia formation	Days required from primordia formation to 1st harvest	Total harvesting period
Rice straw at 250g	15.43 f	5.57 d	4.71 ab	42.71 c
Rice straw at 500g	17.14 d	6.43 b	4.57 abc	42.71 c
Saw dust at 250g	18.14 bc	5.71 cd	4.71 ab	48.57 ab
Saw dust at 500g	17.71 cd	6.43 b	4.86 a	49.57 a
Mustard straw at 250g	14.43 g	5.43 d	4.14 c	41.00 c
Mustard straw at 500g	16.43 e	6.29 b	4.23 bc	41.43 c
Sugarcane baggase at 250g	18.43 b	6.43 b	4.43 abc	49.14 ab
Sugarcane baggase at 500g	19.29 a	7.29 a	4.71 ab	46.57 b
Waste paper at 250g	18.43 b	6.14 bc	4.43 abc	47.86 ab
Waste paper at 500g	19.43 a	6.43 b	4.57 abc	49.57 a
$LSD_{(0.05)}$	0.599	0.542	0.521	2.609
CV (%)	3.21	8.16	10.74	5.31

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

4.1.3.5 Number of primordia/packet

Statistically significant variation was recorded due to interaction effect of substrates and amount of substrate in terms of number of primordia/packet of pink oyster mushroom (Figure 5). The maximum number of primordia/packet (50.71) was observed from mustard straw at 500g substrate/packet followed by rice straw at 500g substrate/packet (46.86). On the other hand, the minimum number (22.71) was found in waste paper at 250g substrate/packet, which was followed by saw dust at 250g substrate/packet (25.00).

4.1.3.6 Number of fruiting body/packet

Number of fruiting body/packet of pink oyster mushroom showed statistically significant differences due to interaction effect of substrates and amount of substrate (Table 6). The maximum number (37.29) of fruiting body/packet was found from mustard straw at 500g substrate/packet followed by rice straw at 500g substrate/packet (35.86) whereas the minimum number (13.00) was recorded in waste paper at 250g substrate/packet, which was followed by saw dust at 250g substrate/packet (29.86).

4.1.3.7 Number of effective fruiting body/packet

Statistically significant variation was recorded due to interaction effect of substrates and amount of substrate in terms of effective fruiting body/packet of pink oyster mushroom (Figure 5). The maximum number of fruiting body/packet (25.86) was observed from rice straw at 500g substrate/packet followed by mustard straw at 500g substrate/packet (25.00). On the other hand, the minimum number (8.00) was found in waste paper at 250g substrate/packet, which was followed by sugarcane bagasse at 250g substrate/packet (12.00).

4.1.3.8 Weight of individual fruiting body

Weight of individual fruiting body of pink oyster mushroom showed statistically significant differences due to interaction effect of substrates and amount of substrate (Table 6). The maximum weight (4.93 g) of individual fruiting body was observed from waste paper at 500g substrate/packet followed by sugarcane bagasse at 250g substrate/packet (4.14 g). On the other hand, the minimum weight of individual fruiting body (3.00g) was found in mustard straw at 250g substrate/packet, which was followed by saw dust at 250g substrate/packet (3.29g).

Table 6. Interaction effect of substrate and amount of substrate on primordia and fruiting body of pink oyster mushroom

Substrate x Amount of substrate	Number of fruiting body/packet	Weight of individual fruiting body (g)
Rice straw at 250g	22.43 c	3.57 d
Rice straw at 500g	35.86 a	3.93 c
Saw dust at 250g	19.86 de	3.29 e
Saw dust at 500g	29.00 b	3.43 de
Mustard straw at 250g	27.29 b	3.00 f
Mustard straw at 500g	37.29 a	3.57 d
Sugarcane baggase at 250g	18.57 ef	4.14 c
Sugarcane baggase at 500g	22.00 cd	4.00 c
Waste paper at 250g	13.00 g	4.43 b
Waste paper at 500g	17.43 f	4.93 a
$LSD_{(0.05)}$	2.216	0.279
CV (%)	8.54	6.80

