INFLUENCE OF SUBSTRATE SUPPLEMENTS ON CONTAMINATION, GROWTH AND YIELD OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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*This is to certify that thesis entitled, "**INFLUENCE OF SUBSTRATE SUPPLEMENTS ON CONTAMINATION, GROWTH 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 requirements for the degree of* MASTER OF SCIENCE in PLANT PATHOLOGY, *embodies the result of a piece of bona-fide research work carried out by **MD. MEHEDI HASAN**, Registration no. 19-10032 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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Dedicated To My
Beloved Parents
And Respected
Teachers

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ABSTRACT

An experiment was conducted at the Mushroom Culture House (MCH) and Plant Pathology Laboratory of the Department of Plant pathology, Sher-e-Bangla Agricultural University (SAU), during July to October 2020, to investigate the impact of enrichment of substrate with different supplements on contaminations, growth and yield of oyster mushroom. The experiment was followed by Completely Randomized Design (CRD) with 3 replications. Sawdust along with different levels of supplement (*viz*: Compost, Vermicompost, Mustard oil cake and their mixtures @ 5, 10 & 15 g) were considered used as different treatments for this present experiment. Application of different doses of supplements along with sawdust showed significant variations in respect of the growth and yield characteristics of mushroom. From the experiment it was revealed that the maximum number of primordia /packet (80), number of fruiting body /packet (54.67), number of effective fruiting body /packet (41), stipe length (2.69 cm), length of the fruiting body (6.56 cm), breadth of the fruiting body (5.67 cm), biological yield (153.67 g), economic yield (116.67 g), dry yield (29.33 g) and biological efficiency (51.22 %) were observed in T_2 (10) g Compost supplemented sawdust spawn packet. While the lowest value was observed in Control. Four contaminants viz *Penicillium sp, Rhizopus sp, Trichoderma sp, Aspergillus sp* were isolated and identified from the contaminated spawn. There was no contamination was observed in T_1, T_2, T_3, T_4, T_{10} and T_{11} during the whole period of experiment whereas the highest contamination severity (77.33%) was recorded from 5g mustard oil cake treated packet at 60 DAT.

LIST OF CONTENTS		
CHAPTER NO.	**TITLE**	**PAGE NO.**
	ACKNOWLEDGEMENT	I
	ABSTRACT	II
	LIST OF CONTENTS	III
	LIST OF TABLES	V
	LIST OF FIGURES	VI
	LIST OF APPENDICES	VII
	LIST OF PLATES	VIII
	LIST OF ABBREVIATION	IX
I	**INTRODUCTION**	1
II	**REVIEW OF LITERATURE**	4
2.1	Supplements	5
2.2	Effect of supplements	5
III	**MATERIALS AND METHODS**	20
3.1	Location of the experimental site	20
3.2	Duration of the experiment	20
3.3	Climate condition during experimentation	20
3.4	Experimental materials	20
3.5	Varietal characteristics of Oyster Mushroom	21
3.6	Treatments of the experiment	21
3.7	Design and layout of the experiment	23
3.8	Preparation of substrates	23
3.9	Preparation of the spawn packets	23
3.10	Sterilization, inoculation and mycelium running in spawn packets	23
3.11	Cultivation of spawn packet	25
3.12	Data collection	25
3.13	Procedure of recording data	26

LIST OF TABLES		
TABLE NO.	**TITLE**	**PAGE NO.**
1	Effect of different levels of supplements on mycelium running rate, days required for completion of mycelium running and primordia initiation of oyster mushroom (*Pleurotus ostreatus*)	35
2	Effect of different levels of supplements on days required from incubation to primordia initiation, primordia initiation to 1st harvest, primordia initiation to final harvest of oyster mushroom	38
3	Effect of chemical treatment of substrate of oyster mushroom on contamination severity	39
4	Effect of different levels of supplements on length of stipe, length of pileus (cm) and breadth of pileus (cm) of oyster mushroom	49
5	Effect of different levels of supplements on biological, economical and dry yield of oyster mushroom (*Pleurotus ostreatus*)	53

LIST OF FIGURES		
FIGURE NO.	**TITLE**	**PAGE NO.**
1	Effect of different levels of supplements on days required from incubation to 1st harvest of oyster mushroom (Vertical bars mention the LSD values at 5% level of probability)	36
2	Effect of different supplements on average number of primordia/packet of oyster mushroom	45
3	Effect of different supplements on average number of fruiting body/packet of oyster mushroom (Vertical bars mention the LSD values at 5 % level of probability)	46
4	Effect of different levels of supplements on average number of effective fruiting body/packet of oyster mushroom	47
5	Effect of different levels of supplements on biological efficiency of oyster mushroom	52
6	Relationship between number of effective fruiting body (g/packet) and economic yield (g) of oyster mushroom (*Pleurotus ostreatus)*	54
7	Relationship between economic yield (g) biological efficiency (%) of oyster mushroom (*Pleurotus ostreatus)*	55

LIST OF PLATE		
PLATE NO.	**TITLE**	**PAGE NO.**
1	Photographs of different treatments **A .** Mustard Oil Cake **B.** Vermicompost Compost **D.** Sawdust	22
2	**A.** Mixing of lime with substrate **B.** Mother of *Pleurotus ostreatus* **C.** Preparation of substrate packets **D-E.** Sterilization of substrate packets by autoclave **F.** Incubation of spawn packets	24
3	Different stages of fruiting bodies **A.** Primordia**, B-D.** Premature fruiting bodies **E.** Matured fruiting bodies	26
4	**A & B**. Different growth stage of mushroom **C.** Measurement of weight of mushroom **D.** Measurement of length of fruiting body **E.** Measurement of breadth of fruiting body **F**. Harvested mushroom	28
5	**A-B.** Boiling of sliced, peeled potatoes (60 g) **B.** Measurement of weight of Dextrose and Agar (6 g each), **D.** Sterilization of glassware in oven **E-F.**PDA solution (300 ml), **G.** Prepared PDA solution poured into sterile Petri dish	31
6	Different contaminated spawn packets during the experiment	40
7	Pure culture of different contaminants obtained from contaminated spawn packet	42
8	Pathogenic structure of different contaminants	43

	LIST OF APPENDICES	
APPENDIX NO.	**TITLE**	**PAGE NO.**
I	Map showing the experimental site under study	68
II	Monthly meteorological information during the period from July to october, 2020.	69
III	Layout of the experiment	70
IV	Analysis of variance on data with the effect of different levels of supplements on mycelium running rate in spawn packet (cm), days required for mother inoculation to completion of mycelium running and days required for stimulation to primordial initiation of oyster mushroom	71
V	Analysis of variance on data with the effect of different levels of supplements on days required from incubation to harvest, days required from 1st primordia initiation to 1st harvest, days required from 1st primordia initiation to final harvest of oyster mushroom (*Pleurotus ostreatus*)	71
VI	Analysis of variance on data with the effect of different levels of supplements on number of primordia /packet, number of fruiting body /packet and number of effective fruiting body/packet of oyster mushroom *(Pleurotus ostreatus)*	72
VII	Analysis of variance on data with the effect of different levels of supplements on length of stripe (cm), length of fruiting body (cm) and breadth of fruiting body (cm) of oyster mushroom *(Pleurotus ostreatus)*	72
VIII	Analysis of variance on data with the effect of different levels of supplements on yield and biological efficiency of oyster mushroom (*Pleurotus ostreatus*)	73

LIST OF ABBREVIATIONS

ABBREVIATIONS	Full word
AEZ	Agro-Ecological Zone
AIS	Agriculture Information Service
Anon.	Anonymous
@	At the rate
BARC	Bangladesh Agricultural Research Council
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
BE	Biological efficiency
BNNC	Bangladesh National Nutrition Council
CV %	Percent of Coefficient of Variance
cv.	Cultivar (s)
eds.	Editors
et al.	And others
etc.	et cetera (and other similar things)
FAO	Food and Agriculture Organization
i.e.	That is
L.	Linnaeus
LSD	Least Significant Difference
MCC	Mushroom Culture Centre
MRR	Mycelium Running Rate
MDI	Mushroom Development Institute
SAU	Sher-e-Bangla Agricultural University
UNDP	United Nations Development Program
var.	Variety
viz.	Namely

CHAPTER I

INTRODUCTION

Mushroom is quite possibly the most diverse organisms on earth and since primitive times have played an indispensable role in human welfare (Martínez-Ibarra *et al*., 2019). 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, normally produced above the ground on soil or on its food substrate. It has been universally utilized as a food and medication by different civilizations science ancient time because of its delicious taste, flavor, dietetic characteristics and a several medicinal properties (Martínez-Ibarra *et al.,* 2019; Kakon *et al.,* 2015 and Ng'etich *et al.,* 2013). The fresh mushroom contains around 85-90% moisture, 3% protein, 4% carbohydrates, 0.3-0.4% fats and 1% minerals and nutrients, just as some medicinal properties like bringing down blood cholesterol level, safeguard against disease and stimulating hair growth (Miah *et al.,* 2017). Edible mushrooms are additionally rich in nutrients like niacin, riboflavin, vitamin D, C, and B complex (Ahmed *et al*., 2009). FAO suggested edible mushrooms as a food to meet protein requirement of developing countries whereas enormous number of populations relies mainly on cereal crops (WB, 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. Mushroom cultivation can help reduce vulnerability to poverty and strengthens livelihoods through the generation of a fast yielding and nutritious source of food and a reliable source of income (Marshall and Nair, 2009). 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. Wildly, 20 types of mushroom are cultivated in the country, of which 5-6 are harmful; furthermore, the suggested species for development are oyster (*Pleurotus spp*.) and white button mushroom (*Agaricus bisporus*) (Banglapedia, 2019).

Bangladesh is one of the most suitable countries in the world for mushroom cultivation owing to its positive environment with low production cost, availability of growing substrates and high market value (Imtiaj and Rahman, 2008; Uddin *et al.,* 2011).

The nutritional status of Bangladeshi people is a matter of great concern as the greater part of the population have been suffering from malnutrition (ICDDRB, 2019). Consumption of mushrooms as food can relieve the suffering from malnutrition to some extent, since they produce in huge amounts within a short period and give more protein per unit area than any other crops (Gupta, 1986). Crop diversification and change of food habit are very vital to very crucial to build up the national health (Dey, 2010). The demand and consumption of mushrooms are expanding day by day and mainly due to reason that small entrepreneurs are coming forward for cultivation, production and marketing of mushrooms and mushroom based products in the country (Sarker *et al.,* (2015). Despite the fact that there is a huge possibility of mushroom production in Bangladesh, there are some issues during cultivation and promoting; which are essential to be addressed and as such steps could be taken to boost production of this crop (Rahman, 2018). Then again, mushrooms lack much consideration as a food items in Bangladesh due to negative attitude of the people thinking it as "toad-stool" and feeling doubt whether it is Halal or not (Easin *et al.,* 2017). In spite of the technology of mushroom cultivations is somewhat recent advancement and consolidation of this non-conventional crop in existing agricultural system can help in improving the social just as economic status of rural farmers and sub-urban dwellers of the country. Considering the importance of mushroom cultivation, Government of Bangladesh has established Mushroom Development Institute (MDI) in 2014 at Savar of Dhaka to impart training and promotion of mushroom farming, previously which was named National Mushroom Development and Extension Centre (NMDEC).

Mushroom can be easily grown on cellulose, hemicellulose and lignin rich natural substrates like sawdust, leaves and other agro by-products (Neupane *et al*., 2018; McGrath, 2003). Sawdust, a by-product of the saw-mill industries, is largely available and has been considered a possible alternative for mushroom cultivation. Sawdust alone can not give rise to commercially viable crops. Additional nitrogen, phosphate and potassium are required for the cultivation of mushrooms with sawdust as the ligno-cellulosic materials in sawdust are generally low in protein content and thus insufficient for the cultivation of mushrooms. It has been well established that deficient supply of proper nutrients with natural lignocellulosic substrates dynamically affects of mushroom cultivation phases (Xing *et al.*, 2006; Naraian *et al*., 2014). The deficiency of several nutrients is improved by the deliberative supplementation of external

compounds changed due to different supplement used in sawdust based substrates (Bhuyan, 2008).

Mushroom supplementation is understood as a farming method based on the physical addition of nutritional amendments to substrate, during the process of subtracting, the mixture of raw materials, at spawning or during casing (Estrada *et al*. 2009; Pardo-Giménez *et al.* 2012a, 2016). Addition of organic and inorganic supplements to the substrates during cultivation shorten the crop period of *Pleurotus spp.* and also increases mushroom productivity (Curvetto *et al.,* 2002; Naraian *et al*., 2009). In this regard, various additives are recommended as supplements (such as compost, vermi-compost, mustard oil cake etc) to the basal substrates for enhancement of oyster mushrooms yield (Ralph and Kurtzman, 1994).

Although supplementing the substrate material leads to improved growth, there are limitations involved. For instance, high supplementation of substrate may lead to contamination (Yildiz *et al.,* 2002) together with reduced yield of the mushrooms (Fanadzo *et al.,* 2010).

Considering the above facts the present study was undertaken to find out the suitable supplements for mushroom production with the following objectives:

- To estimate the severity of contamination of oyster after using of different supplements with substrates
- To identify the contaminants from the contaminated spawn packets
- To determine the impact on growth, yield and yield contributing characters of oyster mushroom

CHAPTER II

REVIEW OF LITERATURE

An attempt was made in this section to collect and study relevant information available regarding the influence of enrichment of substrate with different supplements on contaminations, growth and yield of oyster mushroom to gather knowledge helpful in conducting the present piece of work.

2.1 Supplements used in mushroom production

According to Zied *et al.* (2018) the practice of supplementation has been an important approach for improving yield in the industrial production of the mushroom.

Pardo-Giménez *et al.* (2016) opined that the majority of supplements sold commercially are currently based on nitrogen rich compounds, and it is currently unclear whether the use of low-protein supplements based on carbon-rich sources such as cellulose and hemicellulose components improves the performance of the mushroom equally or even more than nitrogen addition. In comparison to protein-rich components, ingredients with high content of carbohydrates, such as agricultural and commercial waste products, are cheaper and readily available in local producing areas However, it is noteworthy that the use of commercially available protein-based nutrients is a profitable investment since mushroom yield consistently increases and ultimately also mushroom quality. Besides, the use of nutritional additives is a useful tool to partially recycle the SMS (spent mushroom substrate) into new growth cycles, in an effort to building a circular economy involving waste management, and to increase the biological efficiency of alternative cultivated species in order to diversify the industry.

Naraian *et al.* (2009) reported that supplements, which are based on slow nutrient release formulas, can be applied at different points along the mushroom cropping cycle. They are most commonly applied at the end of the substrate preparation, prior to spawning, to promote the vegetative growth throughout the substrate.

2.2 Effect of supplements on growth of mushroom

2.2.1 Mycelium running rate

Hasan *et al.* (2015) conduct a study to evaluate the effect of supplementing of different levels of wheat bran with sugarcane bagasse on the production of pink oyster mushroom (*Pleurotus djamor*) and find out their yield and proximate composition. The sugarcane bagasse was mixed at spawning with 0%, 10%, 20%, 30%, 40%, and 50% of wheat bran supplement and arranged in a complete randomized design with three replications, three spawn packets in each replication and six treatments. Experiment result revealed that the mycelium running rate of oyster mushroom greatly influenced with the supplement of wheat brans in different levels. The highest running rate was observed in 50% (0.96 cm) and lowest in 0% (0.72 cm). The other treatments varied significantly over control.

Nuruddin *et al.* (2010) carried out a study to know the effect of cow dung supplements with rice straw on the yield and proximate composition of *Pleurotus ostreatus*. Five different levels, T_1= 0% (Control), T_2= 5%, T_3= 10%, T_4=15%, T_5= 20% of cow dung were evaluated as the supplement to rice straw substrate of oyster mushroom. The experiment was laid out in Completely Randomized Design with three replications. From the experiment result revealed that the highest mycelium running rate was observed in T_3 (0.70 cm/day) and the lowest running rate of mycelium was observed in T_1 (0.52 cm/day). The other treatments varied significantly over control treatment.

Kulsum *et al.* (2009) noticed that the maximum mycelium running rate was 0.71 cm because of sawdust enhanced with cow compost @ 15%.

