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Adaptation and Cultivation of Different Strain of Shiitake (Lentinus Edodes) Mushroom Under Bangladesh Condition

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ADAPTATION AND CULTIVATION OF DIFFERENT STRAIN OF SHIITAKE (*Lentinus edodes*) MUSHROOM UNDER BANGLADESH CONDITION

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DECLARATION

I, hereby declare that the entire work submitted as a thesis "ADAPTATION AND CULTIVATION OF DIFFERENT STRAIN OF SHIITAKE (*Lentinus edodes*) MUSHROOM UNDER BANGLADESH CONDITION" to the Department of Agronomy and Agricultural Extension, University of Rajshahi, Bangladesh, towards the fulfillment for the degree of **Doctor of Philosophy**, is the result of my own investigation. The thesis has not been submitted in the substance for any other degree.

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CERTIFICATE

This is to certify that the research work presented in this dissertation entitled **"ADAPTATION AND CULTIVATION OF DIFFERENT STRAIN OF SHIITAKE (*Lentinus edodes*) MUSHROOM UNDER BANGLADESH CONDITION"** for the degree of **Doctor of Philosophy**, University of Rajshahi, Rajshahi, Bangladesh is a bonafide research work of Mohammad Golam Mohsin under our suvervision at "National Mushroom Development and Extention Centre", Sobhanbagh, Savar, Dhaka. The information included in this thesis is original and was not submitted before for any other degree.

The advice and guidance received during the course of investigation have been fully acknowledged.

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ABSTRACT

Several experiments were conducted at National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka, Bangladesh, during the period from July 2011 to March 2014 to study the adaptation and cultivation of shiitake mushroom as affected by different strains, seasons, supplement, substrate, opening pattern, amount of substrate and age of spawn packet. All the experiments except seasonal effect were a two factor experiment. These experiments were laid out in the Completely Randomized Design (CRD) with four replications.

In the first experiment 23 strains of shiitake mushroom were cultivated in four seasons (autumn, late autumn, winter and spring) to select the suitable strain and best season for production of shiitake mushroom. The highest mycelium growth rate (7.95 mm/day) and the lowest time required to completion of mycelium running (12.25) was found in autumn season by the strain Le 19. Le 8 gave the highest yield (191.00 g) and highest biological efficiency (109.10 %) in winter season. The strain Le 8 also gave highest yield (145.00 g) and highest biological efficiency (83.29 %) in autumn season. Le 16 gave the highest yield (175.00 g) and highest biological efficiency (100.30%) in late autumn season which was statistically similar to the strain Le 8 and second highest yield (171.80 g) in winter season. Le 12 gave second highest yield (99.00 g) and biological efficiency (56.57 %) in autumn season which was statistically similar to Le 11. Le 1 gave the highest yield (163.50 g) and highest biological efficiency (93.43 %) in spring season but no yield was obtained in autumn season. Le 13 did not produce any fruit body, so no yield was found from the strain in any season.

In second experiment three different types of supplement and two strains of shiitake mushroom (Le 8 and Le 11) were used to identify the best supplement and suitable strain for cultivation of shiitake mushroom. The highest mycelium growth rate (0.51 cm) was found in strain Le 8 and rice bran treatment combination while lowest mycelium growth rate (0.34 cm) was found in Le 8 and wheat bran treatment combination. The highest time required to completion of mycelium running (45 days) was obtained from the strain Le 11 and rice bran + wheat bran (1:1) treatment

combination and the lowest time required to completion of mycelium running (33.25 days) was obtained from the strain Le 8 with wheat bran treatment combination. The maximum time (105.00 days) required for bump formation from the treatment combination of strain Le 11 with wheat bran and the minimum time (81.00 days) was found from strain Le 8 with maize powder treatment combination. The highest number of effective fruiting body (45.50) was recorded in Le 11 with wheat bran treatment combination which was statistically similar to Le 8 with wheat bran. The lowest number of effective fruiting body (11.50) was recorded from strain Le 8 with rice bran treatment combination. The highest yield (159.00 g) was recorded in Le 8 with wheat bran treatment combination. The lowest yield (98.25 g) was recorded from strain Le 11 with rice bran treatment combination.

In third experiment ten different substrates containing sawdust of teak chambul (*Michelia campaca*), ipil-ipil (*Leucaena leucocephala*), teak (*Tectona grandis*), gamari (*Gmelina arborea*), rain tree (*Albizia saman*), mahagony (*Swietenia mahagoni*), mango (*Mangifera indica*), mixed sawdust, mixed woodchips, rice straw and two strains of shiitake mushroom were used to identify the suitable strain and best substrate for cultivation of shiitake mushroom. The highest mycelium growth rate (4.50 mm/day) was found from the treatment combination of Le 8 with mango sawdust and the lowest mycelium growth rate (1.00 mm/day) was found from the treatment combination of the strain Le 12 with woodchips. The lowest time required to completion of mycelium running (23.75 days) was found from the treatment combination of Le 8 with mango sawdust and the highest time required to completion of mycelium running (106.50 days) was found from the treatment combination of the strain Le 12 with woodchips. Time required to bump formation (120.80 days), time required from opening to first harvest (15.50 days) and time required for harvest (136.30 days) was found highest from the treatment combination of Le 8 with teak chambul. The lowest time required to bump formation (90.75 days) was found from the treatment combination Le 8 with rain tree and the lowest time required from opening to first harvest (2.50 days) was found from the treatment combination of Le 12 with sawdust of ipil-ipil. The lowest time required for harvest (99.00 days) was found highest from the treatment combination of Le 8 with gamari. The highest

number of fruiting body (43.75) and the highest number of effective fruiting body (33.75) was also highest in Le 8 when grown on mango sawdust. Yield attributes of two strains such as stalk diameter, pileus diameter and pileus thickness were significantly higher when culture on mango sawdust. The highest yield (189.80 g) and biological efficiency (108.4%) were recorded from the strain Le 8 grown on mixed sawdust followed by mango sawdust. In general, performance of Le 8 was better than Le 12 in terms of yield and yield contributing characters.

In fourth experiment eleven types of opening pattern during incubation for early bump initiation such as top open place on floor, top open place on rack, total open and covered with polypropylene bag place on floor, total open and covered with polypropylene bag place on rack, only cotton plug open and place on floor, no open and place on floor, only cotton plug open and place on rack, no open and place on rack (control), total open and place on rack, total open and place on floor, no open and place on culture house floor were used on growth, yield and yield attributes of two strains (Le 8, Le 16) of shiitake mushroom to find out the appropriate opening pattern and suitable strain. The highest time required for bump formation (120.80 days), highest time required for harvest (129.50 days) was observed from the strain Le 8 with T_8 (no open and place on rack i.e. control) treatment combination and the lowest time required for bump formation (105.00 days), lowest time required for harvest (111.00 days) was observed from the strain Le 16 with T_5 (only cotton plug open and place on floor) treatment combination. The highest number of fruiting body (62.00), the highest number of effective fruiting body (37.25), highest yield (193.00 g) and highest biological efficiency (110.30%) were recorded from the strain Le 16 with treatment T_5 (Only cotton plug open and place on floor). The lowest number of fruiting body (2.00), the lowest number of effective fruiting body (1.25), lowest yield (29.00 g) and lowest biological efficiency (16.57%) were recorded from the strain Le 8 with treatment T_{10} (Total open and place on floor). In general, performance of Le 16 was better than Le 8 in terms of yield and yield contributing characters.

In fifth experiment four different amounts of substrates (300g, 500g, 750g and 1000g) and four strains (Le 8, Le 11, Le 12 and Le 16) of shiitake mushroom were cultivated

to know the optimum amount of substrate and to select the best performing strain. The growth, yield and yield contributing characters of shiitake mushroom were significantly influenced by the different amount of substrate. The time required for mycelium running and total harvest were increased with the increases of amount of substrate. The number of fruiting body was highest (81.50) in 500g size of spawn packet with Le 12 treatment combination and it was lowest (1.25) in 1000g size of spawn packet with the strain of Le 8 and Le 16 treatment combination. The maximum yield (135.80 g) was recorded from the strain Le 12 when cultured on 500g size of spawn packet and the lowest yield (24.50 g) was obtained from 750g size of spawn packet with similar strain. The highest biological efficiency (85.47%) was observed in the treatment combination of 300g spawn packet with Le 11. The biological efficiency decreased with the increase of size of spawn packet. Among the strain Le 12 perform better than other strain and 500 g size of spawn packet was appropriate.

In sixth experiment ten different age (40, 50, 60, 70 80, 90, 100, 110, 120 and 130 days) of spawn packets and two strain (Le 8 and Le 16) of shiitake mushroom were cultured to determine the right age of spawn packet and to select the best strain for shiitake mushroom cultivation. A wide variation was observed in yield and biological efficiency in different ages. The highest yield (179.50 g) and highest biological efficiency (102.60%) were recorded from the treatment combination of 90 days old spawn packet with the strain Le 16 followed by 100 and 110 days old spawn packets with same strain and the lowest yield (36.75 g) and lowest biological efficiency (21.00%) were found in 60 days old spawn packet with Le 16 strain. No yield was obtained from the 40 and 50 days old spawn packets.

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ABBREVIATIONS AND ACRONYMS

:	Colon
^{0}C	Centigrade (in degree)
BE	Biological efficiency
CC	Corn cobs
cm	Centimeter (s)
CMC	Carboxy methyl cellulase
CRD	Completely Randomized Design
CSM	Cotton seed meal
CV	Co-efficient of variation
CW	Cotton waste
DAE	Department of Agricultural Extension
DMRT	Duncan's Multiple Range Test
DP	Diameter of pileus
DS	Diameter of stalk
e.g.	Example
Ed	Edition
et al.	et alli = other people
etc.	Etcetera = and others
Fig.	Figure (s)
g.	Gram (s)
HDTC	Horticulture Demonstration and Training Centre
i.e.	That is
Kg.	Kilogram (s)
Le	*Lentinus edodes*
LS	Length of stalk
LSD	Least Significant Difference
MCH	Mushroom culture house
mg	Milligram (s)
MGR	Mycelium growth rate

mm	Milimetre
MP	Maize powder
NAMDEC	National Mushroom Development & Extension Centre
NFB	Number of fruiting body
NFEB	Number of effective fruiting body
No.	Number
NS	Non significant
OS	Oak wood sawdust
PDA	Potato dextrose agar
pH	Negative Logarithm of $[H^+]$ concentration
PM	Peanut meal
PTC	Plant tissue culture
RB	Rice bran
SBM	Soybean meal
SD	Saw dust
TP	Thickness of pileus
TRBF	Time required for bump formation
TRH	Time required for harvest
TRMR	Time required to completion of mycelium running
TROFH	Time required from opening to first harvest
Viz.	Videli = Namely
WB	Wheat bran
WS	Wheat straw
Wt.	Weight

CHAPTER I

INTRODUCTION

Mushrooms were so far considered as luxury food especially among the rich community because of their unique flavor and excitingly different taste but now they have grown to a common mans food. Mushrooms are traced as special kind of food, since ancient times. The Greeks believed that mushrooms provided strength for warriors in battle and Romans regarded them as “Food of Gods” or “Gods Flesh”, which were served only on festive occasions. The Mexican Indians used mushrooms as hallucinogens in religious ceremonies and in witchcraft and for therapeutic purposes (Shu-Ting and Phillip, 1993). Mushrooms are low in calories but rich in proteins, vitamins and minerals and the nutritionally mushrooms can be placed between meat and vegetable and therefore mushrooms are aptly called as vegetable meat or slimming foods. Mushroom proteins are considered to be intermediate between that of animals and vegetables (Kurtzman, 1976) as it contains all the nine essential amino acids required for human body (Hayes and Haddad, 1976). A protein revolution is possible through mushrooms after the green, white and blue revolutions. Mushrooms being low calorie food with little fat are highly suitable for obese persons. With no starch and very low sugars, they are the “delight of the diabetic”. Mushroom cultivation is an ecofriendly activity where agricultural/industrial wastes are utilized and recycled. It is gaining importance in recent years due to increasing global demand for protein.

Shiitake (*Lentinus edodes)* is an edible mushroom commonly used as food in Asian countries, and also a traditional Chinese medicine (Lin *et al.*, 2008) which is cultivated on a large scale in many countries (Poppe and Hofte, 1995; Chang and Miles, 2004). *Lentinus* belongs to the family Tricholomataceae, order Agaricales, subclass Holobasidiomycetiae and class Basidiomycetes is the second most important edible mushroom in the world from the stand point of production. Shiitake is Japanese name which derived from **“Shii”** means hardwood tree (*Pasania* spp, *Quercus* spp) and **“Take”** means mushroom. It is the most popular fungus cultivated in Japan, China and other East Asian countries. Besides China and Japan, it is also widely cultivated in Taiwan, Thailand, Korea, Singapore as well as Holland, Vietnam, United

States and Canada. Shiitake is among the "Big six" mushrooms in the world accounting for 17% of world production in terms of tonnage (Miles and Chang, 1997; Chang and Miles, 2004). It can grow in winter season and also it can grow all the year in controlled condition. Shiitake has second position (25.4%) on production (Chang, 1999). After the well-known button mushroom (*Agaricus bisporus*), shiitake is the most cultivated of exotic mushroom in the world.

Shiitake mushroom is also known as golden mushroom, black mushroom, black forest mushroom and shiang-ku mushroom. The shiitake mushroom has the distinctive advantage of a much longer shelf-life because they are more commonly sold dried, while most of other mushrooms are sold fresh. It is one of the best known and best characterized mushrooms used for medicinal purposes. Several medicinal properties have been attributed to shiitake in recent years. These properties include antitumor polysaccharides activity (Breene, 1990; Mizuno, 1995a) and glycoproteins, antiviral nucleic acids, platelet agglutination inhibitive substances, and anti-cholesterol active substances (Tokuda *et al*., 1974, Fujii *et al*., 1978, Tokuda and Kaneda, 1978; Wasser, 2002; Mizuno, 1995a; Suzuki, 1979). *Lentinus* is liked by the consumers because of its unique taste and flavour, and presence of a chemical lentinan which reduces plasma-cholesterol level. A famous Chinese doctor, Wu. Shui, during the Ming Dynasty (1368-1644) wrote that the *Lentinus* mushroom was capable, expressed in modern terminology, of generating stamina, curing colds, improving the circulation, and lowering the blood pressure. At the present time numerous scientific investigations have established the nutritive value and medicinal benefits of *Lentinus* in lowering serum cholesterol levels and possessing antitumer and antiviral activities. With the improvement of science and technology, the deeper outstanding of its values and the upgrade of people’s living level, it is predictable that the market demand of *Lentinus* will be larger and larger. The development of *Lentinus* production therefore becomes so necessary and urgent. For all these reasons the demand for *Lentinus* has greatly increases in recent years.

Shiitake mushroom is the second most popular edible mushroom (Chang, 1999 and Chiu *et al*., 1999). It is highly prized in the Orient for its flavor and reputed edible and medicinal value. Many strains of shiitake mushroom are available in the world which is extensively cultivated. The strains of this valuable mushroom vary widely,

particularly in the time required for mycelium colonization, bump formation and fruiting body development. The morphology and productivity of shiitake mushroom vary according to the strains based on the influence of environmental factors (Triratana and Tantikanjana, 1987). The mycelium growth in the vegetative phase involves producing quality fruiting bodies in the reproductive phase. A spawn run of different strains is of ultimate importance for adjusting the reproductive phase. For one strain, 60 days is sufficient to mature, whereas this time would be insufficient for another strain (Miles and Chang, 1989). Substrate selectivity, growth (some strains may produce pre-mature fruiting), quality (shape, size, thickness, color, flavored and aroma etc.) and yield are also strain related (Chen, 2001). Many studies have been carried out in the world to improve the quality and increase the production of *Lentinus edodes.* But, the production of this mushroom is fairly new in Bangladesh.

Mushroom production is completely different from growing green plants because it does not contain chlorophyll and therefore depend on other plant material (substrate) for their food. Therefore, the substrate is an important item for growing mushroom. Sawdust is the most popular basal ingredient in synthetic formulations of substrate used to produce shiitake (Miller and Jong, 1987). Other basal ingredients that may be used include straw and corn cobs or mixtures thereof. Regardless of the main ingredient used, starch-based supplements such as wheat bran, rice bran, millet, rye, corn, etc are added to the mix in a 10 to 40% ratio (dry wt.) to the main ingredient. These supplements serve as nutrients to provide an optimum growing medium (Royse *et al*., 1990). The yield of mushroom might be varied due to the use of heterogeneous mixture of sawdust. It is a kind of medium, which supports good growth of mushroom mycelium but does not encourage growth of competitor. Mushroom substrate may be defined as a kind of lignocelluloses material which supports the growth, development and fruiting of mushroom (Chang and Miles, 1988a). Shiitake mushroom is traditionally cultivated on shii tree or wood log in Japan. Unavailability of shii tree necessitates a search for alternative substrates for shiitake and general mushroom cultivation. Freely available huge amounts of sawdust's of different timber plants offer potential as alternative substrate sources for mushroom cultivation in Bangladesh. In our country, this mushroom is cultivated on sawdust based substrates in polypropylene bags. In the life cycle of shiitake there are two stages- mycelium running, mycelium coat formation, pigmentation and bump formation. To shift the mycelium growth stage to reproductive stage for formation of fruiting body generally

some kinds of stimuli are needed. The physical stimuli are low temperature, high carbon dioxide concentration for bump formation. These stimuli can be initiated by some management practices like opening during incubation and aging of spawn packets, amount of substrate used in spawn preparation etc.

