PERFORMANCE OF DIFFERENT MEDIA FOR MOTHER CULTURE PRODUCTION OF DIFFERENT MUSHROOM SPP.

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*This is to certify that the thesis entitled, "**PERFORMANCE OF DIFFERENT MEDIA FOR MOTHER CULTURE PRODUCTION OF DIFFERENT MUSHROOM SPP.**"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 **TASNIMA HAQUE**, Registration No. **14-06256** 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,2021
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Dedicated to
My
Beloved Husband

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SAU, Dhaka ***The Author***

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ABSTRACT

The experiment was conducted at Mushroom Development Institute, Savar, Dhaka, Bangladesh during the period from June, 2019 to May, 2020. The experiment was laid out following completely randomized design with four replications. Three mushrooms species viz, oyster mushroom, straw mushroom and button mushroom were grown for mother culture production on three different media of rice grain, wheat grain and sawdust respectively. All parameters were significantly influenced by different media with different mushroom variety except length of stalk and diameter of pileus of straw mushroom. Combined effect of straw mushroom and wheat grain media gave the highest potential result in mycelium run rate (0.72cm/day), total number of effective fruiting body (72.8), biological yield (940.10g), economic yield (935.47g), length of stalk (3.20cm), diameter of stalk (1.96cm), and thickness of pileus (1.68cm). In wheat grain media, button mushroom also gave the highest biological yield (830.20g). Oyster mushroom, button mushroom and straw mushroom grown separately in three different media, among these oyster mushroom gave the highest biological yield with rice grain media which was 144.33g and 140.43g, respectively. In wheat grain media, straw mushroom and button mushroom gave the highest biological yield which were 940.10g and 830.20g respectively. All the three mushroom spp. showed lowest biological and economic yield in sawdust media. In case of oyster mushroom the highest biological efficiency was found in rice grain media 72.2% but straw and button mushroom, the highest biological efficiency was found in wheat grain media which was 23.5% and 37.7% respectively. The lowest biological efficiency for oyster, straw and button mushroom was found in sawdust media which were 57.2, 13.7% and 25.2% respectively. According to this result, it was clearly indicated that oyster mushroom gave higher production in rice grain media, straw and button mushroom both gave higher production in wheat grain media and it was more economic than other media substrate.

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ABBREVIATION	ELABORATION
MRR	Mycelium run rate
DCMR	Days reuired 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
DS	Diameter of stalk
LP	Length of pileus
DP	Diameter of pileus
BY	Biological yield
EY	Economic yield
BE	Biological efficiency
CRD	Completely randomized design
et al.	and others
RS	Rice straw
SD	Sawdust
LSD	Least significant difference
kg	Kilogram
No.	Number
%	Percent

CHAPTER I
Introduction

Mushroom is a large reproductive structure of edible fungi which is the most popular nutritious, delicious vegetable in the world. It belong to either Ascomycotina or Basidiomycotina. It is now one of the promising concepts for crop diversification in Bangladesh. Mushrooms supply more protein per unit area than other crops (Gupta, 1986).The climatic condition of Bangladesh is completely suitable for mushroom cultivation. Mushrooms have been cultivated since ancient time for their nutritional value and flavor especially in the far eastern countries. Mushroom can play an important role to meet up the nutritional requirements of the population of Bangladesh (Amin *et al*., 2007). Mushrooms are becoming increasingly important and common in human diets, due to their nutritional (Barros *et al*., 2008; Bernas *et al*., 2006) and medicinal characteristics (Jedinak *et al*., 2010). The nutritional advantages of mushrooms include a low content of calories and a high content of proteins, minerals and dietary fiber (Beluham and Ranogajec, 2011).In many countries, mushroom cultivation and its products yield a lot of income and enhanced dietary meals and improved health of the people (USDA, 2000; Mattila *et al*., 2001; Mau *et al*., 2001; Mau *et al*., 2002; Wasser, 2002; Chu, and Chow 2002 and Akpaja, *et al*., 2003). Mushrooms are good sources of sugars, fibre, proteins and minerals (Senatore, 1990 and Adewusi *et al*., 1993) with comparable amino acid with animal protein (Aletor , 1995). Mushroom protein is intermediate between that of animals and vegetables (Kurtzman, 1976). Mushrooms contain about 85-95% water, 3% protein, 4% carbohydrates, 0.1% fats, 1% minerals and vitamins (Tewari, 1986).Mushrooms contain appreciable amount of potassium, phosphorous, copper and iron but low level of calcium (Anderson. and Feller, 1942). Mushroom also contain appreciable amount of Niacin, pantothenic acid and biotin (Subramanian, 1986).

In Bangladesh since 1987-88, *Pleurotus* species (Oyster Mushroom), *Volvariella volvaceae* (Straw Mushroom) and *Agaricus bisporus* (Button Mushroom) are under commercial cultivation in Bangladesh.

The Oyster mushroom (*Pleurotus ostreatus*), first cultivated in Germany as a subsistence measure during World War I (Eger *et al*., 1976) is now grown commercially around the world for food. Mushroom of *Pleurotus* spp. are rich in medicinal values and useful in preventing disease such as hypertension, hypercholesterolemia (Khatun *et al*., 2007 and Choudhury *et al*., 2008), hyperglycemia and different types of cancer (Nayana and Janardhanan, 2000); some hepatoprotective activity of oyster mushroom (Mishra and Singh, 2010). *Pleurotus* mushrooms, commonly known as oyster mushrooms, grow in the wild in tropical, subtropical and temperate regions and are easily artificially cultivated (Akindahunsi and Oyetayo, 2006). Oyster mushrooms (*Pleurotus* spp) are characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. Oyster mushroom can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, baggasse etc.

Volvariella volvacea (also known as paddy straw mushroom or straw mushroom) is a species of edible mushroom cultivated throughout East and Southeast Asia and used extensively in Asian cuisines. The mushroom was held in such high regard that it was often presented as a tribute to Chinese royalty (Chang ST, 1969; Chang ST 1977). In addition to rice straw, *V. volvacea* also grows on water-hyacinth, palm oil bunch wastes, pericarp wastes, banana leaves, and cotton waste. Considered a health food because of its dietary and medicinal attributes (Chang ST and Buswell JA, 1996), the mushroom is popular in southern China, Thailand, Malaysia and the Philippines. Although *V. volvacea* has been cultivated for ~300 years, multiple problems associated with the practice has greatly restricted development of the industry. The biological efficiency (conversion of the substrate into mushroom fruit bodies) of *V. volvacea* is only ~15% on straw-based substrates and 30–40% on cotton-waste 'composts' (Chang ST, 1980), values which are considerably lower compared to other major cultivated species such as *Agaricus bisporus*, *Lentinula edodes* and *Pleurotus* spp.

Agaricus bisporus is popularly known as the button mushroom. It is an edible basidiomycete mushroom native to grasslands in Europe and North America. Button mushroom is one of the largely growing mushrooms and has the good demand in the market and world trade too. This mushroom is extensively cultivated throughout the world and contributes about 40% of the total world production of mushroom. It has two color states while immature – white and brown – both of which have various names, with additional names for the mature state.

White button mushrooms grow well in nitrogen-rich manure, such as cow or horse manure. The substrate on which button mushroom grows is mainly prepared from a mixture of plant wastes (cereal straw/ sugarcane bagasse etc.), salts (urea, super phosphate / gypsum etc), supplements (rice bran/ wheat bran) and water.

In Bangladesh, white button mushroom can be cultivated only in winter season as the temperature requirement for spawn-run (vegetative growth) is 22 to 25°C and for crop production (fructification) 14 to 18°C. If temperature is too low, spawn-run will be either retarded or arrested and if it is too high, weed-fungi (competitor fungi) will grow. The mushroom requires nearly saturated atmosphere with moisture (relative humidity of 85–90%). However, direct application of water on compost during spawn-run is injurious to the crop.

Agaricus bisporus is used in in fresh, dried or canned form (Bernas *et al*. 2006). In a 100-gram serving, raw white mushrooms provide 93 kilojoules (22 kilocalories) of food energy and are an excellent source (> 19% of the Daily Value, DV) of the B vitamins, riboflavin, niacin, and pantothenic acid. Fresh mushrooms are also a good source (10–19% DV) of the dietary mineral phosphorus. While fresh *A. bisporus* only contains 0.2 micrograms (8 IU) of vitamin D as ergocalciferol (vitamin D_2), the ergocalciferol content increases substantially after exposure to UV light.

In Bangladesh, huge amount of agricultural wastes is produced annually and are of no uses. These wastes could be used as source of food i.e. rice grain, wheat grain, sawdust substrate for mushroom cultivation. Cereal straws such as wheat, rice make a good base for mushroom growth. They're easy to get and fairly cheap. Enriched sawdust is a mushroom substrate works quite well with a variety of different mushrooms.

Contamination of the mushroom growing medium can occur in several ways. The most common contaminants are yeast cultures and bacteria, although other chemical or biological contaminants may affect mushroom growth as well.

Objectives:

- To find out the best/suitable media for mother culture production.
- To find out the best spawn media for ensuring maximum yield of mushroom spp.

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). Mushroom production is different from growing other plants because it is devoid of chlorophyll, and therefore, depends on other plant materials i.e. substrates for their food. The visible mushroom is actually the fruiting body of edible fungus. Mushroom can contribute greatly to poverty alleviation and fight malnutrition in Bangladesh. In addition, mushroom can produce valuable organic manure for the field thus recycling agricultural waste. However available research findings related to the present study are reviewed below is based on the present information about the performance of Oyster mushroom (*Pleurotus djamor*), Straw mushroom (*Volvariella volvaceae*), Button mushroom (*Agaricus bisporus*) and the effect of different kinds of media substrate on mushroom cultivation. The review includes reports of several investigators which appear pertinent in understanding the problem and which may lead to the explanation and interpretation of results of the present investigation.

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 andmost favorable growth was observed at pH 6.

Baby Kumari, *et al*. (2020) studied three kinds of cereals grain (wheat, paddy and maize) were tested as media for spawn production of *Agaricus bisporus* and wheat grain was selected best for spawn production. Wheat grains provide good food base for the fungal species to grow throughout the substrate.

Nuhu Alam, *et al*. (2020) conducted a study on performance of Straw mushroom (*Volvariella volvaceae*) into different media (rice grain, wheat grain, maize grain, mixed chopped rice straw) with hot water sterilization. Preparation of mushroom bed with 25 to 30 days old mother culture give highest growth and yield and the sterilization of rice straw substrate with hot water for one hour was the best sterilization technique for the commercial production of *V. volvacea*.

Yildiz *et al.* (1998) reported that the natural substrates (woods on which *Pleurotus* Species grow) are very poor in nitrogen content, nevertheless the fruit bodies are produced.

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 girthof 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.

Manzi *et al.* (2001) analyzed fresh and processed mushrooms (*Agaricus bisporus*, *Pleurotus djamor* and Boletus group). Results showed that botanical variety, processing and cooking are all effective determinants of mushroom proximate

composition. Dried mushrooms (Boletus group) after cooking show the highest nutritional value, essentially due to insufficient dehydration. Dietary fiber, chitin and beta glucans, all functional constituents of mushrooms are present in variable amounts. Chitin level ranges from 0.3 to 3.9 g/100 g, while beta glucans which are negligible in *Agaricus*, range from 139 to 666 mg/100 g in *Pleurotus djamor* and Boletus group. On an average, a serving (100 g) of mushroom will supply 9 to 40% of the recommended of dietary fiber.

Fujihara *et al.* (2000) found that the nitrogen content of Fruit bodies cultivated on sawdust medium was closely related to that in the medium. Nitrogen content of the sawdust medium was related to amino acid, nucleic acid and chitin contents. No significant relation between lentinic acid in fruit bodies and nitrogen content in the medium was observed. The amount of lentinic acid in fruit bodies cultivated on sawdust medium containing rice bran and corn bran was about two times that cultivated on okara-added medium. Nitrogen content of fruit bodies was affected by nitrogen sources present in the medium. High levels of nitrogen in sawdust medium should decrease carbohydrates in fruit bodies cultivated on the medium, thus making the fruit too soft for eating.

Rathaiah and Shill (1999) in their experiment found that parboiled paddy was as good as wheat for spawn production of oyster mushroom. The spawn prepared from parboiled paddy was also compared with conventionally prepared paddy spawn. The suitability of parboiled paddy for spawn of paddy straw mushroom (*Volvariella volvacea*) was also confirmed.

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 tofind 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.

Suprapti (1987) in an experiment found that wood waste such as sawdust, leaves of legume plants such as turi (*Sesbabia grandiflora* pers.) and lamtoro gung (*Leucaena leucocephala* kam.) used as substrates for oyster mushroom cultivation.The substrate consisted of rubber wood sawdust mixed with turi or lamtoro gung leaves containing 5%, 10%, 15% and 20%, by W/V with distilled water. The highest production was found from sawdust substrate mixed with 10% turi leaf.

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 indicatethat VV-5 strain of *V. volvaceae* is suitable

for the commercial cultivation in Bangladesh.

Ramesh and Ansari (1987) evaluated several locally available substrates such as rice straw, banana leaves, saw dust, oil palm refuse, oil palm bunch refuse or grass straw in Andamans to study conversion efficiency of *Pleurotus sajor-caju*. Rice straw and banana leaves were best substrates, with more than 60% conversion efficiency on dry weight basis. The mean weight of the fruiting body was high (7.1g) on banana leaves compared to other substrates (2.1-5.0 g). The spawn running time was also less with banana leaves, followed by rice straw, grass straw, oil palmbunch refuse, sawdust and oil palm waste. *Pleurotus ostreatus* was successfully grown under local conditions utilizing chopped wheat straw, cotton waste, maize cobs or rice straw as bedding material. Wheat straw and cotton waste gave the highest yields with the shortest incubation period; fruiting bodies were appeared after 15-18 days as compared to 4-5 weeks on the other substrates. The first flush gave the highest yield in all treatments, and was a gradual decline in the yield of successive flushes.

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 sterilized, 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 stipelength 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.

Balasubramanyam *et al,* (1988) used the willow dust as a growing medium for *Pleurotus sajor-caju*. It was first soaked in water and calcium carbonate and calcium sulfate were added at the rate of 4 and 2%, respectively. About 600 g of mushrooms per kg willow dust were obtained in 25 days.

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 variationwas 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- 1 cultivated 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.

Kothandaraman *et al.* (1989) in a study reported that in the split or the logs *Hevea brasiliensis* was inoculated with spawn of *Pleurotus sajor-caju*, *Pleurotus citrinopileatus* and *Pleurotus florida*. They were covered with polythene sheet and kept in darkness at around 26°C until mycelium was visible. Rubber tree sawdust was also investigated as a growing medium; it was soaked in water for 24 hours, then dried to about 70% moisture and mixed with 5% $CaCO_3$; in bottles before inoculation. All 3 species began to grow on the logs within 3 days of inoculation and small fruiting bodies appeared 4 days alter spawn running was completed. However, almost all ceased development shortly afterwards; only 5 (*Pleurotus florida*) reached maturity. Mycelia on sawdust ceased to grow alter penetrating to about three-quarters of the depth of the medium. The reason(s) for the failure to develop fully are not yet known but, since

rubber wood appears to have no inhibitory activity against *Pleurotus spp*., further studies are proposed.

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* (POP2) 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* (POP2) 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.

Khan *et al.* (1991) used sawdust to prepare compost for spawn running amended with lime and different combinations of wheat chaff, wheat bran, paddy straw and cotton waste. Sawdust from *D. sisso* was the most suitable for spawn preparation and all types of sawdust amended with cotton waste were found to give optimum conditions for spawn running.

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.

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. Highestmycelium 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 negativerelationships were found between economic yield and different yield contributing attributes.

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

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

Payapanon *et al.* (1994) mentioned that suitable amount of rice bran added to saw dust medium means the maximum yield of Neuron's *florida* at optimum productioncost. Therefore, investigation on the addition of rice bran to saw dust medium, which consist of 100% saw dust, 0.5% $CaCO_3$ and 0.2% $MgSO_4.7H_20$ by weight was conducted, for the experiment rice bran 0, 1, 5, 10 and 15% respectively was used. *P. florida* was cultivated in plastic bags. Sawdust medium with 10% provided maximum yield. However, the yields obtained by addition of 5, 10 and 15% of rice bran were not significantly different. Yield of *P. florida* cultivation in the saw dust medium with 0% and 1% rice bran were not significantly different. Nevertheless, these were significantly different when compared with the higher rates of rice bran. The recommendation of the appropriate amount of rice bran tobe added in the sawdust medium should be 5-10%.

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

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) andtheir 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 SDsupplemented with MP.