Khan *et al.* (1991) reported that sawdust amended with various natural supplement like wheat chaff, wheat bran, rice straw, cotton waste etc. provided appropriate condition for spawn running.

2.2.2 Completion of mycelium running

Seephueak *et al.* (2019) carried out an experiment to determine the optimum rate of palm oil sludge for *Ganoderma* mushroom (*Ganoderma lucidum* (Fr.) Karst) cultivation. Different concentrations of palm oil sludge (5-20% by dry weight) were mixed with rubber sawdust and used to grow the Ganoderma mushroom in plastic bags.

Experiment revealed that the case in which *G. lucidum* substrate was rubber sawdust supplemented with 15% palm oil sludge gave the fastest mycelial growth. The growth period was 42.85 days, followed by rubber sawdust supplemented with palm oil sludge at 20%, 5%, 8% and 10% in this order, giving 45.25, 45.68, 45.68 and 45.73 days, respectively, without statistically significant difference to the 47.63 days with 5% rice bran. Non-supplemented substrate gave the slowest mycelial growth taking 54.69 days.

Shalahuddin *et al.* (2018) reported that the time required completing mycelium running of oyster mushroom varied significantly due to different chemical nutrients applied on the substrate as nutrient supplements. The highest time (20.40 days) required to complete the mycelium running was recorded from T_1 (only 10 kg straw) while, the lowest time (16.20 days) required to complete mycelium running was found in T_3 (4 g NPK in 10 kg straw) treatment.

Satpal *et al.* (2017) reported that the minimum days for spawn run (18 days) observed starch, which was statistically at par with all other spawns except to dextrose and control spawn (without sugars). The maximum days for spawn run (25 days) were observed in control spawn (without sugars) which was significantly higher than all other treatments. The minimum days for first harvesting (23 days) was observed glucose, which was statistically similar with starch and maltose. The maximum days for first harvesting (30 days) was observed in control spawn (without sugars) which was significantly higher than all other treatments.

Seephueak *et al.* (2014& 2016) reported that 5% or 8% of palm oil sludge was suitable for the mycelial growth of phoenix mushroom (*P. pulmonarius*) and for jew's ear (Auricularia polytricha Mont Sacc.) cultivation.

Tikdari and Bolandnazar (2012) reported that the days required to completion of mycelium running in substrate was significantly affected by different supplements and it ranged from 18.66 to 23 days. The minimum time (18.66 days) was recorded from soybean meal 7.5%, and the maximum time (23 days) was recorded from vermicompost (2.5%) supplements treated with substrate.

Mahjabin *et al*. (2011) reported that the minimum days (13.25) required for completion of mycelial growth was observed in chemical nutrients whereas the highest days (31.75) required for mycelial growth was observed in 5 g NKP in 10 kg straw.

Sobhan (2006) found that the highest duration to complete spawn running was found in mustard oilseed cake at 50% level which might be due to the antifungal properties of mustard oilseed cake.

Kalita *et al.* (2001) observed that time taken for completion of spawn running may required from 17 days to 22 days by use of various substrates.

2.2.3 Stimulation to primordia initiation

Arsia *et al*. (2018) carried out an study to know the effect of supplements on pin head emergence and biological efficiency of three *Pleurotus* spp. Nine different brans and flours *viz.*, gram flour, gram chokar, bajra flour, jawar flour, wheat bran, rice bran, and maize bran were used with wheat straw for supplementation at the rate of 5 per cent on the dry weight basis of substrate and found that *P. flabellatus* takes minimum period (18 days) was recorded with bajra flour whereas, maximum pin head emergence period of 21 days in both control and gram powder was recorded.

Hasan *et al. (*2015) evaluated the effect of supplementing different levels of wheat bran with sugarcane bagasse on the production of pink oyster mushroom (*Pleurotus djamor*) and find out their yield and proximate composition. The sugarcane bagasse was mixed at spawning with 0%, 10%, 20%, 30%, 40%, and 50% of wheat bran supplement. The duration from stimulation to primordia initiation ranged from 3.33 days to 5.50 days. The highest time from stimulation to primordia initiation was observed in 30% (5.50 days) wheat bran supplement. Duration of primordia initiation to first harvest of oyster mushroom was significantly lower as compared to control where no supplement was used and the duration required for total harvest of oyster mushroom increased with the level of supplement used.

Alam *et al.* (2010) carried out an investigated to identify the most suitable supplements and their levels for the commercial cultivation of milky white mushroom. Rice bran, maize powder, and wheat bran with their different levels (10, 20, 30, 40, and 50%) were used as supplements to evaluate the yield and yield contributing characteristics of *C. indica*. Primordia formation was observed in all experimental sets. From the experiment

result revealed that the shortest time (13.5 days) for primordia initiation was recorded in the 40% rice bran supplement, followed by the 20% (14.8 days), and 10% (15.0 days) wheat bran supplement. The longest time (19.3 days) was recorded in 0% level of unsupplemented control rice straw substrate. Rice bran and maize powder are rich in nutrients, which might have promoted the growth and development of *C. indica* primordia and fruiting bodies.

Nuruddin *et al.* (2010) carried out a study to know the effect of cow dung supplements with rice straw on the yield and proximate composition of *Pleurotus ostreatus*. Five different levels, T_1= 0% (Control), T_2= 5%, T_3= 10%, T_4=15%, T_5= 20% of cow dung were evaluated as the supplement to rice straw substrate of oyster mushroom. The experiment was laid out in Completely Randomized Design with three replications. From the experiment result revealed that the the highest time from stimulation to primordia initiation was observed in T_1 (7.23 days) and the lowest time from stimulation to primordia initiation was in the treatment T_4 & T_5 (6.03 days).

Ali (2009) observed that the maximum time from stimulation to primordia initiation was (11.5 days) because of sugarcane bagasse supplemented with wheat bran @ 10%.

2.2.4 Primordia initiation to harvest (days)

Singh *et al*. (2017) carried out an experiment with the aim to evaluate the most effect organic supplement for enhance the growth and yield of oyster mushroom (*Pleurotus djamor*). In the present study five locally available different organic supplements viz. soybean, chickpea gram, black gram, pigeon pea and lentil flour were mixed in wheat straw. The results obtained during the present investigation revealed that the maximum days for cropping period (60.33 days) were observed at control i.e. straw without organic additive which was significantly similar with pigeon pea flour as well as soybean flour. The minimum days for cropping period (55.33 days) were observed at chick pea flour which was significantly lower than all other flours.

Nuruddin *et al.* (2010) carried out a study to know the effect of cow dung supplements with rice straw on the yield and proximate composition of *Pleurotus ostreatus*. Five different levels, T_1= 0% (Control), T_2= 5%, T_3= 10%, T_4=15%, T_5= 20% of cow dung

were evaluated as the supplement to rice straw substrate of oyster mushroom. The experiment was laid out in Completely Randomized Design with three replications. From the experiment result revealed that the time from primordia initiation to harvest was lowest in the treatment T_3 (3.63 days) and it was the highest in the treatment T_1 (5.06 days) followed by T_5 (5.16 days).

Kulsum *et al.* (2009) reported that the lowest time required from primordia initiation to harvest was 3.2 days due to sawdust supplemented with cow dung @ 10%.

2.2.5 Number of primordia

Seephueak *et al.* (2019) carried out an experiment to determine the optimum rate of palm oil sludge for *Ganoderma* mushroom (*Ganoderma lucidum* (Fr.) Karst) cultivation. Different concentrations of palm oil sludge (5-20% by dry weight) were mixed with rubber sawdust and used to grow the *Ganoderma* mushroom in plastic bags. The experiment result revealed that the number of basidiocarps of G. *lucidum* on 850 g substrate in a plastic bag at the 4th flush harvesting with significant differences between the treatments indicated ($P<0.01$). From the first flush to the 3rd flush there were significant differences between supplemented cases and no use of supplement. However, in the 4th flush the number of basidiocarps showed no significant differences. The *G. lucidum* on rubber sawdust supplemented with palm oil sludge gave 4.52-5.04 basidiocarps/bag, which does not significantly differ from the case of sawdust with 5% rice bran giving 5.12 basidiocarps/bag. Non-supplemented sawdust had the lowest average number of basidiocarps at 1.78 basidiocarps/bag.

Hasan *et al.* (2015) reported that the primordia/packet significantly differ due to sugarcane bagasse supplemented with various levels of wheat bran (0, 10,20, 30, 40 & 50 %) application. The highest average number of primordia/packet was observed in 10% (176.3) followed by 20% (159.0), 30% (148.0) and 50% (146.0) and the lowest average number of primordia/packet were in 0% (75.33) *viz* control treatment. The number of primordia significantly varied with the supplements and substrates used in production of oyster mushroom.

Kulsum *et al.* (2009) observed that the highest average number of primordia/packet was 73.21 due to sawdust supplemented with cow dung @ 10%.

Pathan *et al*. (2009) reported that 5 g NPK in 10 kg straw was the best in relation producing average number of primordia per packet.

Amin *et al.* (2007). in his experiment found that the highest number of primordia of oyster mushroom was found in nutrients paddy straw but lowest was found in control treatment.

2.2.6 Number of fruiting body

Salama *et al.* (2019) carried out study in growth chamber on *Pleurotus ostreatus* L. strain 66 to investigate the effect of different levels of supplements on yield quantity and quality of oyster mushroom during the two seasons of 2016/2017 and 2017/2018. Rice straw substrate along with four supplements with three levels of each one was used in this investigation i.e. wheat and rice bran were added the levels of 5, 15 and 25%, while the urea and zinc sulfate were added the levels of 0.5, 1.5 and 2.5% were examined. The obtained results showed that, significant differences in fruit number /bag during the two experimental seasons. The highest fruit number/bag was obtained from wheat bran treatment (17.88 and 17.66), while the second level of supplement gave the best result (12.26 and 12.20) in the both seasons respectively. The interaction between treatments and additive levels exhibited highest fruit number/ bag 23.00 and 22.66) which obtained from wheat bran with the second level in the both seasons respectively.

Bird *et al.* (2017). stated that supplementation of substrates in *A. bisporus* with trace elements has been described as reliable for the production of fruiting bodies enriched with Se, Cu and Zn micronutrients that frequently are deficient in the human diet.

Satpal *et al.* (2017) concluded that the highest number of fruiting body (29.20) was observed in dextrose spawn, which was significantly higher than all other treatments. The minimum number of fruiting body (17.00) was observed control spawn (without sugars) which was significantly lower than all other treatments.

Hasan *et al.* (2015) reported that the fruiting body/packet significantly differ due to sugarcane bagasse supplemented with various levels of wheat bran (0, 10,20, 30, 40 & 50 %) application. The maximum average number of fruiting body/packet was

observed in 10% (77.67) followed by 30% (69.67) and the lowest average number of fruiting body /packet were in 50% (49.00). The number of fruiting body increased with the levels of supplement and continued up to a certain range and decline thereafter.

Jafarpour *et al.* (2012) carried out a study to evaluated combination usage of substrates including wood chips, boll, sugar beet pellet pulp (SBPP) and palm fiber along with wheat bran, rice bran, soya cake powder, Soya Cake Powder + Rice Bran (SCPRB) and carrot pulp as supplements. Substrates with no supplement were regarded as control groups. Results revealed that the minimum and maximum fruiting body number was pertained to palm fiber (17.6) and boll substrate (28.9), respectively. Rice bran supplement and soya cake powder caused to 24.17 and 23.42 fruiting bodies, respectively. The best performance for fruiting bodies was achieved when both substrate and food supplement were applied together (Combination boll substrate with supplement (34.3) and combination of boll substrate with wheat bran supplement (30) fruiting bodies were observed. However, addition of food supplements on boll substrate did not increased fruiting body number which was the only exception for combination usage of substrate and food supplements.

2.2.7 Length of stipe

Sanjel *et al.* (2021) reported that the highest average stipe length was found in molasses supplementation (2.69cm), that was found to be statistically at par (2.18cm) with control (Finger millet husk as substrate with no supplements), rice bran supplementation (2.33 cm) and wheat bran supplementation (2.27 cm). The lowest stipe length was found in mustard oilseed cake supplementation (1.41cm). Therefore, control, molasses supplementation, rice bran supplementation and wheat bran supplementation were found to be statistically different from mustard oilseed cake supplementation.

Salama *et al.* (2019) carried out an study in growth chamber on *Pleurotus ostreatus* L. strain 66 to investigate the effect of different levels of supplements on yield quantity and quality of oyster mushroom during the two seasons of 2016/2017 and 2017/2018. Rice straw substrate along with four supplements with three levels of each one was used in this investigation i.e. wheat and rice bran were added the levels of 5, 15 and 25%, while the urea and zinc sulfate were added the levels of 0.5, 1.5 and 2.5% were examined.

The obtained results showed that, significant differences in stalk length during the two seasons. The tallest stalk length resulted from wheat bran treatment (4.45 and 4.49 cm), in contrast the second level of supplement which gave the longest stalks (3.73 and 3.71 cm) in the first and second seasons. Interaction between treatments and additive levels showed the higher stalk length (5.06 and 4.92 cm) was found from urea with the third level of supplement during the two experimental seasons.

Tikdari and Bolandnazar (2012) carried out an investigate to know the effect of nutritional supplements in substrate on yield of oyster mushroom (*Pleurotus florida*) an experiment was carried out in Completely Randomized Design whit three replications. In present study three nutritional supplements, including alfalfa meal, soybean meal and vermicompost (2.5, 5 and 7.5%) and control were evaluated. Result revealed that the length of stipe differed significantly ($p < 0.05$) between treatments and ranged from 3.86 to 5.86 cm. The highest of stipe was recorded in vermicompost 2.5% (5.86 cm) and the lowest length of stipe was recorded in soybean meal 5% (3.86 cm).

2.2.8 Length of fruiting body

Sanjel *et al.* (2021) reported that the average pileus diameter was found to be highest in molasses supplementation (6.94cm), which was statistically at par with control (Finger millet husk with no supplements) (6.82cm), rice bran supplementation (6.76 cm) and wheat bran supplementation (6.84 cm). The lowest pileus diameter was found in mustard oilseed cake supplementation (6.06 cm). Therefore, control, molasses supplementation, rice bran supplementation and wheat bran supplementation were statistically different from mustard oilseed cake supplementation in terms of average pileus diameter.

Chinara and Mahapatra (2020) reported that the morphological characters (pileus diameter, stipe length and stipe diameter) of mushrooms varied depending upon the application of additives. Maximum pileus diameter (141.1 mm) was recorded in the mushroom harvested from bags (Dry paddy straw substrate) supplemented with groundnut cake which was significantly superior to that of bengal gram (134.3 mm) and maize meal (130.5 mm) supplements.

Salama *et al.* (2019) conducted an experiment in growth chamber on *Pleurotus ostreatus* L. strain 66 to investigate the effect of different levels of supplements on yield quantity and quality of oyster mushroom during the two seasons of 2016/2017 and 2017/2018. Rice straw substrate along with four supplements with three levels of each one was used in this investigation i.e. wheat and rice bran were added the levels of 5, 15 and 25%, while the urea and zinc sulfate were added the levels of 0.5, 1.5 and 2.5% were examined. The obtained results showed that, significant differences in cap diameter during the both experimental seasons. Largest cap diameter resulted from wheat bran treatment (10.55 and 10.63 cm), while the second level of supplement gave the largest one (9.15 and 8.98 cm) during the two seasons. The interaction between treatments and additive levels show best value of cap diameter (13.56 and 13.71 cm) was obtained from wheat bran with the second level of additive during the two seasons, respectively.

Tikdari and Bolandnazar (2012) carried out an investigate to know the effect of nutritional supplements in substrate on yield of oyster mushroom (*Pleurotus florida*) an experiment was carried out in Completely Randomized Design whit three replications. In present study three nutritional supplements, including alfalfa meal, soybean meal and vermicompost (2.5, 5 and 7.5%) and control were evaluated. Result revealed that the maximum length (6.80 cm) was observed in vermicompost 2.5% and the lowest (5.55 cm) was observed from soybean meal 5% without any significant difference with control.

Hasan *et al.* (2015) stated that the 30% wheat bran supplement showed the highest average diameter (6.53 cm) of pileus and the lowest in 50% (5.68 cm).