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

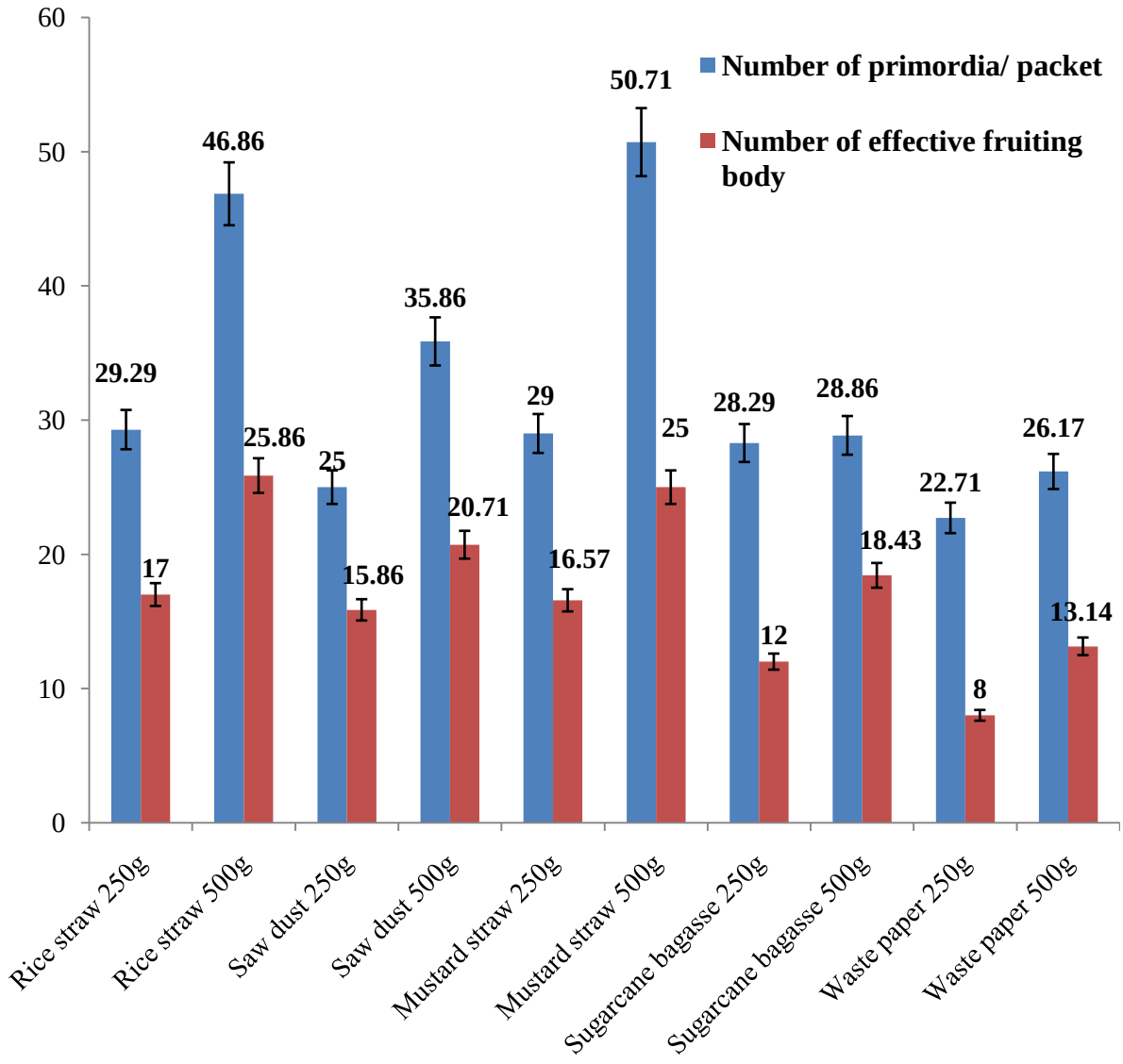

Figure 5. Interaction effect of substrate and amount of substrate on number of primordia/ packet and number of effective fruiting body

4.1.3. 9 Length of stipe

Interaction effect of substrates and amount of substrate have significant differences in terms of length of stipe in oyster mushroom (Table 7). The highest length (1.07cm) of stipe was recorded from mustard straw at 500g substrate/packet. On the other hand, the minimum length (0.50cm) of stipe was found in sugarcane bagasse at 250g substrate/packet, sugarcane bagasse at 500g substrate/packet (0.50cm), mustard straw at 250g substrate/packet (0.50cm) and waste paper at 250g substrate/packet (0.50 cm).

4.1.3.10 Length of pileus

Statistically significant variation was recorded in terms of Length of pileus of pink oyster mushroom due to interaction effect of substrates and amount of substrate (Table 7). The highest length (5.93 cm) of pileus was found from rice straw at 250g substrate/packet and mustard at 500g substrate/packet (5.93cm). While the minimum length (3.50 cm) of pileus was observed in saw dust at 250g substrate/packet.

4.1.3.11 Width of pileus

Interaction effect of substrates and amount of substrate showed statistically significant variation in terms of width of pileus of pink oyster mushroom (Table 7).The highest width (6.21 cm) of pileus was recorded from waste paper at 250g substrate/packet, followed by sugarcane bagasse at 250g substrate/packet (6.14 cm). Whereas the minimum width (5.43 cm) of pileus was found in saw dust at 250g substrate/packet and mustard straw at 250g substrate/packet (5.43 cm).

