EFFECT OF SUBSTRATE OF MOTHER SPAWN ON CONTAMINATION AND YIELD OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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EFFECT OF SUBSTRATE OF MOTHER SPAWN ON CONTAMINATION AND YIELD OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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CERTIFICATE

This is to certify that the thesis entitled, **"EFFECT OF SUBSTRATES OF MOTHER SPAWN ON CONTAMINATION AND YIELD OF OYSTER MUSHROOM (*Pleurotus ostreatus*)"** *submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirement for the degree of* **MASTER OF SCIENCE IN PLANT PATHOLOGY** *embodies the results of a piece of bona fide research work carried out by* **SHUVO MONDOL**, *bearing Registration No.* **19-10336** *under my supervision and guidance. No part of this thesis has been submitted for other degree or diploma, elsewhere in the country or abroad.*

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

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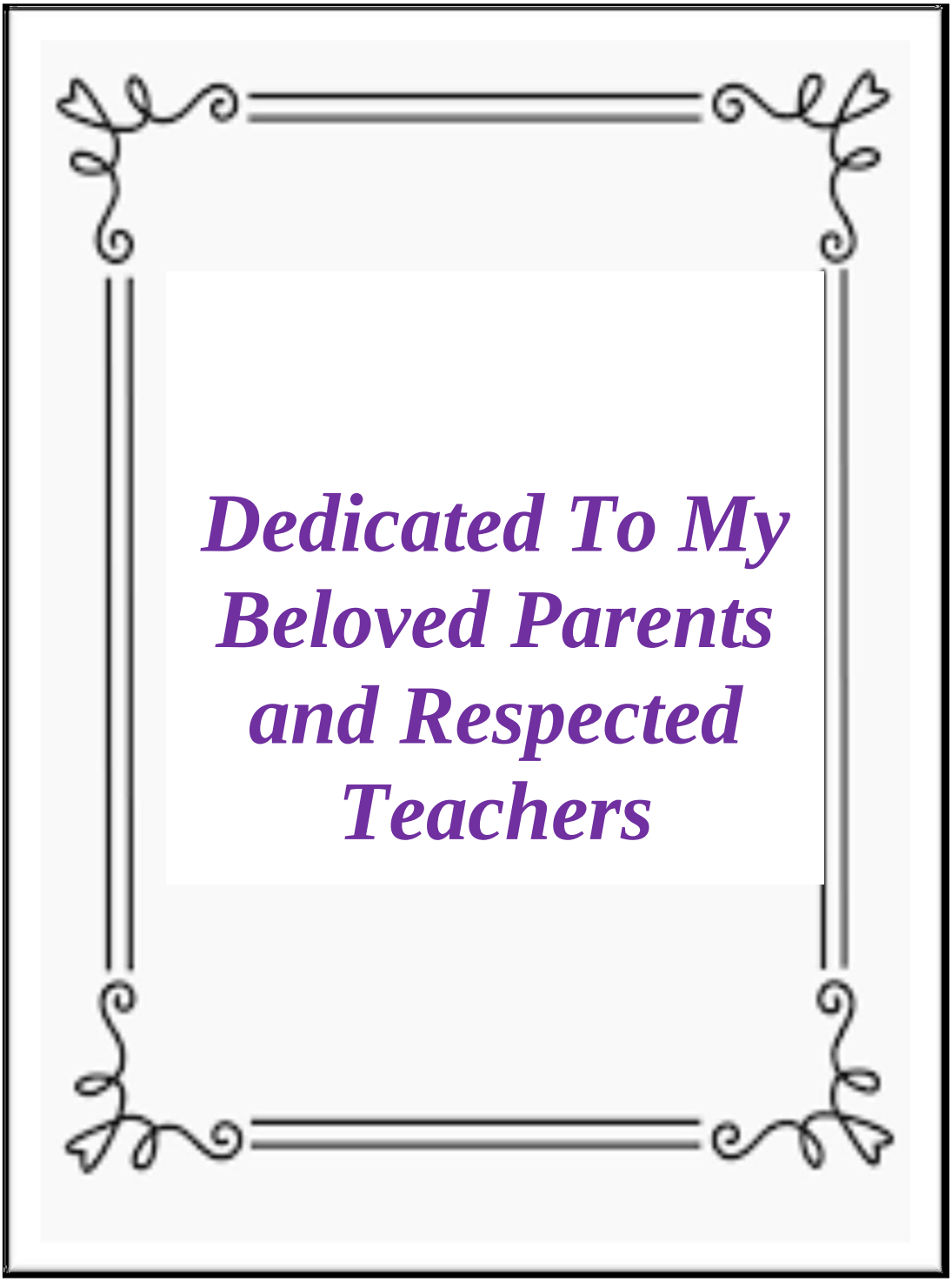
Dedicated To My
Beloved Parents
and Respected
Teachers

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BY

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ABSTRACT

The experiment was carried out during the period from November 2020 to March 2021 to find out the effect of substrate of mother spawn on contamination and productivity of oyster mushroom (*Pleurotus ostreatus*). Nine different treatments were used as viz. T_1 = Mustard; T_2 = Red maize; T_3 = 50% rice straw and 50% sawdust; T_4 = Wheat; T_5 = Millet; T_6 = Gram; T_7 = Pea; T_8 = Rice and T_9 = White maize. The experiment was laid out in single factor Completely Randomized Design (CRD) with replications. The highest mycelium running rate of mother spawn (0.85 cm/day) was observed when the rice grain was used. The minimum duration required from incubation to completion of mycelium running of mother spawn (16.33 days) and maximum mycelium weight (4.08) was observed in rice. The minimum duration required from incubation to completion of mycelium running in yield packets (18.33), from stimulation to primordial initiation (5.89 days), from primordial initiation to 1st harvest (5.67 days) and the lowest total harvesting period (40.00 days) were found when mother spawn of rice was used. Again, the maximum number of primordia (87.33), effective fruiting body per packet (31.00), the highest length of pileus (5.17 cm) and width of pileus (7.49 cm) were found at mother spawn of rice. Oyster mushroom grown on sawdust substrates where mother spawn of rice inoculated gave the highest biological yield (175.67 g), economic yield (168.33 g) and biological efficiency (35.13%). During the cultivation period four contaminants namely *Trichoderma sp*. *Penicillium sp*, *Rhizopus stolonifer* and *Aspergillus niger* were isolated and identified from contaminated substrates. Percent contamination of fungi gradually increased with the increase of days after stimulation. After 45 days after stimulation (DAT), packets inoculated with rice and wheat mother spawn were not contaminated. Among the treatments, the performance of mother spawn of rice was better economically as well as growth and yield performance for the cultivation of oyster mushroom.

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LIST OF ABBREBIATIONS

ABBREBIATION	FULL MEANING
%	Percent
@	At the rate
^{0}C	Degree centigrade
Agril.	Agricultural
BAU	Bangladesh Agricultural University
BBS	Bangladesh Bureau of Statistics
BE	Biological Efficiency
Cm	Centimeter
CRD	Completely Randomized Design
CV	Co-efficient of variation
d. f.	Degrees of freedom
DAS	Days After Stimulation
DMRT	Duncan's Multiple Range Test
e. g.	For example
et. al.	And others
FAO	Food Agriculture Organization
G	Gram
J.	Journal
Kg	Kilogram
LSD	Least significance difference
Mg	Milligram
m^2	Meter square
MRR	Mycelium Running Rate
MDI	Mushroom Development Institute
SAU	Sher-e-Bangla Agricultural University

CHAPTER I

INTRODUCTION

Mushroom is the fleshy large reproductive structure of edible fungi belong to either Ascomycotina or Basidiomycotina typically consisting of a cap (pileus) at the end of a stem arising from an underground mycelium. A mushroom is the fleshy and spore-bearing fruiting body of a fungus and belongs to the class Basidiomycetes under the order Agaricales in fungal classification. FAO recommended ediblemushrooms as a food to meet protein requirement of developing countries where a large number of populations depends mainly on cereal crops (World Bank, 2004). Mushroom is a organic vegetable and the cultivation of mushroom is an eco friendly and beneficial agribusiness yet labour intensive (Chandha and Sharma, 1995). It doesn't need any cultivable land and can be cultivated in room by racking vertically. Mushrooms are being grown on commercial scale in many parts of the world. The business development initially began in Europe with the beginning of last century but the history of mushroom production is very recent in Bangladesh.

There are various types of edible mushrooms such as oyster mushroom, milky white mushroom, shitake mushroom, button mushroom, straw mushroom etc. which are cultivated in our country. Oyster mushroom (Pleurotus sp.) is an edible mushroom and belongs to the family Pleurotaceae (Randive, 2012). The term mushroom applies mainly to those fungi that have stem (stalk), cap (pileus), hymenium (lamellae) and pores on the underside of the cap (Masarirambi et al., 2011). Mushroom spores are produced on the gills and they can fall as a fine powder from underside of the cap. The color of spore print of most oyster mushroom is white and when cultivated produces fruiting bodies (Herlina et al., 2012). Oyster mushrooms are grown from mycelium propagated on steam-sterilized cereal grains (Royse, 2004). Gunde and Cinerman (1995) reported that oyster mushroom has a cap spanning diameter of 5 to 25 cm at maturity. The fruiting body of oyster mushroom differs with respect to stipe length and girth, and pileus width when grown in different farm substrates (Shah et al., 2004).

Mushroom offers for converting lignocellulosic residues from agricultural field, forest into protein rich biomass. Such processing of agro waste not only increase nutrient cycling in the environment but the by-product of mushroom cultivation is also a good source of manure, animal feeds and soil conditioner.

Mushroom is now-a-days one of the promising concepts for crop diversification in Bangladesh. The climatic condition of Bangladesh is completely suitable for mushroom cultivation. It does not require any cultivable land. Mushroom cultivation can be a very interesting hobby with delicious results that could easily become a profitable small business, due to the low cost of inputs and high value of the crop. It requires short time, little capital and easy technique for cultivation. Therefore, all types of people like male and female, youth and old even children and disabled can easily participate in its cultivation. Its cultivation can transfer as a cottage industry and create a good opportunity for export. Therefore, it can generate huge scope of employment opportunities for unemployed people.

Mushroom production in rural communities can alleviate poverty and improve the diversification of agricultural production (Godfrey et al., 2010). For successful cultivation, it is important to select high-yielding strains. However, the production and yield performance of commercial strains of mushrooms tend to decrease after consecutive subculturing (Naraian et al., 2011). Therefore, more information about this genus and its species is necessary to identify good strains to ensure continuous yield improvement (Uhart et al., 2008) and to screen the efficient varieties for Bangladesh.

White Oyster Mushroom Mother Culture or Mother spawn is nothing but the mushroom fungus grown on a grain based medium. Jute stick, paddy grain, kaon, maize, wheat, mustard, rice grain and sawdust can be used as mother spawn media. The fully colonized grain (spawn) is used to seed already prepared substrates such as agricultural and non-agricultural wastes for mushroom production. Grain spawn is in common use because of its ability to the substrate faster and ease of planting (Bahl, 1988). Disease-free grains are used as substrate for growing the spawn materials.

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. (Hassan *et al*., 2011) and this has been reported to influence its growth, yield and composition (Iqbal *et al*., 2005; Kimenju *et al*., 2009; Khare *et al*., 2010). Crop residues

such as grain crop straw are characterised by the predominance of lignocellulose with cellulose, hemicellulose and lignin as the main components (Yildiz *et al*., 2002; Das and Mukherjee, 2007; Jonathan *et al.,* 2012). There are some differences in the nutrient content of the mushroom cultivated on different substrates (Mabrouk and Ahwanyi, 2008; Akinyele *et al*., 2011; Kulshreshtha *et al*., 2013b). However, this change in nutritional content never found to affect their edibility. Now a days among various waste materials sawdust and rice straw are most commonly used for mushroom cultivation in Bangladesh. An ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth (Anonymous, 2008).

In mushroom growing technology, the inoculums are known as the 'spawn'. Spawn is a medium that is impregnated with mycelium made from a pure culture of the chosen mushroom strain. Spawn production is a fermentation process in which the mushroom mycelium will be increased by growing through a solid organic matrix under controlled environmental condition. In almost all cases the organic matrix will be sterilized grain e.g. wheat, maize, sorghum etc (Jain and Vyas, 2005; Jain, 2005)

The availability of good quality mother spawn is vital factor in mushroom production, because the quality and quantity of fruiting bodies depend on mother spawn materials. This was designed to evaluate the suitability of different grain materials for production of spawn.