Generally, people in Bangladesh are still not very aware of nutritional and medicinal importance of mushrooms. The history of mushroom cultivation is very recent in Bangladesh. Only some species of mushrooms are now cultivated in this country and among these *Pleurotus ostreatus, P. sajor-caju, P. florida* and *Calocybe indica* are popular and widely accepted (Amin *et al*., 2007). As there is absolute need for improvement in the yield potentials of shiitake mushroom before its commercial ventures, the current study has mainly focused on to find out the easy, quick and economical method for large scale production of shiitake mushroom with the following objectives:

1. To identify the strains suitable for cultivation in Bangladesh under four seasons
2. To find out the appropriate substrate under Bangladesh condition
3. To determine the optimum supplement level
4. To find out the optimum amount of substrate, age of spawn packets and opening pattern of shiitake mushroom
5. To find out the easy, quick and economical method for large scale production of shiitake mushroom
6. To introduce the production technology of shiitake mushroom under Bangladesh condition

CHAPTER II

REVIEW OF LITERATURE

A series of scientific thoughts involved in mushroom production technology is reviewed under this chapter. The literatures pertaining to shiitake mushroom are meager and the same was recently put a scientific footing in the field of mushroom biology. All relevant literatures concerning to shiitake mushroom cultivation is reviewed here under.

2.1 Effect of strain and season

The spawn run of shiitake requires light intensities of 180-940 lux with an optimum of 550 lux (Han *et al.* 1981) and optimal light intensity during fruiting is 50-100 lux (Chen, 2005). Excessive light exposure can reduce the number of fruit bodies, whereas a lack of light diminishes the diameter of pilei and the length of stipes (Han *et al.* 1981). Furthermore, fruiting bodies apparently develop abnormally and sporulation diminishes when the mushrooms are grown under filtered light using coloured cellophane papers (Tokimoto and Komatsu 1978). Light intensity during a bright sunny day can be more than 100000 lux (Halsted, 1993).

Kuso (1982, cited in Przybylowicz and Donoghue, 1988) reported that shiitake have been found to survive in logs at temperatures from - 30 to 45^0C. The impact of high temperature on the survival of fungi is determined by not only by the temperature, but also on exposure time (Przybylowicz and Donoghue, 1988). The damage caused by a short period of exposure at high temperature corresponds to prolonged exposure at lower temperature. For example, Tokimoto and Komatsu (1978) report mycelium of shiitake can be terminated at 45^0C; however, prolonged exposure of mycelium to 35^0C can also cause mycelial death (Przybylowicz and Donoghue, 1988).

The dramatic change from vegetative mycelial growth to produce macroscopic fruiting bodies in the reproductive phase requires enormous amount of energy reserves. A vigorous spawn run is of ultimate importance. It should be noted that strains vary greatly in duration for mycelial maturation. For one strain, 60 days is sufficient to mature, whereas this would be insufficient time for another strain (Miles and Chang, 1989).

Once fruiting has commenced, logs that are allowed to fruit naturally may continue to repeat fruiting during spring and autumn for another 3 to 5 years (Davis, 1993) or longer up to 7 years (Leatham, 1982). Logs that are grown indoors can fruit more frequently by using a forced fruiting method and requires logs to be immersed in water every 10 to 12 weeks (Sabota, 1998). Forcing reduces the productive life of the logs to 2 or 3 years (Davis, 1993).

Stamets (1993, 2000) summarized the growth parameters for shiitake cultivation. He reported that the optimum temperature required for spawn run 10-16^{0}C, fruiting 16-18^{0}C (cold temperature strains). In case of warm temperature strains induced primordia and fruiting temperature 16-21^{0}C and 21-27^{0}C respectively. For all strains, in case of spawn run temperature required 21-27^{0}C, humidity 95-100% R.H, lighting 50-100 lux, induced primordia 95-100% R.H, 500-2000 lux and fruiting 60-80%, R.H, 500-2000 lux.

In an experiment, Bugarski *et al*. (1994) studied the strains of Oyster mushrooms NS-16 and NS-11 with the aim to determine the best yielding strain, shortest incubation period and fast fructification. The hybrid H_7 was used for check. Three different substrates, Wheat straw, Maize stalks, and Soybean straw were used. First fruiting body appeared on H_7 on 30th to 33rd days depending on substrate. Gupta (1989) found that the fruiting bodies appeared 12-15 days after the bags were removed, and the first crop was harvested 2-3 days later on Wheat straw and *P. sajor-caju* can be successfully cultivated in both hot and spring seasons.

There are many factors promoting fruiting body formation of shiitake including temperature fluctuation, high humidity, water soaking (Royse, 1997: 2-4 hr at 12^{0}C; Stamets, 1993: 24-48 hr), removing of CO_2, or increasing of oxygen supply, physical shocks (agitation, disturbance), stabbing (with a long metal needle) and injecting (with water), turning the blocks up-side-down and beating (e.g. natural logs) (Oei, 1996).

There are three temperature zones which are important for mushroom cultivation: the air temperature outside the growing room, the air temperature inside the growing room and substrate temperature (Oei, 1996). Of these, the substrate temperature (i.e.

within the log) is the most important parameter influencing mycelial growth and fruiting body formation. Despite the importance of substrate temperature, Oei (1996) does not specify the temperature suitable for *Lentinula edodes* although substrate temperatures above 35^0C can cause thermophilic microflora to grow. This activity generates heat which produces an increase in substrate temperature and eventually causes mycelia to terminate.

There is an optimum temperature for mycelial growth above and below which growth is restricted (Przybylowicz and Donoghue, 1988; Miles and Chang, 1997). This may be due to the effect of temperature on enzyme activity and the resulting changes in rates of chemical processes (Miles and Chang, 1997). Temperature also plays an important role in the initiation and development of fruiting bodies and a sudden shift in temperature may be required to induce fruiting of mushrooms (Komatsu, 1961; cited in Przybylowicz and Donoghue, 1988).

Suitable temperatures for mycelial growth and fruiting for a number of species are reported in Kaul (1997). For *Agaricus bisporus*, the optimum temperature for mycelial growth is 23-25^0C, while fruit development is optimal within the range of 14-16^0C. For *Coprinus cinereus* (Schaeff.) Gray, the optimum temperature for vegetative growth is 37^0C; however fruiting does not occur at temperatures above 30^0C. The optimum temperature requirements are more complex for *Flammulina velutipes* (Curt.:Fries) Singer, with the optimum temperature for mycelial growth being 22-26^0C while primordium formation occurs at temperatures of between 10 and 20^0C and fruit development is at 10-15^0C.

Yu (1998) reported that use low temperature, high quality and easily adaptable strains for huagu (flower shiitake) production. Strains towards the lower temperature margin in mid temperature range can also be used. Examples of desirable huagu strains are: L-241-1, Jean-Yin #1, Yee-You #5, 7402, N-06. Strain characteristics should be thoroughly studied before cultivation. For fruiting outdoors, time of spawning should be coordinated with the maturation characteristics in order to benefit from the winter stimulation. For example, strain 7402, N-06, late maturing strains, should be inoculated early during March and April, while 9018, Le 204, early to mid-maturing strains, should be inoculated in May-June in Bi-Yang, China.

The once placed in the fruiting area, mycelium will start to form reproductive nodules under the bark as the logs dries. The emerging primordial fruit bodies are also known as pins (Koske 1998, McCoy and Bruhn 2005, Oei 1996). During formation of primordial fruit bodies, the environment is managed to ensure the right number of pins form and begin to grow out. Recommended moisture content of logs (LMC) varies at this stage of development. Komatsu and Tokimoto (1982, cited in Przybylowicz and Donoghue 1988) and Tokimoto *et al*. (1980) recommend a range of LMC from 35 to 65% with optimum LMC between 55 and 65%. Koske (1998) suggests maintaining the LMC within a much narrower range of 55 to 60%. Humidity around the logs of between 60 to 85% is necessary to avoid premature drying of the primordial.

Chen *et al.* (2000) described shiitake strains are classified into four categories on the basis of fruiting temperature. In case of low temperature 10^0C, mid temperature 10-18^0C, high temperature 20^0C and above wide-range temperature 5-35^0C.

Sabota (2007) suggests that the optimal relative humidity for growth is 80-85%, while Leatham (1982) suggests that relative humidity between 85 and 90% is optimal. Stamets and Chilton (1983) provide humidity levels for various stages of production starting with a high humidity (90-100%) during the spawn run which is reduce to 85% during primordial formation and cropping. Shiitake has been found to survive and grow in relative humidity ranging from 50-70% (Brauer *et al.* 2002), 80-95% (Queiroz *et al*. 2004) and 70-100% (Campbell and Racjan 1999).

Sarker *et al.* (2009) conducted an experiment to determine the performance of nine different strains of shiitake mushroom which were cultivated on sawdust. A wide variation was found in mycelium growth rate and duration to complete mycelium running. The highest mycelium growth rate (0.35cm/day) was obtained in *Lentinula edodes* (Le)-9, which was statistically similar to Le-6, Le-8, Le-10 and Le-4 and the lowest mycelium growth rate was obtained in Le-2. The minimum time required to complete mycelium running (35.17 days) was observed in Le-9 followed by Le-6 and Le-8 whereas maximum time required to complete mycelium running was found in Le-2. Among the nine strains, Le-10 performed best in the respect of total time required from inoculation to bump formation, primordia initiation and first harvest.

Highest number of effective fruiting body (14.33/packet) was recorded in Le-8 which was statistically similar to Le-11 and the lowest number of effective fruiting body was recorded in Le-4. The highest length of stalk (5.50cm), diameter of stalk (1.30cm), diameter of pileus (6.917cm) and thickness of pileus (1.25cm) were observed in Le-5 which was statistically similar to Le-6. Significant variation in biological yield, economical yield and biological efficiency were observed among the selected strains. The highest biological yield, economic yield and biological efficiency were estimated in Le-8 followed by Le-11 and Le-12 where as Le-4 performed very poorly.

Uddin *et al.* (2010) conducted an experiment to determine the suitable seasonal condition for the production of oyster mushroom in Bangladesh. They cultivated four species of oyster mushroom in every season. In all of the selected species of this study, the minimum days required for primordial initiation, and the maximum number of fruiting bodies, biological yield and biological efficiency were found during December to February (14-27^{0}C, 70-80% RH). The production was found minimum during the cultivated time August to October. They suggest cultivation of selected *Pleurotus* spp. in winter (temperature zone 14-27^{0}C with relative humidity 70-80%) for better production and biological efficiency.

2.2 Effect of supplement to substrate

Five years' data were presented from trials with different strains of *P. ostreatus*, *P. pulmonarius* and *P. columbinus* on the effects on yields of adding various amendments to the straw substrate, and of conditioning at different temperatures and for different times. The amendments comprised rice bran, lucerne meal, feather meal, milli champ 3000 (soya meal), poultry manure and gypsum, and were mixed with the straw at filling, when the moisture content of the straw had been raised to between 72 and 78%. The reactions to the amendments varied between strains and even between species. Lucerne meal gave better results than rice bran, which was usually better than straw alone. The *P. ostreatus* strain somycel 3200 grew best without amendments. It did not respond to lucerne meal; poultry manure delayed harvest and resulted in bare patches, while lucerne meal, feather meal, milli champ 3000 and gypsum depressed yields (Visscher, 1989). Sterilized chicken manure was recommended as supplement by Vijoy and Upadhyay (1989) for *P. sajor-caju* and *P. flabellatus* production

Various oilseed meals and cakes, powdered pulse, wheat and rice bran etc. have also been tried in India (Bahukhandi, 1990).

Oyster mushrooms (*P. ostreatus* strain UPLB 503) were grown in polypropylene bags filled with either sawdust (+ 10% rice bran) or sugarcane byproducts: bagasse, press mud, bagasse + press mud (1:1 or 2:1); cane tops. Incubation time needed was typically 4.5 weeks for bagasse, 5 weeks for press mud or cane tops, 6 weeks for the mixtures and 8.5 weeks for sawdust. Yields were highest for bagasse or press mud alone, with 2:1 mixture the next best (Nerona and Latiza, 1990).

To clarify in vitro digestibility of wheat straw and Japanese redwood sawdust substrate, the edible mushroom (*P. ostreatus*) was cultivated under conditions conductive to fruit body production. After adjusting the moisture content to between 65-70%, either chopped straw or sawdust was packed tightly into bottles. The bottles were then sterilized and subsequently inoculated with *P. ostreatus*. Straw substrate, straw substrates mixed with a eutrophic (wheat bran) at 20%, sawdust substrate and sawdust substrate mixed with eutrophics (wheat bran, rice bran and bean curd refuse) at 45% were compared. The incubation period lasted 8 weeks with fruit body formation being induced after 4 weeks, sampling was performed throughout the incubation period. In the sawdust only substrate the spread of the mycelium was arrested at about 4 weeks and fruit body yield was higher when substrates were mixed with eutrophics. Dry matter content of the substrates decreased by between 4.3-22.1% during incubation and was particularly reduced in fruit body harvested substrates (Yoshida *et al*., 1993).

Supplementation of substrate before spawning with various materials recommended to obtaining higher yield. Horse gram powder, cottonseed powder, yeast mud, groundnut cake and rice bran have been tried in the cultivation of different *Pleurotus* spp. All the supplements enhanced the yield and best results were obtained with groundnut cake (Chadha and Sharma, 1995). Biswas *et al*. (1995) investigated the biological efficiency (BE) of *P. florida* in Madhya Pradesh, India. A mixture of paddy straw wheat straw promoted a high BE (106.50%). Supplementation of this substrate with 5% rice flour also promoted BE (125.75%).

Royse (1996) carried out three experiments to determine the effect of millet supplementation on mushroom yield and basidiome size of shiitake when grown on a synthetic substrate. Substrate formulations of sawdust, wheat bran, rye and CaCO3, were amended with white millet (17, 23, 29, 34% on dry weight basis). Addition of 34% millet to the substrate stimulated mushroom yield by 68% compared to a millet addition of only 17%. Biological efficiencies averaged (3crops) 59.1%, 67.1%, 81.7% and 99.6% for millet additions of 17, 23, 29 and 34%, respectively. Thus, biological efficiencies increased an average of 12.5% for each additional 6% increase in the amount of millet supplementation. Alternatively, as millet levels increased, basidiome size decreased from 23 g at 17% millet to 13g per mushroom at 34% millet supplementation.

An and Awan (1996) conducted an experiment to determine the levels of carbon, nitrogen and the pH of the substrates currently being used for oyster mushroom, indigenous farm wastes and to assess the return on investment. Result showed that substrates currently used had 8.12 to 25.5 percent C; 0.805 to 2.205 percent N and at pH 5.2 to 7.3. Carbon level at 17.86, nitrogen level at 1.54 percent and pH 5.6 enhanced the highest yield with the use of Rice straw as the main substrate and 15 percent rice bran and sugar as additive. Conversely, carbon and nitrogen at undetermined level and pH 6.4 affected the lowest yield. The highest return on investment (112.65 percent) was obtained using rice straw + 15 percent rice bran + 15 percent sugar and the lowest (71.05 percent) was obtained using rice straw + 15 percent rice bran + 15 percent chicken dung. Based on the result, addition of 15 percent sugar and rice bran is recommended for *P. florida* production.

Shen and Royse (2001) evaluated the effects of various combinations of wheat bran, rye and millet (at 20% and 30% of total dry substrate wt) on crop cycle time, biological efficiency (BE) and mushroom quality for a commercially used isolate of *Grifola frondosa* (maitake). Supplements were combined with a basal ingredient of mixed oak (primarily red oak) sawdust, and the resulting mixture was pasteurized, cooled, inoculated and bagged with an autoclaving mixer. Times to mushroom primordial formation and mushroom harvest were recorded, and mushroom quality was rated on a scale of 1-4, where 1 was the highest quality and 4 was the lowest

quality. The combinations of 10% wheat bran, 10% millet and 10% rye (BE 47.1%, quality 1.8 and crop cycle 12 weeks) and 10% wheat bran plus 20% rye (BE 44%, quality 1.7 and crop cycle 10 weeks) gave the most consistent yields and best basidiome quality over time.

Pleurotus cornucopiae 608 was grown on a mixture of pasteurized cottonseed hulls (75% dry wt), 24% chopped wheat straw, and 1% ground limestone. The substrate was spawned at various levels (1.25%, 2.5%, 3.75%, or 5% wet wt) and not supplemented or supplemented with commercial delayed release nutrient (Campbell's S-41) at various levels (0%, 3%, 6%, 9%, or 12%). Maximum yield (weight of fresh mushrooms harvested at maturity) was obtained at 3.75-5% spawn level and 6% S-41 supplement. As supplement levels exceeded 6%, yields declined significantly. There was a negative correlation (r=-0.81) between spawn rate and days to production. As the spawn rate increased, the number of days to production decreased. By using a spawn rate of 3.75% of the wet substrate wt, it was possible to reduce the time to production by a mean of 9.2 days compared with a spawn rate of 1.25% (Royse, 2002).