Badshah *et al.* (1994) mentioned that *Pleurotus ostreatus* and *P. florida* were grown on wheat straw, sugarcane bagasse, corn cobs or sawdust, by mixing 120-130 g of spawn with 2 kg of substrate and placing the mixture in sterilized polyethylene bags which were kept in the dark a 25°C for 2-3 weeks. Once the bag became full of mycelial growth, they were removed, leaving the substrate uncovered. Watering was carried out 2-3 times a day. Fruiting bodies were harvested at maturity. *P. ostreatus* and *P. florida* yields ranged from 49.8 and 277.7 g/ 2kg substrate, respectively, on sawdust, to 432.8 and 420.5 g/ 2kg substrate, respectively, on wheat straw. Controls (grown in the field) yielded only 18.5 and 28.5 g/2 kg substrate for *P. ostreatus* and *P, florida*, respectively. In both species, wheat straw and sugarcane bagasse substrates resulted in the highest mushroom ascorbic acid contents, and protein, fat and fiber contents were also affected by substrate. *Pleurotus florida* had higher fat but lower protein contents than *P. ostreatus.*

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* (white snow), *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* (white snow).

Sarawish (1994) found no significantly difference in either the growth of mycelium or the yield of straw mushroom on kaptok residue, chopped-dried water hyacinth, chopped-dried banana stem or chopped-dried water hyacinth, chopped- dried banana stem chopped-dried rice straw as a main substrate.

Jadhav *et al.* (1996) reported that Oyster mushroom (*Pleurotus sajor-caju*) was cultivated on wheat straw, paddy straw, stalks and leaves of maize or cotton, jowar, soyabean straw, groundnut creepers plus wheat straw (1:1), soyabean straw plus groundnut creepers (1:1), or groundnut creepers alone. Cotton stalks and leaves gave the best results with respect to sporophore number, weight of sporophore (5.12 g), and total yield (914 g/kg of dry straw). Yields obtained on other substrates were: 796 g on paddy straw; 557 g on soyabean straw; and 508 g on soyabean + wheat straw. The lowest yield was recorded on groundnut creeper (258 g).

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).

Lim *et al.* (1997) analyzed the cost and return of *Volvariella* and *Pleurotus* mushroom production and found the ROI of 89.16 percent and 51.93 percent respectively. This indicates that mushroom production is economically feasible. The feasibility of low

input mushroom production for upland farmers in reforested areas under the closed canopy high-diversity forest faming system was determined. Agricultural and tree wastes were tested and utilized for spawn and mushroom production. Findings showed that among 10 agricultural/tree wastes tested, mung bean pods, kakawate and cassava leaves, log sawdust, and ipil-ipil leaves, sugarcane bagasse with rice bran, and water hyacinth can be used as alternative substances for *Volvariella* spawn production. Local isolate (VISCA) of *Volvariella volvacea* gave higher yield (2263.65g) compared with *Volvariella* (1574.80 g) isolates from BIOTECH College, Laguna, Philippines. This fruited well in the closed-canopy area than when cultivated in the open area. *Pleurotus* yield was higher (209.60 g/bag) inside mushroom house under closed-canopy area than whengrown inside mushroom house in relatively open area (198.54 g).

Kalita *et al.* (1997) studied the growth of *Pleurotus sajor-caju* in polyethylene hag on different combinations of substrates viz. only rice straw, rice straw plus rice husk mixture (1:1 v/v), water hyacinth, chopped banana leaves, areca nut husk and sugarcane bagasse. They found that only rice straw, rice straw plus rice husk mixture and areca nut husk substrates completed spawn running comparatively within short time (12-14 days.) but other substrates took longer time.

Yildiz *et al.* (1998) mentioned that this study was conducted on the growth and cultivation of *Pleurotus ostreatus* var. *salignus* on local cellulosic wastes. The highest and lowest yields for 100 g material (70% moisture) were obtained with peanut straw (24.8 g) and with sorghum straw (11.3 g), respectively. Protein, pilus/stipe, sporophore weight, 4 dry material, N and C in highest amounts were obtained with peanut straw. The lowest mushroom weight and pilus/stipe ratio were obtained with sorghum, whereas the lowest protein, N and dry material weightwere obtained with wheat straw. In all the *P. osrrealiir* var. *saligrllus* cultivated on peanut and sorghum straw, the most abundant nutrients were protein, potassium and carbon. These results are discussed in relation to the prospect of cultivating *P. ostreatus* var. *salignus* in Diyarbakir, Turkey.

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 (9.93 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 .

Ramjan (2006) in his study found that high concentration of IAA is effective for mycelial growth and mustard straw performed best as a substrate for the production of fruiting bodies of oyster mushroom.

Obodai *et al.* (2000) mentioned that Seasonal effects on spawn run period, time for first appearance of fruiting bodies, number of flushes, morphological characteristics of the first flush and biological efficiency of 7 strains of oyster mushroom (*Pleurotus eous, Pleurotus ostreatus* and *Pleurotus sajor-caju*), grown on composted sawdust of *T. scleroxylon* in Ghana, were studied. *P. eons* strain EM-1 and *Pleurotus sajor-caju* strain ST-6 gave the best yield and biological efficiencies in the wet and dry seasons, respectively. The spawn run period, mycelia growth density and the first appearance of fruiting bodies were not season-dependent.

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

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.

Khan *et al.* (1991) used sawdust to prepare compost for spawn running amended with lime and different combinations of wheat chaff, wheat bran, paddy straw and cotton waste. Sawdust from *D. sisso* was the most suitable for spawn preparation and all types of sawdust amended with cotton waste were found to give optimum conditions for spawn running.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted to find out the performance of different media for mother culture production of different mushroom species. The study was carried out in the laboratory, workshop and culture house of Mushroom Development Institute (MDI), Sobhanbag, Savar, Dhaka from June, 2019 to May, 2020.

3.2 Required materials

Variety/Inoculums

Three selected varieties of three different mushroom such as oyster mushroom (*Pleurotus djamor*), straw mushroom (*Volvariella volvacea*) and *button mushroom (Agaricus bisporus)* were cultivated in the culture house of the Mushroom Development Institute. Mushroom varieties used in this experiment were collected from germplasm centre of Mushroom Development Institute, Savar, Dhaka and was cultured in tissue culture laboratory.

Treatment

The experiment comprised of two factors-

Factor A: Mushroom varieties	Factor B: Media for mother culture production
A_1- Oyster mushroom (*Pleurotus djamor*)	B_1-Rice grain
A_2- Straw mushroom (*Volvariella volvacea*)	B_2-Wheat grain
A_3- *Button mushroom (Agaricus bisporus)*	B_3-Sawdust based substrates

Experimental design

The experiment was laid out in Completely Randomized Design (CRD) with four replications. The experiments with nine treatments each have four replications were conducted to achieve the desired objectives.

Equipments

Laminar air flow 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.

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.

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.

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.

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.

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.

Electric balance

Electric balance was used to measure various chemical and material.

Water bath

Water bath was used to heat potato and culture media.

Test tube

Test tube were used for the experiment. The test tube were washed properly and wipe with propanol.

Microspores

Microspores were used to attach petriplate properly.

Scale and Tape

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

Rubber bands, cotton plug and plastic neck

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

Trolley

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

Rack

The various racks were used to conduct the experiment.

Water Spray Machine

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

Poly propylene (pp) sheet

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

Chemicals

- Dextrose
- Agar
- Urea
- TS
- Gypsum
- Bavistin
- Furadan
- $CaCO_3$

Organic substrates

- Rice grain
- Wheat grain
- Sawdust
- Rice straw
- Wheat bran

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.

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.

Component of PDA media

Following essential components were mixed for one (1) liter of PDA.

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

Procedure for PDA media and pure culture preparation

For one liter PDA Media, 250g potatoes were measured by electric balance. Then potato's pills were removed by knife and sliced into small pieces. The small potato's pieces were boiled with one (1) liter water for 45 min and small potato's pieces were filtrated by thin cloth. All the chemical component of PDA media were 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 test tube. Each test tube was rapped with aluminum foil and autoclaved at 121°C and 15 PSI for 20 min to make it free from contamination. The explants were inoculated under contamination free environment. Laminar air flow was used to inoculate the germplasm in test tube. After surface sterilization the inoculums were laid on the sterile petri dish using sterile forceps by cutting with a sterile scalpel. The test tube were rapped with micro pore under the laminar air flow. Then the test tube were placed for pure culture preparation. After 7-20 days, the test tube were filled with white mycelium. This white mycelium in test tube is called pure culture.

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.

3.4. Preparation of master mother culture

3.4.1 Preparation of master mother culture of oyster mushroom

To prepare master mother culture of the oyster mushroom strain, sawdust and wheat bran were mixed together at 2:1 (v/v) and supplemented with $CaCO_3$ at 0.2% (w/w) of the mixture. The moisture level of the mixed substrate was maintained at 65% with tap water. The substrate was poured into polypropylene bags (7" × 10") at 300g/bag. The substrate in bags was sterilized in an autoclave for 1 h at 121^0C under 1.1 kg/cm^2 pressures and allowed to cool for 24h. Pure cultures of mushroom strains were grown on potato dextrose agar (PDA) following hyphal tip method. A piece of the PDA culture of oyster mushroom strain containing mycelium was placed aseptically in the opening of the mother culture packets. The inoculated packets were placed on a rack in the laboratory at 22 ± 2 ^{0}C for incubation. The substrate of the mother culture was covered by whitish mycelium within 15-20 days after inoculation. The fully colonized packets were used as master mother for inoculation of mother culture.

3.4.2 Preparation of master mother culture of straw mushroom

To prepare master mother culture of straw mushroom strain, rice straw and wheat bran was mixed at the ratio of 70:30. The moisture level of the mixture was maintained at 50% by adding tap water. Calcium carbonate was used at the rate of 0.1% of the mixture. Polypropylene bags of 7" × 10" size were filled with 300g of the above mentioned mixture and packed medium tightly. Then the packets were sterilization, inoculation and incubation in the same process. The inoculated packets were placed on a rack in the laboratory at 22 -28 ^{0}C for incubation. The substrate of the mother culture was covered by whitish mycelium within 10-12 days after inoculation. The fully colonized packets were used as master mother for inoculation of mother culture.

Plate 1: Master mother culture of straw mushroom in wheat grain media

3.4.3 Preparation of master mother culture of button mushroom

To prepare master mother culture of the button mushroom, wheat grains were used as media of master mother culture. At first grains collected which was free from diseases and not broken, old, and insect damaged. The grains were thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses, weeds, etc. Washed grains were then soaked in sufficient water for 7-8 hours and boiled in a container for 10-15 minutes till the skin became soft not started to crack. Excess water from the boiled grains was removed by stirring and heating. Then the grains were thoroughly mixed with calcium carbonate at 0.1% and 0.2% gypsum was also added. This mixing was done on the same container after wearing gloves. The substrate was poured into glass jar at 250-300 g/jar. The jar was covered with lid and tied together by cotton wool. The substrate in bags was sterilized in an autoclave for 1 h at 121^0C under 1.1 kg / cm^2 pressures and allowed to cool for 24 h. Pure cultures of button mushroom strains were grown on potato dextrose agar (PDA) following hyphal tip method. A piece of the PDA culture of button mushroom strain containing mycelium was placed aseptically in the opening of the master mother culture packets. Then the master mother substrate grain was covered by whitish mycelium within 25-30 days after inoculation. The fully colonized packets were used as master mother for inoculation of mother culture.

3.5 Preparation of mother culture

3.5.1 Preparation of mother culture (paddy)

To prepare mother culture of the oyster, straw and button mushroom, paddy grains were used as media of mother culture. At first grains collected which was free from diseases and not broken, old, and insect damaged. The grains were thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses, weeds, etc. Washed grains were then soaked in sufficient water for 7-8 hours and boiled in a container for 20-25 minutes till the skin started to crack. Excess water from the boiled grains was removed by stirring and heating. Then the grains were thoroughly mixed with calcium carbonate at 0.1% so that the pH of the grains was around 7.0 to 7.8. For button mushroom production 0.2% gypsum was also added. This mixing was done on the same container after wearing gloves. The substrate was poured into polypropylene bags (7" × 10") at 250-300 g/bag. The neck of the bags was heat resistant plastic and the neck was plugged with cotton wool, covered with paper piece and then tied together by a rubber band. The substrate in bags was sterilized in an autoclave for 2 h at 121^0C under 1.1 kg / cm^2 pressures and allowed to cool for 24 h. Then the master mother was poured aseptically at 10% in the opening of paddy grain containing mother culture packets and substrate grain was covered by whitish mycelium within 12-25 days according to variety after inoculation. The fully colonized packets were used as mother culture for spawning.

3.5.2 Preparation of mother culture (wheat)

To prepare mother culture of the oyster, straw and button mushroom, wheat grains were used as media of mother culture. At first grains collected which was free from diseases and not broken, old, and insect damaged. The grains were thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses, weeds, etc. Washed grains were then soaked in sufficient water for 7-8 hours and boiled in a container for 10-15 minutes till the skin started to crack. Excess water from the boiled grains was removed by stirring and heating. Then the grains were thoroughly mixed with calcium carbonate at 0.1% and gypsum at 0.2% so that the pH of the grains was around 7.0 to 7.8. This mixing was done on the same container after wearing gloves. The substrate was poured into polypropylene bags (7" × 10") at 250-300 g/bag. The neck

of the bags was heat resistant plastic and the neck was plugged with cotton wool, covered with paper piece and then tied together by a rubber band. The substrate in bags was sterilized in an autoclave for 2 h at 121^0C under 1.1 kg / cm^2 pressures and allowed to cool for 24 h. Then the master mother was poured aseptically at 10% in the opening of wheat grain containing mother culture packets and substrate grain was covered by whitish mycelium within 12-30 days according to variety after inoculation. The fully colonized packets were used as mother culture for spawning.

3.5.3 Preparation of mother culture (sawdust)

To prepare mother culture of the oyster, straw and button mushroom, saw dust was used as media of mother culture by mixing sawdust and wheat bran at the ratio of 2:1. Calcium carbonate was used at the rate of 0.2% of the mixture. The moisture level of the mixture was maintained at 65% by adding tap water. Polypropylene bags of 7" × 10" size were filled with 300g of the above mentioned mixture and packed tightly. The substrate in bags was sterilized in an autoclave for 2 h at 121^0C under 1.1 kg / cm^2 pressures and allowed to cool for 24 h. Then the master mother was poured aseptically at 10% in the opening of saw dust containing mother culture packets and substrate media was covered by whitish mycelium within 12-30 days according to variety after inoculation. The fully colonized packets were used as mother culture for spawning. Then the packets were sterilization, inoculation and incubation in the same process.

3.6 Preparation of spawn

Sawdust was used for oyster mushroom and rice straw based media was used for straw mushroom and compost was used for button mushroom in spawn packets.

3.6.1 Preparation of oyster mushroom spawn packets:

Preparation of spawn packets:

Sawdust was used as a main substrate and wheat bran was used as supplement.8 kg sawdust was mixed with 4 kg wheat bran by mixing sawdust and wheat bran at the ratio of 2:1.Calcium carbonate was used at the rate of 0.2% of the mixture. The moisture level of the mixture was maintained at 65% by adding tap water. The mixture was filled into heat tolerant polypropylene bags of 7"x 10" size at 200 g/bag and their mouth were

plugged by inserting water absorbing cotton and covered with brown paper and tied with a placing rubberband. Then bags were autoclaved at 121^0 C and 15 PSI for 2 hour and then allowed cooling. Each spawn packet was inoculated with the mother culture at the rate of two teaspoonful per packet. Spawning substrate with bags was then incubated for mycelium growth according to the strains.

. **Mycelium running in spawn packets/ Incubation**

The packets were kept at room temperature until the packets become white with the mushroom mycelium. After 20-24 days completion of the mycelium running, the rubber band, 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.

Opening the packet

Two ends, opposite to each other of the upper position i.e. on shoulder of plastic bag were cut in "D" shaped with a blade and opened by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a blade for removing the thin whitish mycelial layer.

Cultivation of spawn packet

The packets of each type were placed separately on the rack of culture room. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3-5 times a day. The light around 150-200 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 5-6 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

Plate 2: Pinhead initiation of oyster mushroom

Collection of produced mushrooms

Oyster mushrooms matured within 5-6 days after primordia initiation. The matured fruiting body was identified by curial margin of the cap, as described by Amin (2002). Mushrooms were harvested by twisting to uproot from the base.

Plate 3: Collection of oyster mushroom

3.6.2 Preparation of straw mushroom bed:

Preparation of substrates:

For straw mushroom bed preparation, the rice straw was chopped to 4-5 cm length and then poured 4-5 kg into cribriform nylon bag. The bags were submerged in water for sometimes and then drained out the excess water. After that bags containing rice straw was kept in a pasteurization chamber at 60-65^{0}C for 1 hour. The bags were kept in same place for 18-20 hours to get cool slowly. After 20 hours the prepared straw was spread over polythene sheet in open place to reduce moisture 63%. After that rice straw was ready for straw mushroom bed preparation.

