EFFECT OF pH LEVEL OF SUBSTRATES AND FREQUENCY OF WATERING ON THE GROWTH AND YIELD OF OYSTER MUSHROOM

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CERTIFICATE

*This is to certify that the thesis titled, "**EFFECT OF pH LEVEL OF SUBSTRATES AND FREQUENCY OF WATERING ON THE GROWTH AND YIELD OF OYSTER MUSHROOM"** submitted to the **Department of Horticulture, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka** in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (M.S.)** in **HORTICULTURE** embodies the result of a piece of bona fide research work carried out by **REBAKA SULTANA, Reg. No. 10-03784** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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Place: Dhaka, Bangladesh

Dedicated to those who

"Working for human nutrition"

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The Author

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ABSTRACT

The experiment was carried out in mushroom research shade house of Olericulture Division, BARI, Gazipur, during the period from July, 2015 to December, 2015 to evaluate the effect of pH levels of substrates and frequency of watering on the growth and yield of oyster mushroom. Spawn of *Pleurotus ostreatus (Jacquin ex Fr.)* was used as test crop. The experiment consisted of two factors *i.e.*, pH level of substrates- P_0: Control (5.5), P_1: 4.9, P_2: 5.2, P_3: 5.8, P_4: 6.1 and P_5: 6.4 and frequency of watering (W_0: Control, W_1: Im + 12 h, W_2: Im + 18 h, W_3: Im + 24 h, W_4: Im + 30 h and W_5: Im + 36 h). The experiment was laid out in two factors Completely Randomized Design with three replications. Results revealed that most of the parameters showed the significant response due to different pH levels of substrate and watering frequency. For pH level of substrate, the treatment P_0 exhibited the better performance in respect of yield and yield contributing characters of mushroom. In case of watering frequency, the treatment W_1 showed higher yield and positive effect on growth parameters as well as on yield parameters. The maximum number of effective fruiting bodies (45.66) and the highest yield of fruiting bodies (187.00 g) were found from P_0W_2. However, statistically the highest yield was obtained from the combined treatment of P_0W_1. So, this combination may be used for higher yield of mushroom.

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LIST OF ACCRONYMS AND ABBREVIATIONS

ACCRONYM	ABBREVAITION
AEZ	Agro-Ecological Zone
Agric.	Agriculture
Agril.	Agricultural
Appl.	Applied
Biol.	Biology
Chem.	Chemistry
cm	Centi-meter
CV	Coefficient of Variance
Dev.	Development
Ecol.	Ecology
Environ.	Environmental
etal	*et alii,* And others
Exptl.	Experimental
g	Gram (s)
HRC	Horticulture Research Centre
i.e.	*id est* (L), that is
IM	Immersion
J.	Journal
LSD	Least Significant Difference
M.S.	Master of Science
m^2	Meter squares
Nutr.	Nutrition
PDA	Potato Dextrose Agar
Physiol.	Physiological
Res.	Research
SAU	Sher-e-Bangla Agricultural University
Sci.	Science
Soc.	Society
viz	*videlicet* (L.), Namely
%	Percentage
@	At the rate of

Chapter I

Introduction

CHAPTER I

INTRODUCTION

Pleurotus spp., popularly known as Oyster mushrooms under the class Basidiomycetes is cultivated and consumed by 97%, of which *Pleurotus ostreatus* alone accounts for 61%. The remaining 3% which belongs to *Agaricus* sp.; *Calocybe* sp.; *Volvareilla* spp. and *Auricularia* spp. are generally called Button, Milky, Paddy straw and Jew's ear or Ear mushrooms, respectively. It was also evident that available carbon source of the substrates for the cultivation of *Pleurotus* spp. was 76% of sawdust where frequencies of flashes were recorded more than five times in 84% cases (Kamal *et al*., 2009). Oyster mushroom (*Pleurotus ostreatus*) is also treated as edible mushroom having excellent flavor and taste. *Pleurotus* species are popular and widely cultivated throughout the world mostly in Asia and Europe owing to their simple and low cost production technology and higher biological efficiency (Mane *et al*., 2007). Cultivation of oyster mushroom has increased tremendously throughout the world because of their abilities to grow at a wide range of temperature and harvested all over the year (Amin *et al*., 2007).

The commercial yield was obtained namely from Oyster mushrooms. On an average, 240 g from each of the spawn-packets which contained 400-500 g of substrate and subsequently on an average 264963 spawn-packets were produced per month, which accounts for 620-675 tons of edible mushrooms production in Bangladesh per annum. It was also estimated that 67% of mushrooms were consumed as fresh, 22% in dried form, 10% in powdered form and 1% in other forms (pickling, frying etc.) as processed or preserved ones. Among the cultivators, 34% cultivated mushrooms on account of its nutritional and medicinal values, 27% to reduce unemployment, 14% for growing mushrooms as more profitable vegetable, 14% to pass leisure time, 10% due to hobby and 1% for other reasons (Kamal *et al*., 2009).

Pleurotus spp. will provide the people with an additional vegetable of high quality, and enrich the diet with high quality proteins, minerals and vitamins which can be of direct benefit to the human health and fitness (Alam and Manjur, 2005).Mushroom reduces serum cholesterol and high blood pressure (Mori, 1986). Edible mushrooms have been treated as important tool in modern medicine for their medicinal values (Kovfeen, 2004). Anti-cancer medicine (Leutinan) is produced recently by some chemical companies from the extract (Polysaccharides) of mushroom (Mori, 1986).

Apart from food value, its medicinal value for diabetics and in cancer therapy has been emphasized (Sivrikaya *et al*., 2002). Many of mushrooms pose a range of metabolites of intense interest to pharmaceutical e.g. antitumour, antigenotoxic, antioxidant, anti-inflammatory, anti-hypertensive, antiplatelet-aggregating, antihyperglycaemic, antimicrobial, antiviral activities and food industries (Chang, 2007). *Pleurotus ostreatus* is one of the most popular oyster mushroom species thatcan grow on different agricultural wastes. *Pleurotus* have the ability to excrete hydrolyzing and oxidizing enzymes (Pathmashini *et al*., 2008) which have capable of utilizing complex organic compounds that occurred agricultural wastes and industrial by-products (Zadrazil *et al*., 1981) with broad adaptability varied agro-climatic conditions (Jandaik and Goyal, 1995). It requires a short growth time in comparison to other edible mushrooms (Kausar, 1988).

As with any agricultural product, mushroom quality and condition are dependent upon environmental conditions such as air quality, soil moisture, pH, temperature, humidity, CO_2 level, and composting and soil conditions (Hanacek *et al*., 1984). Among these factors the response of pH and watering frequency is most important on mycelial development. Some of mushroom growers mention an ideal moist condition is favorable for oyster mushroom cultivation. The availability of growth promoting substances in the substrates of oyster mushroom depends on the pH concentration.

The pH concentration influences the proper growth and development of mushroom under different substrates. The mushroom choice slightly acidic to slightly basic pH of substrates (Chang, 2007). The pH has great response on nutrition and morphological development of mushrooms (Chag and Miles, 1988).The pH influences metabolic processes and consequently the ability of a mushroom species to utilize certain substances as nutrients. In general most of the edible mushroom grows best on a slightly acidic medium. Hong *et al.* (1983) reported that, the optimum range of pH for mycelium growth is about 5.5 and 6.5. Chung *et al.* (1981) reported that, the optimum range of pH was different in different strains of *Pleurotus* spp. Proper moisture condition of substrates verifies the performance of oyster mushroom and watering on mushroom spawn can create different moist condition per day at different frequencies (Rahman *et al*., 2015).

Mushroom cultivation technology is friendly to the environment. In addition, many mushrooms possess multi-functional medicinal properties.In Bangladesh, about 30 million tons of agricultural wastes like paddy straw, wheat straw and sugarcane bagasse are being lost by improper utilization (Ahmed, 2001). Therefore, a culture of mushrooms could be a source of additional income for families on small scale farms with the active participation of members. It has been observed that over 70% of agricultural and of forest products have not been put to total productivity, and have been discarded as waste (Anonymous, 2008).

Mushroom cultivation has a special relevance to Bangladesh, because sawdust and other materials are available to the farmers. The growths of diverse type of mushrooms require different type of substrates and availability of varied type of materials may dictate which type is used (Das and Mukherjee, 2007). Most of the people of Bangladesh have been suffering from malnutrition. Mushrooms could substantiate the suffering from malnutrition to some extent.

Furthermore, the use of these residues in bioprocesses may be one of the solutions to bioconversion of inedible biomass residues into nutritious protein rich food in the form of edible mushrooms (Mshandete and Cuff, 2008).

So, mushroom production could keep great importance on our economy as a whole. It has potential to generate valuable foreign exchange. Mushrooms with their flavor, texture, nutritional value, very high productivity per unit area and time, less dependence on and ability to grow on a variety of residual agricultural wastes, have rightly been identified as a food sources to fight malnutrition in developing countries mostly as Bangladesh.

But, in our country the research on the effects of pH of substrates and frequency of watering on the production of oyster mushroom had not been well established.

So, the present experiment was undertaken with the following objectives:

1. To determine the most suitable pH concentration to adjust the appropriateness of substrates for better growth and yield of mushroom;
2. to evaluate influence of frequency of watering on growth and yield of mushroom; and
3. to assess the most promising combination of pH of substrates and frequency of watering for higheryield of mushroom.