Kapoor *et al.* (2009) coducted an experiment with five organic supplements namely cotton seed meal (CSM), peanut meal (PM), wheat bran (WB), rice bran (RB) and soybean meal (SBM) were supplemented to the wheat straw @ 10% and 20% (on dry weight basis). Mycelial growth and enzyme activity of *Lentinus edodes* strain Le-S were recorded at periodic intervals of 10 d upto 30 d. Maximum mycelial extension rate was obtained with 10% rice bran supplementation and with 20% wheat bran supplementation. CSM and SBM were also found to improve the yield,while a slow growth was observed with PM. Activity of hemicellulases was higher than cellulases. Among the different cellulases; carboxy-methyl cellulases (CMCase) activity was the highest followed by cellobiase (Cbase) and filter paper (Fpase) activity. Similarly WB and CSM were found to improve the CMCase activity. Though RB was effective in stimulating Cbase activity at early stages but maximum activity was observed on CSM and SBM. Fpase activity was also better on SBM and RB supplementation. Supplementation with SBM resulted in maximum xylanase activity followed by WB and CSM that were at par. Laccase activity appeared at later stages (20-30 days after

inoculation) and a very high activity was reported on unsupplemented and PM supplemented wheat straw.

Alam *et al.* (2010**)** investigated 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 initiation was observed between 13.5 and 19.3 days. The results indicated that the 30% maize powder supplement was effective for producing viable fruiting bodies. The maximum diameters of the pileus and stalk were observed with 30% maize powder. The highest biological and economic yield and biological efficiency were also obtained with 30% maize powder as a supplement. The results indicate that increasing the supplement level resulted in less biological efficiency, and that 30% maize powder was the best supplement level for rice straw substrate to cultivate milky white mushrooms.

Moonmoon *et al.* (2011) observed that cultivation of shiitake mushroom (*Lentinus edodes*) is increasing rapidly in Bangladesh due to its nutritional and medicinal importance with excellent flavor and longer shelf life. With the aim of increased production, we have cultivated *L. edodes* on saw dust (SD) supplemented with different levels (10%, 15%, 20%, 25%, 30%, 35% and 40%) of wheat bran (WB), rice bran (RB), maize powder (MP) and their combination (WB+RB+MP=1:1:1) to investigate the growth, yield and quality of this mushroom. Most of the growth, yield and quality parameters varied significantly when mushrooms were cultivated with different levels of supplementation. The yield of mushroom was increased with the level of each supplementation up to a certain level, and then decreased. SD supplemented with 25% WB produced the highest number of fruiting bodies (34.8/500 g packet), highest biological yield (153.3/500 g packet), and biological efficiency (76.6%) of *L. edodes*. But the yield of the best quality mushroom was observed on SD with 40% WB supplementation; however, the qualities were not always supplementation dose dependent. In this study, we report that 25% WB supplementation with SD may be very effective for higher yield and 40% WB supplementation for better quality of *L. edodes.*

Martinez-Guerrero *et al.* (2012**)** studied two genotypes of *Lentinula edodes* (CP-7 and CP-163) selected from 16 strains being used in the region at different levels, in order to assess their mycelial growth rate in Petri dishes, as well as yield (biological efficiency, production rate) and quality of fruit bodies, using 10 different formulations of supplemented sawdust from a common Mexican oak tree *(Quercus acutifolia* Neé). The best mycelial development was 8.5 mm/day for the genotype CP-163 cultivated on 70% *Quercus* sawdust, 10% corn-cobs, 10% maize stubble, 7% wheat bran and 3% rice meal. The highest yield was recorded in the genotype CP-7, using 60% *Quercus* sawdust, 28.5% corn-cobs, 10% maize stubble, 1.5% gypsum, thiamine (100 mg/kg), and magnesium sulfate (20 g/100 kg); reaching a biological efficiency of 103%, a production rate of 1.3, and a high proportion (41.8%) of fruit bodies, having good commercial quality (41 to 70 g fresh weight, > 12 cm cap diameter and 96.5% of regular shape). On the basis of this study, this last genotype and formulation was recommended, as well as to establish a breeding program at the molecular level for shiitake production on a large scale in Mexico or other Latin American countries.

Oseni *et al*. (2012) evaluated the effect of supplementing fermented pine sawdust substrate with different levels of wheat bran on the yield of oyster mushroom (*Pleurotus ostreatus*). The fermented pine sawdust was mixed at spawning with 0%, 5%, 10%, 15% and 20% of wheat bran supplement and arranged in a randomized complete block design with three replications and there were 10 bags per treatment. Result showed significant ($P<0.05$) differences in the number of contaminated bags which ranged from 5 to 30, with the number of contaminated bags increasing with increasing wheat bran level up to 20% supplementation. Number of days to full colonization decreased with increasing wheat bran supplementation, and substrate supplemented with 20% wheat bran took shorter time (33 days) to fully colonize the substrate while it took 43 days in substrate supplemented with 5% wheat bran to full colonization, and the non supplemented substrate failed to colonize due to contamination. The growth of pileus diameter and stipe length were significantly ($P<0.05$) different, being highest on 15% and lowest on 5% wheat bran supplemented substrates, respectively. Significantly ($P<0.05$) higher yields of oyster mushroom (683.9g/500g substrate) and biological efficiency (136.8%) were obtained on substrate supplemented with 15% wheat bran compared to other treatments. Thus,

15% wheat bran supplementation of fermented pine sawdust proved to be a viable option for oyster mushroom and can therefore be recommended for commercial use while any supplementation above this level might reduce the yield of oyster mushroom significantly.

Sharma *et al.* (2013) conducted an experiment to select the best supplement to substrate. Five different strains of *Lentinus edodes* (Le S, OE-38, OE-142, OE-329 and OE-388) were cultivated on wheat straw and saw dust and spawn run time, weight of fruit bodies and biological efficiency were recorded. Wheat straw substrate produced maximum biological efficiency in OE-388 (66.8%) and OE-329 (46.2%) strains. Ten percent supplementation of wheat bran was the best among all the supplements tried. However, in case of sawdust substrate, maximum biological efficiency was obtained at 5 per cent level of wheat bran supplementation in Le S, OE-142 and OE- 388 strains. The days for spawn run were also less in wheat straw substrate as compared to saw dust substrate.

Alam *et al.* (2010) investigated 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 initiation was observed between 13.5 and19.3 days. The results indicated that the 30% maize powder supplement was effective for producing viable fruiting bodies. The maximum diameters of the pileus and stalk were observed with 30% maize powder. The highest biological and economic yield and biological efficiency were also obtained with 30% maize powder as a supplement. The results indicate that increasing the supplement level resulted in less biological efficiency, and that 30% maize powder was the best supplement level for rice straw substrate to cultivate.

2.3 Effect of substrates

In an experiment several locally available substrates such as rice straw, banana leaves, sawdust, oil palm refuse or grass straw in Andamans were evaluated to study conversion efficiency of *P. sajor-caju*. Rice straw and banana leaves were best substrates with more than 60% conversion efficiency on dry weight basis. The mean weight of fruiting body was high (7.1g) on banana leaves, followed by rice straw,

grass straw, oil palm bunch refuse, sawdust and oil palm waste. *Pleurotus ostreatus* was successfully grown under local conditions utilizing chopped wheat straw, cotton waste, maize cobs or rice straw as bedding materials. Wheat straw and cotton waste gave the highest yields with the shortest incubation period. Fruiting bodies were appeared after 15-18 days as compared to 4-5 weeks on the other substrates. A typical flushing pattern was observed in each substrate. The first flush gave the highest yield in all the treatments, and there was a gradual decline in the yield of successive flushes (Ramesh and Ansari, 1987).

Suprapti (1987a) cultivated oyster mushrooms (*P. ostreatus*) on waste wood (sawdust) of 11 species: damar, *Agathis* spp.; durian, *Durio zibethinus*; jeungjing, *Albizia falcata* (*A. falcataria*) Karet or rubber, *Hevea brasiliensis*; kelapa or coconut, *Cocos nucifera*; manii, *Maesopsis eminii*; tusam or pine, *Pinus merkusii*; pulai, *Alstonia scholaris*; sehiye, *Sterculia macrophylla*; shiega, *Celtis latifolia*; and sihara, *Ganophyllum falcatum*. Mushrooms were harvested daily after fruit body growth and yields are given for each wood species. Average yield was 357.25-g/kg dry substrate with the highest yield on Rubber sawdust.

Substrate is an important item for growing mushroom. It is a kind of medium which supports the growth, development and fruiting of mushroom (Chang and Miles, 1988).

In a study it was reported that in the split of the logs of *Hevea brasiliensis* was inoculated with spawn of *Pleurotus sajor-caju, P. citrinopileatus* and *P. florida*. They were covered with polyethene sheeting and kept in darkness at around 26^0C until mycelium was visible. Rubber tree sawdust was also investigated as a growing medium; it was soaked in water for 24 hours, then dried to about 70% moisture and mixed with 5% $CaCO_3$ in bottles before inoculation. All 3 species began to grow on the logs within 3 days of inoculation and small fruiting bodies appeared 4 days after spawn running was completed. However, almost all ceased development shortly afterwards; only 5 (*P. florida*) reached maturity. Mycelia on sawdust ceased to grow after penetrating to about three-quarters of the depth of the medium. The reason(s) for the failure to develop fully are not yet known but, since rubber wood appears to have no inhibitory activity against *Pleurotus* spp., further studies are proposed

(Kothandaraman *et al.,* 1989). Patil (1989) cultivated *P. sajor-caju* on six different substrates, i.e. wheat straw, bajra (*Pennisetum americanum*), maize straw, paddy straw, jower and cotton stick. The results indicated that all the substrates could be used for commercial cultivation of oyster mushroom.

Awasthi and Pande (1989) cultured spawn on 9 different substrates, as follows: waste tea leaves alone; 1:1 (by weight) waste tea leaves and waste paper; 2:1 tea leaves and paper; 1:2 tea leaves and paper; 1:1 maize cobs and paper; wheat grains; maize grains; *Eleusine coracana* grains; and sorghum grains. The highest yield of fruiting bodies of *P. sajor-caju* (Fr.) Sing. was obtained on sorghum grains, followed by *E. coracana*, wheat and maize grains. However, the use of grain spawn is not economical; the next best yield was obtained on the 1:1 mixture of tea leaves and paper and this medium is considered very promising. The yield was lowest where tealeaves alone were used.

Suggestions were given for growing *Pleurotus* on bagasse. Bagasse was cheaper than the conventional substrates (wheat straw and paddy straw), and its powdery form gave a large surface area for fungal growth. Ambient conditions were adjusted to 22-26^{0}C and 80-95% RH, with adequate ventilation. The moisture percentage of substrate was also found to be very critical (Thangamuthu, 1990).

Volvariella volvacea strain PK101 was grown on beds comprising 6.7 kg DW of Sugarcane bagasse alone or bagasse mixed with cotton waste and paddy straw. The cultivation technique is described. Growth rates on various substrates, duration of the spawn run, time taken for production of fruiting bodies, substrate composition, composition of fruiting bodies, and yield were measured. Results are tabulated. The highest yield (3880 g) and biological efficiency (19.4%) were obtained with a substrate containing 50% bagasse and 50% cotton waste. The first flush gave the highest yield (Khan *et al.,* 1991).

Pleurotus sajor-caju, P. citrinopileatus, P. florida, P. platypus and *P. ostreatus* were evaluated for their yield performance on various substrates both for spawn production and cultivation in the plains and in the high ranges of Kerala in the summer and rainy seasons. Sorghum, wheat and paddy grains were equally good for spawn production. *Pleurotus sajor-caju, P. citrinopileatus* and *P. florida* were the most suitable species

for cultivation in both the plains and the high ranges. These three species were successfully cultivated on paddy straw, *Eliocharis plantogena* and rubber wood sawdust, although for commercial cultivation of *P. sajor-caju* rubber wood sawdust was not rated as an ideal medium (Mathew *et al.,* 1991).

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

Murugesan *et al*. (1995) cultivated mushroom, *P. sajor-caju* (Fr.) Sing. on water hyacinth (*Elchhorni crassipe*). They compared water hyacinth with other conventional substrate paddy straw. Total yields for 20 bags of the two substrates were 15.0 and 10.5 kg respectively, although the time taken to reach the pinhead stage was longer on the water hyacinth substrate (17 days in water hyacinth and 10 days in paddy straw). The high yield on water hyacinth attributed to the C:N ratio (24.3 compared with 53.5) and low lignin content (9% compared with 17%) of this substrate. Use of water hyacinth would provide a cheap substrate and a means of eradicating a troublesome aquatic weed.

Poppe and Hofte (1995) tested 40 different types of organic waste, wild herbs and weeds for growing mushroom. More than 80% of them were directly used as substrate for mushroom cultivation and about 20% were used as additives to very poor substrates. Simply moistened or untreated, pasteurized, fermented or sterilized wastes were used. About 30 mushroom species and more than 60 different cultivars were tested on the 40 different available wastes. They reported the efficient use of more than 20 wastes as substrates for more than 20 cultivated species. The highest yields were achieved with straw or corncobs based substrates for *Pleurotus*; Sawdust for Shiitake, *Pholiota* and *Collybia*; grass chaff for *Strophaia* and compost for *Agaricus*.

Singh *et al*. (1995) reported that the *P. florida* was cultivated on Wheat straw, Paddy straw or Sugarcane trash (dried leaves) used either separately or in 1:1 ratio. Yield and biological efficiency were the highest in paddy straw. The profit for small farmer

with 6 crops/year on Sugarcane trash + Cereal straw substrate was calculated as Rs. 12230.

Adamovic *et al*. (1996) studied the impact of *P. ostreatus* mushroom enzymes on wheat straw degradation in laboratory conditions. Chopped and pressured pasteurized straw with 24 dry matters was seeded with a mushroom mycelium. Chemical analyses of the straw were done after 15, 30, 45, 60, 90 and 120 days upon seeding. The mushroom collection was done four times. After seedling, crude fibre, NDF and ADF decreased from 46.93 to 32.40, from 82.42 to 48.53 and from 56.12 to 41.17, respectively. A similar tendency was found for hemicelluloses and celluloses, while it was not so expressed in lignin. The ash content increased from 6.26 to 9.78. The obtained results show that the mushroom enzymes degraded a substantial part of straw dry matter. The effects were most notable on the lignocellulosic complex, which enabled a successful use of the straw as a substrate for mushroom growth.

Jadhav *et al*. (1996) reported that oyster mushroom (*Pleurotus sajor-caju*) was cultivated on wheat straw, paddy straw, stalks and leaves of maize or cotton, jowar, soyabean straw, groundnut creepers plus wheat straw (1:1), soybean straw plus Ground nut creepers (1:1), or Ground nut creepers alone. Cotton stalks and leaves gave the best results with respect to sporophore number, weight of sporophore (5.12 g), and total yield (9.14 g/kg of dry straw). Yields obtained on other substrates were 796 g on paddy straw, 557 g on soybean straw and 508 g on soybean + wheat straw. The lowest yield was recorded on groundnut creeper (258 g).

Hernandez and Lucero (1998) used three bedding materials such as banana leaves, water hyacinth and the combination of banana leaves and water hyacinths for growing of mushroom. There were 3.6 kilograms harvested from banana leaves, 3.2 kilograms from water hyacinth and 3.0 kilograms from the combined banana leaves and water hyacinth.

Yildiz *et al.* (1998) conducted an experiment to study the growth and cultivation of *P. ostreatus* var. salignus on local cellulosic wastes. The highest and lowest yields for 100 g material (70% moisture) were obtained with peanut straw (24.8 g) and with sorghum straw (11.3 g), respectively. Protein, pileus/stip, sporophore weight, % dry

material, N and C in highest amounts were obtained with peanut straw. The lowest mushroom weight and pileus/stip ratio were obtained with sorghum, whereas the lowest protein, N and dry material weight were obtained with wheat straw. In all the *P. ostreatus* var. salignus cultivated on peanut and sorghum straw, the most abundant nutrients were protein, potassium and carbon. These results are discussed in relation to the prospect of cultivating *P. ostreatus* var. *salignus* in Diyarbakir, Turkey.

Shiitake mushrooms grow naturally on decaying wood of hardwood trees and have traditionally been grown on short lengths of freshly-cut logs. Modern commercial production has proved successful on both logs and alternative substrates such as wood shavings and peat moss (Royse, 2001).

Cultivation of the oyster mushroom, *P. sajor-caju*, on rice and wheat straw without nutrient supplementation was investigated. The effects of straw size reduction method and particle size, spawn inoculation level, and type of substrate (rice straw versus wheat straw) on mushroom yield, biological efficiency, bioconversion efficiency, and substrate degradation were determined. Two size reduction methods, grinding and chopping, were compared. The ground straw yielded higher mushroom growth rate and yield than the chopped straw. The growth cycles of mushrooms with the ground substrate were five days shorter than with the chopped straw for a similar particle size. However, it was found that when the straw was ground into particles that were too small, the mushroom yield decreased. With the three spawn levels tested (12%, 16% and 18%), the 12% level resulted in significantly lower mushroom yield than the other two levels. Comparing rice straw with wheat straw, rice straw yielded about 10% a more mushrooms than wheat straw under the same cultivation conditions. The dry matter loss of the substrate after mushroom growth varied from 30.1% to 44.3% (Zhang *et al*., 2002).