Table 7. Interaction effect of substrate and amount of substrates on dimension of fruiting body of pink oyster mushroom

Substrate x Amount of substrate	**Stipe length (cm)**	**Pileus Length (cm)**	**Width of pileus (cm)**
Rice straw at 250g	1.00 b	5.93 a	5.50 b
Rice straw at 500g	1.00 b	5.00 b	5.79 ab
Saw dust at 250g	1.00 b	3.50 e	5.43 b
Saw dust at 500g	1.00 b	4.00 d	4.93 c
Mustard straw at 250g	0.50	5.00 b	5.43 b
Mustard straw at 500g	1.07 a	5.93 a	5.50 b
Sugarcane baggase at 250g	0.50 c	5.00 b	6.14 a
Sugarcane baggase at 500g	0.50 c	5.00 b	6.07 a
Waste paper at 250g	0.50 c	4.50 c	6.21 a
Waste paper at 500g	1.00 b	5.00 b	6.00 a
$LSD_{(0.05)}$	0.064	0.090	0.459
CV (%)	7.40	1.73	7.54

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

4.1.3.12 Biological yield (g)

Statistically significant variation was recorded in terms of biological yield of pink oyster mushroom due to interaction effect of substrates and amount of substrate (Table 8). The highest of biological yield (103.14 g) was found from rice straw at 500g substrate/packet followed by mustard straw at 500g substrate/packet (89.43g) and sugarcane bagasse at 500g substrate/packet (73.43 g). While the lowest biological yield (33.57 g) was recorded in waste paper at 250g substrate/ packet, which was followed by sugarcane bagasse at 250g substrate/packet (48.71 g).

4.1.3.13 Economical yield (g)

Economical yield of pink oyster mushroom showed statistically significant variation due to interaction effect of substrates and amount of substrate (Table 8). The highest of economical yield (86.86 g) was recorded from rice straw at 500g substrate/packet followed by mustard straw at 500g substrate/packet (74.29 g). Whereas the lowest economical yield (28.28 g) was found in waste paper at 250g substrate/packet followed by mustard straw at 250g substrate/packet (39.14 g).

4.1.3.14 Dry yield (g)

Dry yield of pink oyster mushroom showed statistically significant variation due to interaction effect of substrates and amount of substrate (Table 8). The highest dry yield (24.16 g) was recorded from rice straw at 500g substrate/packet followed by mustard straw at 500g substrate/packet (18.57 g). Whereas the lowest dry yield (8.43 g) was found in waste paper at 250g substrate/packet followed by saw dust at 250g substrate/packet (10.14 g).

4.1.3.15 Biological efficiency (%)

Statistically significant variation was recorded in terms of biological efficiency of pink oyster mushroom due to interaction effect of substrates and amount of substrate (Table 8). The highest of biological efficiency (42.38%) was found from

rice straw at 250g substrate/packet followed by saw dust at 250g substrate/packet (34.38%) and rice straw at 500g substrate/packet (34.24%). While the lowest biological efficiency (21.57%) was recorded in waste paper at 500g substrate/ packet followed by saw dust at 500g substrate/packet (22.16%).

Table 8. Interaction effect of substrate and amount of substrate on different yield contributing parameters of pink oyster mushroom

Substrate x Amount of substrate	Biological yield (g)	Economical yield (g)	Dry yield (g)	Biological efficiency (%)
Rice straw at 250g	63.57d	53.14 e	17.64 c	84.76 a
Rice straw at 500g	103.14 a	86.86 a	24.16 a	68.76 b
Saw dust at 250g	51.57 e	41.00 f	10.14 h	68.75 b
Saw dust at 500g	66.50 d	56.29 de	14.79 e	44.33 e
Mustard straw at 250g	49.71 e	39.14 f	11.83 g	66.29 b
Mustard straw at 500g	89.43 b	74.29 b	18.57 b	59.61 c
Sugarcane baggase at 250g	48.71 e	41.57 f	11.71 g	64.95 b
Sugarcane baggase at 500g	73.43 c	63.14 c	15.83 d	48.95 d
Waste paper at 250g	33.57 f	28.28 d	8.43 i	44.76 e
Waste paper at 500g	64.71 d	57.29 d	12.83 f	43.14 e
$LSD_{(0.05)}$	4.760	3.768	0.817	4.177
CV (%)	6.91	6.51	5.23	6.57