Considering the above facts, the present investigation was undertaken with the following objectives:

- To find out the best grain materials for mother spawn preparation to get maximum yield of oyster mushroom species.
- To determine the effect of mother spawn on contamination and yield of oyster mushroom.

CHAPTER II

REVIEW OF LITERATURE

2.1. Definition of mushrooms

Cho and Kang (2004) defined mushroom as "a macrofungus with a distinctive fruiting body which can be either epigeous (growing on or close to the ground) or hypogeous (growing underground)". The word mushroom refers only to the fruit and must be large enough to be seen with the naked eye and to be picked by hand.

Chang and Mills (1992) referred mushroom as a fungus that is seen with the naked eyes and that is picked by other organisms and sometimes used as food. In a broad sense "Mushroom is a macro fungus with a distinctive fruiting body, which can be either epigeous or hypogenous and large enough to be seen with naked eye and to be picked by hand".

Ganopedia (2011) defined that a mushroom is a fungus that has a stem, a cap and gills or pores on the underside of the cap.

Chang (2007) reported that Mushrooms are not only basidiomycetes, but they can also be ascomycetes, grow underground, have a non-fleshy texture and could be inedible. All the poisonous and the non-poisonous fungi that can be seen with the naked eye and can be picked with the hand are described as mushrooms. The various types and shapes of mushrooms that can be picked from the wild include the most common type of umbrella shape with a pileus (cap) and a stipe (stem) i.e., *Lentinula edodes*. There are other species that have different shapes such as volva (cup) in *Volvariella volvacea* or an annulus (ring) in Agarius campestris and some even, like the human ear such as *Plearotus ostreatus*.

Dike et al., (2011) characterized that the life cycle of a mushroom may be traced from a spore which under favorable conditions germinates to form a mass of branched hyphae of mycelia which colonize a substrate. Mushrooms go through two stages, the vegetative stage and the reproductive phase. The vegetative stage ceases when the hyphae fully colonize its substrate. The reproductive phase starts when the hyphae develop primordia. The mushroom is a fruit that results from fully matured primordia of the fungi.

2.2. Classification of mushrooms

Li *et al*. (2021) proposed a system for categorizing mushroom species and assigning a final edibility status. Using this system, they reviewed 2,786 mushroom species from 99 countries, accessing 9,783 case reports, from over 1,100 sources. They identified 2,189 edible species, of which 2,006 can be consumed safely, and a further 183 species which required some form of pretreatment prior to safe consumption or were associated with allergic reactions by some. They identified 471 species of uncertain edibility because of missing or incomplete evidence of consumption, and 76 unconfirmed species because of unresolved, differing opinions on edibility and toxicity. This is the most comprehensive list of edible mushrooms available to date, demonstrating the huge number of mushrooms species consumed. their review highlights the need for further information on uncertain and clash species, and the need to present evidence in a clear, unambiguous, and consistent manner.

Cho and Kang (2004) classified mushrooms by their tropic pattern as saprophytes, parasites or mycorrhizae. The saprophytes are decomposers growing on organic matters like wood, leaves and straw in nature. The parasites on the other hand grow, feed and are sheltered on or in a different organism while contributing nothing to the survival of their host, while mycorrhizas form a symbiotic association of their mycelia with the roots of certain plants.

Ganopedia (2011) reported that There are three groups of mushrooms according to their economic importance; these are edible mushrooms, toxic mushrooms and medicinal mushrooms. Edible mushrooms are mushrooms that have desirable taste and aroma without poisonous effect and are used extensively in cooking; toxic mushrooms produce toxin, mind altering substances, antibiotics and antiviral substances, therefore, ingestion of toxic mushrooms may cause harmful effects that vary from mild symptoms such as gastric upset to severe life-threatening organ-failure which may result in death.

Dzomeku (2009) classified mushrooms according to the substrates they grow on. These include cellulolytic mushrooms, lignocellulolytic and termitomyces. The cellulolytic mushrooms grow mainly on cellulose such as straws; examples include *Vovariella volvacea*, *Agaricus bisporus* etc. The lignocellulolytics grow well on both straws and decaying wood such as sawdust; examples include Pleurotus ostreatus. The

termitomyces grow mainly on anthills and their life cycles are completed by the help of ants or termites; examples include the Termitomyces family.

Chang (2007) had earlier reported that mushrooms can be grouped into four main categories, these include "(1) those which are fleshy and edible fall into the edible mushroom category, e.g., Agaricus bisporus; (2) mushrooms which are considered to have medicinal applications, are referred to as medicinal mushrooms, e.g., Ganoderma lucidum; (3) those which are proven to be, or suspected of being poisonous are named as poisonous mushrooms, e.g., Amanita phalloides; and (4) a miscellaneous category which includes a large number of mushrooms whose properties remain less well defined, which may tentatively be grouped together as 'other mushrooms'."

2.3. Oyster mushroom

Kong (2004) reported that Oyster mushrooms are a diverse group of saprotrophic fungi belonging to the genus Pleurotus.

Kuo (2011) stated that Oyster mushroom belongs to the class Agaricomycetes, order Agaricales, family Pleurotaceae or Tricholomataceae, genus pleurotus and species ostreatus. Scientifically, oyster mushroom is known as *Pleurotus ostreatus*.

Croan (2004) found that oyster mushrooms are a good source of non-starchy carbohydrates, with high content of dietary fiber and moderate quantity of proteins, including most amino acids, minerals, and vitamins. The protein content varies from 1.6 to 2.5%, and the niacin content is about ten times higher than that of any other vegetable.

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

Rosado et al. (2002) reported that Oyster mushroom can grow at moderate temperatures, ranging from 20 to 30°C, and at a humidity of 55-70%, on various agricultural waste materials used as substrate. Because of its flexible nature, the Pleurotus genus is more cultivated than any other mushroom species.

Piska *et al.*, (2017) reported that *Pleurotus ostreatus* is a valuable mushroom of dietary importance. It is rich in primary and secondary metabolites and chemical elements of

physiological significance. One hundred grams of fresh fruiting bodies contains 15% of the recommended daily intake of vitamin C, 40% of niacin, riboflavin, and thiamin, and 0.5 mg of vitamin B12. This species is also characterized by a high content of oleic acid (40%), linolenic acid (55%), and substances responsible for decreasing serum cholesterol levels. High contents of lovastatin and pleuran, have been found in fruiting bodies of this species. It exhibits antiatherosclerotic, hypoglycemic, antioxidant, anticancer and immunomodulatory properties. Due to its wide spectrum of biological activities, *P. ostreatus* is considered a medicinal mushroom. Fruiting bodies and extracts of *P. ostreatus* have found applications in the treatment of civilization – related diseases, especially diabetes, arteriosclerosis and cancer.

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

Deepalakshmi and Mirunalini (2014) reviewed that *Pleurotus ostreatus* is popularly consumed by all over the world due to their taste, flavor, high nutritional values and medicinal properties. Because of the presence of numerous nutritional compositions and various active ingredients in *P. ostreatus*, have been reported to have antidiabetic, antibacterial, anticholestrolic, antiarthritic, antioxidant, anticancer, eye health and antiviral activities. In this review, they particularly expose the high nutritional values of *P. ostreatus*, in relation to their potential medicinal usage which suggest that the *P. ostreatus* mushrooms are the most important nutraceutical functional foods.

Woller (2007) reported that Oyster mushroom is an edible, saprophytic and lignocellulolytic type of mushroom. The fruiting bodies of oyster mushroom are usually

flat with the cap offset from the stalk, or the stalk hardly present at all Pleurotus ostreatus (roughly translating to "beside-the-ear oyster-shaped") predominantly grows on hardwoods such as stumps, logs, and trunks of deciduous trees. It has a pale lilac-grey spore print and a soft fleshy fruiting body that ranges in color from white to grey, brown or even blackish.

Mdconline (2013) found that there is some variability among the species due to the wide distribution and reproductive isolation between continents. The caps of *Plearotus ostreatus* are shell shaped, semicircular to elongate. The margins are smooth and sometimes wavy and are whitish to grayish to tan; the texture is velvety, the flesh is thick and white, gills are narrow, the stalk is short, thick and white and the base being hairy. The spores look narrowly elliptical, smooth and colorless when magnified. On average, the cap width ranges between 2 – 15 cm, stalk length is around 4 cm and stalk width is around 2 cm. *P. ostreatus* fruits year-round, especially after a good rain, if the weather is mild.

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

Woller (2007)reported that the oyster mushroom has four mating types to ensure the chance of successful mating. The new dikaryotic cell multiplies and divides to live as a multi-cellular dikaryotic organism. This is the dominant stage for growing and gathering of nutrients. The dikaryotic mycelia then mature to a mushroom. The mushroom develops dikaryotic basidia within the gills. The nuclei in the basidia finally undergo karyogamy, fusion of the nuclei, and at last form a diploid nucleus that quickly undergoes meiosis. Each diploid nucleus yields four haploid nuclei of different mating types that develop into a basidiospore to repeat the cycle.

2.4. Effect of substrate of mother spawn

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.

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

Jayachandran et. al., (2017) showed that rice grain (10days) taken for spawn, pinhead formation, stalk length, pileus diameter, pileus thickness, yield (383.81±0.24) and bio efficiency (76.76%) were found to be the best substrate for spawn development of *Pleurotus florida* for cultivation paddy straw used as a substrate. Whereas sorghum (12days) yield (303.63±0.01) and bio efficiency (60.72%) and wheat grain (13days) yield (283.21±0.01) and bioefficiency (56.64%) took period of spawn development of Pleurotus florida.

Jatwa et. al., (2017) evaluated that Effect of seed grains viz. Sorghum, Wheat, Rice, Maize, Gram, Mung and Garden pea seeds was used for production and cultivation of Pleurotus spp. (P. florida, P. eous, P. sajor-caju) on rice straw. The minimum days for mycelia run on seed grain was on sorghum grain (9, 8 and 8 days) and maximum number of days on rice grain (17, 18 and 20 days) was noticed in P. florida, P. eous, P. sajor-caju respectively. The spawn substrates showed different response on biological efficiency of Pleurotus spp. (P. florida, P. eous, P. sajor-caju). Maximum biological efficiency of P. florida and P. sajor-caju was noticed in wheat grain used as spawn substrates (170.3 % and 125.8 % respectively) and in P. eous it was noticed on sorghum

grain (166.1 %). The minimum biological efficiency of P. florida, P. eous and P. sajor-caju was noticed on rice grain used as spawn substrate.

Stanley and Awi-Waadu (2010) determined the effects of substrates spawn preparation on mycelial growth of oyster mushroom species. They conducted a factorial experiment design at randomized completely with three replications. In this experiment, first and second factors, respectively were substrates (Wheat, yellow maize, guinea corn, millet, red sorghum and white maize, Bende local and oyster mushroom species (Pleurotus tuber-regium and Pleurotus pulmonarius). The results clearly demonstrated that between various substrates used, maximum and minimum growth rate were recorded for white maize (Bende local) and least mycelial extension and fresh weight on wheat. The second-best grain for both species used was sorghum.

Aminuzzaman et al., (2020) conducted a study to to compare the performance of different substrates and mother culture materials on yield and yield parameters of oyster mushroom (Pleurotus ostreatus). three substrates (sawdust, rice straw, sawdust + rice straw (1:1)) and three mother cultures (rice, maize, sawdust) were used in oyster mushroom cultivation. Among the substrates and mother culture components, using rice straw and sawdust mother spawn, the maximum length of stipe was recorded (23.27 mm and 24.29 mm, respectively). Applying sawdust + rice straw (1:1) and maize mother spawn, the peak diameter of stipe was calculated (9.90 mm and 10.01 mm, respectively). The maximal diameter of pileus was observed in sawdust + rice straw (1:1) and rice mother spawn (72.90 mm and 67.57mm, respectively). With the application of rice straw and maize mother spawn, thickest pileus was viewed (5.60 mm and 5.47mm respectively). The sawdust and sawdust mother spawn delivered peak number of fruiting body (6.67 and 7.33, respectively). Among the substrates, rice straw gave the highest biological yield (44.40 g/packet) and sawdust gave the lowest (41.73 g/packet). Among the mother spawn, sawdust mother spawn presented the highest biological yield (45.47 g/packet) and maize mother spawn gave the lowest (39.16 g/packet). In the comparison of combined effect of substrates and mother spawn, sawdust mother spawn performed best in the biological yield (50.80 g/packet) with rice straw as substrate material and maize mother spawn showed comparatively lower biological yield (37.60 g/packet) with both sawdust and rice straw as substrate material. In the study, they found that Rice straw and sawdust mother spawn can be recommended for its suitability in oyster mushroom (Pleurotus ostreatus) cultivation.