Upadhyay *et al* .(2002) stated oyster mushrooms *Pleurotus* spp draw their nutritional requirement from a host substrate or from the agricultural wastes rich in lignin, cellulose and hemicellulose used for their cultivation. Due to varying nutrients in the substrates, different mushroom yields have been recorded by various workers. Nitrogen is an essential element for cellular functions for growth and various metabolic activities particularly protein and enzymes synthesis. The nitrogen content of mycelium ranges between 3 to 6%. Cereal straw used for cultivation of oyster

mushroom is a poor source of nitrogen (0.5 to 0.8%) and at the time of fructification when most of the nitrogen is utilized for mycelial growth, the depleted nitrogen in the substrate becomes inadequate and limits mushroom yield. In the present studies seven different organic nitrogen sources: wheat bran, rice bran, soybean floor, de-oiled soybean meal, mustard cake, cotton seed cake and cotton seed meal were evaluated for their effect on mushroom yield. Cotton seed cake and de-oiled soybean meal gave significantly higher yield than unsupplemented bags. Mustard cake supplemented bags gave the lowest yield, which obviously was due to its anti-fungal properties. Cotton seed cake and de-oiled soybean meal were further evaluated- on a 1, 2.5, 5, 7.5 and 10 % per dry wt. basis, to find out the minimum dose for optimum yield. Cotton seed cake 2.5%, 5%, 7.5% and 10% gave similar yields although significantly higher than 1%. However, 1% soybean meal was found the best, and all higher rates of supplementation gave lower yields. It could thus be concluded that addition of 1% de-oiled soybean meal and 2.5% cotton seed cake are the optimum doses for these supplements to enhance the yields of *P. ostreatus* var *florida*. Their supplementation also gave higher dry matter than unsupplemented bags.

Philippoussis *et al.* (2002) carried out an experiment with four lignocellulosic substrates (oak-wood sawdust (OS), wheat straw (WS), corn-cobs (CC) and cotton wastes (CW), were comparatively evaluated for the cultivation of the shiitake mushroom *Lentinula edodes.* All five commercial strains (AMRL 118, 119, 120, 121, and 122), examined in 'race-tubes' experiments, demonstrated significantly higher mycelium extension rates on OS and WS than on CC and CW. Strains 121, 118 and 122 proved to be the fastest overall colonizers, but 120 performed notably better on CC and OS. Measurements of pH and electrical conductivity throughout the entire colonization process of substrates with strains 121 and 118 revealed a steady decrease of pH; electrical conductivity values increased until mycelium colonized 60 to 75% of the substrate, and then it slightly declined or remained constant until the end of incubation. Significant differences in electrical conductivity measurements were detected among fully colonized substrates: OS and CC showed the lowest values (0.79-0.93 mS/cm), whereas CW presented almost double salt content. Subjecting tubes fully colonized by strain 118 to cold-shock treatment resulted in fruiting 58-65 days after inoculation in all media tested; WS and CC substrates promoted earlier

sporophore initiation than OS, while CW appeared to be the worst performing substrate. Monitoring of CO_2 concentrations in pilot-scale cultivation of strain 118 on synthetic blocks, revealed higher respiration rates on OS and CC than on WS (peaks on 17th, 20th and 24th day respectively), which are further correlated with substrate colonization rates. Results were evaluated for potential use of OS in combination with WS and CC as substrates for *Lentinus edodes* cultivation.

Obodai, *et al.* (2003) evaluated eight lignocellulosic by-products as substrates for cultivation of the Oyster mushroom, *Pleurotus ostreatus* (Jacq. ex. fr) Kummer. The yields of mushroom on different substrates were 183.1, 151.8, 111.5, 87.8, 49.5, 23.3, 13.0 and 0.0 g for composted sawdust of *Triplochiton scleroxylon*, rice straw, banana leaves, maize stover, corn husk, rice husk, fresh sawdust and elephant grass respectively. The biological efficiency (BE) followed the same pattern and ranged from 61.0% for composted sawdust to 0.0% for elephant grass. The yield of mushroom was positively correlated to cellulose (r2 = 0.6), lignin (r2 =0.7) and fibre (r2 = 0.7) contents of the substrates. Based on the yield and BE of the substrates tested, rice straw appeared to be the best alternate substrate for growing oyster mushrooms.

Spawn running, pin head and fruit body formation and yield of oyster mushroom (*P. ostreatus*) on waste paper supplemented with peat, chicken manure and rice husk (90+10; 80+20 w:w) were studied. The fastest spawn running (mycelia development) (15.8 days), pin head formation (21.4 days) and fruit body formation (25.6 days), and the highest yield (350.2 gr) were realized with the substrate composed of 20% rice husk in weight. In general, increasing the ratio of rice husk within the substrate accelerated spawn running, pin head and fruit body formation, and resulted in increased mushroom yields, while more peat and chicken manure had a negative effect on growing of mushroom (Baysal *et al.,* 2003).

Shah *et al*. (2004) investigated the cultivation of oyster mushroom on different substrates. Mushroom cultivation is a profitable agribusiness. Incorporation of non conventional crops in existing agricultural system can improve the economic status of the farmer. Mushrooms are the source of protein, vitamins and minerals and are anticancerous, anticholesteral, and antitumorous. Sawdust produced highest yield,

biological efficiency and number of fruiting bodies, recommended as a best substrate for oyster mushroom cultivation.

In an experiment *Lentinula edodes* strain S4080 was grown by Philippoussis *et al.* (2004) on seven oak-wood sawdust substrates (OS), supplemented with wheat straw (WS) or corn-cobs (CC) in order to examine the influence of the wastes on mycelium growth and sporophore production characteristics. Colonization rate measurements demonstrated faster colonization on OS supplemented with WS or CC in a ratio of 1:2 (OS:supplements). Similarly, higher sporophore yields were obtained on OS+CC mixtures, especially in the supplementation ratios 1:1 and 1:2. However, substrates with high OS content (2:1 ratio) appeared to promote mushroom quality and high protein content of the sporophores. These results are evaluated in the view of bioconverting OS, WS and CC wastes into *Lentinula* mushrooms, an added value food with medicinal properties.

In a study Iqbal *et al.* (2005) investigated the cultivation of oyster mushroom, *Pleurotus ostreatus* (local & exotic strains) and *P. sajarcaju* were conducted to find out the growth and yield performance on different substrates. Results regarding the time required for completion of spawn running, formation of pin-heads and maturation of fruiting bodies on different substrates showed that in all the three cases, they appeared earlier on sugarcane bagasse followed by cotton waste and the maximum number of flushes were obtained from wheat straw and banana leaves followed by cotton boll locules and cotton waste. Furthermore, the results revealed that the minimum flush to flush interval was obtained on millet followed by wheat straw and sugarcane leaves and the maximum yield percentage on fresh and dry weight basis was obtained from banana leaves followed by paddy and wheat straw.

In a study Nwanze *et al.* (2005) was cultured *Lentinus squarrosulus*, an indigenous mushroom species commonly found growing on dead logs in the Zaria environ of Kaduna State on six different media which were inoculated separately with three different spawn grains and amended with six different oils at five different rates. The various media, oil type and rate all had a highly significant effect ($p<0.01$) on the stipe length, stipe and pileus diameters and carpophore wet and dry weights of *L. squarrosulus*. Spawn grains, however, had a significant effect on all the above

parameters except pileus diameter. The results reveal that corn and millet spawn induced comparable carpophore wet weights which were superior to that induced by wheat spawn. Corn spawn induced the longest stipe length and heaviest carpophore dry weight while millet induced the widest stipe diameter. The oil rate of 0.014 ml/g induced the longest stipe length, heaviest carpophore wet weight and widest pileus diameter while the highest oil rate (0.028 ml/g) induced the widest stipe diameter and heaviest carpophore dry weight. Coconut oil induced superior results for all the parameters tested except stipe diameter. Animal bedding and rice media induced optimum results for all the parameters.

Pathmashini *et al.* (2008) conducted an investigation to evaluate the efficacy of four different types of grain spawns viz; kurakkan (*Eleusine coracana*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), and paddy (*Oryza sativa*) on oyster (*Pleurotus ostreatus*) mushroom production. Spawn types were based on their effects on mushroom solids (%), biological efficiency (BE %), size (g), number of sporophores, yield, yield ratio and substrate weight loss (%). Locally available kurakkan (*Eleusine coracana*), maize (broken) (*Zea mays*), sorghum (*Sorghum bicolor*), and paddy (*Oryza sativa*) were used for spawn production. The experiment was designed as a complete randomized design with three replicates. Four types of spawns were tested on a medium based on sawdust. The growing media (substrate) inoculated with kurakkan spawns showed the highest biological efficiency of 27.82± 0.12 (%) , mean yield of 52.94 ± 0.67 g, mush-room size of 7.73 ± 0.33 g and yield ratio of 23.19 ± 0.01. The harvests obtained from paddy spawn showed the highest mushroom solids of 9.97 ± 0.26 (g/100g of harvest). Highest mean numbers of sporophore (fruiting bodies) were noticed in the harvests obtained from sorghum spawn (7.67 ± 0.66). The kurakkan spawn significantly. Enhanced biological efficiency and increased size and yield, when com-pared with other spawn types viz; maize (*Zea mays*), sorghum (*Sorghum bicolor*), and paddy (*Oryza sativa*).

Ashrafuzzaman *et al.* (2009) reported that shiitake mushroom was cultivated on sawdust of timber plants babla, champa, garzon, ipil-ipil, jackfruit,mango, segun, shimul, shisoo, rain tree, sawdust mixtures and rice straw to test for growth and fruiting characteristics. Cultivation on jackfruit resulted in significantly faster

mycelial growth compared to other substrates. Shiitake mushroom required 43 days to complete mycelial growth on jackfruit sawdust while requiring up to 55 days for other substrates. However with respect to fructification, jackfruit produced the first pinhead (primordium) significantly early compared to other substrates used. Number of primordia and effective fruiting body was also highest in mushroom raised on jackfruit sawdust. Yield attributes of shiitake mushroom such as stalk length, stalk diameter, pileus diameter and pileus thickness were significantly higher in mushroom raised on jackfruit sawdust. Biological efficiency, yields (biological yield, economic yield and dry yield) at first harvest and final harvest were highest on jackfruit substrate. The lowest biological and economic yields were found in mushroom raised on teak chambul. Rice straw, surprisingly, did not produce any fruiting bodies as well as showing no yield attributes.

Stanley (2010**)** stated that spawn quality is the most important factor in the production of edible mushroom (which is fast gaining prominence in Nigeria and Africa at large). In order to determine the effects of substrates spawn preparation on mycelial growth of oyster mushroom species, the experiment was conducted in a factorial experiment design at randomized completely with three replications. In this experiment, first and second factors respectively were substrates (Wheat, yellow maize, Guinea Corn, Millet, Red Sorghum and White Maize (Bende local)) and oyster mushroom species (*Pleurotus tuber-regium* and *pelurotus pulmonarius*). The results clearly demonstrated that between various substrates used, maximum and minimum growth rate were recorded for white maize (Bende Local) and least mycelial extension and fresh weight on wheat. The second best grain for both species used was Red Sorghum.

Hasan *et al.* (2010) conducted an experiment from April 20 to June 28, 2010 at Microbial Biotechnology Laboratory of Genetic Engineering and Biotechnology Department, Khulna University, Khulna, Bangladesh. Twelve different treatments with lime were evaluated to find out the growth and yield of mushroom. The mycelium running time and days required completion of full running of mycelium. Time required for the initiation of primordia to harvesting and number of primordia and number of effective primordia, Biological yield of mushroom were greatly influenced by different pretreated substrates. The highest yield (119 gm) and return (12.85Tk) were obtained from the treatment of rice straw + 10% poultry litter + 1%

lime. The highest mycelium running rate was observed in the treatment of banana leaf mid ribs + 10% horse dung + 1% lime. The minimum duration of mushroom production found in banana leaf mid ribs + 10% cow dung + 1% lime. However, the rice straw + 10% poultry litter + 1% lime and rice straw + 10% horse dung + 1% lime were the best treatments for the growing of oyster (*Pleurotus ostreatus*) mushroom and they are economically viable.

Fanadzo *et al.* (2010*)* conducted an experiment 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*). Wheat straw had superior performance over maize stover and thatch grass when cultivating *P. sajor-caju*. However, maize stover was more suitable for *P. ostreatus* than wheat straw. Supplementation with cottonseed hull improved yields when cultivating *P. ostreatus* using wheat straw. These findings suggest that at 25% inclusion rate, farmers should not supplement with maize bran, as this would reduce yields significantly. Further investigations are needed to test both lower and higher rates of inclusion of supplements.

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

In an experiment Narh *et al.* (2011**)** used sorghum and millet grains to assess as single treatments and combined in various proportions to determine their suitability for production of spawns and carpophores of *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer.

The mycelia growth rates on the grains, one of the parameters assessed, were measured from the third to the ninth day of incubation. In addition, for each replicate of the various treatments, the days from inoculation of the bottles till total colonization were recorded. The combination of sorghum and millet grains in a 3:1 (w/w) ratio showed fastest mycelial growth of 16 days followed by sorghum only recording a value of 18 days. These were however not significantly different (P>0.5). The best grain treatments were used as inocula on composted sawdust of *Triplochiton scleroxylon* to compare their yield characteristics. Parameters assessed during fruiting included the number and weight of carpophores obtained, flush number and biological efficiency (BE). No significant difference in BE was observed. Based on the results obtained, for large-scale *P. ostreatus* spawn production, a combination of sorghum and millet grains in a 3:1 ratio would be most appropriate for use as substrate.

Puri (2011) stated that *Lentinula edodes* or Shiitake is a white rot wood decay fungus that produces flavourful brown sporocarps with medicinal properties. According to a Chinese folk fare, this mushroom is an "elixir of life", capable of generating stamina, curing colds, improving circulation, and preventing premature aging. Many countries have developed production technology of this mushroom but detailed accounts are not available in literature. Therefore, present investigations were undertaken to use locally available poplar and teak sawdust, sorghum and wheat grains in combination with chalk powder and gypsum for selection of an ideal material for shiitake spawn production. *Lentinula edodes* had maximum and faster linear growth rate in test tubes having sorghum and its combinations. It colonized sorghum substrate rapidly without any contamination and the spawn prepared with sorghum had maximum yield and biological efficiency @ 6-8% spawn doses. The results showed that sorghum is an ideal material for shiitake spawn production.

Puri *et al*. (2011) conducted an experiment with two different strains of *Lentinula. edodes* fungus (L_1 and L_2) were cultivated on different saw dusts and agricultural wastes viz., wheat straw, coir pith, poplar saw dust, teak saw dust and sal sawdust etc. alone and in combinations with one another and their yield, biological efficiency, numbers and size of sporocarps and spawn runtime were recorded. Substrate of wheat-straw gave significantly higher yield (80.4g) with 45.9% biological efficiency for strain L_1. Ten percent supplementation of wheat bran was the best among all the

supplements tried. The wheat straw substrate produced the heaviest and beautiful brown sporocarps with maximum number of fruiting bodies. No satisfactory yield was obtained from coir pith and sal saw dust either alone or in combination. The minimum time for colonization (55 days) was recorded in the mixture of wheat straw and poplar saw dust and maximum (85 days) in the mixture of sal and coir pith.

Ajonina and Tatah (2012) reported that mushrooms are increasingly becoming an important component of diets worldwide and it is of paramount importance to choose appropriate substrates in a given place to grow them. The experiment was conducted at the Cameroon Society for Sustainable Development of Natural Resources and Environmental Projection (CASSDNREP) mushroom department farm. The main goal was to evaluate the growth performance of oyster mushroom (*Pleurotus ostreatus*) on some locally available substrate material compositions as well as to find out the best substrate for mushroom cultivation. Bags were sterilized in 1000 litres iron containers for 5h at 100^0C, cooled for 6 h and then inoculated with actively growing mushroom mother culture on rice grains obtained from Mushroom Cameroon, Bamenda. The bags were incubated until mycelium had fully colonized the substrate and then taken to the cropping house. The highest mycelium running rate was found on corn cobs and palm cones (1:1) but the lowest in control. Completion of mycelium running time was lowest in (1:3, 3:1 and palm cones). Number of total primordia and effective primordia, found highest in control but the highest pileus thickness was measured from corn cobs. Highest biological yield (146.1 g and 172.1 g) was obtained from corn cobs.