Chapter II

Review of literature

CHAPTER II

REVIEW OF LITERATURE

A number of literatures relating to the performance of different substrate with different pH levels and watering frequency on mushroom cultivation were available separately but the performances of pH of substrate with combination of watering frequency were not available. The review includes reports of several investigators which appear pertinent in understanding the results of the present investigation. So, in this chapter some important research works have been reviewed related to present study for oyster mushroom production.

2.1 Response to pH of substrates

Akinyele and Adetuyi (2005) reported that pH range of 5.5 to 8.5 recorded the maximum mycelia yield and the highest mycelia weight was recorded at pH 6.5 of *Volvariella volvacea*. The mycelia yield decreased at pH above 6.5 while poor mycelia growth of the mushroom and the least mycelia weight was recorded at pH 2.0.

Fasidi (1996) reported that *Volvariella esculenta*was able to tolerate temperature range of 20-30ºC and pH range of 3-10 in substrates. He also said that, the optimum temperature for the growth of the mushroomwas found to be 35ºC while the optimum pH was 6.0.This probably explains the ability of the mushroom toflourish very well on various agricultural wastes in thetropics. The pH range for the growth of *V. volvacea* was found to be 5.5-8.5, while the optimum pH was found tobe 6.5 and the mushroom was able to tolerate atemperature range of 27-40ºC with the optimum temperature of 30ºC.

Khan *et al*. (2013) reported that the pH is an important factor for good production of oyster mushroom. Most of the mushrooms grow and perform well at pH near to neutral or light basic.

Lime ($CaCO_3$) is an important constituent in mushroom cultivation, commercial cultivation of mushroom depends upon proper adjustment of pH of substrate. Most of the substrates used for the cultivation of mushroom have pH approximately near to neutral *i.e.*, 7 in Pakistan. They told that, Oyster mushroom (*Pleurotus spp.)* was grown on cotton waste with different levels of lime in substrate like 0%, 2%, 4% and 6% of substrate weight at pH 7.2, 7.8, 8.2 and 8.7 respectively.

Oei (2003) said that additives like $CaCO_3$ and $CaSO_4$. $2H_2O$ have a positive effect on the structure of the substrate and stabilize the pH. The use of lime as an additive to adjust the pH concentration in substrates is an important thing for the proper development of mushroom.

Lokuge*et al*. (2012) carried out a research to investigate optimum pH values for the cultivation of oyster mushrooms (*Pleurotus ostreatus*). Accordingly, initial pH values of the nine different growth substrates were adjusted as 5.4, 5.6, 7.1, 7.3, 7.5, 8.1, 8.2, 8.6, and 8.8 and the final values upon completion of the sterilization procedure was also measured. Sawdust was used as the basal ingredient for substrate preparation with the addition of rice bran, green gram powder, $CaCO_3$ and $MgSO_4$ as supplements. Average yield (g), piles diameter (cm), stalk length (cm) and number of flowers per flush were measured. Average yield was the highest in the media with pH 5.6. With the decreasing pH in the medium mushroom yield increased and after pH of 5.6 it was decreased. However, no significant difference was observed between the treatments on piles diameter, stalk length and number of flowers per flush.

Odero*et al*. (2011) reported that as pH of the substrate is one of the key factors that affect its mycelial growth and subsequently the sporophore yields. They conducted a research to determine the effect of varying pH on growth parameters of *P. ostreatus* cultivated on different substrates with pH of 6.0, 6.5 and 7.0 were chosen as treatments.

The results indicated that pH had significant effect (P≤0.05) on yield, average biological efficiency (ABE), number of pileus, days to pinning and flushing interval among all the four substrates tested.

Saya-An and Tad-Awan (1995) conducted a research to determine the levels of carbon, nitrogen and the pH of the substrates currently being used for oyster mushroom. Result showed that substrates currently used had 8.12 to 25.5 percent C; 0.805 to 2.205 percent N and at pH 5.2 to 7.3. Carbon level at 17.86, nitrogen level at 1.54 percent and pH 5.6 enhanced the highest yield with the use of rice straw as the main substrate 15 percent rice bran and sugar as additive. Conversely, carbon and nitrogen at undetermined level and pH 6.4 affected the lowest yield. The highest return on investment (112.65 percent) was obtained using rice straw + 15 percent rice bran + 15 percent sugar and the lowest (71.05 percent) was obtained using rice straw + 15 percent rice bran + 15 percent chicken dung. Based on the result, addition of 15 percent sugar and rice bran is recommended for *Pleurotus florida* production.

Kumla *et al.*(2013) conducted the research on the cultures of *P. giganteus*CMU54-1 on PDA. They reported that, the strain was incubatedin darkness at 15, 20, 25, 30, 35, 40 and45^{o}C. The pH of the medium was adjusted to from 2.0-9.0 with 1N HCl or 1NNaOH before autoclaving. After inoculation,cultures were incubated at 25^{o}C inthe darkness and said that, all the parameters studied showed the significant variation.

Nagy (2010) investigated onthe pH change of the substrate on the production of *Pleurotus ostreatus* and said that, the pH value of the substrate quickly decreased parallel with the mycelial growth, for the 9 day of the incubation the substrate reached the pH range for the mycelial growth of *Pleurotus ostreatus* of 5.5, 6 in all cases.

Sardar *et al*. (2016) conducted a series of experiments to investigate the effects of various growth conditions on growth and development of *Pleurotus* species.

It was noted that all the species exhibited maximum mycelial growth at pH 6, whereas the minimum growth was recorded at pH 4.

2.2 Response to frequency of watering

Rahman *et al.*(2015) supplemented sawdust with different times of watering has profound effect on chemical composition of pink oyster mushroom. Three times watering per days on mushroom had highest amount of carbohydrate (42.42%) and lipid (4.66%) whereas moisture (90.39%), dry matter (10.01%) and crude fiber (21.81%) was highest at watering frequency of four times. Protein content was highest (32.37%) at two times per days of watering. Mineral content of the fruiting body found to be significantly varied on watering frequency. Increasing watering frequency was negatively correlated with the decreasing one. Nitrogen, phosphorus, potassium and magnesium contents were highest when watering frequencies were one to two times per day. But Copper, Manganese and Zinc were obtained at highest levels for the three to four times of watering.

Mutale (2002) conducted a research to determine the response of watering frequency on the yield and flushing intervals of button mushroom. The mean total yields obtained were 1089.7 g, 1113.9 g and 1530.4 g for the daily, 2-interval and 3- interval watering frequencies, respectively. There was no significant difference in yields of button mushroom for the three watering frequencies. The treatments had no significant effects on the number of days to first, second and third flushes. The treatments had also little effects on the watering intervals.

Rama (2000) said that in the first stage of mushroom cultivation, the spawn is mixed with compost so that it can successfully compete with other microorganisms for nutrients. The mixing of spawn in compost is called spawning. After spawning, the compost surface is covered with old newspaper sheets, and is sprinkled with water to provide humidity (no water is added directly to the compost during spawn run).

Atkins (1972) reported that as mushrooms exceed half an inch in diameter, watering should be reduced to a minimum. This is because the caps will tend to stain unless they are dried quickly.

Flegg (1999) observed that applying 10, 20, 30 and 40 liters of water per square meter to mushroom beds from casing layer was ruffled (about one week) had no significant effect on crop yields. Measurements of the water content of the compost showed that the upper layer of the compost losses water and the bottom layer become wetter. The higher levels of watering hardly affected the water contents of the upper and middle layers of compost but the bottom layer became increasingly wetter with the heavier watering. It was concluded that, mush of excess water applied drained through to the bottom layer. Even though the bottom compost layer reached water content of 80% yields of mushrooms were not affected. Adding a water-absorbent polymer to the upper layer (5 cm) of compost to increase its moisture content did not affect yield.

Mushaim (2004) reported that watering after primodium formation to maintain optimal moisture content in substrate is very important for the production of high yields of high quality oyster mushrooms. Disease usually increases with too much watering on cultivation beds (excessive moisture content). Too little watering reduces yields and induces abnormal shapes in fruiting bodies. Substrate blocks shrink and fruiting bodies become brown on dry cultivation beds, and new mycelia grow and many small new fruiting bodies are formed on old mushroom fruiting bodies.

Flegg (1974) reported that the amount of water applied was positively correlated with crop weight from about the second week of cropping and increasing the amount of water applied reduced the drying of the compost and casing during cropping. The watering treatments affected the character of the mushroom mycelium developing in the casing layer.

Flegg (1975) pointed out that delaying the start of watering, without affecting the total amount given, retarded the start of cropping slightly, reduced yield and allowed more mushroom mycelium to cover the casing layer. Where the casing was covered extensively with mycelium, fruiting was reduced even when subsequent watering removed some of the mycelial covering. In the more productive areas of the trays with excessive mycelial overlay the mushrooms were closely packed and thus of poor quality and difficult to pick. Comparison of various ways of apportioning the total amount of water applied between pre-cropping and cropping showed that yield was optimum when watering was spread regularly and evenly throughout pre-cropping and cropping. Cased trays which were not watered produced in 6 weeks a yield of mushrooms about two-thirds of that from trays watered regularly, thus showing that there is a considerable reserve of water in the compost and casing on which the crop can draw.