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

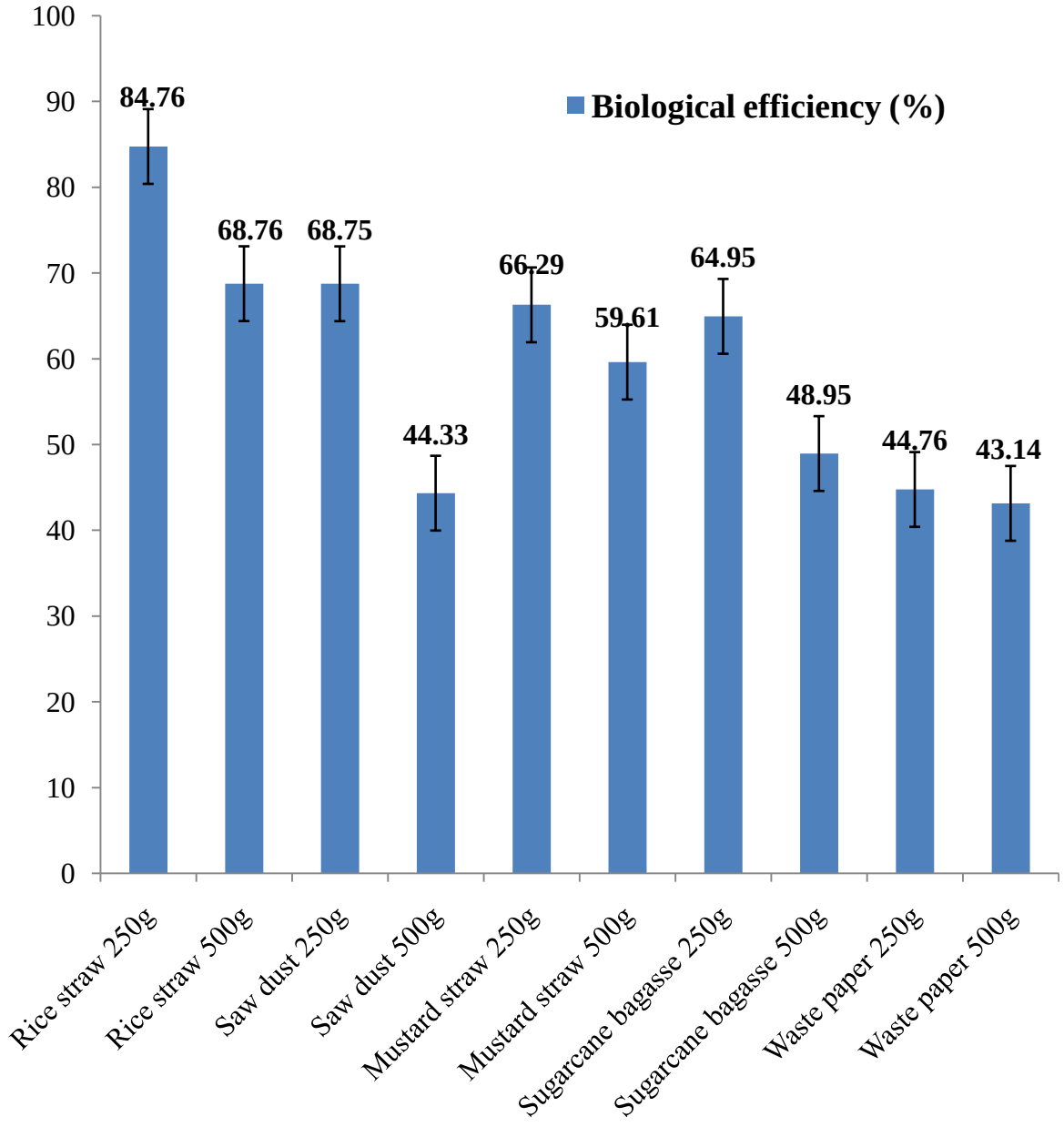

Figure 6. Interaction effect of substrate and amount of substrate on biological efficiency

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

The economic yield of oyster mushroom was correlated positively with the biological efficiency. Strong linear correlation (R2 = 0.973) was observed between economic yield and biological efficiency, where the equation was y = 0.813x+ 6.135, stated that the biological efficiency increased gradually at the rate of 0.81% (Figure 7).