Puri (2012**)** stated that *Lentinula edodes* or shiitake is an edible and medicinal mushroom, widely used for preparation of dietary supplements and other delicacies. Rich diversity of mushrooms is found in Uttarakhand state of India, besides other flora fauna. Two strains of *L. edodes* fungus were collected from Uttarakhand hills. To study the physiological and yield attribute, mycelial growth and yield of *L. edodes* isolates were compared on different media, temperature, pH and agricultural wastes like sugarcane bagasse, paddy straw, poplar sawdust, coir pith, teak and sal sawdust alone and in combinations of 1:1. Potato dextrose agar (PDA) medium was found to be superior in terms of radial growth. The fungus showed maximum radial growth at 25^0C and acidic pH. The cultivation of mycelium was successful at all the substrates

tested. On the basis of morphological, cultural and yield characteristics, strain L1 was superior to L2.

Sawdust of different woods which included Kikar, Mango, Simbal and Kail used as substrates were investigated for cultivation of Oyster mushroom. The maximum linear mycelial growth after 5, 10 and 15 days respectively were observed on Kikar (*Acacia nilotica*). The minimum linear mycelial growth after 5, 10 and 15 days respectively were observed on kail (*Pinus wallichiana).Pleurotus* species artificially cultivated worldwide especially in South East Asia, India, Europe and Africa. Oyster mushroom showed relatively more linear mycelial growth in control treatment of cotton waste as compared to other substrates. The data regarding 25%, 50%, 75%, 100% of spawn running of *Pleurotus ostreatus,*the significantly effective substrate was sawdust of Kikar followed by the other substrates. It was observed that *Pleurotus ostreatus* gave the maximum yield in the first flush followed by second and third flush. The maximum yield was obtained on Kikar sawdust 282.2g followed by Mango sawdust 257.7g, mixed sawdust 233g, Simbal sawdust 216.5g and Kail 200.5g. Oyster mushroom showed relatively more yield on control treatment of cotton waste as compared to other substrates. The maximum biological efficiency was obtained in kikar sawdust which was 70.56 %. The lowest biological efficiency was obtained in kail sawdust which was 50.12 %. Among all substrates, sawdust of Kikar proved the best substrates for the effective cultivation of Oyster mushroom (Nasir *et al.,* 2012).

Uddin *et al.* (2012) conducted an experiment at the mushroom growth house and tissue culture laboratory, Horticulture Demonstration and Training Centre (HDTC), Kewatkhali, Mymensingh during February to May, 2006 to investigate the effect of different substrate on growth and yield of button mushroom (*Agaricus biporus)*. The substrates which were used in the experiment were wheat: paddy (1:1) straw compost, paddy straw compost and decomposed cowdung. The parameters observed in first and second flush were number of primordia, number of fruiting bodies and fresh weight of mushroom. Data revealed that different substrate significantly affected the production of number of primordia, number of fruiting bodies and fresh weight. In both the first and second flush, all the three observed parameters were obtained highest in wheat: paddy straw compost and the lowest were in decomposed cowdung.

A study was conducted by Ng'etich *et al*. (2013) to examine the growth and yield performance on oyster mushroom (*Pleurotus ostreatus*) using different organic

substrates namely, elephant grass, cotton seed husks, sugarcane bagasse, corn cobs, beans straw, mixture of bagasse + maize cobs (1:1) and bagasse + beans straw (1:1) to determine the substrate that could optimize production. The substrates were treated with the spawn of edible mushrooms before incubation. The experimental units were arranged in randomized complete block design (RCBD) with four replications both at incubation and cropping chamber. Data gathered were number of days for complete mycelia growth, stem height (cm), stem circumference (cm), cap diameter (cm) at harvesting, days to harvesting in the cropping chamber and total edible yield (kg). The substrates significantly ($p \leq 0.05$) influenced both growth and yield of mushroom differently. Cotton seed husks not only showed a maximum yield of 118 kg/m2 but influenced greatly stem circumference (49.0%), mushroom height 69% and cap diameter (16.6%) compared to the control (elephant grass). It is suggested therefore that cotton seed husks should be recommended to farmers as the best substrates for maximum production.

2.4 Effect of opening pattern

Kakon *et al.* (2012) conducted an experiment to find out the influence of opening methods, soaking in water and covering materials on growth, yield and yield attributes of reishi mushroom (*Ganoderma lucidum*). Considerable variations on different parameters related to yield and yield attributes were recorded. The lowest duration (4.50 days) from opening of spawn packet to primordial initiation, duration from opening to harvesting (33.25 days), number of fruiting body (2.25), dry weight (4.25g/packet) and biological efficiency (10.50%) were observed in treatment combinations of no covering, no soaking and top opening, which were statistically similar to control. The highest number of fruiting body (4.00), dry mushroom weight (7.25 g/ packet) and biological efficiency (16.21%) were recorded from the treatment combination, newspaper covering + soaking in water + top opening. Size of fruiting body increased where spawns packets were covered and soaked in water. Results suggested that covering, soaking in water and top opening combinations was the best cultural practices for commercial production and yield improvement of reishi mushroom.

2.5 Effect of amount of substrate

Hossain *et al.* (2001) conducted an experiment to know the appropriate amount of

substrate i.e. bag size of spawn packet. They used five different sizes (500g, 750g, 1000g, 1250g and 1500g) of spawn packet for the cultivation of shiitake mushroom. The growth, yield and yield contributing characters of shiitake mushroom were significantly influenced by the size of spawn packet. The time required for mycelium running and total time required for harvest was increased with the increases of packet size. The number of fruiting body was highest in 1500g size of spawn packet and it was lowest in 500g size of spawn packet. The maximum yields of both biological (245.50g) and economic (229.50g) were recorded from 1500g size of spawn packet and the lowest yields were obtained in 500g size of spawn packet. The biological efficiency showed 60.38% in 500g size of spawn packet which was better than others. The biological efficiency decreased with the increase of size of spawn packet.

2.6 Effect of Age of Spawn

In an experiment Pani (2011) studied the effect of age of spawn (14, 21, 30, 37, 45 and 60 days after inoculation) and quantity (100, 200, 300, 400 and 500 g per kg dry substrate) on sporophore production of *Calocybe indica* was investigated. Quickest substrate colonization and primordial initiation as well as highest number and weight of sporophores were recorded in 21 days- old spawn. Mushroom yield decreased with increase in spawn age. A spawn dose of 200 g/ kg of dry substrate was found optimum.

Ahmed *et al.* (2010) evaluated different age, opening pattern and water soaking method of spawn packet to determine the right spawn age, opening patterns and water soaking method for shiitake mushroom cultivation. A wide variation was observed in yield and biological efficiency indifferent ages, opening patterns and water soaking methods. The highest yield and biological efficiency were recorded in 110 days old spawn packet followed by 120 and 130 days old spawn packets and the lowest yield and biological efficiency were found in 80 days old spawn packet. The full opened spawn packet gave the highest yield followed by top opened spawn packet and no yield was obtained from the packets which were not opened. Water soaking after opening of spawn packet has a negative effect on yield and biological efficiency.

CHAPTER III

GENERAL MATERIALS AND METHODS

The chapter deals with the materials and methods that were used in conducting the experiments. Six experiments were conducted in tissue culture laboratory and the mushroom culture house of NAMDEC, Savar, Dhaka during August 2011 to March 2014. Different steps involved in the implementation of the experiments are described below:

3.1 Environmental condition

The temperature of tissue culture laboratory was maintained within the range of 20-25^{0}C by air cooler. The temperature and relative humidity of mushroom culture house was maintained by spraying water and the mean maximum temperature was 27.37^{0}C and the mean minimum temperature was 19.26^{0}C and the mean maximum relative humidity was 92.70% and the mean minimum relative humidity was 46.31%. The environmental data including temperature in incubation room as well as temperature and humidity in culture house are given in Appendix-I.

3.2 Preparation of pure culture

Pure culture of selected mushroom strain was prepared on potato dextrose agar (PDA) medium containing 200 g peeled and sliced potato, 20 g dextrose and 20 g agar per liter. The mixture was boiled on gas burner until the agar dissolved. The medium was poured into test tube at 10 ml/tube. The medium in test tube was sterilized in an autoclave for 20 minutes at 121 ^{0}C under 1.5 kg/cm^2 pressure. After sterilization and solidification, the tubes were inoculated separately with the inoculants of selected shiitake mushroom strains. Pieces of inner tissues of the joint of stalk and pileus were used as inocula. A fresh and full grown sporophore of shiitake mushroom was surface sterilized with 70% ethanol by rubbing cotton soaked in the alcohol. The stalk was peeled from out site. Tissues were collected from inner region of the joint of stalk and pileus. The tissues were cut into small pieces and placed on the solidified tubes containing PDA. After inoculation, the tubes were covered with cotton plug. All operations were done under sterile condition in a clean bench. The inoculated tubes were incubated in a growth chamber at 22 ± 2 ^{0}C for 10-12 days. After completion of

the whitish mycelium, this10 days culture was used for inoculation of mother culture (Plate 1a-1f).

Plate 1a: Peeled and sliced potato

Plate 1b: Mixture was boiled on gas burner

Plate 1c: Medium was poured into test tube and covered with foil paper

Plate 1d: The medium was poured in an autoclave

Plate 1e: The medium in test tube was sterilized in an autoclave

Plate 1f: Tubes were inoculated separately with the inoculants

3.3 Preparation of mother culture

To prepare mother culture of test mushroom sawdust and wheat bran mixed together at the ratio of 2:1 (v/v). Water was added to adjust moisture content at 65% and $CaCO_3$ was mixed at the rate of 0.2% of the mixture. Polypropylene bags of 18 cm ×

25 cm size were filled with 300 g of the above prepared mixture and packed tightly. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the volume of the bag was made for space to put the inoculums. The neck was plugged with cotton and covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for one (1) hour at 121^0C under 1.5 kg/cm^2 pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. A piece of pure PDA culture medium containing mycelium of shiitake mushroom was placed aseptically in the hole of mother culture packet and again plugged the packet as mentioned above. The inoculated packets were placed on a rack in the laboratory at 22 ± 2^0C temperatures for incubation. The substrate of the mother culture was colonized by the growth of whitish mycelium within 15-20 days after inoculation. The fully colonized packets were used for spawning.

Plate 2: Preparation of mother culture

3.4 Preparation of spawn packet

The substrate of spawn packets were prepared using sawdust and wheat bran mixture at the ratio of 2:1. Water was added to make the moisture level about 65% and $CaCo_3$ was added at 0.2% (w/w) of the mixture. The substrate mixture was poured into 18 cm × 25 cm polypropylene bags at 500 g/bag. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the bag was made for

space to introduce the inocula. The neck of each poly bags was plugged with cotton, covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for 2 h at 121^0C under 1.1 kg/cm^2 pressures. After sterilization, the packets were cooled and transferred to an inoculation chamber. The packets were inoculated separately with the mother culture of the twenty three strains at the rate of two tea spoonful per packet. The inoculated packets were incubated at 22 ± 2^0C.

Plate 3a: Preparation of spawn packets

Plate 3b: Sterilization of spawn packets

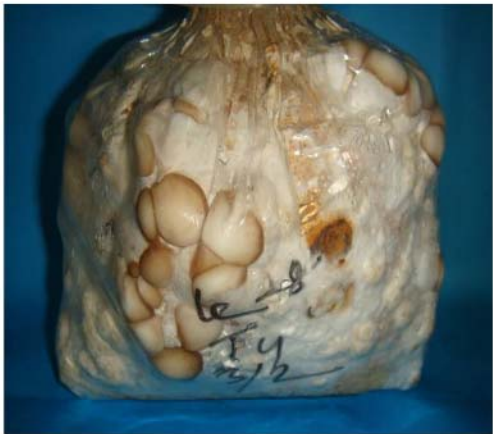
3.5 Mycelial colonization and bump formation

During incubation period, whitish mycelia started to grow in the substrate. All the strains showed optimal mycelial growth at 22 ± 2^0C temperatures and 60-70% relative humidity. After full colonization of spawn packets, a thick mycelial coat formed on the outer surface of colonized substrate. Clumps of mycelia appeared as blister like bumps of various sizes on the surface of the mycelial coat in each packet. Bumping usually started when colonization of white mycelia changed to brown. There are four phase steps involved in mycelial growth phase such as mycelium running, mycelium coat formation, pigmentation and bump formation.

Mycelium running **(20-30 days)**

Mycelium coat formation (30-50 days)

Pigmentation (Browning) and hardening (50-75 days)



Bump formation (75-110 days)

Plate 4: Mycelial growth phage of shiitake mushroom

3.6 Cultivation conditions for fruiting

After mycelium maturation and bump formation, all the packets were fully opened by removing the polypropylene bag. Then the packets were placed separately on the rack of culture house. Temperature, relative humidity were maintained season wise and light was maintained at 10-20 lux. Sufficient water was applied per day and proper aeration was maintained in culture house for the release of excess CO_2 and supply of sufficient O_2 as required for the development of primordia and fruiting body.

Packet opening Water spraying

Plate 5: Packet opening and water spraying in spawn packet

3.7 Data collection

Data on the following parameters were collected following standard procedures as described here after.

3.7.1 Mycelium Growth Rate (mm or cm/day): Mycelium growth rate (MGR) for each packet was measured after the mycelium colony crossed the shoulder of the packet. The linear length was measured at four different places of packet.

$$\text{MGR} = \frac{L}{N} \text{ cm/day or mm/day}$$

Where, L = Average length of mycelium running of four different places (mm/cm) and

N = Number of days

3.7.2 Time required for completion of mycelium running (days): Days required from inoculation to completion of mycelium running, was recorded.

3.7.3 Time required for bump formation (days): Days required from completion of mycelium running to bump formation was recorded.

3.7.4 Time required from opening to first harvest (days): Days required from opening to primordial initiation and harvesting, was recorded.

3.7.5 Time required for harvest (days): Days required from inoculation to harvest, was recorded.

3.7.6 Number of fruiting body (NFB): Total number of well-developed fruiting body was recorded. Dry and pinheaded fruiting body as well as twisted and tiny fruiting body was included during counting.

3.7.7 Number of effective fruiting body (NEFB): Number of well-developed fruiting body (NEFB) was recorded. Dry, pinheaded, twisted and tiny fruiting body was discarded during counting.

3.7.8 Length of stalk (LS): Length of stalk of four randomly selected fruiting bodies was recorded using a scale.

3.7.9 Diameter of stalk (DS): Diameter of the stalk of four randomly selected fruiting bodies was recorded using a slide caliper.

3.7.10 Diameter of pileus (DP): Diameter of pileus, of four randomly selected fruiting bodies was recorded using a scale.

3.7.11 Thickness of pileus (TP): Thickness of the pileus of four randomly selected fruiting bodies was recorded using a slide caliper.

3.7.12 Yield (g): Yield in g/500 g packet was recorded by weighing all the fruiting bodies in a packet after removing the lower hard and dirty portion.

3.7.13 Biological efficiency (%): Biological efficiency (%) was determined by the following formula:

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

3.8 Statistical analysis

Data on various parameters were statistically analyzed using the MSTAT-C computer program. All the characters were subjected to analysis for variance (ANOVA). Means were compared using Duncan's Multiple Range Test. Correlations among different characters were also determined (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 SEASONAL EFFECT ON THE PERFORMANCE OF DIFFERENT STRAINS OF SHIITAKE MUSHROOM (*Lentinus edodes*) AVAILABLE IN BANGLADESH