2.5. Contamination of spawn

Adhikari and Jha (2017) studied samples of *Pleurotus ostreatus* and *Pleurotus florida* were collected from three major vegetable market of Kathmandu city which revealed presence of 21 fungi and worked to control of fungal contaminants in mushrooms during the postharvest storage.

Pervez*, et al.,* (2010) carried out the study to identify weed mycoflora associated with *Pleurotus ostreatus* (*Oyster mushroom*) substrate during culture in the spawn packet and to evaluate Formalin and Bavistin (Cabendazim) 50WP against the weed mycoflora. A total of 50 spawn packets colonizing substrate of *Pleurotus ostreatus* were collected randomly at different growth stages Ten weed mycoflora namely *Aspergillus flavus, A. fumigatus, A. niger, A. nidulans, A. terreus, Penicillium citrinum, P. thiersii*, *Penicillium. sp*., *Rhizopus stolonifer* and *Trichoderma harzianum* were found to be associated with the substrate.

Biswas (2014) revealed that the occurrence of seven contaminants in mushroom beds and out of which *Trichoderma harzianum, Penicillium notatum, Sclerotium rolfsii* and *Coprinus* spp. were found to be most dominant fungal contaminants and occurrence was high during June and July (28.4 & 35.8 %) causing maximum loss to mushroom yield. Among the botanicals tested for management of competitors moulds, *Azadirachta indica* (neem) showed its supremacy and exhibited maximum inhibitory effect (54.1 to 71.6 %) against *Aspergillus* spp., *Trichoderma* spp., *Coprinus* spp., and *Penicillium* spp. and was found to be less effective against *Sclerotium rolfsii in vitro* followed by extracts of *Pongamia pinnata* (42.4 to 61.3%). A range of 35.3 to 62.4% reduction in inky caps (*Coprinus sp.*) and 26.3 to 68.4% in green moulds (*Trichoderma* spp) were recorded with different phyto-extracts. The botanicals except *Acacia nilotica* reduced the incidence of competitor moulds (18.18 to 70.91%) in mushroom beds which increase the yield up to 21.3 %. The study will provide the idea of appropriate cultivation time as well as provide an alternative method of surface sterilization. 18

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

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

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted to study the effect of different mother spawn on the productivity and contaminationof oyster mushroom (*Pleurotus ostreatus*). The chapter includes a brief description of the location of experiment, materials used for the experiment, design of the experiment, preparation of substrates, preparation of packets, cultivation of spawn packet, collection of produced mushrooms, proximate analysis of the mushrooms, data collection and data analysis procedure which are presented below under the following headings-

3.1. Experimental site

The field experiment was conducted at 'Mushroom Culture House (MCH)' of Sher-e-Bangla Agricultural University, Dhaka. On the other hand, the laboratory experiment was done in 'Plant Pathology laboratory', of Sher-e-Bangla Agricultural University, Dhaka and 'Biochemistry laboratory' of Mushroom Development Institute (MDI), Savar, Dhaka. Details of the meteorological data during the period of the experiment was collected from the Bangladesh Meteorological Department, Agargaon, Dhaka and presented in Appendix I.

3.2. Duration of the experiment

The experiment was carried out during the period from November, 2020 to March, 2021.

3.3. Experimental materials

Test tube Mother spawn of snow white (WS) variety of oyster mushroom was collected from Mushroom Development Institute (MDI), Savar, Dhaka. (Plate 1.A)

3.4. Varietal characteristics of oyster mushroom

Oyster mushroom (*Pleurotus ostreatus*) has a light to dark whitish colored cap depending upon the strain and growing conditions. Primordia and young mushrooms are light white but become less intensely colored as the mushroom matures. Oyster mushroom is characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. Their fruiting bodies are shell or spatula

shaped with white color. If the temperature increases above 32^0 C, its production markedly decreases.

3.5. Treatments of the experiment

Nine different treatments were used with three replications to achieve the desired objectives. The treatments were as follows:

T_1 – Mustard grain
T_2 – Red maize grain
T_3 – 50% rice straw and 50% sawdust
T_4 – Wheat grain
T_5 – Kaon grain
T_6 – Gram grain
T_7 – Pea grain
T_8 – Rice grain
T_9 – White maize grain

Figure 1. Treatments of the experiments

3.6. Design and layout of the experiment

The experiment was laid out in single factor Completely Randomized Design (CRD). The experiment included nine treatments with three replications.

3.7. Preparation of packet for mother spawn

The grains were soaked in water for 12 hours. Then spawn packets were prepared by mixing the grains, wheat bran (50g) and $CaCO_3$ (1g per packet). The grain materials (300 g) were taken in a plastic bowl and mixed thoroughly by hand. Then the mixture of grain was filled into polypropylene bag and their mouth were plugged by absorbing cotton and covered with brown paper. Finally, these bags were tied with a rubber band. (Plate 1.C) The packets were sterilized about at 121ºC and 15 PSI for 1 hour and then these were kept for cooling. After cooling, mycelium (5g) of snow-white (WS) variety of oyster mushroom was inoculated into the packets of grain in the laminar airflow cabinet.

3.8. Incubation of mushroom mother spawn

The mother spawn packets were kept in the incubation chamber at 20-22°C temperature until the packets become white with the mushroom mycelium. After completion of the mycelium running, these mother spawns were used for the preparation of spawn packets for cultivation.

3.9. Preparation of spawn packet for cultivation

Spawn packets were prepared by using sawdust as substrate. $CaCO_3$ was mixed with sawdust at the rate of 1 g per packet and moisture was increased by adding water at the rate of 65% which was measured by using the moisture meter. The substrates were filled into 7×11 inch polypropylene bag @ 500 g and their mouth were plugged by absorbing cotton and covered with brown paper. Finally, these bags were tied with a rubber band.

Plate 1. Preparation of spawn packets for mother spawn **A.** Mother of snow-white (WS) **B.** Mixing of lime with substrate, **C.** Preparation of substrate packets, **D.** Sterilization of substrate packets by autoclave, **E.** Incubation of spawn packets

3.10. Sterilization, inoculation and incubation of spawn

Therefore, the packets were sterilized about at 121ºC and 15 PSI for 1 hour and then these were kept for cooling. After cooling, spawn packets were inoculated with 10g mother in the laminar airflow cabinet. Spawns were kept in the incubation room at 20-22°C temperature until mushroom mycelium running completed. After completion of mycelium running the packets were transferred in cultivation room.

3.11. Cultivation of spawn packet

Opposite two ends of each other of the upper position of plastic bag were cut in "D" shape with a blade and opened by removing the plastic sheet. Then the opened surface of the substrate was scraped slightly with a teaspoon for removing the thin whitish mycelial layer. Then the spawn packets were soaked in water and inverted to remove excess water for another 15 minutes. The packets were placed separately on the rake of culture room and covered with newspaper. The moisture of the culture room was maintained 80-85% relative humidity by spraying water three times in a day. The light around 300-500 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22°C to 25°C.

3.12. Harvesting of produced mushrooms

The matured fruiting body was identified by curial margin of the cap, as described by Amin (2004). Mushrooms were harvested by twisting to uproot from the base. After first harvesting, again the packets were scraped at the place of “D” shaped cut as like as previous method. In this way, the harvesting was continued until fruiting bodies were flushed.

3.13. Data collection

3.13.1. Following data were collected from mother spawn

3.13.1.1. Mycelium Running Rate (MRR) in mother spawn

Mycelium running rate (MRR) for each spawn packets was measured after the mycelium colony cross the shoulder of the packet. The linear length was measured at different places of packet using the following formula (Sarker, 2004):

Mycelium Running Rate (MRR) = *L*/D cm/day

Where, L = Average length of mycelium running for different places (cm)

D = Number of days

Plate 2. Mycelium running in mother spawn. (A-B). Mustard grain packets, C. White maize grain packets, D. Rice grain packets

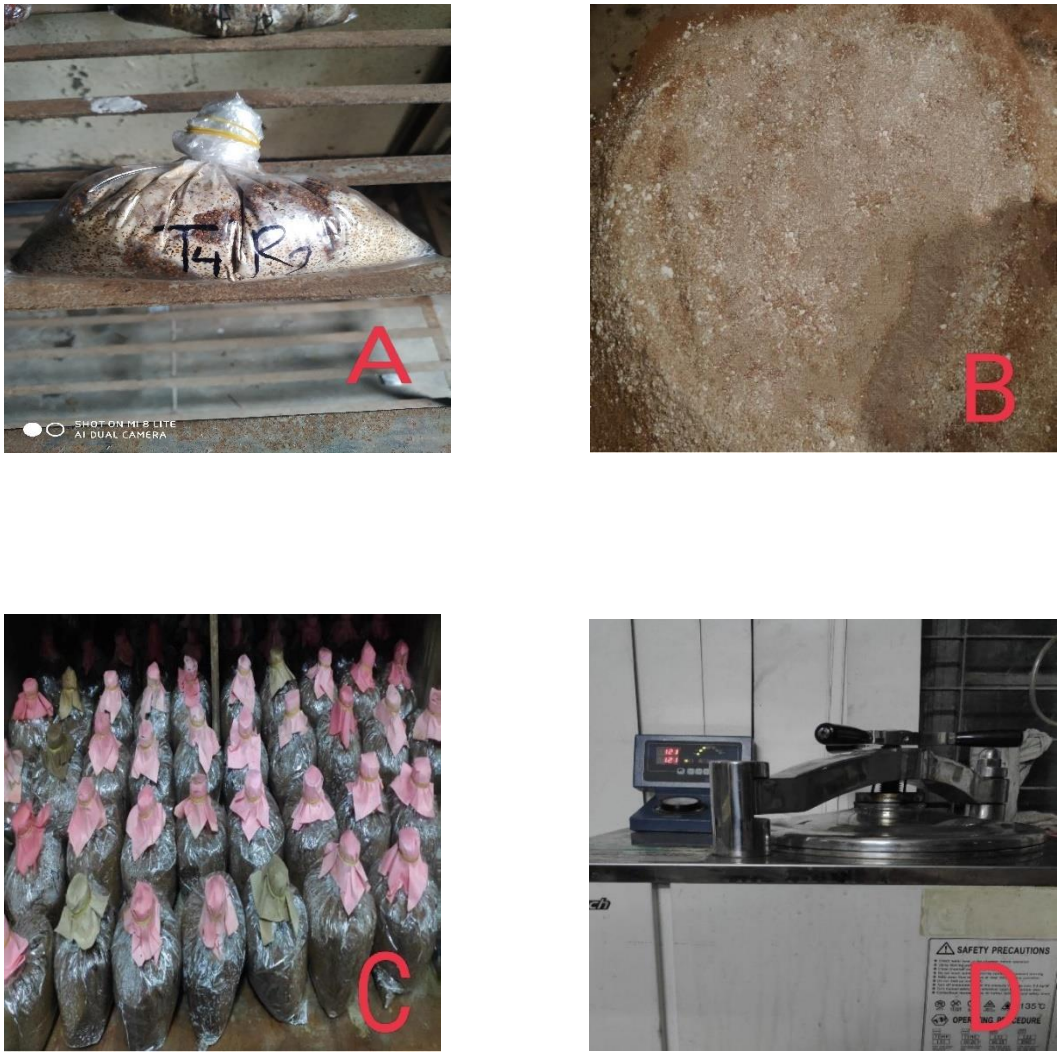


Plate 3. Preparation of packets for mushroom cultivation **A**. Prepared mother spawn, **B.** Mixing of lime with substrate, **C.** Preparation of substrate packets, **D.** Sterilization of substrate packets by autoclave, **E.** Incubation of spawn packets

3.13.1.2. Days required from incubation to mycelium initiation

Days required from incubation to mycelium initiation was recorded.

3.13.1.3. Days required from incubation to completion of mycelium running

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

3.13.1.4. Weight of mycelium in spawn packets

Weight of mycelium in spawn packets was recorded.

