EFFECT OF DIFFERENT SUBSTRATES AND MOTHER CULTURES ON YIELD AND YIELD PARAMETERS OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*)

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CERTIFICATE

This is to certify that the thesis entitled "**EFFECT OF DIFFERENT SUBSTRATES AND MOTHER CULTURES ON YIELD AND YIELD PARAMETERS OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*)**" submitted to the Department of Plant Pathology, 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**, embodies the result of a piece of bona fide research work carried out by **MUBASSHIRA MUYEED**, Registration No. **13-05708** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Dated: 30.09.2020
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Dedicated To

My Respected Parents
Who always picked me on time
And encouraged me
&
My Beloved Husband

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ABSTRACT

This study was conducted to determine the effects of different substrates and mother culture materials on yield and yield parameters of oyster mushroom (*Pleurotus ostreatus*) in the Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University and Mushroom Research Institute, Savar, Dhaka. Three substrates namely sawdust, rice straw and sawdust + rice straw (1:1) and three mother cultures namely rice, maize and sawdust were tested for their efficacy in oyster mushroom production. Among the substrates the highest length of the stipe, thickness of pileus and biological yield were found in rice straw (23.27 mm, 5.60 mm and 44.40 g/packet, respectively). The highest diameter of stipe and diameter of pileus were measured in sawdust + rice straw (1:1) (9.90 mm and 72.90 mm, respectively). The highest number of fruiting body/packet was counted in sawdust (6.67). Among the mother cultures the highest length of the stipe, number of fruiting body/packet and biological yield were found in sawdust mother spawn (23.27 mm, 6.67 and 45.47 g/packet, respectively). The highest diameter of stipe and thickness of pileus were observed in maize mother spawn (9.90 mm and 5.60 mm, respectively). The highest diameter of pileus was measured in rice mother spawn (67.57mm). In the combined effect of substrates and mother spawn materials the highest biological yield was recorded in sawdust mother spawn (50.80 g/packet) in which the substrate material was rice straw. The result revealed the good growth performance of oyster mushroom in terms of substrates and mother spawn. So, rice straw substrate and sawdust mother spawn can be recommended for the cultivation of oyster mushroom (*Pleurotus ostreatus*) among the tested substrates and mother spawn culture materials.

CONTENTS

LIST OF ABBREVIATIONS

Abbreviation		Full word
@	=	At the rate
°C	=	Degree Centigrade
Anon.	=	Anonymous
BAU	=	Bangladesh Agricultural University
BBS	=	Bangladesh Bureau of Statistics
CV	=	Coefficient of Variance
DMRT	=	Duncan's Multiple Range Test
e.g.	=	For example
et al.	=	And Others
etc.	=	Etcetera
g	=	Gram
hr	=	Hour (s)
i.e.	=	That is
kg	=	Kilogram
LSD	=	Least Significant Difference
No.	=	Number
SAU	=	Sher-e-Bangla Agricultural University
T	=	Treatment
MRR	=	Mycelium Running Rate
MCC	=	Mushroom Culture Centre
mg	=	Milligram
Conc.	=	Concentration

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CHAPTER 1

INTRODUCTION

Mushroom is a macro fungus. Macro fungi form large fructifications visible without the help of a microscope and have easily observable spore-bearing structures. Macro fungi have a long interest in scientists and the public due to their significant roles in human life, such as their beneficial and devastating impacts on forests, their use in the pharmacology and food industry, and their vital role in biodegradation (Stojchev *et al*., 1998). Mushrooms are becoming increasingly prominent and common in human diets due to their nutritional (Barros *et al*., 2008) and medicinal characteristics (Jedinak *et al*., 2010). Mushrooms are a significantly high potential non-green crop in Bangladesh. It can also play a vital role in improving the nutritional status of the population. It can also significantly improve employment opportunities and the empowerment of the women population in this country. Mushrooms are recognized as an alternative source of good quality protein and can produce the highest quantities of protein per unit area and time from the worthless agro wastes (Chanda and Sharma, 1995). Mushrooms are not only sources of nutrients but also reported as therapeutic and functional foods.

As Bangladesh is a small country with a vast population, mushroom cultivation can play a promising role in the national economy due to its vertical expansion. *Pleurotus* mushrooms, commonly known as oyster mushrooms, grow well in tropical, subtropical, and temperate regions and are easily artificially cultivated. Among all mushrooms, *Pleurotus ostreatus* is a very popular oyster mushroom in Bangladesh because it can be cultivated artificially for suitable weather and climatic conditions. Oyster mushroom (*Pleurotus ostreatus*) belonging to class Basidiomycetes and family Agaricaceae. The popularity of oyster mushroom has been escalating due to its ease of cultivation, high yield potential, and high nutritional value (Banik and Nandi, 2004; Gregori, 2007). Oyster mushrooms help to eliminate the toxicity produces by the agro wastes (Fan, 2000; Murthy and Manonmani, 2008). Mushrooms of *Pleurotus* spp. are also rich in medicinal components and useful in preventing hypertension, hypercholesterolemia (Khatun *et al.*, 2007), hyperglycemia, and different types of cancer (Jose and Janardhanan, 2000). Oyster mushroom production in Bangladesh is expanding rapidly day by day. The cultivation of oyster mushroom makes a significant contribution to the national economy and provides an effective alternative to traditional farming. Nevertheless, during this period of rapid expansion, microbial contamination becomes a threat for oyster mushroom cultivation.

As a vegetable, Mushroom can play a significant role in meeting Bangladesh's population's nutritional requirements. A healthy person requires 200-250g vegetables per day. Nevertheless, in Bangladesh, only 40-50 g vegetable per day is available to people. To get rid of this situation, it is essential to increase the production of vegetables, which need and substantial land areas. Mushroom may be used to reduce the shortage of vegetables since it required minimum land area.

Oyster mushrooms are broadly cultivated because of low-cost and simple production technology with high biological efficiency. The cultivation of *Pleurotus* spp. can be done by using several lignocelluloses waste materials of agricultural and forestry origin. These techniques include a cheap way of reducing waste materials, contributing to safe waste disposal, and conserving our natural environment (Jain, 2005). The present study deals with the cultivation of *Pleurotus ostreatus* on some familiar and abundantly available waste for conversion in food and to check its yield potential to grow on that substrate. Generally, lignocellulosic materials like paddy straw, wheat straw, maize stalks, etc. are used as substrates for the commercial production of oyster mushrooms in our country (Jain and Vyas, 2002). The use of sawdust and rice straw as the principal substrate for mushroom culture has been disseminated broadly in Bangladesh. Despite this, the experimentations done to ascertain the fitness of these locally available lignocellulosic wastes for the advancement of *P. ostreatus* are not sufficient. Hence finding out the most cost compatible strains in this environmental condition has become a significant challenge. With the help of advanced growing technology and controlled temperature, this strain can be made most required out of all *Pleurotus* spp. due to its unique texture and shelf life.

It is a significant concern that commercial production of oyster mushrooms can be seriously affected by the substrates used for mushroom cultivation. This study was conducted to select suitable substrate for mushroom cultivation. In this study we determined the effects of different substrates and mother cultures on yield and yield contributing characters of oyster mushroom *Pleurotus ostreatus* with the following objectives:

i. To study the effect of selected substrates on yield and yield parameters of oyster mushroom (*Pleurotus ostreatus*)
ii. To study the effect of selected mother cultures on yield and yield parameters of oyster mushroom (*Pleurotus ostreatus*)
iii. To study the interaction of the effect of selected substrates and mother cultures on yield and yield parameters of oyster mushroom (*Pleurotus ostreatus*)

CHAPTER 2

REVIEW OF LITERATURE

Rambey *et al*. (2020) carried out a research to estimate the effect of the composition of rice straw mixture on white oyster mushroom growing media on the growth and productivity of white oyster mushrooms (*Pleurotus ostreatus*) and get the best composition of planting media for the growth and productivity of white oyster mushrooms (*P. ostreatus*). *Pleurotus ostreatus* can be grown in a medium derived from wood dust or lignin, which has been weathered and wrapped in plastic. The treatment used is the planting media composition with a mixture of rice straw 0, 10, 20, 30, 40, and 50%. In this study it has been revealed that the rice straw mixture resulted in mycelium growth, and the harvest age began to be longer. The composition of the best growing media for growth and productivity of white oyster mushrooms is a mixture of 30% rice straw (300-gram rice straw + sawdust 550 grams).

