EFFECT OF SPAWN AGE ON YIELD AND YIELD ATTRIBUTES OF DIFFERENT OYSTER MUSHROOM SPECIES

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EFFECT OF SPAWN AGE ON YIELD AND YIELD ATTRIBUTES OF DIFFERENT OYSTER MUSHROOM SPECIES

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CERTIFICATE

*This is to certify that the thesis entitled, "**EFFECT OF SPAWN AGE ON YIELD AND YIELD ATTRIBUTES OF DIFFERENT OYSTER MUSHROOM SPECIES**." Submitted to the **DEPARTMENT OF HORTICULTURE** Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in HORTICULTURE** embodies the result of a piece of bonafide research work carried out by **FAHMIDA AKTER**, Registration No. **09-03403** 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 been duly acknowledged by him.

Dated: June, 2015
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Dedicated to
My
Beloved Parents

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ABSTRACT

The experiment was conducted at Mushroom Development Institute, Savar, Dhaka during the period from January to June in 2014 with a view to determine the effect of spawn age on yield and yield attributes of different oyster mushroom species. The experiment was laid out following completely randomized design with two factors, they were ; Factor A : Four varieties of mushroom *i.e.*V_1 *(Pleurotus djamor*), V_2 (*Pleurotus ostreatus var. white snow)*, V_3*(Pleurotus ostreatus)*, V_4 *(Pleurotus salmoneostramineus)*and Factor B : seven types of age of spawn T_1 (1 day old), T_2(5 days old), T_3(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old), T_7 (30 days old). Except days required for complete mycelium running all parameters were significantly influenced by the age of spawn. All varieties were given the maximum yield at 1 day age of spawn and the minimum yield at 30 days age of spawn. Maximum yield 135.3g/500g packet, 111.5 g/500g packet, 126.5 g/500g packet and 137.0 g/500g packet at one day old of spawn were found from V_1, V_2, V_3 and V_4 respectively and the lowest yield 41.00 g/500g packet was recorded in V_1T_7.The highest biological efficiency was recorded in V_4 T_1 (68.63%) and the lowest in V_1T_7 (20.50%).So the variety *Pleurotus salmoneostramineus* with one day old spawn was the suitable combination for mushroom production.

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DCMR = Days required to complete mycelium running

DRPI = Days required to pinhead initiation

NFB = Number of fruiting body

NEFB = Number of effective fruiting body

LS = Length of stalk

DP = Diameter of pileus

TP = Thickness of pileus

DS = Diameter of stalk

BY = Biological Yield

BE = Biological Efficiency

CRD = Completely randomized design

cm = Centimeter

^{0}C = Degree Centigrade

et al. = and others

g = gram (s)

% = Percent

RH=Relative Humidity

RS = Rice Straw

SD = Sawdust

LSD = Least Significant Difference

Chapter I

Introduction

CHAPTER I

INTRODUCTION

Oyster mushroom (*Pleurotus sp.*) is an edible mushroom that belongs to the family Pleurotaceae (Randive, 2012). The term mushroom applies mostly to those fungi that have stem (stalk), cap (pileus), hymenium (lamellae) and pores on the underside of the cap (Masarirambi *et al.,* 2011). Mushroom spores (spawn) are produced on the gills and they can fall as a fine powder from underside of the cap. The colour of spore print of most oyster mushroom is white and when cultivated produces fruiting bodies (Herlina *et al.,* 2012). Oyster mushroom can be cultivated in different farm substrates. They are mushrooms of wide adaptability. Royse (2004), reported that oyster mushrooms are grown from mycelium propagated on steam-sterilized cereal grains. Their fast mycelia growth and multilateral enzyme system can biodegrade nearly all types of waste makes them grow on the largest varieties of wastes (Dlamini *et al.,* 2012). Gunde and Cinerman (1995) reported that oyster mushroom has a cap spanning diameter of 5 to 25 cm at maturity. The fruiting body of oyster mushroom differs with respect to stipe length and girth, and pileus width when grown in different farm substrates (Shah *et al.,* 2004).

Among the various cultivated mushrooms, oyster mushroom is easy to cultivate due to its strong enzymatic action towards the utilization of various kinds of organic substrates. It has gained importance only in the last decade and is now cultivated in many countries in the subtropical and temperate zones. This mushroom is a good source of non-starchy carbohydrates, with high content of dietary fiber and moderate quantity of proteins, including most amino acids, minerals, and vitamins. The protein content varies from 1.6 to 2.5%, and the niacin content is about ten times higher than that of any other vegetable. Oyster mushroom significantly reduced serum triglyceride and serum cholesterol in diabetic subjects. Oyster mushroom diet effectively prevented the progress of hypercholesterolemia (decreased by 38%) and cholesterol accumulation in liver (decrease by 25%) that were induced by the cholesterol diet in rats. This mushroom is gaining popularity day by day considering the nutritional and medicinal importance of this mushroom, an attempt was made to evaluate different strains for their physiological requirements and the substrate suited for their production . Oyster mushroom contains 20-35% protein in dry weights which makes its protein higher than that of vegetables and fruits and is good as ingredients of functional foods.

Mushroom cultivation has been reported as an alternative way of alleviating poverty in developing countries due to its possibility of low cost of production, high profit and quick returns (Masarirambi *et al*., 2011). Farmers can utilize agricultural wastes, such as dried sugar cane leaves, saw dust, maize stover and banana leaves as substrates for mushroom production. Fresh mushrooms have traditionally been imported into Swaziland (Anon, 2007).

Oyster mushroom can grow at moderate temperatures, ranging from 20 to 30°C, and at a humidity of 55–70%, on various agricultural waste materials used as substrate. Because of its flexible nature, the *Pleurotus* genus is more cultivated than any other mushroom species (Rosado *et al.,* 2002). The climatic conditions and seasonal diversity of Bangladesh is ideal for the cultivation of the oyster mushroom (Amin *et al.,* 2007b). Mushrooms are not dependent on weather conditions such as rainfall and can be grown all year round in a cropping house.
The collection of the Mushroom Development Institute in Bangladesh includes different strains of oyster mushrooms. In recent years, four new strains have been introduced: *Pleurotus high-king* (PHK), *P. ostreatus* (PO3), and *P. geesteranus* (PG1 and PG3). However, the performances of these strains have not yet been properly investigated in the climatic conditions of Bangladesh.

Pleurotus sp. Are relatively new to the mushroom industry but has gained popularity at a tremendous pace and today it is cultivated in about 25 countries of far-east Asia, Europe and America . It is the 3rd largest cultivated mushroom in the world and its annual world production is around 797000 tonnes (Chang, 1996). China along contributes 88% of the total world production. Now a days Bangladesh also play important role in mushroom cultivation . Most of the world's poor are in, or employed mainly on family farms. Strengthening mushroom production sector could be essential in order to enable the rural economy to keep its vibrancy and development, increasing and diversifying business and employment opportunities in the rural areas, and providing income opportunities for disadvantageous groups, small family farms. Also, mushroom production gives additional / alternative income to farmers looking for a value-added product and a way to supplement farm income while making use of by products or coproducts from other crops.

This experiment will help to improve the production of oyster mushroom. From this experiment farmers would find the maximum yield oyster mushroom. Thus the total production would increase by fulfilling the local market demand .Grower can export it and earn foreign currency. Quality and quantity of spawn play an important role in the successful production of any mushroom species. In the present study, the effect of spawn age on different oyster mushroom species may lead to better performance of the mushroom species. Considering the present situations and above facts the present investigation was undertaken with the following objectives:

Objectives

- To find out the best variety for ensuring the maximum yield of oyster mushroom species
- To determine the optimum age of spawn of oyster mushroom to increase the production.
- To find out the suitable combination of age of spawn of oyster mushroom with quality production of oyster mushroom species.

Chapter II

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Mushrooms represent one of the world's greatest untapped resources of nutritious food. Cultivation of saprophytic edible mushrooms may be the only current economical biotechnology for lignocellulose organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution (Obodai *et al.*, 2003). The oyster mushrooms (*Pleurotus spp*.) are in the third place after the white button and shiitake among the world mushroom production (Gyorfi and Hajdu, 2007). Mushroom is newly introduced horticultural crop. For that a very few studies on the age of spawn on yield and quality have been carried out in our country as well as many other countries of the world. Therefore, the research work so far done in Bangladesh is not adequate and conclusive. Nevertheless, some of the important informative works and research findings related to yield and quality of mushroom have been found at home and abroad . Research findings related to the present study have been reviewed here.

Effect of Mushroom species, yield and yield related characters on Quality Mushroom production

Nusrat *et al.* (2014) conducted to screening the suitable conditions for mycelial growth and phylogenetic relationship of the selected strains of *Pleurotus florida*. Suitable temperature for the mycelial growth was obtained at 25ºC and minimum mycelial growth was found at 10ºC. This mushroom has a broad pH range for its mycelial growth and most favorable growth was observed at pH 6.

Chukwurah *et al.* (2013) conducted a study on performance of *Pleurotus ostreatus* to correlate the stipe length, pileus width and stipe girth of oyster mushroom grown in different farm substrates. The experiment was laid out in a completely randomized design with eight treatments and four replications. The farm substrates (treatments) were composed of mixtures of different types of agricultural wastes with lime and water as additives to each substrate. Also single agricultural waste supplemented with lime and water was also used to prepare some farm substrates. Higher mean values of stipe length, pileus width and stipe girth were obtained from mushrooms grown in the substrates composed of two different types of agricultural wastes while lower values were obtained from those grown in the substrate composed of single agricultural waste. Highest coefficient of determination was obtained from the correlation between biological efficiency and pileus width. The changes in the stipe length, pileus width and stipe girth of the mushrooms grown in the different farm substrates depended on the type of agricultural wastes, single or mixtures of two different agricultural wastes used in preparing the farm substrates. Biological efficiency was highest (97.9%) in the substrate made from maize cob and palm kernel cake. Farm substrates that were composed of two different agricultural wastes were recommended. The use of single agricultural waste for farm substrate production is not encouraged.

Moonmoon *et al,* (2013) conducted an investigation on the performance of seven oyster mushroom variety such as *Pleurotus ostreatus* (PO-2), *Pleurotus ostreatus* (WS) *P. djmour* (POP-1 & POP-2), *P. salmoniostraminus* (PSS) and *P. florida* (FLO-1 & FLO-2*)* with five locations like Dhaka, Dinajpur, Rangamati, Faridpur and Jessore were carried to find out the growth and yield performance. Considerable variations on different parameters related to yield and yield attributes were recorded. The minimum days required from opening to harvesting (2.67 days), was observed from the treatment combination Savar and PO-2. The maximum days (53.00) required from opening to first harvest were observed from Faridpur with WS variety. The highest number of fruiting

body (51.75) was observed from the treatment combination Savar and PSS variety. The lowest number of fruit body was observed in Faridpur and FLO-1. The highest yield (151.30g) and biological efficiency (75.63%) were found in Dinajpur with the strain of PSS followed by Dinajpur and POP-2 and the lowest yield (50.00) and biological efficiency were found at Rangamati with the strain of FLO-1. Results suggested that all varieties are not suitable for all locations or each variety is suitable for specific location.

Sanjuti *et al.* (2013) initiated to evaluate the favorable vegetative growth and to determine molecular phylogenetic relationship in five different strains of *P. sajor-caju*. Optimum temperature for the mycelial growth was obtained at 30^0C. This mushroom grew well at acidic condition and pH 5.0 was the most favorable. Considering growth phenotype of mycelia, glucose peptone, was the favorable, while Hennerberg was the unfavorable media.

Singha *et al.* (2013) studied to assess the performance of eight different strains i.e. VV-1, VV-2, VV-3, VV-4, VV-5, VV-6, VV-7, and VV-8 of *Volvariella volvaceae* for their appropriate vegetative growth, yield and yield contributing characters. Among the tested strains mycelial growth and run rate were observed on PDA medium and mother culture. Highest days required for completing the mycelial growth and run rate were recorded in VV-4 (8.55 days) and VV-1 (22.75 days), while lowest days required for completing the mycelial growth and run rate were observed in VV-7 (7.00 days) and VV-2 (17.72 days), respectively. Minimum days required to primordia initiation (DRPI) was found in both VV-3 and VV-5 (6.33 days), whereas maximum DRPI was recorded in VV-6 (9.33 days). Optimum days required for first harvest was recorded in VV-7 (10 days). The lowest and highest numbers of effective fruiting bodies were observed in VV-2 (85.00) and VV-8 (147.30) respectively. Maximum length (3.83 cm) and diameter (2.20 cm) of fruiting bodies were observed in VV-6 strain. Highest biological yield (1045.10 g) and biological efficiency (26.13%) were observed in VV-5, whereas lowest biological yield and biological efficiency were recorded in VV-1 strain of *V. volvaceae*. These results indicate that VV-5 strain of *V. volvaceae* is suitable for the commercial cultivation in Bangladesh.

Dlamini *et al.* (2012) observed that the growth and yield of *Pleurotus ostreatus* was evaluated by the use of four replicated bags of sugarcane tops, maize stover, maize stover with cobs and banana leaves as substrates. The moist substrates were sterilised, packed in heat-resistant plastic bags, seeded with 2-4% spawn and incubated for 3-3.5 months. Yield of each mushroom flush, marketable yield, pileus diameter and stipe length were measured and recorded. For the first flash the significantly ($p<0.05$) highest yield was obtained from maize stover and cobs followed in decreasing order by banana leaves, sugarcane tops and lastly maize stover gave the least yield. The trend was similar for the second and third flash except that in the third flash sugar cane tops produced mushroom of higher yield than banana leaves, similar trends were measured for the other mushroom attributes. The maize stover and cobs substrate gave the highest yield which was 221.7, 189.2 and 107.9 g in the first, second and third flashes, respectively. While Kumari & Achal (2008) cultivated *P. ostreatus* on different substrates and reported the highest yield on wheat straw, followed by the combination of paddy and wheat straw.

Moonmoon *et al,* (2012) studied on the performance of 23 varieties of oyster mushroom on two substrates were studied in summer season. The objective of this study was to identify suitable variety and substrate for summer season in Bangladesh. A wide variation was observed in yield and yield attributes in different varieties as well as substrates. Among the varieties, the highest yield (235.0 g/packet), number of fruiting body (50.3), diameter of pileus (5.75 cm) were recorded when Po-10 cultivated on rice straw followed by sawdust. The highest (4.10 cm) diameter of stalk and thickness of pileus was found in Pcys-1 in rice straw substrate. The highest days (33.75) required to complete mycelium running when Po-4 cultivated on sawdust whereas the lowest (13.50days) in Pop-1cultivated on rice straw. The lowest (112.8 g/packet) yield was observed in Po-4 mushroom on sawdust. The lowest (0.30 cm) diameter of stalk was found in Po-1 on sawdust and the lowest (0.26 cm,) thickness of pileus was observed in Po-8 on rice straw substrate. Considering yield and yield attributes, strain Po-10 cultivated on both substrates may be recommended summer season in Bangladesh.

Howlader *et al.* (2011) observed the performance of different strains of *Pleurotus cystidiosus* (Pcys) were studied in National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh. Significant variation was observed in the growth, yield and yield contributing characters of these strains. The highest mycelial growth (0.58 cm/day) was observed in Pcys-4 which was followed by Pcys-5, Pcys-2 and Pcys-1. The lowest mycelial growth (0.22 cm/day) was observed in Pcys-3. The highest biological and economic yield, 196.3 and 189.0 g/packet respectively were obtained from Pcys-1, which followed by Pcys-2, Pcys-4 and Pcys-5. The lowest biological and economic yields were observed in Pcys-6. The number of effective fruiting bodies was the highest (37.25) in pcys-1, while, the weight of individual fruiting body was the highest (26.88 g) in Pcys-6. The highest length of stipe (4.95cm) and thickness of pileus (1.35cm) were observed in Pcys-1. The strain also showed the highest biological efficiency (BE) among the strains.