Jang *et al.* (2003) investigated the favorable watering frequency and amount for the good form and yield of *P. ostreatus*, the watering of 0, 1, 3 times a day and amount 0, 0.8, 2 l, 4 l/3.3 m^2 per watering time were done. And the yield and morphological characteristics of *P. ostreatus* on different watering times a flush, watering after 1-2 flush and conventional watering were investigated. When the cultures were sprinkled at the watering amount of 0.8 *l*/day, the yield was intensive and intends to decrease with increase watering amounts. When the cultures were sprinkled at the watering of once a day, yield was the worst, but the conventional watering gave higher yields than other watering.But individual weight of none-watering was lesser than other watering substrates including conventional watering. Stripe of some fruit bodies became brownish by drying and regeneration of secondary fruit body formation occurred in none-watering substrates. This result suggested that the optimum watering of *P. ostreatus* should sprinkling at fruit body before drying of substrates regardless of watering frequencies and amounts during the cultivation periods.

Sarker *et al*. (2007) pointed out that time required from stimulation to primordial initiation, stimulation to first harvest and for total harvest was 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.

Finally it may be taken that, the adjustment of pH of substrates may provide more nutrition than that of conventional application of substrates for better quality mushroom. The frequency of watering may act as better growth enhancing factor for the better quality mushroom.

Chapter III

Materials and methods

CHAPTER III

MATERIALS AND METHODS

The experiment was carried out to find out the response of pH of substrates and frequency of watering on the production ofoyster mushroom.This chapter deals with a brief description on location and design of experiment, experiments and treatments, preparation of substrates, preparation of packets, cultivation of spawn packet, collection of produced mushrooms, data recording and their analysis under the following headings and sub-headings:-

3.1 Site of experimentation

The present study was carried out in the mushroom research shade house of Olericulture Division, HRC (Horticulture Research Centre), Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during the period from July, 2015 to December, 2015 (shown in Appendix I). The Geo position of Gazipur district is $23^{\circ}53'$ to $24^{\circ}20'$ N latitudes and between$90^{\circ}9'$ to $90^{\circ}42'$ E longitude and it also situated under Madhupur tract (AEZ-28).

3.2 Planting materials

Spawn of *Pleurotus ostreatus(Jacquin ex Fr.)* in a bottle has been collected from Savar farm and used to inoculate the substrate with 5% of spawn for each bag.

3.3 Varietal characteristics of Oyster Mushroom

Oyster mushrooms (*Pleurotus ostreatus (Jacquin ex Fr.)* are characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. Their fruiting bodies are shell or spatula shaped white color. If the temperature increases above 32°C, its production markedly decreases.

3.4 Experimental treatment: The present study was consisted of two factors *i.e.*, pH level of substrates and frequency of watering which are as follows-

Factor A: pH level of substrates (Level-6)

P_0= Control (5.5)
P_1= 4.9
P_2= 5.2
P_3= 5.8
P_4= 6.1
P_5= 6.4

Factor B: Frequency of watering (Level-6)

W_0= Control
W_1= Im + 12 h
W_2= Im + 18 h
W_3= Im + 24 h
W_4= Im + 30 h
W_5= Im + 36 h

3.5 Treatment combination: There were thirty six (36) treatment combinations used under present study mentioned as follows-

$P_0 \times W_0$,$P_0 \times W_1$, $P_0 \times W_2$, $P_0 \times W_3$, $P_0 \times W_4$, $P_0 \times W_5$, $P_1 \times W_0$, $P_1 \times W_1$, $P_1 \times W_2$, $P_1 \times W_3$, $P_1 \times W_4$, $P_1 \times W_5$, $P_2 \times W_0$, $P_2 \times W_1$, $P_2 \times W_2$, $P_2 \times W_3$, $P_2 \times W_4$, $P_2 \times W_5$, $P_3 \times W_0$, $P_3 \times W_1$, $P_3 \times W_2$, $P_3 \times W_3$, $P_3 \times W_4$, $P_3 \times W_5$, $P_4 \times W_0$, $P_4 \times W_1$, $P_4 \times W_2$, $P_4 \times W_3$, $P_4 \times W_4$, $P_4 \times W_5$, $P_5 \times W_0$, $P_5 \times W_1$, $P_5 \times W_2$, $P_5 \times W_3$, $P_5 \times W_4$ and$P_5 \times W_5$.

3.6 Design and layout of the experiment

The experiment was laid out in two factors Completely Randomized Design (CRD) with three replications.

3.7 Preparation of substrates

Spawn packets was prepared with waste paper amended with wheat bran at 2:1 ratio and 0.57% calcium carbonate in polypropylene bags. The mushroom house was provided with well ventilation for easy flow of natural air.

3.8 Adjustment pH of substrates

Six different levels of pH *viz.*, Control (5.5), 4.90, 5.20, 5.80, 6.10 and 6.40 were tested to determine the best levels of pH for Oyster Mushroom cultivation. For P_0 treatment the farmers substrates was collected and just pH was observed and which was 5.5. Rest levels of pH in substrates were adjusted by using $CaCO_3$ and HCl. The pH of substrate was determined using water extract of the materials with a pH meter (HORIBA M. 8_L).

3.9 Managing the frequency of watering

Water was applied following two methods *viz.*, immersion in water for 15 minutes and water was sprayed on the spawn packets placed on the shelves of mushroom house. For immersion spawn packets was immersed in a bucket of water after scraping and opening at every harvest. Water was sprayed to the spawn packets once at 12, 18, 24, 30 and 36 hours intervals.

3.10 Preparation of spawn packets

The mixed substrates were filled into 7×11 inch polypropylene bag @ 500 g. The filled polypropylene bags were prepared by using plastic neck and plugged the neck with cotton and covered with brown paper placing rubber band to hold it tightly in place.

3.11 Sterilization, inoculation and mycelium running in spawn packets

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

3.12 Cultivation of spawn packets

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

3.13 Collection of produced mushrooms

Oyster mushrooms matured within 2-3 days after primordia initiation. The matured fruiting body was identified by curial margin of the cap. Mushrooms were harvested by twisting to uproot from the base.

3.14 Data recording

3.14.1 Days required for development of pin head

Growth of mushroom was recorded daily for all the treatments. When such bags become full of growth and pin-heads started appearing, the bags were mouth opened to facilitate the development of fruiting bodies. The appearing days were deducted from the days of inoculation.

3.14.2 Days required from pin head to first harvest

Growth of mushroom pins was keenly observed daily for all the treatments. The days of first harvest of mushroom was deducted from the days of inoculation.

3.14.3 Number of fruiting bodies produced/packet

Number of well-developed fruiting body was recorded. Dry andpinheaded fruiting bodies were discarded but tiny fruiting bodies were included in counting.

3.14.4 Number of harvest/packet

The well-developed mushroom was harvested each time and total number of harvest was recorded per treatment.

3.14.5 Length of individual stripe

Stipe length was measured in centimeter using transparent ruler from the base of the stipe to the pileus and divided by the number of samples. Then final mean value was worked out per bag.

3.14.6 Breadth of individual stripe

Stipe breadth was measured in centimeter using measuring tape and divided by the number of samples. Then final mean value was worked out per bag.

3.14.7 Number effective fruiting bodies

Number of very well-developed fruiting body was recorded. Tiny fruiting bodies were discarded from counting.

3.14.8 Yield of fruiting bodies/packet

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

3.15Statistical analysis

The data obtained for different characters were statistically analyzed following the analysis of variance techniques by using MSTAT-C computer package and the treatment means were compared by Least Significant Difference (LSD) at 5% level of probability (Gomez and Gomez, 1984).

Chapter IV

Results and discussion

CHAPTER IV

RESULT AND DISCUSSION

The present investigation was attempted to evaluate the response of pH of substrates and frequency of watering on different characteristics of oyster mushroom. In this chapter; figures, tables and appendices have been used to present, discuss and compare the findings obtained from the present study. The ANOVA (analysis of variance) of data in aspects of all the quantitative characteristics have been presented in Appendix (II-V). The revelation and all possible interpretations were given under the following headings:

4.1 Days required for development of pin head

In respect of days required for the development of pin head due to different levels of pH was found statistically significant (Appendix II). A gradual decreasing trend was found with the increasing of pH level. The longest (30.52 days) period was required by the mushroom produced from P_0.The shortest (29.83 days) period was required from P_2which was statistically similar to P_1, P_3,P_4 and P_5 (Table 1). The increasing of pH from moderate acidic to slightly basic has lengthening the period of pinning on mushroom (Hao *et al*., 2015).

In respect of days required for the development of pin head due to different frequency of watering was found statistically significant (Appendix II). A gradual increasing trend was found with the increasing of frequency of watering. The longest (31.50 days) period required for development of pin head was found from $W_5$and the shortest (27.91 days) was from W_2 (Table 2). Gislerod (1987) pointed out that, the lower non intermittent application of water decreased the pin formation times from spawn packet. This result is in agreement with findings of present study.

Significant variation was found due to different combinations of pH of substrates and frequency of wateringon days required for development of pin head of mushroom from seed in packets (Appendix II).Results showed that, the longest (32.66 days) period required for development of pin head of mushroom from seed in packets was found from $P_0 \times W_5$ which was statistically similar to $P_0 \times W_4$ (32.00 days) and $P_3 \times W_5$ (32.00 days**)** while, the shortest (27.36 days)was found from $P_1 \times W_2$ (Table 3).