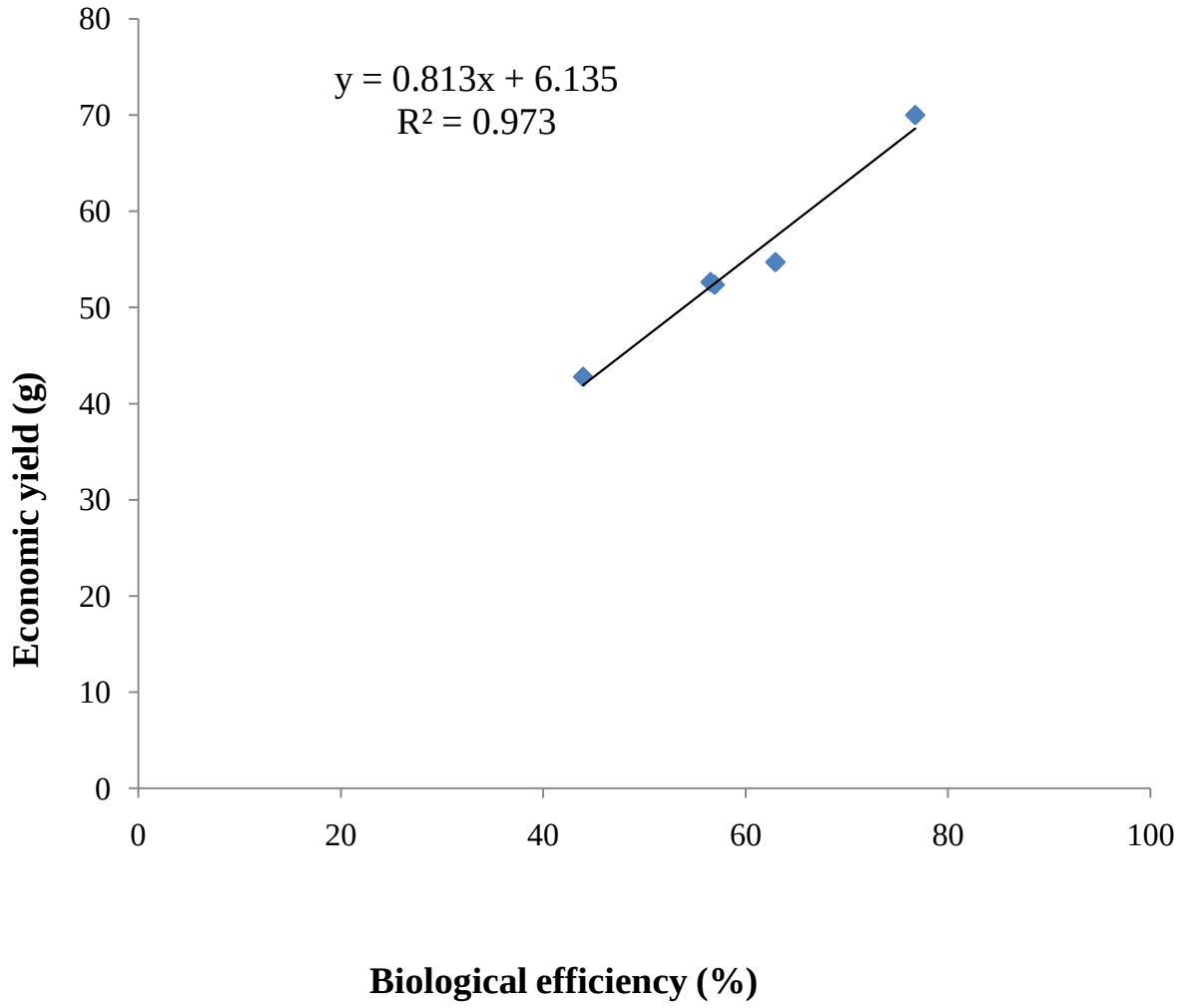

Figure 7. Relationship between economic yield and biological efficiency

4.2. Interaction effect of substrates and amount of substrate on contamination severity

The contamination with fungi was found saw dust at 500g/packet only at first harvest where the severity was 3.57%. The contamination severity was found with rice straw at 500g/packet, mustard straw at 250g/packet, sugarcane bagasse at 500g/packet only at 2nd harvest where the severity was 1.43%, 5% and 7.14% respectively. At 3rd harvest contamination was found with rice straw at 500g/packet, saw dust at 500g/packet, mustard straw at 250g/packet and sugarcane bagasse at 500g/packet, the contamination severity were observed 5.71%, 9.29%, 8.57% and 10.71% respectively, where in other cases the spawn packets were free from contamination (Table 9)

Table 9. Interaction effect of substrate and amount of substrate on severity of contamination

Substrate x amount of substrate	Contamination severity (%)		
	1st harvest	2nd harvest	3rd harvest
Rice straw at 250g	0.0	0.0	0.0
Rice straw at 500g	0.0	1.43%	5.71%
Saw dust at 250g	0.0	0.0	0.0
Saw dust at 500g	3.57%	5.71%	9.29%
Mustard straw at 250g	0.0	5	8.57%
Mustard straw at 500g	0.0	0.0	0.0
Sugarcane baggase at 250g	0.0	0.0	0.0
Sugarcane baggase at 500g	0.0	7.14%	10.71%
Waste paper at 250g	0.0	0.0	0.0
Waste paper at 500g	0.0	0.0	0.0

4.4 Identified contaminants from contaminated spawn

Based on the morphological characters commonly three pathogens were identified, these are *Trichoderma harzianum*, *Penecillium* sp.and *Aspergillus niger*.