Alam *et al.* (2010) reported that the maximum diameter of the pileus (7.1 cm) was obtained with the 30% maize powder supplement, followed by 30% wheat bran (6.9 cm) and 50% rice bran supplements (6.6 cm) for rice straw substrate.

2.2.9 Breadth of fruiting body

Seephueak *et al.* (2019) reported that the average thickness of pileus was the highest at 1.10 cm./basidiocarp on non-supplemented substrate, followed by the case of rubber sawdust supplemented with 5% rice bran at 1.04 cm./basidiocarp. Using 5- 20% palm oil sludge resulted in 0.89-0.97 cm./basidiocarp, without significant differences.

2.2.10 Biological yield

Sanjel *et al.* (2021) reported that the highest total fresh mushroom yield was obtained from rice bran supplementation (793.04g/bag) with highest biological efficiency (137.92%) which was closely followed by control (780.59g/bag) with biological efficiency(135.75%), molasses supplementation (763.21g/bag) with biological efficiency (132.73%), wheat bran supplementation (721.9g/bag) with biological efficiency(125.54%), whereas the lowest fresh mushroom yield was obtained from mustard oilseed cake supplementation (521.84g/bag) with lowest biological efficiency (90.75%). Therefore, control (Finger millet husk with no supplements), molasses supplementation, rice bran supplementation and wheat bran supplementation are statistically at par with each other for total fresh mushroom yield and biological efficiency whereas they are significantly different with mustard oilseed cake supplementation.

Seephueak *et al.* (2019) reported that the highest yield was 74.82 g/ bag (B.E.=22.01%) obtained on rubber sawdust supplemented with 5% palm oil sludge, followed by 8%, 10%, 15% and 20% of palm oil sludge supplement with the yields 74.75 g, 71.88 g, 69.44 g and 65.06 g/bag, respectively.

Shalahuddin *et al.* (2018) conducted a study to investigate the effect of different chemical nutrients (NPK) on the production and proximate composition of oyster mushroom (*Pleurotus ostreatus*). Mother culture of oyster mushroom was used as test crop for this experiment. The experiment consists of four different mixers of chemical nutrients *viz.* T_1 (only 10 kg straw); T_2 (2 g NPK in 10 kg straw); T_3 (4 g NPK in 10 kg straw) and T_4 (6 g NPK in 10 kg straw). Where NPK was kept as 2:1:1. The highest biological yield (282.36 g) was attained from T_3 treatment.

Satpal *et al.* (2017) conducted a study with the aim to find out the most favorable sugar for the improvement of spawn quality and production in minimum days and its effect on yield and growth of oyster mushroom (*Pleurotus djamor*). In the present Study five different sugars viz. Dextrose, Maltose, Starch, Sucrose and Glucose were mixed as a supplement with wheat grain for spawn production and effects of its spawn on the yield of sporophores and growth of *Pleurotus djamor* were observed. Result revealed that the effects of different sugars added spawn, the data revealed that, maximum yield (613.33g/Kg of dry substrate with 61.33% B.E.) was observed in glucose spawn which was significantly similar with starch spawn. It was followed by sucrose and maltose spawn (500 g/kg, 500g /kg of dry substrate with 50, 50.00% B.E.). The minimum yield was recorded in control spawn (without sugars) (40g/Kg of dry substrate with 40.00% B.E.) which was significantly lower than all other treatments.

Pardo-Giménez *et al.* (20167 2018) reported that, compost supplementation with defatted pistachio meal and defatted almond meal significantly improved the quality of white button mushroom, *A. bisporus,* (larger mushrooms with firmer texture and greater content in dry weight and protein) and increased more than 30% the yield in oyster mushroom, *P. ostreatus,* in comparison to non-supplemented substrates.

Krupodorova and Barshteyn (2015) reported that the slight growth of mushroom species on the mustard oilseed cake that contains 37% protein, 6% carbohydrate and 11% fat indicating that probably the high fat content in the cake doesn't contribute to the mushroom growth and also probably due to the limitation of the essential components.

Sharma (2009) in her study found that supplementation of 2% wheat bran and 2% rice bran resulted 48.1 % and 48.3% increase in yield of *P. ostreatus* respectively over the control.

Viziteu (2004) in his research reported that supplementation of *P. ostreatus* with additives didn't increase the productivity significantly. The decreased yield may be due to the higher dose of supplements i.e. greater than 2-3%.

Randle (1983) observed that 2-20% increases in mushroom yield are possible with the addition of delayed-release nutrient supplements.

Gupta and Vijay (1991) reported that supplementation above 2% resulted in under heating of compost which may be the cause for reduced yield.

2.2.11 Economic yield

Shalahuddin *et al.* (2018) conducted a study to investigate the effect of different chemical nutrients (NPK) on the production and proximate composition of oyster mushroom (*Pleurotus ostreatus*). Mother culture of oyster mushroom was used as test crop for this experiment. The experiment consists of four different mixers of chemical nutrients *viz.* T_1 (only 10 kg straw); T_2 (2 g NPK in 10 kg straw); T_3 (4 g NPK in 10 kg straw) and T_4 (6 g NPK in 10 kg straw). Where NPK was kept as 2:1:1. The highest economic yield (267.38 g) was recorded in T_3 treatment, again the lowest economic yield (208.11 g) was observed in T_1.

Zied *et al.* (2018). stated that in addition, waste materials, including agro-industrial waste (provided by peanut and acerola juice) and noble grains, a mix with bran of soybean, corn, and cotton have been proved effective to increase the industrial yield, which highlights materials with high S, Cu, and Mn contents as ideal supplements.

Moonmoon *et al.* (2011) reported that Sawdust supplemented with different levels of wheat bran, rice bran or maize powder improved yield and quality of *Lentinula edodes*, with 25% wheat bran and 40% wheat bran reported as the best rate to obtained highest yield and best quality respectively.

Kalmis *et al.* (2008) reported that the wheat substrate and supplementation with 25% olive mill effluent gives economic mushroom yield.

2.2.12 Dry matter

Hasan *et al.* (2015) stated the dry yield of the oyster mushroom, grown on sugarcane bagasse responded significantly with the different levels of supplement (0,10, 20,30,40 and 50 % of wheat bran). The dry yield of mushroom was higher (12.47.40 g) in 10% and minimum (8.637 g) control wheat bran.

2.2.13 Biological efficiency

Arsia *et al*. (2018) carried out a study to know the effect of supplements on pin head emergence and biological efficiency of three *Pleurotus spp*. Nine different brans and

flours *viz.*, Gram flour, gram chokar, bajra flour, jawar flour, wheat bran, rice bran and maize bran were used with wheat straw for supplementation at the rate of 5 per cent on the dry weight basis of substrate and found that the maximum biological efficiency of *Pleurotus flabellatus* was 98.5% with jowar flour supplementation followed by maize bran (95.0%) with wheat straw (as substrate). The height biological efficiency (93.5%) of *P. florida* was recorded with rice bran supplementation followed by maize bran (93.5%) and gram flour (87.5%) supplementation. While, in *P. sajor-caju*, it the maximum (89.5%) BE was recorded with gram chokar followed by (88.0%) bajra flour supplementation with wheat straw and minimum (70.5%) was obtained in control (wheat straw only).

Picornell-Buendía *et al.* (2016) observed that substrate formulations with material based on wheat straw and spent *Pleurotus* substrates supplemented with wheat bran and the commercial supplement (Calprozime®) have shown good agronomic performance for *P. ostreatus*, with supplemented mixtures producing mushrooms of higher protein and ash content. In addition, quantitative parameters, such as good biological efficiency (BE), high quantity of mushrooms and an excellent unit weight of the fruiting bodies have been achieved with this supplemented SMS (spent mushroom substrate) when employed to re-grow *P. ostreatus*. Therefore, spent mushroom substrates supplemented with protein-rich additives can be potentially employed as a cheap base material to grow *P. ostreatus* and simultaneously implement a circular economy based on the integral management of wastes.

Rugolo *et al.* (2016) reported that the mushroom cultivation on substrates which were supplemented with 20–40% composted or 20% raw two-phase olive mill waste ("alperujo") revealed a great potential for the cultivation of *Pleurotus spp*. and *Agrocybe cylindracea* while valorizing environmentally hazardous agricultural waste in Greece. *Flammulina velutipes* has been also cultivated in substrates with a high amount of alperujo, resulting in good biological efficiencies while minimizing the highly phytotoxic properties of this contaminating by-product.

Singh and Singh (2014) reported that the enhanced biological efficiency, protein and essential amino acids of oyster mushroom were observed when grown on paddy straw

substrate and supplemented with different vegetable waste including pea pod shell, cauliflower leaves, radish leaves and brassica straw.

Fanadzo *et al.* (2010) led an experiment was conducted to determine the effects of different substrates namely wheat straw (*Triticum aestivum*), maize stover (*Zea mays* L), thatch grass (*Hyparrhenia filipendula*) and oil/protein rich supplements (maize bran, cottonseed hull [*Gossypium hirsutum*]) on biological efficiency of two oyster mushroom species (*Pleurotus sajor-caju* and *P. ostreatus*). from the experiment result revealed that the analysis of the changes in BE with a switch from wheat straw (control) to the supposedly inferior substrates indicated that maize straw with no supplement was superior to wheat straw when cultivating *P. ostreatus*. Maize straw resulted in the highest BE increase of 112%. However, supplementation of the wheat straw with cottonseed hull resulted in a significant improvement of 54% BE.

Alam *et al.* (2007) who observed that the biological efficiency ranged from 45.21% to 125.70% in case of oyster mushroom.

2.2.14 Microbial contamination

Bhatta and Bist (2017) carried out an experiment to identify the effects of supplements in rice straw on oyster mushroom (*Pleurotus florida*) production. The experiment consisted of five treatments with different additives with four replications each were arranged in Completely Randomized Design (CRD). The Treatments are- T_1: Rice Straw Only (Control); T_2: Rice Straw + Wheat Bran (10:1 dws); T_3: Rice Straw + Rice Bran (20:1 dws); T_4: Rice Straw + Gram Flour (25:1 dws); T_5: Rice Straw + Maize Cob (10:1 dws). [dws = dry weight of substrate]. Result expressed that most severe contamination by saprophyte (*Trichoderma*) was recorded in gram flour supplemented bags whereas mild contamination was seen in bags treated with rice bran. The reason behind this finding can be attributed to the higher percentage composition of easily soluble nutrients in the supplements which is favourable for saprophyte growth. Treatments with wheat bran, maize cob and control were devoid of any contamination. Fast growth of mycelium was helpful to avoid the attack by saprophyte in wheat bran supplemented samples.

Biswas and Kuiry (2013) reported that *Aspergillus niger, Coprinus sp, Penicillium sp* and *Sclerotium rolfsii* were the most predominant fungal contaminant of mushroom beds of *P. florida.*

Jaivel and Marimuthu (2010) reported that *Trichoderma, Aspergillus and Rhizopus* on oyster mushroom bed were predominant microorganisms and especially occurrence of these was severe in summer and spring seasons than autumn and winter.

Spilman (2002) recognized *Trichoderma* as green mould on the production bed of oyster mushroom.

Kananen *et al*. (2000) reported that the lower incidence of disease and shorter spawn runs (rapid colonization of the composted substrate) for substrates inoculated with mushroom spawn-supplement.

Castle *et al.* (1998) reported that *Trichoderma harzianum, Aspergillus spp., Penicillium spp., Monilia sitophila, Stemonitis spp. and Coprinus spp.* were the major contaminants of *Pleurotus spp.* These species become prevalent in *Pleurotus* cultures if the substrate has not been uniformly or properly pasteurized. Among these contaminants, *Trichoderma harzianum* was reported to be the most damaging one, competing aggressively with the mycelium of *P. pulmonarius* and *P. ostreatus* in- vitro and reducing the production surface from 30 to 50%.

CHAPTER III

MATERIALS AND METHODS

This chapter presents a concise depiction about the experimental period, climatic condition, crop or planting materials that were being used in the experiment, treatments, experimental design and layout, data collection and statistical analysis.

3.1 Location of the experimental site

The experiment was conducted at the Plant pathology laboratory and Mushroom Culture House (MCH) of the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar Agargaon, Dhaka, 1207. The experimental site is geographically situated at 23°77′ N latitude and 90°33′ E longitude at an altitude of 8.6 meters above sea level (Anon., 2004). For better understanding, the location of the experimental site has been shown in the Map of AEZ of Bangladesh in Appendix-I.

3.2 Duration of the experiment

The experiment was conducted during the period from July, 2020 to November, 2020.

3.3 Climate condition during experimentation

The experimental area was under the subtropical climate and was characterized by high temperature, high humidity and heavy precipitation with occasional gusty winds during the period from March to August, but scanty rainfall associated with moderately low temperature prevailed during the period from March to August (Edris *et al*., 1979). The detailed meteorological data in respect of Maximum and minimum temperature, relative humidity and total rainfall were recorded by the meteorology center, Dhaka for the period of experimentation have been presented in Appendix II.

3.4 Experimental materials

Mother culture of oyster mushroom was collected from Mushroom Development Institute (MDI), Savar, Dhaka.

3.5 Varietal characteristics of oyster mushroom

Oyster mushrooms have a white to light brown to a darker brown, funnel-shaped cap, with whitish-yellow gills running up a short off-center stem. The flesh is white. The cap is usually 5 to 25cm (2 to 10 inches) across and it grows in a shelf-like formation often with overlapping clusters. Gills are white and decurrent. The stem is very short, stout, lateral, and they are somewhat hairy near the base. spore are white to grey or slightly lilac grey. If the temperature increases above 32°C, its production markedly decreases. The nutrition in oyster mushrooms is very high.

3.6 Treatments of the experiment

The experiment was consisted of a single factor with 13 treatments with three replication, were conducted to achieve the desired objectives.

Factor A: Various levels of supplements

T_0 : Sawdust (Control) =500g

T_1 : Sawdust + 5 g Compost

T_2 : Sawdust + 10 g Compost

T_3 : Sawdust + 15 g Compost

T_4 : Sawdust + 5 g Vermicompost

T_5 : Sawdust + 10 g Vermicompost

T_6 : Sawdust + 15 g Vermicompost,

T_7 : Sawdust + 5 g Mustard oil cake

T_8 : Sawdust + 10 g Mustard oil cake

T_9 : Sawdust + 15 g Mustard oil cake

T_{10} : Sawdust + 5 g (Co+Vc+Mc)

T_{11} : Sawdust + 10 g (Co+Vc+Mc) and

T_{12} : Sawdust + Co+Vc+Mc)-15 g, [Co: Compost, Vc: Vermicompost and Mc: Mustard oil cake]

A. Mustard Oil Cake

B. Vermicompost

C. Compost

D. Sawdust

Plate 1. Photographs of different treatments **A.** Mustard oil cake **B.** Vermicompost **C.** Compost **D.** Sawdust

3.7 Design and layout of the experiment

The experiment was conducted with 13 treatments with three replications and laid out in single factor Completely Randomized Design (CRD).

3.8 Preparation of substrates

At first weight of dry sawdust was taken. Then, the sawdust was soaked in water over night. Subsequently the sawdust was taken off from water and left on a perforated sieve for eliminating the excess water for few hours. Then, at that point the supplements were added according to the treatments requirement. $CaCO_3$ was also added with spawn preparing substrate @ 1% on dry weight basis. The measured materials were taken in a plastic bowl and blended completely by hand and moisture was increased by adding water. Moisture was estimated by using the moisture meter and adjusted the moisture.

3.9 Preparation of the spawn packets

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

3.10 Sterilization, inoculation and mycelium running in spawn packets

The packets were sterilized at autoclave at 15PSI for 1 hour and afterward these were kept for cooling. After cooling, 5 g mother spawn was inoculation into the packets in the laminar airflow cabinet and the packets were kept at 20-22°C temperature until the packets become white with the mushroom mycelium. After completion of the mycelium running, the rubber band, brown paper, cotton plug and plastic neck of the mouth of spawn packet were removed and the mouth was tightly with rubber band. Then, at that point these spawn packets were transferred to the culture house.