4.2 Days required from pin head to first harvest

A day required from pin head to first harvest due to different levels of pH was found statistically significant (Appendix II). Results showed that, the maximum (4.47 days)required from pin head to first harvest was found from P_5 which was statistically similar to P_4 (4.20 days) and P_1(4.22 days) while, the minimum (4.11 days)was found fromP_0 (Table 1). Gislerod (1987) said that, the pH near about basic condition had lengthening the period of harvest of spawn. This result is in agreement with findings of present study.

Days required from pin head to first harvest due to different frequency of watering were found statistically significant (Appendix II). A gradual increasing trend was found with the increasing of frequency of watering. The maximum (4.58 days) required from pin head to first harvest was found from W_5 which was statistically similar to W_4 (4.52 days) and W_0 (4.41 days). The minimum (3.61 days)was found from W_0 (Table 2). Gislerod (1987) also said that, the long interval application of water on spawn packets has increased the duration of first harvest of spawn from packets.

No significant variation was found due to different combinations of pH of substrates and frequency of wateringon days required from pin head to first harvest (Appendix II) of oyster mushroom. But numerically, the maximum (5.20 days) required from pin head to first harvest was found from $P_4 \times W_5$and the minimum (3.16 days and 3.16 days)was found from $P_0 \times W_1$ and $P_4 \times W_1$, respectively, (Table 3).Result was also supported by Sarker *et al*. (2007).

4.3 Number of fruiting bodies produced/packet

In case of number of fruiting bodies produced due to different levels of pH was found statistically significant (Appendix III). A gradual decreasing trend was found with the increasing of pH level from P_0 up to P_3 and thereafter increased as similar to P_0. Results showed that, the maximum (43.27) number of fruiting bodies produced/packet from P_4which was statistically similar to P_0 (42.66) while, the minimum (33.33) was from P_2 (Table 1).

In case of number of fruiting bodies produced due to different frequency of watering was found statistically significant (Appendix III). A gradual decreasing trend was found with the increasing of frequency of watering. The maximum (43.90) number of fruiting bodies produced/packet was found from W_1 and the minimum (34.50) was from W_5 (Table 2).

Significant variation was found due to different combinations of pH of substrates and frequency of wateringon number of fruiting bodies produced/packet (Appendix III) of oyster mushroom. Results showed that, the maximum (52.33) number of fruiting bodies produced/packet was found from $P_0 \times W_2$ which was statistically similar to $P_0 \times W_1$ (51.66) and the minimum (30.66) was found from $P_3 \times W_5$(Table 3).

4.4 Number of harvest/packet

In respect of number of harvest due to different levels of pH was foundstatistically significant (Appendix III). Results showed that, the maximum (4.36) number of harvest/packet from P_3which was statistically similar to P_0 (4.35), P_1(4.33) and P_4 (4.23) while, the minimum (4.02) was from P_2 (Table 1). Litar *et al*. (2000) said that, the acidic condition (above 5.4) has increased the duration and frequency of harvest of Nigerian edible fungi. This result is in agreement with findings of present study.

In respect of number of harvest due to different frequency of watering was found statistically significant (Appendix III).A gradual increasing trend was found with the increasing the frequency of watering from W_0 up to W_2 and

thereafter decreased towards W_5. Results showed that, the maximum (4.63) number of harvest/packet from W_1which was statistically similar to W_2 (4.49) while, the minimum (3.80) was found from W_5 (Table 2). Ismail *et al*. (2008) also observed that, increases in spawn harvest number with higher daily irrigation frequency compared to once-a-day irrigation when plants were grown in soil conditions.This result is in agreement with findings of present study.

Significant variation was found among different combinations of pH of substrates and frequency of watering on number of harvest/packet (Appendix III) of oyster mushroom. Results showed that, the maximum (5.13) number of harvest/packet was found from $P_3 \times W_1$ which was statistically similar to $P_4 \times W_1$ (5.00), $P_3 \times W_2$ (4.90) and $P_1 \times W_1$ (4.83**)** while, the minimum (3.00) was found from $P_3 \times W_5$ (Table 3).

Table 1. Response of pH levels of substrates on days required for development of pin head and pin head to first harvest, number of fruiting bodies and number of harvest

pH level	Days required for development of pin head	Days required from pin head to first harvest	No. of fruiting bodies produced/ packet	No. of harvest/ packet
P_0	30.52 a	4.11b	42.66 a	4.35 a
P_1	29.89 b	4.22ab	37.55 b	4.33ab
P_2	29.83 b	4.05 b	33.33 d	4.02 c
P_3	29.88 b	3.97 b	35.55 c	4.36 a
P_4	29.88 b	4.20ab	43.27 a	4.23ab
P_5	29.94 b	4.47 a	36.72 b	4.15bc
LSD (0.05)	0.29	0.32	1.12	0.18
CV (%)	1.48	11.65	4.42	6.64

Numbers in columns followed by the same letter are not statistically different at $P_{0.05.}$
P_0: Control, P_1: 5.00, P_2: 5.30, P_3: 5.80, P_4: 6.10 and P_5: 6.40.

Table 2. Response of frequency of watering on days required for development of pin head and pin head to first harvest, number of fruiting bodies and number of harvest

Frequency of watering	Days required for development of pin head	Days required from pin head to first harvest	No. of fruiting bodies produced/ packet	No. of harvest/ packet
W_0	31.00 b	4.41ab	35.88d	4.31 b
W_1	28.69 d	3.61 c	43.90a	4.63 a
W_2	27.91 e	3.67 c	40.44b	4.49ab
W_3	30.03 c	4.22 b	38.05c	4.11c
W_4	30.83 b	4.52ab	36.27d	4.10 c
W_5	31.50 a	4.58 a	34.50e	3.80 d
LSD (0.05)	0.29	0.32	1.12	0.18
CV (%)	1.48	11.65	4.42	6.64

Numbers in columns followed by the same letter are not statistically different at $P_{0.05.}$

W_0: Control, W_1: Im + 12 h, W_2: Im + 18 h, W_3: Im + 24 h, W_4: Im + 30 h and W_5: Im + 36 h.

Table 3. Combined effect of pH levels of substrates and frequency of watering on days required for development of pin head and pin head to first harvest, number of fruiting bodies and number of harvest

Treatment combination	Days required for development of pin head	Days required from pin head to first harvest	No. of fruiting bodies produced/ packet	No. of harvest/ packet
$P_0 \times W_0$	31.83 b	4.50	35.33 j-m	3.66jk
$P_0 \times W_1$	28.46 h-j	3.16	51.66 a	4.66 b-e
$P_0 \times W_2$	27.83jk	3.66	52.33 a	4.83 a-d
$P_0 \times W_3$	30.33ef	4.00	40.00 f-h	4.33 e-h

Table 3. Cont'd

Treatment combination	Days required for development of pin head	Days required from pin head to first harvest	No. of fruiting bodies produced/packet	No. of harvest/packet
$P_0 \times W_4$	32.00ab	4.66	39.66 g-i	4.66 b-e
$P_0 \times W_5$	32.66a	4.66	37.00 i-k	3.93 h-k
$P_1 \times W_0$	30.83 d-f	4.26	37.33 h-j	4.33 e-h
$P_1 \times W_1$	28.66 g-i	3.93	41.33 e-g	4.83 a-d
$P_1 \times W_2$	27.36 k	3.66	37.33 h-j	4.33 e-h
$P_1 \times W_3$	30.66 d-f	4.50	39.33 g-i	4.33 e-h
$P_1 \times W_4$	30.16 f	4.66	36.33 j-l	4.00 g-j
$P_1 \times W_5$	31.66bc	4.33	33.66 l-n	4.16 f-i
$P_2 \times W_0$	30.33ef	4.33	31.33 n-p	4.50 c-f
$P_2 \times W_1$	29.36 g	3.50	35.33 j-m	4.16 f-i
$P_2 \times W_2$	28.33ij	3.50	34.33 k-m	4.00 g-j
$P_2 \times W_3$	30.16 f	4.16	33.66 l-n	3.50 k
$P_2 \times W_4$	30.50ef	4.16	35.66 j-m	3.83 i-k
$P_2 \times W_5$	30.33ef	4.66	29.66 p	4.16 f-i
$P_3 \times W_0$	30.83 d-f	4.33	34.33 k-m	4.66 b-e
$P_3 \times W_1$	29.16gh	3.66	44.667cd	5.13 a
$P_3 \times W_2$	28.33ij	3.33	38.00 h-j	4.90 a-c
$P_3 \times W_3$	28.00 i-k	4.00	34.33 k-m	4.33 e-h
$P_3 \times W_4$	31.00 c-e	4.50	31.33 n-p	4.16 f-i
$P_3 \times W_5$	32.00ab	4.00	30.66 op	3.00 l
$P_4 \times W_0$	31.66bc	4.00	37.00 i-k	4.33 e-h
$P_4 \times W_1$	28.33ij	3.16	48.66 b	5.00ab
$P_4 \times W_2$	27.33 k	3.66	42.66 d-f	4.50c-f
$P_4 \times W_3$	30.33ef	4.33	46.66bc	4.00 g-j
$P_4 \times W_4$	30.66 d-f	4.83	41.66 e-g	3.93 h-k
$P_4 \times W_5$	31.00 c-e	5.20	43.00 de	3.66jk
$P_5 \times W_0$	30.50ef	5.06	40.00 f-h	4.40 d-g
$P_5 \times W_1$	28.16ij	4.23	42.00 d-g	4.00 g-j
$P_5 \times W_2$	28.30 ij	4.23	38.00 h-j	4.40 d-g
$P_5 \times W_3$	30.70 d-f	4.33	34.33 k-m	4.16 f-i
$P_5 \times W_4$	30.66 d-f	4.33	33.00 m-o	4.03 g-j
$P_5 \times W_5$	31.33 b-d	4.66	33.00 m-o	3.90 h-k
LSD (0.05)	0.72	-	2.74	0.45
CV (%)	1.48	11.65	4.42	6.64

Numbers in columns followed by the same letter are not statistically different at $P_{0.05}$.
P_0: Control, P_1: 5.00, P_2: 5.30, P_3: 5.80, P_4: 6.10 and P_5: 6.40.
W_0: Control, W_1: Im + 12 h, W_2: Im + 18 h, W_3: Im + 24 h, W_4: Im + 30 h and W_5: Im + 36 h.