Kakon *et al.,* (2011) conducted to evaluate the effect of nutrient on the mycelial growth rate of some commercially important oyster mushroom. The fastest mycelium growth rate and the minimum days required for completion of mycelium running in Petri plate were observed when *Pleurotus djmour* (POP_2) inoculated in PDA media at the ratio of 15 : 150 (Dextrose: Potato). Minimal mycelial growth rate and maximum days required for the completion of mycelium running were observed when *Pleurotus djmour* (POP_2) inoculated in PDA media at the ratio of 25 : 250 (Dextrose : Potato). No mycelial growth was observed when all variety inoculated in PDA media at the ratio of 0:0.

Mahjabin *et al.* (2011) evaluated the effect of different temperature and pH on mycelial growth and substrates on yield of oyster mushroom var. white snow, six different temperatures- 10^0C, 15^0C, 20^0C, 25^0C, 30^0C and 35^0C, ten different pH levels- 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 and eight different substrates- sawdust, coir pith, lentil straw, chickpea straw, lentil + chickpea straw, wheat straw, rice straw (autoclaved)

and rice straw (hot water treated) were tested. The highest mycelium growth rate was observed at 20^0C temperature and 6.5 pH level and the highest yield (242.8 g/ 500g substrate) obtained from hot water treated rice straw.

Mamiro *et al.* (2011) carried out an experiment on Two crops of *Pleurotus ostreatus* were grown on rice straw as the basal substrate. In crop I, rice straw was mixed at spawning with 0%, 25%, 50%, 75% and 100% of banana leaves or *Leucaena leucocephala* or maize bran or maize cobs. In crop II, rice straw was supplemented at spawning with 0%, 1%, 2%, 3%, 4%, and 5% of sunflower or cotton seed cake. Mushroom yield (1,040.0 g) and Biological efficiency (BE) (98.5%) were greater on a 50/50 mixture of rice straw and banana leaves. Rice straw supplemented with 2% sunflower seed hulls (yield 1,087.5 g, BE 103.3%) gave similar yield and BE to rice straw supplemented with 2% cotton seed hulls (yield 1,073.8 g, BE 101.8%), and were significantly greater than ($p < 0.001$) other supplement ratios. By comparison, mushroom yield on banana leaves were 786.5 g, on rice straw were 582.5 g, on *Leucaena leucocephala* were 534.5 g, on maize cobs were 468.5 g, on rice bran were 406.0 g and on maize bran were 305.3 g. The largest mushrooms (21.0 g) were obtained from non-supplemented rice straw.

Stanley and Nyenke (2011) studied that *Pleurotus* species are characterized by a white spore pint attached to recurrent gills, often with an eccentric (off center) stipe, or no stipe at all.The common name “oyster mushroom” comes from the white shell-like appearance of the fruiting body.

Uddin *et al* (2011) conducted a experiment on Oyster mushrooms (*Pleurotus* spp.) are widely cultivated all over the world. Its production is remarkably affected by the environmental conditions like temperature and relative humidity. In this study, we 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 0C) and relative humidity (% RH) of culture house in

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, 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. We suggest cultivation of selected *Pleurotus* spp. in winter (temperature zone 14-27 0C with relative humidity 70-80%) for better production and biological efficiency.

Mondal *et al.* (2010) conducted a study on *Pleurotus florida* in different substrate compositions as well as to find out the better substrate for mushroom cultivation. Highest mycelium running rate was found in banana leaves and rice straw (1:1) but the lowest in control. Completion of mycelium running time was lowest in banana leaves and rice straw (1:3 and 3:1). Number of total primordia and effective primordia, found highest in control but the maximum pileus thickness was measured from rice straw. Highest biological yield and economic yield (164.4 g and 151.1 g) was obtained from rice straw which was much higher than control. From the graphical view, both positive and negative relationships were found between economic yield and different yield contributing attributes.

Moonmoon *et al*. (2010) conducted to find out an effective supplement, *Pleurotus citrinopileatus* was grown on sawdust (SD), cotton waste (CW) and paddy straw (PS) and their combinations supplemented with rice bran (RB), wheat bran (WB), maize powder (MP) and sesame oil seed cake (SOSC) at 1% level. The days required from opening to first harvest (DROFH) varied from 3.00 to 15.50 days and it was maximum (15.50) in SD + CW (1:1) supplemented with SOSC. The minimum DROFH (3.00) was required in SD + PS (1:1) supplemented with SOSC. The maximum yield (166.00g/ 200g dry substrate) was obtained from SD supplemented with SOSC and the lowest yield (24.00g/ 200g dry substrate) was obtained from SD supplemented with MP. The highest biological

efficiency (BE) (83.0%) was recorded in SD supplemented with SOSC that was significantly higher than all other treatments. The lowest BE (12.0%) was recorded in SD supplemented with MP.

Shelly *et al.* (2010) studied on ten different species of oyster mushroom have been cultivated on rice straw and the yield and yield related attributes were compared. The minimum days required from stimulation to primordia initiation (DRSPI) (3.50) was recorded in *Pleurotus ostreatus* (whitesnow), *Pleurotus geesteranus* and *Pleurotus citrinopileatus* and the maximum DRSPI (10.25) was recorded in *Pleurotus erryngi* and *Pleurotus sajor-caju.* The minimum days required from stimulation to first harvesting (DRSFH) was found in *Pleurotus citrinopileatus* (5.50) and the maximum DRSFH (15.25) was found in *Pleurotus erryngi* closely followed by *Pleurotus sajor-caju* (14.25). The number of effective fruiting bodies was highest (47.00) in *Pleurotus citrinopileatus* and it was lowest (1.00) in *Pleurotus erryngi*. The length of stipe ranged from 3.45 to 6.80 cm. The highest length of stipe (6.80cm) was found in *Pleurotus erryngi* followed by *Pleurotus ostreatus* (5.50cm) and the lowest length of stipe was found in *Pleurotus sajor-caju* (3.45cm). The diameter of stipe, pileus and thickness of pileus ranged from 0.35 to 4.60 cm; 4.00 to 11.00 cm and 0.35 to 1.40 cm respectively. The highest diameter of stipe (4.60 cm) and pileus (11.00 cm) were found in *Pleurotus erryngi*. The biological and economic yields and biological efficiency were the highest, 191.00g and 183.5g and 127.30% respectively, in *Pleurotus ostreatus* (whitesnow).

Five different types of substrates were investigated to determine the growth and yield of *P. ostreatus*. Weekly mycelial extension on different substrates was observed. The fastest mycelia extension was observed in rice straw substrate followed by mixture of rice plus wheat straw, sugarcane bagasse, mixture of rice straw plus paper and sawdust, respectively. Mycelial growth is a preliminary step that creates suitable internal

conditions for fruiting. Thus, outstanding growth of mycelium is a vital factor in mushroom cultivation (Pokhrel *et al.* 2009).

Dundar *et. al.* (2008) determined tha Harvesting periods of three mushroom spices .The longest harvesting periods after three harvests were 85.27 days for *P. eryngii*, 82.64 days for *P. ostreatus* and the shortest was 67.46 days for *P. sajor-caju*. Total fresh mushroom yield obtained with 100 g material (70% moisture) after the three harvests were determined. *P. sajor-caju* gave the highest yield as 20.2 g, *P. ostreatus* was 17.9 g and the lowest yield was obtained from *P. eryngii* as 4.5 g. This study also showed the energy, protein, fat, carbohydrate, dietary fibre, moisture and ash contents of the mushrooms. The energy(kcal/100 g dried matter) values of mushrooms are 229.22, 243.66 and 276.33 for *P. sajor-caju*, *P. ostreatus* and *P. eryngii*, respectively. While the highest energy value was obtained from *P. eryngii*, the lowest was obtained from *P. sajor-caju*. The fat content values are 1.15, 2.60 and 7.50 g for *P. sajor-caju*, *P. ostreatus* and *P. eryngii*, respectively. The carbohydrate values are 37.72, 37.87 and 39.85 (g/100 g dried matter) in *P. sajor-caju*, *P. ostreatus*and *P. eryngii*, respectively. Dietary fibre values are 28.45, 30.25 and 30.67 (g/100 g dried matter) in *P. eryngii*, *P. ostreatus* and *P. sajor-caju*, respectively. Bonatti *et al*. (2004) found 5.58 and 6.13 g of ash in *P. ostreatus* cultivated in banana straw and rice straw, respectively. In this study, we found the ash content values as 4.78, 4.89 and 5.84 in *P. ostreatus*, *P. eryngii*, and *P. sajor-caju*, respectively. The moisture contents are 7.23 in *P. eryngii*, 7.39 in *P. ostreatus* and 7.42 in *P. sajor-caju.* Cultivating different mushroom species affects amino acid content significantly ($P < 0.05$). Each amino acid content generally changes due to mushroom species. Hydroxy-L-proline was not detec-ted in the mushrooms and histidine was found only in *P. eryngii*. In *P. eryngii* and *P. sajor-caju* the highest amount of amino acid was from aspartic acid and the lowest was from methionine. The highest and the lowest amino acid amounts in *P. ostreatus* were from glutamicacid and methionine, respectively.

Khan *et al.* (2008) said that the nutritional composition of six species of oyster mushrooms such as *Pleurotus sajor-caju, P. ostreatus, P. florida, P. cystidiosus, P. highking 51* and *P. geestaranus* was determined. The protein content was found highest in *P. sajor-caju* (24.5g/100g of dry weight) followed by *P. ostreatus, P. highking 51, P. florida, P. geestaranus* and *P. cystidiosus.* The highest lipid content was found in *P. cystidiosus* (5.5g/100g dry sample) followed by *P. highking 51, P. sajor-caju, P. florida, P. geestaranus* and *P. ostreatus.* The carbohydrate content was found highest in *P. geestaranus* (45.9g/100g dry sample) followed by *P. cystidiosus, P. florida, P. ostreatus, P. sajor-caju* and *P. highking 51*. The fiber content was found highest in *P. highking 51* (30.3 g/100g dry sample) followed by *P. ostreatus, P. florida, P. geestaranus, P. sajor-caju* and *P. cystidiosus.* The total ash content was found highest in *P. florida* (8.3 g/100g dry sample) followed by *P. sajor-caju, P. ostreatus, P. cystidiosus, P. highking 51* and *P. geestaranus.* Following these data the highest metabolizable energy was found in *P. cystidiosus* (262.8 kcal/100g dry sample) followed by *P. sajor-caju* (254.1 kcal/100g), *P. geestaranus* (252.7 kcal/100g), *P. florida* (250.1 kcal/100g), *P. highking 51*(249.7 kcal/100g) and *P. ostreatus* (242.6 kcal/100g). The moisture content of fresh oyster mushrooms was found 85-88%.

Saw dust and rice straw are the most available agricultural wastage in Bangladesh. For this, in this study, these two substrates were used for the production of three different strains of *P. eryngii.* The mycelium run rate (MRR) was highest for Pe-1 in both substrates but MRR of each strain was slightly lower on RS than SD, this was not significant. This result is similar to the findings of Khandakar *et al.* (2008), who investigated the mycelial growth on different culture media. Number of primordia varied from 3.7 to 4.5 among the strains on two substrates without any significance.

Amin *et al.* (2007) found the maximum number of fruiting bodies of different oyster mushroom species on SD when compared with RS. Although king oyster gives small number of fruiting body, texture and shelf life is very higher than other *Pleurotus sp*.

Similar result was found in shiitake mushroom (Sarker *et al*., 2009). He also did not find any significant variation of primordial initiation number of *Pleuratus sp.* between SD and RS. The DRPI of different strains of *P. eryngii* ranged 11.3– 17.0 days. These periods were shorter than the data from the study of Kirbag and Akyuz (2008), who reported that the time need for primordial initiation of *P. eryngii* was 26.2–44.2 days, depending on the type of substrate used and the rate of additive matter. Days required to first harvest of *P. eryngii* was also found different from other study (Akyuz and Yildiz, 2007). In this study, the number of fruiting bodies of different strains on SD was significantly higher than corresponding strains on RS.

Ruhul Amin *et al*. (2007) noted that history of mushroom cultivation is very recent in Bangladesh. Only some species of mushrooms are now cultivated in this country and among these *Pleurotus ostreatus, P. sajor-caju, P. florida* and *Calocybe indica* are popular and widely accepted .

Sarker *et al*. (2007a) tested the effect of eight different levels of pH, *viz.* 5.04, 5.21, 5.40, 5.62, 5.85, 6.05, 6.27 and 6.51 on *Pleurotus ostreatus* to determine the best level for growth and yield . These levels of pH in substrates were obtained by using CaCO3 at the rate of 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0g/500g packet. The pH levels significantly influenced the mycelial growth and the time required to complete mycelium running in spawn packet. Number of fruiting bodies, weight of fruiting bodies, biological efficiency, biological yield and economic yield were also significantly influenced by different pH levels. The number of fruiting bodies was increased with the increase of pH levels up to 5.40 and then decreased. The biological efficiency, biological yield and economic yield were increased with the increase of pH levels up to 5.04 and then decreased. The highest economic yield (241.90 g/packet) was obtained from 5.40 pH level and the second highest economic yield (231.50 g/packet) was observed in 5.62 pH level. The lowest economic yield was obtained from 6.51 pH level. The optimum pH level for production

of oyster mushroom is 5.40 which can be maintained by using 1.0 g of CaCO3 per 500g packet of substrate.

Sarker *et al*. (2007b) observed that time required from stimulation to primordia initiation, stimulation to first harvest and for total harvest were influenced significantly by frequency of watering. Significant variation was observed in the number of fruiting bodies and the weight of fruiting bodies by the frequency of watering. Maximum number of fruiting bodies (59.75) was recorded when the spawn packets were immersed in water and watered once daily. The length of stalk, diameter of stalk, diameter of pileus and thickness of pileus were not influenced significantly by the frequency of watering. Biological efficiency was significantly influenced by different frequencies of watering. The biological efficiency increased gradually with the increase of frequencies of watering. The highest biological efficiency (172.56%) was observed when the spawn packets were immersed in water and watered four times daily. The biological yield and economic yield per packet of *Pleurotus ostreatus* were also significantly influenced by different frequencies of watering. The highest economic yield (258.11 g/packet) was recorded when the spawn packets were immersed in water and watered four times daily. But the dry yield of oyster mushroom decreased with the increase of watering more than once daily. No significant difference was observed in dry yield between the one and two times of watering per day.

Yildiz *et al.* (2002) reported that the natural substrates (woods on which *Pleurotus* species grow) are very poor in nitrogen content, nevertheless the fruit bodies are produced.