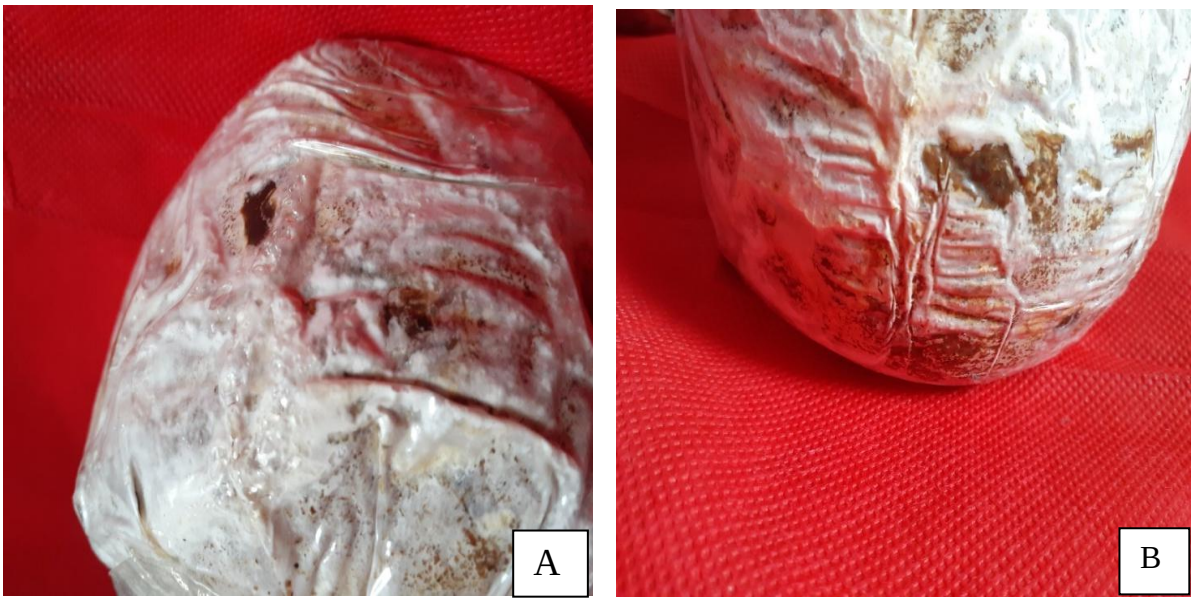

Plate 5. A-B; Different contaminated spawn

4.4.1. *Trichoderma harzianum*

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

4.4.2. *Penicillium sp*.

Initially, *Penicillium* appeared as a white colored powder on the substrates of oyster mushroom and later turned into green as timed passed, it is called blue green mold. Pure culture of *Penicillium* was prepared on PDA from collected contaminated spawn (Plate 6 C-D). Conidiophores are hyaline, smooth or rough walled arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides. Conidia hyaline or brightly colored in mass, chain of single celled conidia are produced in basipetal succession from a specialized conidiogenous cell called a phialide.

4.4.3. *Aspergillus niger*

Aspergillus niger was found in the contaminated the spawn packets in the growing house. It produced black colored spores so it was called black mold (plate 6 E-F). Initially fungal colonies were whitish which quickly became quite black. The hyphae were hyaline and septate. The conidia produced were globose, single celled, pale to dark brown on maturity. The conidiophores were erect, unbranched, straight, hyaline to light brown, long and aseptate.