4.5 Length of individual stripe

In aspect of length of individual stripe due to different levels of pH was found statistically significant (AppendixIV).The maximum (4.92 cm) length of individual stripe was found from P_0 which was statistically similar to P_5 (4.91 cm), P_2 (4.91 cm) and P_1 (4.89 cm) while, the minimum (4.64 cm) was found from P_4 (Table 4). Result is also supported by Malavi-tau *et al*. (1997) because, they told that, medium acidic condition of growing medium (5.5) has increased the stripe nodal distance from the root base of *Agaricus*.

The length of individual stripe due to different frequency of watering was found statistically significant (Appendix IV). A gradual decreasing trend was found with the increasing of frequency of watering other than control. The maximum (5.26 cm) length of individual stripe was found from W_1 which was statistically similar to W_2 (5.21 cm) and the minimum (4.32 cm) was from W_5 (Table 5).The frequent and short interval application of water on spawn packets may be the reasons for proper moist condition by which the stripe length was increased.

Significant variation was found due to different combinations of pH of substrates and frequency of wateringon length of individual stripe (Appendix IV) of oyster mushroom. Results showed that, the maximum (5.46 cm) length of individual stripe was found from $P_2 \times W_1$ which was statistically similar to $P_0 \times W_1$ (5.40 cm), $P_0 \times W_2$(5.36 cm), $P_1 \times W_1$(5.33 cm), $P_2 \times W_2$ (5.33 cm), $P_5 \times W_2$ (5.30 cm) and $P_4 \times W_2$ (5.23 cm) while, the minimum (3.86 cm) was found from $P_4 \times W_0$ (Table 6). Result was also supported by Sarker *et al*. (2007).

4.6 Breadth of individual strip

In aspect of breadth of individual stripe due to different levels of pH was found statistically significant (Appendix IV).

A gradual decreasing trend was found with the increasing of pH level of substrates. Results showed that, the maximum (4.66 cm) breadth of individual stripe was found from P_0 and the minimum (4.27 cm)was found from P_4 (Table 4). Result is also supported by Result is also supported by Malavi-tau *et al.* (1997) because, they told that, moderate acidic condition of growing medium (bellow 5.0) has increased the stripe apical diameters *Agaricus*.

In case of breadth of individual stripe due to different frequency of watering was found statistically significant (Appendix IV). A gradual decreasing trend was found with the increasing of frequency of watering other than control. The maximum (4.91 cm) breadth of individual stripe was found from W_2 which was statistically similar to W_1 (4.82 cm) and the minimum (3.99 cm) was found from W_5 (Table 5). The frequent and short interval application of water on spawn packets may be the reasons for proper moist condition by which the stripe length was increased.

Significant variation was found among different combinations of pH of substrates and frequency of wateringon breadth of individual stripe (Appendix IV) of oyster mushroom. Results showed that, the maximum (5.06 cm)breadth of individual stripe was found from $P_3 \times W_1$ which was statistically similar to $P_0 \times W_0$ (5.03 cm), $P_0 \times W_2$ (5.03 cm), $P_2 \times W_2$ (5.03 cm), $P_1 \times W_2$ (5.00 cm), $P_2 \times W_1$ (50.00 cm), $P_5 \times W_2$ (5.00 cm), $P_1 \times W_1$ (4.93 cm), $P_4 \times W_2$ (4.93 cm), $P_0 \times W_1$ (4.86 cm) and $P_2 \times W_3$ (4.86 cm**)** while, the minimum (3.63 cm)was found from $P_4 \times W_0$(Table 6). Result was also supported by Sarker *et al.* (2007).

Table 4. Response of different pH levels of substrates on length and breadth of individual stripe

pH Level	Length of individual stripe (cm)	Breadth of individual stripe (cm)
P_0	4.92 a	4.66 a
P_1	4.89 a	4.53 b
P_2	4.91 a	4.56 b
P_3	4.73 b	4.42 c
P_4	4.64 b	4.27 d
P_5	4.91 a	4.52 b
LSD (0.05)	0.11	0.09
CV (%)	3.45	3.05

Numbers in columns followed by the same letter are not statistically different at $P_{0.05.}$
P_0: Control, P_1: 5.00, P_2: 5.30, P_3: 5.80, P_4: 6.10 and P_5: 6.40.

Table 5. Response of frequency of watering on average length and breadth of individual stripe

Frequency of watering	Length of individual stripe (cm)	Breadth of individual stripe (cm)
W_0	4.63 c	4.38 c
W_1	5.26 a	4.82 a
W_2	5.21 a	4.91 a
W_3	4.98 b	4.58 b
W_4	4.61 c	4.27 d
W_5	4.32 d	3.99 e
LSD (0.05)	0.11	0.09
CV (%)	3.45	3.05

Numbers in columns followed by the same letter are not statistically different at $P_{0.05.}$
W_0: Control, W_1: Im + 12 h, W_2: Im + 18 h, W_3: Im + 24 h, W_4: Im + 30 h and W_5: Im + 36 h.

4.7 Number effective fruiting bodies

Thenumber of effective fruiting bodies due to different levels of pH was found statistically significant (Appendix V). A gradual decreasing trend was found with the increasing of pH level from P_0 up to P_3 and thereafter increased as similar to P_0. Results showed that, the maximum (36.44) number of effective fruiting bodies found from P_0 which was statistically similar to P_4 (36.22) while, the minimum (26.94)was from P_2 (Figure 1). Hoa *et al*. (2015) pointed out that, the pH (5.86) has increased the shoot primordial activity of lettuce in field condition and as a result the number of shoot was increased per unit area. Tabalu *et al.* (2006) also said that, the slightly acidic to slightly basic pH has increased the fruit body of edible *Agaricus spp*. This result is in agreement with findings of present study.

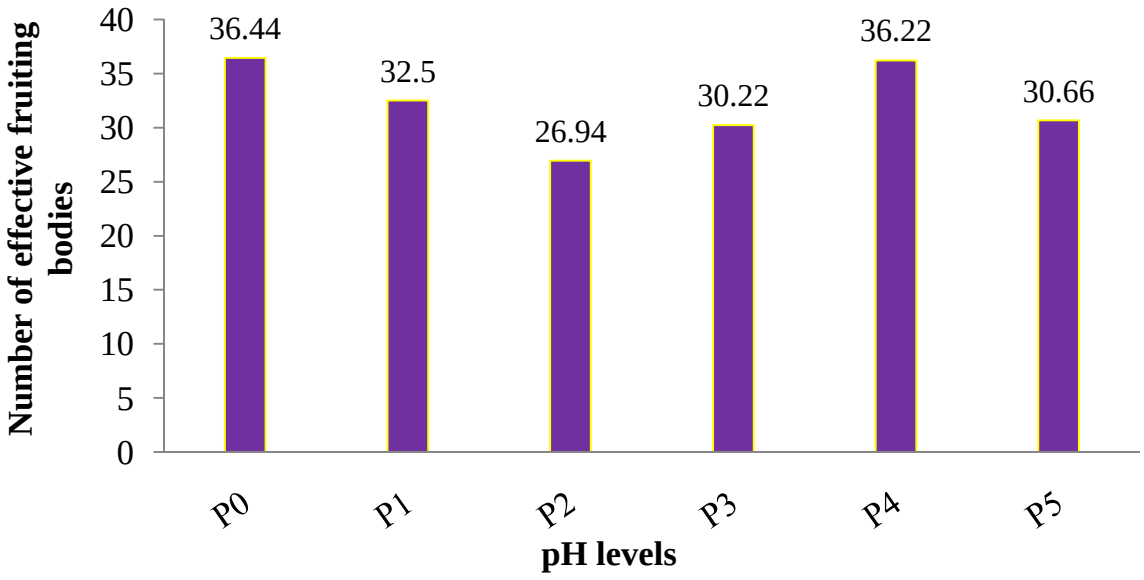

Figure 1. Response of pH levels of substrates on number of effective fruiting bodies (LSD value=0.86)

P_0: Control, P_1: 5.00, P_2: 5.30, P_3: 5.80, P_4: 6.10 and P_5: 6.40.

In respect of number of effective fruiting bodies due to different frequency of watering was found statistically significant (Appendix V). The maximum (36.61) number effective fruiting bodies was found from W_1 and the minimum (28.61) was from W_5 (Figure 2).