Bughio (2001) cultivated the oyster mushroom, *Pleurotus ostreatus* on combination of wheat straw, cotton boll locules, paddy straw, sugarcane and sorghum leaves at 1:1 ratio in polythene bags (650 g/bag) using sorghum grain spawn @ 30 grams per bag, followed by boiling of substrates and sterilization of bags. He also reported 43.25 to 53.00 days

after spawning by using sorghum grains @ 30 g per 650 g in case of using wheat straw, sugarcane and sorghum leaves at 1:1 ratio on substrate dry weight basis. He reported that maturation of fruiting bodies took 5 to 6 days after pinhead formation. The minimum period (6.33 days) between flushes was taken by using 20 g per kg substrate dry weight basis, followed by 40 g (8.16 days), 30 g (12.42 days), 80 g (14.05 days), 70 g (15.11 days), 90 g (15.67 days), 70, 50 and 100 g (16.72 days). However, only one flush was harvested by using spawn at 10 g per kg on substrate dry weight basis, hence, no days were recorded between flushes. Lozano (1990) reported that seven harvesting were carried during 60 days. Jiskani *et al.,* (1999) reported 7.5 days, but Bughio (2001) recorded 8.53 to 14.33 days between flushes. The results obtained for percentage yield of oyster mushroom on fresh (wet) and dry weight basis are highly significant at LSD 0.05. The results reveals that the maximum percentage yield (45.40% on fresh and 4.63% on dry weight basis) was obtained by using spawn at 70 g/kg on substrate dry weight basis, which is near to 60 g spawn per kg substrate (44.27% fresh and 4.10% dry). These spawn rates were found to be the best followed by 80, 90, 100, 50, 40, 30, 20 and 10 g per kg (39.93 and 3.96%, 38.27 and 3.72%, 33.40 and 3.70%, 32.00 and 3.30%, 27.20 and 2.65%, 15.67 and 1.62%, 15.13 and 1.55% and 10.53 and 1.15%) fresh and dry yield respectively .

Quimio *et al.* (1999) reported that good harvest of *P. ostreatus* was 3-4 weeks after incubation. In our results, colonization and harvest time are not consistent with their results.

Cangy & Peerally (1995) used spawning rates 0.75, 1.50, 3.00 and 6.00% of substrate fresh weight for 10 species of *Pleurotus*. Results showed that 1% spawning rate was found to be adequate when using the smaller bags (yields >16% of spawned substrate weight) at mean temperature 18^0C (range 13-23^0C).

Moorthy & Mohanan (1991) recorded 332 to 474 g/bag yield from polyethylene bags containing 1.2 kg dry substrate/bag when inoculated with 150 g spawn/bag using a multi-layered spawning technique.

Visscher (1989) reported that many strains of *P. eryngii* are available in the world, which are extensively cultivated. Different strains of king oyster mushroom response differently to different substrates, supplements, supplementation amount and environmental factors in the aspects of mycelium run, average yield and quality .

Szili and Vessey (1980) observed that growing technology will be developed and temperature may be control, that can make this strain most demanded out of all *Pleurotus sp.* due to its excellent texture and shelf life . After completion of mycelial growth, the bottles of sawdust were uncapped and soaked in water for 3–5 min. But the spawn bags of rice straw were opened by square shaped (100 · 100) cut on the different place in a culture house. The temperature, relative humidity and light were maintained at 13–22^{0}C, 70–85% and about 180–250 lux, respectively. Carbon dioxide concentration was not monitored and controlled instrumentally. Mushroom were harvested when the mushroom cap surface were flat to slightly up-rolled at the cap margins. One flush of mushroom in each bottle or bag was harvested. The yield of mushrooms and their different quality parameters were recorded regularly.

Zadrazil (1978) reported that the growth of *Pleurotus* spp. was greatly dependent on temperature and humidity, which also varied from species to species. A temperature range of 20-26^{0}C and relative humidity of 70-90% were ideal for *P. sajorcaju* and a fairly good crop can be obtained at up to 30^{0}C.

Effect of Age of Spawn on Mushroom production

Age of spawn play an important role in the successful cultivation of any mushroom species. Age of four selected species of oyster mushroom such as,

Pleurotus ostreatus, P. djamor, P. ostreatus var. white snow and *P. salmoneostramineus* is an indicator of its productivity.

Subramanian *et al*. (2015) analyzed the effect of spawn maturity period on the growth and yield of *Agrocybe aegerita*, black poplar mushroom. The spawn of *Agrocybe aegerita* was prepared and used at three different days of maturity viz. 25 days, 35 days and 45 days. The days of spawn run, pin headed appearance, first harvest, second harvest and third harvest were observed and recorded. Similarly, the effect of different days of spawn maturity on the yield and bio-efficiency of the mushroom were also analysed. The study showed that, beds inoculated with 35 days spawn gave more yield of mushrooms with higher bio-efficiency when compared to other two groups of spawn inoculated beds. This mushroom possess potent anti-cancer biochemical and phytochemical compounds which on cultivation yields great benefit to the society.

Pani (2011) showed that quality and quantity of spawn play an important role in the successful cultivation of any mushroom species. In his study, the effect of age of spawn (14, 21, 30, 37, 45 and 60 days after inoculation) and quantity (100, 200, 300, 400 and 500 g per kg dry substrate) on sporophore production of *Calocybe indica* was investigated. Quickest substrate colonization and primordial initiation as well as highest number and weight of sporophores were recorded in 21 days- old spawn. Mushroom yield decreased with increase in spawn age.A spawn dose of 200 g/ kg of dry substrate was found optimum. Quickest substrate colonization (15 days) and primordial initiation (30 days) as well as highest number (6) and weight of sporophores (70.5 % BE) was recorded in 21 days old spawn . This finding is in concurrence with the report of (Purkayastha and Nayak,1981) .The average pileus diameter and stipe length were 14.6 and 16.7 cm, respectively.A single sporophore weighed about 117.5 g. The viable and active mycelia of younger spawn ramified faster in the substrate and had better chance of withstanding adverse conditions in comparison to older spawn. The mushroom yields

obtained in response to 21 and 30 days spawns were statistically but significantly higher than other treatments. Increase in spawn age beyond 21 days reduced the number of sporophores which could have been due to apparent loss of vigour and viability of fungal mycelia. Two month old spawn sustained the least weight of sporophores (305.3 g) but supported maximum individual weight of fruiting body (152.6 g). Least weight of sporophore (374.6 g, 37.4 % BE) was recorded in the substrate supplemented with minimum spawn dose (100 g). Spawning of substrate with 200 g spawn resulted in 701.3 g of fresh mushrooms (70.1%BE) which was significantly superior to the yield obtained with 100 g of spawn. It was observed that there was a steep increase in mushroom yield when the spawn dose increased from 100 g to 200 g. But when the spawn dose was raised from 200 g to 500 g, there was only a gradual increase in productivity and these resultant yields were not statistically different from each other. It was also observed that there was quicker substrate colonization, earlier pinhead appearance and higher number of sporophores as the amount of spawn increased in the cultivation substrate. Kuforiji and Fasidi similarly observed that high spawning rate led to more rapid colonization of substrate. This prevented other unwanted micro-organisms from becoming established in the substrate. It was noted that the rate of primordial mortality increased with increased spawn doses especially in the range of 400-500 g/bag ostensibly due to fierce competition among the developing primordia for space and nutrition in the cultivation substrate. Higher mycelial load might have also created problems in the proper escape of respiratory gases. As a result the rate of increase in productivity could not keep pace with the rate of increase in spawn quantity. The results are in agreement with earlier findings .It was concluded from the study that 200 g of 21 days-old wheat grain spawn should be used per kg of dry paddy straw substrate in 60cmX40cm cylindrical polythene bags to get optimum yield of milky mushroom.

Khandakar *et al.,* (2008) evaluated that five media such as Potato Dextrose Agar, Yeast Extract Agar, Malt Extract Agar, Wheat Extract Agar and Cane Juice Agar were used in this study to find out the suitable media for the mycelial growth of six edible mushroom

species such as *Pleurotus ostreatus, Pleurotus highking-51, Pleurotus geesteranus, Pleurotus eryngii, Lentinus edodes* and *Hypsizygus tessulatus.* Maximum mycelial growth of *P. ostreatus* (0.475 cm/day), *P. highking 51* (0.47 cm/day), *P. eryngii* (0.51 cm/day), *Hypsizygus tessulatus* (0.60 cm/day) was recorded on Malt Extract Agar and incase of *P. geesteranus* (0.83 cm/day) and *Lentinus edodes* (0.55 cm/day) maximum growth was observed on Wheat Extract Agar and Potato Dextrose Agar respectively. Minimum days required for completion of mycelium in petriplates were observed in *P. ostreatus* (7.75days), *P. highking* 51 (8.25 days), *P. eryngii* (10.5days), *Hypsizygus tessulatus* (9.25 days) on Malt Extract Agar and *P. geesteranus* (7days), *Lentinus edodes* (11days) on Wheat Extract Agar and Potato Dextrose Agar respectively.

Shah *et al.* (2004) reported that primordial formation of *P. ostreatus* appears 27-34 days of inoculation which is consistent with the results of this study. The size of fruiting bodies was higher in case of rice straw (control) than in all other substrates. This is probably due to higher degradation of various constituents of the substrate rice straw and consumption of high nutrients by *P. ostreatus.*

Fan *et al.* (2000) carried out the studies with 2.5-25% spawn rates, 25% spawn rate appeared superior, but recommended 10% spawn rate in view of the process economics. The first fructification occurred after 20-23 days of inoculation and the biological efficiency reached about 90-97% after 50-60 days.

Patra and Pani (1995) cultivated five different species of *Pleurotus* in polythene [polyethylene] bags containing chopped paddy straw (2 kg) + spawn (200 g) + boiled wheat (200 g). The fungi took 13-16 days for complete mycelial run in the bags and 20-24 days for initiation of fruiting bodies, producing the heaviest (12.2 g), and the lightest (6.9 g) fruiting bodies.

Chapter III

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

3. Materials and methods

3.1 Experimental site

The experiment was conducted to find out the effect of age of spawn on yield and yield attributes of different oyster mushroom.The study was carried out in the laboratory, workshop and culture house of Mushroom Development Institute (MDI), Sobhanbag, Savar, Dhaka from January to June in 2014.

3.2 Required materials

3.2.1 Inoculums

Four selected species of oyster mushroom such as, *Pleurotus ostreatus, Pleurotus djamor*, *Pleurotus ostreatus var. white snow* and *Pleurotus salmoneostramineus* were cultivated in the culture house of the Mushroom Development Institute. Mushroom varieties used in the experiment were collected from germplasm centre of Mushroom Development Institute (MDI), Savar, Dhaka. This was cultured in biotechnology laboratory and cultivated in the culture house of MDI.

3.2.2 Treatment

The experiment comprised of two factors-

Factor A: mushroom varieties	**Factor B: Spawn age**
V_1 (*Pleurotus djamor*) **V_2 (*Pleurotus ostreatus var. white snow*)** **V_3 (*Pleurotus ostreatus*) and** **V_4 (*Pleurotus salmoneostramineus)*.**	**T_1(1 day old)** **T_2(5 days old)** **T_3(10 days old)** **T_4(15 days old)** **T_5(20 days old)** **T_6 (25 days old)and** **T_7 (30 days old)**

There were in total 28 (4×7) treatment combinations.

3.2.3 Laminar airflow transfer cabinet

Laminar air-flow transfer cabinet was used to inoculate and transfer of the experimental materials in aseptic condition. It presents a gentle flow of filtered air over the cabinet and fitted with UV light for decontamination of the inner space.

3.2.4 Spirit lamp

Small glass laboratory lamps with a wick were used to sterilize instruments or to flame the opening of the petriplate and other containers.

3.2.5 Disinfectants

Ethyl alcohol (70%) was used to wipe surfaces of working areas, to rinse hands and to dip instruments, with or without subsequent flaming. It's also used to disinfect the surface of fruiting body.

3.2.6 Forceps, knife, tweezers and scalpels

These were provided from scientific supply houses and were in various sizes, shapes and models. These were sterilized before use.

3.2.7 Labels and marking pencil

Marking pencils made of wax and useful for writing on glass surfaces were used. Various kinds of gum labels were also used.

3.2.8 P^H meter

P^H meter was used in measuring P^H of the medium and the P^H was adjusted with the help of 0.1N HCl or 0.1 N NaOH solutions.

3.2.9 Electric balance

Electric balance was used to measure various chemical and material.

3.2.10 Water bath

Water bath was used to heat potato and culture media.

3.2.11 Petri plates

There are six replication petriplate were used for each experiment. The petriplates were washed properly and wipe with propanol.

3.2.12 Microspores

Microspores were used to attach petriplate properly.

3.2.13 Scale and Tape

Scale and Tape were used to measure mycelium run rate and size fruiting body.

3.2.14 Brown paper, rubber bands, cotton plug and plastic neck

These materials were used to prepare mother culture and spawn packet.

3.2.15 Trolley

A trolley was used to move mother culture and spawn packet from one place to another.

3.2.16 Rack

The various racks were used to conduct the experiment.

3.2.17 Water Spray Machine

A Water Spray Machine was used for watering the spawn packet.

3.2.18. Poly propylene (pp) sheet

The PP sheet were used for packaging of mother culture and spawn packet.

3.2.19 Chemicals

Dextrose

Agar

Tetracycline

$CaCO_3$

Asparagines

3.2.20 Organic Substrates

Saw dust

Rice husk

Wheat bran

3.2.21 Basic organization and facilities including equipment

The experiment was performed in a clean room having provision for working space, free of dust and convection current that carry spores of microorganisms. The preparation room was equipped with the following:

- Cabinet and shelf space for safe storage of chemical and dust free storage for clean glassware.
- Transfer areas for aseptic manipulation.
- Analytical and top loader balances.
- Steam autoclave and oven for sterilizing media, solution, water, culture vessels and instruments.
- Culture room where cultures were incubated under controlled light and temperature.
- Various instruments and appliances.

3.2.22 Precaution to Ensure Aseptic Conditions

- All inoculations and aseptic manipulations were carried out in a laminar air flow cabinet.
- The cabinet was made on for half an hour before use and cleaned with 70% ethyl alcohol to reduce the chances of contamination.
- Surgical operations were taken with care as usual to obtain possible contamination free conditions.

3.3 Preparation of media and pure culture

PDA (potato, dextrose & agar) media were used to culture and observe mycelium growth rate.

3.3.1 Component of PDA media

For one (1) liter of PDA media following essential components were mixed.

Component	Amount
Potato	250 g
Dextrose	20 g
Agar	20 g
Aspersing	250 mg
Tetracycline	250 mg

3.3.2 Procedure for PDA media and pure culture preparation

For one (1) litter PDA Media, 250g potatoes were measured by electric balance. Then potato' pills were removed by knife and sliced into small pieces. The small potato's pieces were boiled with one (1) litter water for 45 min and small potato' pieces were filtrated by thin cloth. All the chemical component of PDA mediawere mixed with remaining 1 L water. Then the mixed solution was also boiled for 15 min and sometimes agitated with stick. After boiling, 10 ml solution was poured in each contamination free petriplate. Each petriplate were rapped with aluminum foil and autoclaved at 121˚C and 15 PSI for 45 min to make it free from contamination.

The explants were inoculated under contamination free environment. Laminar air flow was used to inoculate the germplasminpetriplate. After surface sterilization the inoculums were laid on the sterile Petri dish using sterile forceps by cutting with a sterile scalpel. The petriplates were raped with micropore under the laminar air flow. Then the

petriplates were placed for pure culture preparation. After 5-10 days, the petriplates were filled with white mycelium. This white mycelium in petriplate is called pure culture.

3.3.3 p^H

P^H of the medium was usually adjusted to 5.0-6.0 before autoclaving. In the present findings P^H of the medium was adjusted to 5.8 with the help of P^H meter by adding 0.1N NaOH or 0.1N HCl whichever was necessary.

3.3.4 Culture condition

After inoculation petriplates were carried out to laboratory of Mushroom Development Institute (MDI). The relative humidity was 70-85% and the temperature was maximum 27˚C and minimum 22˚C. The relative humidity (RH %) and the temperature were maintained by watering fourth time daily.

3.4 Preparation of Mother Culture

Saw dust mother cultures were used to observe mycelium growth rate.