4.1.1 INTRODUCTION

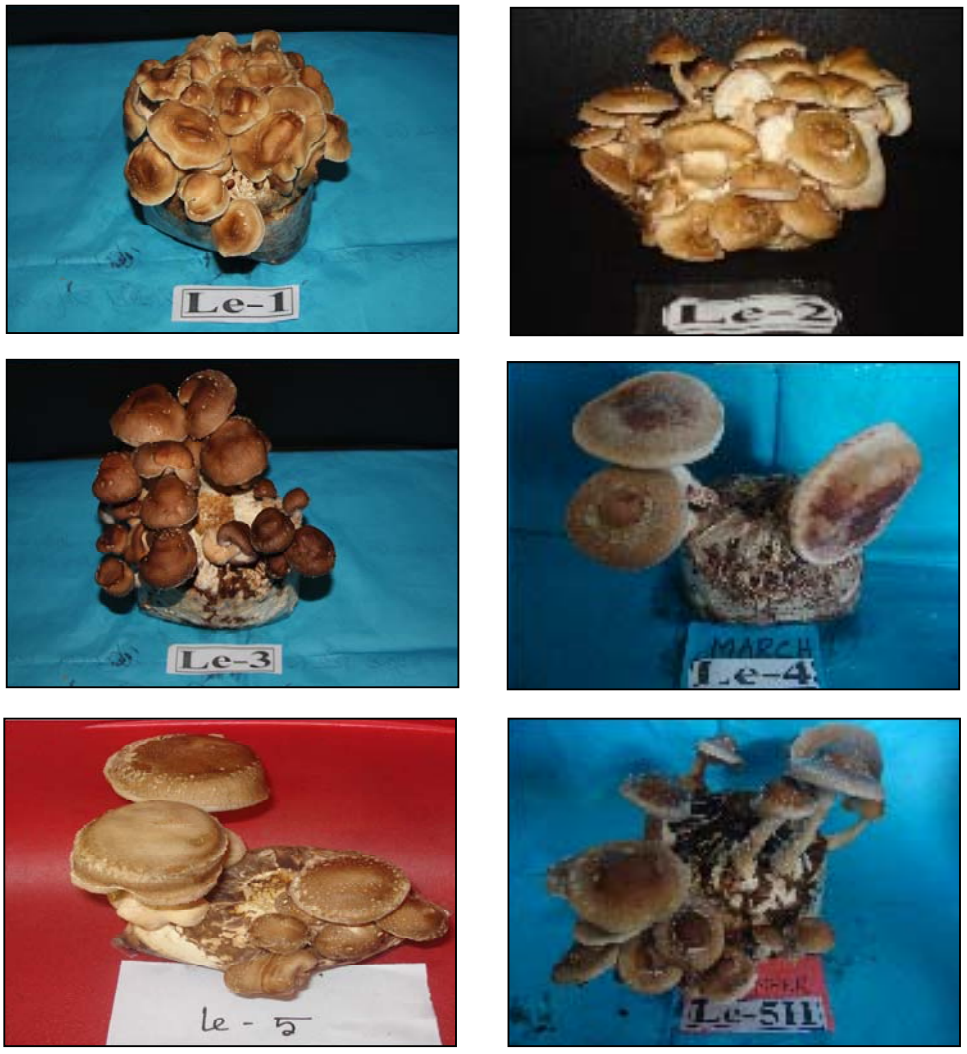
Lentinus edodes, a mushroom primarily of temperate climate, is indigenous in the Far East. It has been cultivated for centuries in China, Korea, Japan, Singapore, Thailand and other Asian countries (Ciesla, 2002). The word 'shiitake' was originally derived from Japanese words: *shii* which means oak and *take* which means mushroom, reflecting the importance of oak wood as the natural host of the fungus (Davis, 1993; Royse, 2001). There are many commercial strains of shiitake selected and propagated for their adaptability to different log species, the time taken to fruit after inoculation and the size, colour, taste and shape of the mushroom (Stamets, 1993). Shiitake mycelium growth and development are clearly influenced by environmental factors. Moisture and temperature are the two most important factors (Kaul, 1997) although the log itself plays an important role in buffering the environmental factors and protecting the fungi from extremes. The availability of nutrients, either as drawn from the substrate or provided as a supplement in supplied water, is also important. Different strains respond differently to forcing and produce mushrooms with different characteristics (McCoy and Bruhn, 2005). Different strains are often categorized by fruiting temperature requirements. Przybylowicz and Donoghue (1988) indicate that temperature has a strong influence on survival, growth rate, time of fruiting, yield and the shape of the mushroom produced. The optimum temperature for mycelial development of *Lentinula edodes* is ranged from 24-28^{0}C reported by Tokimoto and Komatsu, 1982 or 15-24^{0}C (Sabota, 2007). The temperature required for fruiting ranges from 5 to 30^{0}C (Sabota, 2007), while the optimum range is from 10 to 25^{0}C (Przybylowicz and Donoghue, 1988). However, the optimum temperature for fruiting may vary with the particular strain used for cultivation. For example, cold weather strains will fruit when temperatures are between 7 and 15^{0}C (Przybylowicz and

Donoghue, 1988). In contrast, warm weather strains will fruit when temperatures are between 10 and 28^0C (Sabota, 2007) and wide-range fruiting strains will produce fruiting body between 10 and 27^0C. Based primarily on the Chinese system, strains are classified into four categories according to their fruiting temperatures. Faced with massive imports, the Japanese developed a number of new shiitake strains with large and thick basidiocarps (Watanabe, 2001). Performance and stability of superior strains are both important. Experienced growers know the potential problems of strain attenuation. For example repeated subcultures and prolonged storage of the stock culture may result in smaller fruiting bodies and lower yield. Shiitake strains vary widely, particularly in fruiting temperature and mycelial maturation (early or late; shorter or longer production time). Substrate selectivity, growth rate (some fast strains may produce pre-mature fruiting), quality (shape, size, thickness, color, flavor and aroma, etc.), yield and ecological adaptability to extreme temperature (usually cold tolerance) are also strain-related.

Lentinus edodes, no doubt, is the most important specialty mushroom. Despite increasing interest in growing this species, successful cultivation is a challenge. Appropriate strains must be used particularly in fruiting temperature and mycelial maturation. Strains vary greatly not only in fruiting temperature and the time required for spawn maturation but also other traits. Close attention should be given to crucial stages during transition from intricate vegetative phase to reproductive phase. In order to cultivate shiitake mushroom, it is important to first understand the biology, environmental and nutritional requirements of shiitake. The present study was undertaken to evaluate the influence of seasonal variation in different strains of shiitake mushroom available at National mushroom Development and Extension Centre (NAMDEC) and to identify the best strain and season that can be highly productive and suitable for culture conditions in Bangladesh.

4.1.2 MATERIALS AND METHODS

An experiment in completely randomized design with 4 replications was carried out at the National Mushroom Development and Extension Centre, Savar, Dhaka during the period from June 2011 to April 2012. The first factor was different season and second factor was different strains of shiitake mushroom available in Bangladesh. Four different seasons namely autumn (August to October), late autumn (October to December), winter (December to February) and spring (February to April) and twenty three strains of shiitake mushroom viz. Le 1, Le 2, Le 3, Le 4, Le 5, Le 5(H), Le 6, Le 8, Le 9, Le 10, Le 10(H), Le 11, Le 12, Le 13 Le 14, Le 15, Le 16, Le 17, Le 18, Le 19, Le 20, Le 21, Le JR, were used in the experiment (Plate 6).

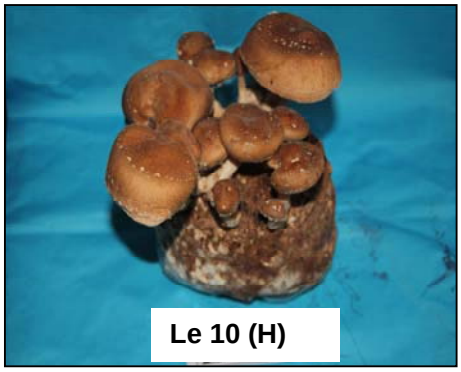


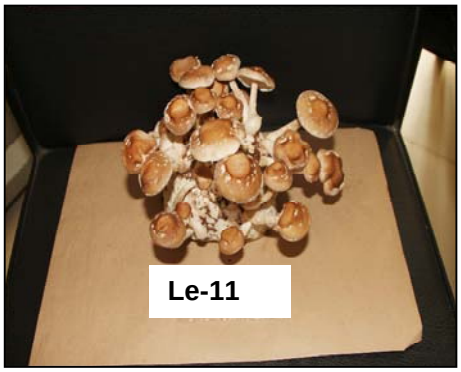

Le-6


Le-8


Le-9


Le-10


Le 10 (H)


Le-11


Le-12


NOVEMBER
Le-14

Plate 6: Different strains of shiitake mushroom

4.1.2.1 Preparation of pure culture, mother culture and spawn packets

Pure culture of twenty three strains were prepared on potato dextrose agar (PDA) medium containing 200 g peeled and sliced potato, 20 g dextrose and 20 g agar per liter. The procedures for preparation of pure culture, mother culture and spawn packets were same as described in chapter III.

4.1.2.2 Mycelial colonization and bump formation

During incubation period, whitish mycelia started to grow in the substrate. All the strains showed optimal mycelial growth at 22 ± 2^0C temperatures and 60-70% relative humidity under control condition. After full colonization of spawn packets, a thick mycelial coat formed on the outer surface of colonized substrate. Clumps of mycelia appeared as blister like bumps of various sizes on the surface of the mycelial coat in each packet. Bumping usually started when colonization of white mycelia changed to brown.

4.1.2.3 Cultivation conditions for fruiting

After mycelium maturation and bump formation, all the packets were fully opened by removing the polypropylene bag. Then the packets were placed separately on the rack of culture house under natural condition. Temperature, relative humidity was recorded season wise and light was maintained at 10-20 lux. Temperature of culture house was recorded at autumn (25.8^0C-32.0^0C), late autumn (19.6^0C-29.1^0C), winter (14.6^0C-25.9^0C) and spring (21.9^0C-32.8^0C). Similarly relative humidity was recorded at autumn (82.35% - 84%), late autumn (77.20% - 82.35%), winter (72.50% - 78.45%) and spring (68.50% -79.55%). Water was applied per day as required and proper aeration was maintained in culture house for the release of excess CO_2 and supply of sufficient O_2 as required for the development of primordia and fruiting body.

4.1.2.4 Data collection and analysis

Data were recorded on mycelium growth rate (MGR), time required for completion of mycelium running (TRMR), time required for bump formation (TRBF), time required from opening to first harvest (TROFH), time required for harvesting (TRH), number of fruiting body (NFB), number of effective fruiting body (NEFB), length of stalk

(LS), diameter of stalk (DS), diameter of pileus (DP), thickness of pileus (TP), yield (g), biological efficiency (BE %).

Yield was recorded after removing lower hard and dirty portion of fruiting bodies and biological efficiency (%) was determined by the following formula:

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

Data were analyzed following MSTAT-C computer program. Means were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

4.1.3 RESULTS AND DISCUSSION

4.1.3.1 Effect of different strains of shiitake mushroom in four seasons on the following thirteen characters were studied in this experiment

4.1.3.1.1 Mycelium growth rate (mm/day)

Mycelium growth rate of different strains of shiitake mushroom in autumn, late autumn, winter and spring season was recorded. In case of growth rate of mycelium, most of the strains showed higher growth in autumn season compare to other seasons. On the other hand, growth rate was found poor in winter season and gradually increased with the increase of temperature in spring season. Growth rate of mycelium in autumn, late autumn, winter and spring season ranged from 1.80-7.95, 1.93-4.95, 1.83-5.03 and 1.85-4.93 mm/day, respectively (Table 1). The strain Le 19 showed the highest growth rate 7.95 mm/day in autumn season and the strain Le JR showed the lowest 1.80 mm/day in same season. The strain Le 12 showed the second highest (5.03 mm/day) growth rate in winter season (Table 1).

Table 1: Mycelium growth rate of different strains of shiitake mushroom in different growing seasons under controlled condition

Strains	Mycelium growth rate (mm/day)			
	Autumn	Late autumn	Winter	Spring
Le 1	3.00	2.75	3.38	3.45
Le 2	3.05	2.50	3.05	3.15
Le 3	2.75	2.60	3.40	3.43
Le 4	2.98	3.10	3.68	3.68
Le 5	3.85	3.63	3.65	3.65
Le 5 (H)	2.90	2.70	2.50	3.00
Le 6	3.60	2.45	3.53	3.53
Le 8	5.50	4.50	3.05	3.15
Le 9	3.20	3.50	3.23	3.23
Le 10	4.90	4.78	3.20	3.20
Le 10 (H)	2.20	1.95	2.13	2.08
Le 11	5.23	4.95	2.70	2.65
Le 12	4.48	4.35	5.03	2.73
Le 13	2.28	2.20	2.63	4.93
Le 14	4.13	4.03	3.60	2.63
Le 15	3.55	3.45	3.30	3.60
Le 16	3.98	3.60	2.85	2.78
Le 17	4.30	3.13	3.05	2.65
Le 18	3.30	3.23	3.85	3.60
Le 19	7.95	3.65	3.88	3.83
Le 20	6.73	4.45	3.10	3.13
Le 21	3.90	3.20	3.00	3.63
Le JR	1.80	1.93	1.83	1.85

4.1.3.1.2 Time required to completion of mycelium running (Days)

Completion of mycelium running in autumn, late autumn, winter and spring ranged from 12.25-58.25, 21.50-57.25, 28.50-56.75 and 25.75-57.50 days, respectively. The maximum time (58.25 days) required to complete mycelium running was recorded from the strain Le JR in autumn season while minimum time (12.25days) required to complete mycelium running was recorded from the strain Le 19 in same season (Table 2).

Table 2: Time required to completion of mycelium running of different strains of shiitake mushroom in different growing seasons under controlled condition

Strains	Time required to completion of mycelium running (days)			
	Autumn	Late autumn	Winter	Spring
Le 1	49.75	50.75	32.75	27.50
Le 2	32.50	38.25	34.50	30.50
Le 3	47.50	49.25	32.50	25.75
Le 4	47.50	47.00	34.25	26.00
Le 5	32.25	32.75	34.25	34.25
Le 5 (H)	33.25	35.25	38.50	32.00
Le 6	26.75	39.00	33.50	33.00
Le 8	43.75	27.00	33.75	31.00
Le 9	30.00	43.00	35.00	34.00
Le 10	19.75	20.50	32.75	31.00
Le 10 (H)	48.75	48.50	44.25	47.50
Le 11	55.50	51.75	32.00	30.75
Le 12	40.00	37.25	28.50	27.25
Le 13	42.75	43.50	55.00	52.75
Le 14	23.50	24.50	37.00	35.00
Le 15	47.50	27.75	49.50	47.50
Le 16	47.50	41.50	33.50	47.75
Le 17	44.25	47.75	50.75	47.50
Le 18	29.25	45.75	35.00	45.75
Le 19	12.25	26.50	40.00	38.75
Le 20	22.50	21.50	45.25	44.50
Le 21	40.00	40.75	43.00	39.25
Le JR	58.25	57.25	56.75	57.50

4.1.3.1.3 Time required for bump formation (days):

Time required for bump formation in different seasons ranged from 88.00-108.00, 81.00-121.00, 84.00-138.00 and 75.25-170.00 days, respectively (Table 3). The strain Le 10(H) developed bump earlier (75.25 days) in spring season. On the other hand, Le 8 required highest (170.00 days) duration to form bump in same season compare to other seasons and strains. The strain Le 8 developed bump earlier in autumn season followed by winter and late autumn while developed bump late in spring season.

Table 3: Time required for bump formation of different strains of shiitake mushroom in different growing season under controlled condition

Strains	Time required for bump formation (days)			
	Autumn	Late autumn	Winter	Spring
Le 1	-----	109.00	98.00	120.50
Le 2	-----	81.00	114.00	135.50
Le 3	-----	90.00	91.50	90.00
Le 4	-----	97.00	96.00	106.80
Le 5	-----	121.00	121.00	95.50
Le 5 (H)	-----	-----	130.00	110.00
Le 6	-----	90.00	115.30	122.00
Le 8	88.00	108.00	107.00	170.00
Le 9	-----	94.00	103.00	119.80
Le 10	-----	100.00	103.00	86.00
Le 10 (H)	-----	-----	90.00	75.25
Le 11	106.50	92.25	105.00	115.00
Le 12	108.00	100.30	107.00	105.00
Le 13	-----	-----	-----	-----
Le 14	-----	90.00	138.00	113.50
Le 15	-----	89.50	112.00	109.00
Le 16	108.00	94.00	91.00	100.80
Le 17	-----	106.00	102.00	107.50
Le 18	-----	103.00	104.00	96.50
Le 19	-----	-----	84.00	88.00
Le 20	108.00	112.00	119.00	152.80
Le 21	-----	89.50	90.50	119.50
Le JR	-----	101.00	114.00	110.80

4.1.3.1.4 Time required from opening to first harvest (days):

The time required from opening to first harvest in different strains of shitake mushroom was non significant in autumn season but highly significant in other seasons (Appendix II-V) The duration from opening of spawn packet to first harvest in different seasons ranged 5.0-6.0, 4.0-11.0, 4.25-13.50 and 3.25-14.50 days respectively. The strain Le 12 required maximum days (14.50 days) from opening to

first harvest in spring season while Le 8 required minimum days (3.25 days) in same season (Table 4).

Table 4: Time required from opening to first harvest of different strains of shiitake mushroom in different growing season under natural condition

Strains	Time required from opening to first harvest (days)			
	Autumn	Late autumn	Winter	Spring
Le 1	-----	7.00c-f	13.50a	5.00efg
Le 2	-----	11.00a	7.00efg	6.25c-f
Le 3	-----	8.00b-e	7.00efg	8.25c
Le 4	-----	10.00ab	7.00efg	6.25c-f
Le 5	-----	10.00ab	10.00bc	7.00cde
Le 5 (H)	-----	-----	7.50def	6.00def
Le 6	-----	8.00b-e	4.75gh	7.75cd
Le 8	5.00a	5.00fg	5.00gh	3.25g
Le 9	-----	8.00b-e	6.00fgh	4.75fg
Le 10	-----	10.00ab	11.00b	8.00cd
Le 10 (H)	-----	-----	7.00efg	7.00cde
Le 11	6.00a	6.00d-g	8.00c-f	8.25c
Le 12	5.75a	5.00fg	9.00b-e	14.50a
Le 13	-----	-----	-----	-----
Le 14	-----	6.00d-g	9.00b-e	6.00def
Le 15	-----	8.25bcd	9.00b-e	6.00def
Le 16	5.00a	5.75efg	4.25h	7.25cd
Le 17	-----	9.00abc	6.50fgh	7.00cde
Le 18	-----	4.00g	7.00efg	7.00cde
Le 19	-----	-----	11.00b	11.00b
Le 20	6.00a	4.00g	9.50bcd	6.75c-f
Le 21	-----	5.00fg	6.50fgh	5.00efg
Le JR	-----	6.00d-g	10.00bc	6.25c-f
LSD(0.05)	**1.86**	**2.02**	**2.03**	**1.72**
CV (%)	**22.19**	**19.97**	**18.03**	**17.38**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.1.3.1.5 Time required for harvest (days):

Time required for harvest was highly significant in different strains of shiitake mushroom under four seasons (Appendix II-V). Time required for harvest in autumn, late autumn, winter and spring ranged from 93.00 -114.00, 92.00-131.00, 95.00-147.00 and 94.00-173.30 days, respectively (Table 5). The strain Le 8 required the maximum time (173.30 days) in spring season and the strain Le 2 required minimum time (92.00 days) in late autumn season.