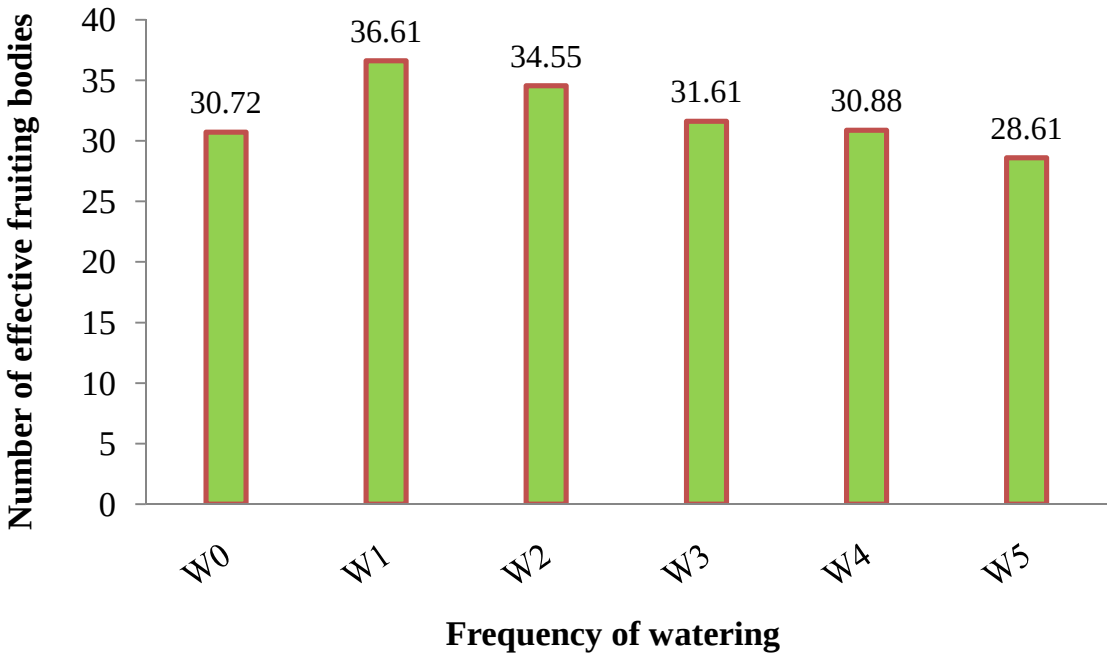

Figure 2. Response of frequency of watering on number of effective fruiting bodiesof oyster mushroom (LSD value=0.86)

W_0: Control, W_1: Im + 12 h, W_2: Im + 18 h, W_3: Im + 24 h, W_4: Im + 30 h and W_5: Im + 36 h.

Significant variation was found among different combinations of pH of substrates and frequency of wateringon number of effective fruiting bodies (Appendix V) of oyster mushroom. Results showed that, the maximum (45.66)number of effective fruiting bodies was found from $P_0 \times W_2$and the minimum (24.66) was found from $P_2 \times W_5$ which were statistically similar to $P_2 \times W_3$ (25.00) in (Table 6). Result was also supported by Sarker *et al*. (2007). Because they told that, fruiting body was highest number when the watering was applied on spawn packets at twice or thrice time per day. Hoa *et al*. (2015) pointed out that, the combination of lower pH that 4.8 and proper moist condition has increased fruiting body number per spawn packet. This result is in agreement with findings of present study.

4.8 Yield of fruiting bodies

In respect of yield of fruiting bodies due to different levels of pH was found statistically significant (Appendix V).

Results showed that, the maximum (161.33 g) yield of fruiting bodies/packet from P_0 followed by P_4 (159.17 g) and the minimum (129.22 g) was from P_5 (Figure 3).The optimum pH range (5.57-6.09) has increased the yield of mushroom on pine wood substrates because this pH has increased the nutrient availability for fruiting body yield by more dry matter partitioning (Tabalu *et al*., 2006). This result is in agreement with findings of present study.

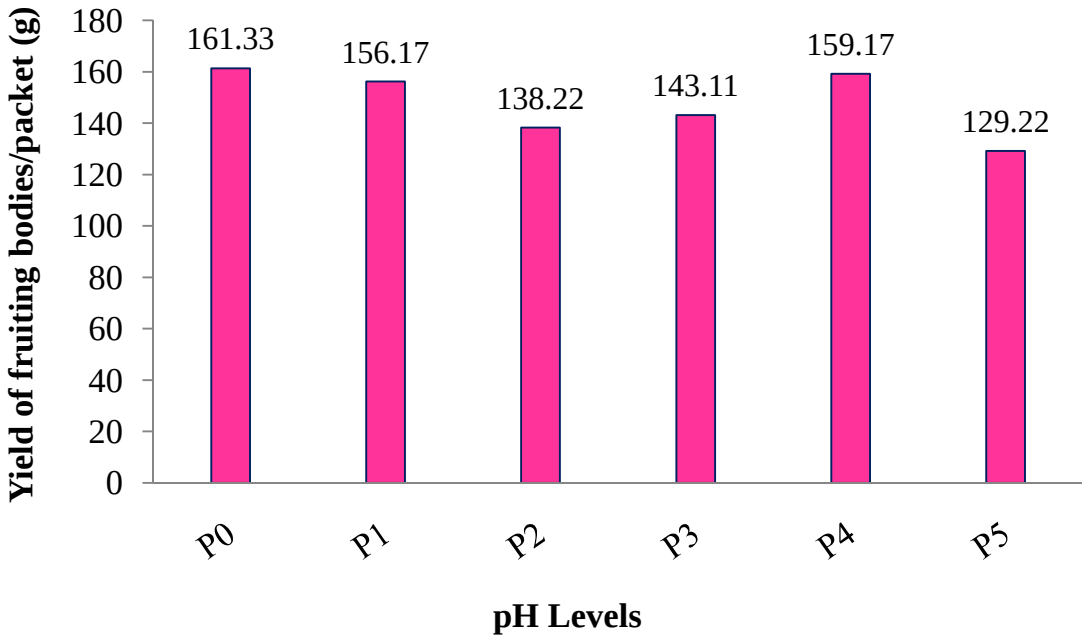

Figure 3. Response of pH level of substrates on yield of fruiting bodies/packet (LSD value=1.98)

P_0: Control, P_1: 5.00, P_2: 5.30, P_3: 5.80, P_4: 6.10 and P_5: 6.40.

The yield of fruiting bodies due to different frequency of watering was found statistically significant (Appendix V). A gradual decreasing trend was found with the increasing of frequency of watering other than control. Results showed that, the maximum (162.83 g) yield of fruiting bodies/packet from W_1 and the minimum (131.11 g) was found from W_5 (Table 6).The reasons behind the present results is the proper and sufficient moisture was present in second and third watering so the yield was highest at earlier levels.

Ismail *et al*. (2008) also observed that, increases in spawn yield with higher daily irrigation frequency compared to once-a-day irrigation when plants were grown in soil conditions.

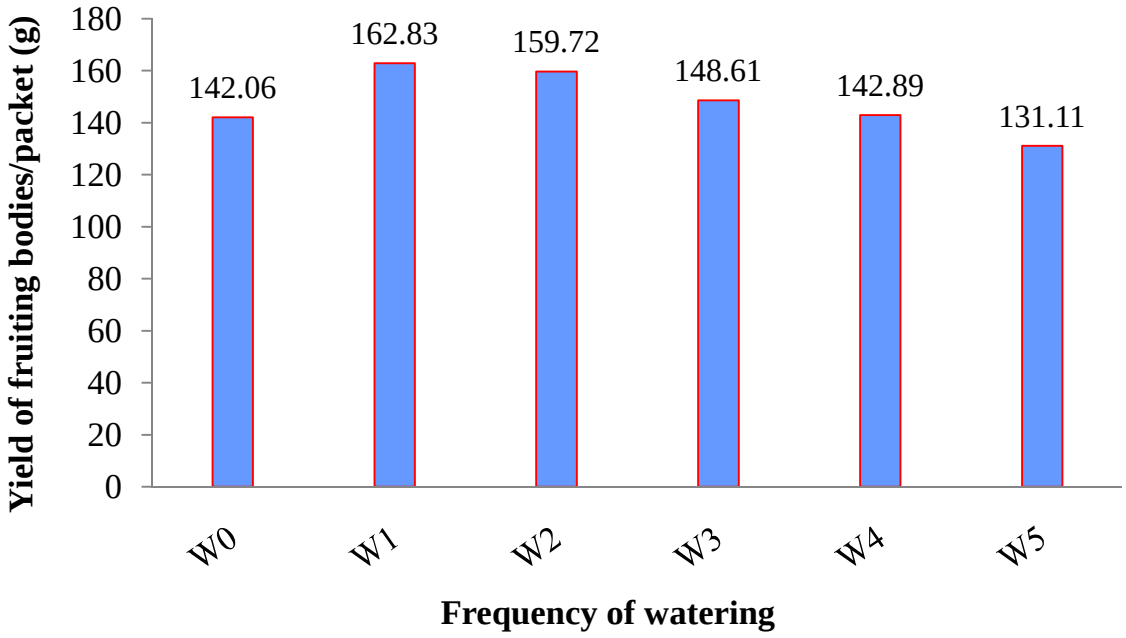

Figure 4. Response of frequency of watering on yield of fruiting bodies/packet (g) (LSD value=1.98)

W_0: Control, W_1: Im + 12 h, W_2: Im + 18 h, W_3: Im + 24 h, W_4: Im + 30 h and W_5: Im + 36 h.