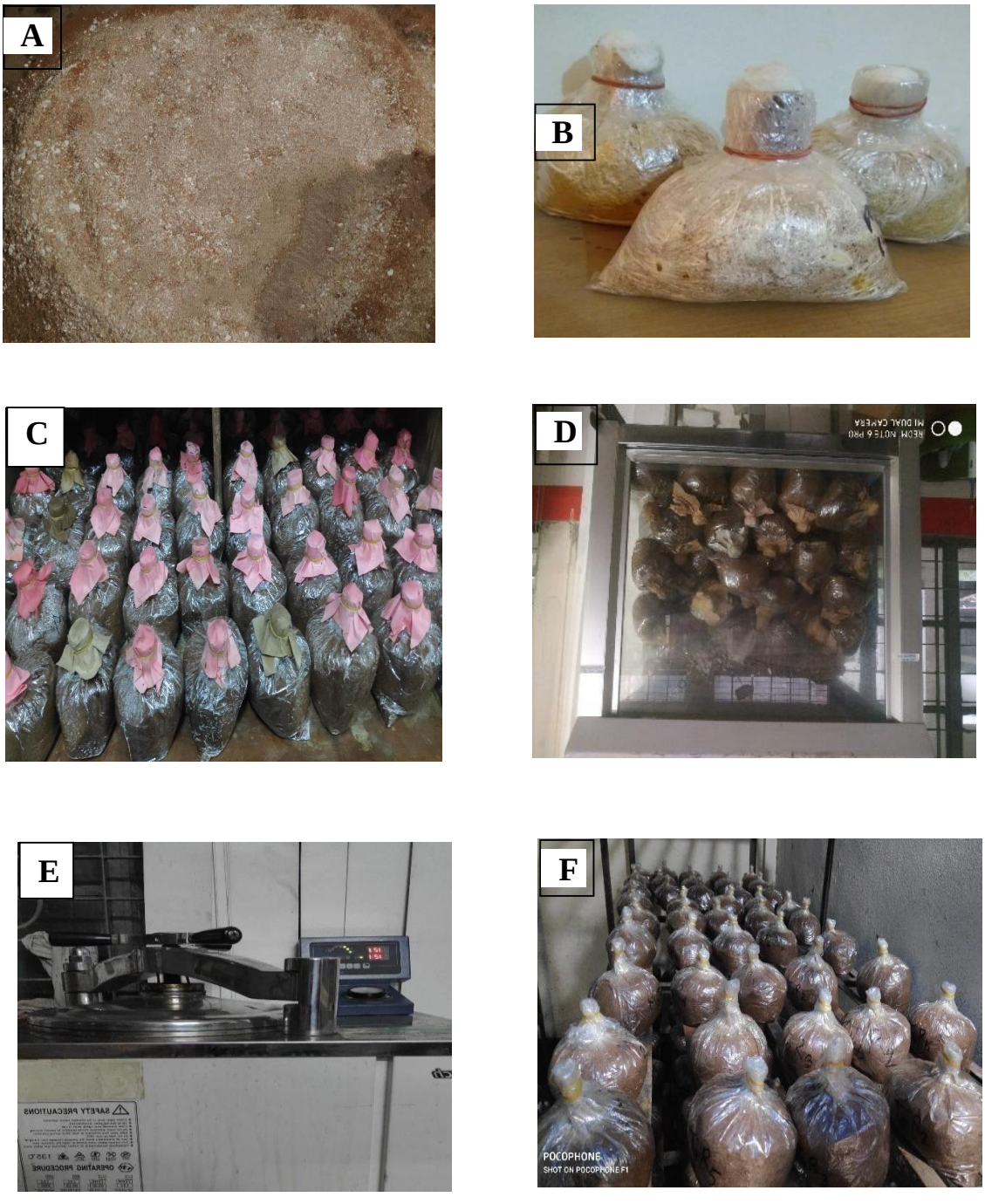


Plate 2. A. Mixing of lime with substrate **B.** Mother of *Pleurotus ostreatus* **C.** Preparation of substrate packets **D-E**. Sterilization of substrate packets by autoclave **F.** Incubation of spawn packets

3.11 Cultivation of spawn packet

The packets of each type were put separately on the rack of culture room and covered with newspaper. The moisture of the culture room was kept 80-85% relative humidity by spraying water 3 times a day. The light around 300-500 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was kept up 22°C to 25°C. The first primordia appeared 3-4 days subsequent to scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

3.12 Data collection

The data were recorded on the following parameters

i. Mycelium running rate in spawn packet (cm)
ii. Days required for mother inoculation to completion of mycelium running
iii. Days required for stimulation to primordia initiation
iv. Days required from incubation to 1st harvest
v. Days required from primordia initiation to 1st harvest
vi. Days required from primordia initiation to final harvest
vii. Average number of primordia /packet
viii. Average number of fruiting body /packet
ix. Average number of effective fruiting body/packet
x. Length of stripe (cm)
xi. Length of fruiting body (cm)
xii. Breadth of fruiting body (cm)
xiii. Biological yield (g)
xiv. Economic yield (g)
xv. Dry yield (g)
xvi. Biological efficiency (%)

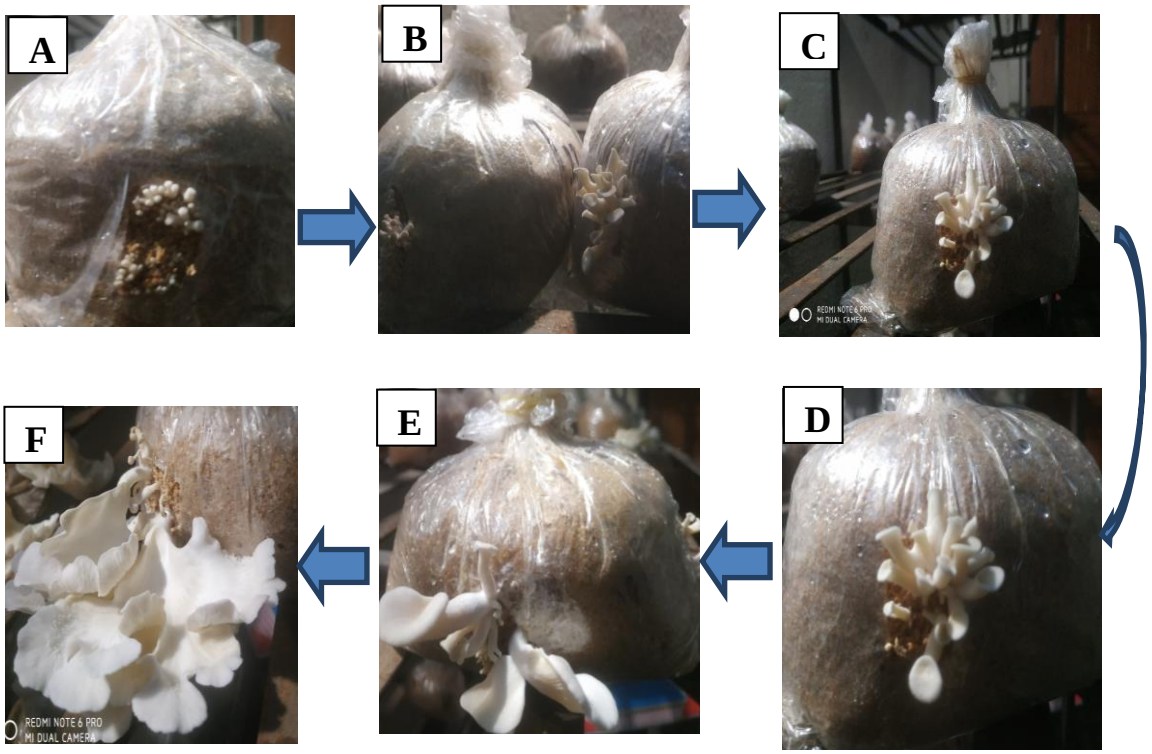


Plate 3. Different stages of fruiting bodies **A-C.** Primordia **D-E.** Premature fruiting bodies **F.** Matured fruiting bodies

3.13 Procedure of recording data

i. Mycelium running rate in spawn packet (cm)

Mycelium running rate (MRR) for each kind of substrate was estimated after the mycelium colony cross the shoulder of the packets. The straight length was estimated at different places of packet using the following equation (Sarker, 2004)

$$MRR = \frac{\text{Average length (L)}}{\text{Number of days (N)}} \text{ cm/Days}$$

ii. Days required for mother inoculation to completion of mycelium running

Days required for mother inoculation to completion of mycelium running was recorded.

iii. Days required for stimulation to primordia initiation.

Days required for stimulation to primordia initiation was recorded.

iv. Days required from incubation to 1st harvest.

Days required from incubation to 1st harvest was recorded.

v. Days required from primordia initiation to 1st harvest.

Days required from primordia initiation to 1st harvest was recorded.

vi. Days required from primordia initiation to final harvest

Days required from primordia initiation to final harvest was recorded.

vii. Average number of primordia /packet

An average number of primordia /packet was recorded.

viii. Average number of fruiting body /packet

Well-developed fruiting body number were counted then the average were estimated and data were recorded. Dry and pinheaded fruiting bodies were discarded but tiny fruiting bodies were included in counting.

ix. Average number of effective fruiting body/packet

Well-developed effective fruiting body number were counted then the average were estimated and data were recorded. Dry and pinheaded fruiting bodies as considered as non-effective and were discarded.

x. Length of the stipe (cm)

Length of the stripe of the mushroom bodies was measured using a slide calipers data were recorded.

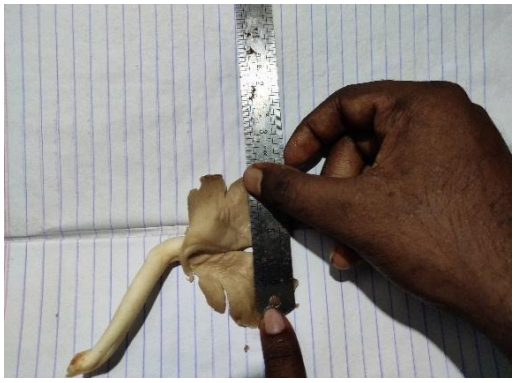
A

B

C

D

E



F

Plate 4. A & B. Different growth stage of mushroom **C.** Measurement of weight of mushroom **D.** Measurement of length of fruiting body **E.** Measurement of Breadth of fruiting body **F**. Harvested mushroom

xi. Length of fruiting body (cm)

Length of the fruiting body of the mushroom bodies was measured using a slide calipers data were recorded.

xii. Breadth of fruiting body (cm)

Breadth of fruiting body of the mushroom bodies was measured using a slide calipers data were recorded.

xiii. Biological yield (g)

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

xiv. Economic yield:

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

xv. Dry yield (g)

About 50g of randomly selected mushroom sample was taken in a paper wrap and was weighed accurately. The mushroom was oven dried at 72ºC temperature for 24 hours and weighed once more. The weight of blank envelop was subtracted from both the weight. The dry yield was determined by using the following equation:

$$\text{Dry yield (g/500g packet)} = \text{Economic yield} \times \frac{\text{Oven dry weight of the sample (g)}}{\text{Fresh weight of the sample (g)}}$$

xvi. Biological efficiency

Biological efficiency was determined by using the following equation:

$$\text{Biological efficiency (\%)} = \frac{\text{Total biological weight per packet (g)}}{\text{Total dry weight substrate used per packet (g)}} \times 100$$

3.14 Experimental design = CRD with 3 replication data analysis technique

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique with the help of a computer package program name Statistics 10 data analysis software and the mean differences were adjusted by Least Significant Difference (LSD) test at 5% level of probability (Gomez and Gomez, 1984).

3.15 Preparation and composition of agar media

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 1 liter 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 lbs. pressure (121°C) for 45 minutes. After autoclaving the media, the conical flask are then taken into the laminar airflow chamber in order to avoid contamination. The laminar airflow chamber must be wiped thoroughly with cotton cloth dipped in 70% ethyl alcohol. So prepared agar media is then poured into the sterile petri plates at equal volumes. After the agar is poured into the sterile petri plates, it is allowed to cool down volumes. After the agar is poured into the sterile petri plates, it is allowed to cool down.

3.16 Isolation and purification of competitor moulds from collected spawn

10 g 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 27º C (±2) º C 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 sub culturing were done by hyphal tip technique.

Plate 5. A-B. Boiling of sliced, peeled potatoes (60 g) **C.** Measurement of weight of Dextrose and Agar (6 g each), **D**. Sterilization of glassware in oven, **E-F.** PDA solution (300 ml), **G.** Prepared PDA solution poured into sterile Petridish

3.17 Identification and isolation of contaminating 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 literature. The microphotograph of pathogens was also taken using microscope. The morphological characteristics of individual fungus were recorded and compared with appropriate key book like CMI description of fungi to identify each fungus (Barnett, 1972).

CHAPTER IV

RESULTS AND DISCUSSION

The data on different parameters were recorded to find out the suitable supplements for mushroom production. The results have been presented and discussed and possible explanation have been given under the following headings:

4.1 Effect of supplement growth characters of oyster mushroom

4.1.1 Mycelium running rate in spawn packet (cm)

Mycelium running rate in spawn packet was found to be significantly varied from 0.30-0.64cm/day due to various supplements used with substrates compared to control (Table-1). The maximum mycelium running rate in spawn packet was observed in T_6 treatment. The minimum mycelium running rate was observed in treatment, T_{10} and T_{12} statically similarest T_{11} The result obtained from the present study was similar with the findings of Hasan *et al.,* (2015); Nuruddin *et al.,* (2010); Kulsum *et al.,* (2009) and Khan *et al.,* (1991). Hasan *et al.,* (2015) reported that the mycelium running rate of oyster mushroom greatly influenced with the supplement of wheat bran in different levels. The highest running rate (0.96cm) was observed in 50% and lowest (0.72 cm) in 0% wheat brans used as supplement on sugarcane bagasse (as substrate). Kulsum *et al*. (2009) noticed that the maximum mycelium running rate (0.71 cm) was observed in because of sawdust treated with cow compost @ 15%. Khan *et al.* (1991) reported that sawdust amended with various natural supplement like wheat chaff, wheat bran, rice straw, cotton waste etc. provided appropriate condition for spawn running. The mycelium running rate (0.62cm) of T_2 and T_5 was statically similarly, again, T_{10}, T_{11} and T_{12} gave also statistically similar result.

4.1.2 Days required from mother inoculation to completion of mycelium running

Various supplements significantly effect on duration from mother inoculation to completion of mycelium running (Table-1). Days required from mother inoculation to completion of mycelium running ranged from 23 days to 28 days. The maximum days required from mother inoculation to completion of mycelium running was observed in

T_2 (28 days) treatment which was statistically similar with T_9 (28 days) treatment. Whereas the minimum days required from mother inoculation to completion of mycelium running was observed in T_0 (23 days) treatments which was statistically similar with T_5 (23 days) and T_8 (23 days) treatments. The others treatments varied significantly in term of days required for mother inoculation to completion of mycelium running. The findings of the present study corroborate with the findings of Seephueak *et al.* (2014, 2016) and Tikdari and Bolandnazar (2012). Tikdari and Bolandnazar (2012) reported that the days required to completion of mycelium running in substrate was significantly affected by different supplements and it ranged from 18.66 to 23 days. The minimum time (18.66 days) was recorded from soybean meal 7.5%, and the maximum time (23 days) was recorded from vermicompost (2.5%) supplements treated with substrate. The findings also match with the findings of Sobhan (2006) who found that the highest duration to complete spawn running was found in mustard oilseed cake at 50% level which might be due to the antifungal properties of mustard oilseed cake.

4.1.3 Days required from stimulation to primordia initiation

Various supplements influenced significantly on days required from stimulation to primordia initiation compared to control (Table-1). Days required from stimulation to primordia initiation was ranged from 5-7 days. The maximum days (6.89 days) required from stimulation to primordia initiation was observed in T_{12} treatment. Whereas the minimum days T_5 (5.43 days) required from stimulation to primordia initiation was observed in which was statistically similar with and T_6 (5.43 days). The findings also match with the findings of Ali (2009) who observed that the maximum time (11.5 days) from stimulation to primordia initiation was observed sugarcane bagasse supplemented with wheat bran @ 10%. In the experiment the time from stimulation to primordia initiation needed higher comparable to the control treatment which was due to the antifungal properties of the supplements which may increasing the time of stimulation to primordia initiation for cultivation of mushroom.