Preparation of straw mushroom bed:

The bed size was 1m x 30cm x 30cm. Four layer of rice straw placed vertically. Each layer was 2 inch in height. Mother culture of straw mushroom was inoculated among those layers. The whole bed was covered with a transparent polythene sheet. After 8 days, the sheet was removed for sufficient aeration, light, temperature and humidity, so that fructifications could be ensured.

Culture condition:

The moisture of the culture room was maintained 85-95% relative humidity by spraying water 3-5 times a day. The light around 150-200 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 25°C to 30°C. The first primordia appeared 15-20 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

Collection of produced mushrooms

Straw mushrooms matured within 3-4 days after primordia initiation. The matured fruiting body was identified by curial margin of the cap, as described by Amin (2002). Mushrooms were harvested by twisting to uproot from the base.

3.6.3 Preparation of button mushroom spawn packets

Preparation of substrates:

For button mushroom compost preparation, 300 kg of rice straw, 30 kg wheat bran, 10 kg $CaCO_3$, 9 kg urea, 6 kg TSP,6 kg TSP, 3 kg MP,15kg Gypsum, 150 gm bavistin and 250 gm furadan were used. At first rice straw was chopped 2-3 inch length and divided

into five equal portion. One fifth of the portion of straw was placed in container 5 ft by 5 ft and soaked with water .Then one fifth of the other components were mixed with this wet rice straw. Following this process 2^{nd}, 3^{rd} and 4^{th} layer were prepared. In 5^{th} layer a portion of straw was kept separated and it was used for covering at the top of 5^{th} layer, some water was sprayed at the top of layer. This layer should be 5ft in length, width and height .Within 24-48 hours compost temperature was 60-70^{0}C for 1^{st} to 5^{th} days. On 6^{th} day, compost was turned to ensure proper aeration of compost and water was added. Following this way, on 10, 13, 16, 19, 22, 25^{th} day, turning was done. Gypsum was added on 13^{th} day and furadan was added on 16^{th} day and 25^{th} day. On 28^{th} day, compost was ready.

Preparation of spawn packets

The button mushroom spawn packets were prepared using heat tolerant polypropylene bags 12"x 18" size at 2-2.5 kg/bag. Compost was placed 4-5 layer mixed with spawn in each bag. Spawn was mixed at 0.5-0.75% with compost in each bag.

Mycelium running in spawn packets/ Incubation

These mushroom packets were placed in growing chamber, where temperature range between 22-25ºC and 65-70% RH (Relative Humidity).The paper over the packets were sprayed regularly with water to prevent drying out and humidity was built up by frequently watering the floor and walls. Formalin solution of 0.5-1% was sprayed to protect from mushroom disease and infection. The room was kept closed as only a small amount of fresh air recirculating within the room for maintaining the carbon dioxide levels. Mushroom packets were completely colonized by mushroom mycelium within (20-31days). The compost becomes lighter in color and the mycelium seen as thin white-threads. Caging was done by mixing 2 year old compost, cowdung and loamy soil (2:1:1) and it was placed 3-4 cm thick layer in each packet after mycelium run complete. These spawn packets were transferred to the culture house.

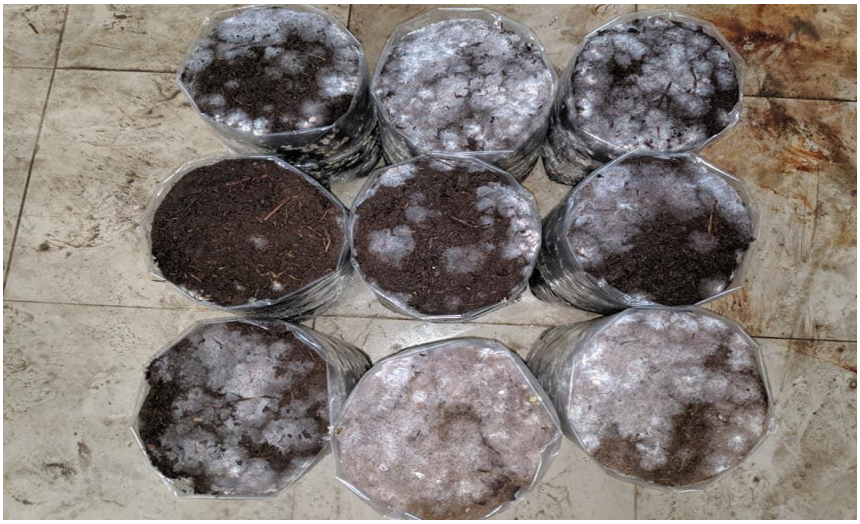

Plate 4: Mycelium running in spawn packets of button mushroom

Cultivation of spawn packet

The packets of each type were placed separately on the rack of culture room. The moisture of the culture room was maintained 85-90% relative humidity by spraying water 3-5 times a day. The light around 150-200 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 16°C to 18°C. The first primordia appeared 8-10 days after caging depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

Plate 5: Pinhead initiation of button mushroom

Plate 6: Fruiting body of button mushroom

Collection of produced mushrooms

Button mushrooms matured within 2-3 days after primordia initiation. The matured fruiting body was identified by curial margin of the cap, as described by Amin (2002). Mushrooms were harvested by twisting to uproot from the base.

Plate 7: Collection of button mushroom

3.7 Yield measurement process:

Data collection

Data required for mycelium run rate per day, mycelium running complete days, days required for pin head initiation, Days required from pin head initiation to harvest, number of fruiting bodies, effective number of fruiting body, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus,yield were recorded.

Data on mycelium run rate (cm/day)

Mycelium run rate was recorded from date of inoculation to complete mycelium running in spawn packet.

Data on days required to complete mycelium running

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

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.

Days required from pin head initiation to harvest

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

Number of fruiting body

Number of total fruiting body was recorded.

Number of effective fruiting body

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

Thickness of pileus (cm)

Thickness of the pileus of fruiting bodies were recorded by using a slide calipers.

Diameter of pileus

Diameter of pileus of fruiting bodies were recorded by using a slide calipers.

Length of stalk

Length of stalk of fruiting bodies were recorded by using a slide calipers.

Diameter of stalk

Diameter of stalk of fruiting bodies were recorded by using a slide calipers.

Biological yield (g)

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

Economic yield (g)

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

Biological Efficiency (%)

The Biological Efficiency was determined by using the following formula:

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

3.8 Statistical analysis

The experiment was laid out in double factor Complete Randomized Design (factorial experiment). The experiment considered 9 treatments with 4 replications. The data for the characters considered in the present experiments were statistically analyzed by Complete Randomized Design (CRD) using MSTAT software. The analysis of variance was conducted and means were compared following the significance of the difference was evaluated by Duncan's Multiple Range Test (DMRT) or Least significant difference (LSD) according to Gomez and Gomez, (1984) for interpretation of the results at 5% level of probability.

CHAPTER IV

RESULTS AND DISCUSSION

An experiment was conducted to find out the performance of different media for mother culture production of different mushroom species. Results of the present study have beenpresented and discussed in this chapter under the following headings.

4.1 Mycelium run rate (cm/day)

Mycelium run rate (cm/day) was significantly varied among oyster mushroom (*Pleurotus djamor*), straw mushroom (*Volvariella volceae*) and button mushroom *(Agaricus bisporus)* (Fig.1). The highest mycelium run rate was observed in straw mushroom (A_2) which was 0.60 cm/day followed by Oyster mushroom (A_1) which was 0.57cm/day and the lowest mycelium run rate was observed in button mushroom (A_3) which was 0.51 cm/day (Fig-1).

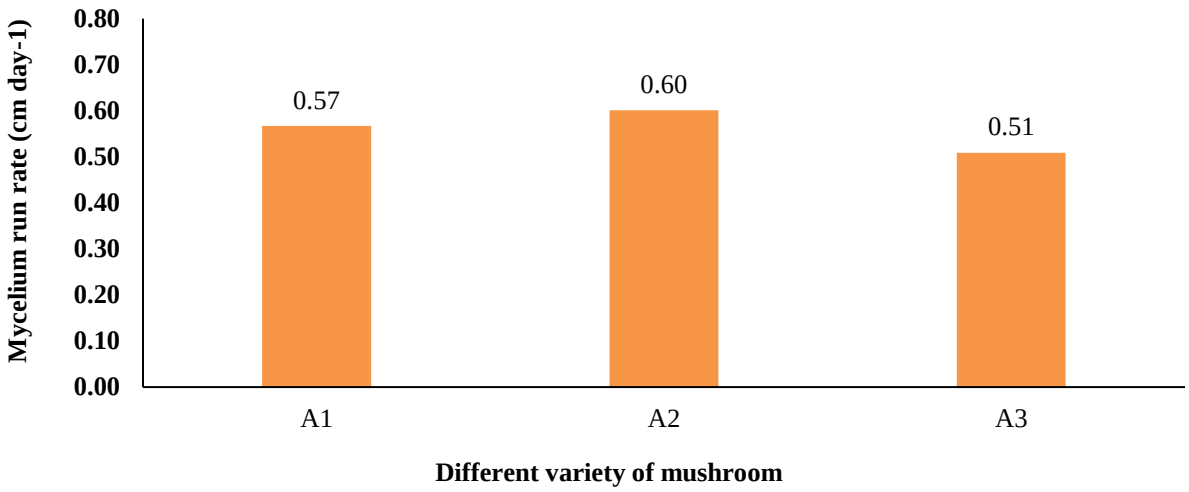

Fig. 1. Effect of different variety on mycelium run rate of mushroom,
(A_1: Oyster Mushroom, A_2: Straw mushroom and A_3: Button mushroom)

Mycelium run rate showed significant variation to different media (Fig.2). The highest mycelium run rate was observed in wheat grain (B_2) which was 0.64 cm/day followed by rice grain (B_1) which was 0.59 cm/day and the lowest mycelium running rate was

observed in sawdust substrate (B_3) which was 0.45 cm/day. Khan *et al*. (1991) reported that wheat grain with different organic supplement provided suitable condition for spawn running. Sarker (2004) found that the mycelium running rate of oyster mushroom greatly influenced with the supplement of wheat brans in different levels.

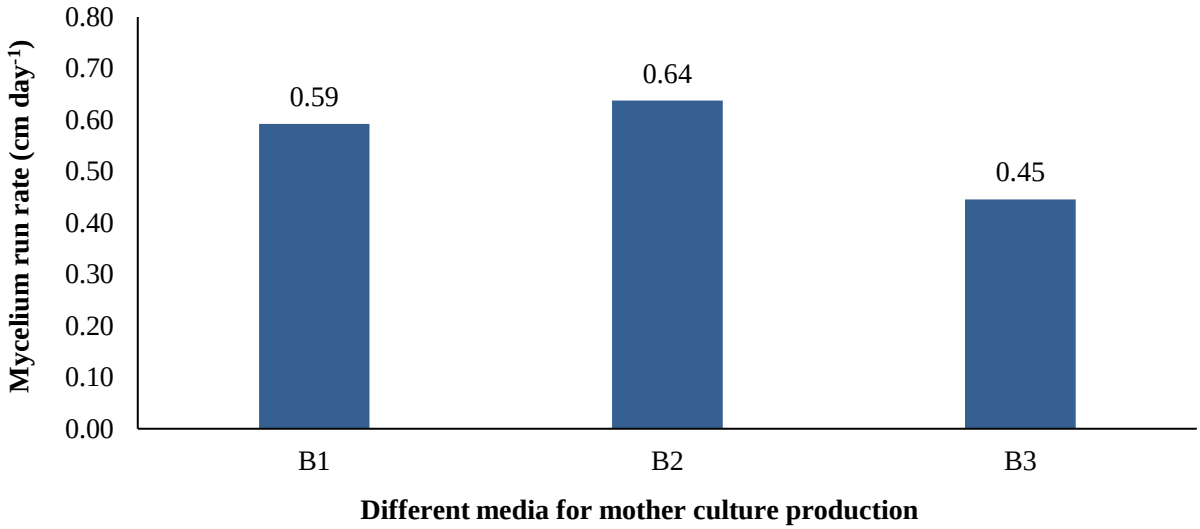

Fig. 2. Effect of different media on mycelium run rate of mushroom,
(B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

Combined effect of different mushroom variety with different media showed significant variation on mycelium run rate (cm/day).

In case of oyster mushroom with three different media, A_1B_1 (oyster mushroom with rice grain media) showed the highest mycelium run rate which was 0.71 cm/day followed by A_1B_2 (oyster mushroom with wheat grain media) which was 0.54 cm/day and A_1B_3 (oyster mushroom with saw dust substrate) showed the lowest mycelium run rate which was 0.46 cm/day. Sarker (2004) found that the mycelium running rate of oyster mushroom greatly influenced with the supplement of rice brans in different levels. Bhuyan (2008) also found similar result as found in the present experiment (Fig.3). In case of straw mushroom with three different media, A_2B_2 (straw mushroom with wheat grain media) showed the highest mycelium run rate which was 0.72 cm/day followed by A_2B_1 (straw mushroom with rice grain media) which was 0.55 cm/day and significantly varied from A_2B_3 (straw mushroom with saw dust substrate), showed the

lowest mycelium run rate which was 0.45 cm/day (Fig.3). Anita Tripathy (2010) found that the mycelium running rate of paddy straw mushroom were highest in wheat+rice bran media compared with sawdust based media.

For button mushroom with three different media, A_3B_1 (button mushroom with rice grain media) showed the highest mycelium run rate which was 0.65 cm/day followed by A_3B_2 (button mushroom with wheat grain media) which was 0.53 cm/day and significantly varied from A_3B_3 (button mushroom with saw dust substrate) showed the lowest mycelium run rate which was 0.43 cm/day (Fig.3).

Among these three varieties of mushroom with three different media, the result showed that the highest mycelium run rate in A_2B_2 (straw mushroom with wheat grain media) which was 0.72 cm/day. It is significant from all other combination.

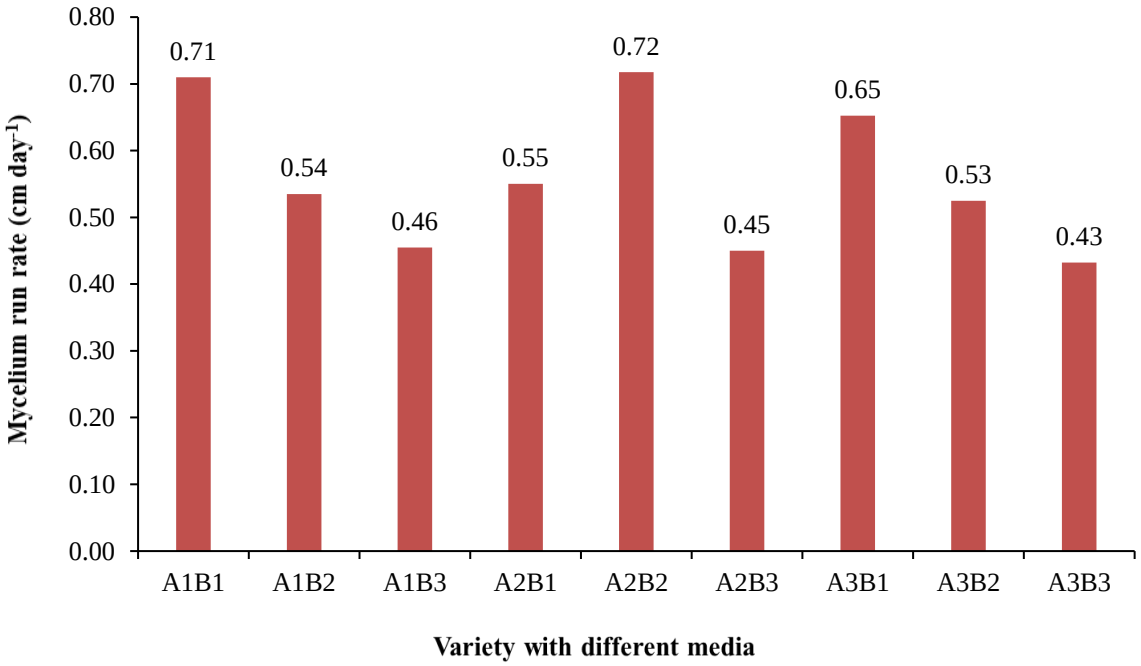

Fig. 3. Combined effect of variety and different media on mycelium run rate of different mushroom spp.,

(A_1: Oyster mushroom, A_2: Straw mushroom, A_3: Button mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

4.2 Days required for complete mycelium running

Days required for complete mycelium running (DCMR) was significantly varied among oyster mushroom (*Pleurotus djamor*), straw mushroom (*Volvariella volceae*) and button mushroom *(Agaricus bisporus)* (Fig.4). The highest value required for complete mycelium running was 32.9 days obtained from button mushroom (A_3) followed by oyster mushroom (A_1) which was 22.8 days and the lowest value required for complete mycelium running was 18.3 days obtained from straw mushroom (A_2).