3.13.2. Following data were collected from spawn packets for cultivation

3.13.2.1. Mycelium Running Rate (MRR) in spawn packet

Mycelium running rate (MRR) for each type of substrate was measured after the mycelium colony cross the shoulder of the packet. The linear length was measured at different places of packet using the following formula (Sarker, 2004):

Mycelium Running Rate (MRR) = *L*/D cm/day

Where, L = Average length of mycelium running for different places (cm)

D = Number of days

3.13.2.2. Days required from incubation to mycelium initiation

Days required from incubation to mycelium initiation was recorded.

3.13.2.3. Days required from incubation to completion of mycelium running

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

3.13.2.4. Days Required from Stimulation to Primordia Initiation (days)

Days Required from Stimulation to Primordia Initiation (days) was recorded.

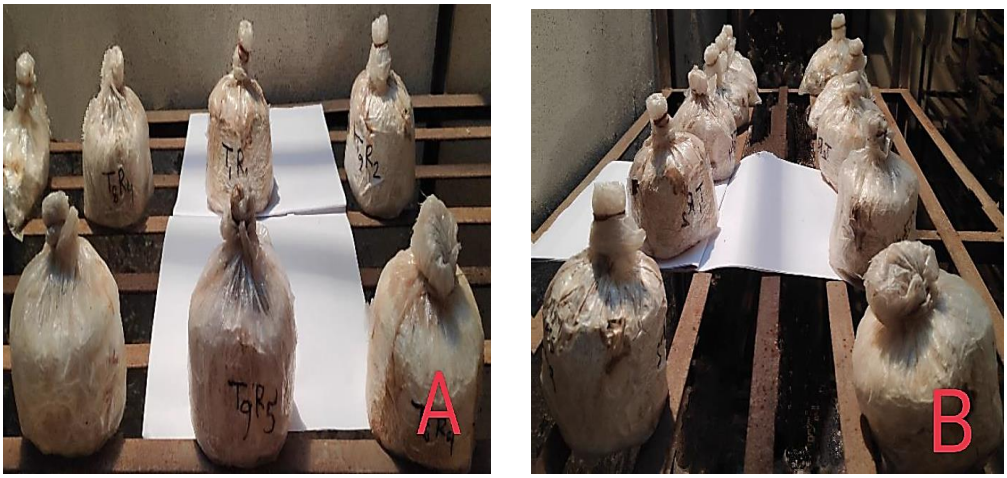


Plate 4. Mycelium running during incubation

3.13.2.5. Days Required from Primordia Initiation to 1st Harvest (days)

Days required from primodia formation to first harvest was recorded.

3.13.2.6. Total harvesting period (days)

Days required from primordia formation to final harvest was recorded.

3.13.2.7. Data on yield contributing parameters

Number of primordia and well-developed fruiting bodies was recorded. Dry, undesired fruiting bodies were discarded. Data on the following parameters were also recorded:

a) Number of primordia per packet

b) Number of fruiting bodies per packet

c) Number of effective fruiting bodies per packet

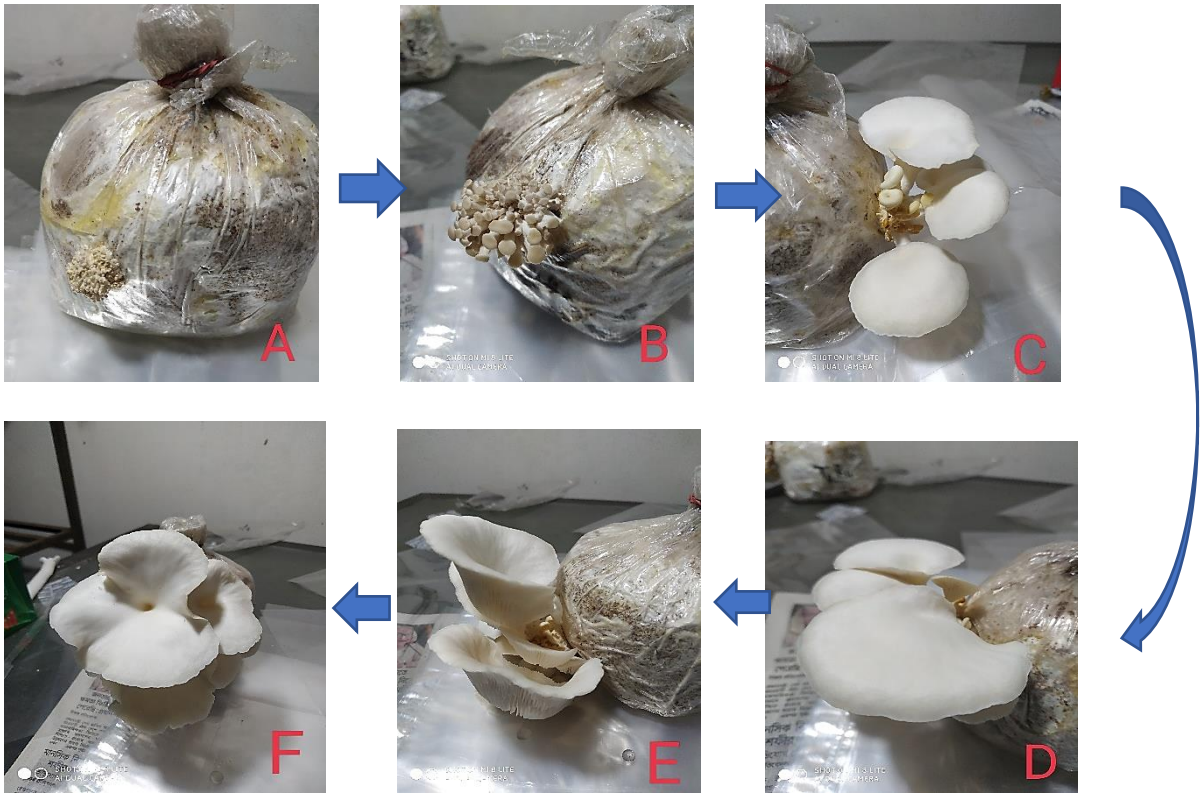


Plate 5. Different stages of fruiting bodies **A-B**. Primordia formation **C-D.** Premature fruiting bodies **E-F.** Matured fruiting bodies

3.13.2.8. Dimension of fruiting body (stipe and pileus)

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

3.13.2.9. Biological yield (g)

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

3.13.2.10. Economic yield (g)

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

Plate 6. Cultivation of mushroom **A.** Premature fruiting body **B.** Matured fruiting body

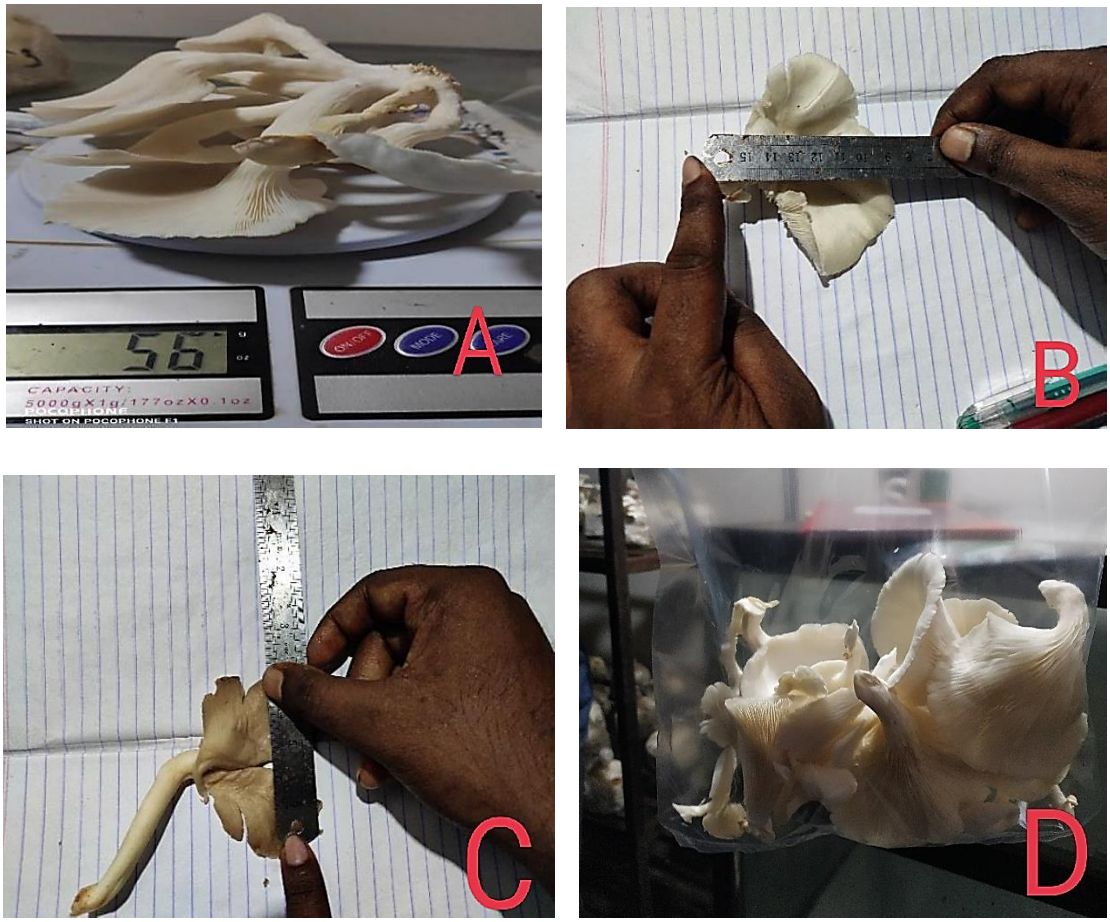


Plate 7. Measurement of different growth parameters of oyster mushroom **A.** Measurement of weight of mushroom **B.** Measurement of length of fruiting body **C.** Measurement of Breadth of fruiting body **D.** Harvested mushroom

3.13.2.11. Biological efficiency (%)

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

$$\text{Biological Efficiency} = \frac{\text{Total Biological Weight}}{\text{Total Dry Weight of Substrares used}} \text{ X } 100$$

3.13.2.12. Severity of contamination (%) on spawn packets

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

Grade 0: 0% – Free from contamination

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

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

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

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

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

$$\text{Severity of contamination (\%)} = \frac{\text{Sum of total score}}{\text{Total number of observation x Maximum grade of the scale}} \text{ X } 100$$

3.14. Analysis of data

All the data collected on different parameters were statistically analyzed by following the analysis of variance (ANOVA) technique and mean differences were adjusted by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) using the MSTAT-c computer package program. The mean differences among the treatments were compared by least significant difference (LSD) test at 5% level of significance.

3.15. Collection of contaminated spawn packet

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

3.15.1. Composition and preparation of agar media

Ingredients	Amount (per liter)
Potato	200 g
Dextrose	20 g
Agar	20 g
Water	1 L

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

Plate 8. Preparation of agar media(**A-B.)** Boiling of sliced, peeled potatoes (60 g), **C.** Measurement of the weight of Dextrose and Agar (6 g each), **D.** Sterilization of glassware in the oven, **E.** PDA solution (300 ml)

3.15.2. Isolation and purification of competitor molds from collected spawn

10g of substrate samples were taken from the contaminated packets and mixed with 100 ml sterile distilled water. A series of dilutions were made by taking 1 ml from the stock solution to add with 9 ml sterile water and shaken thoroughly to obtain the dilution. From the each of the substrate dilutions 0.5 ml volumes were pipetted on PDA media and incubated at 27ºC (±2) ºC for 3-4 days. The pathogen grown as the mixed colony then individual culture plates of substrate samples were isolated. To prepare pure culture sufficient number of subculturing were done by hyphal tip technique (Hyakumachi, 1994). All the pure cultures were kept in refrigerator at 4ºC for preservation.

3.15.3. Identification of pathogens

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

CHAPTER IV

RESULTS

4.1. Effect of mother spawn materials on contamination severity

All the spawn packets under different treatments were free from contamination during incubation period (Table 1). During cultivation at 15 days after stimulation (DAS), similar contamination (6.67%) was recorded from T_1 (Mustard) and T_3 (50% rice straw and 50% saw dust) while the rest mother spawn was free from contamination. At 30 DAS, contamination severity was identical (6.67%) which was recorded from T_2 and T_5. Similarly, 13.33% severity was recorded in case of T_6 and T_9. At 45 DAS, the highest contamination severity (46.67%) was recorded from T_1 (Mustard) and Similar (26.68%) contamination was observed at T_5 and T_9 while there was no contamination was observed in T_4 and T_8 during whole harvesting period.