3.4.1 Components of Mother Culture

For preparing mother cultures following essential component were used. (300g/packet)

Component	**Amount**
Saw dust	100 g
Wheat husk	50 g
Calcium Carbonate ($CaCO_3$)	1 g
Water	150 ml

The brown paper, rubber bands, cotton plug and plastic neck are used to prepare mother culture packet.

3.4.2 Procedure of mother culture preparation

Sawdust was used as a main substrate and wheat bran was used as supplement. For each 300 g mother packet above component were mixed. The mixture was filled into heat tolerant polypropylene bags of 7"x 10" size and their mouth were plugged by inserting water absorbing cotton and covered with brown paper and tied with a rubber band. Then bags were autoclaved at 121^0 C and 15 PSI for 1 hour and then allowed for cooling. Each mother packet was inoculated with the pure culture at the rate of 5 square mm per packet. The activities were done under total aseptic condition in mother culture house.

3.4.3 Culture condition

After inoculation mother packets were carried out to cultivate in Mushroom Development Institute (MDI) mother culture house. The relative humidity was 70-85% and the temperature was maximum 27˚C and minimum 22˚C. The relative humidity (RH %) and the temperature were maintained by watering fourth time daily.

3.5 Preparation of spawn packets

Sawdust was used to observe mycelium growth rate in spawn packets.

3.5.1 Components of spawn packets

For preparing spawn packets following essential component were used. (500g/packet)

Component	**Amount**
Saw dust	150 g
Rice bran	50 g
Calcium Carbonate ($CaCO_3$)	1 g
Water	300 ml

The brown paper, rubber bands, cotton plug and plastic neck are used to prepare mother culture packet.

3.5.2 Procedure of spawn packets preparation

Sawdust was used as a main substrate and wheat bran was used as supplement. For each 500 g spawn packet above component were mixed. The mixture was filled into heat tolerant polypropylene bags of 7"x 10" size and their mouth were plugged by inserting water absorbing cotton and covered with brown paper and tied with a placing rubber band. Then bags were autoclaved at 121^0 C and 15 PSI for 1 hour and then allowed cooling. Each spawn packet was inoculated with the mother culture at the rate of two teaspoonfuls per packet. Spawning substrate with bags was then incubated for mycelium growth according to the strains.

3.5.3 Culture condition

After inoculation spawn packets were carried out to cultivate in Mushroom Development Institute (MDI) spawn culture house. The relative humidity was 70-85% and the temperature was maximum 27°C and minimum 22°C. The relative humidity (RH %) and the temperature were maintained by watering four times daily.

3.5.5 Spawn packet culture in culture house

The spawn packets with complete mycelium were transferred to the culture house and the brown paper, rubber bands, cotton plug and plastic neck of the spawn packets were removed and the mouths of the polypropylene bags were wrapped and tied with rubber bands. The plastic bags were opened by "D" shaped cut on the shoulder side and removed the sheet. The opened surface of substrate was scraped slightly with a blade for removing the thin whitish mycelial layer. The packets were placed separately side by side on the rack in the culture house.

The relative humidity (RH %) was maintained by watering four times daily. The average temperature (22-27^0 C) and relative humidity (70-85%) were measured.

3.6 Yield measurement process:

Data collection

Data on days required for complete mycelium running ,days required for pin head initiation, days required for first harvesting, number of fruiting bodies, effective number of fruiting body, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, yield and biological efficiency were recorded.

3.6.1 Data on days required to complete mycelium running

Complete mycelium running was recorded from date of inoculation to date of spawn packet opening.

3.6.2 Days required for pin head initiation

It was recorded by counting the days which were required to conk formation from the date of spawn packet opening.

3.6.3 Days required for first harvest

It was determined by counting the days which were required for well developed fruiting body formation from pin head initiation.

3.6.4 Number of fruiting body

Number of total fruiting body was recorded.

3.6.5 Number of effective fruiting body

Number of well developed fruiting body was recorded. Dry and pinheaded fruiting body was discarded but twisted and tiny fruiting body was included during counting.

3.6.6 Thickness of pileus (cm)

Thickness of the pileus of four randomly selected fruiting bodies was recorded by using a slide calipers.

3.6.7 Diameter of pileus

Diameter of pileusof four randomly selected fruiting bodies was recorded by using a slide calipers.

3.6.8Length of stalk

Length of stipe of four randomly selected fruiting bodies was recorded by using a slide calipers,

3.6.9Diameter of stalk

Diameter of stipe of four randomly selected fruiting bodies was recorded by using a slide calipers.

3.6.10 Biological Yield (g)

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

3.6.11 Biological Efficiency (%)

The Biological Efficiency was determined by using the following formula:

$$\text{Biological Efficiency (\%)} = \frac{\text{Total biological yield (g)}}{\text{Total dried substrate used (g)}} \times 100$$

3.7 Statistical analysis

The experiment was laid out following completely randomized design (CRD) with 4 varieties and 4 replications and each replication contained twelve population. Data were analyzed following Gomez and Gomez (1984) using MSTAT-C computer program and Excel software. Mean separation was computed following Least Significant Difference (LSD) using the same computer program.

Chapter IV

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

An experiment was conducted to find out the effect of age of spawn on yield and yield attributes of different oyster mushroom species. Results of the present study have been presented and discussed in this chapter under the following headings.

4.1 Days required for complete mycelium running

Days required for complete mycelium running (DCMR) was significantly varied among different oyster mushroom species ranged from 22.29 - 25.07 days (Fig.1 and Appendix. I). The highest days required for complete mycelium running was 25.07 days obtained from V_4 *(Pleurotus salmoneostramineus)* which was significantly differed from V_1 *(Pleurotus djamor)*, V_2 *(Pleurotus ostreatus var. white snow*) and V_3 *(Pleurotus ostreatus)*. On the other hand, V_1*(Pleurotus djamor*), V_2 (*Pleurotus ostreatus var. white snow)* and V_3*(Pleurotus ostreatus)* required same days (22.29 days) for complete mycelium running.

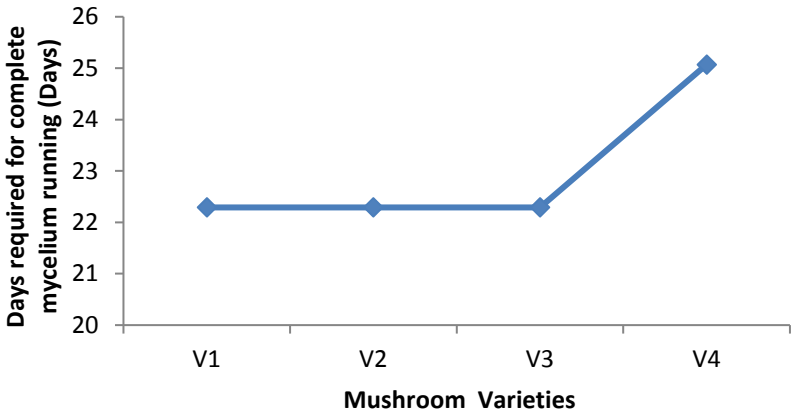

Figure 1. Effect of varietal variation on the days required for complete mycelium running of oyster mushroom species (LSD $_{(0.05)}$ = 0.29). V_1 *(Pleurotus djamor*), V_2 (*Pleurotus ostreatus var. white snow)*, V_3 *(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

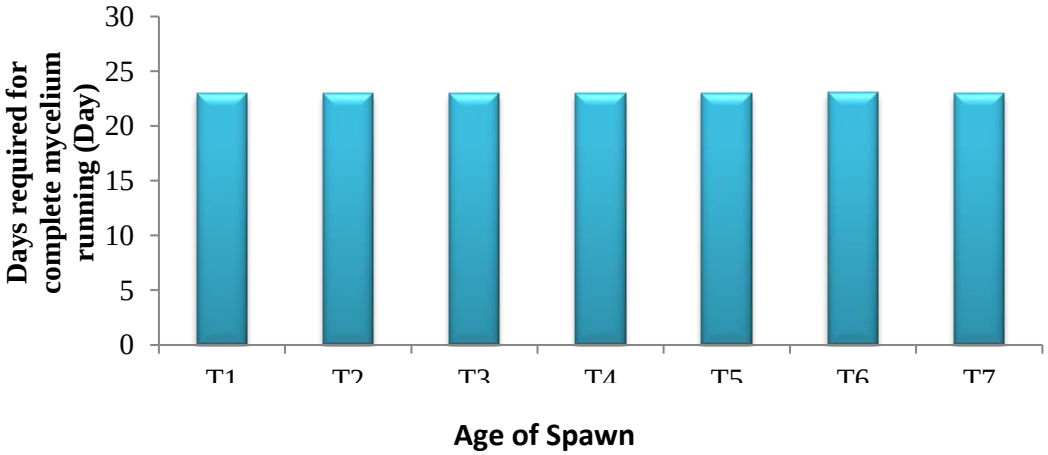

Figure 2. Effect of age of spawn on the days required for complete mycelium running of oyster mushroom species (LSD $_{(0.05)}$ = NS). Spawn age: T_1 (1 day old) ,T_2(5 days old), T_3 or control (10 days old), T_4 (15 days old), T_5 (20 days old), T_6 (25 days old) and T_7 (30 days old).

There had no significant variation existed within the oyster mushroom varieties and age of spawn combination in case of days required for complete mycelium running (DCMR) 23 days (Fig.02 and Appendix. I). T_1 (1 day old) ,T_2 (5 days old),T_3 (10 days old), T_4 (15 days old), T_5 (20 days old), T_6 (25 days old) and T_7 (30 days old) and they were statistically similar.

Due to combined effect of different varieties and age of spawn showed significant variation on days required to complete mycelium running (Table.1 and Appendix. I).The treatment combination of V_4T_1, V_4T_5 and V_4T_7 took the maximum time (25.25 days) required to complete mycelium running. On the other hand, V_1T_1 (22.00 days) and V_3T_1 (22.00 days) took the lowest number of days to complete mycelium running. This result was also in agreement with Shah *et al*. (2004) who reported that the mycelium running took 16.67- 25.00 days after inoculation in case of *Pleurotus ostreatus* . All substrates were inoculated at the same day. It also agrees with the findings of Tan (1981) who reported that the spawn running took three weeks.

4.2. Days required for pin head initiation

Days required for pinhead initiation (DRPI) significantly influenced by varieties and ranged from 2.78 to 3.85 days (Fig.03 and Appendix. I). Variety V_1 required the highest (3.85) days for pin head initiation which was significantly different from other varieties. The lowest period of 2.78 days was required for pin head initiation in V_4 *(Pleurotus salmoneostramineus).* These results are in agreement with Ahmad (1986) who stated that *Pleurotus ostreatus* completed spawn running in 17-20 days on different substrates and time for pinhead formation was noted as 23-27 days means pinhead initiation required (3-6 days).

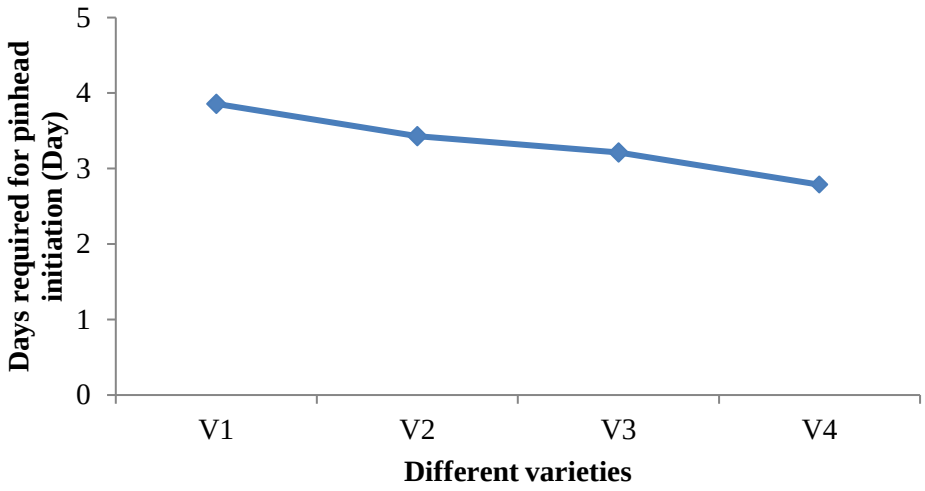

Figure 03. Effect of varietal variation on the days required for pinhead initiation of oyster mushroom species (LSD $_{(0.05)}$ = 0.12). V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

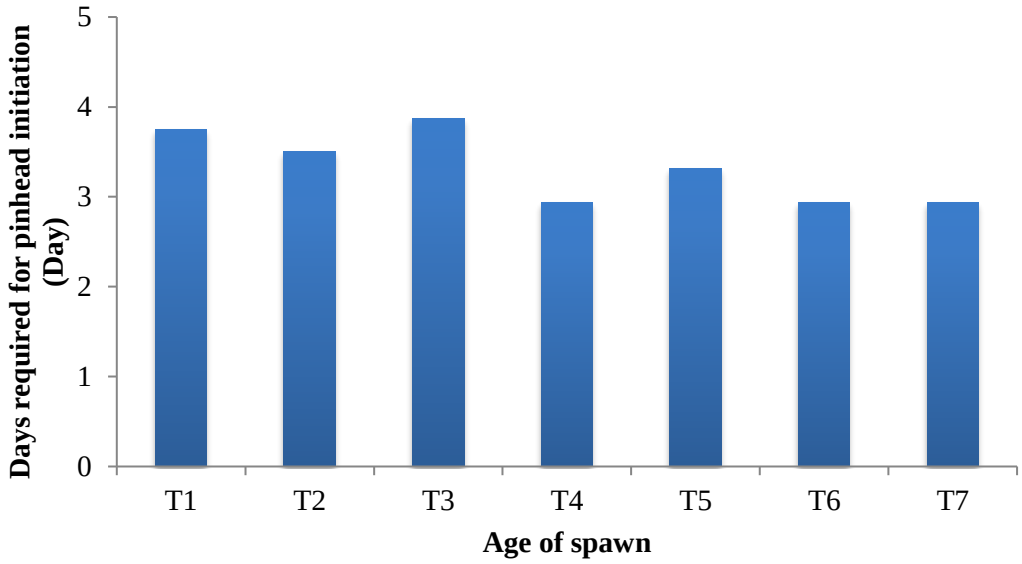

Figure 04. Effect of age of spawn on the days required for pinhead initiation of oyster mushroom species (LSD $_{(0.05)}$ = 0.16). Spawn age: T_1(1 day old) ,T_2(5 days old), T_3 or control(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old) and T_7 (30 days old).

Age of spawn significantly varied on days required to pinhead initiation of oyster mushroom and it ranged from 2.93 to 3.87 days (Fig. 4 and Appendix. I). The highest days were required in T_3 or control (3.87 days) for DRPI which was statistically similar to T_1 (3.75 days) whereas, the lowest value (2.93 days) was found in T_4, T_6 and T_7 .

Combined effect of varieties and age of spawn showed significant variation on days required to pinhead initiation (Table.1 and Appendix. I). The treatment combination V_1T_2 and V_3T_1 took the maximum period (5-50 days) for days required to pinhead initiation which was statistically similar to V_1T_3 (5.25 days). And the lowest value was found in V_3T_4 and V_3T_7 (2.00 days) which was statistically similar to V_4T_2(1.75 days). This result was also coroborated with Obodai *et al.* (2003) who reported that pinhead formation took four to six days after the completion of spawn running, with harvest after 10 to 12 days in the case of *P. ostreatus.*

4.3 Days required to first harvest

Significant variation was observed due to the effect of varieties on days required to first harvest (DFH) of oyster mushroom (Fig. 5 and Appendix. I). The highest (2.96 days) number of days required for first harvest was recorded in V_2 and the lowest was found in V_4 (2.28 days).