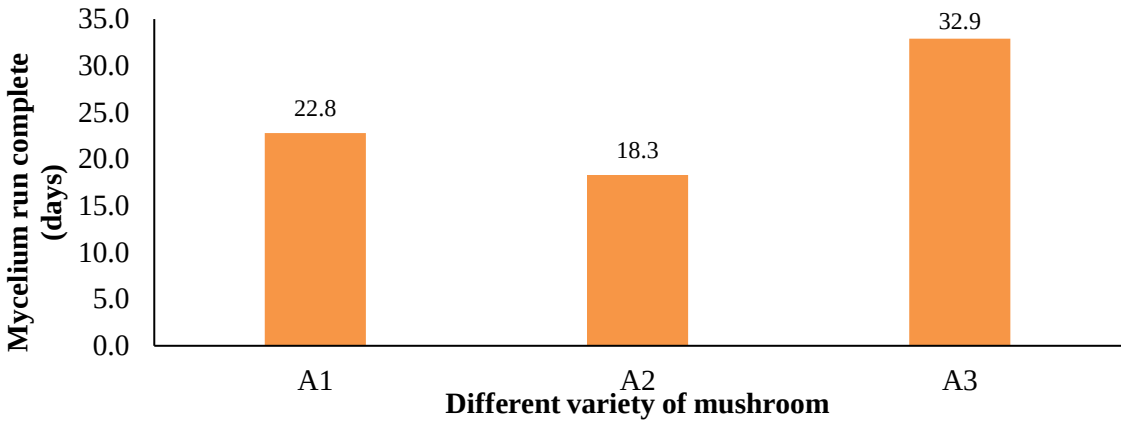

Fig. 4. Effect of different variety on mycelium run complete (days)
(A_1: Oyster mushroom, A_2: Straw mushroom and A_3: Button mushroom)

Days required for complete mycelium running showed significant variation to different media (Fig.5). The highest value required for complete mycelium running was 26.1 days obtained from sawdust substrate (B_3) followed by B_2 (wheat grain substrate) which was 24.5 days and the lowest value required for complete mycelium running was 23.5 days obtained from B_1 (rice grain).

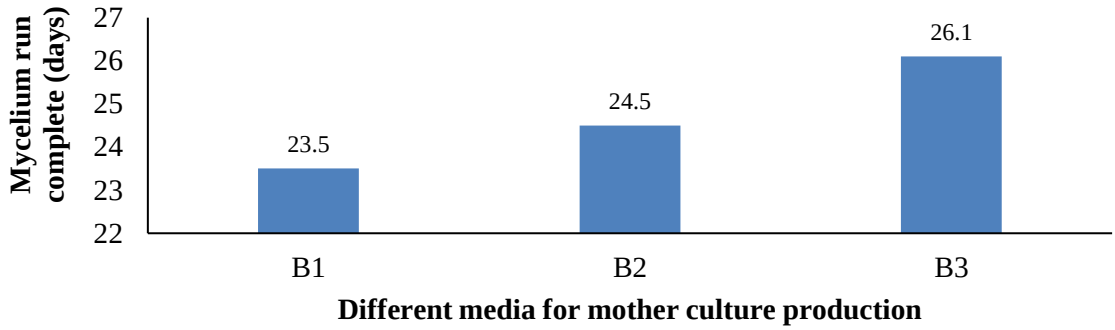

Fig. 5. Effect of different media on mycelium run complete (days) of mushroom,
(B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

Combined effect of different mushroom variety with different media showed significant variation on days required for complete mycelium running (Fig.6).

In case of oyster mushroom with three different media, A_1B_3 (oyster mushroom with saw dust substrate) showed the highest value which was 24 days followed by A_1B_2 (oyster mushroom with wheat grain substrate) which was 23 days and A_1B_1 (oyster mushroom with rice grain media) showed the lowest value for completing mycelium running days which was 21.5. 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 spp.*

In case of straw mushroom with three different media, A_2B_2 (straw mushroom with rice grain substrate) showed the highest value which was 19.5 days followed by A_2B_3 (straw mushroom with sawdust substrate) which was 19.0 days and significantly varied from A_2B_2 (straw mushroom with wheat grain media), showed the lowest value which was 16.5 days.

For button mushroom with three different media, A_3B_3 (button mushroom with saw dust substrate) showed the highest value which was 34.8 days followed by A_3B_2 (button mushroom with wheat grain substrate) which was 33.0 days and significantly varied from A_3B_1 (button mushroom with rice grain media), showed the lowest value which was 31 days.

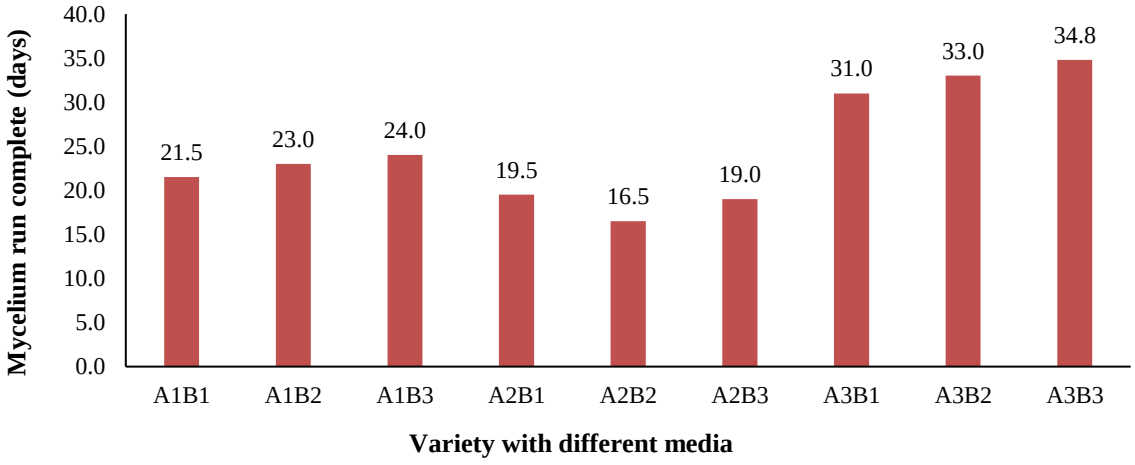

Fig. 6. Combined effect of variety and different media on mycelium run complete (days) of different mushroom spp.,
(A_1: Oyster mushroom, A_2: Straw mushroom, A_3: Button mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

4.3 Days required for pinhead initiation

Days required for pinhead initiation (DRPI) significantly influenced by varieties and substrate media (Fig.7 and Appendix. II). Button mushroom (A_3) required the highest 9.4 days for pin head initiation followed by straw mushroom (A_2) which value was 7.5 days and the lowest period of 4.7 days was required for pin head initiation in Oyster mushroom (A_1). Patra and Pani (1995) who found that oyster mushroom took 4-8 days for initiation of pinhead. The difference among the findings may be due to the difference of genetic make up of the varieties.

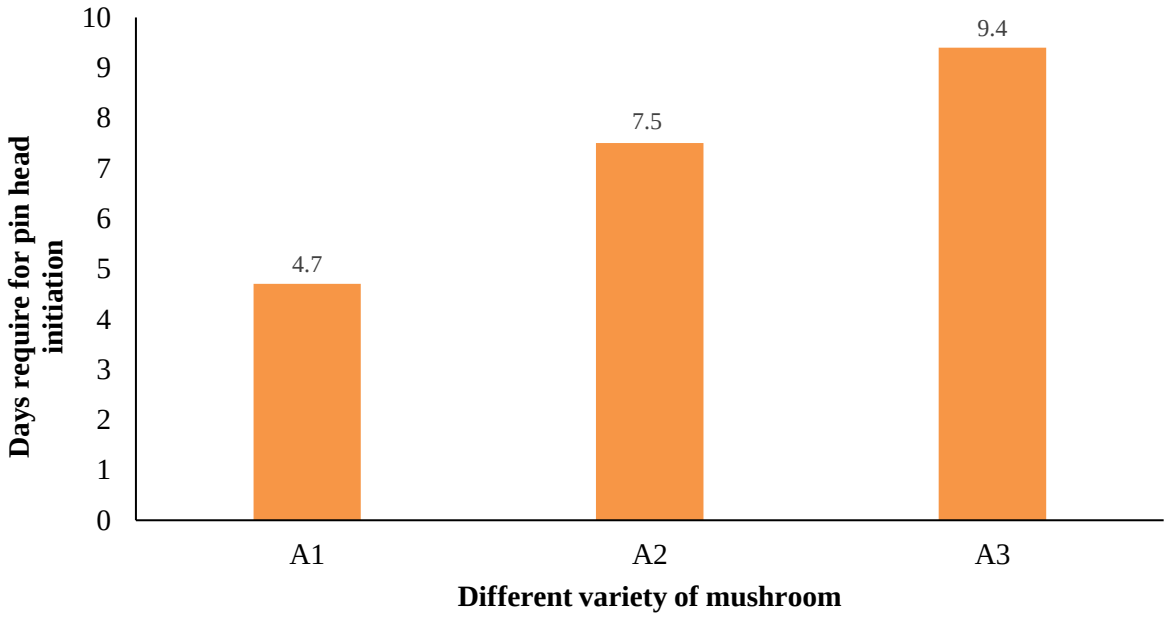

Fig. 7. Effect of different mushroom variety on pinhead initiation (days)
(A_1: Oyster mushroom , A_2: Straw mushroom and A_3: Button mushroom)

Days required for pin head initiation showed significant variation to different media (Fig.8 and Appendix. II). The highest value required for pin head initiation was 8.2 days obtained from sawdust substrate (B_3) followed by rice grain (B_1) which was 6.8 days and the lowest value required for complete pin head initiation was 6.5 days obtained from wheat grain (B_2).

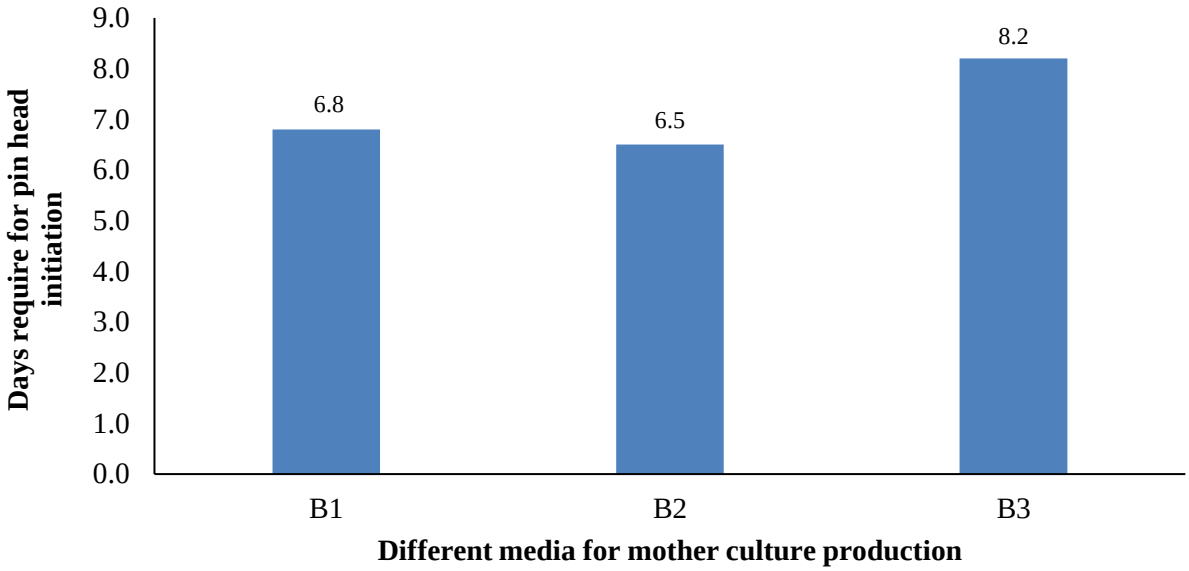

Fig. 8. Effect of different media on pinhead initiation (days) of mushroom,
(B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

Combined effect of different mushroom variety with different media substrate showed significant variation on days required for pin head initiation (Fig.9 and Appendix. II). In case of oyster mushroom with three different media, A_1B_3 (oyster mushroom with sawdust substrate) showed the highest value which was 5.5 days followed by A_1B_2 (oyster mushroom with wheat grain substrate) which was 5.0 days and A_1B_1 (oyster mushroom with rice grain media) showed the lowest value for pin head initiation days which was 3.5. These results are in agreement with Ahmad (1986) for one aspect who stated that oyster mushroom (*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.

In case of straw mushroom with three different media, A_2B_3 (straw mushroom with saw dust substrate) showed the highest value which was 8.0 days followed by A_1B_2

(straw mushroom with wheat grain substrate) which was 7.5 days and significantly varied from A_2B_1 (straw mushroom with rice grain media), showed the lowest value which was 6.0 days. Anita Tripathy (2010) found that days required for pinhead initiation of paddy straw mushroom were highest in sawdust based media compared with wheat + rice bran media.

For button mushroom with three different media, A_3B_3 (button mushroom with saw dust substrate) showed the highest value which was 10.5 days followed by A_3B_3 (button mushroom with rice grain substrate) which was 9.0 days and significantly varied from A_3B_2 (button mushroom with wheat grain media), showed the lowest value which was 8.5 days.

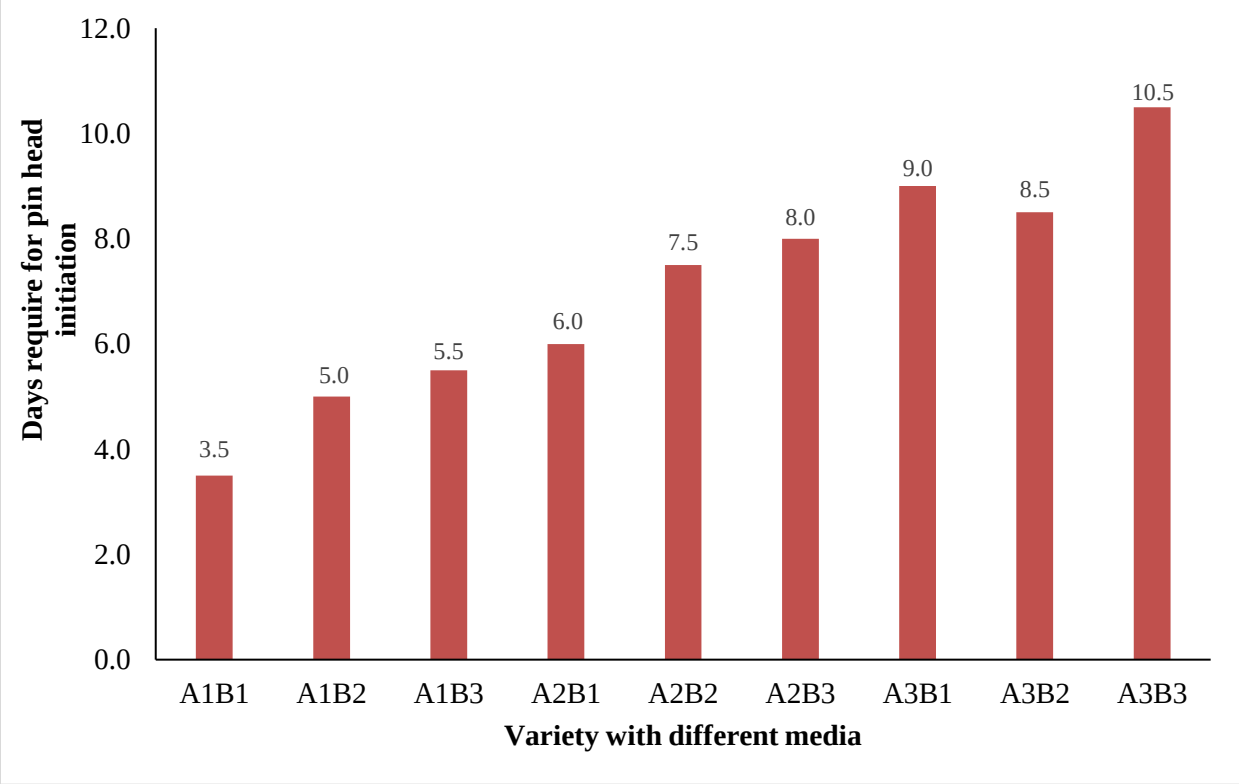

Fig. 9. Combined effect of variety and different media on pinhead initiation (days) of different mushroom spp.,

(A_1: Oyster mushroom, A_2: Straw mushroom, A_3: Button mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

4.4 Days required from pinhead initiation to harvest

Mushroom varieties and substrate media significantly influence on pinhead initiation to harvest days. (Fig.10 and Appendix. II). Oyster mushroom (A_1) required the highest 6.0 days for pin head initiation to harvest followed by straw mushroom (A_2) which was 3.8 days. The lowest period of 3.6 days was required for pin head initiation to harvest in Button mushroom (A_3).