Table 1. Effect of mother spawn materials on the severity of contamination at different days after stimulation (DAS)

Treatments	Cultivation Period (%)		
	15 DAS	30DAS	45DAS
T_1	6.67	26.68	46.67
T_2	0	6.67	13.33
T_3	6.67	20	40
T_4	0	0	0
T_5	0	6.67	26.68
T_6	0	13.33	33.33
T_7	0	0	6.67
T_8	0	0	0
T_9	0	13.33	26.68

[T_1 = Mustard; T_2 = Red maize; T_3 =50% rice straw and 50% saw dust; T_4 = Wheat; T_5 = Kaon; T_6 = Gram; T_7 = Pea; T_8 = Rice; T_9 = White maize]

4.2. Identified contaminants from contaminated spawn

Moulds are competitor to *Pleurotus spp*. Based on the morphological characters commonly four pathogens were identified, namely *Trichoderma sp.*, *Penicillium* sp. *Rhizopus stolonifer* and *Aspergillus niger*.

Plate 9. Different contaminated spawn packets

4.2.1. *Trichoderma sp.*

Green color growth of mycelium was observed in contaminated spawn packet due to heavy sporulation of causal agent. Colonies are usually fast growing and initially whitish in color that later turn into bright green color (Plate 10 A-B). *T. harzianum* had occasionally concentric condition with whitish yellow conidial area. Conidiophores are branched that cluster into fascicles. Normally branches are formed near 90º with the main branch. The conidiophores terminated with one or few phialides that usually rise from the axis near the tip.

4.2.2. *Rhizopus stolonifer*

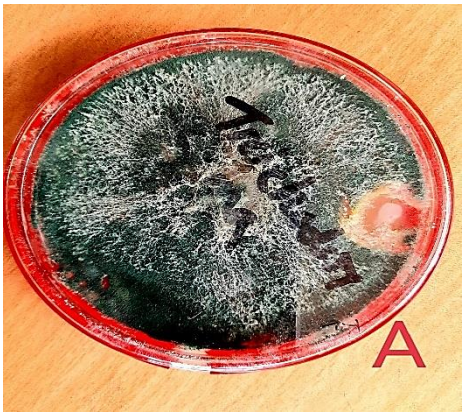
Rhizopus stolonifer is common contaminant. What makes *Rhizopus* tricky to deal with is that it grows extremely fast. Similar in appearance to pin mould, *Rhizopus* develops hair-like sporophores with a tiny head (Plate 10 C-D). Sporangia which are bulbous structures that sprout from the vegetative hyphae and hold the haploid spores. *Rhizopus* has a sour odour. Sometimes, the smell of the fungus may also resemble alcohol.

4.2.3. *Aspergillus niger*

Aspergillus niger produced black colored spores so it was called black mold (plate 11-A-B). Initially fungal colonies were whitish which quickly became dark black. *Aspergillus flavus* produced green spores, it is also called green mold. The hyphae were hyaline and septate. The conidia produced were globose, single celled, pale to dark brown on maturity. The conidiophores were erect, unbranched, straight, hyaline to light brown, long aseptate and darker near vesicle. The vesicle was globose, thick walled and brown to black.

4.2.4. *Penicillium sp.*

Initially, *Penicillium* appeared as a white colored powder on the substrates of oyster mushroom and later turned into green as timed passed, it is called blue green mold (Plate 11 C). Pure culture of *Penicillium* was prepared on PDA from collected contaminated spawn (Plate 11 D). Conidiophores are hyaline, smooth or rough walled arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides. Conidia hyaline or brightly colored in mass, chain of single celled conidia is produced in basipetal succession from a specialized conidiogenous cell called a phialide.



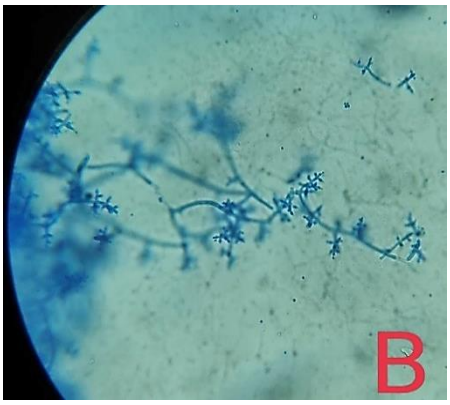


Plate 10. A. Pure culture of *Trichoderma sp.*, **B.** Pathogenic structure of *Trichoderma sp.*, **C.** Pure culture of *Rhizopus stolonifer*, **D.** Pathogenic structure of *Rhizopus stolonifer*,

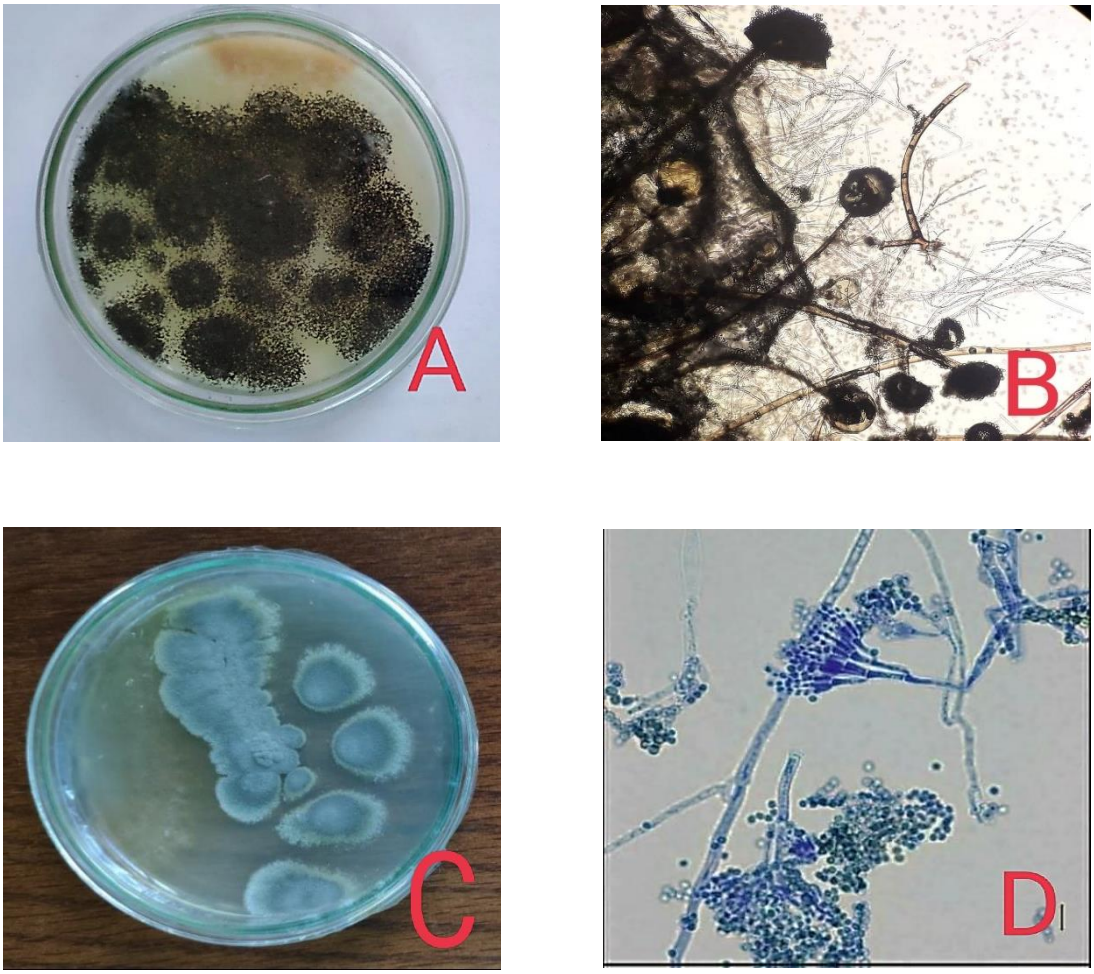


Plate 11. A. Pure culture of *Aspergillus niger*, **B.** Pathogenic structure of *Aspergillus niger*, **C.** Pure culture of *Penicillium sp*., **D.** Pathogenic structure of *Penicillium sp.*

4.3. Effect of different grains on growth contributing characters of mother spawn

4.3.1. Effect of different grains on mycelium running rate of mother spawn for cultivation of oyster mushroom

Mycelium running rate of mother spawn of oyster mushroom (*Pleurotus ostreatus*) showed statistically significant variation due to different grains under the present trial (Figure 1 and Appendix II). The highest mycelium running rate (0.85 cm/day) was recorded from T_8 (Rice), which was statistically similar with T_4 (Wheat), while the lowest mycelium running rate (0.62 cm/day) was observed in T_1 (Mustard).

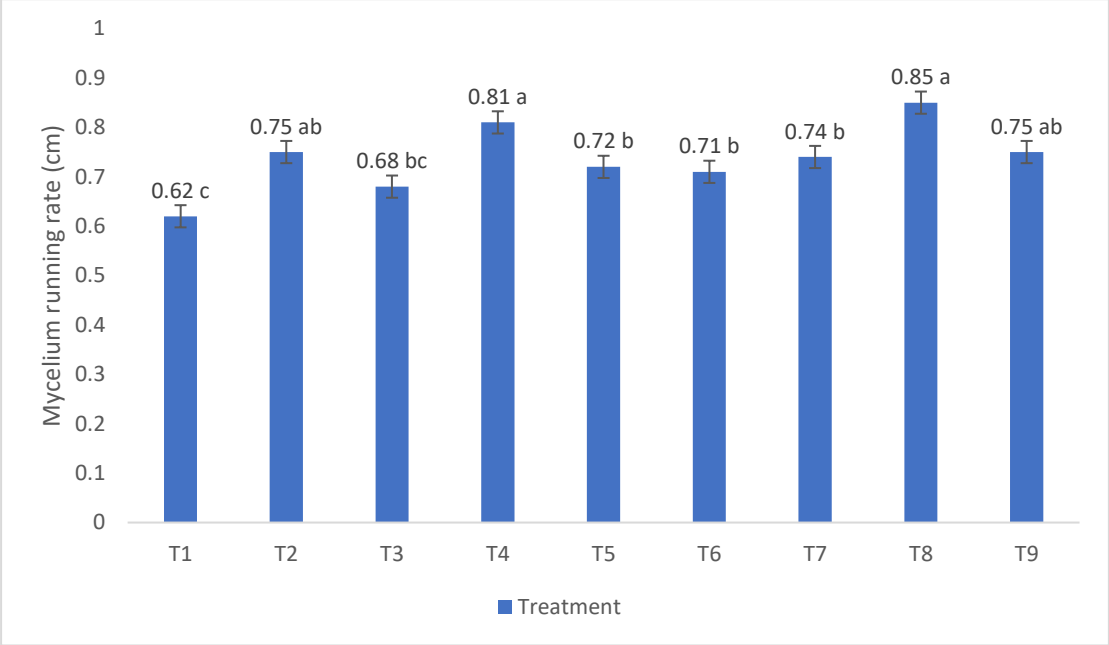


Figure 2. Effect of different grains on mycelium running rate of mother spawn for cultivation of oyster mushroom

[T_1 = Mustard; T_2 = Red maize; T_3 =50% rice straw and 50% saw dust; T_4 = Wheat; T_5 = Kaon; T_6 = Gram; T_7 = Pea; T_8 = Rice; T_9 = White maize]

4.3.2. Effect of different grains on time required from incubation to initiation

There was significant variation in terms of time from incubation to mycelium initiation of mother spawn due to different grains (Table 2 and Appendix II). The highest time (16.67 days) from incubation to mycelium initiation was found in T_1 (Mustard) followed by T_3 (50% rice straw and 50% saw dust), whereas the lowest time (10.67 days) from incubation to mycelium initiation was recorded in T_8 (Rice).

4.3.3. Effect of different grains on time required from incubation to completion of mycelium running

Days required from incubation to completion of mycelium running of mother spawn varied significantly due to different grains (Table 2 and Appendix II). The highest time (22.33 days) required for mycelium running was recorded in T_1 (Mustard) followed by T_3 (50% rice straw and 50% saw dust), whereas the lowest time (16.33 days) required for mycelium running was recorded in T_8 (Rice).