Table 5: Time required for harvest of different strains of shiitake mushroom in different growing season under natural condition

Strains	Time required for harvest (days)			
	Autumn	Late autumn	Winter	Spring
Le 1	-----	116.00b	111.50hi	125.50e
Le 2	-----	92.00i	121.00ef	141.80c
Le 3	-----	98.00fgh	98.50kl	98.25k
Le 4	-----	107.00de	103.00jk	113.00h
Le 5	-----	131.00a	131.00c	102.50j
Le 5 (H)	-----	-----	137.50b	116.00gh
Le 6	-----	98.00fgh	120.00efg	129.80d
Le 8	93.00b	113.00bc	112.00hi	173.30a
Le 9	-----	102.00ef	109.00ij	124.50e
Le 10	-----	110.00cd	114.00ghi	94.00l
Le 10 (H)	-----	-----	97.00kl	82.25m
Le 11	112.50a	98.25fgh	113.00hi	123.30e
Le 12	113.80a	105.30de	116.00fgh	119.50f
Le 13	-----	-----	-----	-----
Le 14	-----	96.00ghi	147.00a	119.50f
Le 15	-----	97.75fgh	121.00ef	115.00gh
Le 16	112.80a	99.75fg	95.25l	108.00i
Le 17	-----	115.00b	108.50ij	114.50gh
Le 18	-----	107.00de	111.00hi	103.50j
Le 19	-----	-----	95.00l	99.00k
Le 20	114.00a	116.00b	108.50ij	159.50b
Le 21	-----	94.50hi	97.00kl	124.50e
Le JR	-----	107.00de	124.00de	117.00fg
LSD(0.05)	**2.77**	**4.48**	**6.04**	**2.85**
CV (%)	**1.68**	**3.00**	**3.75**	**1.70**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.1.3.1.6 Number of total fruiting body:

Number of total fruiting body in four seasons ranged from 14.00–55.00, 1.50-55.00, 1.75-52.00 and 2.0-40.00 per packet respectively. Their differences were highly significant (Appendix II-V). The highest number (55.00) of fruiting body was recorded from the strain Le 12 in late autumn season and the lowest number (1.50) of fruiting body was recorded from the strain Le 9 in same season. The second highest number (52.00) of fruiting body was recorded from the strain Le 11 in winter season (Table 6).

Table 6: Number of total fruiting body of different strains of shiitake mushroom in different growing season under natural condition

Strains	Number of total fruiting body			
	Autumn	Late autumn	Winter	Spring
Le 1	-----	32.25d	23.75d	32.75c
Le 2	-----	5.50ij	28.50c	11.00g
Le 3	-----	5.25ij	9.75fgh	17.00e
Le 4	-----	5.25ij	9.25fgh	8.75hi
Le 5	-----	5.00ij	18.25e	7.75ij
Le 5 (H)	-----	-----	6.00ghi	6.00jk
Le 6	-----	6.00hi	10.00fgh	10.25gh
Le 8	40.50b	50.50b	45.25b	40.00a
Le 9	-----	1.50j	1.75i	4.50kl
Le 10	-----	20.25f	31.00c	14.25f
Le 10 (H)	-----	-----	4.00i	4.25klm
Le 11	14.00d	19.50f	52.00a	18.00e
Le 12	55.00a	45.50c	47.50b	36.00b
Le 13	-----	-----	-----	-----
Le 14	-----	12.00g	6.50fghi	7.25ij
Le 15	-----	4.50ij	5.25hi	3.00lmn
Le 16	21.00c	55.00a	44.00b	21.75d
Le 17	-----	25.75e	27.75cd	7.50ij
Le 18	-----	1.75j	2.75i	2.25mn
Le 19	-----	-----	3.75i	3.00lmn
Le 20	4.00e	12.75g	4.25i	2.00n
Le 21	-----	10.00g	11.25f	6.25jk
Le JR	-----	9.50gh	10.75fg	7.75ij
LSD (0.05)	**2.67**	**3.53**	**4.20**	**1.98**
CV (%)	**6.58**	**14.41**	**16.23**	**11.42**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.1.3.1.7 Number of effective fruiting body:

Number of effective fruiting body in different strains of shitake mushroom in different seasons was highly significant (Appendix II-V). Number of effective fruiting body in autumn, late autumn, winter and spring ranged from 3.25–39.50, 1.25-36.25, 1.50-37.00 and 1.00-32.25 per packet respectively. The highest number (39.50) of effective fruiting body was recorded from the strain Le 12 followed by Le 11 in winter season and the lowest number (1.00) of effective fruiting body was recorded from the strain Le 20 in spring season (Table 7).

Table 7: Number of effective fruiting body of different strains of shiitake mushroom in different growing season under natural condition

Strains	Number of effective fruiting body			
	Autumn	Late autumn	Winter	Spring
Le 1	-----	26.50b	16.50e	27.75b
Le 2	-----	3.00gh	22.50d	9.25e
Le 3	-----	2.50gh	6.75hijk	12.00d
Le 4	-----	4.75gh	7.25ghij	5.75gh
Le 5	-----	5.00g	12.75f	6.75fg
Le 5 (H)	-----	-----	4.25jklm	4.25hi
Le 6	-----	4.00gh	8.00ghi	7.50fg
Le 8	35.00b	37.50a	35.75ab	32.25a
Le 9	-----	1.25h	1.50m	3.25ij
Le 10	-----	18.75c	29.25c	8.25ef
Le 10 (H)	-----	-----	3.50lm	4.00i
Le 11	5.25d	15.25d	37.00a	13.50d
Le 12	39.50a	35.75a	36.75ab	28.50b
Le 13	-----	-----	-----	-----
Le 14	-----	6.00fg	6.25ijkl	3.00ij
Le 15	-----	4.25gh	3.50klm	2.50ijk
Le 16	15.75c	36.25a	33.75b	18.75c
Le 17	-----	18.00cd	20.50d	6.00g
Le 18	-----	1.25h	2.25m	1.75jk
Le 19	-----	-----	2.75m	2.75ij
Le 20	3.25d	11.25e	3.75klm	1.00k
Le 21	-----	9.00ef	9.50gh	3.50ij
Le JR	-----	8.75ef	10.00fg	7.25fg
LSD (0.05)	**3.19**	**3.19**	**2.91**	**1.60**
CV (%)	**10.72**	**17.23**	**14.43**	**11.89**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.1.3.1.8 Length of stalk (cm):

The length of stalk in different strains of shiitake mushroom under four seasons was highly significant (Appendix II-V). Ranges of length of stalk under different strains with different seasons were 4.25-7.25, 2.45-9.50, 3.15-6.18 and 3.15-7.65cm respectively. Most of the strains produced shorter stalk in winter season compare to other seasons. On the other hand, the length of stalk gradually increased with the increase of temperature in three seasons. The strain Le 20 produced longest (7.25 cm) stalk when grow in autumn season and the strain Le 1 produced shortest stalk (2.45 cm) in late autumn season (Table 8).

Table 8: Length of stalk of different strains of shiitake mushroom in different growing season under natural condition

Strains	Length of stalk (cm)			
	Autumn	Late autumn	Winter	Spring
Le 1	-----	2.45j	3.38ij	3.18j
Le 2	-----	3.80i	4.75cdef	5.15e
Le 3	-----	4.12hi	5.00bcd	4.80f
Le 4	-----	5.78c	5.17bcd	6.30c
Le 5	-----	4.20ghi	4.08efghi	4.75f
Le 5 (H)	-----	-----	4.75cdef	7.65a
Le 6	-----	4.95defg	4.63defg	4.18gh
Le 8	6.28b	4.58efghi	3.15j	4.05hi
Le 9	-----	4.85defgh	3.80ghij	5.50d
Le 10	-----	5.43cd	5.07bcd	5.50d
Le 10 (H)	-----	------	4.03efghi	4.45fg
Le 11	4.63cd	4.53fghi	4.88bcde	4.50fg
Le 12	4.25d	4.25ghi	4.88bcde	3.15j
Le 13	-----	-----	-----	-----
Le 14	-----	4.55fghi	5.53abc	7.23b
Le 15	-----	9.50a	4.98bcd	5.13f
Le 16	4.88c	5.35cde	3.70hij	4.63f
Le 17	-----	3.88i	4.05efghi	6.00c
Le 18	-----	4.05hi	5.70ab	6.03c
Le 19	-----	------	4.58defgh	3.75i
Le 20	7.25a	8.20b	6.18a	5.23de
Le 21	-----	5.15cdef	3.80ghij	3.33j
Le JR	-----	3.80i	3.98fghij	4.00hi
LSD (0.05)	**0.48**	**0.70**	**0.76**	**0.32**
CV (%)	**5.84**	**10.80**	**11.77**	**4.53**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.1.3.1.9 Diameter of stalk (cm):

Differences in diameter of stalk in twenty three strains of shiitake mushroom in four seasons were 1% level of significant (Appendix II-V). The maximum stalk diameter 2.70cm of Le 5(H) was found in spring season whereas lowest diameter of 0.75 cm was obtained from the strain Le 8 in same season (Table 9).

Table 9: Diameter of stalk (cm) of different strains of shiitake mushroom in different growing season under natural condition

Strains	Diameter of stalk (cm)			
	Autumn	Late autumn	Winter	Spring
Le 1	-----	1.28e-i	1.08g	1.25ef
Le 2	-----	1.58cde	1.28fg	1.08fg
Le 3	-----	1.50def	1.25fg	1.33e
Le 4	-----	1.93bc	2.00cd	2.08b
Le 5	-----	1.45d-h	1.83cde	0.88ghi
Le 5 (H)	-----	-----	1.35fg	2.70a
Le 6	-----	1.80bcd	2.48ab	0.95ghi
Le 8	1.07bc	1.65cd	1.25fg	0.75i
Le 9	-----	2.55a	2.48ab	1.60cd
Le 10	-----	1.28e-i	1.15g	0.93ghi
Le 10 (H)	-----	------	1.15g	1.63c
Le 11	1.18ab	1.65bcd	1.28fg	1.60cd
Le 12	0.90c	0.95i	1.43efg	1.55cd
Le 13	-----	-----	-----	-----
Le 14	-----	1.20f-i	1.53efg	2.13b
Le 15	-----	2.00b	1.83cde	1.03gh
Le 16	1.38a	1.13ghi	1.20fg	1.40de
Le 17	-----	1.07i	1.23fg	1.65c
Le 18	-----	1.10hi	2.15bc	2.10b
Le 19	-----	------	2.65a	2.63a
Le 20	1.10bc	1.10hi	2.00cd	1.95b
Le 21	-----	1.50def	1.65def	1.65c
Le JR	-----	1.48d-g	1.53efg	0.85hi
LSD (0.05)	**0.20**	**0.31**	**0.40**	**0.19**
CV (%)	**12.20**	**15.27**	**17.59**	**8.81**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.1.3.1.10 Diameter of pileus (cm):

The diameter of pileus in different strains in autumn, late autumn, winter and spring season ranged from 4.25-6.68, 2.48-9.50, 5.05-10.38 and 5.30-10.80 cm respectively with highly significant difference (Appendix II-V). The highest diameter of pileus

(10.80 cm) was obtained from the strain Le 18 in spring season and the lowest diameter of pileus (2.48cm) was obtained from the strain Le 18 in late autumn season (Table 10).

Table 10: Diameter of pileus of different strains of shiitake mushroom in different growing season under natural condition

Strains	Diameter of pileus (cm)			
	Autumn	Late autumn	Winter	Spring
Le 1	-----	5.00hi	5.70fg	6.86hi
Le 2	-----	9.50a	6.68def	6.98h
Le 3	-----	9.13a	5.75fg	7.33g
Le 4	-----	7.13bcd	7.65bcd	8.68d
Le 5	-----	5.23ghi	7.75bcd	6.65hij
Le 5 (H)	-----	-----	7.88bcd	7.88ef
Le 6	-----	7.90b	8.50bc	6.58ijk
Le 8	5.85c	6.40def	5.95efg	4.63m
Le 9	-----	7.38bc	10.38a	9.00cd
Le 10	-----	6.58cdef	6.00efg	7.50g
Le 10 (H)	-----	-----	5.05g	7.60fg
Le 11	6.18b	6.38df	5.13g	7.40g
Le 12	4.25e	4.45i	7.45cd	5.30l
Le 13	-----	-----	-----	-----
Le 14	-----	6.88cde	6.70def	9.15c
Le 15	-----	6.88cde	7.58bcd	8.20e
Le 16	5.45d	6.45def	6.08efg	6.25k
Le 17	-----	5.83fgh	5.45g	9.05c
Le 18	-----	2.48j	10.05a	10.80a
Le 19	-----	------	10.25a	9.78b
Le 20	6.68a	5.15hi	8.65b	8.18e
Le 21	-----	6.43def	7.05de	7.60fg
Le JR	-----	6.05efg	7.03de	6.38jk
LSD (0.05)	**0.17**	**0.79**	**1.03**	**0.33**
CV (%)	**4.78**	**8.80**	**10.14**	**3.10**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.1.3.1.11 Thickness of pileus (cm):

The thickness of pileus in different strains of shiitake mushroom as well as different seasons differed significantly (Appendix II-V) and ranged from 0.80-1.40, 0.88-1.44, 1.05-2.50 and 0.65-2.40cm respectively. The highest thickness of pileus (2.50 cm) was obtained from the strain Le 9 in winter season and the lowest thickness of pileus (0.65 cm) was obtained from the strain Le 8 in spring season (Table 11).

Table 11: Thickness of pileus of different strains of shiitake mushroom in different growing season under natural condition

Strains	Thickness of pileus (cm)			
	Autumn	Late autumn	Winter	Spring
Le 1	-----	1.00hi	1.28ghij	1.15jk
Le 2	-----	1.88bcd	1.23hij	1.50gh
Le 3	-----	1.08ghi	1.05j	1.30hij
Le 4	-----	2.05ab	2.25ab	1.78def
Le 5	-----	1.58def	1.60efg	1.10jkl
Le 5 (H)	-----	-----	1.45fghi	2.40a
Le 6	-----	2.13ab	1.60efg	0.98kl
Le 8	0.90bc	1.28fgh	1.13ij	0.65m
Le 9	-----	2.30a	2.50a	1.68efg
Le 10	-----	1.25fgh	1.23hij	1.08jkl
Le 10 (H)	-----	-----	1.15hij	1.60fg
Le 11	0.95bc	1.35e-h	1.25hij	1.23j
Le 12	1.05b	0.88i	1.33ghij	1.28ij
Le 13	-----	-----	-----	-----
Le 14	-----	1.90bcd	1.48fgh	1.48ghi
Le 15	-----	1.63cde	1.85cde	0.90l
Le 16	1.40a	1.30e-h	1.23hij	1.08jkl
Le 17	-----	1.08ghi	1.08j	2.05bc
Le 18	-----	1.93bc	2.10bc	2.13b
Le 19	-----	------	1.85cde	1.85cde
Le 20	0.80c	1.40efg	2.05bcd	1.95bcd
Le 21	-----	1.08ghi	1.75def	1.58fg
Le JR	-----	1.28fgh	1.35ghij	0.93kl
LSD (0.05)	**0.19**	**0.30**	**0.29**	**0.205**
CV (%)	**12.40**	**14.52**	**13.23**	**9.96**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.1.3.1.12 Yield (g):

The yield was highly significant in different strains of shiitake mushroom in different seasons (Appendix II-V). Total 23 strains were grown in different season. Among the strains, maximum number of strain produces fruit body in winter and spring season and minimum in autumn season. Out of 23 strain only five strain produce fruit body in autumn season sixteen in late autumn and twenty two in winter as well as spring season (Fig.1) Most of the strains gave higher yield in winter season compare to other seasons while yield performance was poor in autumn season (Table 12). Yield of different strains of shiitake mushroom in autumn, late autumn, winter and spring season ranged from 42.25-145.80g, 18.75-175.50g, 191.00-40.50g and 39.50-163.50g, respectively (Table 12). The highest yield (191.00g) obtained from the strain Le 8 in

winter season and the lowest yield (18.75g) gave the strain Le 18 in late autumn season. Each strain gave different yield on season based. Le 1 gave highest yield (163.50g) in spring season while no yield (0.0 g) obtained in autumn season. Among the strains four strains were commercially cultivated round the year and yield variation was observed in four seasons (Fig. 2). Le 8, Le 11 and Le 12 gave highest yield in winter season but Le 16 gave highest yield in late autumn. On the other hand Le 8 and Le 12 gave lowest yield in spring season as well as Le 11 and Le 16 gave lowest yield in autumn season.