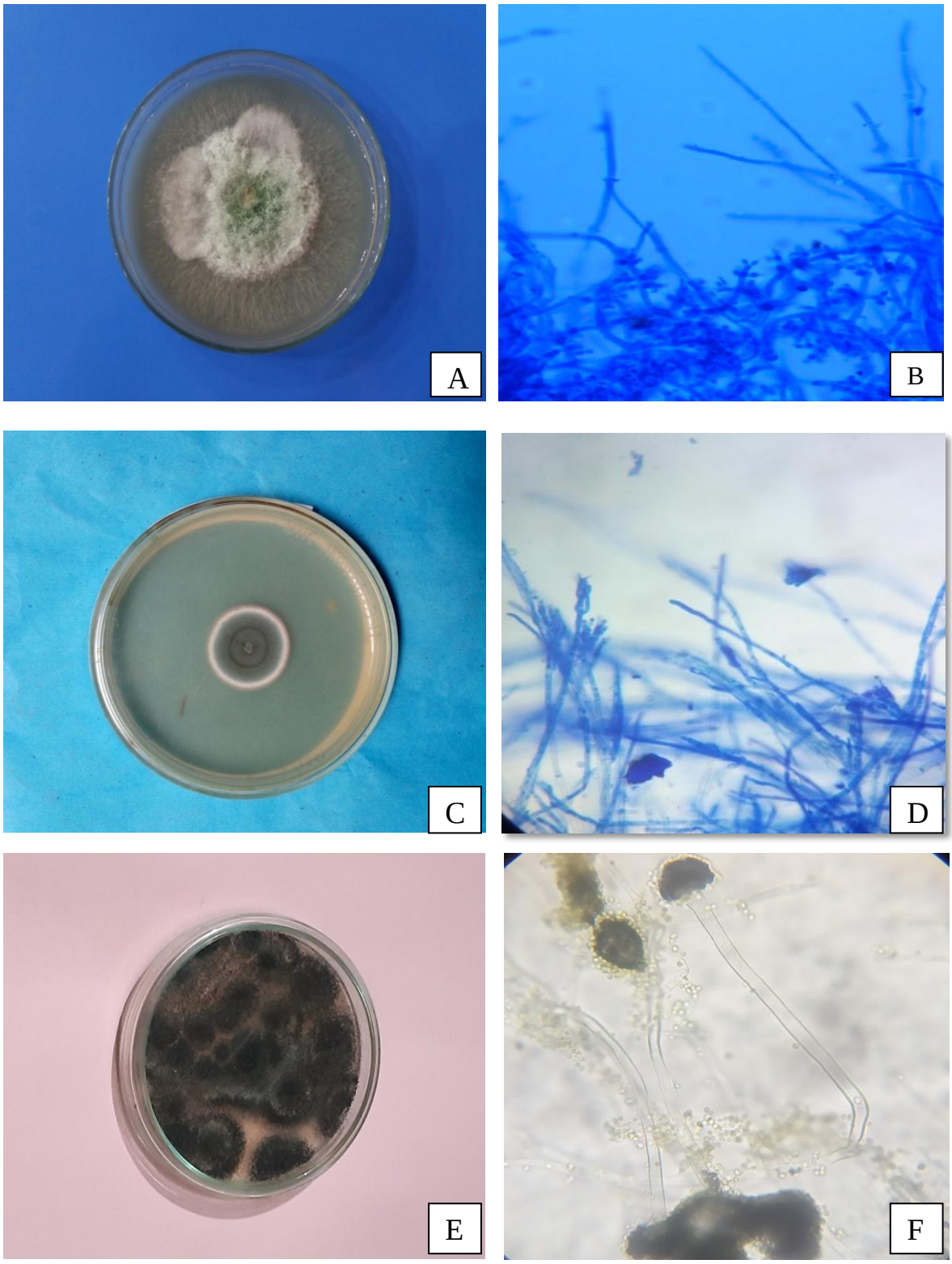

Plate 6. **A.** Pure culture of *Trichoderma* **B.** Pathogenic structure of *Trichoderma sp.*; **C.** Pure Culture of *Penicillium*, **D**. Microscopic Structure of *Penicillium*; **E.** Pure culture of *Aspergillus niger*, **F.** Pathogenic structure of *Aspergillus niger*

CHAPTER V

DISCUSSIONS

The present experiment was conducted to evaluate the effect of different substrates and different spawn packet size on pink oyster mushroom cultivation and severity of contaminants on different substrates. Five substrates namely rice straw, mustard straw, saw dust, sugarcane bagasse and waste paper were used for oyster mushroom cultivation. Among them maximum days for mycelium running was required for waste paper (18.96 days), which was statistically similar with sugarcane bagasse (18.86 days). Days required for primordia initiation was highest in sugarcane bagasse (25.72nd days), which was statistically similar with waste paper. Days required for primordia formation was ranged from 20-25 days. More or less similar findings have been reported by previous scientists. According to Jegadeesh *et al.*, (2018) found that, for the cultivation of pink oyster mushroom, *Pleurotus djamor* var. roseus, different substrates viz. paddy straw, sugarcane bagasse, coir pith, sorghum straw, ragi straw and mixed bed were used. Primordium initiation was observed on 17-22nd day after spawning. Namdev *et al.* (2006) observed that the number of days required for spawn run was significantly less than 14 days to 20 days in different straw substrates (gram straw, parthenium straw, sugarcane straw and wheat straw, sunflower stalk, mustard straw and paddy straw). Sarker *et al*., (2007 a) revealed that, the duration to complete mycelium running was 17.75 days in waste paper. Similar kind of results has been recorded in the present study. The maximum biological and economic yield was found in rice straw among the five substrates and highest number of effective fruiting body was also found in rice straw in the present study, which is more or less similar with reports of previous scientists. According to Sonali (2012) the development of Oyster mushroom (Grey and pink) on agricultural waste like Paddy straw and wheat straw gave very high yield. Maniruzzaman (2004) utilized wheat, maize, rice and sawdust for the production of mother spawn in oyster mushroom and

found that substrate rice was the best for mother spawn production of oyster mushroom. Jegadeesh *et al.,* (2018) found that, maximum yield of *P. djamor* var. roseus was obtained using paddy straw. Dhoke *et al.*,(2001) also found significant effect of different agro-wastes on yield of oyster mushroom. However, an ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth (Anonymous, 2008). Hassan *et al*., (2011) reported that oyster mushroom can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, bagasse etc. and this has been reported to influence its growth, yield and composition (Iqbal *et al*., 2005; Kimenju *et al*., 2009; Khare *et al*., 2010). Ashraf *et al*., (2013) conducted an experiment to compare the effect of different agricultural wastes on growth and yield of mushroom production, three species of *Pleurotus* viz. *P. sajor-caju*, *P. ostreatus*, and *P. djmor* were grown on three different substrates cotton waste, wheat straw and paddy straw. Paddy straw showed maximum yield in 1st flush. Which is similar to the results of present study. According to Sarker *et al*., (2007 a) the highest economic yield was estimated from the waste paper. The economic yield on sugarcane bagasse was statistically identical to that grown on rice straw, which does not support the present study. In the present experiment highest economic yield is estimated from rice straw. In the present study the biological efficiency (BE) was highest in rice straw due to presence of enough cellulosic material and other factors. Biological efficiency varies from substrate to substrate. Similar results were found by scientist previously. According to Bernardi *et al.,* (2007) the productivity and biological efficiency will vary according to different strains and various kinds of substrates used. This confirms the finding of Mandeel *et al*., (2005) that B.E is highly affected by the quality of the spawn of the cultivated mushroom strain. Different substrates have been used to grow *Pleurotus* sp. with BE values varying from 32.10 - 79.18% (Dhanda *et al*., 1994).