Dubey *et al*. (2019) conducted an experiment to determine the effect of different substrates on the performance of oyster mushrooms. In this research, various substrates used as treatments were rice straw (T1), wheat straw (T2), banana leaves (T3), and sugarcane bagasse (T4), each of 4.5 kg and replicated for 4 times. This study revealed that the highest yield (1515 gm) with the highest stipe length (4.86 cm) and cap diameter (5.14 cm) was obtained from the rice straw, followed by other substrates. So this analysis showed that rice straw was best suitable for oyster mushroom production in terms of economic return than other agricultural residues with a B-C ratio of 3.498.

Elattar *et al*. (2019) conducted an experiment. In the current study, the oyster mushroom was grown using various agricultural wastes, including wheat straw, rice straw, sawdust, and water hyacinth, either single or mixed with wheat straw (rice straw + wheat straw, sawdust + wheat straw, and water hyacinth + wheat straw) at a ratio of 1:1 (w/w), to ascertain their significance on growth, composition, and consumer acceptance. The experimental results showed that the highest mushroom yield from the harvesting periods was obtained from rice straw + wheat straw and single rice straw (7600 g and 6650 g, respectively). The mushrooms grown on a mixture of rice straw and wheat straw had the highest yield. It was revealed to be a rich source of protein, minerals, and fibers. It could be established that oyster mushroom developed on a blend of rice straw and wheat straw is nutritious and rich in pharmaceutical-type products.

Ibrahim *et al*. (2015) conducted a research to determine the growth performance, yield, and postharvest quality of different oyster mushroom species cultivated on sawdust and oil palm frond (OPF). Combination of Sawdust and OPF with rice bran and calcium carbonate in 100:10:1 ratio was used as substrates to produce oyster mushroom. In this study, the growth performance in terms of mycelium growth, pinhead emergence, and fruiting body formation was estimated. The shortest time for mycelium growth, mycelium fills up the bag, pinhead emergence, and fruiting body formation was observed in Yellow oyster mushroom on OPF. Although, oyster mushrooms grown on sawdust had higher biological efficiency (29.34 to 60.76%) than on OPF (21.49 to 45.08%). White oyster mushrooms had the highest biological efficiency, followed by yellow, grey, and pink. So this experiment revealed that oyster mushrooms grown on sawdust produced better yield. However, OPF gave better growth performance and physical properties of color and firmness.

Gimay *et al*. (2016) carried out an experiment in which four substrates (cotton seed, paper waste, wheat straw, and sawdust) were tested for their efficacy in oyster mushroom production. In this research, it has been revealed that cotton seed, paper waste, sawdust, and wheat straw can be counted as proper substrates for oyster mushroom production with varying growth performances. The study results have shown that the highest percentage of biological efficiency and biological and economic yield of oyster mushrooms have been obtained from cotton seed among the substrates, while the least was from sawdust. So in this experiment, cotton seed has been recommended as suitable substrates to cultivate oyster mushroom followed by paper waste.

Fekadu (2014) conducted an experiment to assess the growth and yield of *Pleurotus ostreatus* on teff straw. In this experiment, teff straw was shown as the best quality of media (substrate) for the production of oyster mushrooms. This is because a high number of primordial was formed, resulting in the high number of the fruiting body (edible part of mushroom) and high yielding. In conclusion, this study was considered the baseline information to cultivate mushrooms other edible mushrooms on this substrate and the other substrate used for cultivation.

Ashraf *et al*. (2013) conducted an experiment to show the comparison effect of different agricultural wastes on yield and growth of oyster mushroom. In this experiment, 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). This study revealed that, maximum number of fruiting bodies, the fastest spawn running, harvesting stage and primordial initiation was observed in cotton waste within minimum number of days. So among all the treatments cotton waste was found most favourable for mashroom cultivation in this experiment.

Dey *et al*. (2013) carried out a study to find out the suitable substrate for the cultivation of oyster mushroom. Different substrates viz. paddy straw, sugarcane bagasse, and mustard straw were used in this study. These different substrates significantly affected the amount of fresh weight or yield, the number of primordia, and fruiting bodies of oyster mushroom. The highest number of primordia and fruiting bodies, and the amount of fresh weight was obtained with sugarcane bagasse in all flushes whereas the lowest with mustard straw.

Soniya *et al*. (2013) conducted an experiment in which the performance of *Pleurotus ostreatus* was investigated on various substrates such as rice straw, rice straw + paper, rice straw + wheat straw, sawdust, and sugarcane bagasse. Except for rice straw, all the substrates were supplemented with 10% rice bran. The analysis was done on the impacts of various substrates on mycelial growth, primordial initiation time, colonization time, mushroom yield, mushroom size, biological efficiency (BE), and chemical composition. The rice straw was found as the best substrates among all aspects with yield (381.85 gm) and BE (95.46%) to produce mushrooms, followed by paper waste and rice plus wheat straw. The nutritional composition of mushrooms grown on rice straw was also better.

Sonali and Randive (2012) carried out an experiment to investigate the growth of oyster mushrooms on various agricultural waste substrates and their nutrient analysis. The Mushrooms are a good cash crop. The development of Oyster mushroom (Grey and pink) production methodologies on agricultural waste like Paddy straw and wheat straw gives very high yield and nutritional, containing like carbohydrate, protein, ash, calcium, magnesium, crude fibers, and lipid.

Fatema *et al*. (2011) carried out an experiment to study the cultivation of *Pleurotus* sp. on wheat straw, water hyacinth, and their combinations. The results revealed that the best response in the form of pinhead emergence and productivity of mushroom came from the bags containing wheat straw only (3.1 kg), followed by the 3:1 combination of wheat straw + water hyacinth (2.6 kg), 1:1 combination of wheat straw + water hyacinth (1.9 kg), 1:3 combinations of wheat straw + water hyacinth (1.5 kg) and only water hyacinth (0.77 kg) respectively where it took 16, 20, 25, 30 and 40 days for the appearance of pinheads, respectively.

Deepika and Varenyam (2008) conducted an experiment to investigate the effect of five different substrates viz. paddy straw, wheat straw, a mixture of wheat straw and paddy straw (in the ratio of 1:1), lawn grasses, and bamboo leaves for the production of edible oyster mushroom (*Pleurotus ostreatus*). The earliest colonization of fungus was recorded in wheat straw and a mixture of paddy and wheat straw. The highest yield of *Pleurotus ostreatus* was recorded on wheat straw (29.27 g fresh weight/kg substrate), followed by the combination of wheat straw and paddy straw (27.96 g fresh weight/kg substrate). Non-enzymatic antioxidant activities were also obtained by estimating vitamins A, C, and E. Significant amount of vitamin E was detected in both fresh (7.23 mg/g) and dry fruit body (5.93 mg/g) of *P. ostreatus*.

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

Sarker *et al*. (2007) carried out an experiment to determine the performance of different cheap agricultural households' by-products, grasses, and weeds as substrate available in Bangladesh. In different substrates, mycelium growth rate and time required to complete mycelium running in the spawn packet differed significantly. The minimum duration to complete mycelium running was 17.75 days in waste paper, which differed significantly from all other substrates. There was significant variation found in duration from stimulation to primordial initiation, primordial initiation to the first harvest, and stimulation to the first harvest in different substrates. The minimum duration required from stimulation to the first harvest was observed in sugarcane bagasse (6.75 days), which was statistically similar to that in waste paper, wheat straw, and sawdust (7.00 days). The number of the fruiting body was positively correlated with biological efficiency, biological yield, and economic yield of oyster mushroom. The number of fruiting body grown on different substrates differed significantly, and the highest number of the fruiting body per packet (183.25) was recorded on waste paper, which was significantly higher than all other substrates. The lowest number of the fruiting body (19.25) was observed in water hyacinth. Significant variation in biological efficiency, biological yield, and economic yield of oyster mushrooms were observed in different substrates. 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 the same as that grown on rice straw (183.28 g/packet) and kash (182.93 g/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. The performances of the substrates were analyzed based on the benefit-cost ratio (BCR). The highest BCR (6.50) was assessed when wheat straw was used as a substrate, followed by sugarcane bagasse (5.90), waste paper (5.65), rice straw (5.58), and kash (5.25). The lowest BCR was obtained from water hyacinth (1.05), followed by ulu (4.74) and sawdust (4.90).

Namdev *et al*. (2006) ascertained the effect of different straw substrates on spawn growth and oyster mushroom yield. The number of days needed for spawn running was significantly less (14 days) in gram straw, parthenium straw, sugarcane straw, and wheat straw, compared with 20 days for sunflower stalk, mustard, and paddy straw. The yield was highest on paddy straw (666 g/500 g), followed by wheat straw and mustard straw (427 and 400 g/500 g, respectively). The yield was lowest on parthenium straw (95 g/500 g dry substrates).