Significant variation was found among different combinations of pH of substrates and frequency of wateringon yield of fruiting bodies/packet of oyster mushroom (Appendix V). Results showed that, the maximum (187.00 g) yield of fruiting bodies/packet was found from $P_0 \times W_2$ which was statistically similar to $P_0 \times W_1$ (186.67 g) and the minimum (120.33 g) was found from $P_3 \times W_5$ (Table 6).

Table 6. Combined effect of pH levels of substrates and frequency of watering on length and breadth of individual stripe, number of effective fruiting bodies and yield of fruiting bodies

Treatment combination	Length of individual stripe (cm)	Breadth of individual stripe (cm)	No. of effective fruiting bodies	Yield of fruiting bodies/packet (g)
$P_0 \times W_0$	5.00 c-f	5.03 a	31.00 g-i	162.00 d
$P_0 \times W_1$	5.40 ab	4.86 ab	43.33 b	186.67 a
$P_0 \times W_2$	5.36 ab	5.03 a	45.66 a	187.00 a
$P_0 \times W_3$	5.00 c-f	4.60 cd	32.33 e-i	150.67 gh
$P_0 \times W_4$	4.43 j-l	4.43 d-g	34.66 d	151.33 gh
$P_0 \times W_5$	4.36 k-m	4.00 jk	31.66 f-i	130.33 k
$P_1 \times W_0$	4.78 f-i	4.36 e-g	31.66 f-i	128.67 kl
$P_1 \times W_1$	5.33 ab	4.93 ab	37.66 c	173.33 bc
$P_1 \times W_2$	5.30 ab	5.00 a	32.66 e-i	162.00 d
$P_1 \times W_3$	5.00 c-f	4.60 cd	33.00 d-g	162.00 d
$P_1 \times W_4$	4.36 k-m	4.30 e-h	31.33 f-i	159.33 de
$P_1 \times W_5$	4.60 h-k	4.03 ij	28.66 jk	151.67 g
$P_2 \times W_0$	4.93 d-g	4.23 g-i	28.00 k	152.00 fg
$P_2 \times W_1$	5.46 a	5.00 a	28.66 jk	152.67 fg
$P_2 \times W_2$	5.33 ab	5.03 a	26.66 k-n	146.67 h
$P_2 \times W_3$	5.00 c-f	4.86 ab	25.00 n	131.67 k
$P_2 \times W_4$	4.70 g-j	4.46 d-f	28.66 jk	124.67 l-n
$P_2 \times W_5$	4.03 no	3.80 kl	24.66 n	121.67 mn
$P_3 \times W_0$	4.66 g-j	4.60 cd	28.66 jk	136.67 ij
$P_3 \times W_1$	5.36 ab	5.06 a	37.66 c	163.67 d
$P_3 \times W_2$	4.76 f-i	4.50 de	31.33 f-i	156.67 ef
$P_3 \times W_3$	4.73 f-i	4.26 f-h	30.66 h-j	151.00 gh
$P_3 \times W_4$	4.73 f-i	4.13 h-j	27.66 kl	130.33 k
$P_3 \times W_5$	4.16 l-n	4.00 jk	25.33 mn	120.33 n
$P_4 \times W_0$	3.86 o	3.63 l	31.66 f-i	141.00 i
$P_4 \times W_1$	4.86 e-h	4.36 e-g	38.33 c	161.00 de

Table 3. Cont'd

Treatment combination	Length of individual stripe (cm)	Breadth of individual stripe (cm)	No. of effective fruiting bodies	Yield of fruiting bodies/packet (g)
$P_4 \times W_2$	5.23 a-c	4.93 ab	37.66 c	175.00 b
$P_4 \times W_3$	5.00 c-f	4.40 d-g	38.33 c	171.00 bc
$P_4 \times W_4$	4.73 f-i	4.30 e-h	37.33 c	168.67 c
$P_4 \times W_5$	4.16 mn	4.00 jk	34.00 de	138.33 i
$P_5 \times W_0$	4.56 i-k	4.46 d-f	33.33 d-f	132.00 jk
$P_5 \times W_1$	5.13 b-e	4.73 bc	34.00 de	139.67 i
$P_5 \times W_2$	5.30 ab	5.00 a	33.33 d-f	131.00 k
$P_5 \times W_3$	5.16 b-d	4.76 bc	30.33 ij	125.33 lm
$P_5 \times W_4$	4.73 f-i	4.03 ij	25.66 l-n	123.00 mn
$P_5 \times W_5$	4.60 h-k	4.13 h-j	27.33 k-m	124.33 l-n
CV (%)	3.45	3.05	4.04	2.02
LSD (0.05)	0.27	0.22	2.11	4.86

Numbers in columns followed by the same letter are not statistically different at $P_{0.05.}$
P_0: Control, P_1: 5.00, P_2: 5.30, P_3: 5.80, P_4: 6.10 and P_5: 6.40.
W_0: Control, W_1: Im + 12 h, W_2: Im + 18 h, W_3: Im + 24 h, W_4: Im + 30 h and W_5: Im + 36 h.

4.9 Functional relationship between number effective fruiting bodies and yield of fruiting bodies

The direct linear relation is obtained between number of effective fruiting bodies and average yield of mushroom by plotting yield against number of effective fruiting bodies. The figure indicates that average yield of mushroom per spawn packet increased as raising the number of fruiting bodies. The results show that about 50% (R^2=0.4475) of yield is affected by number of effective fruiting bodies (Figure-5). The present result is also supported by Das *et al.* (2014) and Sarker *et al*. (2007).

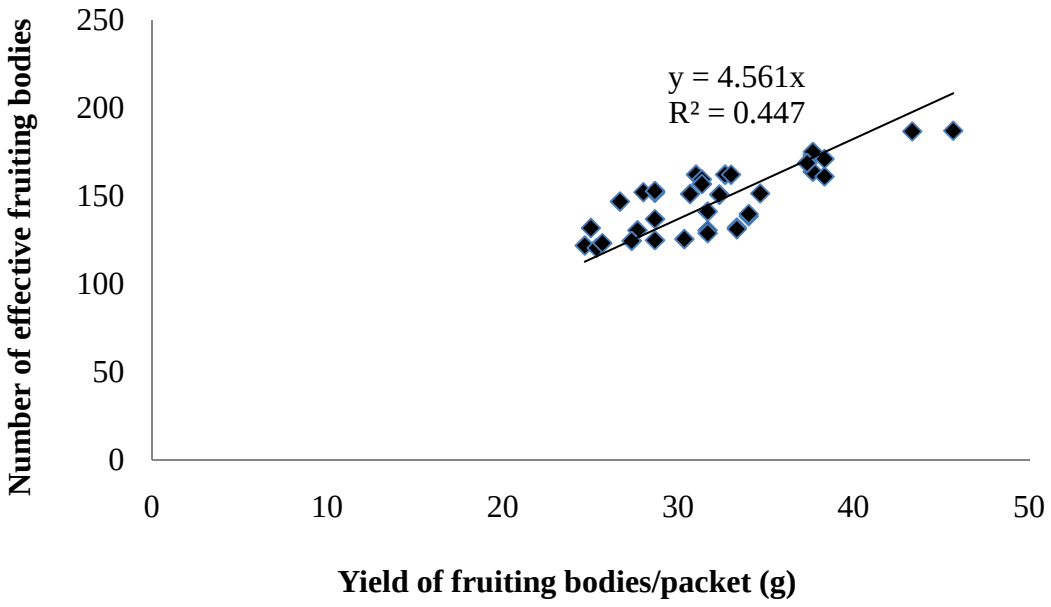

Figure 5. Functional relationship between number of effective fruiting bodies and yield of fruiting bodies

Chapter V

Summary and conclusion

CHAPTER V

SUMMARY AND CONCLUSION

The present study was carried out in the mushroom research shade house of Olericulture Division, HRC (Horticulture Research Centre), Bangladesh Agricultural Research Institute, Joydebpur, Gazipur (shown in Appendix I) during the period from July, 2015 to December, 2015 to evaluate the effects of pH levels of substrates and frequency of watering on the growth and yield of oyster mushroom. The Geo position of Gazipur district is 23˚53′ to 24˚20′ N latitudes and between90˚9′ to 90˚42′ E longitude and it also situated under Madhupur tract (AEZ-28).Spawn of *Pleurotus ostreatus (Jacquin ex Fr.)* was used as test crop under present study. The present study was consisted of two factors *i.e*., pH level of substrates (p) such as P_0: Control (5.5), P_1: 4.90, P_2: 5.20, P_3: 5.8, P_4: 6.1 and P_5: 6.40 and frequency of watering (w) *viz*., W_0: Control, W_1: Im + 12 h, W_2: Im + 18 h, W_3: Im + 24 h, W_4: Im + 30 h and W_5: Im + 36 h, respectively. There were thirty six (36) treatment combinations under present study.