Table 1. Effect of different levels of supplements on mycelium running rate, days required for completion of mycelium running and primordia initiation of oyster mushroom (*Pleurotus ostreatus*)

Treatments	Mycelium running rate in spawn packet (cm)/day	Days required from mother inoculation to completion of mycelium running	Days required from stimulation to primordia initiation
T_0	0.30 h	23 f	5.55 d
T_1	0.56 d	25 d	5.55 d
T_2	0.56 d	28 a	6.11 b
T_3	0.62 b	27 b	5.43 e
T_4	0.59 c	23 f	5.56 d
T_5	0.62 b	23 f	5.45 e
T_6	0.64 a	26 c	5.45 e
T_7	0.56 d	23.67 e	5.55 d
T_8	0.37 g	23 f	6.00 c
T_9	0.43 f	28 a	5.98 c
T_{10}	0.51 e	24 e	5.55 d
T_{11}	0.52 e	24 e	5.55 d
T_{12}	0.51 e	25.67 c	6.89 a
$LSD_{(0.05)}$	0.01	0.66	0.07
CV(%)	1.19	1.58	0.68

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

T_0 : Control, T_1 : Compost - 5 gm, T_2 : Compost - 10 gm, T_3 : Compost - 15 gm, T_4 : Vermicompost - 5 gm, T_5 : Vermicompost - 10 gm, T_6 : Vermicompost - 15 gm, T_7 : Mustard oil cake- 5 gm, T_8 : Mustard oil cake- 10 gm, T_9 : Mustard oil cake- 15 gm, T_{10} : (Co+Vc+Mc)- 5 gm, T_{11} : (Co+Vc+Mc)- 10 gm and T_{12} : (Co+Vc+Mc)-15 gm [Co: Compost, Vc: Vermicompost and Mc: Mustard oil cake]

4.1.4 Days required from incubation to 1st harvest

Significant variation was observed in days required from incubation to 1st harvest due to application of various supplements on sawdust (Figure-1 and Table-2). Days required from incubation to 1st harvest ranges from 25 days to 31 days. The maximum days required from incubation to 1st harvest was observed in T_2 (30.33 days) treatment which was statistically similar with T_1 (30 days), T_5 (30 days) and T_6 (30 days) treatment. Whereas the minimum days required from incubation to 1st harvest was observed in T_0 (25 days) snd T_7 (25 days) treatment, The others treatment varied significantly in term of days required from incubation to 1st harvest. In the present study some of the supplements required maximum days from incubation to 1st harvest comparable to the control treatment due to the reason that antifungal properties and slow releasing nutrients capability of the supplements increasing the day's requirement from incubation to 1st harvest.

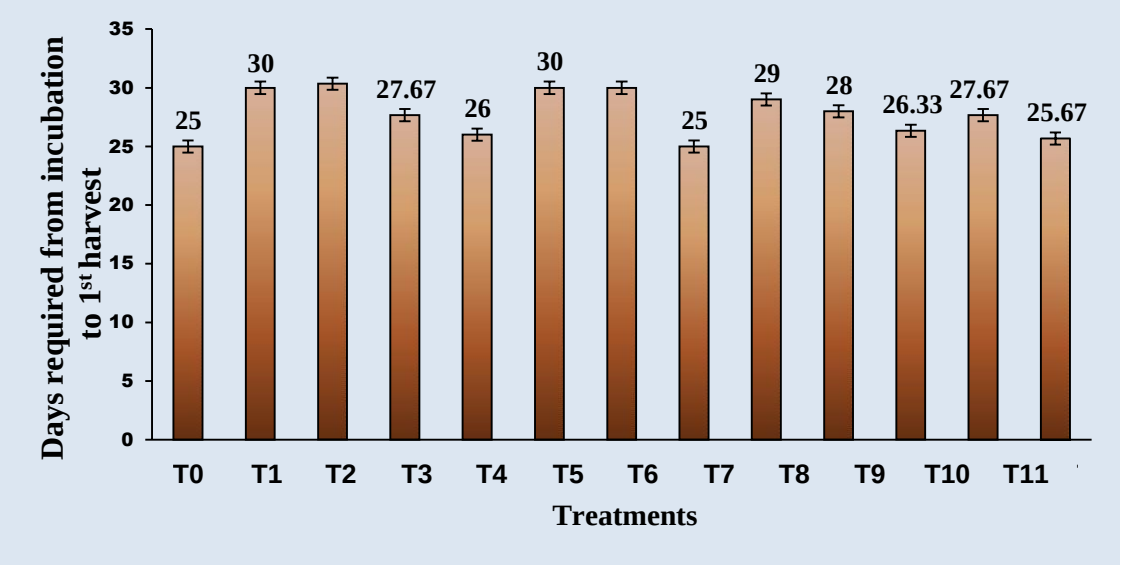


Figure.1. Effect of different levels of supplements on days required from incubation to 1st harvest of oyster mushroom (Vertical bars mention the LSD values at 5 % level of probability)

T_0: Control, T_1: Compost - 5 g, T_2: Compost - 10 g, T_3: Compost - 15 g, T_4: Vermicompost - 5 g, T_5: Vermicompost - 10 g, T_6: Vermicompost - 15 g, T_7: Mustard oil cake- 5 g, T_8: Mustard oil cake- 10 g, T_9: Mustard oil cake- 15 g, T_{10}: (Co+Vc+Mc)- 5 g, T_{11}: (Co+Vc+Mc)- 10 g and T_{12}: (Co+Vc+Mc)-15 g [Co: Compost, Vc: Vermicompost and Mc: Mustard oil cake]

4.1.5 Days required from primordia initiation to 1st harvest

Significant variation was observed in days required from primordia initiation to 1st harvest due to application of various supplements on sawdust (Table-2). Days required from primordia initiation to 1st harvest ranged from 4 days to 8 days. The maximum days required from primordia initiation to 1st harvest was observed in T_{10} (7.67 days) treatment. Whereas the minimum days required from primordia initiation to 1st harvest was observed in T_5 (4.67 days) treatment. The others treatment varied significantly in term of days required from incubation to 1st harvest.

4.1.6 Days required from primordia initiation to final harvest

Significant variation was observed in days required from primordia initiation to final harvest due to application of various supplements on sawdust (Table-2). Days required from primordia initiation to final harvest ranged from 32 days to 48 days. The maximum days required from primordia initiation to final harvest was observed in T_6 (47.67 days) treatment. Whereas the minimum days required from primordia initiation to final harvest was observed in T_0 (32 days) treatment which was statistically similar with T_9 (32 days) treatment. The others treatment varied significantly in term of days required from primordia initiation to final harvest. Due to the some anti-fungal properties and slow nutrient releasing capability of the supplements days required from primordia initiation to final harvest required more time comparable to control treatment.

Table 2. Effect of different levels of supplements on days required from incubation to primordia initiation, primordia initiation to 1st harvest, primordia initiation to final harvest of oyster mushroom

Treatments	Days required from		
	Incubation to primordia initiation	Primordia initiation to 1st harvest	Primordia initiation to final harvest
T_0	25.00 f	6.67 d	32.00 g
T_1	30.00 a	7.00 c	45.00 b
T_2	30.33 a	7.00 c	44.67 b
T_3	27.67 c	6.67 d	43.33 c
T_4	26.00 de	6.33 e	43.00 c
T_5	30.00 a	4.67 f	44.00 bc
T_6	30.00 a	6.67 d	47.67 a
T_7	25.00 f	6.33 e	39.33 d
T_8	29.00 b	6.33 e	45.00 b
T_9	28.00 c	7.00 c	32.00 g
T_{10}	26.33 d	7.67 a	40.00 d
T_{11}	27.67 c	6.33 e	34.67 f
T_{12}	25.67 e	7.33 b	38.00 e
$LSD_{(0.05)}$	0.52	0.07	1.32
CV(%)	1.12	0.66	1.93

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

T_0: Control, T_1: Compost - 5 g, T_2: Compost - 10 g, T_3: Compost - 15 g, T_4: Vermicompost - 5 g, T_5: Vermicompost - 10 g, T_6: Vermicompost - 15 g, T_7: Mustard oil cake- 5 g, T_8: Mustard oil cake- 10 g, T_9: Mustard oil cake- 15 g, T_{10}: (Co+Vc+Mc)- 5g, T_{11}: Co+Vc+Mc)- 10g and T_{12}: Co+Vc+Mc)-15, [Co: Compost, Vc: Vermicompost and Mc: Mustard oil cake]

Severity of contamination in spawn packet

The contamination with fungi was found under mustard oil cake treated packet from 15 days after incubation where the severity was ranged 2-77.33%. The maximum contamination severity was found with 5 g mustard oil cake treated packet in T_7 at 60 DAI. At 15, 30.45 and 60 DAI contamination severity was 2.67, 14.33, 36.33 and 72%, respectively under T_9. The contamination severity was 2%, 17%, 31%, and 52%, found under T_{12} when substrate was treated with the combination of Vermicompost and mustard oil cake, respectively. In other cases, the spawn packets under (T_1, T_2, T_3, T_4 and T_{11}) the spawn packets were free from contamination (Table 3). Akhter (2017) reported that maximum contamination found in mixed substrates (rice straw + saw dust + waste paper) and the severity of the contamination increased gradually with gradual process of days after incubation (DAI) that ranged from 9.30 to 26.73.

Table 3. Effect of chemical treatment of substrate of oyster mushroom on contamination severity

Treatments	Contamination of spawn (%)			
	15 DAI	30 DAI	45 DAI	60 DAI
T_0	0	0	5	10
T_1	0	0	0	0
T_2	0	0	0	0
T_3	0	0	0	0
T_4	0	0	0	0
T_5	0	5	13	30
T_6	0	7	19	29
T_7	5.67	15.33	38.33	77.33
T_8	2.00	12.00	34.00	65.67
T_9	2.67	14.33	36.33	72.00
T_{10}	0	0	0	0
T_{11}	0	0	0	0
T_{12}	2.0	17.00	31.00	52.00

DAI-Days after incubation

T_0: Control, T_1: Compost - 5 g, T_2: Compost - 10 g, T_3: Compost - 15 g, T_4: Vermicompost - 5 g, T_5: Vermicompost - 10 g, T_6: Vermicompost - 15 g, T_7: Mustard oil cake- 5 g, T_8: Mustard oil cake- 10 g, T_9: Mustard oil cake- 15 g, T_{10}: (Co+Vc+Mc)- 5g, T_{11}: Co+Vc+Mc)- 10g and T_{12} : Co+Vc+Mc)-15, [Co: Compost, Vc: Vermicompost and Mc: Mustard oil cake]

Contaminants Isolated and identified from contaminated spawn

Based on the morphological characters commonly four pathogens were identified. These were *Trichoderma harzianum, Penicillium* sp, *Rhizopus sp* and *Aspergillus niger.* Contamination of spawn and identification of major contaminants has been worked out by Akhter (2017); Fletcher and Gaze (2008) and observed that *Trichoderma sp., Penicillium sp, Rhizopus sp, Aspergillus niger, Aspergillus flavus, Coprinus sp. And Sclerotium sp.* are some of the important fungal contaminants of mushroom that are associated with several disease and deteriorated the quality and reduced the production of mushroom.

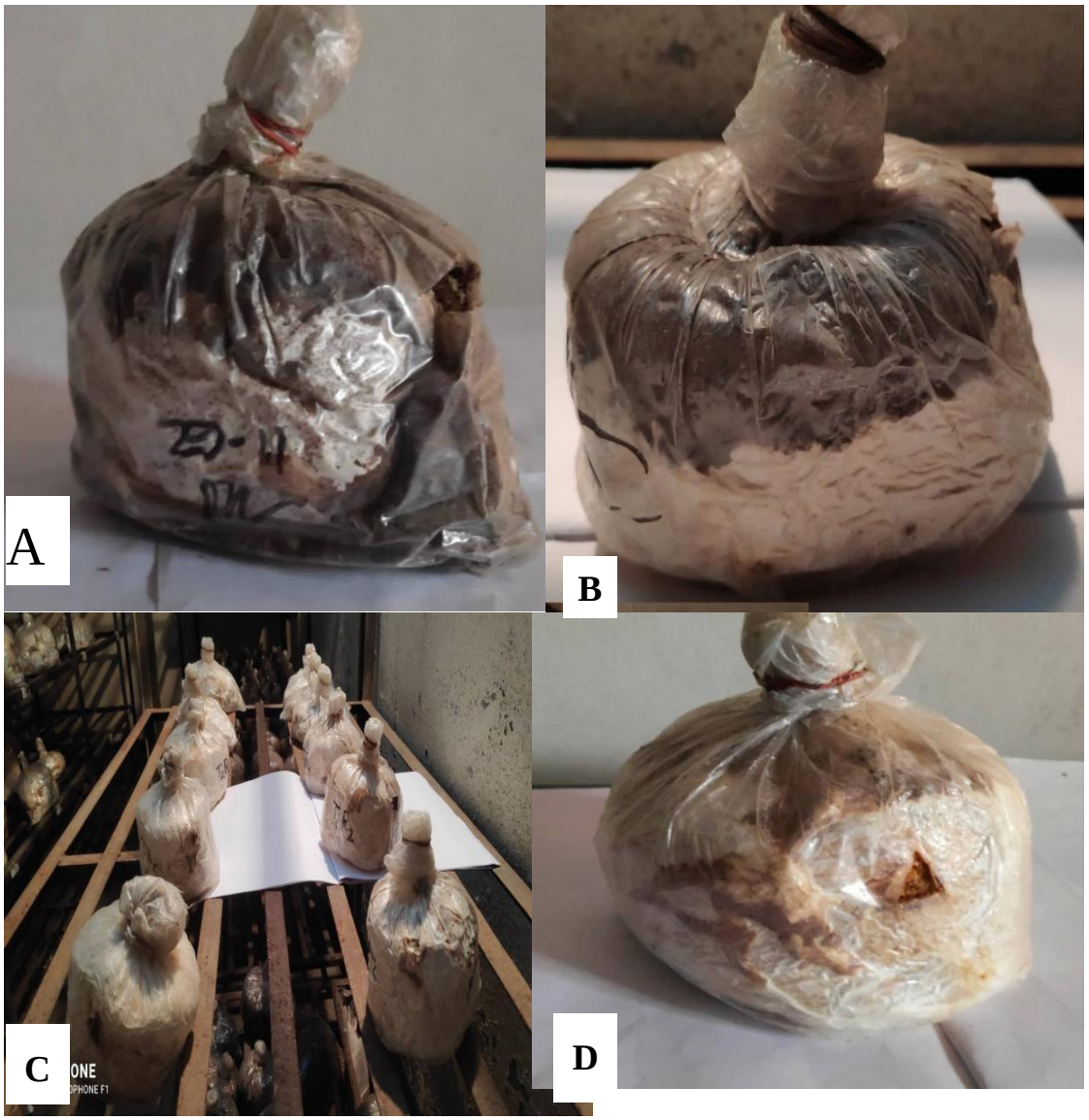


Plate 6. A-D. Different contaminated spawn packets during the experiment

Morphological characterization of isolated fungal contaminants

Trichoderma sp

Green colour growth of mycelium was observed in contaminated spawn packet due to heavy sporulation of causal agent (Plate 7-A). Colonies are usually fast growing and initially whitish in color that later turn into bright green color. *T. harzianum* had occasionally concentric conidiation with whitish yellow conidial area (Plate 8-A). 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.

Aspergillus niger

Aspergillus niger produced black colored spores so it was called black mold (plate 7-B & 8-B). Initially fungal colonies were whitish which quickly became quite 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.

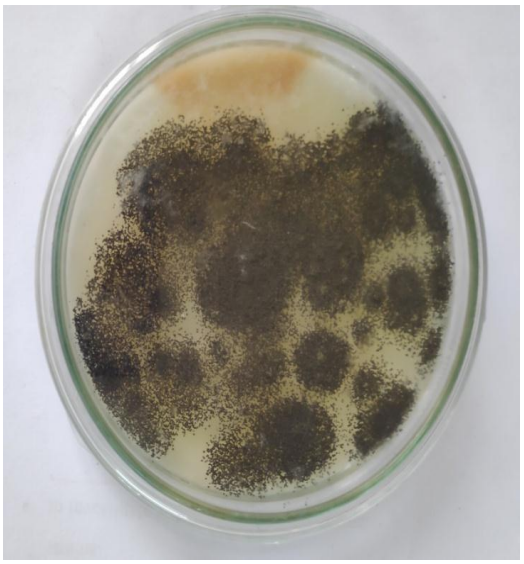
Penicillium sp.