Habib (2005) carried out an experiment to evaluate different substrates such as rice straw, sawdust, wheat straw, sugarcane bagasse, and waste paper to produce oyster mushroom in a polypropylene bag. The number of fruiting bodies, the number of primordia, and fresh weight or yield of oyster mushroom were affected significantly by different substrates. This experiment showed that the highest number of primordia and fruiting bodies were found in waste paper, 43.75 and 31.00, respectively. The highest amount of fresh weight was also observed in waste paper, 94.25 g.

Iqbal *et al*. (2005) conducted an experiment to ascertain the growth and yield performance of oyster mushroom, *Pleurotus ostreatus* (local & exotic strains), and *P. sajar-caju* on different substrates. The time required to complete spawn running, the formation of pin-heads, and maturation of fruiting bodies on different substrates showed that they appeared earlier on sugarcane bagasse followed by cotton waste. The maximum number of flushes were obtained from wheat straw and banana leaves, followed by cotton waste. Moreover, the results exposed that the minimum flush to flush interval was obtained on millet followed by wheat straw and sugarcane leaves. The maximum yield percentage on fresh and dry weight was obtained from banana leaves followed by paddy and wheat straw.

Al Amin (2004) in his experiment showed that the highest number of primordia of oyster mushroom was found in sterilized paddy straw at first flush and the lowest number of primordia was obtained from sawdust.

Moni *et al*. (2004) showed that the oyster mushroom (*Pleurotus sajor-caju*) was cultivated on paddy straw, banana leaves, sugarcane bagasse, water hyacinth, and beetle nut husk. The fruiting bodies were sun-dried and investigated for various nutritional parameters. There were considerable variation found in the composition of fruiting bodies grown on different substrates was analyzed. Moisture content varied from 88.15 to 91.64%. The percentage of nitrogen and crude protein varied from 4.22 to 5.59 and 18.46 to 27.78%, respectively, and carbohydrate from 40.54 to 47.68% on a dry matter basis. The variation in crude fat content and crude fiber ranged from 1.49 to 1.90 and 11.72 to 14.49%, respectively whereas, the energy value of fruit bodies was between 310.00 and KCal/100 g of fruit body weight.

Maniruzzaman (2004) used wheat, maize, rice straw, and sawdust in his study for the production of oyster mushroom spawn packets and revealed that rice straw was the best for spawn production of oyster mushroom.

Shah *et al*. (2004) carried out an experiment to study the performance of oyster mushroom on the mixture of different substrates. The substrates used in this experiment were: sawdust, wheat straw, leaves, 50 % sawdust + 50 % wheat straw, 50 % wheat straw + 50 % leaves, and 75 % sawdust + 25 % leaves. The temperature was maintained at 25o C for spawn running and 17-20o C for fruiting body formation. The data were recorded in terms of the time for the completion of mycelial growth, pinheads' appearance, and maturation of fruiting bodies on different substrates. The spawn running was observed 2-3 weeks after inoculation, while small pinhead like structures formed within 6-7 days after spawn running. The fruiting bodies appeared 27-34 days after spawn inoculation and 3-6 weeks after pinhead formation. The results revealed that sawdust produced the highest yield (646.9 g) with the highest biological efficiency (64.69 %), and the highest number of fruiting bodies (22.11). Hence, in this study, sawdust acclaimed as the best substrate for oyster mushroom cultivation.

Obodai *et al*. (2003) conducted an experiment to evaluate eight lignocellulosic by-products as the substrates to cultivate oyster mushroom (*Pleurotus ostreatus*). The various substrates used in this study were: rice straw, composted sawdust of *Triplochiton scleroxylon*, banana leaves, corn husk, maize stover, rice husk, Elephant grass, and fresh sawdust and the yields of mushroom on different substrates were: 151.8, 183.1, 111.5, 49.5, 87.5, 23.3, 0.0 g, and 13.0 respectively. The biological efficiency (BE) was the highest in composted sawdust (61.0%). Based on the yield and BE of oyster mushrooms on the substrates tested, rice straw was considered the best alternate substrate for growing oyster mushrooms.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental site and experimental period

The laboratory experiments were conducted in the Department of Plant Pathology laboratory, Sher-e-Bangla Agricultural University, Dhaka and Mushroom Development Institute, Savar, Dhaka during the period from January 2019 to February 2020.

3.2 Experimental materials

The fruiting body of oyster mushroom (*Pleurotus ostreatus*) was collected from Mushroom Development Institute, Savar, Dhaka, to produce mother culture. Sawdust and rice straw used in preparing spawn packets was also collected from Mushroom Development Institute, Savar, Dhaka. Rice grain, maize grain and sawdust were used to prepare mother spawn packets.

3.3 Design layout and treatments of the experiment

The experiment was laid out in a Factorial Completely Randomized Design (CRD). In this experiment, three mother cultures were prepared. Three treatments with five replications were considered under each mother culture. The three mother cultures were produced by using rice grain, maize grain, and sawdust. The thee treatments were-

Factor A: Three mother cultures

M_1: Rice mother spawn packets

M_2: Maize mother spawn packets

M_3: Sawdust mother spawn packets

Factor B: Three substrates

T_1: Sawdust

T_2: Rice straw

T_3: Sawdust + Rice straw (1:1)

3.4 Varietal characteristics of oyster mushroom

The oyster mushroom (*Pleurotus ostreatus*) was characterized by rapid mycelial growth and high saprophytic colonization activity on cellulosic substrates. They are of different colors viz. white, cream, pink, grey, yellow, light brown, etc. Their fruiting bodies are shell or spatula-shaped. In this experiment, the variety PO2 was used, which is white color.

3.5 Production of tissue mother

To produce tissue mother, two steps were followed. Tissue culture of oyster mushroom was done in sterilized PDA (Potato dextrose agar) media. This tissue mother is used to produce the mother culture of oyster mushroom (*Pleurotus ostreatus*).

3.5.1 Preparation of PDA (Potato Dextrose Agar) media

To prepare 1000 ml PDA media, 250 g potatoes were washed, peeled, and sliced at first. Then peeled and sliced potatoes were boiled in water to prepare them soft and filtered through a cheesecloth. To get 1000 mL media more water was added. After that, 20 g dextrose and 20 g agar were added. Then, the prepared solution was heated and stirred for about 45 minutes. Then 10 mL media was taken into each test tube, and the mouths of the test tubes were plugged with cotton and aluminium foil. After that, all the test tubes were sterilized in an autoclave for 20 minutes at 121 °C and 1.5 kg/cm^2. The test tubes were taken out and kept in a slanting position for maximum space for the organism in pure culture proliferate.

3.5.2 Tissue Culture

After collecting a small portion of tissue from the mushroom's fruiting body *Pleurotus ostreatus* it is placed on the sterilized PDA medium under an aseptic condition in a laminar flow cabinet so that a pure culture can be obtained. To ensure adequate mycelial growth, the inoculated PDA medium was kept for 7-10 days in an incubator at 25 °C. These are mentioned pure cultures of *Pleurotus ostreatus* was used for the entire experiment (Figure 1).

Figure 1. Tissue mother of Oyster mushroom

3.6 Production of mother culture

To produce the mother culture of oyster mushroom, different grains or substrates were used. First of all, the grains or substrates were sterilized, and then the inoculation of tissue mother was done. The mother cultures (Figure 2) were ready to use after running full mycelium, and it turned into white color.

3.6.1 Preparation and sterilization of the grains/substrates

For the production of the mother culture of *P. ostreatus,* three selected grains were taken. Production of the mother was carried out on Rice grain, Maize grain, and sawdust substrate. Two kg of each grain was taken separately. The grains were cleaned thoroughly, washed, and soaked in water for 4 to 6 hrs. 0.5 gm carbendazim was mixed with each 2 kg of the rice grain, 2 kg of maize grains, and 1.5 kg of sawdust. The grains were cooked until they become soft. The cooked grains were decanted on the sieve to remove excess water and evenly spread on muslin cloth for cooling. The cooled grains were mixed with calcium carbonate (10g/kg) to avoid clumping of grains and maintained the pH. These prepared grains and substrate were taken (250 g each) in poly bags and plugged with non-absorbent cotton and sterilized in an autoclave for 1.5 hrs.

3.6.2 Inoculation of tissue mother

Twelve packets of each grain/substrate and a total of 36 packets were prepared. 5 mm diameter mycelial disc of *P. ostreatus* was inoculated in each bag and incubated at 25 ºC for mycelial growth.