4.3.4. Effect of different grains on weight of mycelium of mother spawn of oyster mushroom

Weight of mycelium varied significantly due to different grains (Table 2 and Appendix II). The highest weight was recorded in T_8 (Rice) and lowest was recorded from T_1 (Mustard) followed by T_3 (50% rice straw and 50% saw dust). T_2, T_5, T_6 and T_7 were statistically same but significantly different.

Table 2. Performance of different grains on time required from incubation to initiation, completion of mycelium running and weight of mycelium

Treatments	Time required from incubation		
	to mycelium initiation (days)	to completion of mycelium running (days)	Weight of Mycelium (g)
T_1	16.67a	22.33a	1.84c
T_2	12.33bc	17.33cd	3.08b
T_3	13.67b	20.67ab	2.08c
T_4	11.67cd	17.00cd	4.02a
T_5	13.33b	18.67bcd	2.71b
T_6	13.33b	19.33bc	2.61b
T_7	12.67bc	18.00cd	2.89b
T_8	10.67d	16.33d	4.08a
T_9	12.33bc	17.33cd	3.96a
CV (%)	7.27	7.69	9.38
LSD $_{(0.05)}$	1.61	2.44	0.48

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

[T_1 = Mustard grain; T_2 = Red maize grain; T_3 =50% rice straw grain and 50% saw dust; T_4 = Wheat grain; T_5 = Millet grain; T_6 = Gram grain; T_7 = Pea grain; T_8 = Rice grain; T_9 = White maize grain]

4.4. Growth and yield contributing characters

4.4.1. Effect mother spawn materials on mycelium running rate (cm/day)

Mycelium running rate of oyster mushroom (*Pleurotus ostreatus*) showed statistically significant variation due to mother spawn materials under the present trial (Figure 3 and Appendix III). The highest mycelium running rate (0.84 cm/day) was recorded from T_8 (Rice), which was statistically similar with T_4 (Wheat), while the lowest mycelium running rate (0.61 cm/day) was observed in T_1 (Mustard).

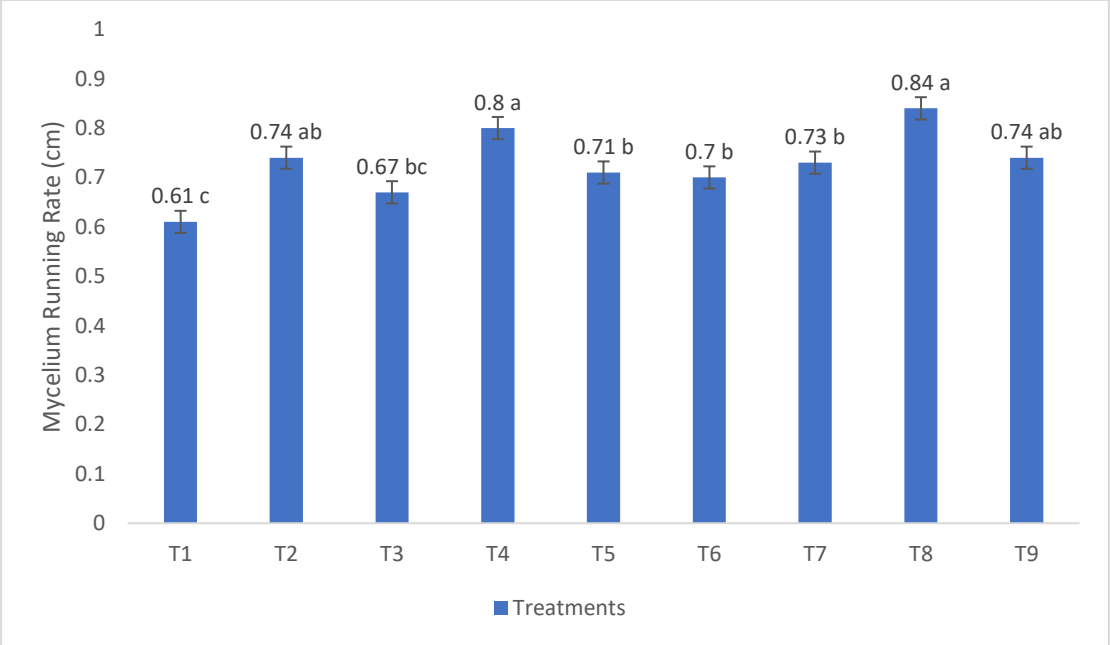


Figure 3. Effect of mother materials on mycelium running rate of oyster mushroom [T_1 = Mustard grain; T_2 = Red maize grain; T_3 =50% rice straw grain and 50% Saw dust; T_4 = Wheat grain; T_5 = Millet grain; T_6 = Gram grain; T_7 = Pea grain; T_8 = Rice grain; T_9 = White maize grain]

4.4.2. Effect of mother spawn on time required from incubation to mycelium initiation

There was significant variation in terms of time from incubation to mycelium initiation of oyster mushroom (*P. ostreatus*) due to mother spawn materials (Table 3 and Appendix III). The highest time (8.67 days) from incubation to mycelium initiation was found in T_1 (Mustard) followed by T_3 (50% rice straw and 50% saw dust), whereas the lowest time (6.00 days) from incubation to mycelium initiation was recorded in T_8 (Rice). The duration from incubation to mycelium initiation in T_2, T_4, T_5, T_7 and T_9 was statistically same but significantly different.

4.4.3. Effect of mother spawn materials on time required from incubation to completion of mycelium running

Days required from incubation to completion of mycelium running of oyster mushroom varied significantly due to mother spawn materials (Table 3 and Appendix III). The highest time (24.33 days) required for mycelium running was recorded in T_1 (Mustard) followed by T_3 (50% rice straw and 50% saw dust), whereas the lowest time (18.33 days) required for mycelium running was recorded in T_8 (Rice). The duration from incubation to completion of mycelium running in T_2, T_4, T_7 and T_9 was statistically identical but significantly different.

Table 3. Performance of mother spawn materials on time required from incubation to initiation and completion of mycelium running

Treatments	Time required from incubation	
	to mycelium initiation (days)	to completion of mycelium running (days)
T_1	8.67a	24.33a
T_2	6.67cd	19.33cd
T_3	8.33ab	22.67ab
T_4	7.33bc	20.67bcd
T_5	7.00cd	21.33bc
T_6	6.67cd	20.00cd
T_7	6.33cd	19.00cd
T_8	6.00d	18.33d
T_9	6.67cd	19cd
CV (%)	8.60	6.94
LSD $_{(0.05)}$	0.914	2.44

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

[T_1 = Mustard grain; T_2 = Red maize grain; T_3 =50% rice straw grain and 50% saw dust; T_4 = Wheat grain; T_5 = Millet grain; T_6 = Gram grain; T_7 = Pea grain; T_8 = Rice grain; T_9 = White maize grain]

4.4.4. Effect of mother spawn materials on time required from stimulation to primordia initiation (days)

There was significant variation in terms of time from stimulation to primordial initiation of oyster mushroom due mother spawn materials (Table 4 and Appendix IV). The time from stimulation to primordial initiation ranged from 5.89 days to 7.00 days. The highest time from stimulation to primordial initiation (7.00 days) was observed in T_1 (Mustard), whereas the lowest time (5.89 days) from stimulation to primordia initiation was in the treatment T_8 (Rice). The other treatments varied significantly in terms of time from stimulation to primordia initiation.

4.4.5. Effect of mother spawn materials on time required from primordial initiation to 1st harvest (days)

Data revealed that time from primordial initiation to 1st harvest of oyster mushroom was statistically significant compared to control due to mother spawn materials (Table 4 and Appendix IV). The maximum time (7.33 days) from primordia initiation to harvest was observed in T_1 (Mustard), whereas the lowest time (5.67 days) from primordia initiation to harvest was in T_8 (Rice).

4.4.6. Total harvesting period (days)

Statistically significant variation was recorded in terms of days for final harvest of oyster mushroom due to mother spawn materials (Table 4 and Appendix IV). The highest harvesting period (48.33 days) was recorded in T_1 (Mustard) which was statistically similar with T_3 followed by T_5 and T_6. On the other hand, the lowest harvesting period (40.00 days) was recorded in T_8 (Rice).

Table 4. Performance of mother spawn materials on time required from stimulation to primordia initiation, primordial initiation to 1st harvest and total harvesting period

Treatments	Time required from stimulation to primordia initiation (days)	Time required from primordial initiation to 1st harvest (days)	Total harvesting period (days)
T_1	7.00a	7.33a	48.33a
T_2	6.11b	6.33abc	46.67ab
T_3	6.67ab	7.00ab	48.33a
T_4	6.11b	6.00bc	42.00bc
T_5	6.45ab	6.33abc	47.33ab
T_6	6.11ab	6.67abc	47.11a
T_7	6.11b	6.67abc	45.33ab
T_8	5.89b	5.67d	40.00c
T_9	6.22ab	6.00bc	44.67abc
CV (%)	7.35	8.96	7.36
LSD $_{(0.05)}$	0.79	0.99	4.86

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

[T_1 = Mustard grain; T_2 = Red maize grain; T_3 =50% rice straw grain and 50% saw dust; T_4 = Wheat grain; T_5 = Millet grain; T_6 = Gram grain; T_7 = Pea grain; T_8 = Rice grain; T_9 = White maize grain]

4.4.7. Effect of mother spawn materials on average number of primordia per packet

Average number of primordia per packet varied from 87.33 to 65.00 significantly due to the mother spawn materials under the present trial (Figure 4 and Appendix V). The maximum average number of primordia per packet was observed from T_8 (Rice) which was statistically similar with T_4 (Wheat); T_9 (Maize white); T_7 (Pea); T_2 (Maize red) and T_5 (kaon). On the other hand, the minimum number of primordia per packet was found in T_1 (Mustard) which was statistically identical with T_3 (50% rice straw and 50% saw dust) and T_6 (Gram).

4.4.8. Effect of mother materials on average number of effective fruiting body

Statistically significant variation was recorded due to mother spawn materials in terms of effective fruiting body per packet of oyster mushroom (Figure 4 and Appendix V). The maximum number of effective fruiting body per packet (31.00) was recorded from T_8 (Rice) followed by T_4, T_7 and T_9 whereas the minimum average number of effective fruiting body per packet (18.33) was observed in T_1 (Mustard) Number of effective fruiting body of T_3 was statistically similar with T_6.

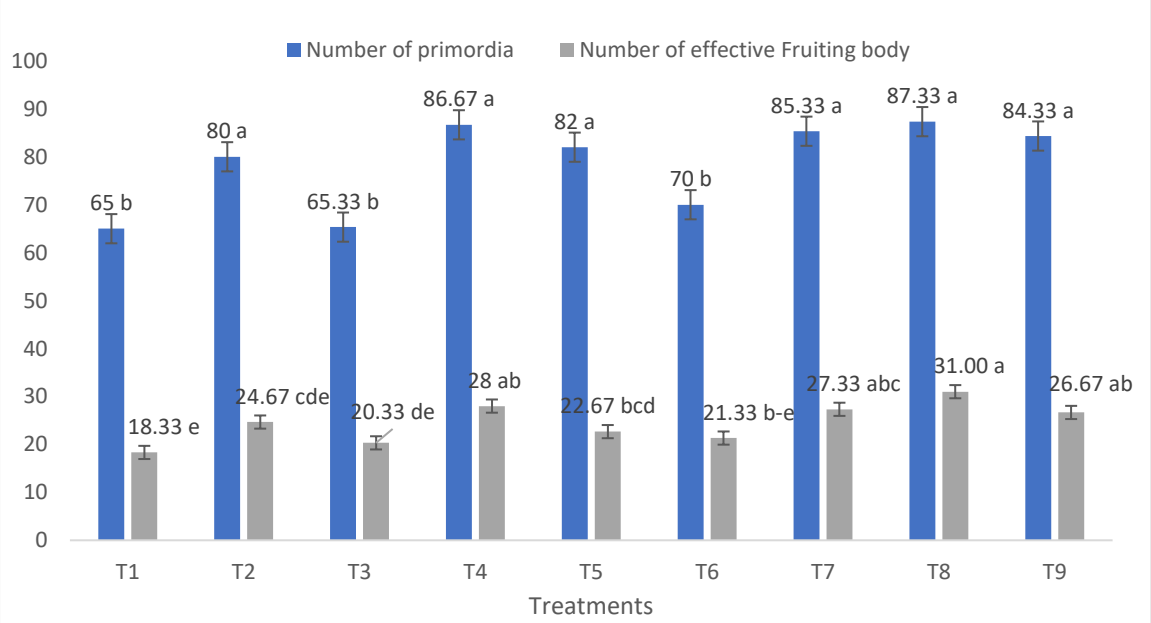


Figure 4. Effect of mother spawn materials on number of primordia/packets and number of effective fruiting body/packet of oyster mushroom

[T_1 = Mustard grain; T_2 = Red maize grain; T_3 =50% rice straw grain and 50% saw dust; T_4 = Wheat grain; T_5 = Millet grain; T_6 = Gram grain; T_7 = Pea grain; T_8 = Rice grain; T_9 = White maize grain]

4.5. Dimension of fruiting body (cm)

4.5.1. Length of pileus

Length of pileus of oyster mushroom varied from 4.09 to 5.17 cm significantly due to mother spawn materials under the present trial (Table 5 and Appendix VI). The highest length of pileus was recorded from T_8 (Rice), whereas the lowest length of pileus was found in T_1 (Mustard). Statistically similar result was found in case of T_2, T_3, T_4, T_5, T_6, T_7 and T_9 which were significantly different.