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 7 C). Pure culture of *Penicillium* was prepared on PDA from collected contaminated spawn (Plate 8 C). 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 are produced in basipetal succession from a specialized conidiogenous cell called a phialide.

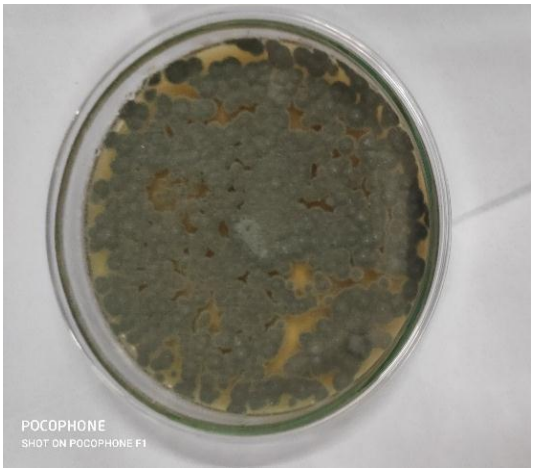
Rhizopus sp.

Similar in appearance to pin mould, *Rhizopus* develops hair-like sporanpgiophare with a tiny head (Plate 7-D and 8-D). Sporangia which are bulbous structures that sprout from the vegetative hyphae and hold the haploid spores. *Rhizopus* has a sour odour.

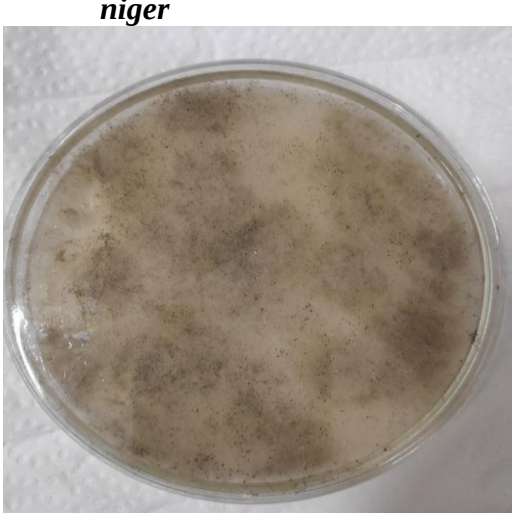
A. Pure culture of *Trichoderma sp.*



B. Pure culture of *Asprgillus niger*



C. Pure culture of *Penicillium sp.*



D. Pure culture of *Rhizopus sp.*

Plate 7. Pure culture of different contaminants obtained from contaminated spawn pack

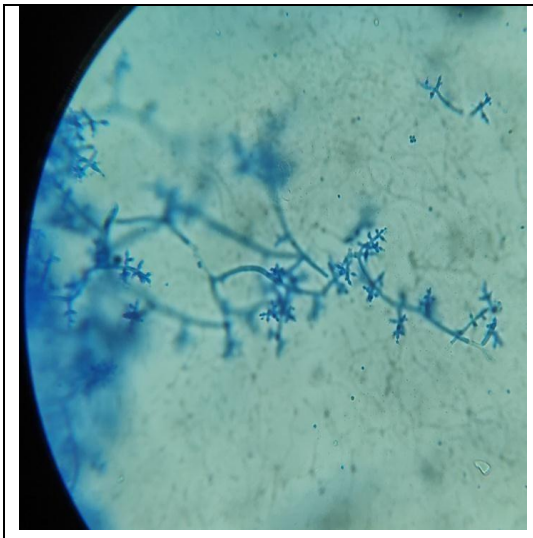
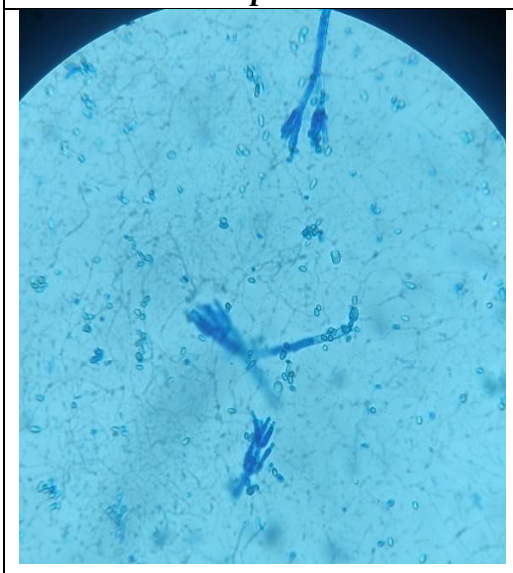
	
A. *Trichoderma sp*	**B. *Asprgillus niger***
	
C. *Penicillium sp*	**D. *Rhizopus sp***

Plate 8. Pathogenic structure of different contaminants component microscope (10x)

4.2 Yield contributing characters

4.2.1 Average number of primordia/packet

Application of different supplements on sawdust significantly influence on averaged number of primordia/packet (Figure 2) compared to control. From the experiment result revealed that the maximum number of primordia /packet was observed in T_2 (80). While the minimum number of primordia /packet was observed in T_0 (32.33). The present findings corroborated with the findings of previous workers (Seephueak *et al.,* 2019; Hasan *et al.,* 2015; Kulsum *et al.,* 2009; Pathan *et al*., 2009 and Amin *et al.,* 2007). Seephueak *et al.,* (2019) reported that the production of *G. lucidum* on rubber sawdust supplemented with palm oil sludge gave 4.52-5.04 basidiocarps/bag, which does not significantly differ from the case of sawdust with 5% rice bran giving 5.12 basidiocarps/bag. Non-supplemented sawdust had the lowest average number of basidiocarps at 1.78 basidiocarps/bag. Hasan *et al.,* (2015) reported that the number of primordia significantly varied with the supplements and substrates used in production of oyster mushroom. Kulsum *et al,.* (2009) observed that the highest average number of primordia/packet was 73.21 due to sawdust supplemented with cow dung @ 10%. Pathan *et al*., (2009) reported that 5 g NKP in 10 kg straw was the best in relation producing average number of primordia per packet. Amin *et al,.*(2007). in his experiment found that the highest number of primordia of oyster mushroom was found in nutrients paddy straw but lowest was found in control treatment.

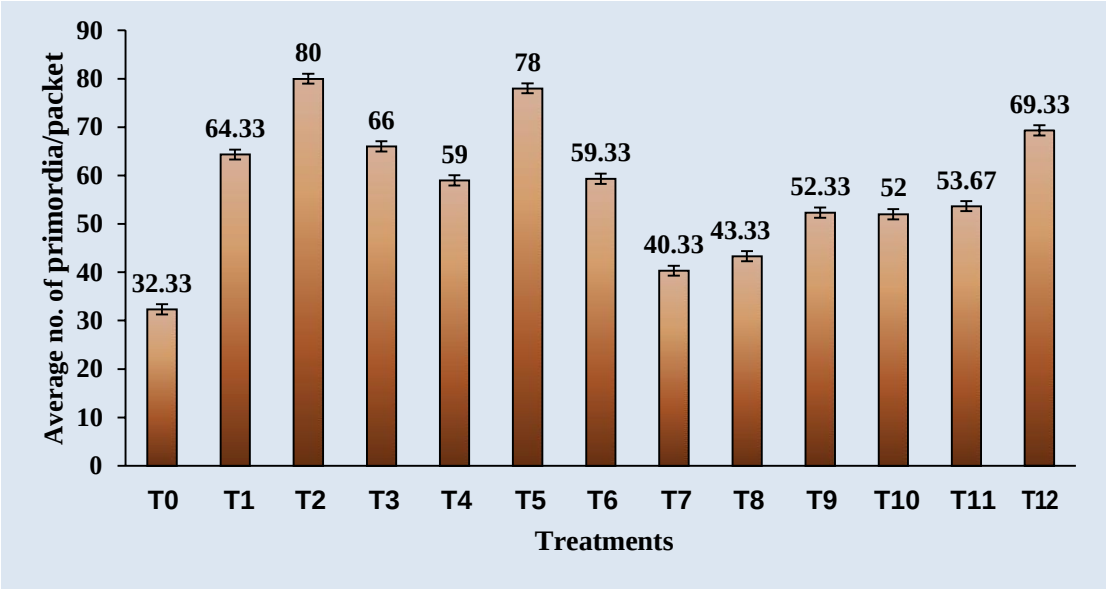


Figure 2. Effect of different supplements on average number of primordia/packet of oyster mushroom

T_0: Control, T_1: Compost - 5 g, T_2: Compost - 10 g, T_3: Compost - 15 g, T_4: Vermicompost - 5 g, T_5: Vermicompost - 10 g, T_6: Vermicompost - 15 g, T_7: Mustard oil cake- 5 g, T_8: Mustard oil cake- 10 g, T_9: Mustard oil cake- 15 g, T_{10}: (Co+Vc+Mc)- 5 g, T_{11}: (Co+V_C+Mc)- 10 g and T_{12}: (Co+V_C+Mc)-15 g [Co: Compost, V_C: Vermicompost and Mc: Mustard oil cake]

4.2.2 Average number of fruiting body /packet

Fruiting bodies are fungal structures that contain spores. Application of different supplements on sawdust significantly influenced on number of fruiting body /packet (Figure 3). From the experiment result revealed that the maximum number of fruiting body /packet (54.67) was observed in T_2. While the minimum number of fruiting body /packet T_0 (10) was observed in The others treatment varied significantly in term of number of primordia /packet comparable to control treatment. Satpal *et al.*, (2017) concluded that the highest number of fruiting body (29.20) was observed in dextrose spawn, which was significantly higher than all other treatments. The minimum number of fruiting body (17.00) was observed control spawn (without sugars) which was significantly lower than all other treatments. Hasan *et al.* (2015) reported that the maximum average number of fruiting body/packet was observed in 10% (77.67) followed by 30% (69.67) and the lowest average number of fruiting body /packet were

in 50% (49.00) of wheat bran application as supplements on sugarcane bagasse use as substrate. The number of fruiting body increased with the levels of supplement and continued up to a certain range and decline thereafter.

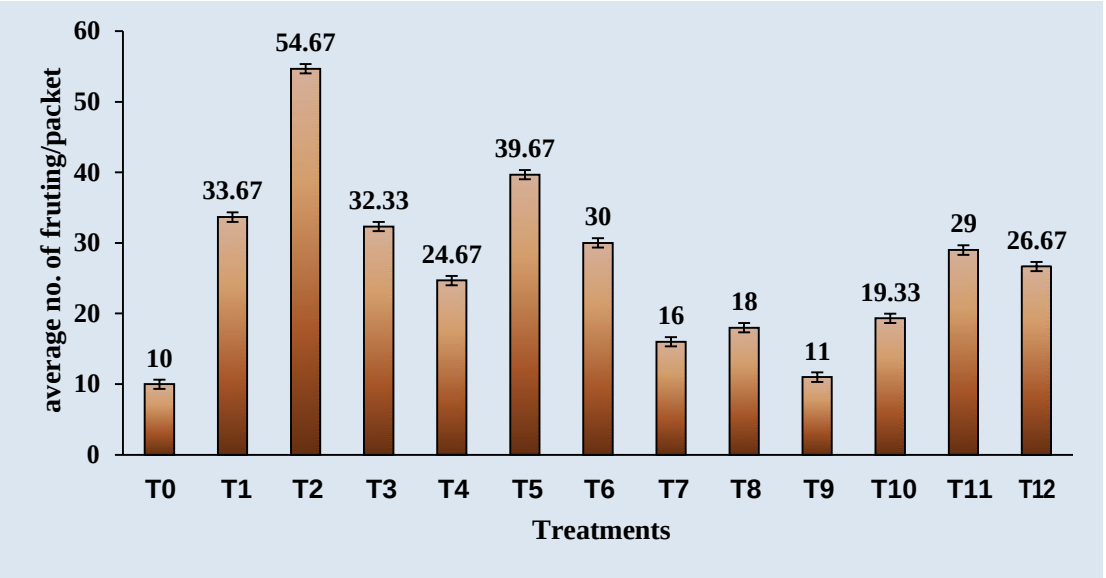


Figure. 3. Effect of different supplements on average number of fruiting body/packet of oyster mushroom (Vertical bars mention the LSD values at 5 % level of probability)

$T_{0:}$ Control, $T_{1:}$ Compost - 5 g, $T_{2:}$ Compost - 10 g, $T_{3:}$ Compost - 15 g, $T_{4:}$ Vermicompost - 5 g, $T_{5:}$ Vermicompost - 10 g, $T_{6:}$ Vermicompost - 15 g, $T_{7:}$ Mustard oil cake- 5 g, $T_{8:}$ Mustard oil cake- 10 g, $T_{9:}$ Mustard oil cake- 15 g, $T_{10:}$ (Co+Vc+Mc)- 5 g, $T_{11:}$ (Co+Vc+Mc)- 10 g and $T_{12:}$ (Co+Vc+Mc)-15 g [Co: Compost, Vc: Vermicompost and Mc: Mustard oil cake]**.**

4.2.3. Average number of effective fruiting body/packet

Fruiting bodies are fungal structures that contain spores. Application of different supplements on sawdust significantly influenced on number of effective fruiting body /packet (Figure 4). From the experiment result revealed that the maximum number of effective fruiting body /packet (41) was observed in T_2. While the minimum number of effective fruiting body /packet (7.33) was observed in T_0. The others treatment varied significantly in term of number of primordia /packet comparable to control treatment.

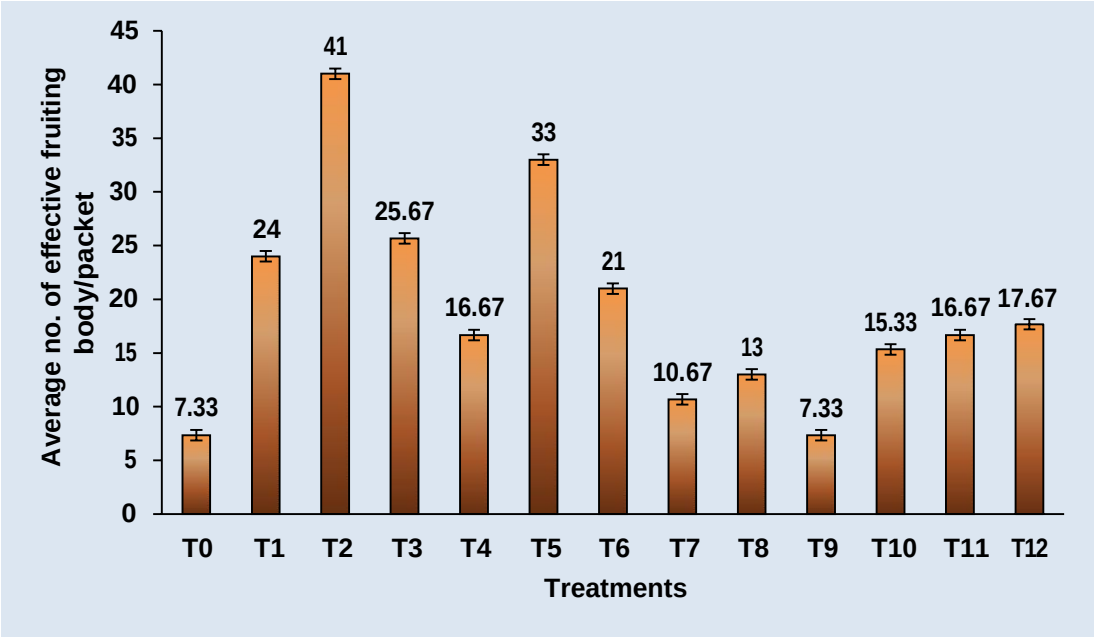


Figure 4. Effect of different levels of supplements on average number of effective fruiting body/packet of oyster mushroom

T_0 : Control, T_1 : Compost - 5 g, T_2 : Compost - 10 g, T_3 : Compost - 15 g, T_4 : Vermicompost - 5 g, T_5 : Vermicompost - 10 g, T_6 : Vermicompost - 15 g, T_7 : Mustard oil cake- 5 g, T_8 : Mustard oil cake- 10 g, T_9 : Mustard oil cake- 15 g, T_{10} : (Co+Vc+Mc)- 5 g, T_{11} : (Co+Vc+Mc)-10 g and T_{12} : (Co+Vc+Mc)-15 g [Co: Compost, Vc: Vermicompost and Mc: Mustard oil cake]

4.2.4 Length of stipe (cm)

Supplementation of sawdust with different supplement had great effect on stipe length of mushroom (Table 4). From the experiment result revealed that the maximum stripe length of mushroom (2.69 cm) was observed in which (2.69 cm) was statistically similar with T_9 While the minimum stripe length of mushroom (2.02 cm) was observed in T_0. The others treatment varied significantly in term of stripe length of mushroom comparable to control treatment. Sanjel *et al.,* (2021) reported that the highest average stipe length was found in molasses supplementation (2.69cm), that was found to be statistically at par (2.18 cm) with control (Finger millet husk as substrate with no supplements), rice bran supplementation (2.33 cm) and wheat bran supplementation

(2.27 cm). The lowest stipe length was found in mustard oilseed cake supplementation (1.41cm). Salama *et al.*, (2019) found similar result which supported the present study. Tikdari and Bolandnazar (2012) also reported that the length of stipe differed significantly ($p < 0.05$) between treatments and ranged from 3.86 to 5.86 cm. The highest of stipe was recorded in vermicompost 2.5% (5.86 cm) and the lowest length of stipe was recorded in soybean meal 5% (3.86 cm) as supplementation application on substrate.