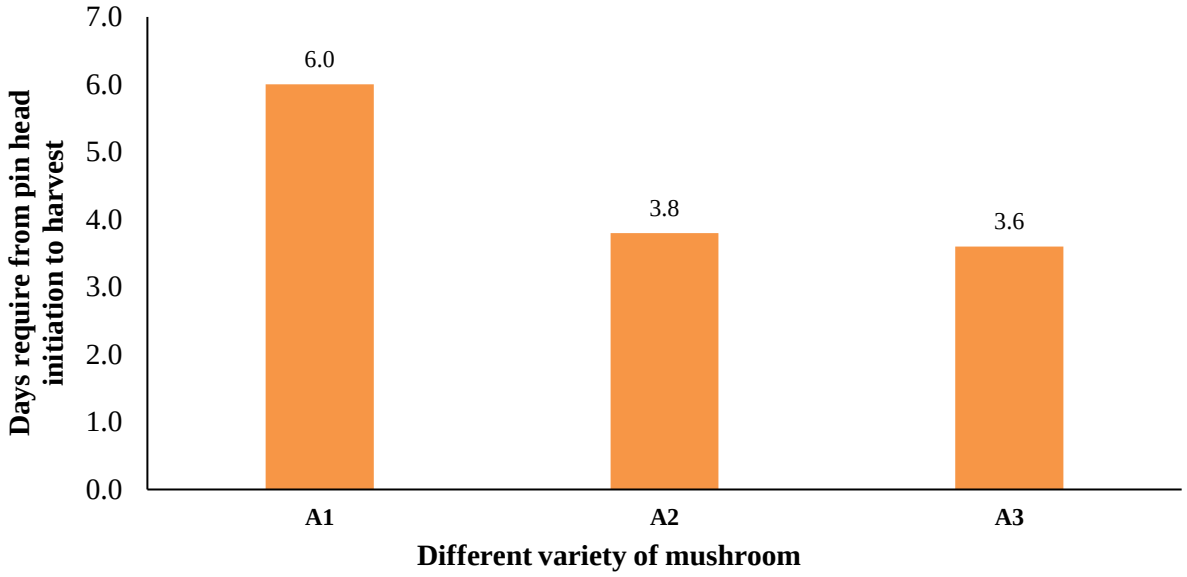

Fig. 10. Effect of different variety on pinhead initiation to harvest (days)
(A_1: Oyster mushroom, A_2: Straw mushroom and A_3: Button mushroom)

Days required for pin head initiation to harvest showed significant variation to different media (Fig.11 and Appendix. II). The highest value required for pin head initiation to harvest was 5.2 days obtained from sawdust substrate (B_3) followed by rice grain media (B_1) which was 4.2 days and the lowest value required for complete pin head initiation to harvest was 4.1 days obtained from wheat grain (B_2).

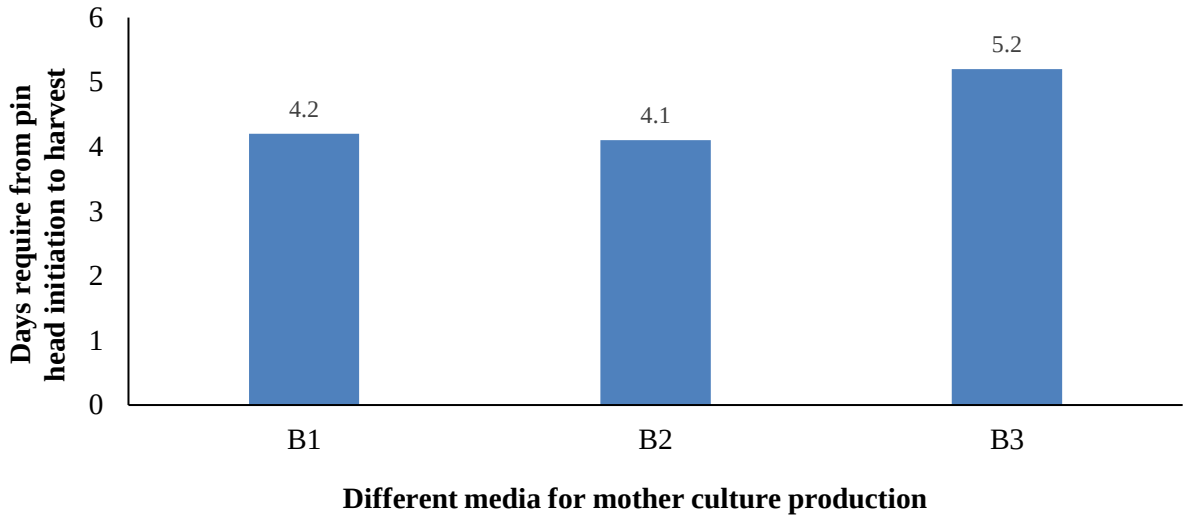

Fig. 11. Effect of different media on pinhead initiation to harvest (days) of mushroom, (B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

Combined effect of different mushroom variety with different media substrate showed significant variation on days required for pin head initiation to harvest (Fig.12 and Appendix. II).

In case of oyster mushroom with three different media, A_1B_3 (oyster mushroom with sawdust substrate) showed the highest value which was 6.8 days followed by A_1B_3 (oyster mushroom with rice grain substrate) which was 6.3 days and A_1B_2 (oyster mushroom with wheat grain media) showed the lowest value for pin head initiation to harvest days which was 5.0. Ruhul Amin *et al*. (2007) also found significant differences among the level of different supplements used for preparing the substrates for oyster mushroom.

In case of straw mushroom with three different media, A_2B_3 (straw mushroom with saw dust substrate) showed the highest value which was 4.5 days followed by A_2B_2 (straw mushroom with wheat grain substrate) which was 4.0 days and significantly varied from A_2B_1 (straw mushroom with rice grain media), showed the lowest value which was 3.0 days.

For button mushroom with three different media, A_3B_3 (button mushroom with saw dust substrate) showed the highest value which was 4.3 days followed by A_3B_1 (button mushroom with rice grain substrate) which was 3.3 days and significantly varied from

A_3B_2 (button mushroom with wheat grain media), showed the lowest value which was 3.2 days.

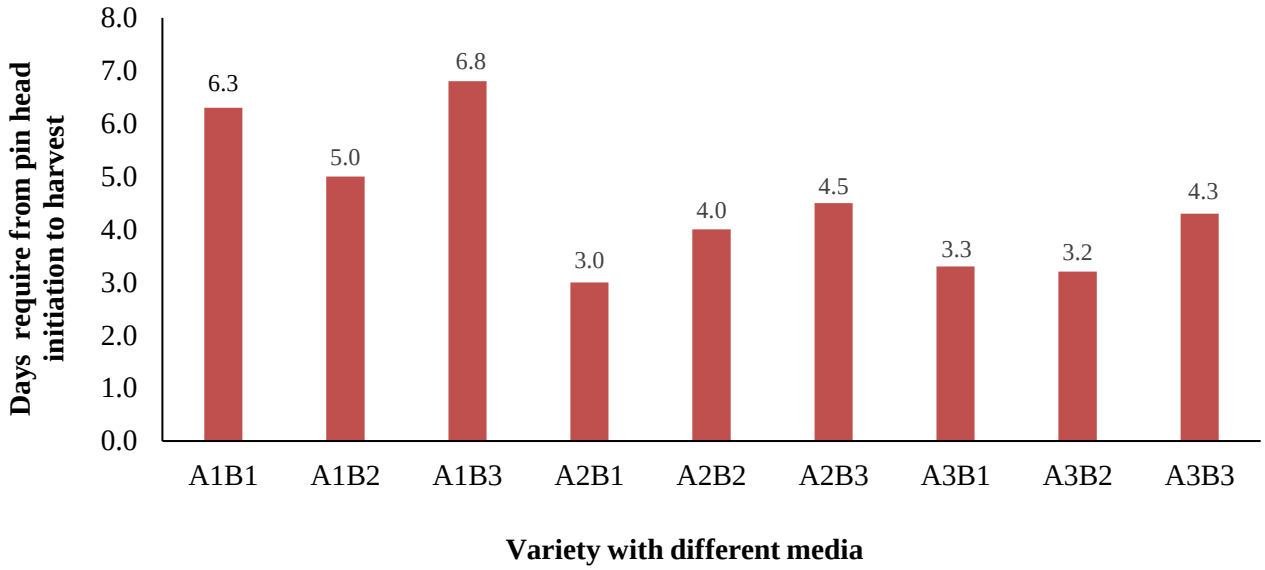

Fig. 12. Combined effect of variety and different media on pinhead initiation to harvest (days) of different mushroom spp.,
(A_1: Oyster mushroom, A_2: Straw mushroom, A_3: Button mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

4.5 Number of fruiting body

The number of fruiting body significantly varied among different mushroom varieties and substrate media (Table 1 and Appendix II). In case of 1st harvest, the highest number of fruiting body was found in straw mushroom (A_2) which was 36.1 followed by button mushroom (A_3) which was 29.9 and lowest was 27.8 in oyster mushroom (A_1). In case of 2nd harvest, similar type result found. The highest number of fruiting body was found in straw mushroom (A_2) which was 42.3 followed by button mushroom (A_3) which was 29.9 and the lowest 32.6 was in oyster mushroom (A_1).

The number of fruiting body significantly influenced by different substrate media (Table 2 and Appendix II). In 1st harvest, the highest (33.5) number of fruiting body was found in rice grain media (B_1) followed by wheat grain (B_2) which was 33.1 and the lowest (27.3) numbers of fruiting body was found in sawdust media (B_3). In case of 2nd harvest, the highest (40.2) number of fruiting body was found in B_2 (wheat grain media) followed by rice grain (B_1) which was 40.0 and the lowest (31.3) was in

sawdust media (B_3).

Significant variation was recorded due to combined effect of mushroom varieties and substrate media (Table 3 and Appendix II).

In case of 1st harvest, oyster mushroom with three different substrate combination, A_1B_1 (oyster mushroom cultivated with rice grain media) showed the highest number of fruiting body was 33.0 followed by A_1B_3 (oyster mushroom cultivated with sawdust media) which was 25.5 and the lowest number of fruiting body was found in A_1B_2 (oyster mushroom with wheat grain media) which was 25.

Straw mushroom with three different substrate combination, A_2B_2 (straw mushroom cultivated with wheat grain media) showed the highest number of fruiting body was 38.3 followed by A_2B_1 (straw mushroom cultivated with rice grain media) 37.5 which was and the lowest number of fruiting body was found in A_2B_3 (straw mushroom with sawdust media) which was 32.5.

Button mushroom with three different substrate combination, A_3B_2 (button mushroom cultivated with wheat grain media) showed the highest number of fruiting body was 37.3 followed by A_3B_1 (button mushroom cultivated with rice grain media) which was 28.8 and the lowest number of fruiting body was found in A_3B_3 (button mushroom with sawdust media) which was 23.8.

In case of 2nd harvest, oyster mushroom with three different substrate combination, A_1B_1 (oyster mushroom cultivated with rice grain media) showed the highest number of fruiting body was 39.5 followed by A_1B_2 (oyster mushroom cultivated with wheat grain media) which was 30.8 and the lowest number of fruiting body was found in A_1B_3 (oyster mushroom with sawdust media) which was 27.5. Amin *et al*. (2007) found the maximum number of fruiting bodies of different oyster mushroom species on rice straw when compared with sawdust.

Straw mushroom with three different substrate combination, A_2B_2 (straw mushroom cultivated with wheat grain media) showed the highest number of fruiting body was 46.3 followed by A_2B_1 (straw mushroom cultivated with rice grain media) which was 45.3 and the lowest number of fruiting body was found in A_2B_3 (straw mushroom with

sawdust media) which was 35.3.

Button mushroom with three different substrate combination, A_3B_2 (button mushroom cultivated with wheat grain media) showed the highest number of fruiting body was 43.0 followed by A_3B_1 (button mushroom cultivated with rice grain media) which was 35.8 and the lowest number of fruiting body was found in A_3B_3 (button mushroom with sawdust media) which was 31.3.

4.6 Number of effective fruiting body

The number of effective fruiting body is also significantly varied among Mushroom varieties and substrate media (Table 1 and Appendix II). In case of 1st harvest on different mushroom varieties, the highest number of effective fruiting body was found in straw mushroom (A_2) which was 31.2 followed by button mushroom (A_3) which was 25.7 and the lowest was 23.3 in oyster mushroom (A_1). In case of 2nd harvest, the highest number of effective fruiting body was found in straw mushroom (A_2) which was 36.2 followed by button mushroom (A_3) which was 31.3 and the lowest 25.2 was in oyster mushroom (A_1).

The number of effective fruiting body significantly influenced by different substrate media (Table 2 and Appendix II). In 1st harvest, the highest (29.0) number of fruiting body was found in wheat grain media (B_2) followed by rice grain media (B_1) which was 28.5 . The lowest (22.7) numbers of fruiting body was found in sawdust media (B_3). In case of 2nd harvest, the highest (34.5) number of fruiting body was found in wheat grain media (B_2) followed by rice grain media (B_1) which was 32.8 and the lowest (25.4) was in sawdust media (B_3).

Significant variation recorded for combined effect of mushroom varieties and substrate media (Table 3 and Appendix II).

In case of 1st harvest, oyster mushroom with three different substrate combination, A_1B_1 (oyster mushroom cultivated with rice grain media) showed the highest number of fruiting body was 30.5 followed by A_1B_2 (oyster mushroom cultivated with wheat grain media) which was 20.0 and the lowest number of fruiting body was found in

A_1B_3 (oyster mushroom with sawdust media) which was 19.5.

Straw mushroom with three different substrate combination, A_2B_2 (straw mushroom cultivated with wheat grain media) showed the highest number of fruiting body was 33.3 followed by A_2B_1 (straw mushroom cultivated with rice grain media) which was 31.5 and the lowest number of fruiting body was found in A_2B_3(straw mushroom with sawdust media) which was 28.8.

Button mushroom with three different substrate combination, A_3B_2 (button mushroom cultivated with wheat grain media) showed the highest number of fruiting body was 32.3 followed by A_3B_1 (button mushroom cultivated with rice grain media) which was 25.0 and the lowest number of fruiting body was found in A_3B_3(button mushroom with sawdust media) which was 19.5.

In case of 2^{nd} harvest,

oyster mushroom with three different substrate combination, A_1B_1 (oyster mushroom grown in rice grain media) showed the highest number of fruiting body was 34.5 followed by A_1B_2 (oyster mushroom grown in wheat grain media) which was 21.5 and the lowest number of fruiting body was found in A_1B_3 (oyster mushroom with sawdust media) which was 19.5.

Straw mushroom with three different substrate combination, A_2B_2 (straw mushroom cultivated with wheat grain media) showed the highest number of fruiting body was 39.5 followed by A_2B_1 (straw mushroom cultivated with rice grain media) which was 38.5 and the lowest number of fruiting body was found in A_2B_3 (straw mushroom with sawdust media) which was 30.5.

Button mushroom with three different substrate combination, A_3B_2 (button mushroom cultivated with wheat grain media) showed the highest number of fruiting body was 37.3 followed by A_3B_1 (button mushroom cultivated with rice grain media) which was 30.5 and the lowest number of fruiting body was found in A_3B_3 (button mushroom with sawdust media) which was 26.3.

According to a findings of Mondal *et. al.* (2010), the number of effective fruiting body of different mushroom ranged from 8.5 to 37.25 which is similar to this experiment and among different substrate media, sawdust give them higher effective fruiting body.