The experiment was laid out in two factors Completely Randomized Design (CRD) with three replications. Spawn packets was prepared with waste paper amended with wheat bran at 2:1 ratio and 0.57% calcium carbonate in polypropylene bags. The mushroom house was provided with well ventilation for easy flow of natural air. For P_0 treatment the farmers substrates was collected and just pH was observed and which was 5.5. Rest levels of pH in substrates were adjusted by using $CaCO_3$ and HCl. The pH of substrate was determined using water extract of the materials with a pH meter (HORIBA M. 8_L). Water was applied following two methods *viz.,* immersion in water for 15 minutes and water was sprayed on the spawn packets placed on the shelves of mushroom house.

For immersion spawn packets was immersed in a bucket of water after scraping and opening at every harvest. Water was sprayed to the spawn packets once at 12, 18, 24, 30 and 36 hours intervals. The sterilization, inoculation, cultivation and collection of mushroom were done as per methods described in methodology. Data on days required for development of pin head of mushroom from seed in packets, days required from pin head to first harvest, number of fruiting bodies produced/packet, number of harvest/packet,length of individual stripe (cm), breadth of individual stripe (cm), number effective fruiting bodies and yield of fruiting bodies/packet (g) were collected under present study.The data obtained for different characters were statistically analyzed following the analysis of variance techniques by using MSTAT-C computer package and the treatment means were compared by Least Significant Difference (LSD) at 5% level of probability.

The results revealed that, all the parameters showed significantly variation by pH level of substrates and frequency of watering on mushroom growth and yield. The longest period (days) was required by the mushroom produced to develop pin head from P_0 (30.52 days) while, the shortest days was required from P_2 (29.83 days) which was statistically similar to P_1, P_3, P_4 and P_5.The maximum days required for development of pin head of mushroom from seed in packets was found from W_5 (31.50 days) and the minimum was from W_2 (27.91 days). The maximum days required for development of pin head of mushroom from seed in packets was found from $P_0 \times W_5$ (32.66 days) which was statistically similar to $P_0 \times W_4$ (32.00 days) and $P_3 \times W_5$ (32.00 days**)** while, the minimum was found from $P_1 \times W_2$ (27.36 days).

The maximum days required from pin head to first harvest was found from P_5 (4.47 days) which was statistically similar to P_4 (4.20 days) and P_1 (4.22 days) while, the minimum was found from P_0 (4.11 days). The maximum days required from pin head to first harvest was found from W_5 (4.58 days) which was statistically similar to W_4 (4.52 days) and W_0 (4.41 days). The minimum was found from W_0 (3.61 days).

No significant variation was found among different combinations of pH of substrates and frequency of watering on days required from pin head to first harvest.

The maximum number of fruiting bodies produced/packet from P_4 (43.27) which was statistically similar to P_0 (42.66) while, the minimum was from P_2 (33.33).The maximum number of fruiting bodies produced/packet was found from W_1 (43.90) and the minimum was from W_5 (34.50). The maximum number of fruiting bodies produced/packet was found from $P_0 \times W_1$ (51.66) which was statistically similar to $P_0 \times W_2$ (52.33) and the minimum was found from $P_3 \times W_5$ (30.66).

The maximum number of harvest/packet found from P_3 (4.36) which was statistically similar to P_0 (4.35), P_1 (4.33) and P_4 (4.23) while, the minimum was from P_2 (4.02). The maximum number of harvest/packet from W_1 (4.63) which was statistically similar to W_2 (4.49) while, the minimum was found from W_5 (3.80).The maximum number of harvest/packet was found from $P_3 \times W_1$ (5.13) which was statistically similar to $P_4 \times W_1$ (5.00), $P_3 \times W_2$ (4.90) and $P_1 \times W_1$ (4.83**)** while, the minimum was found from $P_3 \times W_5$ (3.00).

The maximum length of individual stripe was found from P_0 (4.92 cm) which was statistically similar to P_5 (4.91 cm), P_2 (4.91 cm) and P_1 (4.89 cm) while, the minimum was found from P_4 (4.64 cm). The maximum length of individual stripe was found from W_1 (5.26 cm) which was statistically similar to W_2 (5.21 cm) and the minimum was from W_5 (4.32 cm). The maximum length of individual stripe was found from $P_2 \times W_1$ (5.46 cm) which was statistically similar to $P_0 \times W_1$ (5.40 cm), $P_0 \times W_2$ (5.36 cm), $P_1 \times W_1$ (5.33 cm), $P_2 \times W_2$ (5.33 cm), $P_5 \times W_2$ (5.30 cm) and $P_4 \times W_2$ (5.23 cm) while, the minimum was found from $P_4 \times W_0$ (3.86 cm).

The maximum breadth of individual stripe was found from P_0 (4.66 cm) and the minimum was found from P_4 (4.27 cm). The maximum breadth of individual stripe was found from W_2 (4.91 cm) which was statistically similar to W_1 (4.82 cm) and the minimum was found from W_5 (3.99 cm).

The maximum breadth of individual stripe was found from $P_3 \times W_1$ (5.06 cm) which was statistically similar to $P_0 \times W_0$ (5.03 cm), $P_0 \times W_2$ (5.03 cm), $P_2 \times W_2$ (5.03 cm), $P_1 \times W_2$ (5.00 cm), $P_2 \times W_1$ (50.00 cm), $P_5 \times W_2$ (5.00 cm), $P_1 \times W_1$ (4.93 cm), $P_4 \times W_2$ (4.93 cm), $P_0 \times W_1$ (4.86 cm) and $P_2 \times W_3$ (4.86 cm**)** while, the minimum was found from $P_4 \times W_0$ (3.63 cm).

The maximum number of effective fruiting bodies found from P_0 (36.44) which was statistically similar to P_4 (36.22) while, the minimum was found from P_2 (26.94). The maximum number of effective fruiting bodies was found from W_1 (36.61) and the minimum was found from W_5 (28.61). The maximum number of effective fruiting bodies was found from $P_0 \times W_2$ (45.66) and the minimum was found from $P_2 \times W_5$ (24.66) which was statistically similar to $P_2 \times W_3$ (25.00).

The maximum yield of fruiting bodies/packet from P_0 (161.33 g) followed by P_4 (159.17 g) and the minimum was from P_5 (129.22 g). The maximum yield of fruiting bodies/packet from W_1 (162.83 g) and the minimum was found from W_5 (131.11 g).The maximum yield of fruiting bodies/packet was found from $P_0 \times W_2$ (187.00 g) which was statistically similar to $P_0 \times W_1$ (186.67 g) and the minimum was found from $P_3 \times W_5$ (120.33 g).

Conclusion

1. From the present study it was revealed that most of the parameters showed the significant response due to different pH levels of substrate and watering frequency.
2. For pH level of substrate, the treatment P_0(5.5) exhibited the better performance in respect of yield and yield contributing characters of mushroom.
3. In case of watering frequency, the treatment W_1 (Im + 12 h) showed higher yield and positive effect on growth parameters as well as on yield parameters.

4. The highest yield was obtained from the combined treatment $P_0 \times W_1$ *i.e*., pH of substrate 5.5 in combination with watering frequency Im + 12 h. So, this combination may be used for higher yield of mushroom.

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Appendices

APPENDICES

Appendix I. Monthly average of lab temperature and relative humidity ofthe experimental site during the period from July to December

Month	Temperature(^{0}C)		Relative humidity (%)
	Minimum	Maximum	
July, 2015	10	25	90
August, 2015	10	28	90
September, 2015	15	25	90
October, 2015	10	25	90
November, 2015	10	25	90
December, 2015	11	25	90

Appendix II. Mean square values for days required for development of pin headand days required from pin head to first harvest

Source of variation	Degrees of freedom	Mean square value	
		Days required for development of pin head	Days required from pin head to first harvest
pH levels	5	1.2150**	0.55637*
Frequency of watering	5	35.9648**	3.31770**
pH levels × frequency of watering	25	1.5120**	0.28575^{NS}
Error	72	0.1968	0.23627

* Significant at 5 % level of provability
** Significant at 1 % level of provability

NS, non-significant

Appendix III. Mean square values for number of fruiting bodies and number of harvest

Source of variation	Degrees of freedom	Mean square value	
		No. of fruiting bodies produced/packet	No. of harvest/packet
pH levels	5	284.437**	0.32356**
Frequency of watering	5	218.815**	1.61511**
pH levels × frequency of watering	25	31.708**	0.42960**
Error	72	2.846	0.07952

** Significant at 1 % level of provability

Appendix IV. Mean square values for length of individual stripe and breadth of individual stripe

Source of variation	Degrees of freedom	Mean square value	
		Length of individual stripe (cm)	Breadth of individual stripe (cm)
pH levels	5	0.25492**	0.32225**
Frequency of watering	5	2.52161**	2.17816**
pH levels × frequency of watering	25	0.17392**	0.18087**
Error	72	0.02780	0.01888

** Significant at 1 % level of provability

Appendix V. Mean square values for number effective fruiting bodies and yield of fruiting bodies

Source of variation	Degrees of freedom	Mean square value	
		No. effective fruiting bodies	Yield of fruiting bodies/packet (g)
pH levels	5	245.378**	3028.24**
Frequency of watering	5	151.667**	2535.86**
pH levels × frequency of watering	25	24.151**	384.89**
Error	72	1.685	8.94

** Significant at 1 % level of provability

Plate 1. Preparation of substrates

Plate 2. Preparation of spawn packets

Plate 3. Cultivation of mushroom

Plate 4. Full maturity for harvest

Plate 5. Growth of mushroom at better combination ($P_0 \times W_1$)

Plate 6. Weighing of mushroom