4.2.5 Length of fruiting body (cm)

Length of pileus of fruiting body was significantly varied due to supplementation of sawdust with different supplement (Table-4). From the experiment result revealed that the maximum length of pileus of mushroom was observed in T_2 (6.56 cm), while the minimum length of fruiting body of mushroom was observed in T_0 (2.53 cm). Sanjel *et al.* (2021) reported that the average pileus diameter was found to be highest in molasses supplementation (6.94 cm), which was statistically at par with control (Finger millet husk with no supplements) (6.82 cm), rice bran supplementation (6.76 cm) and wheat bran supplementation (6.84 cm). The lowest pileus diameter was found in mustard oilseed cake supplementation (6.06 cm). Chinara and Mahapatra (2020) reported that the morphological characters (pileus diameter, stipe length and stipe diameter) of mushrooms varied depending upon the application of additives. Maximum pileus diameter (141.1 mm) was recorded in the mushroom harvested from bags (Dry paddy straw substrate) supplemented with groundnut cake which was significantly superior to that of bengal gram (134.3 mm) and maize meal (130.5 mm) supplements. Salama *et al.* (2019) and Tikdari and Bolandnazar (2012) also found similar result which supported the present study. Alam *et al.* (2010) also reported that the maximum diameter of the pileus (7.1 cm) was obtained with the 30% maize powder supplement, followed by 30% wheat bran (6.9 cm) and 50% rice bran supplements (6.6 cm) for rice straw substrate.

4.2.6 Breadth of fruiting body (cm)

Breadth of mushroom was significantly varied due to supplementation of sawdust with different supplement (Table-4). From the experiment result revealed that the maximum breadth of pileus of mushroom (5.67 cm) was observed in T_2 treatment which was statistically similar with T_2 (5.63 cm). While the minimum breadth of fruiting body

of mushroom was observed in T_0 (3.03 cm). The others treatment varied significantly in term of breadth of fruiting body of mushroom comparable to control treatment. Seephueak *et al.*, (2019) reported that the average thickness of pileus was the highest at 1.10 cm./basidiocarp on non-supplemented substrate, followed by the case of rubber sawdust supplemented with 5% rice bran at 1.04 cm./basidiocarp. Using 5-20% palm oil sludge resulted in 0.89-0.97 cm./basidiocarp, without significant differences.

Table 4. Effect of different levels of supplements on length of stipe, length of pileus (cm) and breadth of pileus (cm) of oyster mushroom

Treatments	Length of stipe (cm)	Length of pileus (cm)	Breadth of pileus (cm)
T_0	2.02 i	2.53 l	3.03 j
T_1	2.28 e	4.55 d	4.32 g
T_2	2.69 a	6.56 a	5.67 a
T_3	2.60 b	4.89 c	4.21 h
T_4	2.38 d	3.86 e	4.74 e
T_5	2.23 f	3.27 h	5.43 c
T_6	2.16 g	5.02 b	5.63 ab
T_7	2.09 h	2.88 k	3.08 j
T_8	2.51 c	3.14 j	5.56 b
T_9	2.69 a	3.28 h	4.46 f
T_{10}	2.50 c	3.54 g	4.98 d
T_{11}	2.62 b	3.20 i	5.37 c
T_{12}	2.21 f	3.63 f	4.09 i
$LSD_{(0.05)}$	0.04	0.05	0.09
CV(%)	0.93	0.75	1.20

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

T_0: Control, T_1: Compost - 5 g, T_2: Compost - 10 g, T3: Compost - 15 g, T4: Vermicompost - 5 g, T5: Vermicompost - 10 g, T6: Vermicompost - 15 g, T7 : Mustard oil cake- 5 g, T8 : Mustard oil cake- 10 g, T_9 : Mustard oil cake- 15 g, T_{10} : (Co+Vc+Mc)- 5g, T_{11} : Co+Vc+Mc)- 10g and T12 : Co+V+Mc)-15, [Co: Compost, Vc: Vermicompost and Mc: Mustard oil cake]

4.3 Yield characters

4.3.1 Biological yield (g)

Application of different supplements on sawdust significantly influenced on biological yield of mushroom (Table-5). The maximum biological yield (153.67 g) was observed in T_2. While the minimum biological yield was observed in T_0 (23.33 g) which (24.33 g) was statistically similar with T_7. The other treatments varied significantly as compared with control in terms of biological yield. Sanjel *et al*. (2021); Seephueak *et al.* (2019); Shalahuddin *et al.* (2018) and Pardo-Giménez *et al.* (2016, 2018) found similar result which supported the present study. Sharma (2009) in her study found that supplementation of 2% wheat bran and 2% rice bran resulted 48.1 % and 48.3% increase in yield of *P. ostreatus* respectively over the control. Viziteu (2004) in his research reported that supplementation of *P. ostreatus* with additives didn't increase the productivity significantly. The decreased yield may be due to the higher dose of supplements i.e. greater than 2-3%. Randle (1983) observed that 2-20% increases in mushroom yield are possible with the addition of delayed-release nutrient supplements.

4.3.2 Economic yield (g)

In the present experiment economic yield ranged due application of different supplements on sawdust (Table-5). From the experiment it was record that the maximum economic yield was observed in T_2. While the minimum economic yield was observed in T_0 which (17. 00 g) was statistically similar with T_7. The result obtained from the present study was similar with the findings of Shalahuddin *et al.* (2018). Zied *et al.* (2018). stated that in addition, waste materials, including agro- industrial waste (provided by peanut and acerola juice) and noble grains, a mix with bran of soybean, corn, and cotton have been proved effective to increase the industrial yield, which highlights materials with high S, Cu, and Mn contents as ideal supplements. Moonmoon *et al.* (2011) reported that Sawdust supplemented with different levels of wheat bran, rice bran or maize powder improved yield and quality of Lentinula edodes, with 25% wheat bran and 40% wheat bran reported as the best rate to obtained highest yield and best quality respectively. Kalmis *et al.* (2008) reported that the wheat substrate and supplementation with 25% olive mill effluent gives economic mushroom yield.

4.3.3 Dry yield (g)

The dry yield of the oyster mushroom grown on sawdust was influenced significantly with application of different supplements (Table-5). From the experiment it was revealed that the maximum dry yield (29.33 g) was observed in $T_{2.}$ Whereas the minimum dry yield (5.33 g) was observed in T_0 which was statistically similar with T_7 (5.67 g). The other treatments varied significantly as compared with control in terms of dry yield. Hasan *et al.* (2015) stated the dry yield of the oyster mushroom, grown on sugarcane bagasse responded significantly in terms of dry yield with the different levels of supplement (0,10, 20,30,40 and 50 % of wheat bran). The dry yield of mushroom was higher in 10% (12.47.40 g) and minimum in 0% (8.637 g).

4.3.4 Biological efficiency (%)

The maximum biological efficiency (51.22 %) was observed in T_2 while the minimum biological efficiency (7.78 %) was observed in $T_{0,}$ which was statistically similar with T_7 (8.11) The result of biological efficiency and statistically varied significantly as compared with control in terms of biological efficiency (Figure 5). Arsia *et al*. (2018) reported that The maximum biological efficiency of *Pleurotus flabellatus* was 98.5% with jowar flour supplementation followed by maize bran (95.0%) with wheat straw (as substrate). Biological efficiency of *P. florida* with Rice bran recorded maximum (93.5%) followed by maize bran (93.5%) and gram flour (87.5%) supplementation. While, in *P. sajor-caju,* it was maximum (89.5%) with gram chokar supplementation followed by (88.0%) bajra flour supplementation with wheat straw and minimum (70.5%) was obtained in control (wheat straw only). Picornell-Buendía *et al.* (2016 b); Rugolo *et al.* (2016); Singh and Singh (2014) and Fanadzo *et al.* (2010) also found similar result which supported the present study. Alam *et al.* (2007) reported that the biological efficiency ranged from 45.21% - 125.70% in case of oyster mushroom which also supported the result of present experiments.

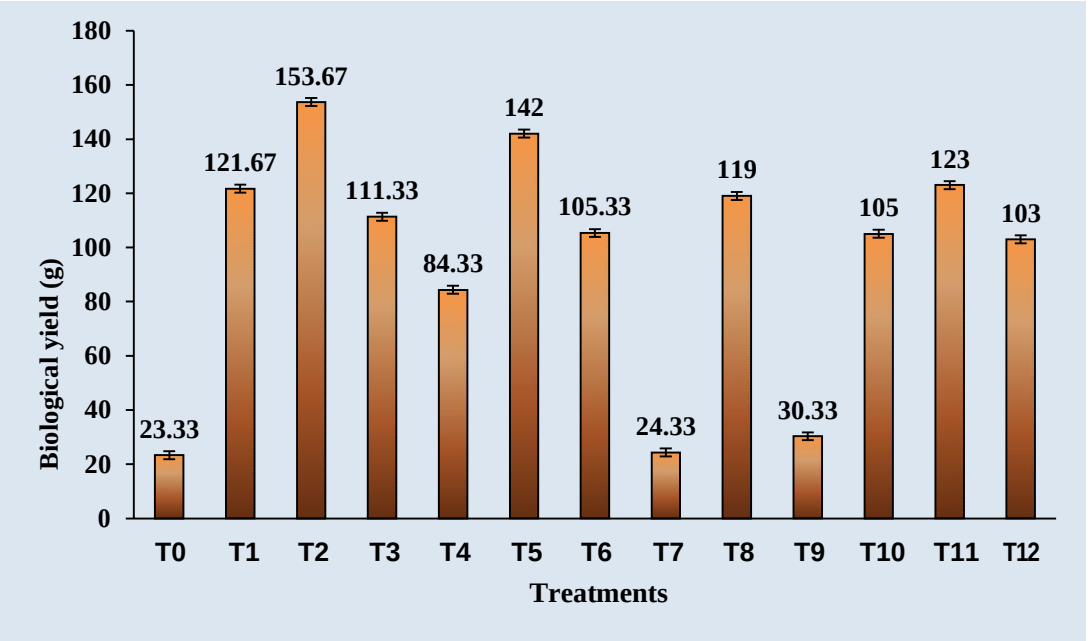


Figure 5. Effect of different levels of supplements on biological efficiency of oyster mushroom

T_0: Control, T_1: Compost - 5 g, T_2: Compost - 10 g, T_3: Compost - 15 g, T_4: Vermicompost - 5 g, T_5: Vermicompost - 10 g, T_6: Vermicompost - 15 g, T_7: Mustard oil cake- 5 g, T_8: Mustard oil cake- 10 g, T_9: Mustard oil cake- 15 g, T_{10}: (Co+Vc+Mc)- 5g, T_{11}: Co+Vc+Mc)- 10g and T_{12}: Co+Vc+Mc)-15, [Co: Compost, Vc: Vermicompost and Mc: Mustard oil cake.

Table 5. Effect of different supplements on biological, economic yield and dry yield of oyster mushroom (*Pleurotus ostreatus*)

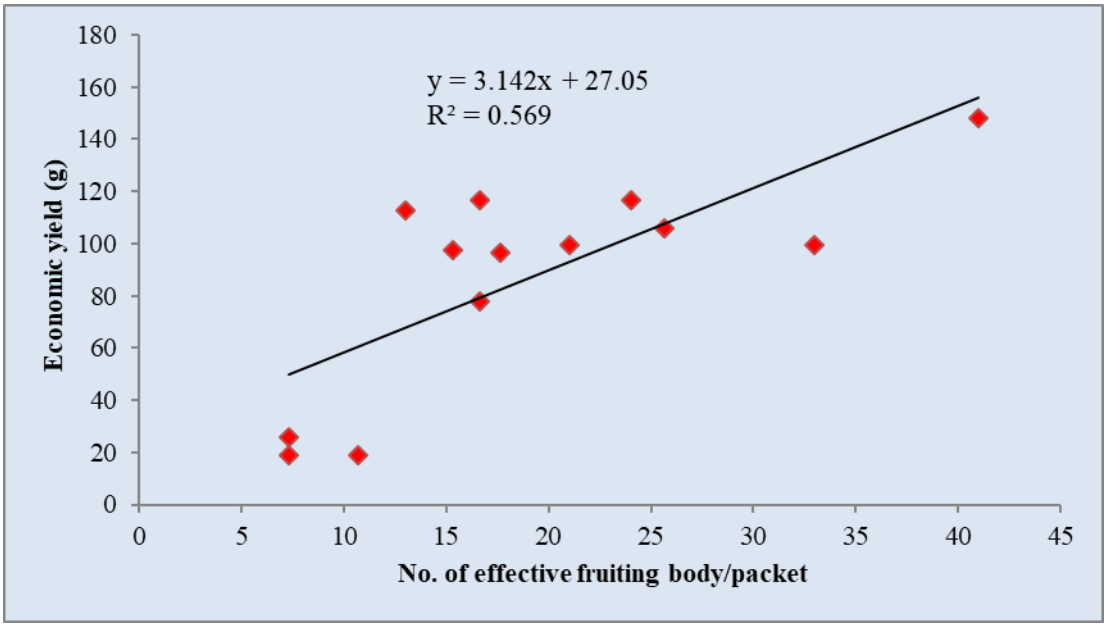
Treatments	Biological yield (g)	Economic yield (g)	Dry yield (g)
T_0	23.33 j	19.00 j	5.33 k
T_1	121.67 c	116.67 b	20.33 d
T_2	153.67 a	148.00 a	29.33 a
T_3	111.33 e	106.00 d	19.33 e
T_4	84.33 h	78.00 h	14.00 i
T_5	142.00 b	99.67 e	23.00 c
T_6	105.33 f	99.33 ef	16.67 h
T_7	24.33 j	19.00 j	5.67 k
T_8	119.00 d	112.67 c	20.67 d
T_9	30.33 i	26.00 i	7.33 j
T_{10}	105.00 f	97.67 fg	17.33 g
T_{11}	123.00 c	116.67 b	23.67 b
T_{12}	103.00 g	96.67 g	18.00 f
$LSD_{(0.05)}$	1.47	1.92	0.47
CV(%)	0.91	1.31	1.64

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

T_0 : Control, T_1 : Compost - 5 g, T_2 : Compost - 10 g, T_3 : Compost - 15 g, T_4 : Vermicompost - 5 g, T_5 : Vermicompost - 10 g, T_6 : Vermicompost - 15 g, T_7 : Mustard oil cake- 5 g, T_8 : Mustard oil cake- 10 g, T_9 : Mustard oil cake- 15 g, T_{10} : (Co+Vc+Mc)- 5g, T_{11} : Co+Vc+Mc)- 10g and T_{12} : Co+Vc+Mc)-15, [Co: Compost, Vc: Vermicompost and Mc: Mustard oil cake]

4.4 Relationship between number of effective fruiting body and economic yield

Correlation study was done to establish the relationship between the number of effective fruiting body and economic yield of mushroom using different supplements on sawdust substrate. From the study it was revealed that, significant correlation was observed between the number of effective fruiting body and economic yield of mushroom due to application of different supplements. It was evident from the (Figure 6) the regression equation y = 3.142x + 27.05 gave a good fit to the data and the co-efficient of determination (R^2 = 0.569) that, fitted regression line had a significant regression co-efficient. From this regression analysis, it was evident that there was a strongly positive relationship between number of effective fruiting body and economic yield of mushroom. Economic yield depended on number of effective fruiting body. Variation of number of effective fruiting body of mushroom significantly influenced the economic yield due to the application of different



supplements on sawdust.