Days required to first harvest after pinhead initiation was significantly influenced by age of spawn and it ranged from 2.50 to 2.75 days (Fig. 6 and Appendix. I). T_1 and T_5 took the highest period (2.75 days) which was statistically similar to T_4 (2.68 days) whereas T_6 took the lowest period (2.5 days).

Combined effect of varieties and age of spawn showed significant variation on days required to first harvest (Table. 1 and Appendix. I). The highest days was recorded in V_2T_4, V_2T_5 and V_3T_1(3.25 days) to first harvest .The lowest value was found in V_1T_2, V_1T_4 ,V_1T_6 and V_4T_3 (2.00 days). The result of the present

experiment are is agreement with the findings of Bugarski *et al.*, (1994) who found that the first harvest required different days depending on substrate.

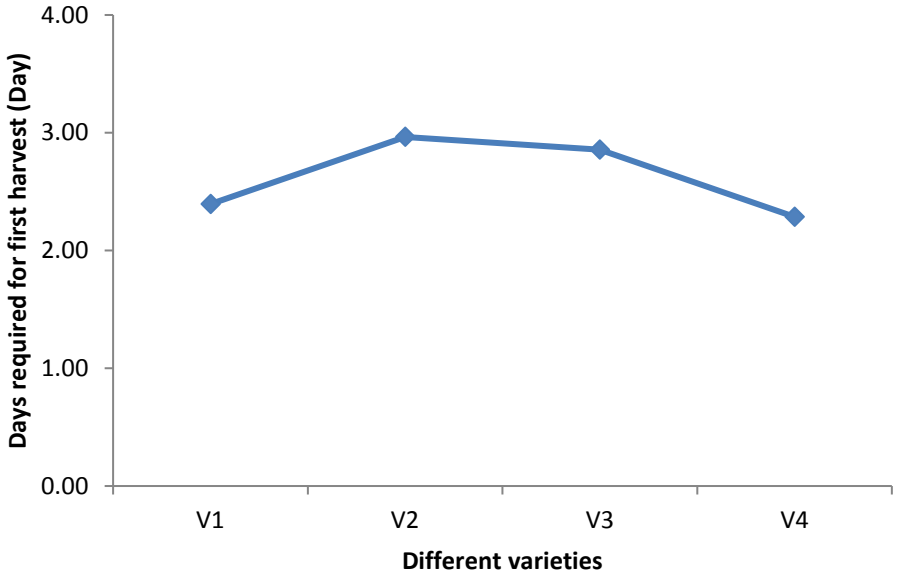

Figure 05. Effect of varietal variation on the days required for first harvest of oyster mushroom species (LSD $_{(0.05)}$ = 0.09). V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

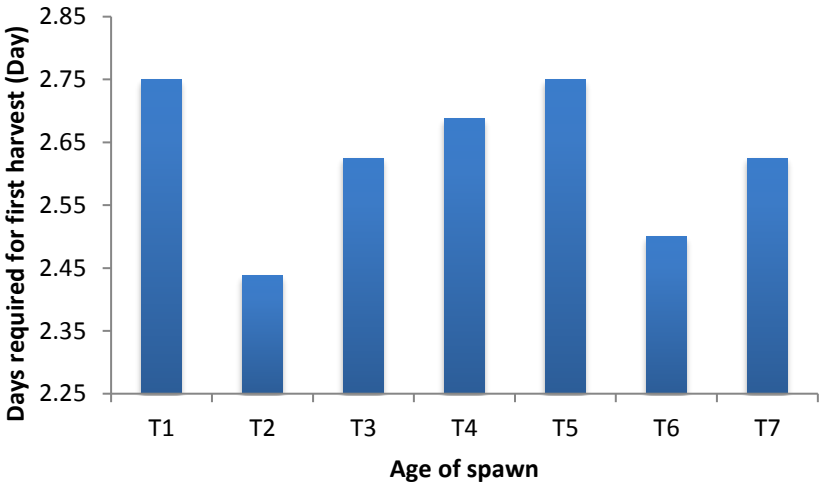

Figure 06. Effect of age of spawn on the days required for first harvest of oyster mushroom species (LSD $_{(0.05)}$ = 0.12). Spawn age: T_1(1 day old) ,T_2(5 days old), T_3 or control(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old) and T_7 (30 days old).

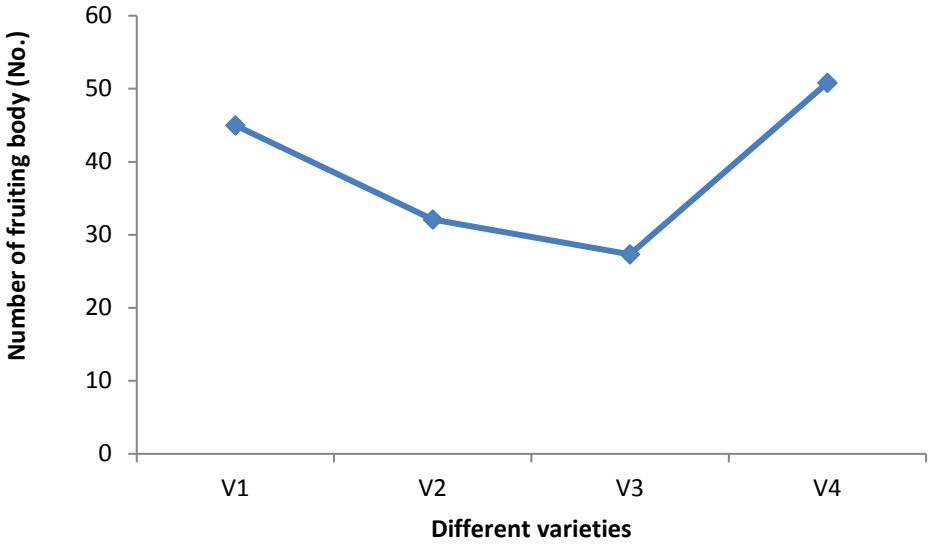

Figure 07. Effect of varietal variation on the number of fruiting body of oyster mushroom species (LSD $_{(0.05)}$ = 0.81). V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

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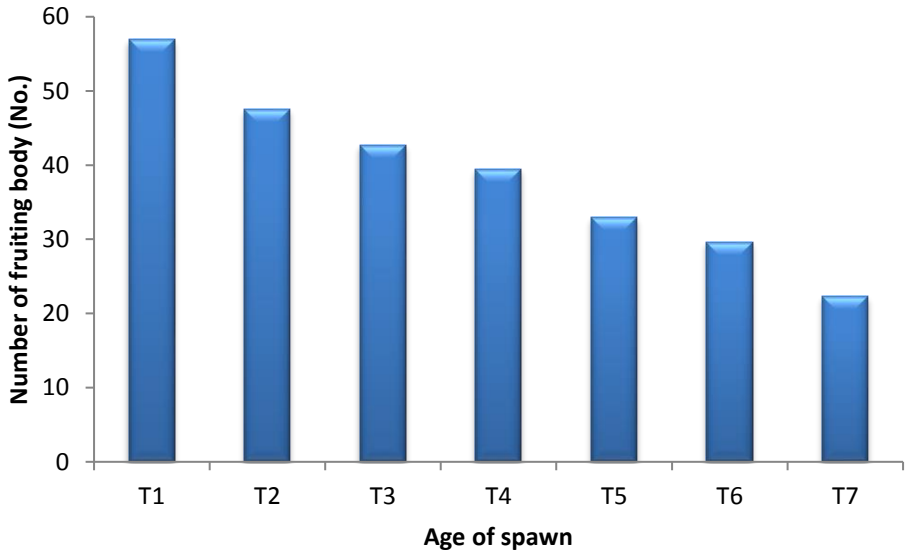

Figure 08. Effect of age of spawn on the number of fruiting body of oyster mushroom species (LSD $_{(0.05)}$ = 1.07). Spawn age: T_1(1 day old) ,T_2(5 days old), T_3 or control(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old) and T_7 (30 days old).

4.4 Number of fruiting body

The number of fruiting body significantly differed among the different strains (Fig.07 and Appendix. I). The highest (50.82) number of fruiting body was found in V_4. In case of V_1 and V_2 the number of fruiting bodies were 27.11 and 20.68 respectively. The lowest (27.32) number of fruiting body was found in V_3.

The number of fruiting body significantly influenced by age of spawn and it ranged from 22.31 – 56.94 (Fig.08 and Appendix. I). The highest (56.94) number of fruiting body was found in T_1. The lowest (22.31) numbers of fruiting body was found in T_7. Here, T_3 was considered as control because farmers normally opened spawn packet at 10 days old. In the present study T_1 was gave the highest number of fruiting body than control.

Significant variation was recorded due to combined effect of oyster mushroom varieties and age of spawn on number of fruiting body and it ranged from 12.00 to 81.25 (Table. 1 and Appendix. I). The highest number of fruiting body was recorded in V_4T_1 (81.25) and the lowest (12.00) number of fruiting body was found in V_3T_7. Amin *et al.* (2007) found the maximum number of fruiting bodies of different oyster mushroom species on SD when compared with RS. Although king oyster gives lower number of fruiting bodies, texture and shelf life is very higher than other Pleurotus spp. Similar result was found in shiitake mushroom (Sarker *et al.*, 2009).

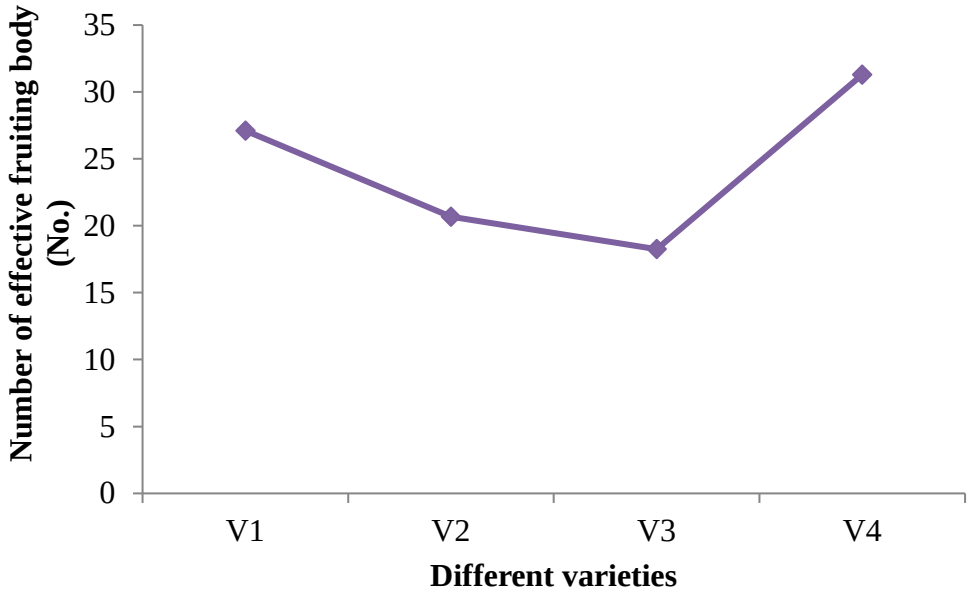

Figure 09. Effect of varietal variation on the number of effective fruiting body of oyster mushroom species (LSD $_{(0.05)}$ = 0.76). V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

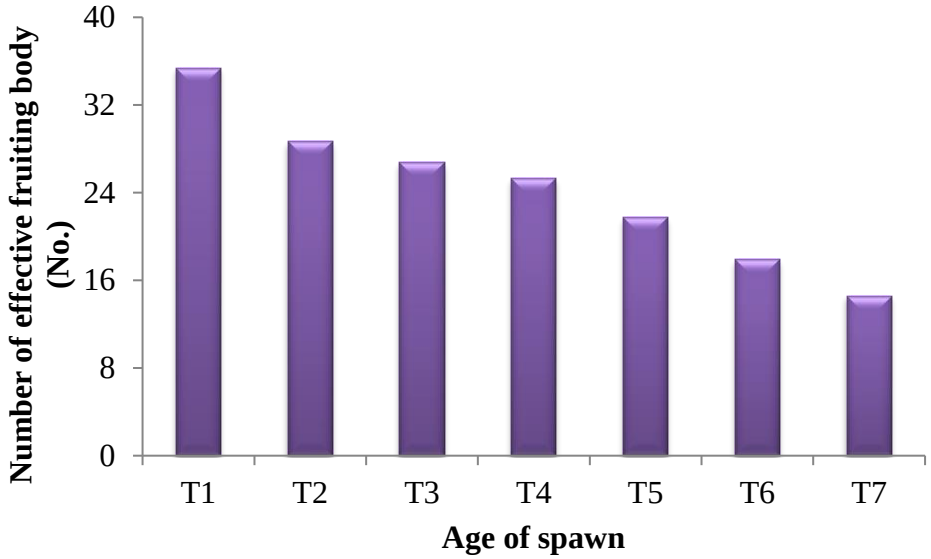

Figure10. Effect of age of spawn on the number of effective fruiting body of oyster mushroom species (LSD $_{(0.05)}$ = 1.00). Spawn age: T_1(1 day old) ,T_2(5 days old), T_3 or control(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old) and T_7 (30 days old).

4.5 Number of effective fruiting body

The number of effective fruiting body was significantly influenced by different varieties (Fig. 09 and Appendix. I). The highest number of fruiting body (31.32) was found in V_4. The lowest (18.25) number of fruiting body was found in V_3.

The number of effective fruiting body was significantly influenced by the age of spawn and ranged from 14.56 – 35.38 (Fig.10 and Appendix. I). The highest (35.38) number of fruiting body was found in T_1. This number was not statistically similar to the other age of spawn. The lowest (14.56) number of fruiting body was found in T_7.

Due to the combined effect of varieties and age of spawn significant variation was found on number of effective fruiting body (Table.01 and Appendix. I).The highest number of fruiting body was recorded in V_4T_1 (51.00) and the lowest (7.75) number of fruiting body was found in V_3T_7.According to Mondal *et. al.*(2010) the number of effective fruiting body ranged from 8.5 to 37.25 and the maximum number of effective fruiting body was recorded on sawdust which agreed with the present study.