3.6.3 Mycelium running in the mother culture

Days for mycelium initiation on grains/substrate, days for complete colonization of mycelium on grains/substrate were carried out. The mother culture packets were kept at 22-25°C temperature until they became white with the mushroom mycelium. After completion of the mycelium running, the mother cultures were ready to use for spawn packets preparation (Figure 2).

Figure 2. Mother cultures of Oyster mushroom

3.7 Production of spawn packets

Fifteen spawn packets of rice mother, 15 of maize mother, and 15 of sawdust mothers were prepared. Each packet was 500 g in weight, which contained 400 g substrate and 100 g mother.

3.7.1 Preparation and sterilization of the substrates

Sawdust and rice straw was pasteurized in the pasteurization chamber for an hour. Then rice straw was chopped into small pieces and mixed with sufficient water to make moist. Sawdust was also mixed with water to make it moist. Then a total of 45 spawn packets were prepared.

3.7.2 Inoculation of mother culture in the spawn packet

The produced mother cultures were mixed with the substrate to prepare spawn packets. The substrates were 400g in weight, and 100g mother culture was mixed with the substrates to prepare each spawn packet. Then the mouth of the spawn packets was tightly wrapped with plastic neck, rubber, and cotton. Fifteen spawn packets for each mother and a total of 45 spawn packets were prepared. The prepared spawn packets were kept in the culture room for mycelium growth. Observations were recorded with three treatments and five replications.

3.7.3 Mycelium running in the spawn packets

Observation on days for mycelium initiation on grains/substrate, days for complete colonization of mycelium on grains/substrate were carried out. The prepared spawn packets were kept in the culture room for mycelium growth. After completion of the full mycelium running, the spawn packets become white color (Figure 3 and Figure 4).

Figure 3. Spawn packets before mycelium running

Figure 4. Spawn packets after mycelium running

3.7.4 Cultivation of the spawn packets

The two ends, opposite each other of the plastic bag's upper position, were cut in "D" shape with a blade and pierced by removing the plastic sheet. After that, the opened surface of the substrate was scraped slightly with a teaspoon for removing the thin whitish mycelia layer. The spawn packets were soaked in water for 15 minutes and invested to remove excess water for 15 minutes. Each type's packets were arranged separately on the floor of the culture room and covered with newspaper. The culture room moisture was controlled by 80-85% relative humidity by spraying water three times a day. The light around 300-500 lux and ventilation of the culture house was controlled uniformly. The temperature of the culture house was maintained at 22-25 °C. The first primordial appeared 3-5 days after removing the plastic sheet depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate (Figure 5).

Figure 5. Spawn packet with fruiting body of Oyster mushroom

3.7.5 Harvesting of the oyster mushroom (*Pleurotus ostreatus*)

After primordial initiation, oyster mushrooms developed within 2-3 days. The cap's cural margin identified the matured fruiting body, as reported by Sarker *et al*. (2007). Mushrooms were harvested by twisting to uproot from the bottom. Mushrooms were collected three times from a packet. The packets were scrapped again after the first harvest at the place where the "D" shaped cut had been done and then placed in the culture house. Water was sprayed regularly to keep the packets moist. The primordial developed 9-10 days after the first harvest and 7-8 days after the second harvest. Water was sprayed until the mushrooms were ready to be harvested. Then the harvested mushroom samples were subjected to growth and yield analysis. The whole cultivation procedure of oyster mushroom is presented by flow chart (Figure 6 and Figure 7).

3.8 Data collection

Data were recorded on the following parameters:

- Length of the stipe (mm)
- Diameter of the stipe (mm)
- Diameter of the pileus (mm)
- Thickness of pileus (mm)
- Number of the fruiting body
- Biological yield: Biological yield per 500 g packet was measured by weighing the whole fruiting body cluster without removing the lower hard and dirty portion.

3.9 Statistical analysis of data

All the data collected on different parameters were statistically analyzed by following the analysis of variance (ANOVA) technique, and mean differences were adjusted by Least Significant Difference Test (LSD) (Gomez and Gomez, 1984) using the MSTAT-C computer package program. The mean differences among the treatments were compared by the least significant difference (LSD) at 5% level of significance.

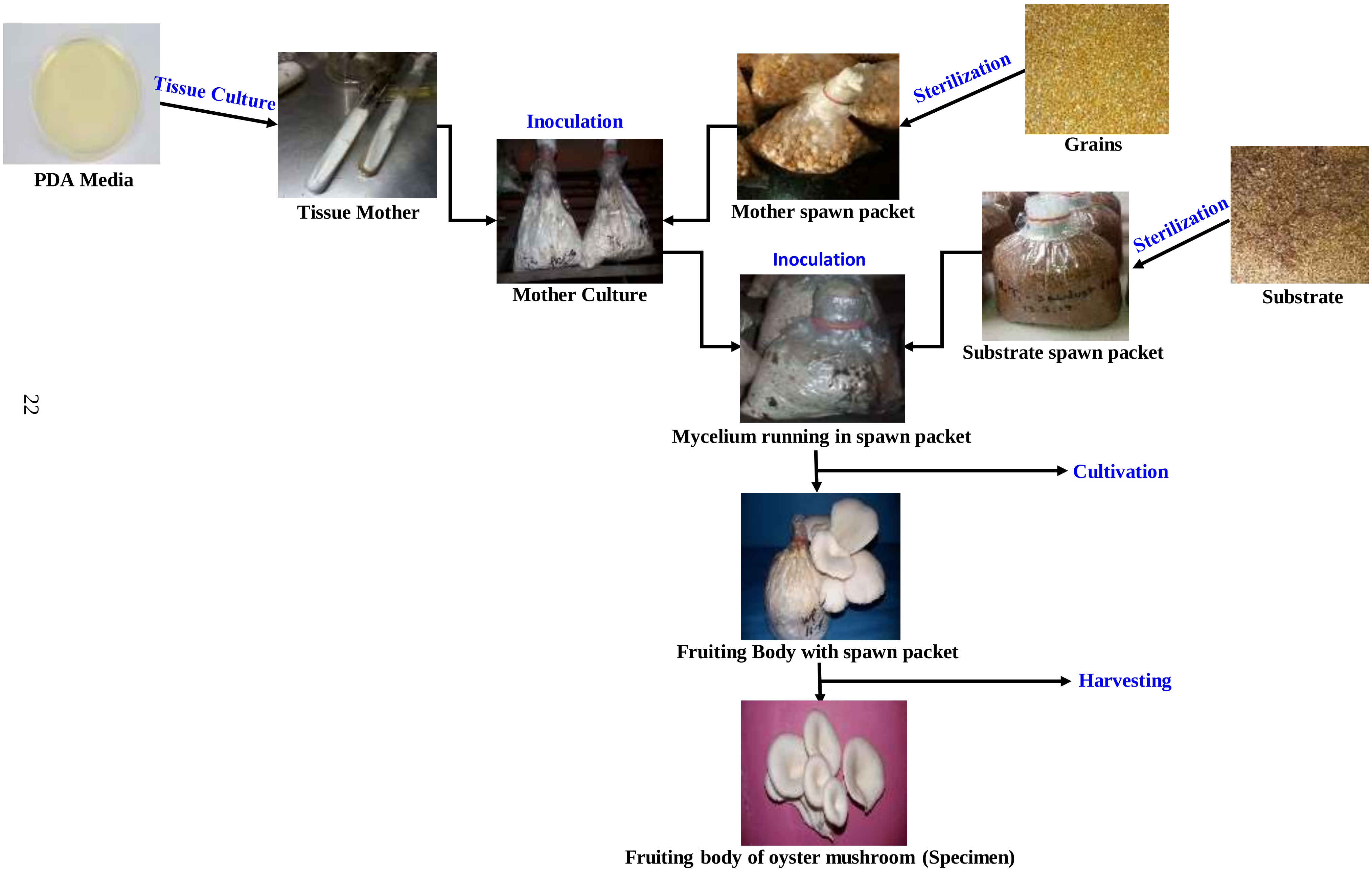

Figure 6. Flow chart of Oyster Mushroom (*Pleurotus ostreatus*) Cultivation

Figure 7. Photograph of experiment set up for Oyster mushroom production

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of substrate materials on yield and yield parameters of oyster mushroom (*Pleurotus ostreatus*)

Significant variations of yield and yield parameters were found in Oyster mushrooms due to the effect of substrate materials. The result of yield and yield parameters are presented in table 1.

4.1.1 Length of stipe (mm)

The highest average length of stipe was observed in rice straw (23.27 mm) and the lowest average length of stipe was in the mixture of sawdust + rice straw (22.33 mm). There was no statistical significant difference among the treatments in terms of average length of stipe.