During cultivation period three contaminants namely *Trichoderma harzianum, penicillium* sp. *and Aspergillus niger* were isolated and identified from contaminated substrates. According to Pervez *et al.,* (2010) weed mycoflora namely *Aspergillus* spp, *Penicillium* spp., *Rhizopus stolonifer* and *Trichoderma harzianum* were found to be associated with the substrate of oyster mushroom at different growth stages. Mazumder and Rathaiah (2001) found *Trichoderma harzianum, Aspergillus spp.* and *Penicillium spp.* As the three most dominant fungal contaminants during spawn production in oyster mushroom. Mejía and Albertó (2013) reported that the low contamination might have occurred due to quality of a substrate. Akhter (2017) found that, *Trchoderma, Rhizopus, Aspergillus, Penicillium, Alternaria, Ceratocytis, Coprinus, Chaetomium sp.* were associated as contaminants where green mold was detected as the major one. In present findings, saw dust promoted maximum contamination due to containing protein and starch content and pore space, which helps to growth and penetration of mycelium of microorganisms. In present experiment 500g substrate packets were more contaminated comparing to 250g substrate packet. It might be due to amount of substrate is high, so the contamination rate is high. Similar findings have been reported by Akhter (2017). She found that, the severity of contamination in 1000g substrate packet was higher than in 500g substrate packet. The amount of substrates is a matter of discussion as it affects the yield largely (Zhang *et al.,* 2002). Jebunnahar *et al.*,(2007) reported that the yield of button mushrooms have increased with increasing the amount of substrates. Amin *et al.*,(2008) reported that the biological efficiency for the cultivation of oyster mushroom was highest in 500g rice straw and in this experiment the highest biological efficiency is in 250g rice straw.

In the present study it was found that higher contamination was observed in large sized substrates bags might be due larger surface area of substrates having more space for mycelial growth of contaminants. Tinoco *et. al.,* (2001) however found

that larger the surface area and pore of substrates, more is the mycelial growth rate of fungi. Choi *et al*., (2010) also isolated and identified *Trichoderma, Mucor racemosus* f. *racemosus*, *Aspergillus tubingensis* from *Pleurotus ostreatus* substrates for molecular and morphological characterization. Urmi (2019) isolated 5 fungi namely *Sclerotium rolfsii, Trichoderma harzianum, Fusarium oxysporum, penicillium sp. and Aspergillus niger* from contaminated packet of oyster mushroom. Oxaley (1985) and Earanna (1991) reported that the spawn contamination was occupied to be caused by the air-borne microflora present inside the incubation room. During the transfer of the mother spawn into the autoclaved bags, air carrying air-borne microflora might enter into the bags quickly and lead to contamination of spawn during incubation. Similar kind of results has been recorded in the present study. Contamination of spawn and identification of major contaminants has been worked out by Akhter (2017), Mazumder and Rathaiah (2001), Mazumder *et al*., (2005), Kurtzman (2010) and Kumar (2015) and their results agreed with the result of the present experiment. Biswas (2016) suggest cultivation of *Pleurotus spp.* in temperature zone 14-27 ◦C with 70-80% relative humidity for better production and biological efficiency, which was maintained in the present experiment.

CHAPTER VI
SUMMARY AND CONCLUSION

A series of experiments were conducted on different substrates, such as rice straw, waste paper, saw dust, mustard straw and saw dust were used to observe the growth and yield of pink oyster mushroom considering two size (500g and 250 g) of substrate packets.

Time required for completing mycelium running varied in different substrates. The maximum days (19.43 days) required for mycelium running was recorded from waste paper at 500g substrate/packet whereas the minimum days (14.43 days) found in mustard straw at 250g substrate/packet. The maximum days (4.86 days) required for primordia initiation to 1st harvest was observed from saw dust at 500g substrate/packet whereas the lowest days (4.14 days) was observed in mustard straw at 250g substrate/packet. The highest width (5.21 cm) of pileus was recorded from waste paper at 250g substrate/packet, whereas the minimum width (4.93 cm) of pileus was found in saw dust at 500g substrate/packet. The highest of biological yield (103.14 g) was found from rice straw at 500g substrate/packet. Mustard straw at 500g substrate/packet showed significantly best performance with the total number of fruiting body and rice straw at 500g substrate/packet showed best performance in yield per spawn packets. The better biological efficiency (84.76%) was obtained from rice straw at 250g substrate/packet.

It was revealed that, Percent contamination of fungi gradually increased from incubation stage to 3rd flash stage. Severity of contamination was observed 10.71% in sugarcane bagasse at 500g substrate/packet and 9.29% in saw dust at 500g substrate/packet at 3rd harvest. Waste paper was not contaminated. Large size substrate packets (500g) were contaminated highly comparatively with small size packets (250g)

From the present research work it may be concluded that,

- Considering yield contributing characters in case of yield and biological efficiency it can be concluded that rice straw is suitable as substrate for pink oyster mushroom (*Pleurotus djamor*) cultivation in Bangladesh.
- From the contaminated spawn packets total 3 fungi namely *Trichoderma harzianum*, *penicillium sp*. and *Aspergillus niger* were isolated and identified.
- Considering the severity of contamination, less contamination was observed in 250g substrate packet compared to 500g substrate packet during the cultivation of mushroom.

CHAPTER VII
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CHAPTER VIII

APPENDICS

Appendix 1: Temperature and relative humidity of culture house and outside during pink oyster mushroom cultivation

Duration	**Average Temperature (◦C) of culture house**	**Average RH (%) of culture house**	**Average temperature of outside of culture house (◦C)**	**Average RH (%) of outside of culture house**
February	20	75%	25	42%
March	21	85%	28	45%
April	22	87%	30	50%

RH: Relative humidity

Appendix 2: Fruiting bodies of pink oyster mushroom in the spawn packets of different substrates