Table 1. Combined effect of varietal variation and age of spawn on the days required for complete mycelium running, days required for pinhead initiation, days required for first harvest, no. of fruiting body and no. of effective fruiting body of oyster mushroom

Treatment	Days required for complete mycelium running (Day)	Days required for pinhead initiation (Day)	Days required for first harvest (Day)	No. of fruiting body (No.)	No. of effective fruiting body (No.)
V_1T_1	22.00 b	2.50 hi	2.75 c	66.00 b	38.00 b
V_1T_2	22.25 b	5.50 a	2.00 f	49.50 e	30.00 d
V_1T_3	22.25 b	5.25 a	3.00 b	46.00 f	29.75 de
V_1T_4	22.50 b	3.50 de	2.00 f	42.25 g	28.50 def
V_1T_5	22.25 b	3.50 de	2.50 d	41.75 g	25.75 gh
V_1T_6	22.50 b	3.25 ef	2.00 f	39.50 h	19.00 j-m
V_1T_7	22.25 b	3.50 de	2.50 d	29.75 k	18.75 klm
V_2T_1	22.50 b	3.75 cd	2.75 c	44.50 f	27.75 efg
V_2T_2	22.25 b	3.50 de	2.50 d	40.75 gh	26.75 fgh
V_2T_3	22.25 b	4.25 b	3.00 b	32.25 j	20.25 i-l
V_2T_4	22.25 b	3.25 ef	3.25 a	29.50 kl	21.00 ij
V_2T_5	22.25 b	3.00 fg	3.25 a	27.25 m	17.50 mno
V_2T_6	22.25 b	3.00 fg	3.00 b	27.50 lm	16.25 op
V_2T_7	22.25 b	3.25 ef	3.00 b	22.75 no	15.25 p
V_3T_1	22.00 b	5.50 a	3.25 a	36.00 i	24.75 h
V_3T_2	22.25 b	3.25 ef	2.75 c	35.25 i	22.00 i
V_3T_3	22.25 b	2.50 hi	2.50 d	31.25 jk	20.75 ijk
V_3T_4	22.50 b	2.00 jk	3.00 b	31.00 jk	17.50 mno
V_3T_5	22.25 b	4.00 bc	3.00 b	23.75 no	17.25 m-p
V_3T_6	22.50 b	3.25 ef	2.75 c	22.00 o	17.75mno
V_3T_7	22.25 b	2.00 jk	2.75 c	12.00 p	7.750 q
V_4T_1	25.25 a	3.25 ef	2.25 e	81.25 a	51.00 a
V_4T_2	25.00 a	1.75 k	2.50 d	64.75 b	36.00 bc
V_4T_3	25.00 a	3.50 de	2.00 f	61.25 c	36.25 bc
V_4T_4	24.75 a	3.00 fg	2.50 d	55.25 d	34.25 c
V_4T_5	25.25 a	2.75 gh	2.25 e	39.00 h	26.50 fgh
V_4T_6	25.00 a	2.25 ij	2.25 e	29.50 kl	18.50 lmn
V_4T_7	25.25 a	3.00 fg	2.25 e	24.75 n	16.50 nop
CV (%)	2.35	6.84	6.55	3.91	5.87

Variety: V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).* Spawn age: T_1(1 day old) ,T_2(5 days old), T_3 or control(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old) and T_7 (30 days old).

4.6 Length of stalk

The length of stalk was significantly differed by the effect of different varieties and it ranged from 1.67 to 3.03 cm (Fig.11 and Appendix. II). The highest length of stalk was found in V_2 (3.03cm) and the lowest length of stalk was found in V_1 (1.67cm).

The length of stalk showed significant difference due to the effect of spawn (Fig.12 and Appendix. II). The highest length of stalk was found in T_5 (2.77 cm) and the lowest length of stalk was found in T_3 (1.88cm).

Significant variation was recorded on length of stalk due to the combined effect of varieties and age of spawn. The length of stalk ranged from 0.90 to 4.63 cm (Table.02 and Appendix. II). The highest length was recorded in V_2T_5 (4.63 cm) and the lowest length was found in V_1T_3 (0.90 cm), and V_1T_6 (0.90 cm). Ahmed *et.al.* (2013) observed differences in stalk length (2.43 to 3.24 cm) for *P. ostreatus*, whereas Monadal *et.al.* (2010) reported a decrease in the storage quality of the oyster mushroom with the increase of the pileus and stalk diameters.

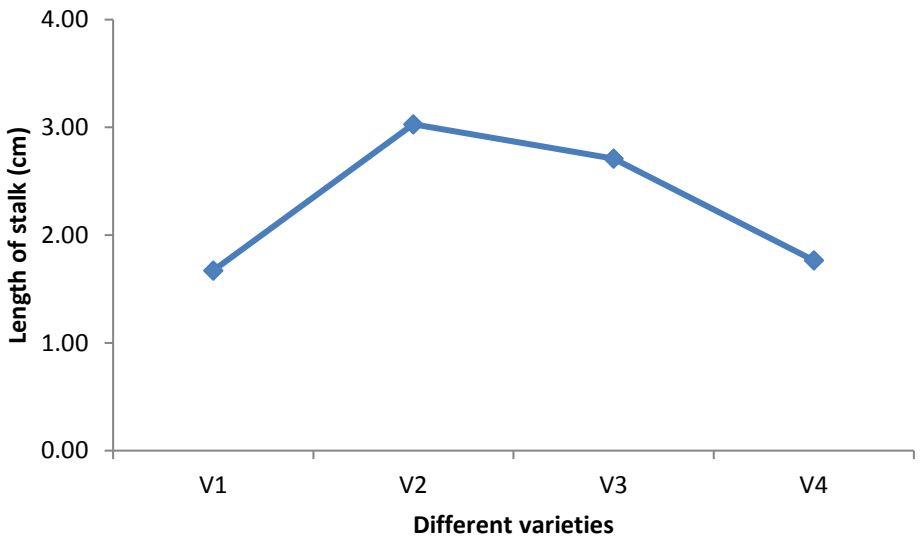

Figure 11. Effect of varietal variation on the length of stalk of oyster mushroom species (LSD $_{(0.05)}$ = 0.06). V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

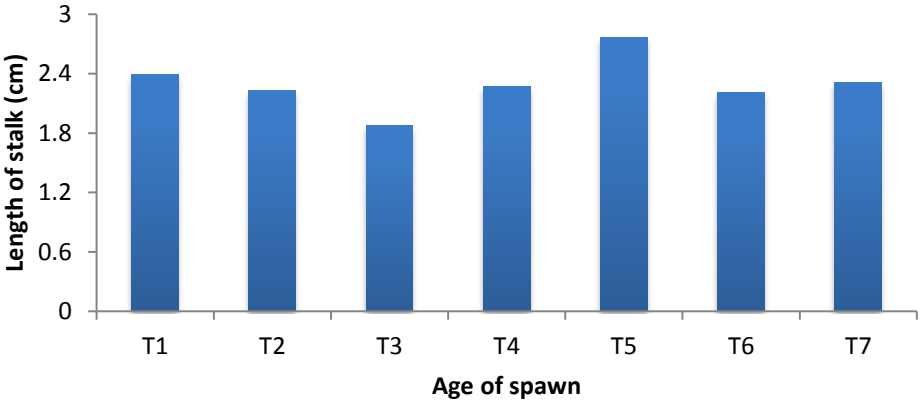

Figure 12. Effect of age of spawn on the length of stalk of oyster mushroom species (LSD $_{(0.05)}$ = 0.07) Spawn age: T_1(1 day old) ,T_2(5 days old), T_3 or control(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old) and T_7 (30 days old).

4.7 Diameter of pileus

Due to the effect of different varieties of oyster mushroom significant differences was found in diameter of pileus (Fig.13 and Appendix. II). The highest diameter of pileus was found in V_3 (6.75cm) followed by V_4 (6.07cm) and the lowest diameter of pileus was found in V_1 (5.85 cm).

Significant difference was found in diameter of pileus which ranged from 5.56 to 6.85 cm (Fig.14 and Appendix. II). The highest diameter of pileus was found in T_3 (6.85cm) followed by T_1 (6.68cm). The lowest diameter of pileus was found in T_7 (5.56 cm) which was statistically similar to T_5 (5.60 cm) and T_6 (5.62 cm).

Combined effect of different oyster mushroom varieties and age of spawn showed significant variation on diameter of pileus (Table.02 and Appendix. II). The highest diameter was recorded in V_3T_3 (9.50 cm) and the lowest diameter was found in V_1T_4 (4.98 cm) which was statistically similar to V_1T_5 (5.00 cm), V_1T_6 (5.00 cm) and V_3T_7(5.03 cm) and similar results were found by Monadal *et al.* (2010).

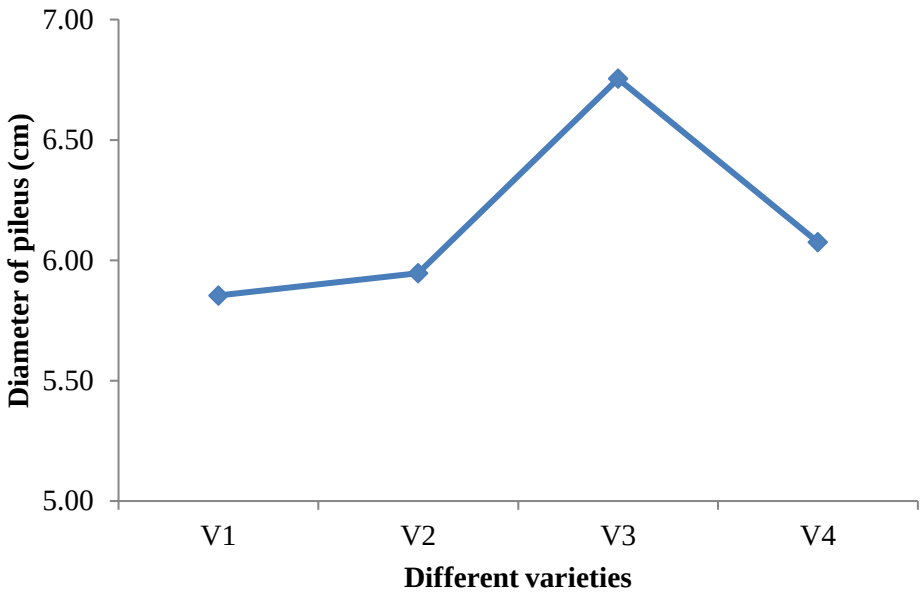

Figure 13. Effect of varietal variation on the diameter of pileus of oyster mushroom species (LSD $_{(0.05)}$ = 0.07). V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

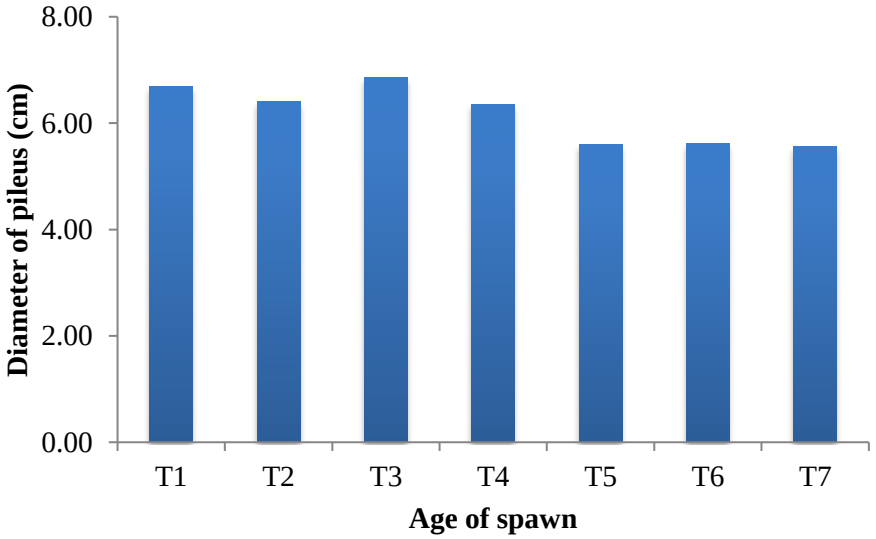

Figure 14. Effect of age of spawn on the diameter of pileus of oyster mushroom species (LSD $_{(0.05)}$ = 0.09). Spawn age: T_1(1 day old) ,T_2(5 days old), T_3 or control(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old) and T_7 (30 days old).

4.8 Thickness of pileus

There had a significant differences on thickness of pileus due to the effect of different varieties of mushroom which ranged from 0.60 to 0.90 cm (Fig.15 and Appendix. II). The highest thickness was found in V_3 (0.90 cm) which was statistically similar to V_2 (0.89 cm) and the lowest thickness of pileus (0.60 cm) was found in V_1.

Significant variation was found on thickness of pileus due to the effect of age of spawn and ranged from 0.66 to 0.87 cm (Fig.16 and Appendix. II). The highest thickness was found in T_3 (0.87 cm) and thickness of pileus was the lowest in T_5(0.66 cm).

The combined effect of age of spawn and mushroom varieties was significantly influenced on thickness of pileus (Table.02 and Appendix. II). The highest thickness of pileus was recorded in V_3T_3 (1.23 cm) which statistically differed from other treatments. However, the lowest thickness of pileus was recorded in V_1T_2 (0.53 cm) which was statistically similar to V_1T_3(0.55 cm), V_1T_6(0.55 cm) and V_4T_5(0.55 cm). Sarker *et al.* (2007b) found an average thickness of pileus of 0.5 to 0.8 cm for oyster mushroom which agree with the current study.

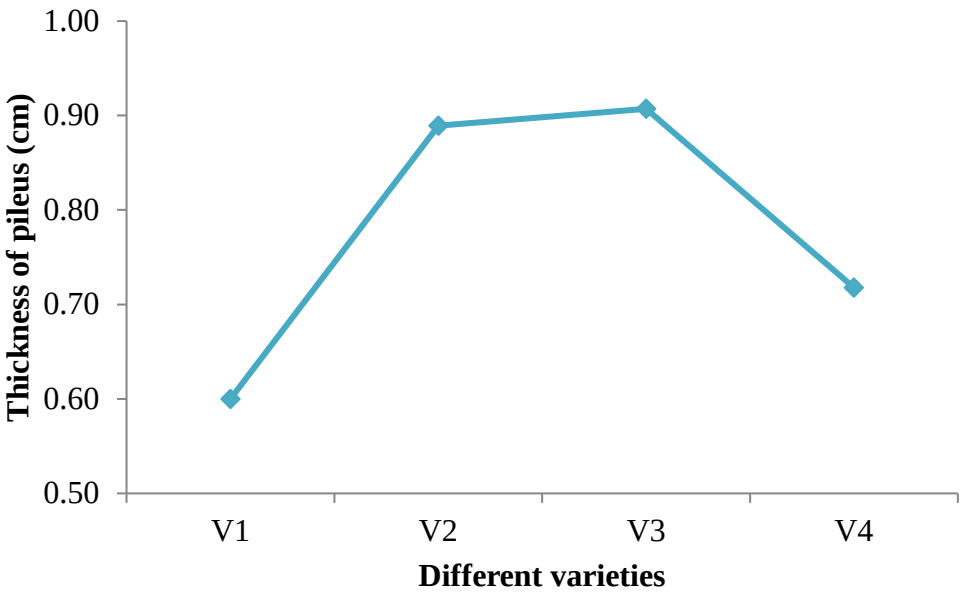

Figure 15. Effect of varietal variation on the thickness of pileus of oyster mushroom species (LSD $_{(0.05)}$ = 0.03). V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

Figure 16. Effect of age of spawn on the thickness of pileus of oyster mushroom species (LSD $_{(0.05)}$ = 0.04). Spawn age: T_1(1 day old) ,T_2(5 days old), T_3 or control(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old) and T_7 (30 days old).

4.9 Diameter of stalk

Due to the effect of different varieties significant variation was found on diameter of stalk which ranged from 0.54 to 0.68 cm (Fig.17 and Appendix. II). However the highest diameter of stalk was found in V_4 (0.68 cm) which was statistically similar to V_2 (0.66 cm). The lowest diameter of stalk was found in V_1 (0.54 cm).

Significant differences was found on diameter of stalk (cm) due to the effect of age of spawn. The range of diameter of stalk was from 0.60 to 0.76 cm (Fig.18 and Appendix. II) .The highest diameter was found in T_1 (0.76 cm) which was statistically similar to T_3 (0.71 cm) and diameter of stalk was the lowest in T_5(0.60 cm) and T_2(0.60 cm) which was statistically similar to T_7 (0.63 cm).

The combined effect of age of spawn and mushroom varieties showed significant variation on diameter of stalk (Table.02 and Appendix. II). The highest diameter of stalk was recorded in V_3T_3 (0.98 cm). The lowest diameter of stalk was recorded in V_1T_4(0.45 cm) which was statistically similar to V_1T_3(0.50 cm), V_1T_6(0.51 cm) and V_4T_4(0.52 cm). Moonmoon *et. al.* (2012) reported that the diameter of stalk ranged from 0.30 to 0.41 in different oyster mushroom species.