Figurer 6. Relationship between number of effective fruiting body (g/packet) and economic yield (g) of oyster mushroom (*Pleurotus ostreatus)*

4.5 Relationship between economic yield and biological efficiency

A positive linear relationship was observed between economic yield and biological efficiency of mushroom. the regression equation y = 0.344x + 1.859 gave a good fit to the data, and the co-efficient of determination (R^2 = 0.946) showed the, fitted regression line which had a significant regression co-efficient. From this regression analysis, it was evident a strong positive exit relationship between economic yield and biological efficiency of oyster

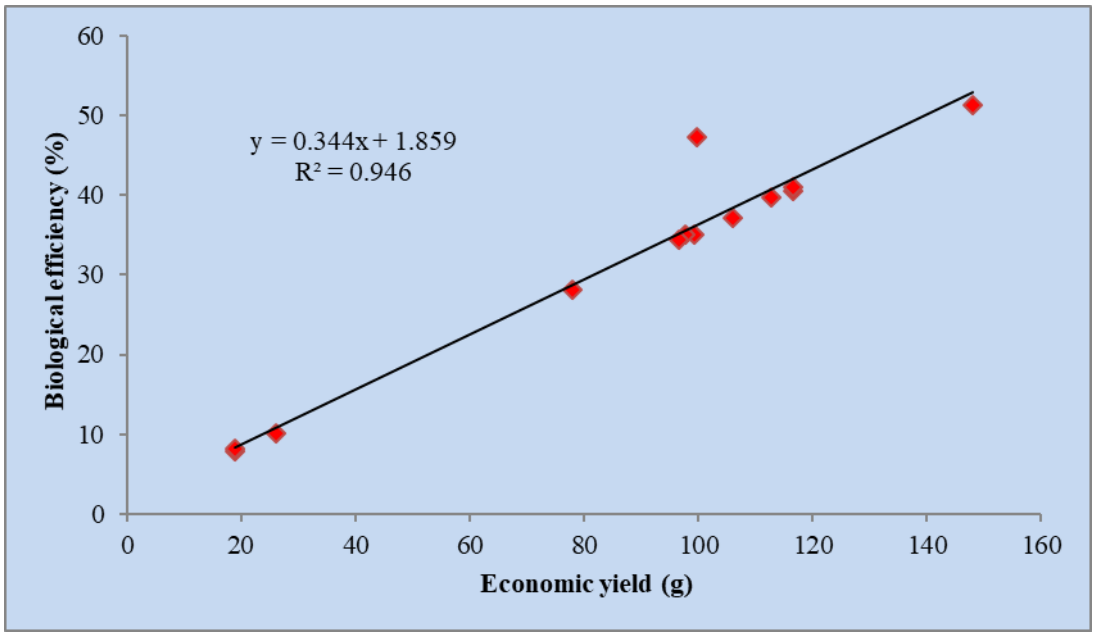


Figure 07. Relationship between economic yield (g) biological efficiency (%) of oyster mushroom (*Pleurotus ostreatus)*

CHAPTER V

SUMMARY AND CONCLUSION

The present piece of work was carried out at the Plant Pathology Laboratory and Mushroom Culture House (MCH) of the Department of Plant Pathology, Sher-e- Bangla Agricultural University (SAU), Sher-e-Bangla Nagar Agargaon, Dhaka, 1207, during July to October- 2020, to investigate the ability of enrichment of substrate with different supplements on contaminations, growth and yield of oyster mushroom. The experiment consisted of single factor, and followed Completely Randomized Design (CRD). Factor A: (13) *viz*: T_0 : Sawdust (Control), T_1 : Sawdust + Compost - 5 g, T_2 : Sawdust + Compost - 10 g, T_3 : Sawdust + Compost - 15 g, T_4 : Sawdust + Vermicompost - 5 g, T_5 : Sawdust + Vermicompost - 10 g, T_6 : Sawdust + Vermicompost - 15 g, T_7 : Sawdust + Mustard oil cake- 5 g, T_8 : Sawdust + Mustard oil cake- 10 g, T_9 : Sawdust + Mustard oil cake- 15 g, T_{10} : Sawdust + (Co+Vc+Mc)- 5 g, T_{11} : Sawdust + (Co+Vc+Mc)- 10 g and , T_{12} : Sawdust + Co+Vc+Mc)-15 g, [Co: Compost, Vc: Vermicompost and Mc: Mustard oil cake]. Data on different growths, yield contributing characters and yield were recorded to find out the effect of different levels of supplements on sawdust for the production of mushroom cultivation.

Different levels of supplements application along with sawdust showed significant variations in respect of most of the characteristics of mushroom. From the experiment result revealed that the maximum mycelium running rate in spawn packet was observed in T_6 (0.64 cm/day) treatment, the maximum days required from mother inoculation to completion of mycelium running was observed in T_2 (28 days), the maximum days required from stimulation to primordia initiation was observed in T_{12} (6.89 days), the maximum days required from incubation to 1st harvest was observed in T_2 (30.33 days) , the maximum days required from primordia initiation to 1st harvest was observed in T_{10} (7.67 days), the maximum days required from primordia initiation to final harvest was observed in T_6 (47.67 days) . The maximum number of primordia /packet (80.00), number of fruiting body /packet (54.67), number of effective fruiting body /packet (41), stripe length (2.69 cm), length of fruiting body (6.56 cm), breadth of fruiting body (5.67 cm), biological yield (153.67 g), economic yield (116.67 g), dry yield (29.33 g) and biological efficiency (51.22 %) were observed in T_2 (Sawdust + 10 g Compost) treatment. On the other hand, the minimum mycelium running rate in spawn packet

(0.30 cm/day) and days required from mother inoculation to completion of mycelium running (23 days) were observed in T_0 treatment. The minimum days required from stimulation to primordia initiation was observed in T_3 (5.43 days) treatment. The minimum days required from incubation to 1st harvest was observed in T_0 (25 days) treatment. The minimum days required from primordia initiation to 1st harvest was observed in T_5 (4.67 days) treatment. The minimum days required from primordia initiation to final harvest (32 days), number of primordia /packet (32.33), number of fruiting body /packet (10), number of effective fruiting body/packet (7.33), stripe length (2.02 cm), length of fruiting body (2.53 cm), breadth of fruiting body (3.03 cm), biological yield (23.33 g), economic yield (19g), dry yield (5.33 g) and biological efficiency (7.78 %) were observed in T_0 (Control).

Conclusion

There was no contamination found in T_1, T_2, T_3, T_4, T_{10} and T_{11} whereas the highest contamination was observed in case of T_7 (5g mustered oil case spawn packet). Maximum mycelium running rate and the highest total harvesting period were recorded where the spawn also enriched with 10g compost. Among the treatments, T_2 (Sawdust enriched with 10 g compost) performed best in yield contributing characters such as maximum number of primordia/packet, effective fruiting body/packet, pileus and stipe length, pileus breadth of fruiting body as well as the highest biological yield, economic yield and biological efficiency.. Four contaminants namely *Penicillium sp., Aspergillus niger, Trichoderma sp.* and *Rhizopus sp.* were isolated and identified from contaminated spawn during cultivation of mushroom.

Recommendation

In this experiment, sawdust along with 10 g Compost supplementation (T_2) performed better in respect of growth, yield and yield contributing characteristics of oyster mushroom. Therefore, sawdust along with Compost - 10 g supplementation (T_2) can be recommended for wide range cultivation of oyster mushroom.

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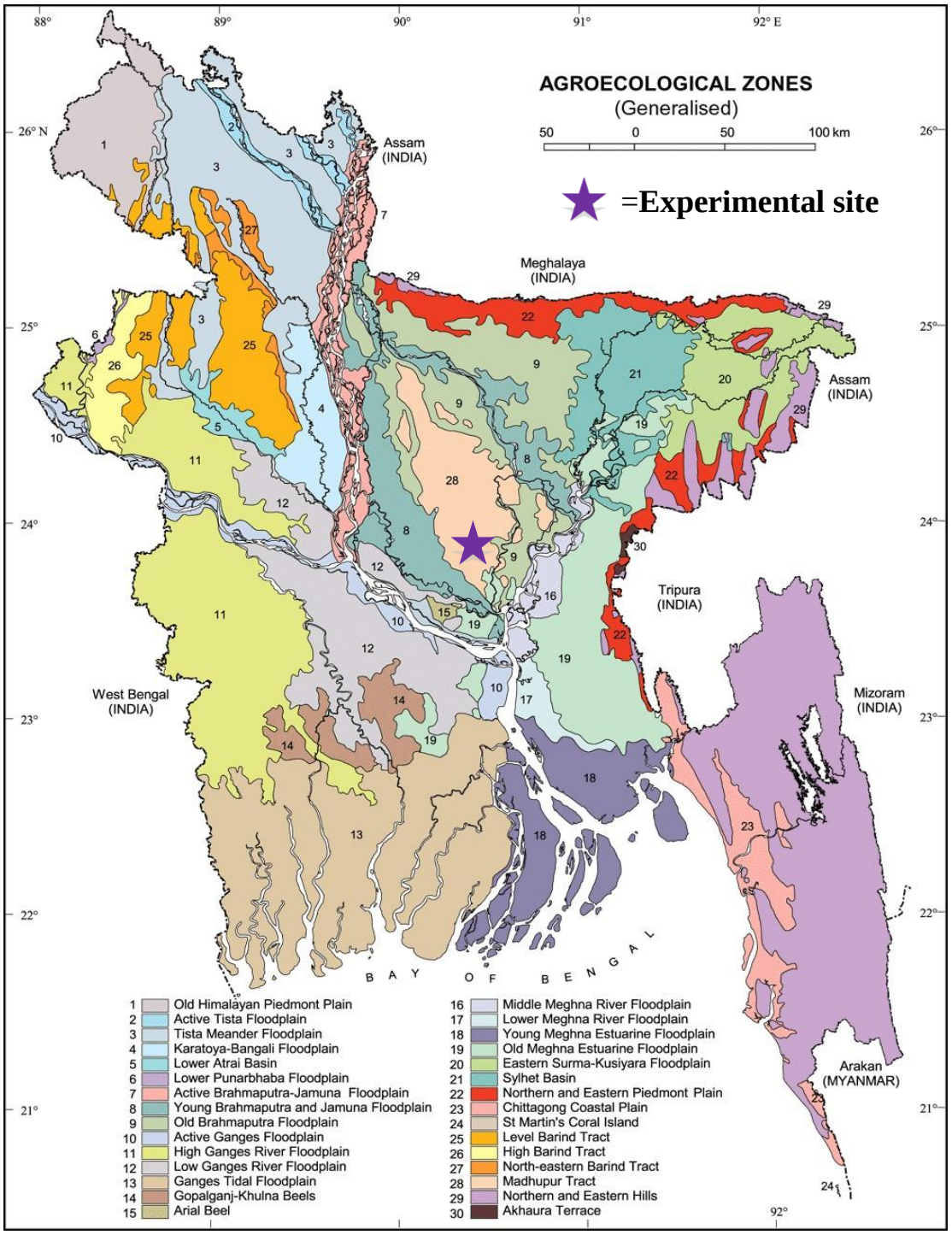
APPENDICES

Appendix I. Map showing the experimental site under study



Appendix II. Monthly meteorological information during the period from July to October, 2020.

Year	Month	Air temperature (^{0}C)		Relative humidity (%)	Total rainfall (mm)
		Maximum	Minimum		
2020	July	32.6°C	26.8°C	81%	114mm
	August	32.6°C	25.5°C	80%	106mm
	September	32.4°C	25.7°C	80%.	86mm
	October	31.2°C	23.9°C	76%.	52mm

Source: Metrological Centre, Agargaon, Dhaka (Climate Division

Appendix III. Layout of the experiment

R_1	R_2	R_3
T_0	T_{12}	T_0
T_1	T_{11}	T_1
T_2	T_{10}	T_2
T_3	T_9	T_3
T_4	T_8	T_4
T_5	T_7	T_5
T_6	T_6	T_6
T_7	T_5	T_7
T_8	T_4	T_8
T_9	T_3	T_9
T_{10}	T_2	T_{10}
T_{11}	T_1	T_{11}
T_{12}	T_0	T_{12}

1m

1m

LEGENDS

T_0 : Control, T_1 : Compost - 5 gm, T_2 : Compost - 10 gm, T_3 : Compost - 15 gm, T_4 : Vermicompost - 5 gm, T_5 : Vermicompost - 10 gm, T_6 : Vermicompost - 15 gm,, T_7 : Mustard oil cake- 5 gm, T_8 : Mustard oil cake- 10 gm, T_9 : Mustard oil cake- 15 gm, T_{10} : (Co+Vo+Mc)- 5gm, T_{11} : Co+Vo+Mc)- 10gm and T_{12} : Co+Vo+Mc)-15, [Co: Compost, Vo: Vermicompost and Mc: Mustard oil cake]

Appendix IV. Analysis of variance on data with the effect of different levels of supplements on mycelium running rate in spawn packet (cm), days required for mother inoculation to completion of mycelium running and days required for stimulation to primordial initiation of oyster mushroom

Source	DF	Mean square of		
		Mycelium running rate in spawn packet (cm)	Days required for mother inoculation to completion of mycelium running	Days required for stimulation to primordia initiation
Treatment	**12**	0.03081**	10.4677**	0.51545**
Error	**26**	0.00004	0.1538	0.00154
Total	**38**			

**: Significant at 1% level of probability

Appendix V. Analysis of variance on data with the effect of different levels of supplements on days required from incubation to harvest , days required from 1st primordia initiation to 1st harvest, days required from 1st primordia initiation to final harvest of oyster mushroom (*Pleurotus ostreatus*)

Source	DF	Mean square of		
		Days required from incubation to harvest	Days required from 1st primordia initiation to 1st harvest	Days required from 1st primordia initiation to final harvest
Treatment	**12**	12.0029**	1.54648**	80.4506**
Error	**26**	0.0962	0.00192	0.6154
Total	**38**			

**: Significant at 1% level of probability

Appendix VI. Analysis of variance on data with the effect of different levels of supplements on number of primordia /packet, number of fruiting body /packet and number of effective fruiting body/packet of oyster mushroom *(Pleurotus ostreatus)*

Source	DF	Mean square of		
		Number of primordia /packet	Number of fruiting body /packet	Number of effective fruiting body/packet
Treatment	**12**	597.812**	455.166**	289.233**
Error	**26**	0.385	0.154	0.085
Total	**38**			

**: Significant at 1% level of probability

Appendix VII. Analysis of variance on data with the effect of different levels of supplements on length of stripe (cm), length of fruiting body (cm) and breadth of fruiting body (cm) of oyster mushroom *(Pleurotus ostreatus)*

Source	DF	Mean square of		
		Length of stripe (cm)	Length of fruiting body (cm)	Breadth of fruiting body (cm)
Treatment	**12**	0.16351**	3.67337**	2.45327**
Error	**26**	0.00049	0.00084	0.00315
Total	**38**			

**: Significant at 1% level of probability

Appendix VIII. Analysis of variance on data with the effect of different levels of supplements on yield and biological efficiency of oyster mushroom (*Pleurotus ostreatus*)

Source	DF	Mean square of			
		Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)
Treatment	**12**	5669.22**	5017.51**	157.695**	629.838**
Error	**26**	0.77	1.31	0.078	0.385
Total	**38**				

**: Significant at 1% level of probability