4.5.2. Diameter of pileus

Significant difference was recorded in terms of diameter of pileus of oyster mushroom due to different mother spawn materials (Table 5 and Appendix VI). The highest width of pileus (7.49 cm) was observed in T_8 (Rice) which was statistically similar with T_4 (6.87 cm). On the other hand, the lowest width of pileus (5.29 cm) was found in T_1 (Mustard).

4.5.3. Length of stipe

Length of stipe of oyster mushroom ranged 1.71 to 3.14 cm significantly due to mother spawn materials (Table 5 and Appendix VI). The highest length of stipe was observed in T_8 (Rice) whereas, the lowest length of stipe was found in T_1 (Mustard).

Table 5. Effect of mother spawn materials on the dimension of fruiting body of oyster mushroom

Treatments	Length of Pileus (cm)	Diameter of pileus (cm)	Length of stipe (cm)
T_1	4.09b	5.29c	1.71c
T_2	4.73ab	6.66ab	2.76ab
T_3	4.39ab	5.45bc	2.37bc
T_4	4.29ab	6.87a	2.79ab
T_5	4.76ab	6.59ab	2.49abc
T_6	4.44ab	6.23abc	2.48abc
T_7	4.37ab	6.55ab	2.76ab
T_8	5.17a	7.49ab	3.14a
T_9	4.90ab	6.79ab	2.83ab
CV (%)	7.45	10.76	15.57
LSD $_{(0.05)}$	0.89	1.06	0.72

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

[T_1 = Mustard grain; T_2 = Red maize grain; T_3 =50% rice straw grain and 50% saw dust; T_4 = Wheat grain; T_5 = Millet grain; T_6 = Gram grain; T_7 = Pea grain; T_8 = Rice grain; T_9 = White maize grain]

4.6. Yield parameter (g)

4.6.1. Biological yield (g)

Due to mother spawn materials, biological yield of oyster mushroom showed statistically significant variation (Table 6 and Appendix VII). The highest biological yield (175.67 g) was recorded from T_8 (Rice) followed by T_4 (170.00 g) and T_9 (166.67 g). While the lowest biological yield (120.33 g) was recorded in T_1 (Mustard) which was statistically similar with T_3 (124.00 g).

4.6.2. Economic yield (g)

Statistically significant variation was recorded in terms of economic yield of oyster mushroom due to mother spawn materials (Table 6 and Appendix VII). The highest economic yield (168.33 g) was recorded from T_8 (Rice) followed by T_4 (164.67 g) and T_9 (160.00 g), whereas the lowest economic yield (114.67 g) was found in T_1 (Mustard) which was statistically similar with T_3 (118.67 g).

Table 6. Effect of mother spawn materials on yield parameters of oyster mushroom

Treatments	Biological yield (g)	Economic yield (g)
T_1	120.33f	114.67f
T_2	154.33cd	149.00cd
T_3	124.00f	118.67f
T_4	170.00ab	164.67ab
T_5	147.67d	141.67d
T_6	135.67e	130.33e
T_7	162.33bc	156.33bc
T_8	175.67a	168.33a
T_9	166.67ab	160.00ab
CV (%)	3.55	3.85
LSD $_{(0.05)}$	9.17	9.55

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

[T_1 = Mustard grain; T_2 = Red maize grain; T_3 =50% rice straw grain and 50% saw dust; T_4 = Wheat grain; T_5 = Millet grain; T_6 = Gram grain; T_7 = Pea grain; T_8 = Rice grain; T_9 = White maize grain]

4.6.3. Biological efficiency (%)

Remarkable differences were observed in biological efficiency and it was ranged from 24.06% to 35.13%. The highest of biological efficiency (35.13%) was observed in T_8 followed by T_4, while the lowest (24.06%) biological efficiency was recorded in T_1 was statistically similar with T_3 treatment.

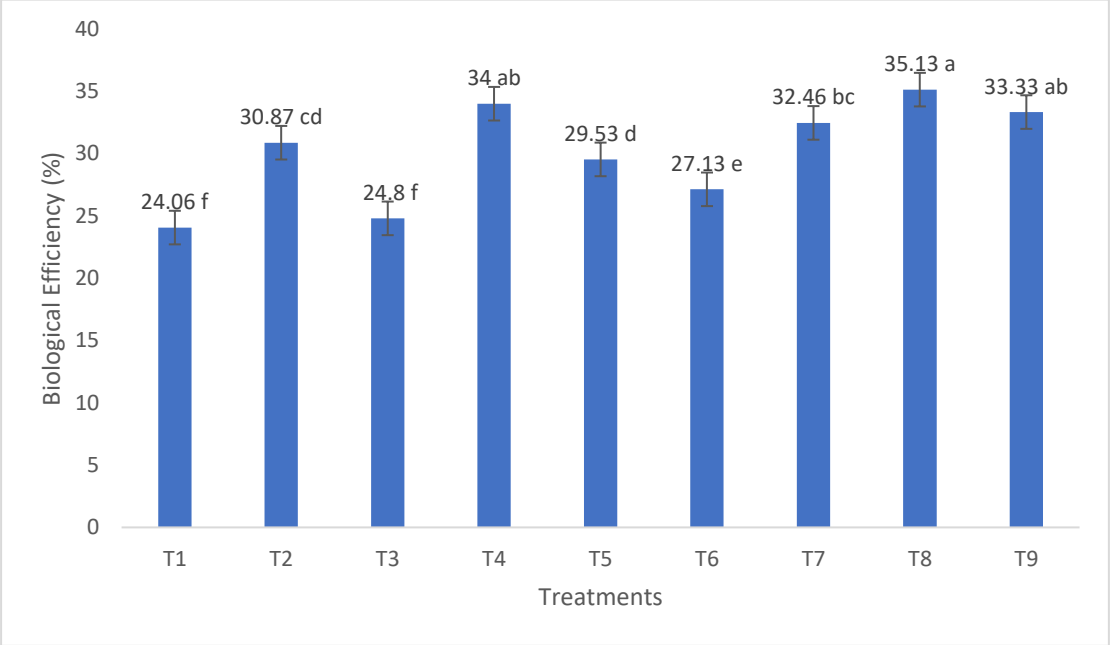


Figure 5. Effect of different mother spawns on biological efficiency of oyster mushroom

[T1 = Mustard grain; T2 = Red maize grain; T3 =50% rice straw grain and 50% saw dust; T4 = Wheat grain; T5 = Millet grain; T6 = Gram grain; T7 = Pea grain; T8 = Rice grain; T9 = White maize grain]

4.7. Relationship between economic yield and different parameters of oyster mushroom

4.7.1. Relationship between the number of primordia per packet and economic yield of oyster mushroom

A significant and linear positive correlation between economic yield and number of primordia per packet of oyster mushroom when different mother spawn materials were used (Figure 5). The relationship between economic yield and number of primordia per packet of oyster mushroom could be expressed by the regression equation, y = 2.1066x – 20.395 (R^2 = 0.9438) where y = economic yield and x = number of primordia per packet. The R^2 value indicated that 94.38% of economic yield of oyster mushroom (*Pleurotus ostreatus*) was attributed to the number of primordia per packet.

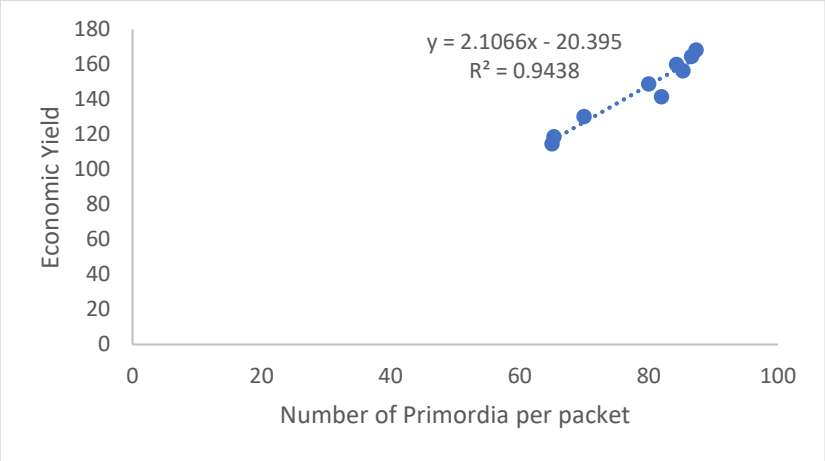


Figure 6. Relationship between the number of primordia per packet and economic yield of oyster mushroom as influenced by different mother spawn materials

4.7.2. Relationship between the number of effective fruiting body and economic yield of oyster mushroom

A highly significant and linear positive correlation between economic yield and number of effective fruiting body per packet of oyster mushroom when different mother spawn materials were used (Figure 6). The relationship between economic yield and number of effective fruiting body per packet of oyster mushroom could be expressed by the regression equation, y = 3.3956x + 33.179 (R_2 = 0.7522) where y = economic yield and x = number of effective fruiting body per packet. The R^2 value indicated that 75.22% of economic yield of oyster mushroom (*Pleurotus ostreatus*) was attributed to the number of effective fruiting body per packet.

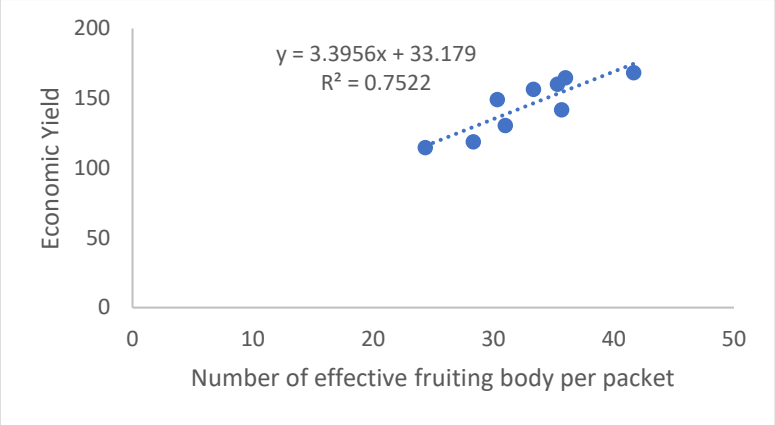


Figure 7. Relationship between the number of effective fruiting bodies per packet and economic yield of oyster mushroom as influenced by different mother spawn materials

4.7.3. Relationship between biological efficiency and economic yield of oyster mushroom

A highly significant and linear positive correlation between economic yield and biological efficiency of oyster mushroom when different mother spawn materials were used (Figure 7). The relationship between economic yield and biological efficiency of oyster mushroom could be expressed by the regression equation, y = 4.6678x + 30.578 (R^2 = 0.9444) where y = economic yield and x = biological efficiency. The R^2 value indicated that 94.44% of economic yield of oyster mushroom (*Pleurotus ostreatus*) was attributed to the biological efficiency.