Table 1. Effect of different variety on number of fruiting body and effective fruiting body of mushroom

Treatments	Number of fruiting body		Number of effective fruiting body	
	1st harvest	2nd harvest	1st harvest	2nd harvest
A_1	27.8 c	32.6 c	23.3 c	25.2 c
A_2	36.1 a	42.3 a	31.2 a	36.2 a
A_3	29.9 b	36.7 b	25.7 b	31.3 b
$LSD_{0.05}$	**1.23**	**1.05**	**1.13**	**1.24**
CV (%)	**4.69**	**3.36**	**5.01**	**4.76**

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability,
(A_1: Oyster mushroom, A_2: Straw mushroom and A_3: Button mushroom)

Table 2. Effect of different media on number of fruiting body and effective fruiting body of mushroom

Treatments	Number of fruiting body		Number of effective fruiting body	
	1st harvest	2nd harvest	1st harvest	2nd harvest
B_1	33.5 a	40.0 a	28.5 a	32.8 b
B_2	33.1a	40.2 a	29.0 a	34.5 a
B_3	27.3 b	31.3 b	22.7 b	25.4 c
$LSD_{0.05}$	**1.23**	**1.05**	**1.13**	**1.24**
CV (%)	**4.69**	**3.36**	**5.01**	**4.76**

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability,
(B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

Table 3. Combined effect of different variety and media on number of fruiting body and effective fruiting body of mushroom

Treatments	Number of fruiting body		Number of effective fruiting body	
	1st harvest	2nd harvest	1st harvest	2nd harvest
A_1B_1	33.0 b	39.5 c	30.5 bc	34.5 c
A_1B_2	25.0 d	30.8 e	20.0 e	21.5 f
A_1B_3	25.5 d	27.5 f	19.5 e	19.5 f
A_2B_1	37.5 a	45.3 a	31.5 ab	38.5 ab
A_2B_2	38.3 a	46.3 a	33.3 a	39.5 a
A_2B_3	32.5 b	35.3 d	28.8 c	30.5 d
A_3B_1	28.8 c	35.8 d	25.0 d	30.5 d
A_3B_2	37.3 a	43.0 b	32.3 ab	37.3 b
A_3B_3	23.8 d	31.3 e	19.5 e	26.3 e
$LSD_{0.05}$	**2.14**	**1.82**	**1.95**	**2.15**
CV (%)	**4.69**	**3.36**	**5.01**	**4.76**

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability,
(A_1: Oyster mushroom, A_2: Straw mushroom, A_3: Button mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

4.7 Length of stalk (cm)

The length of stalk was found significant among the varieties with different media substrate. Among the three different mushroom variety, the longest stalk length (3.18 cm) was obtained from straw mushroom (A_2) followed by oyster mushroom (A_1) which was 1.80 cm and the shortest stalk length (1.44 cm) was obtained from button mushroom (A_3) (Table 4 and Appendix III). The maximum (2.31 cm) length of stalk was recorded from wheat grain media (B_2) which is statistically similar with rice grain media(B_1) having length of 2.25 cm, while sawdust media (B_3) gave the minimum (1.87 cm) length of stalk (Table 5 and Appendix III).

Combined effect of different mushroom variety with different media substrate showed significant variation on length of stalk (cm) (Table 6 and Appendix III).

In case of oyster mushroom with three different media, A_1B_1 (oyster mushroom with rice grain substrate) showed the highest value which was 2.2 cm which is statistically similar to A_1B_2 (oyster mushroom with wheat grain substrate) which was 2.1 cm and A_1B_3 (oyster mushroom with sawdust media) showed the lowest value for length of

stalk which was 1.2 cm. Ahmed *et.al.* (2013) observed differences in stalk length (2.43 to 3.24 cm) for *P. ostreatus*, whereas Mondal *et.al.* (2010) reported a decrease in the storage quality of the oyster mushroom with the increase of the pileus and stalk diameters.

In case of straw mushroom with three different media, the value of length of stalk was non-significant. A_2B_2 (straw mushroom with wheat grain substrate) and A_2B_3 (straw mushroom with saw dust substrate) showed the same value which was 3.2 cm and non significant from A_2B_1 (straw mushroom with rice grain media), showed the lowest value which was 3.1 cm.

For button mushroom with three different media, A_3B_2 (button mushroom with wheat grain substrate) showed the highest value which was 1.7 cm followed by A_3B_1 (button mushroom with rice grain substrate) which was 1.5 and significantly varied from A_3B_3 (button mushroom with sawdust media), showed the lowest value which was 1.2 cm. For overall performance in relation to length of stalk revealed that straw mushroom perform better in wheat grain substrate than those of other treatment combinations.

4.8 Diameter of Stalk (cm)

A significant variation in the diameter of stalk was found among the varieties with different media substrate. Among the three different mushroom variety, the highest value of the diameter of Stalk (1.78 cm) was obtained from straw mushroom (A_2) followed by button mushroom (A_3) which was 1.15 cm and the lowest value of the diameter of Stalk (0.55 cm) was obtained from oyster mushroom (A_1) (Table 4 and Appendix III). The maximum (1.31 cm) diameter of stalk was recorded from wheat grain media (B_2) followed by rice grain media (B_1) having stalk diameter of 1.16 cm, while sawdust media (B_3) gave the minimum (1.01 cm) diameter of stalk (Table 5 and Appendix III).

Combined effect of different mushroom variety with different media substrate showed significant variation on diameter of stalk (cm) (Table 6 and Appendix III).

In case of oyster mushroom with three different media, A_1B_1 (oyster mushroom with rice grain substrate) showed the highest value which was 0.61 cm followed by A_1B_2 (oyster mushroom with wheat grain substrate) which was 0.55 cm and A_1B_3 (oyster

mushroom with sawdust media) showed the lowest value for diameter of pileus which was 0.51 cm.

In case of straw mushroom with three different media, the value of diameter of stalk was significant. A_2B_2 (straw mushroom with wheat grain substrate) showed the highest value which was 1.96 cm followed by A_2B_1 (straw mushroom with rice grain substrate) which was 1.77 cm and significant from A_2B_3 (straw mushroom with saw dust substrate) showed the lowest value which was 1.61 cm.

For button mushroom with three different media, A_3B_2 (button mushroom with wheat grain substrate) showed the highest value which was 1.42 cm followed by A_3B_1 (button mushroom with rice grain substrate) which was 1.13 cm and significantly varied from A_3B_3 (button mushroom with sawdust media), showed the lowest value which was 0.93 cm.

4.9 Diameter of Pileus (cm)

The diameter of pileus was found significant among the varieties with different media substrate. Among the three different mushroom variety, the highest diameter of pileus (6.5 cm) was obtained from oyster mushroom (A_1) followed by button mushroom (A_3) which was 4.6 cm and the shortest diameter of pileus (1.7 cm) was obtained from straw mushroom (A_2) (Table 4 and Appendix III). Similar results were found by Mondal *et al.* (2010) for oyster mushroom.

The highest (4.51 cm) diameter of pileus was recorded from wheat grain media (B_2) followed by rice grain media (B_1) which was 4.46 cm while sawdust media (B_3) gave the minimum (3.7 cm) diameter of pileus (Table 5 and Appendix III).

Combined effect of different mushroom variety with different media substrate showed significant variation on length of stalk (cm) (Table 6 and Appendix III).

In case of oyster mushroom with three different media, A_1B_1 (oyster mushroom with rice grain substrate) showed the highest value which was 7.5 cm followed by A_1B_2 (oyster mushroom with wheat grain substrate) which was 6.1 cm and A_1B_3 (oyster mushroom with sawdust media) showed the lowest value for diameter of pileus which was 5.8 cm.

In case of straw mushroom with three different media, the value of diameter of pileus was non significant. A_2B_2 (straw mushroom with wheat grain substrate) showed the highest value which was 1.7 cm followed by A_2B_1(straw mushroom with rice grain substrate) which was 1.6 and non significant from A_2B_3 (straw mushroom with sawdust substrate) showed the lowest value which was 1.5 cm.

For button mushroom with three different media, A_3B_2 (button mushroom with wheat grain substrate) showed the highest value which was 5.6 cm followed by A_3B_1 (button mushroom with rice grain substrate) which was 4.2 and significantly varied from A_3B_3 (button mushroom with sawdust media), showed the lowest value which was 3.8 cm.

4.10 Thickness of pileus

There had a significant differences on thickness of pileus due to the effect of different media substrate with different varieties of mushroom. Among the three different mushroom variety, the highest thickness was found in straw mushroom (A_2) which was 1.33 cm followed by button mushroom (A_3) which was 0.61 cm and the lowest thickness of pileus (0.58 cm) was found in oyster mushroom (A_1) (Table 4 and Appendix III). 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.

Significant variation was found on thickness of pileus due to the effect of different media substrate. The highest thickness was found in wheat grain media (B_2) which was 0.95 cm followed by rice grain media (B_1) which was 0.86 cm and thickness of pileus was the lowest in sawdust media (B_3) which was 0.69 cm (Table 5 and Appendix III).

The combined effect of mushroom varieties with three different media substrate was significantly influenced on thickness of pileus (Table 6 and Appendix III).

In case of oyster mushroom with three different media, A_1B_1 (oyster mushroom with rice grain substrate) showed the highest value which was 0.63 cm followed by A_1B_3 (oyster mushroom with sawdust substrate) which was 0.55 cm and A_1B_2 (oyster

mushroom with wheat grain media) showed the lowest value for thickness of pileus which was 0.54 cm.

In case of straw mushroom with three different media, the value of thickness of pileus was significant. A_2B_2 (straw mushroom with wheat grain substrate) showed the highest value which was 1.68 cm followed by A_2B_1 (straw mushroom with rice grain substrate) which was 0.60 cm and significant from A_2B_3 (straw mushroom with saw dust substrate) showed the lowest value which was 0.58 cm.

For button mushroom with three different media, A_3B_1 (button mushroom with rice grain substrate) showed the highest value which was 1.35 cm followed by A_3B_3 (button mushroom with sawdust substrate) which was 0.95 cm and significantly varied from A_3B_2 (button mushroom with wheat grain media), showed the lowest value which was 0.64 cm.

Table 4. Effect of different variety on size of fruiting body of mushroom

Treatments	Length of Stalk (cm)	Diameter of Stalk (cm)	Diameter of Pileus (cm)	Thickness of Pileus (cm)
A_1	1.80 b	0.55 c	6.5 c	0.58 b
A_2	3.18 a	1.78 a	1.7 a	1.33 a
A_3	1.44 c	1.15 b	4.6 b	0.61 b
$LSD_{0.05}$	**0.11**	**0.05**	**0.15**	**0.035**
CV (%)	**6.35**	**4.89**	**3.37**	**4.91**

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability,
(A_1: Oyster mushroom, A_2: Straw mushroom and A_3: Button mushroom)

Table 5. Effect of different media on size of fruiting body of mushroom

Treatments	Length of Stalk (cm)	Diameter of Stalk (cm)	Diameter of Pileus (cm)	Thickness of Pileus (cm)
B_1	2.25a	1.16 b	4.46 b	0.86 b
B_2	2.31 a	1.31 a	4.51 a	0.95 a
B_3	1.87 b	1.01 c	3.7 c	0.69 c
$LSD_{0.05}$	**0.11**	**0.05**	**0.15**	**0.035**
CV (%)	**6.35**	**4.89**	**3.37**	**4.91**

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability,
(B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

Table 6. Effect of different variety and media on size of fruiting body of mushroom

Treatments	Length of Stalk (cm)	Diameter of Stalk (cm)	Diameter of Pileus (cm)	Thickness of Pileus (cm)
A_1B_1	2.2 b	0.61 g	7.5 d	0.63 de
A_1B_2	2.1 b	0.55 gh	6.1 b	0.54 d
A_1B_3	1.2 e	0.51 h	5.8 c	0.55 fg
A_2B_1	3.1 a	1.77 b	1.6 a	0.60 def
A_2B_2	3.2 a	1.96 a	1.7 a	1.68 a
A_2B_3	3.2 a	1.61 c	1.5 a	0.58 efg
A_3B_1	1.5 d	1.13 e	4.2 e	1.35 b
A_3B_2	1.7 c	1.42 d	5.6 c	0.64 d
A_3B_3	1.2 e	0.93 f	3.8 f	0.95 c
$LSD_{0.05}$	**0.2**	**0.08**	**0.25**	**0.06**
CV (%)	**6.35**	**4.89**	**0.2521**	**4.91**

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability,
(A_1: Oyster mushroom, A_2: Straw mushroom, A_3: Button mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

4.11 Biological Yield (g):

Oyster mushroom with three different substrate combination, A_1B_1 (oyster mushroom grown in rice grain media) showed the highest biological yield which was 144.33g followed by A_1B_2 (oyster mushroom grown in wheat grain media) which was 129.3 and the lowest biological yield was found in A_1B_3 (oyster mushroom with sawdust media) which was 114.18g.

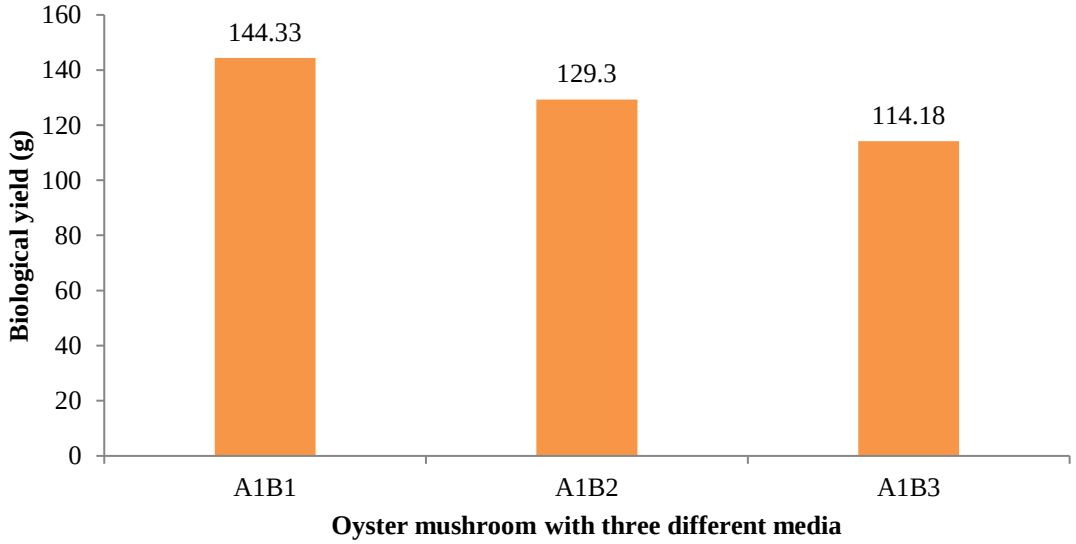

Fig. 13. Effect of oyster mushroom on biological yield for three different media
(A_1: Oyster mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

In case of straw mushroom with three different substrate combination, A_2B_2 (straw mushroom grown in wheat grain media) showed the highest biological yield which was 940.1g followed by A_2B_1 (straw mushroom grown in rice grain media) which was 782.4g and the lowest biological yield was found in A_2B_3 (straw mushroom with sawdust media) which was 548.42g.

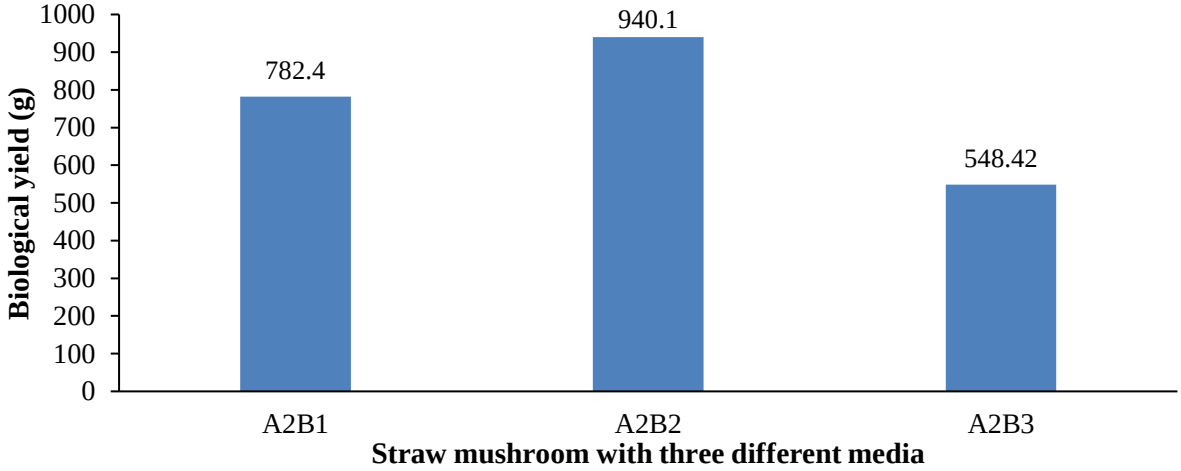

Fig. 14. Effect of straw mushroom on biological yield for three different media
(A_2: Straw mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

For button mushroom with three different substrate media, A_3B_2 (button mushroom grown in wheat grain media) showed the highest biological yield which was 830.22g followed by A_3B_1 (button mushroom grown in rice grain media) which was 617.6g and the lowest biological yield was observed in A_3B_3 (button mushroom with sawdust media) which was 554.38g.