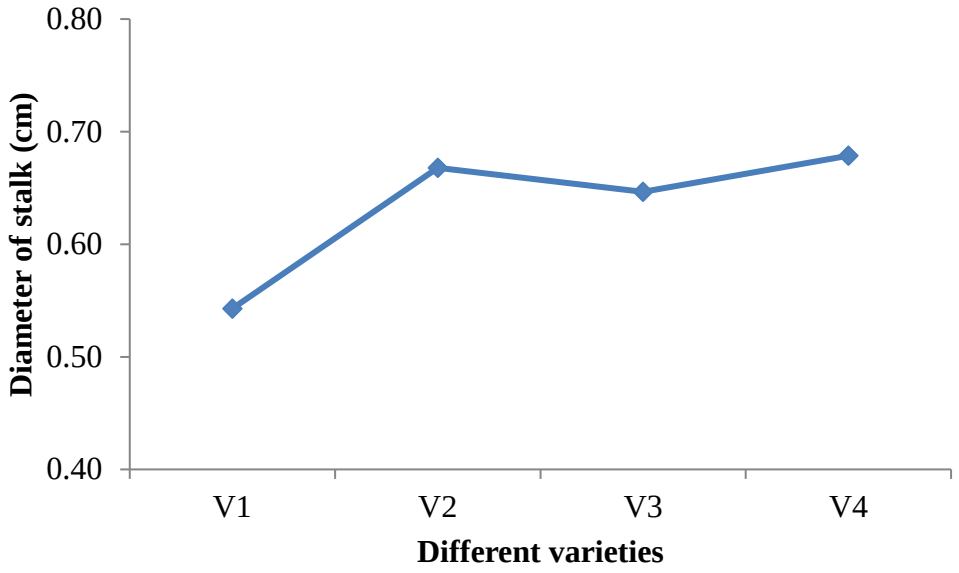

Figure 17. Effect of varietal variation on the diameter of stalk of oyster mushroom species (LSD $_{(0.05)}$ = 0.03). V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

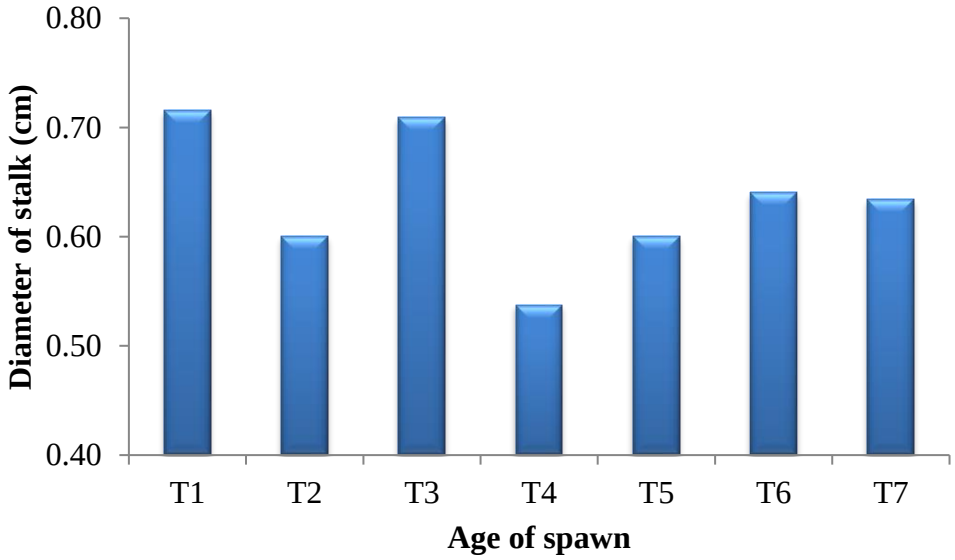

Figure18. Effect of age of spawn on the diameter of stalk of oyster mushroom species (LSD $_{(0.05)}$ = 0.04) Spawn age: T_1(1 day old) ,T_2(5 days old), T_3 or control(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old) and T_7 (30 days old).

4.10 Yield

Variation of yield per packet was significantly influenced by different varieties of oyster mushroom (Fig.19 and Appendix. II). The highest yield (104.8g/packet) was found in V_4 followed by V_2 (91.14 g/ packet) and the lowest yield was found in V_1 (77.39 g/packet). The recorded yield of V_3 was 89.75 g/packet.

Significant variation was observed in yield per packet due to effect of age of spawn (Fig.20 and Appendix. II). The highest yield (127.6 g/500g packet) was found in T_1 followed by T_2 (109.3 g/500g packet) and the lowest yield was found in T_7 (62.06 g/500g packet).

Significant variation was recorded due to the combined effect of different varieties of oyster mushroom and age of spawn (Table.02 and Appendix. II). The highest yield (137.3 g/500g packet) was found in V_4T_1 which was statistically similar to V_1T_1 (135.3 g/500g packet). Whereas, the treatment combination of V_1T_7 gave the lowest (41.00g/500g packet) yield. So the strain of *Pleurotus salmoneostramineus* with one day old spawn had given maximum mushroom yield. Pani (2011) observed his study that Mushroom yield decreased with increase in spawn age. This statement also matched with the present study. In this current study, the lowest yield was found in 30 days old spawn of V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow),* V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

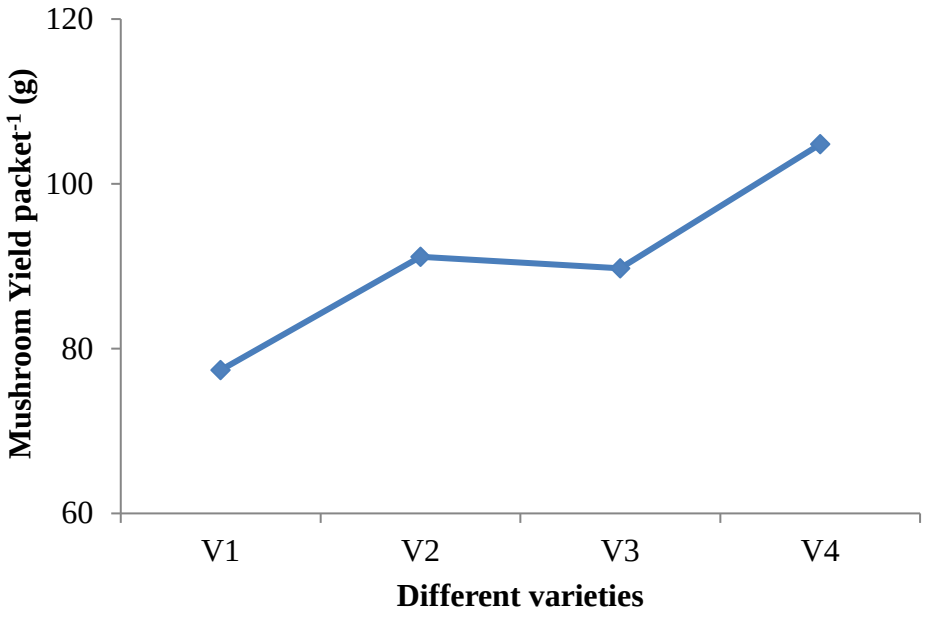

Figure 19. Effect of varietal variation on the mushroom yield packet^{-1} of oyster mushroom species (LSD $_{(0.05)}$ = 1.12). V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

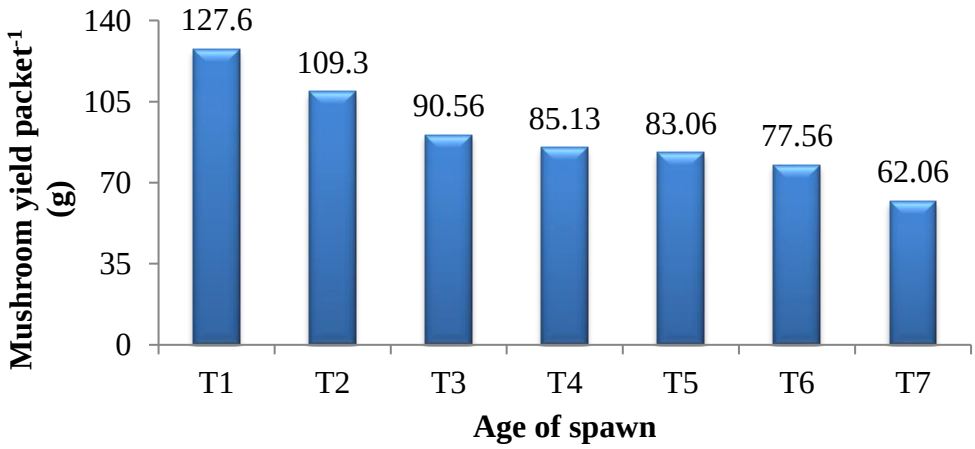

Figure 20. Effect of age of spawn on the mushroom yield packet^{-1} of oyster mushroom species (LSD $_{(0.05)}$ = 1.48). Spawn age: T_1(1 day old) ,T_2(5 days old), T_3 or control(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old) and T_7 (30 days old).

4.11 Biological Efficiency

Significant variation was observed on biological efficiency due to the effect of different varieties of oyster mushroom (Fig.21 and Appendix. II). The highest biological efficiency (52.38%) was found in V_4 followed by V_2 (45.51%) and the lowest biological efficiency was found in V_1 (38.70%). The recorded biological efficiency of V_3 was 44.88%. Kirbag and Akyuz (2008) found 48.05% biological efficiency on wheat straw. In this study, the BE was found higher with difference among strains and substrates. Peng *et al.* (2001) also reported the different biological efficiency in different strains on sawdust.

Significant variation was observed on biological efficiency due to the effect of different age of spawn (Fig.22 and Appendix. II). The highest biological efficiency (63.81%) was found in T_1 followed by T_2 (54.66%) and the lowest biological efficiency was found in T_7 (31.03%).

Biological efficiency was significantly influenced by the combined effect of different varieties of oyster mushroom and age of spawn which ranged from 20.50 to 68.63% (Table.02 and Appendix. II). The highest biological efficiency (68.63%) was found in V_4T_1 . which was statistically similar to V_1T_1(67.63%) and the lowest biological yield was found in V_1T_7 (20.50%). Shah *et al* (2004) showed in their study that substrate saw dust provided the best biological efficiency (64.69%) in case of oyster mushroom which was also found in the current study.

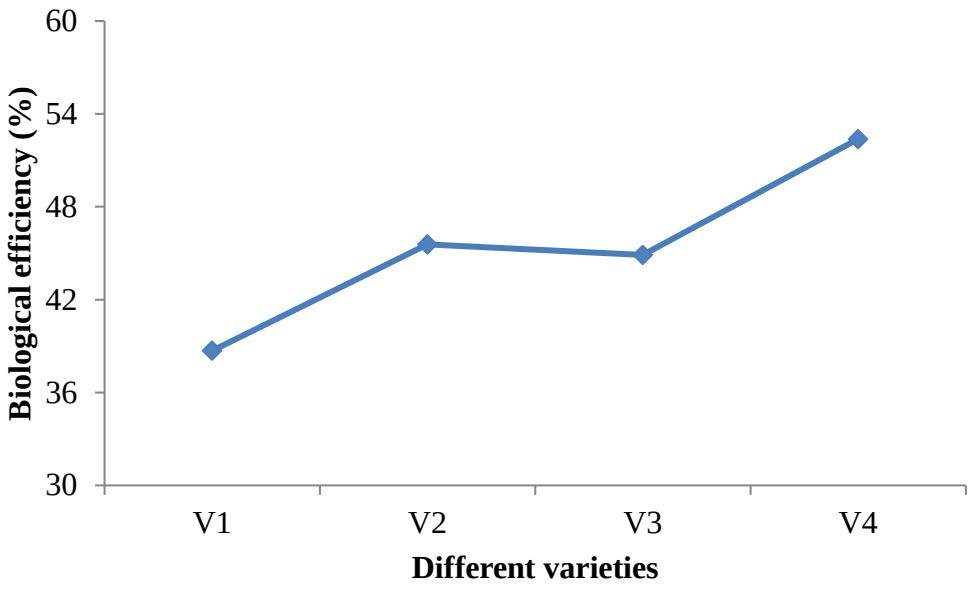

Figure 21. Effect of varietal variation on the biological efficiency of oyster mushroom species (LSD $_{(0.05)}$ = 0.56).Variety: V_1*(Pleurotus djamor*), V_2 (*P. ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*

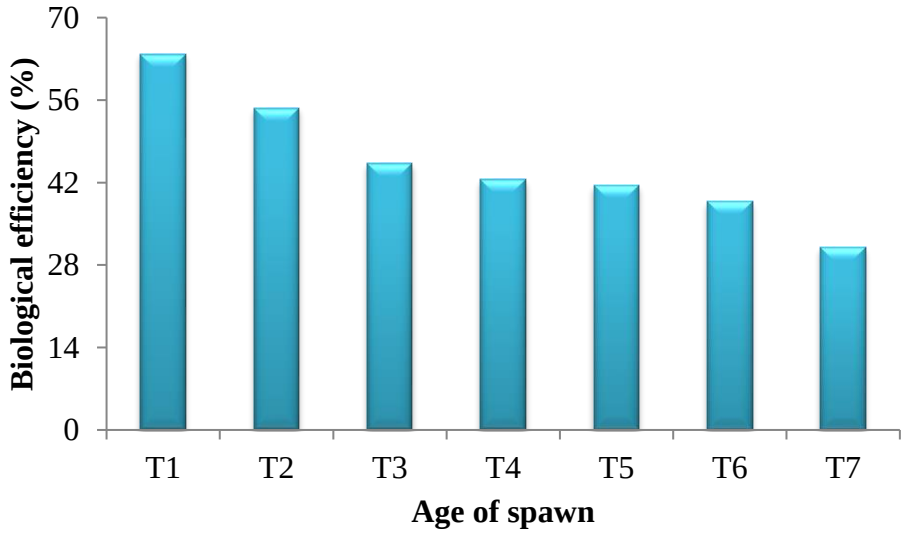

Figure 22. Effect of age of spawn on the mushroom biological efficiency of oyster mushroom species (LSD $_{(0.05)}$ = 0.74). Spawn age: T_1(1 day old) ,T_2(5 days old), T_3 or control(10 days old), T_4(15 days old), T_5(20 days old), T_6 (25 days old) and T_7 (30 days old).