4.1.2 Diameter of stipe (mm)

The highest average diameter of stipe was observed in the mixture of sawdust + rice straw (1:1) (9.90 mm) and the lowest average diameter of stipe was in sawdust (7.13 mm). The treatments differed significantly in terms of average diameter of stipe.

4.1.3 Diameter of pileus (mm)

The highest average diameter of pileus was observed in the mixture of sawdust + rice straw (1:1) (72.90 mm) and the lowest average diameter of pileus was in sawdust (56.27 mm). The treatments differed significantly in terms of average diameter of pileus.

4.1.4 Thickness of pileus (mm)

The highest average thickness of pileus was observed in rice straw (5.60 mm) and the lowest average thickness of pileus was in sawdust (5.20 mm). There was no statistical significant difference among the treatments in terms of average thickness of pileus.

4.1.5 Number of fruiting body

The highest average number of fruiting body was observed in sawdust (6.67) and the lowest average number of fruiting body was observed in rice straw (6.33). There was no statistical significant difference among the treatments in terms of number of fruiting body.

Table 1. Effect of substrate materials on length and diameter of stipe, diameter and thickness of pileus and number of fruiting body of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Length of stipe (mm)	Diameter of stipe (mm)	Diameter of pileus (mm)	Thickness of pileus (mm)	Number of fruiting body/packet
Sawdust	22.40	7.13 b	56.27 b	5.20	6.67
Rice straw	23.27	9.33 a	67.13 a	5.60	6.33
Sawdust + Rice straw (1:1)	22.33	9.90 a	72.90 a	5.33	6.60
$LSD_{(0.05)}$	--	2.107	7.399	--	--
CV(%)	16.18	32.38	15.27	17.75	30.27

LSD = Least Significant Difference

CV = Coefficient of Variance

4.1.6 Effect of substrate materials on biological yield (g/packet)

The following graph (Figure 8) shows that, the highest average biological yield was observed in rice straw (44.40 g/packet) and the lowest average biological yield was observed in sawdust (41.73 g/packet).

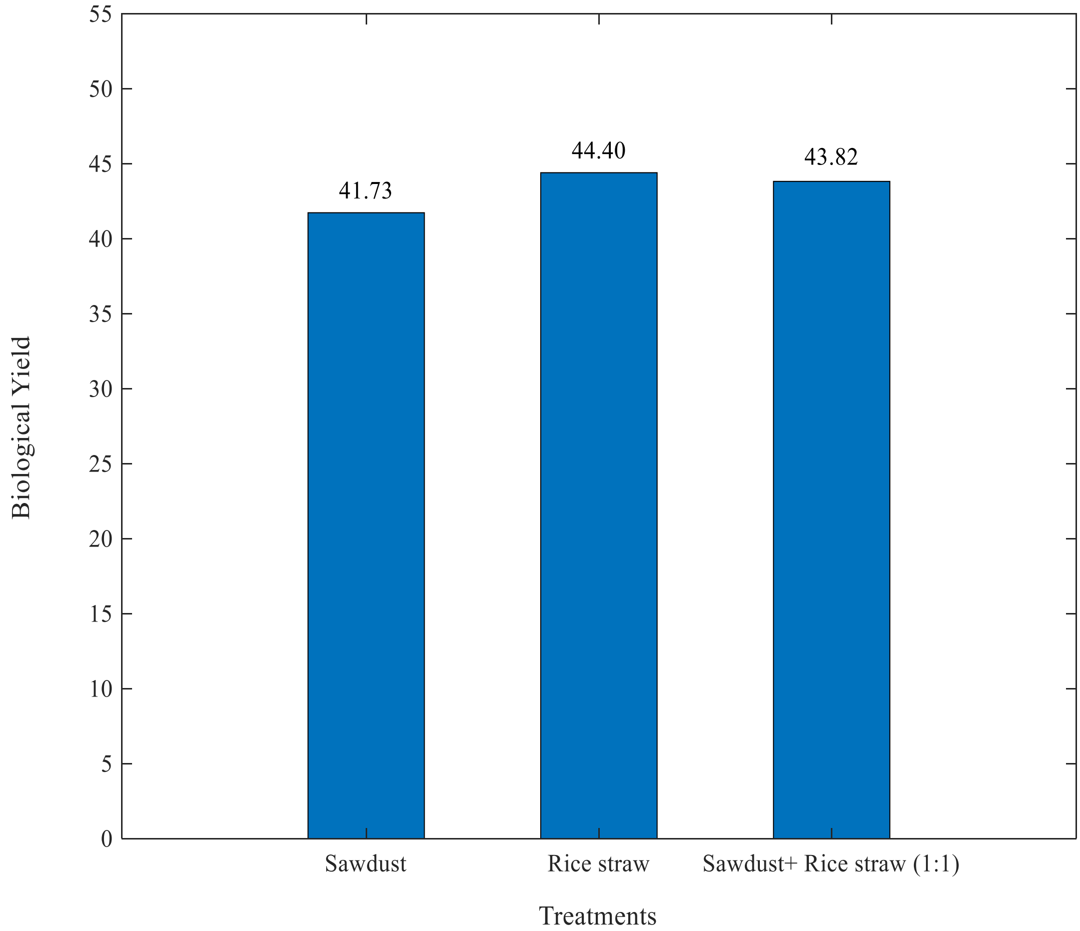

Figure 8. Effect of substrate materials on biological yield (g/packet) of Oyster mushroom (*Pleurotus ostreatus*)

4.2 Effect of mother spawn packet materials on yield and yield parameters of oyster mushroom (*Pleurotus ostreatus*)

Significant variations of yield and yield parameters were found in Oyster mushrooms due to the effect of mother spawn. The result of yield and yield parameters are presented in table 2.

4.2.1 Length of stipe (mm)

The highest average length of stipe was observed in sawdust mother spawn packets (24.29 mm) and the lowest average length of stipe was in maize mother spawn packets (20.00 mm). The treatments differed significantly in terms of average length of stipe.

4.2.2 Diameter of stipe (mm)

The highest average diameter of stipe was observed in maize mother spawn packets (10.01 mm) and the lowest average diameter of stipe was sawdust mother spawn packets (7.72 mm). There was no statistical significant difference among the treatments in terms of average diameter of stipe.

4.2.3 Diameter of pileus (mm)

The highest average diameter of pileus was observed in rice mother spawn packets (67.57mm) and the lowest average diameter of pileus was in sawdust mother spawn packets (62.56 mm). There was no statistical significant difference among the treatments in terms of average diameter of pileus.

4.2.4 Thickness of pileus (mm)

The highest average thickness of pileus was observed in both rice mother and maize mother spawn packets (5.47mm) and the lowest average thickness of pileus was in sawdust mother spawn packets (5.20 mm). There was no statistical significant difference among the treatments in terms of average diameter of pileus.

4.2.5 Number of fruiting body

The highest average number of fruiting body was observed in sawdust mother spawn packets (7.33) and the lowest average number of fruiting body was observed in maize mother spawn packets (5.67). There was no statistical significant difference among the treatments in terms of average number of fruiting body.

Table 2. Effect of mother spawn packet materials on length and diameter of stipe, diameter and thickness of pileus and number of fruiting body of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Length of stipe (mm)	Diameter of stipe (mm)	Diameter of pileus (mm)	Thickness of pileus (mm)	Number of fruiting body/packet
Rice mother spawn packets	23.71 a	8.64	67.57	5.47	6.60
Maize mother spawn packets	20.00 b	10.01	66.17	5.47	5.67
Sawdust mother spawn packets	24.29 a	7.72	62.56	5.20	7.33
$LSD_{(0.05)}$	2.716	--	--	--	--
CV(%)	16.18	32.38	15.27	17.75	30.27

LSD = Least Significant Difference

CV = Coefficient of Variance

4.2.6 Effect of mother spawn packet materials on biological yield (g/packet)

The highest average biological yield was observed in sawdust mother spawn packets (45.47 g/packet) and the lowest average biological yield (39.16 g/packet) was observed in maize mother spawn packets which are shown in figure 9.