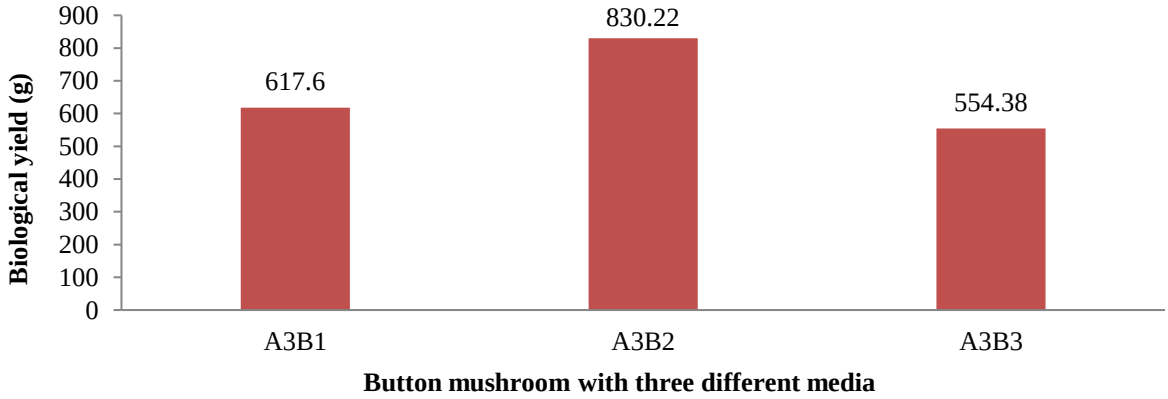

Fig. 15. Effect of button mushroom on biological yield for three different media
(A_3: Button mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

Economic Yield (g):

Oyster mushroom with three different substrate combination, A_1B_1 (oyster mushroom grown in rice grain media) showed the highest economic yield which was 140.43g followed by A_1B_2 (oyster mushroom grown in wheat grain media) which was 124.68g and the lowest economic yield was found in A_1B_3 (oyster mushroom with sawdust media) which was 109.5g.

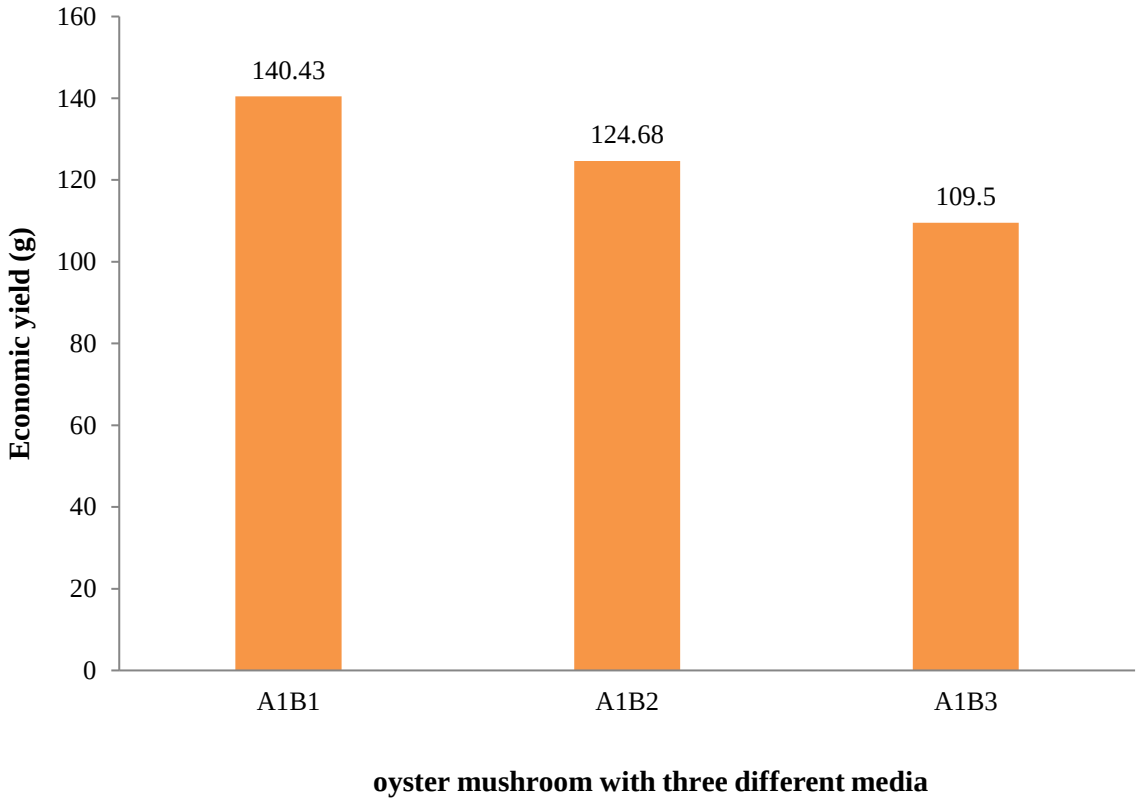

Fig. 16. Effect of oyster mushroom on economic yield for three different media
(A_1: Oyster mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

For straw mushroom with three different substrate combination, A_2B_2 (straw mushroom grown in wheat grain media) showed the highest economic yield which was 935.47g followed by A_2B_1 (straw mushroom grown in rice grain media) which was 777.5g and the lowest economic yield was found in A_2B_3 (straw mushroom with sawdust media) which was 543.5g.

Fig. 17. Effect of straw mushroom on economic yield for three different media
(A_2: Straw mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

In case of button mushroom with three different substrate media, A_3B_2 (button mushroom grown in wheat grain media) showed the highest economic yield which was 825.5g followed by A_3B_1 (button mushroom grown in rice grain media) which was 612.3g and the lowest economic yield was observed in A_3B_3 (button mushroom with sawdust media) which was 549.8g.

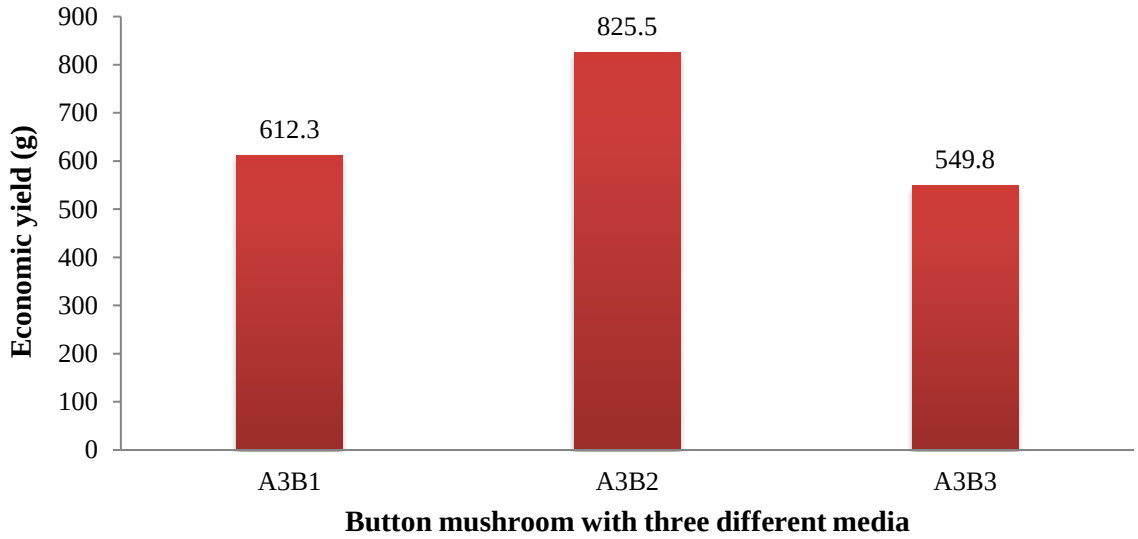

Fig. 18. Effect of button mushroom on economic yield for three different media
(A_3: Button mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

4.13 Biological efficiency:

Significant variation was observed on biological efficiency among Oyster mushroom (*Pleurotus djamor*), Straw mushroom (*Volvariella volceae*) and Button mushroom (*Agaricus bisporus*) (Fig.19). The highest biological efficiency was found in Oyster mushroom (A_1) which was 64.7% followed by button mushroom (A_3) which was 30.3% and the lowest biological efficiency was found in Straw mushroom (A_2) which was 18.9%. Peng *et al.* (2001) also reported the different biological efficiency in different strains on sawdust.

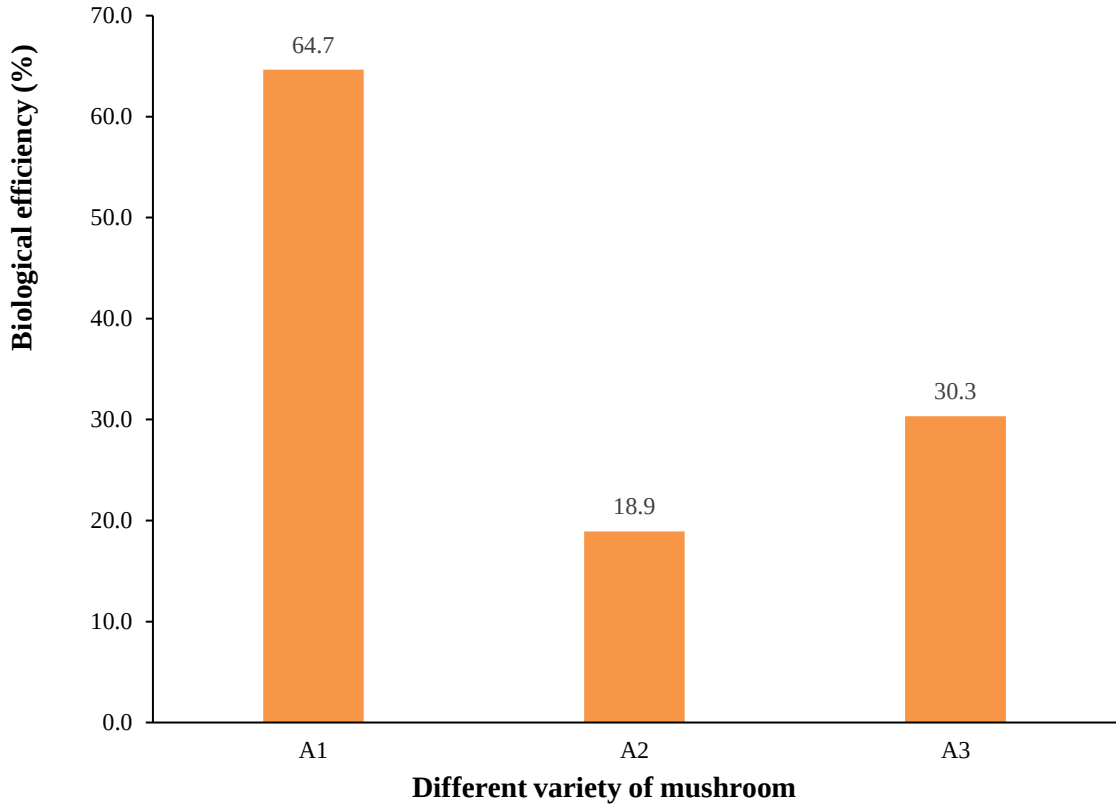

Fig. 19. Effect of different mushroom variety on biological efficiency
(A_1: Oyster mushroom, A_2: Straw mushroom, A_3: Button mushroom)

Biological efficiency was also significantly influenced by different substrate media (Fig.20). The highest biological efficiency was found in B_2 (wheat grain media) which was 42% followed by B_1 (rice grain media) which was 39.9% and the lowest biological efficiency was found in B_3 (sawdust media) which was 32%.

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.

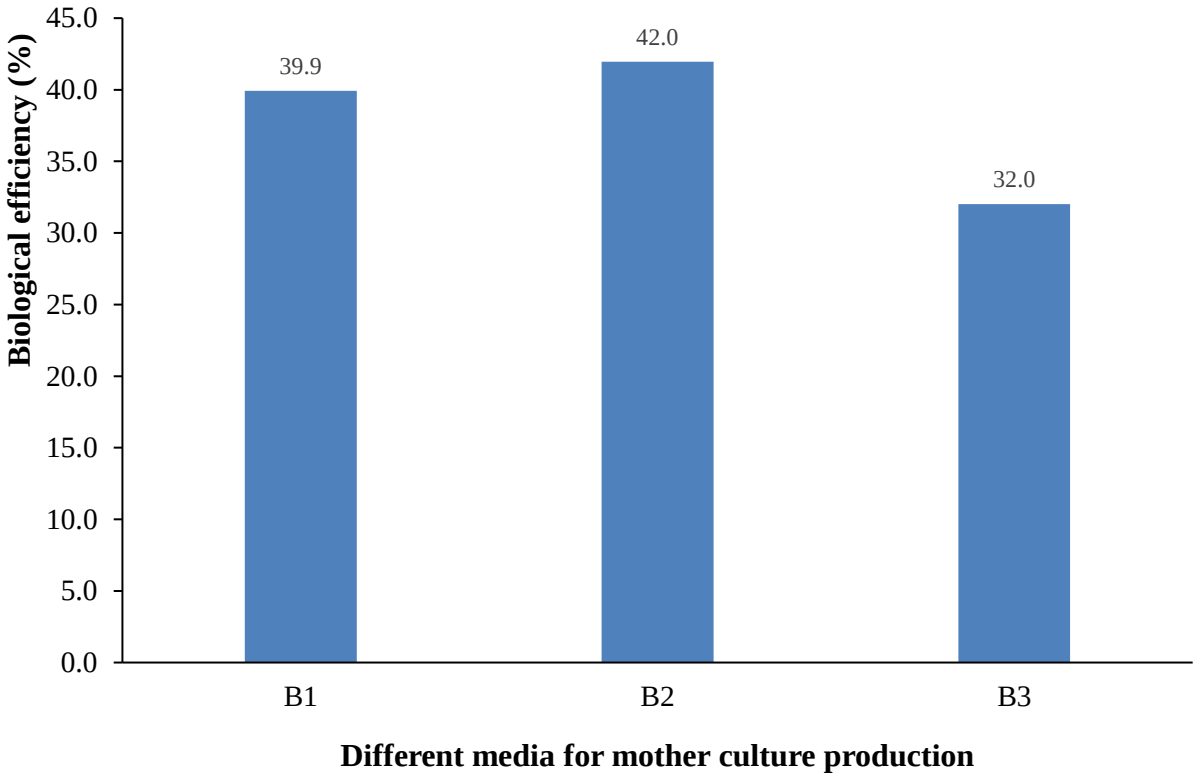

Fig. 20. Effect of different substrate media on biological efficiency
(B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

Biological efficiency was significantly influenced by the combined effect of different mushroom variety with different media substrate (Fig.21). The highest biological efficiency (72.2%) was found in A_1B_1 which is oyster mushroom with rice grain media and the lowest biological efficiency was found in A_2B_3 (13.7%) which is straw mushroom with sawdust media. Shah *et al* (2004) showed in their study that the best biological efficiency with different substrate combination in case of oyster mushroom was 64.69%. The current study also found near about similar result. .