Table 02. Combined effect of varietal variation and age of spawn on the length of stalk, diameter of pileus, thickness of pileus, diameter of stalk, yield of mushroom packet^{-1} and biological efficiency of oyster mushroom species

Treatment	Length of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Diameter of stalk (cm)	Yield of mushroom packet^{-1} (g)	Biological efficiency (%)
V_1T_1	2.30 hi	8.05 b	0.63 ij	0.60 f-i	135.3 a	67.63 a
V_1T_2	2.20 ij	5.93 ij	0.53 k	0.55 h-j	108.0 d	54.00 d
V_1T_3	0.90 o	6.13 f-h	0.55 jk	0.50 jk	69.75 l	34.88 l
V_1T_4	1.50 mn	4.98 m	0.70 hi	0.45 k	63.00 m	31.50 m
V_1T_5	2.10 jk	5.00 m	0.63 ij	0.65 e-g	63.75 m	31.88 m
V_1T_6	0.90 o	5.00 m	0.55 jk	0.51 jk	61.00 m	30.50 m
V_1T_7	1.80 l	5.90 ij	0.63 ij	0.54 ij	41.00 o	20.50 o
V_2T_1	2.03 k	5.70 k	0.98 b	0.74 cd	111.5 c	55.75 c
V_2T_2	2.60 f	6.63 cd	0.93 bc	0.65 e-g	108.3 d	54.13 d
V_2T_3	2.80 e	6.00 hi	0.98 b	0.74 cd	92.25 g	46.13 g
V_2T_4	3.03 bc	6.08 g-i	0.93 bc	0.63 f-h	87.25 hi	43.63 hi
V_2T_5	4.63 a	5.93 ij	0.73 gh	0.60 f-i	84.50 ij	42.25ij
V_2T_6	3.13 b	6.00 hi	0.88 c-e	0.68 d-f	83.00 j	41.50 j
V_2T_7	3.00 bc	5.30 l	0.83 d-f	0.65 e-g	71.25 l	35.63 l
V_3T_1	2.95 cd	6.70 c	0.93 bc	0.68 d-f	126.5 b	63.25 b
V_3T_2	2.38 h	6.65 c	0.95 bc	0.55 h-j	96.00 f	48.00 f
V_3T_3	2.43 gh	9.50 a	1.23 a	0.98 a	94.00 fg	47.00 fg
V_3T_4	2.80 e	8.08 b	0.90 b-d	0.55 h-j	88.00 h	44.00 h
V_3T_5	2.83 de	6.08 g-i	0.73 gh	0.58 g-j	83.75 j	41.88 j
V_3T_6	3.05 bc	5.25 l	0.80 e-g	0.58 g-j	82.00 j	41.00 j
V_3T_7	2.55 fg	5.03 m	0.83 d-f	0.63 f-h	58.00 n	29.00 n
V_4T_1	2.30 hi	6.30 ef	0.80 e-g	0.85 b	137.3 a	68.63 a
V_4T_2	1.75 l	6.45 de	0.75 f-h	0.65 e-g	125.0 b	62.50 b
V_4T_3	1.38 n	5.80 jk	0.73 gh	0.63 f-h	106.3 d	53.13 d
V_4T_4	1.75 l	6.30 ef	0.63 ij	0.53 i-k	102.3 e	51.13 e
V_4T_5	1.53 m	5.43 l	0.55 jk	0.58 g-j	100.3 e	50.13 e
V_4T_6	1.78 l	6.23 fg	0.78 f-h	0.80 bc	84.25 j	42.13 j
V_4T_7	1.88 l	6.03 hi	0.80 e-g	0.73 c-e	78.00 k	39.00 k
CV (%)	4.49	2.1	7.35	8.52	2.32	2.32

Variety: V1*(Pleurotus djamor*), V2 (*P. ostreatus var. white snow)* , V3*(Pleurotus ostreatus)* and V4 *(Pleurotus salmoneostramineus).* Spawn age: T1(1 day old) ,T2(5 days old), T3 or control(10 days old), T4(15 days old), T5(20 days old), T6 (25 days old) and T7 (30 days old).

Chapter V

Summery and Conclusion

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted to find out the effect of age of spawn on yield and yield attributes of different oyster mushroom species. The study was carried out in the laboratory, workshop and culture house of Mushroom Development Institute (MDI), Savar, Dhaka from January to June, 2014. Four selected species of oyster mushroom such as, *Pleurotus ostreatus*, *Pleurotus djamor*, *Pleurotus ostreatus var. white snow* and *Pleurotus salmoneostramineus*were cultivated in the culture house of the Mushroom Development Institute. Mushroom varieties used in the experiment was collected from germplasm centre of Mushroom Development Institute (MDI), Savar, Dhaka. This was cultured in biotechnology laboratory and cultivated in the culture house of MDI.

The experiment was laid out following completely randomized design (CRD) with 4 varieties and 4 replications and each replication contain twelve population. Data were analyzed following Gomez and Gomez (1984) using MSTAT-C computer program and Excel software. Means separation were computed following Least Significant Difference (LSD) using the same computer program.There were two factors in this experiment.Factor A: Variety, V_1*(Pleurotus djamor*), V_2 (*Pleurotus ostreatus var. white snow)* , V_3*(Pleurotus ostreatus)* and V_4 *(Pleurotus salmoneostramineus).*And Factor B: Age of spawn , T_1(1 day old),T_2 (5 days old), T_3 (10 days old), T_4 (15 days old), T_5(20 days old), T_6 (25 days old)and T_7 (30 days old).

Different parameters were selected for data collection and data were collected ondays required for complete mycelium running, days required for pinhead initiation, days required for first harvest,no. of fruiting body, no. of effective fruiting body, the length of stalk, diameter of pileus, thickness of pileus, diameter of stalk, yield of mushroom packet^{-1} and biological efficiency of oyster mushroom species.

In case of varietal variation, the highest days required for complete mycelium running was recorded in V_4 (25.07 days) andthe lowestamong V_1, V_2 and V_3.The highest days required for pinhead initiationwas recorded in V_1 (3.85 days) and the lowest in V_4 (2.78 days). The highestdays required for first harvestwas recorded in V_2 (2.96 days) and the lowest in V_4 (2.28 days). The highestno. of fruiting body was recorded in V_4 (50.82) and the lowest in V_3 (27.32). The highestno. of effective fruiting body was recorded in V_4 (31.32) and the lowest in V_3 (18.25). The highest length of stalk was recorded in V_2 (3.03 cm) and the lowest in V_1 (1.67 cm). The highestdiameter of pileus was recorded in V_3 (6.75 cm) and the lowest in V_1 (5.85 cm). The highestthickness of pileus was recorded in V_3 (0.90 cm) and the lowest in V_1 (0.60 cm).The highestdiameter of stalk was recorded in V_4 (0.68 cm) and the lowest in V_1 (0.54 cm). The highestYield was recorded in V_4 (104.8 g/packet) and the lowest in V_3 (89.75 g/packet). The highestbiological efficiency was recorded in V_4 (52.38%) and the lowest in V_3 (44.88%).

In case of age of spawn, the highest days required for complete mycelium running was recorded among T_1, T_2, T_3, T_4, T_5,$T_6$and T_7(23 days). The highest days required for pinhead initiationwas recorded in T_3 (3.87 days) and the lowest among T_4, T_6 andT_7(2.93 days). The highestdays required for first harvestwas recorded between T_2 and T_5 (2.75 days) and the lowest in T_6 (2.50 days). The highestno. of fruiting bodywas recorded in T_1 (56.94) and the lowest in T_7 (22.31). The highestno. of effective fruiting body was recorded in T_1 (35.38) and the lowest in T_7(14.56). The highest length of stalk was recorded in T_5 (2.77 cm) and the lowest in T_3 (1.88 cm). The highestdiameter of pileus was recorded in T_3 (6.85 cm) and the lowest in T_7(5.56 cm). The highestthickness of pileus was recorded in T_3 (0.87 cm) and the lowest in T_5(0.66 cm). The highestdiameter of stalk was recorded in T_1(0.76 cm) and the lowest betweenT_2andT_5 (0.60 cm). The highestYield was recorded in T_1(127.6 g/500g packet) and the lowest in T(62.06 g/500g packet). The highest biological efficiency was recorded in T_1(63.81%) and the lowest in T_3 (31.03%).

In case of combined effect of variety and age of spawn, the highestdays required for complete mycelium running was recorded between V_4T_1 and V_4T_7 (25.25 days) and the lowest between V_1T_1 and V_3T_1(22.00 days).The highestdays required for pinhead initiation was recorded between V_1T_2 and V_3T_1 (5.50 days) and the lowest between V_3T_4 and V_3T_7 (2.00days). The highestdays required for first harvest (3.25 days) was recorded among V_2T_4, $V_2T_5$and V_3T_1 and the lowest (2.00 days) was recorded among V_1T_2, V_1 V_4T_1 , V_1T_6 and V_4T_3.The highestno. of fruiting body (81.25) was recorded in V_4T_1 and the lowest in V_3T_7(12.00). The highestno. of effective fruiting body (51.00) was recorded in V_4T_1 (31.32) and the lowest in V_3T_7(7.75). The highest length of stalk was recorded in V_2 T_5(4.62 cm) and the lowest (0.90 cm) recordedbetweenV_1T_3 and V_1T_6.The highestdiameter of pileus was recorded in V_3T_3(9.50 cm) and the lowest in V_1T_4(4.97 cm). The highestthickness of pileus was recorded in V_3T_3(1.22 cm) and the lowest in V_1T_2(0.53 cm). The highestdiameter of stalk was recorded in V_3T_3(0.97 cm) and the lowest in V_1T_4(0.45 cm). The highestYield was recorded in V_4T_1 (137.3 g/500g packet) and the lowest in V_1T_7(41.00 g/500g packet). The highestbiological efficiency was recorded in V_4T_1(68.63%) and the lowest in V_1T_7(20.50%).

Based on the result of current study, it could be concluded that all varieties gavethe maximum yield at 1 day age of spawn and the minimum yield at 30 days age of spawn. V_1, V_2, V_3 and V_4 gave maximum yield of 135.3g/500g packet , 111.5 g/500g packet, 126.5 g/500g packet and 137.0 g/500g packet at one day old of spawn respectively and the the lowest yield 41.00 g/500g packet was recorded in V_1T_7. The best combined effect was found from V_4T_1. So the variety *Pleurotus salmoneostramineus*with one day old spawn gavethe maximum mushroom yield.

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Appendices

APPENDICES

Appendix I. Analysis of variance of the data on the days required for complete mycelium running, days required for pinhead initiation, days required for first harvest, no. of fruiting body and no. of effective fruiting body of mushroom varieties as influenced by age of spawn and their combinations.

Source of variation	df	Mean square values				
		Days required for complete mycelium running	**Days required for pinhead initiation**	**Days required for first harvest**	**No. of fruiting body**	**No. of effective fruiting body**
Variety (A)	3	54.321*	5.571*	3.155*	3355.747*	993.009*
Age of spawn (B)	6	0.036^{NS}	2.571*	0.229*	2164.640*	777.411*
Variety (A) X Age of spawn (B)	18	0.127*	3.571*	0.356*	184.219*	61.669*
Error	84	0.292	0.052	0.030	2.301	2.039

*Significant at 5% level of significance

^{NS} Non significant

Appendix II. Analysis of variance of the data on the length of stalk, diameter of pileus, thickness of pileus, diameter of stalk, yield of mushroom packet^{-1} and biological efficiency mushroom varieties as influenced by age of spawn and their combinations

Source of variation	df	Mean square values					
		Length of stalk (cm)	**Diameter of pileus (cm)**	**Thickness of pileus (cm)**	**Diameter of stalk (cm)**	**Yield (g/packet)**	**Biological efficiency (%)**
Variety (A)	3	12.893*	4.658*	0.601*	0.108*	3505.295*	2628.971*
Age of spawn (B)	6	1.126*	4.858*	0.072*	0.064*	7445.301*	11167.951*
Variety (A) X Age of spawn (B)	18	1.140*	3.686*	0.038*	0.038*	341.447*	1536.513*
Error	84	0.011	0.017	0.003	0.003	4.438	93.188

*Significant at 5% level of significance

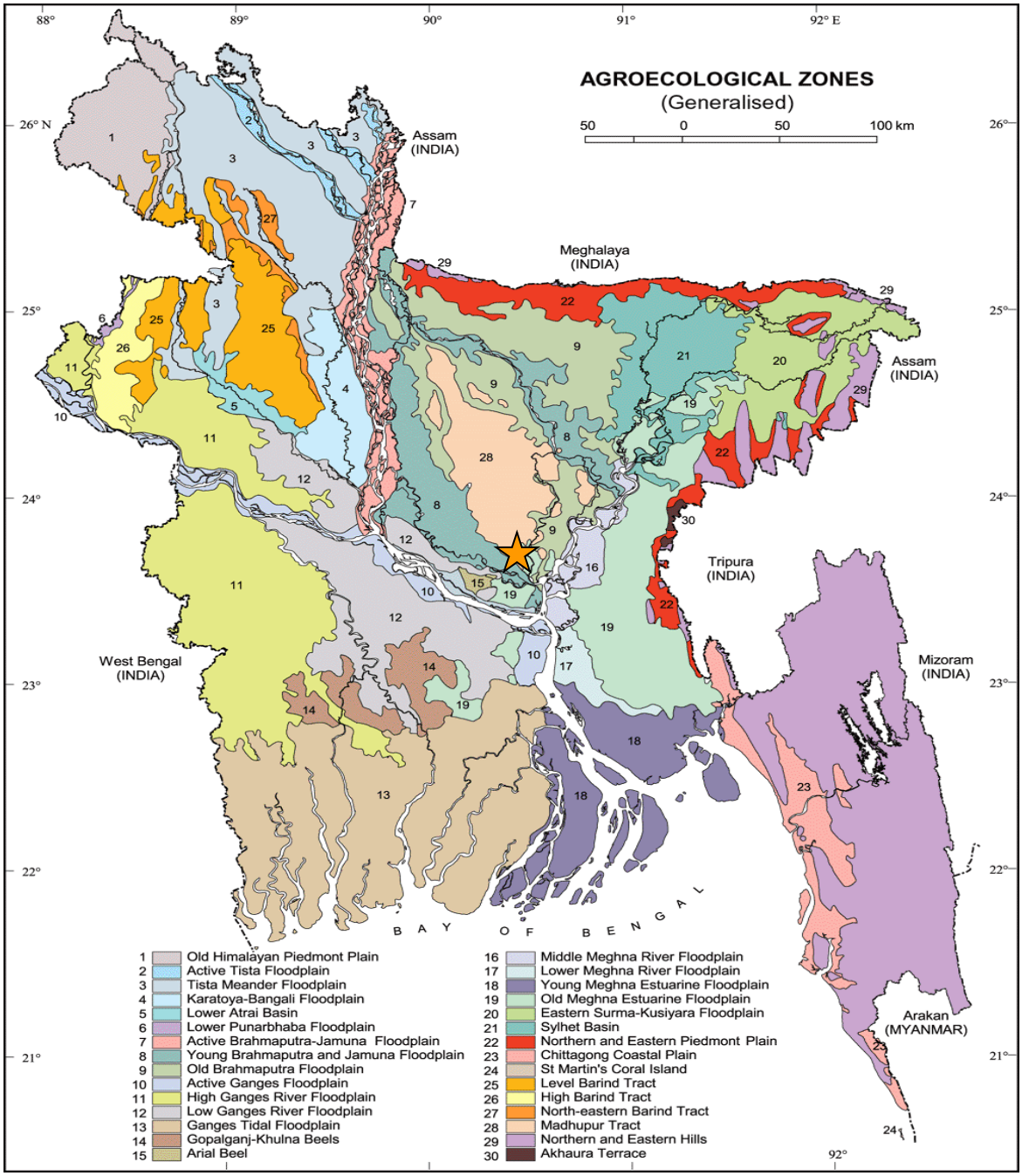
^{NS} Non significant

Appendix III. Temperature and Relative humidity of culture house and outside during oyster mushroom cultivation throughout the year

	Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec
Temp.(^{0}C) of culture house	14-25	14-25	22-30	22-33	22-33	24-30	23-30	25-30	25-30	24-30	20-28	16-27
RH(%) of culture house	72-80	70-80	70-78	72-78	73-79	75-88	90-95	85-90	80-90	80-90	65-70	70-80
Outside temp.(^{0}C)	16-28	20-32	23-34	24-33	26-32	26-32	26-31	26-31	26-31	24-31	20-29	14-26
Outside RH(%)	50-58	47-51	42-48	50-60	68-76	77-81	75-85	76-80	75-81	70-74	65-67	60-66

RH: Relative humidity

Appendix IV: Map showing the experimental sites under study.



★ **The experimental site under study**