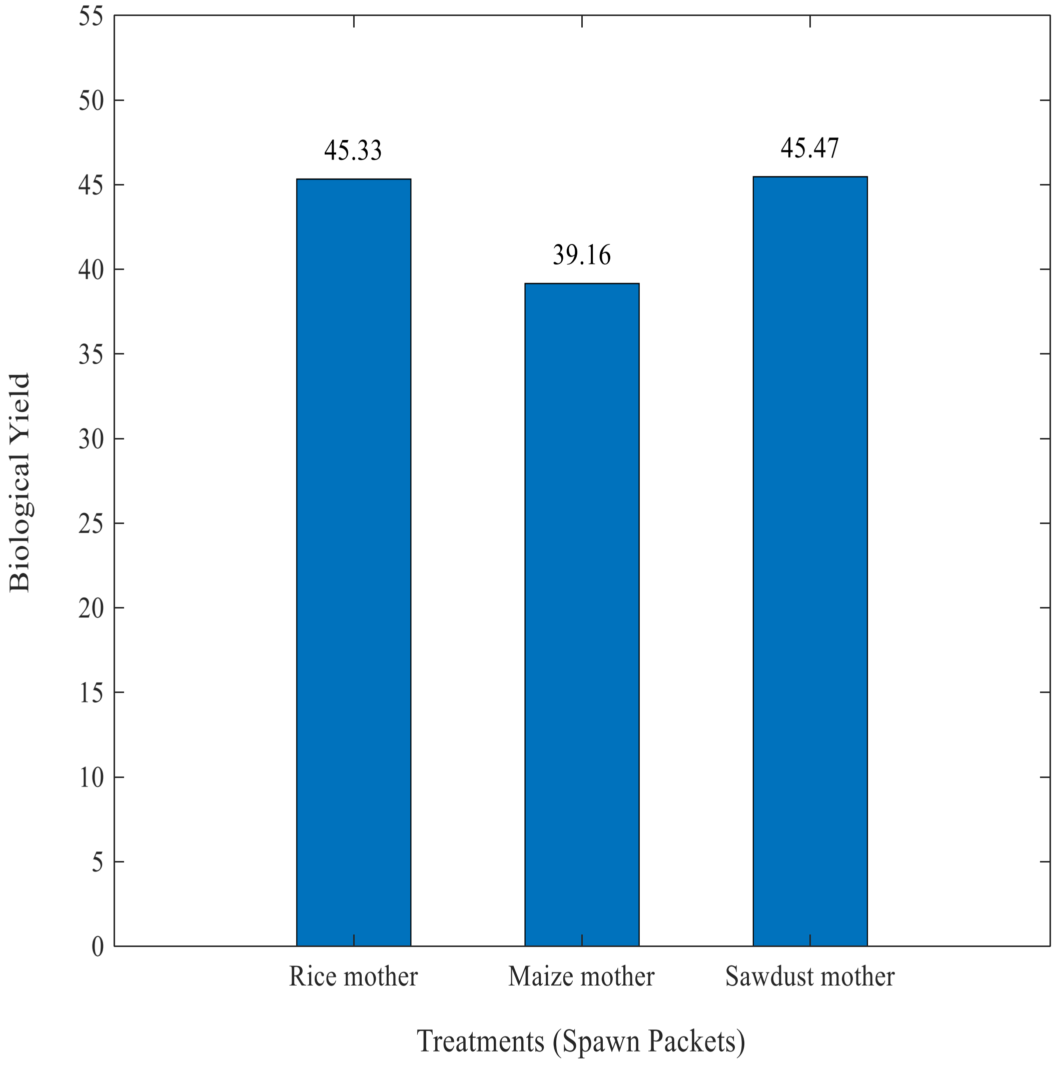

Figure 9. Effect of mother spawn packet materials on biological yield (g/packet) of Oyster mushroom (*Pleurotus ostreatus*)

4.3 Combined effect of substrate and mother spawn packet materials on yield and yield parameters of oyster mushroom (*Pleurotus ostreatus*)

The result of combined effect of substrate and mother spawn packet materials on yield and yield parameters of Oyster mushroom (*Pleurotus ostreatus*) are shown in table 3.

4.3.1 Length of stipe (mm)

The highest average length of stipe was observed in T_1M_3 (26.80 mm) which was sawdust mother spawn packet and the substrate material was sawdust and the lowest average length of stipe was in T_1M_2 (17.40 mm) maize mother spawn packet and the substrate material was sawdust. The other treatments differed significantly in terms of average length of stipe.

4.3.2 Diameter of stipe (mm)

The highest average diameter of stipe was observed in T_3M_2 (11.62mm) which was maize mother spawn packet and the substrate material was sawdust + rice straw (1:1) and the lowest average diameter of stipe was in T_1M_3 (6.40 mm) which was sawdust mother spawn packet and the substrate material was sawdust. There was no statistical significant difference among the treatments in terms of average diameter of stipe.

4.3.3 Diameter of pileus (mm)

The highest average diameter of pileus was observed in T_3M_2 (74.90 mm) which was maize mother spawn packet and the substrate material was sawdust + rice straw (1:1) and the lowest average diameter of pileus was inT_1M_3 (54.00 mm) which was sawdust mother spawn packet and the substrate material was sawdust. There was no statistical significant difference among the treatments in terms of average diameter of pileus.

4.3.4 Thickness of pileus (mm)

The highest average thickness of pileus was observed in both T_3M_1 and T_2M_2 (5.80 mm). The T_3M_1 was rice mother spawn packet and the substrate material was sawdust + rice straw (1:1) and T_2M_2 was maize mother spawn packet and the substrate material was rice straw. The lowest average thickness of pileus was observed in T_1M_1 (5.00 mm) which was rice mother spawn packet and the substrate material was sawdust. There was no statistical significant difference among the treatments in terms of average thickness of pileus.

4.3.5 Number of fruiting body

The highest average number of fruiting body was observed in T_2M_3 (8.00) which was sawdust mother spawn packet and the substrate material was rice straw and the lowest average number of fruiting body was observed in T_2M_2 (6.33) which was maize mother spawn packet and the substrate material was rice straw. There was no statistical significant difference among the treatments in terms of number of fruiting body.

Table 3. Combined effect of substrate and mother spawn packet materials on length and diameter of stipe, diameter of pileus, thickness of pileus and number of fruiting body of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Length of stipe (mm)	Diameter of stipe (mm)	Diameter of pileus (mm)	Thickness of pileus (mm)	Number of fruiting body/packet
T_1M_1	23.00ab	7.40	57.00	5.00	7.00
T_1M_2	17.40 c	7.60	57.80	5.40	6.00
T_1M_3	26.80 a	6.40	54.00	5.20	7.00
T_2M_1	24.20ab	8.40	71.40	5.60	6.00
T_2M_2	19.80bc	10.80	65.80	5.80	5.00
T_2M_3	25.80 a	8.80	64.20	5.40	8.00
T_3M_1	23.94ab	10.12	74.32	5.80	6.80
T_3M_2	22.80ab	11.62	74.90	5.20	6.00
T_3M_3	20.26bc	7.96	69.48	5.00	7.00
$LSD_{(0.05)}$	4.704	--	--	--	--
CV(%)	16.18	32.38	15.27	17.75	30.27

LSD = Least Significant Difference

CV = Coefficient of Variance

T_1 = Sawdust, M_1 = Rice mother spawn packets, T_2 = Rice straw, M_2 = Maize mother spawn packets, T_3 = Sawdust + Rice straw (1:1), M_3 = Sawdust mother spawn packets

4.3.6 Combined effect of substrates and mother spawn packet materials on biological yield (g/packet)

The combined effect of substrates and mother spawn packets materials on biological yield (g/packet) is shown in figure 10. The highest average biological yield was observed in sawdust mother spawn packets (50.80 g/packet) in which the substrate material was rice straw and the lowest average biological yield was recorded in maize mother spawn packets (37.60 g/packet) in which the substrate material was both sawdust and rice straw (Figure 10).