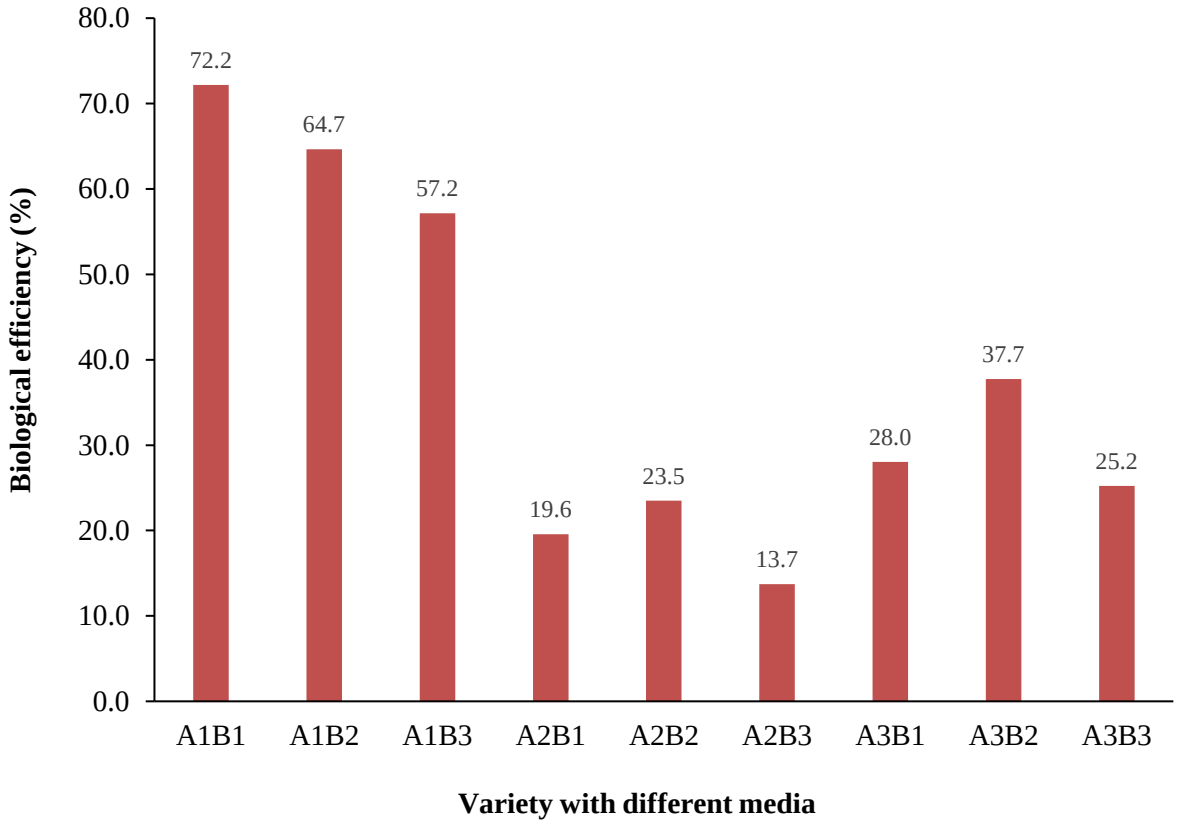

Fig. 21. Combined effect of three different substrate media with three different mushroom variety on biological efficiency
(A_1: oyster mushroom, A_2: straw mushroom, A_3: button mushroom, B_1: Rice grain, B_2: Wheat grain and B_3: Sawdust substrate)

CHAPTER V

SUMMARY AND CONCLUSION

The present study was carried out in the laboratory and culture house of Mushroom Development Institute (MDI), Sobhanbag, Savar, Dhaka during the month of June, 2019 to May,2020 to investigate the performance of different media for mother culture production of different mushroom species. Oyster mushroom (*Pleurotus djamor*), Straw mushroom (*Volvariella volceae*) and *Button mushroom (Agaricus bisporus)* were selected to cultivate in the culture house of the Mushroom Development Institute. Three culture media were used: Rice grain media, Wheat grain media and Sawdust media.

The experiment was laid out following completely randomized design (CRD) with 9 treatments and each had 4 replications. Duncan's Multiple Range Test (DMRT) and Least significant difference (LSD) used for mean separation.

The experiment has two factor - Factor A: Mushroom varieties, A_1- Oyster mushroom (*Pleurotus djamor*), A_2- Straw mushroom (*Volvariella volceae*), A_3- *Button mushroom (Agaricus bisporus)* and Factor B: Media for mother culture production, B_1-Rice grain, B_2-Wheat grain, B_3-Sawdust based substrates.

Different parameters were selected for data collection and data were collected on mycelium run rate (cm/day), days required for complete mycelium running, days required for pinhead initiation, days required for pinhead initiation to first harvest, no. of fruiting body, no. of effective fruiting body, the length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, yield of mushroom packet.

In case of varietal variation, the highest mycelium run rate (cm/day) was recorded in straw mushroom (A_2=0.60 cm/day) and the lowest in button mushroom (A_3=0.51cm/day), the highest days required for complete mycelium running was recorded in button mushroom (A_3=32.9 days) and the lowest in straw mushroom (A_2=18.3 days). The highest days required for pinhead initiation was recorded in button

mushroom (A_3=9.4 days) and the lowest in oyster mushroom (A_1=4.7 days). The highest days required from pinhead initiation to first harvest was recorded in oyster mushroom (A_1=6 days) and the lowest in button mushroom (A_3=3.6 days). In case of 1st harvest, the highest number of fruiting body was recorded in straw mushroom (A_2=36.1) and lowest was oyster mushroom (A_1=27.8). In case of 2nd harvest, similar type result found. The highest number of fruiting body found in straw mushroom (A_2=42.3) and lowest was oyster mushroom (A_1=32.6). In case of 1st harvest, the highest number of effective fruiting body was recorded in straw mushroom (A_2=31.2) and lowest was in oyster mushroom (A_1=23.3). In case of 2nd harvest, similar type result found. The highest number of effective fruiting body found in straw mushroom (A_2=36.2) and lowest was found in oyster mushroom (A_1=25.2).The highest length of stalk was recorded in straw mushroom (A_2=3.18cm) and the lowest in button mushroom (A_3=1.44cm). The highest diameter of stalk was recorded in straw mushroom (A_2=1.78cm) and the lowest in oyster mushroom (A_1=0.55cm). The highest diameter of pileus was recorded in oyster mushroom (A_1=6.5 cm) and the lowest in straw mushroom (A_2=1.7 cm).The highest thickness of pileus was recorded in straw mushroom (A_2=1.33cm) and the lowest in oyster mushroom (A_1=0.58cm).

In case of different culture media, the highest mycelium run rate (cm/day) was recorded in wheat grain (B_2=0.64 cm/day) and the lowest in sawdust (B_3=0.45cm/day). The highest days required for complete mycelium running was recorded in sawdust (B_3=26.1 days) and the lowest in rice grain (B_1=23.5 days). The highest days required for pinhead initiation was recorded in sawdust (B_3=8.2 days) and the lowest in wheat grain (B_2=6.5 days). The highest days required from pinhead initiation to first harvest was recorded in sawdust (B_3=5.2 days) and the lowest in wheat grain (B_2=4.1 days). In case of 1st harvest, the highest number of fruiting body was recorded in rice grain (B_1=33.5) and lowest was sawdust (B_3=27.3). In case of 2nd harvest, the highest number of fruiting body was recorded in wheat grain (B_2=40.2) and lowest was recorded in sawdust (B_3=31.3). In case of 1st harvest, the highest number of effective fruiting body was recorded in wheat grain media (A_2=29.0) and lowest was in sawdust

media (A_3=22.7). In case of 2nd harvest, similar type result found. The highest number of effective fruiting body found wheat grain media (A_2=34.5) and lowest was in sawdust media (A_3=25.4). The highest length of stalk was recorded in wheat grain (B_2=2.31cm) and the lowest in sawdust (B_3=1.87cm). The highest diameter of stalk was recorded in wheat grain (B_2=1.31 cm) and the lowest in sawdust (B_3=1.01cm). The highest diameter of pileus was recorded in wheat grain (B_2=4.51cm) and the lowest in sawdust (B_3=3.7 cm).The highest thickness of pileus was recorded in wheat grain (B_2=0.95cm) and the lowest in sawdust (B_3=0.69cm).

In case of combined effect of different mushroom variety with different culture media, the highest mycelium run rate (cm/day) was recorded in straw mushroom with wheat grain media (A_2B_2=0.72cm/day) and the lowest in button mushroom with sawdust media (A_3B_3=0.43cm/day). The highest days required for complete mycelium running was recorded in button mushroom with sawdust media (A_3B_3=34.8 days) and the lowest in straw mushroom with wheat grain media (A_2B_2=16.5cm/day) The highest days required for pinhead initiation was recorded in button mushroom with sawdust media (A_3B_3=10.5 days) and the lowest in oyster mushroom with rice grain media (A_1B_1=3.5 days). The highest days required from pinhead initiation to first harvest was recorded in oyster mushroom with sawdust media (A_1B_3=6.8 days) and the lowest in straw mushroom with wheat grain media (A_2B_1=3.0 days). In case of both 1st and 2nd harvest, the highest number of fruiting body recorded in straw mushroom with wheat grain media (A_2B_2) which was 38.3 and 46.3 respectively and lowest was in case of 1st harvest found on button mushroom with sawdust media (A_3B_3=23.8) and for 2nd harvest, lowest number of fruiting body recorded in oyster mushroom with sawdust media (A_1B_3=27.5). In case of both 1st and 2nd harvest, the highest number of effective fruiting body recorded in straw mushroom with wheat grain media (A_2B_2) which was 33.3 and 39.5 respectively and lowest value in case of 1st harvest found on both straw mushroom with sawdust media (A_1B_3=19.5) and button mushroom with sawdust media (A_3B_3=19.5) and for 2nd harvest, lowest number of fruiting body recorded in oyster mushroom with sawdust media (A_1B_3=19.5). The highest length of stalk was

recorded in straw mushroom with both wheat grain and sawdust media which was 3.2cm and the lowest value found in both oyster mushroom with sawdust media (A_1B_3) and button mushroom with sawdust media (A_3B_3) which was 1.2cm. The highest diameter of stalk was recorded in straw mushroom with wheat grain media (A_2B_2=1.96cm) and the lowest value found in oyster mushroom with sawdust (A_1B_3=0.51cm). The highest diameter of pileus was recorded in oyster mushroom with rice grain media (A_1B_1=7.50cm) and the lowest value found in straw mushroom with sawdust media (A_2B_3=1.5cm).The highest thickness of pileus was recorded in straw mushroom with wheat grain media (A_2B_2=1.68cm) and the lowest value found in oyster mushroom with wheat grain media (A_1B_2=0.54cm). For oyster mushroom with three different substrate combination, the highest biological yield recorded for oyster mushroom grown in rice grain media (A_1B_1) which was 144.33g and the lowest biological yield was found in oyster mushroom with sawdust media (A_1B_3) which was 114.18g. In case of straw mushroom with three different substrate combination, straw mushroom grown in wheat grain media (A_2B_2) showed the highest biological yield which was 940.1g and the lowest biological yield was found in straw mushroom with sawdust media (A_1B_3) which was 548.42g. For button mushroom with three different substrate media, button mushroom grown in wheat grain media (A_3B_2) showed the highest biological yield which was 830.22g and the lowest biological yield was observed in button mushroom with sawdust media (A_3B_3) which was 554.38g. For oyster mushroom with three different substrate combination, the highest economic yield recorded for oyster mushroom grown in rice grain media (A_1B_1) which was 140.43g and the lowest economic yield was found in oyster mushroom with sawdust media (A_1B_3) which was 109.5g. In case of straw mushroom with three different substrate combination, straw mushroom grown in wheat grain media (A_2B_2) showed the highest economic yield which was 935.47g and the lowest economic yield was found in straw mushroom with sawdust media (A_2B_3) which was 543.5g.For button mushroom with three different substrate media, button mushroom grown in wheat grain media (A_3B_2) showed the highest economic yield which was 825.5g and the

lowest economic yield was observed in button mushroom with sawdust media (A_3B_3) which was 549.8g.Among the three different variety of mushroom, the highest biological efficiency was found in Oyster mushroom (A_1) which was 64.7% and the lowest biological efficiency was found in Straw mushroom (A_2) which was 18.9%.For different substrate media, The highest biological efficiency was found in B_2 (wheat grain media) which was 42% and the lowest biological efficiency was found in B_3 (sawdust media) which was 32%. Oyster mushroom in three different media combination, the highest biological efficiency was found in rice grain media which was 72.2% and lowest biological efficiency was found in sawdust media which was 57.2%. For straw mushroom in three different media combination, the highest biological efficiency was found in wheat grain media which was 23.5% and the lowest biological efficiency was found in sawdust media which was 13.7%. For button mushroom in three different media combination, the highest biological efficiency was found in wheat grain media which was 37.7% and the lowest biological efficiency was found in sawdust media which was 25.2%.

Conclusion:

Based on the result of current study, it is could be concluded that oyster mushroom gives higher production in rice grain media while straw and button mushroom both showed higher production efficiency in wheat grain media and it is more economic than other media substrate.

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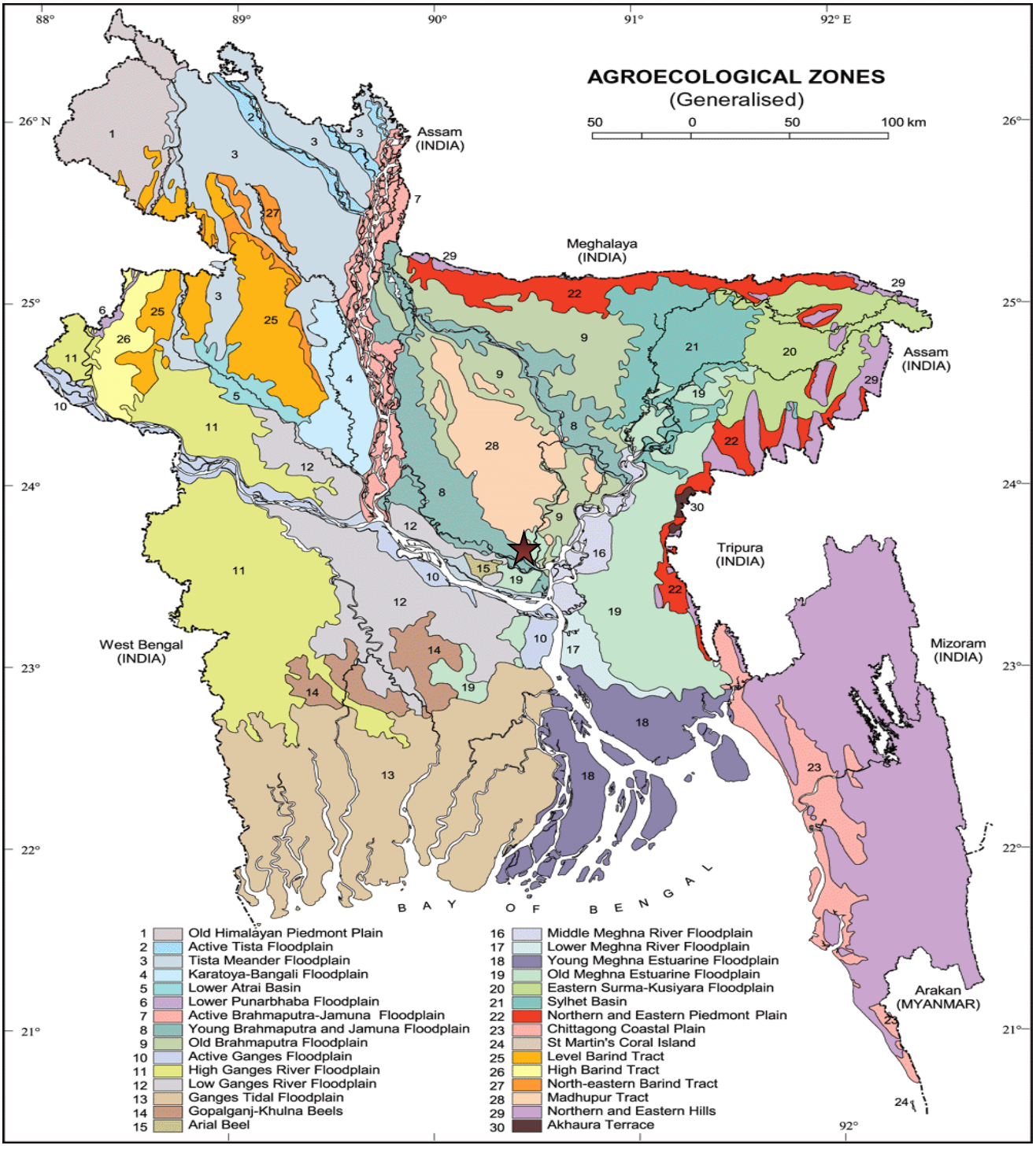
APPENDICES



Appendix I: Mark showing experimental area

Appendix II. Analysis of variances of the data on number of fruiting body and effective fruiting body of mushroom

Source of variation	Degrees of freedom (df)	Mean square for number of fruiting body		Mean square for number of effective fruiting body	
		1st harvest	2nd harvest	1st harvest	2nd harvest
Replication	3	0.778	1.667	3.667	0.222
Factor A	2	220.861*	282.583*	194.111*	364.778*
Factor B	2	146.528*	306.333*	148.778*	278.694*
Interaction (A×B)	4	79.611*	68.167*	91.861*	103.528*
Error	24	2.153	1.563	1.792	2.16

*****: Significant at 0.05 level of probability

Appendix III. Analysis of variances of the data on yield related attributes of mushroom

Source of variation	Degrees of freedom (df)	Mean square for length of stalk	Mean square for diameter of stalk	Mean square for diameter of pileus	Mean square for thickness of pileus
Replication	3	0.0277	0.00546	0.0255	0.00323
Factor A	2	10.1508*	4.50205*	16.9975*	2.15444*
Factor B	2	0.6908*	0.26852*	3.1633*	0.20868*
Interaction (A×B)	4	0.3442*	0.05832*	2.0733*	0.16613*
Error	24	0.0185	0.00323	0.0298	0.00168

*****: Significant at 0.05 level of probability