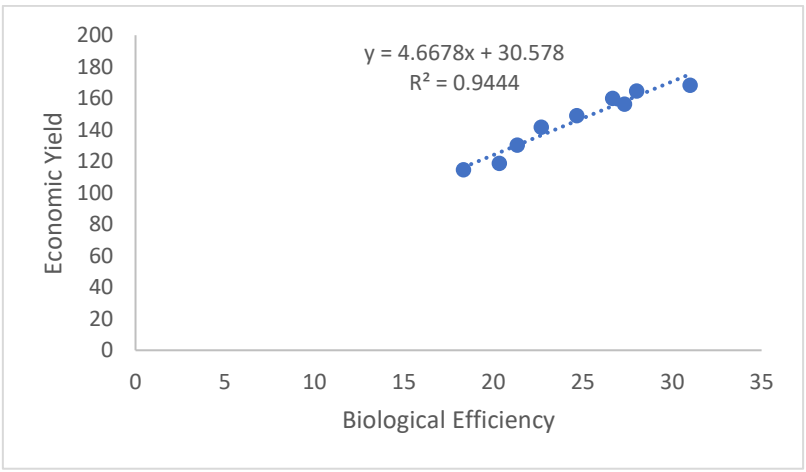


Figure 8. Relationship between biological efficiency and economic yield of oyster mushroom as influenced by different mother spawn materials

CHAPTER V

DISCUSSION

The present experiment was conducted to evaluate the effect of mother culture of different grains on the productivity and contamination of oyster mushroom. Nine grains namely Rice, Maize red, 50% rice straw and 50% saw dust, Mustard, Kaon, Gram, Pea, Wheat, Maize white for comparison were used for oyster mushroom cultivation. Using mother cultures of different grains increased the yield, yield attributes, quality and nutrient contents of mushroom over control in the present study. Grains can be easily used by fungi because grains have more food and nutrients, which allow the fungi to obtain more energy for mycelial growth and mushroom formation. In the present study, during cultivation period four contaminants namely *Trichoderma harzianum*, *Penecillium* sp. *Rhizopus stolonifer* and *Aspergillus niger* were isolated and identified from contaminated substrates. Several researches from all around the world have illustrated the devastating effects of green mould disease in mushroom production caused by *Trichoderma* species like *T. citrinoviride, T. harzianum, T. aggressivum, T. pleuroti, T. viride, T. polysporum, T. longibrachiatum, T. koningii,* and *T. pleuroticola* (Innocenti *et al.,* 2018, Akhter, 2017, Hatvani *et al.,* 2017, Kumar *et al.,* 2017, Kim *et al.,* 2012). Singh *et al.* (2006) recognized *T. harzianum* as the most important species of *Trichoderma* capable of causing green mould disease in many instances and resulting in potential yield losses. *Trichoderma* spp. are very dominant to all fungus and possess high outbreak capacity (Bhandari *et al.,* 2021). *Penicillium* competes for preoccupancy with green spores and inhibits the formation of fruiting bodies, resulting in the spores spreading out in the middle and top portion of the mushrooms bottles (Choi *et al.,* 2003). *Trichoderma* spp., *Aspergillus niger*, *Coprinus* spp., *Penicillium* spp., *Sclerotium rolfsii, Mycogyone perniciosa, Lecanicillium fungicola, Cladobotryum* spp. are some of the important fungal contaminants of mushrooms that are associated with several economically important diseases like green mould, dry bubble, wet bubble, cobweb, etc. (Biswas and Kuiry, 2013; Fletcher and Gaze, 2008). These contaminants deteriorate quality and damage basidiomycetes ultimately leading to reduced production and sometimes complete failure of the crop (Gea *et al.,* 2021). During the transfer of the mother spawn into the autoclaved bags, air carrying air-borne microflora might enter into the bags quickly and lead to contamination of spawn during incubation.

Similar kind of results has been recorded in the present study. Contamination of spawn and identification of major contaminants has been worked out by Akhter (2017), Mazumder and Rathaiah (2001), Kurtzman (2010) and Kumar (2017) and their results agreed with the result of the present experiment.

The highest mycelium running rate was recorded from Rice grain (0.84 cm/day) and lowest rate was found from Mustard treatment (0.6 1cm/day). The present findings corroborated with the findings of previous workers (Maniruzzaman, 2004). Jayachandran *et. al*., (2017) reported that highest mycelium running rate was recorded from rice.

Among the treatments, the maximum days for incubation to mycelium initiation was required for Mustard treatment (7.67 days) and the minimum days was required for rice treatment (5.00 days). The result of the present findings was found similar with Maniruzzaman (2004). Jayachandran *et. al*., (2017) observed that duration from incubation to mycelium initiation was lowest in rice grain. But *Jatwa et. al*., (2017) found that that duration from incubation to mycelium initiation was highest in rice and lowest in sorghum.

Days required for stimulation to primordia initiation ranged from 5.89 days to 7.00 days and the minimum time required for primordia initiation was recorded from rice grain. The result of the present study keeps in with the findings of previous workers (Maniruzzaman, 2004). Maniruzzaman (2004) observed that duration from stimulation to primordia initiation of oyster mushroom was significantly lower in rice as compared to other treatments and the duration required for total harvest of oyster mushroom increased when mustard treatment was used. In the present study, the time required for total harvest decreased with Rice grain. *Jayachandran et. al.,* (2017) showed that rice grain required lowest harvesting period than other grains.

Minimum 5.67 days was required to harvest from primordia initiation due to use of Rice grain. The result of the present study keeps in with the findings of several workers. Maniruzzaman (2004) observed that duration from primordia initiation to first harvest of oyster mushroom was significantly lower in rice as compared to other treatments. Jayachandran *et. al*., (2017) also found the similar result. The maximum number of primordia per packet was found rice (87.33). The result of the present findings keeps in with the findings of previous). The present findings corroborated with the findings of

previous workers (Maniruzzaman, 2004). *Jayachandran et. al*., (2017) reported that highest number of primordia was recorded from rice.

The maximum number of fruiting body per packet (41.67) and maximum effective fruiting body (31) was recorded from rice grain. More or less similar result found from the study of Maniruzzaman, 2004 and Jayachandran et. al., (2017). In the experiment, the highest length of pileus (5.17 cm), width of pileus (7.49 cm) and length of stipe (3.14 cm) was recorded from rice grain and lowest recorded from mustard. More or less similar findings have been reported by previous scientists. 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. *Jayachandran et. al.*, (2017) showed that rice grain (10days) taken for spawn, pinhead formation, stalk length, pileus diameter, pileus thickness.

The maximum biological (175.67) and economic yield (168.33) was observed in Rice among the nine grains. Jayachandran et. al., (2017) showed that rice grain (10days) taken for yield (383.81±0.24) was found to be the best substrate for spawn development of *Pleurotus florida*. Maniruzzaman , 2004 also found that highest biological and economic yield were found in rice grain.

The highest biological efficiency (35.13%) was recorded from rice grain. 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 biological efficiency of oyster mushroom. Jayachandran et. al., (2017) showed that bio efficiency (76.76%) were found in rice grain and 56.64% was found in wheat grain.

CHAPTER VI

SUMMARY AND CONCLUSION

The field experiment was conducted at 'Mushroom Culture House (MCH)' of Sher-e-Bangla Agricultural University, Dhaka. On the other hand, the laboratory experiment was done in 'Plant Pathology Laboratory', of Sher-e-Bangla Agricultural University, Dhaka during the period from November 2020 to March 2021 to study the effect of different mother cultures on the productivity, contamination, nutrient composition and cost benefit ratio of oyster mushroom (*Pleurotus ostreatus*). The experiment consists of nine different type of mother cultures of different grains with three replications to achieve the desire objectives. The treatments are T_1: Mustard; T_2: Red maize; T_3: 50% rice straw and 50% saw dust; T_4: Wheat; T_5: Kaon; T_6: Gram; T_7: Pea; T_8: Rice; T_9: White maize. The experiment was laid out in single factor Completely Randomized Design (CRD). The results obtained in the study of mother culture preparation have been summarized below. The highest mycelium running rate of mother spawn (0.85 cm/day) was recorded when rice grain was used for mother preparation, while the lowest mycelium running rate (0.62 cm/day) was observed in the mother spawn of mustard grain. But maximum time required for incubation to initiation of mother packets (16.67) was found in Mustard and lowest (10.67) was found in rice. Minimum time (16.33) was required for incubation to completion of mycelium running of mother packets in rice and maximum (22.33) was found in mustard. The highest weight of mycelium (4.08) was recorded in rice but lowest (1.84) found in mustard grain spawn. Now the results obtained in the study of yield of oyster mushroom have been summarized below. The highest mycelium running rate (0.84 cm/day) was recorded when rice grain spawn was used for mother preparation, while the lowest mycelium running rate (0.61 cm/day) was observed in mustard. But maximum time required for incubation to initiation (8.67) was found in mustard and lowest (6.00) was found in rice. Minimum time (18.33) was required for incubation to completion of mycelium running in rice and maximum (24.33) was found in mustard. The maximum time required for primordia initiation to 1st harvest (7.33 days) was found in mustard and lowest (5.67) was recorded in rice. The maximum number of effective fruiting body per packet (31.00) was found from rice and the minimum number of effective fruiting (18.33) body was found from mustard. The highest length (5.17 cm) and width (7.49 cm) of pileus

was observed in rice and the minimum length and width of pileus was observed in mustard. The highest biological yield (175.7 g) was recorded from rice and lowest (124.00) was found in mustard. The highest economic yield (168.33) was found in rice and lowest (114.67) was found in mustard.

Percent contamination of fungi were gradually increased with the increase of days after stimulation. After 45 days after stimulation (DAT), the spawn packets of wheat and rice were not contaminated. But the severity of contamination (46.67 %) was observed in mustard treatment, 40.00% in 50% rice straw and 50% sawdust mixturet and 33.33% in gram.

Based on the experimental results, it may be concluded that-

- Rice grain is suitable for oyster mushroom (*Pleurotus ostreatus*) cultivation in Bangladesh based on growth and yield contributing characters.
- Comparatively less contamination severity was observed in the spawn packets prepared from wheat and rice during the cultivation of mushroom.
- Four fungi namely *Trichoderma sp.*, *Penicillium* sp., *Rhizopus stolonifer* and *Aspergillus niger* were isolated and identified from the contaminated spawn.

CHAPTER VII

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CHAPTER VIII

APPENDICES

Appendix I. Temperature and relative humidity of culture house and outside during oyster mushroom cultivation

Duration	Average Temperature (0C) of culture house	Average RH (%) of culture house	Average temperature of outside house (0C)	Average RH (%) of outside of culture house
November	21	85%	28	50%
December	20	80%	25	45%
January	20	75%	25	45%
February	20	75%	27	50%
March	21	85%	28	55%

RH- Relative Humidity

Appendix II. ANOVA table for Mycelium Running Rate (cm/day), days required from incubation to mycelium initiation and days required from incubation to completion of mycelium running (days) of mother spawn for cultivation

Source of variation	Degrees of freedom	Mean Square			
		Mycelium Running Rate	Days Required from Incubation to Mycelium Initiation	Days Required from Incubation to Completion of Mycelium Running	Weight of Mycelium
Between	8	0.0137	8.37	11.25	2.08
Within	18	0.0005	0.89	2.037	0.08
Total	26				

Appendix III. ANOVA table for Mycelium Running Rate (cm/day), days required from incubation to mycelium initiation and days required from incubation to completion of mycelium running (days)

Source of variation	Degrees of freedom	Mean Square		
		Mycelium Running Rate	Days Required from Incubation to Mycelium Initiation	Days Required from Incubation to Completion of Mycelium Running
Between	8	0.0137	2.398	11.25
Within	18	0.0005	0.370	2.03
Total	26			

Appendix IV: ANOVA table for time required from stimulation to primordia initiation, primordial initiation to 1st harvest and Total harvesting period

Source of variation	Degrees of freedom	Mean Square		
		Time required from stimulation to primordia initiation	Time required from primordial initiation to 1st harvest	Total harvesting period
Between	8	0.361	0.833	14.45
Within	18	0.214	0.33	11.11
Total	26			

Appendix V. ANOVA table for number of primordia per packet and number of effective fruiting body per packet

Source of variation	Degrees of freedom	Mean Square	
		Number of primordia per packet	Number of effective fruiting body per packet
Between	8	250.42	51
Within	18	28.63	9.70
Total	26		

Appendix VI: ANOVA table for dimension of fruiting body of oyster mushroom

Source of variation	Degrees of freedom	Mean Square		
		Length of pileus	Width of pileus	Length of stipe
Between	8	0.336	1.311	0.489
Within	18	0.281	0.396	0.186
Total	26			

Appendix VII: ANOVA table for yield parameters of oyster mushroom

Source of variation	Degrees of freedom	Mean Square	
		Biological yield	Economic yield
Between	8	1226.06	1177
Within	18	28.59	31.04
Total	26		

Appendix VIII: Fruiting bodies of snow-white variety of oyster mushroom