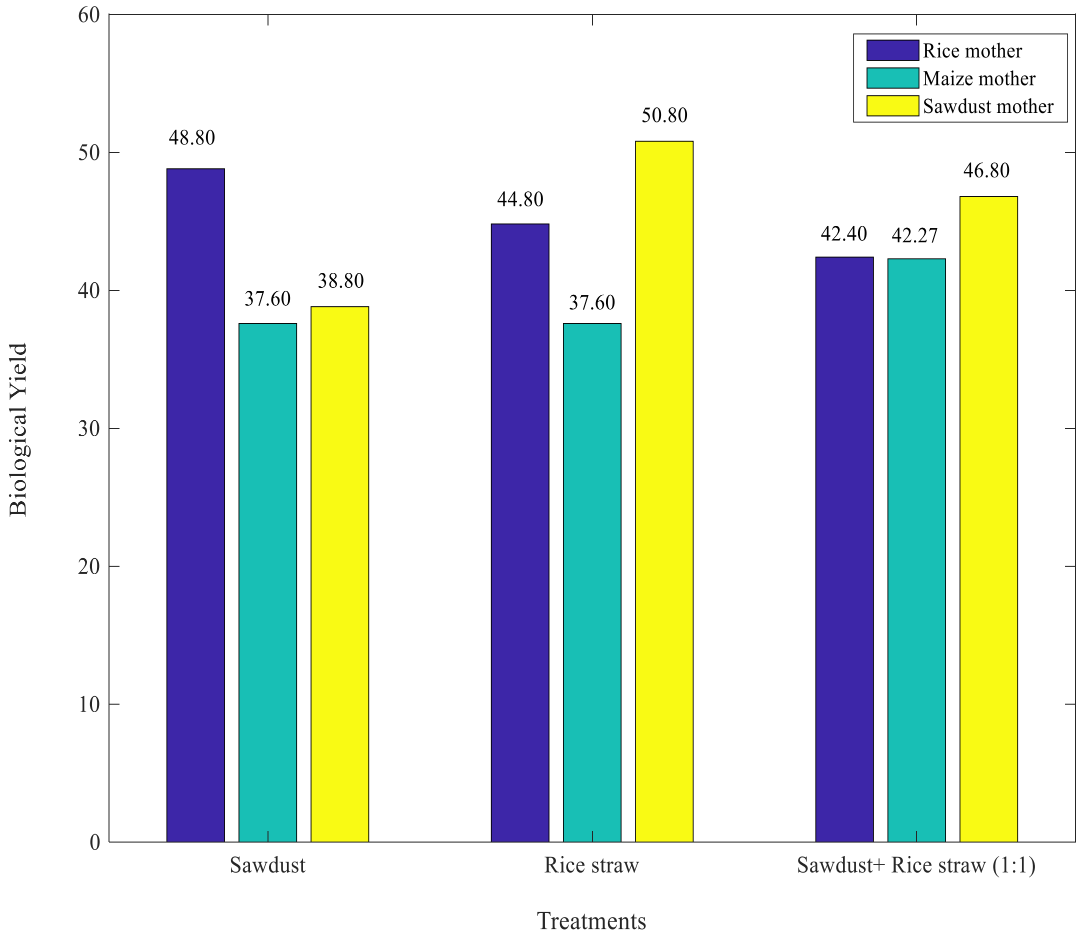

Figure 10. Combined effect of substrates and mother spawn packet materials on biological yield (g/packet) of Oyster mushroom (*Pleurotus ostreatus*)

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Mushroom is fruiting body of macro fungi. These are highly valued for their rich characteristic flavor, potent nutritional properties and possess various types of dietary supplements. Mushrooms are low in calorific value but rank very high for their vitamins, minerals and protein contents (Beetz and Greer, 1999). A macro fungus is a species whose basidiocarp is visible in naked eye. The number of recognized mushroom species has been reported to be 14,000, which is about 10% of the total estimated mushroom species on the earth (Cheung, 2008). Some of those are edible and some are toxic. Mushroom production is an appropriate technology for the management of agricultural and agro-industrial residues. It can play an important role in improving the nutritional status of the population. Mushrooms are recognized as an alternative source of good quality protein and are capable of producing the highest quantities of protein per unit area and time from the worthless agro wastes (Chanda and Sharma, 1995). Mushroom production could be a possible option to alleviate poverty and improve the lifestyle of vulnerable people (Imtiaj and Rahman, 2008). More than 2,000 species composed of 31 genera are identified to be edible over the world (Moore, 2005). Twelve species are commonly grown for food and/or medicinal purposes, across tropical and temperate zones. *Pleurotus ostreatus*, commonly known as oyster mushrooms, are edible fungi cultivated worldwide especially in South East Asia, India, Europe and Africa. China produces 64% edible mushrooms in the world and 85% of all oyster mushrooms all over the world. Mushroom cultivation provides an environmentally friendly and economical way of converting agricultural and forest wastes into nutritious food.

The three most commonly cultivated mushrooms are *Agaricus bisporus* (button mushroom), *P. ostreatus* (oyster mushroom) and *Lentinul aedodes* (shiitake mushroom). Oyster mushrooms are the second largest commercially produced mushroom in the world (Sánchez, 2010; Mohamed *et al*., 2011) next to *A. bisporus*. Shorter growth time is required to oyster mushrooms in comparison to other edible mushrooms. It converts a high percentage of the substrates to fruiting bodies and hence increases profitability and low-cost cultivation technology (Baysal *et al*., 2003).

The consumption of oyster mushrooms has an advantage of preventing as well as reducing diseases such as diabetes, heart disease, high blood cholesterol level, gastric cancer, hepatitis B, liver illness, kidney problems, hypertension, microbial infection, chronic fatigue syndrome and impaired immune response (Ooi, 2000). These mushrooms are known to contain therapeutic ingredients such as dietary fibers and phenolic compounds various bioactive compounds. Oyster mushrooms are saprophytes that decompose agricultural plant by-product as they have the ability to use cellulose, hemicelluloses, and lignin materials as a source of their nourishment (Ragunathan *et al*., 1996). Oyster mushroom is known for its ability to degrade lignocelluloses residues from agricultural fields and forests and convert them into protein-rich biomass (Rowel *et al*., 2000). Species of oyster mushroom show good adaptability to a wide range of temperature, making it possible to grow this mushroom almost all year round without controlled climatic conditions (Chadha, 2001; Baysal *et al*., 2003). Oyster mushroom production is a useful method of environmental waste management and waste disposal. The cultivation of oyster mushroom adds value to the economy, environmental restoration and food security (provision) worldwide.

There is a number of study conducted on utilization efficiency of agricultural waste by the mushroom. Hence, this study was conducted to evaluate the best substrate materials for oyster mushroom cultivation using locally available agro-industrial by-products. Oyster mushroom can be grown on various substrates including rice straw, maize stalks/cobs, vegetable plant residues, bagasse etc. (Hassan *et al*., 2011). This has been reported to influence its growth, yield, and composition (Iqbal *et al*., 2005). However, an ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth (Khare *et al*., 2010). Quimio (1987) stated that cellulose rich organic substance has been reported to be of good substrates for the cultivation of mushrooms. Substrates with high lignin and phenolic content decreased the activity of cellulase, but less lignin would enhance enzyme activity and thus ensure higher yield of mushrooms (Sivaprakasam, 1980). So, oyster mushroom cultivation can play a vital role in managing organic wastes. In Bangladesh, about 30 million tons of agricultural wastes like sawdust, rice straw, wheat straw and sugarcane bagasse are available (Ahmed, 2001), which can be used for mushroom cultivation.

In this experiment, sawdust, rice straw, and sawdust + rice straw (1:1) were used as substrate materials. The spawn packets were prepared by using three different mothers – Rice mother, Maize mother, and Sawdust mother.

During this experiment, the effects of both substrate materials and mother spawn packets materials were observed on mushroom yields and yield contributing characters like length of stipe, diameter of stipe, diameter of pileus, thickness of pileus, number of fruiting body/packet, and biological yield (g/packet). The average length of stipe was observed highest in rice straw (23.27 mm) and lowest was in sawdust + rice straw (22.33 mm). The average diameter of stipe was observed in sawdust + rice straw (9.90 mm) and lowest in sawdust 7.13 mm). This study corroborates the result of Sarker *et al*. (2007) where he observed that, the diameter of stipe ranged from 7.00 mm to 9.90 in case of oyster mushroom. The highest average diameter of pileus was observed in the mixture of sawdust + rice straw (72.90 mm) and the lowest average diameter of pileus was in sawdust (56.27 mm). Sarker *et al*. (2007) observed that, the average diameter of pileus ranged from 40.00 mm to 75.00 mm. The highest average thickness of pileus was observed in rice straw (5.60 mm) and the lowest average thickness of pileus was in sawdust (5.20 mm). Sarker, (2004) observed that, the thickness of pileus of oyster mushroom was ranged from 5.20 mm to 6.20 mm. The highest average number of fruiting body was observed in sawdust (6.67) and the lowest average number of fruiting body was observed in rice straw (6.33). The large sized fruit bodies are considered to be of good quality and rated highly in mushroom production (Onyango *et al*., 2011).

In case of biological yield, the effect of substrate materials was observed. The highest average biological yield was observed in rice straw (44.40 g/packet) and the lowest average biological yield was observed in sawdust (41.73 g/packet). Oyster mushroom mycelia invasion took the minimum number of days (14) for spawn running on sugarcane bagasse followed by sorghum (16 days), barley (17 days). Sugarcane bagasse may be an appropriate source of carbon and energy for mycelia colonization and spawn production. The growth of Oyster mushroom (*P. ostreatus*) mycelia was relatively faster on a combination of sawdust + teff straw wastes as compared to the remaining three combinations (Besufekad *et al*., 2020). Similarity, Tsegaye and Tefera (2017) reported the production of spawn on sugarcane bagasse took the shortest time (14 days) compared to other grains sorghum and millet that took (16 to 17 days). The result further supported by (Rana *et al*., 2007) on oyster mushroom showed significantly rapid growth on different grains as compared to the rest of other mushroom species. Bisaria *et al.* (1987) who reported that, the *Pleurotus ostreatus* yield with rice straw was 11.7% higher than wheat straw. Baysal *et al*. (2003) found the highest yield of oyster mushroom (*Pleurotus ostreatus*) with the substrate composed of 20% rice husk in weight. Khanna and Garcha (1981) recorded cumulative yield of 32% in 104 days from rice straw. Dubey *et al*. (2019) conducted an experiment by growing *Pleurotus ostreatus* on four different substrates i.e. paddy straw, wheat straw, sugarcane bagasse and banana leaves. Among all the treatments, rice straw was found most favorable for mushroom cultivation in terms of yield (1515 g) than other substrates banana leaves, wheat straw and sugarcane bagasse. Kulsum *et al*. (2009) found that the yield was 21.27 g due to sawdust. In this experiment the biological yield in sawdust was 41.73 g/packet. Khan *et al.* (1991) reported that sawdust amended with different organic supplement like rice straw, wheat chaff, wheat bran, cotton waste etc. provided suitable condition for spawn running. The highest mycelium colonization, primordial initiation, fruiting bodies formation, and fresh weight were obtained from sawdust + teff straw with a yield of 730 g/kg (Besufekad *et al*., 2020). Adjapong *et al*. (2015) reported about 32.99 g of fruiting bodies of mushroom harvested per crop on maize husk.

Tsegaye and Tefera (2017) reported highest fresh yield of mushroom (790 g/kg) harvested from a combination of cotton waste + coffee pulp.

Mushroom cultivation requires carbon, nitrogen and inorganic compounds as their nutritional sources, and main nutrients are carbon sources such as cellulose and lignin. Oyster mushrooms require less nitrogen and more carbon source. Thus, most organic matters containing cellulose, hemicellulose and lignin can be used as the mushroom substrate. The ability of oyster mushroom to grow successfully on the combination of sawdust and teff straw associated with the essential chemical composition of selected substrates is important for the growth of mushroom. Variations observed in the number of fruiting bodies produced may be associated with the physical nature of the substrates as well as the nature of the mushroom species. The number of fruit bodies recorded is related to their mycelia colonization (Besufekad *et al*., 2020).

In case of mother spawn packets, the highest average length of stipe was observed in sawdust mother spawn packets (24.29 mm) and the lowest average length of stipe was in maize mother spawn packets (20.00 mm). The highest average diameter of stipe was observed in maize mother spawn packets (10.01 mm) and the lowest average diameter of stipe was sawdust mother spawn packets (7.72 mm). The highest average diameter of pileus was observed in rice mother spawn packets (67.57 mm) and the lowest average diameter of pileus was in sawdust mother spawn packets (62.56 mm). The highest average thickness of pileus was observed in both rice mother and maize mother spawn packets (5.47 mm) and the lowest average thickness of pileus was in sawdust mother spawn packets (5.20 mm). The highest average number of fruiting body was observed in the mixture of sawdust mother spawn packets (7.33) and the lowest average number of fruiting body was observed in maize mother spawn packets (5.67). The large sized fruit bodies are considered to be of good quality and rated highly in mushroom production (Onyango *et al*., 2011). The biological yield was observed highest in sawdust mother spawn packets (45.47 g/packet) and the lowest in maize mother spawn packets (39.16 g/pack). The maximum value of fresh weight is obtained when we have used sawdust + teff straw and cotton seed + teff straw with a yield of 291.02 g and 279.90 g, respectively. While the least yield of fresh weight obtained from a combination of teff straw and enset waste with the yield of 151.08 g. The growth parameters such as ring diameter and stipe length showed the non-significance level at probability less than 5% (pet) (Besufekad *et al*., 2020).

The combined effect of substrate materials and mother spawn packet materials on biological yield of oyster mushroom has been showed in this experiment. The highest average biological yield was observed in sawdust mother spawn packets (50.80 g/packet) in which the substrate material was rice straw and the lowest average biological yield was observed in maize mother spawn packets (37.60 g/packet) in which the substrate material was both sawdust and rice straw. In another study, the highest mean value of growth and yield parameters are obtained from the combination of sawdust and teff straw. While the combination of teff straw and onset waste gives the lowest mean value of growth and yield parameters (Besufekad *et al*., 2020).

So, the findings of the present study clearly indicated that, among the substrates tested the rice straw is the best substrate for oyster mushroom (*Pleurotus ostreatus*) production as yield is higher in rice straw. On the other hand, sawdust mother spawn gave the best result in mushroom production with highest yield.

CHAPTER 5

SUMMARY AND CONCLUSION

The present research was conducted to evaluate the effect of different substrates in oyster mushroom cultivation (*Pleurotus ostreatus*). This research confirmed that the use of different substrates brought about significant effect on yield of oyster mushroom. This study was carried out with three treatments of both for the substrate materials and mother spawn packet materials with four replications. The effect of different substrates was observed in yield and yield contributing characters such as length of stipe (mm), diameter of stipe (mm), diameter of pileus (mm), thickness of pileus (mm), number of fruiting body/packet and biological yield (g/packet) of oyster mushroom (*Pleurotus ostreatus*).

Many researchers found different effects of substrates on yield and yield parameters of oyster mushroom. It could be summarized from this study that, the substrate materials and mother spawn packet materials have significant effects on mushroom yield. Different yield parameters were observed and revealed that, the mushroom growing substrates have significant effects on yield and yield contributing characters. In case of substrate materials, the highest average length of stipe was found in rice straw (23.27 mm) and lowest was in sawdust + rice straw (22.33 mm). The diameter of the stipe observed highest in sawdust + rice straw (9.90 mm) and lowest in sawdust (7.13 mm). The diameter of pileus was observed also highest in sawdust + rice straw (72.90 mm) and the lowest in sawdust (56.27 mm). The highest average thickness of pileus was observed in rice straw (5.60 mm) and the lowest average thickness of pileus was in sawdust (5.20 mm). The number of fruiting body was recorded highest in sawdust (6.67) and the lowest average number of fruiting body was observed in rice straw (6.33). In case of mother spawn packet materials, the observations showed that, the highest average length of stipe was observed in sawdust mother spawn packets (24.29 mm) and the lowest in maize mother spawn packets (20.00 mm).

The average diameter of pileus was observed highest in maize mother spawn packets (10.01 mm) and the lowest in sawdust mother spawn packets (7.72 mm). The highest average diameter of pileus was observed in rice mother spawn packets (67.57 mm) and the lowest in sawdust mother spawn packets (62.56 mm).The thickness of pileus was observed highest in both rice mother and maize mother spawn packets (5.47 mm) and the lowest in sawdust mother spawn packets (5.20 mm). The highest average number of fruiting body was recorded in sawdust mother spawn packets (7.33) and the lowest in maize mother spawn packets (5.67). The large sized fruiting bodies are considered to be of good quality and rated highly in mushroom production.

The biological yield of oyster mushroom (*Pleurotus ostreatus*) was found significant variations due to use of different substrates and mother spawn packet materials. In case of substrates the highest average biological yield (44.40 g/packet) of oyster mushroom was observed in rice straw and the lowest average biological yield (41.73 g/packet) was observed in sawdust. On the other hand, the highest average biological yield (45.47 g/packet) was observed in sawdust mother spawn packets and the lowest average biological yield was observed in maize mother spawn packets (39.16 g/packet).

The combined effect of substrate materials and mother spawn packet materials was observed in this experiment. The combination of rice straw and sawdust mother gave the highest biological yield of oyster mushroom (*Pleurotus ostreatus*).

Based on the above discussion, it can be concluded that, oyster mushroom can be grown on different substrates with different mother. The performance of oyster mushroom grown in different substrates with respect to length of stipe, diameter of stipe, diameter of pileus, thickness of pileus and number of fruiting body depended on which substrate the mushroom has been cultivated. The yield parameters are also dependent on the mother culture used in the substrate spawn packets. The result revealed that the rice straw can be recommended as the preferred substrate for oyster mushroom cultivation. In addition, sawdust mother is preferred to be suitable for oyster mushroom cultivation.

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