Table 12: Yield performance of different strains of shiitake mushroom in different growing season under natural condition

Strains	Yield(g)			
	Autumn	Late autumn	Winter	Spring
Le 1	-----	83.00g	105.50f	163.50a
Le 2	-----	53.00h	151.00cd	80.00h
Le 3	-----	57.75h	50.75j	146.00b
Le 4	-----	88.75	120.00e	108.80e
Le 5	-----	82.75fg	122.50e	66.00i
Le 5 (H)		-----	50.00j	95.00f
Le 6	-----	90.00g	115.50ef	64.00i
Le 8	145.80a	172.00a	191.00a	123.80c
Le 9	-----	31.50i	88.25g	64.00i
Le 10	-----	121.80bc	162.50bc	144.30b
Le 10 (H)	-----	-----	40.50j	41.25k
Le 11	97.25b	126.50b	151.80cd	120.00d
Le 12	99.00b	110.30de	148.50d	94.00f
Le 13	-----	-----	----	-----
Le 14	-----	125.50b	63.50i	97.75f
Le 15	-----	107.50e	70.25hi	57.75j
Le 16	85.25c	175.50a	171.80b	110.00e
Le 17	-----	115.50cd	152.50cd	95.50f
Le 18	-----	18.75j	86.50g	80.00h
Le 19	-----	-----	83.75g	87.50g
Le 20	45.25d	115.00cd	77.00gh	64.75i
Le 21	-----	96.00f	105.30f	39.50k
Le JR	-----	90.00fg	88.50g	86.50g
LSD (0.05)	**4.97**	**6.99**	**11.48**	**3.65**
CV (%)	**3.51**	**5.05**	**7.46**	**2.80**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

----- = Not produce fruit body.

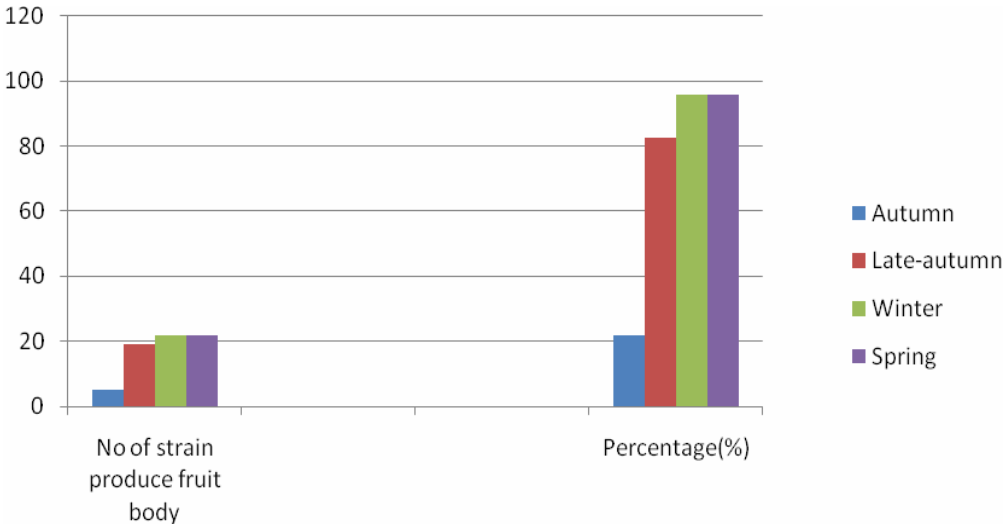


Fig.1: Number and percentage of strain of shiitake mushroom gave fruit body in four seasons

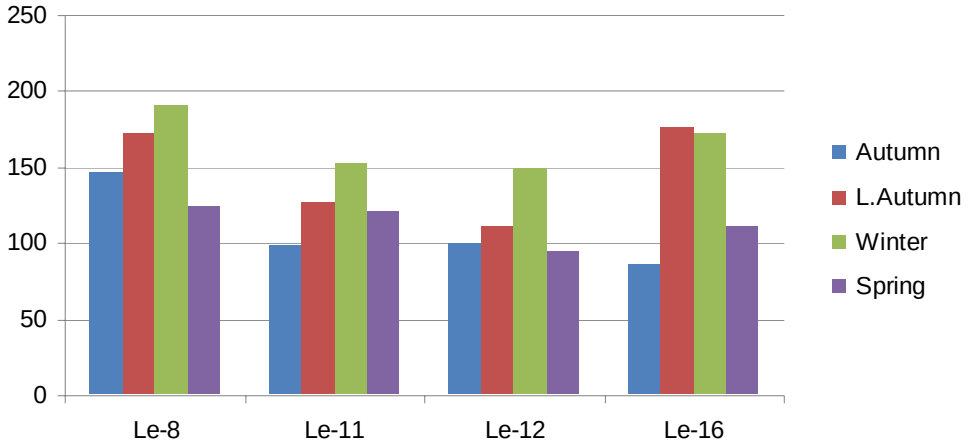


Fig.2: Comparative yield performance of commercially cultivated shiitake strain in Bangladesh under four seasons.

4.1.3.1.13 Biological Efficiency (%):

Differences in this parameter under those four seasons were highly significant (Appendix II-V). Biological efficiency was higher in winter season in maximum strain. Biological efficiency gradually increased with the decrease of temperature in winter season. The highest biological efficiency (109.10%) was obtained from the

strain Le 8 grow in winter season and the lowest biological efficiency (10.72%) was obtained from the strain Le 18 in late autumn (Table 13).

Table 13: Biological efficiency (%) of different strains of shiitake mushroom in different growing season under natural condition

Strains	Biological Efficiency (%)			
	Autumn	Late autumn	Winter	Spring
Le 1	-----	47.43g	60.28f	93.43a
Le 2	-----	30.28h	85.78d	45.72h
Le 3	-----	33.00h	29.00j	83.43b
Le 4	-----	50.72fg	68.57e	62.14e
Le 5	-----	47.28g	70.00e	37.71i
Le 5 (H)	-----	-----	28.57j	54.28f
Le 6	-----	51.43fg	66.00ef	36.57i
Le 8	83.29a	98.28a	109.10a	70.71c
Le 9	-----	18.00i	50.43g	36.57i
Le 10	-----	69.57bc	92.85bc	82.43b
Le 10 (H)	-----	-----	23.14j	23.57k
Le 11	55.57b	72.28b	88.21cd	68.57d
Le 12	56.57b	63.00de	84.85d	53.71f
Le 13	-----	-----	-----	-----
Le 14	-----	71.71b	36.29i	55.85f
Le 15	-----	61.43e	40.14hi	33.00j
Le 16	48.71c	100.30a	98.14b	62.85e
Le 17	-----	66.00cd	87.14cd	54.57f
Le 18	-----	10.72j	49.43g	45.72h
Le 19	-----	-----	47.86g	50.00g
Le 20	24.15d	65.71cd	44.00gh	37.00i
Le 21	-----	54.85f	60.14f	22.57k
Le JR	-----	51.43fg	50.57g	49.43g
LSD (0.05)	**2.84**	**3.99**	**6.14**	**2.08**
CV (%)	**3.51**	**5.05**	**7.29**	**2.80**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Results of the present experiment reveal that there are appreciable variations in growth and yield contributing attributes with the variation of season (temperature, humidity) and different strains of shiitake mu*shroom.* In terms of yield and yield attributes Le 8, Le 11, Le 12 and Le 16 produce fruit body round the year. Performance of the strain Le-1 was better in spring season than other seasons. Among the seasons autumn suitable for mycelium running late autumn, winter and spring suitable for cultivation. Many other investigators also found variations in effect of temperature, moisture, strain on growth, yield and yield contributing characters of shiitake mushroom.

Many strains of shiitake mushroom are available in the world which is extensively cultivated. The strains of this valuable mushroom vary widely, particularly in the time required for mycelium colonization, bump formation and fruiting body development. The morphology and productivity of shiitake mushroom vary according to the strains based on the influence of environmental factors (Triratana andTantikanjana, 1987).

Under natural condition, fruiting occurs primarily in spring and autumn due to seasonal rains and temperature changes (Leatham, 1982). While, in forced fruiting, mushrooms can be produced more frequently and even during winter and summer by carefully managing temperature and humidity conditions (Anderson and Marcouiller, 1990).

Different strains are often categorized by fruiting temperature requirements. Shiitake from strains of cool season, wide range and warm season will generally fruit at log temperatures between 5 and 20^0C, 10 and 27^0C, and 10 and 30^0C, respectively. Productivity, appearance, mushroom size and length of time it takes to fruit will also differ as a result of different strain employed (Sabota, 1998). Whilst, requirement of LMC for fruiting is over 40%, however, higher LMC is favourable (Leatham, 1982). During formation of primordial fruit bodies, the environment is managed to ensure the right number of pins form and begin to grow out. Recommended moisture content of logs (LMC) varies at this stage of development. Komatsu and Tokimoto, 1982 (cited in Przybylowicz and Donoghue, 1988) and Tokimoto *et al*., (1980) recommend a range of LMC from 35 to 65% with optimum LMC between 55 and 65%. Koske (1998) suggests maintaining the LMC within a much narrower range of 55 to 60%.

The appropriate temperature for the formation of fruiting boy primordia ranges from 15-25^{0}C, although each strain has its own optimum temperature (Komatsu and Tokimoto, 1982; Tokimoto and Komatsu, 1982).

All shiitake strains show optimal mycelial growth at 25^{0}C (Chen, 2000). The duration of spawn run is usually 1-4 months depending on strains and methodology (Oei, 1996). There is an optimum temperature for mycelial growth above and below which growth is restricted (Przybylowicz and Donoghue, 1988; Miles and Chang, 1997). This may be due to the effect of temperature on enzyme activity and the resulting changes in rates of chemical processes (Miles and Chang, 1997).

Clumps of mycelia appear as blister- or popcorn-like bumps of various sizes on the surface of the mycelial coat in most strains. This usually begins when colonization of white mycelia covers the entire substrate in the bag, or sometimes earlier. Primordia are produced at the tips of some of these bumps. However, most bumps are aborted and never develop into fruiting bodies. Time of bump formation varies with strains, substrate and temperature. Usually bumps form 10 days faster at 25^{0}C than at 15^{0}C (Miles and Chang, 1989). Fluctuation of temperature and high CO_2 concentration encourage bump formation. Lower the CO_2 in the bag, when bumps become too numerous by cutting slits on the bag. In any case, some aeration should be provided when bumps are formed.

As the shiitake mycelium spread through a log it secretes exoenzymes that degrade the dead wood in order to obtain nutrients (Andrade *et al*., 2008). The production of mushroom fruiting bodies (sporophores) starts when the logs are fully colonized. Under natural conditions heavy rains and an associated drop in temperature stimulates mushroom production (Shiomi *et al*., 2007).

There is an optimum temperature for mycelial growth above and below which growth is restricted (Przybylowicz and Donoghue, 1988; Miles and Chang, 1997). This may be due to the effect of temperature on enzyme activity and the resulting changes in rates of chemical processes (Miles and Chang, 1997). Temperature also plays an important role in the initiation and development of fruiting bodies and a sudden shift in temperature may be required to induce fruiting of mushrooms (Komatsu, 1961; cited in Przybylowicz and Donoghue, 1988).

Sarker *et al.,* 2009, observed that duration to complete mycelium running of different strains of shiitake mushroom on sawdust varied greatly. Mycelium growth rate (MGR) in spawn packet ranged from 0.23 to 0.35 cm/day among the strains. He also observed that shiitake mushrooms took 65.83 to 98.33 days for bump formation during incubation period. This result supports the findings of Kawai *et al.* (1997) who reported that 60 to 90 days is required for incubation period of shiitake mushroom. Sarker *et al.,* 2009 also reported that among the strains, a wide variation was observed in the duration from inoculation to primordia initiation and first harvest. Days required for primordia initiation ranged 69.83-103.5 days and for first harvest ranged 78.67-111.2 days. The highest biological yield was found in Le 8 which was followed by Le 11, Le 12, Le 10 and Le 2. The lowest yield was recorded from Le 4, which was followed by Le 9, Le 5 and Le 6. The result is in line with the report of Przybylowicz and Donoghue (1990) who found the biological efficiency of shiitake mushroom to vary between 50 and 80% for 2-5 harvests. The strain Le 4 performed very poorly.

Low temperatures induce fruiting body development which accompanies the enhancement of enzyme activities such as acid protease and the accumulation of nutrients around a developing fruiting body (Tokimoto *et al.,* 1984; Tokimoto and Fukuda, 1997). Temperature during fruiting body development also affects the shape and yield of fruiting bodies and each strain has its own optimum temperature (Kawai and Kashiwagi, 1968; Ohira *et al*., 1982). When the temperature is lower or higher than the optimum for the strain, smaller fruiting bodies are produced. High temperature strains are induced to produce fruiting bodies with a shorter exposure of low temperature, and may need only several hours of cold. However, low or medium temperature strains require much longer exposure to low temperature to induce fruiting by exposure to a brief low temperature.

Temperature also affects mushroom shape of *Lentinula edodes* (Ohira *et al.,* 1982, cited in Przybylowicz and Donoghue, 1988). Mushrooms developed under higher temperatures tend to form long stems and thin cap, whereas those cultivated under cooler temperature have short stems and thick caps (Tokimoto and Komatsu, 1978).

4.2 EFFECT OF DIFFERENT SUPPLEMENTS AND THEIR COMBINATIONS ON THE GROWTH AND YIELD OF SHIITAKE (*Lentinus edodes*) MUSHROOM

4.2.1 INTRODUCTION

Shiitake mushroom *(Lentinula edodes)* is the second most popular edible mushroom (Chang, 1999 and Chiu *et al*., 1999). After the button mushroom, shiitake is the most cultivated mushroom in the world (Chang and Miles, 2004). It can grow in winter season and also it can grow all the year in controlled condition. *Lentinus* is liked by the consumers because of its unique taste and flavour, and presence of a chemical lentinan which reduces plasma-cholesterol level. It also has considerable high nutritional value and medicinal value. With the improvement of science and technology, the deeper outstanding of its values and the upgrade of people's living level, it is predictable that the market demand of *Lentinus* will be larger and larger. So the development of *Lentinus* production therefore becomes so necessary and urgent.

Lentinus edodes is traditionally cultivated on tree logs, which have been partly replaced by bag cultivation by using sterilized sawdust to enhance the biological efficiency and to reduce the production cycle (Kalberer, 1998). Sawdust is the most popular basal ingredient used in synthetic substrate formulations for producing shiitake spawn. Supplementation of the substrate with various materials is recommended prior to spawning for enhancement of the yield of mushrooms (Hadwan *et al*., 1997). Different starch based supplements, such as wheat bran, rice bran, millet, rye and maize powder are suggested to add to saw dust to serve as major nutrients to provide optimum growth medium (Royse, 1997 and 2001). These supplements improve the production, quality, flavor, and shelf life of cultivated mushrooms (Mau *et al*., 2002; Isikhuemhen *et al*., 2000; Chiroro, 2004 and Okhuoya *et al*., 2005).

Many strains of shiitake mushroom are available in the world which is extensively cultivated in Bangladesh. The strains of this valuable mushroom vary widely, particularly in the time required for mycelium colonization, bump formation and fruiting body development. The morphology and productivity of shiitake mushroom vary according to the strains based on the influence of environmental factors

(Triratana and Tantikanjana, 1987). The mycelial growth in the vegetative phase involves producing quality fruiting bodies in reproductive phase. Spawn run of different strains is of ultimate importance for adjusting the reproductive phase. Sixty days is sufficient to mature for one strain whereas this time would be insufficient for another strain (Miles and Chang, 1989). Substrate selectivity, growth (some strains may produce pre-mature fruiting), quality (shape, size, thickness, color, flavored and aroma etc.) and yield are also related to strain (Chen, 2001). Many studies have been carried out in the world to improve the quality and increase the production of *Lentinus edodes*. But, the production of this mushroom is fairly new in Bangladesh. The purpose of the present study was to find out the suitable strain and supplement as well as their combination of sawdust for higher production of shiitake mushroom.

4.2.2 MATERIALS AND METHODS

The present study was carried out at National Mushroom Development and Extension Centre, Savar, Dhaka during the period from August 2011 to March 2012. Two strains of shiitake namely S_1 = Le 8 and S_2 = Le 11 as well as three different supplements and their combinations namely T_1 = Wheat bran, T_2 = Rice bran, T_3 = Maize powder, T_4 = Wheat bran + Rice bran (1:1), T_5 = Wheat bran + Maize powder (1:1) and T_6 = Wheat bran + Rice bran + Maize powder (1:1:1) were used in the experiment as treatments.

4.2.2.1 Preparation of pure and mother culture:

The procedures for preparation of pure culture and mother culture were same as described in chapter III.

4.2.2.2 Preparation of spawn packets:

Sawdust used as a main substrate was supplemented with wheat bran, rice bran, maize powder and their combinations, wheat bran + rice bran (1:1), wheat bran + maize powder (1:1) and wheat bran + rice bran + maize powder (1:1:1) at the ratio of 2:7:1 (dry weight basis). The amount of supplement was used as 27.27 % (65 g/packet) for each treatment. Rice husk also mixed (10.86 g/packet) with substrate and supplement. For each 500 g spawn packet substrate, supplement (wheat bran, rice bran, maize powder, and their combinations) and rice husk were mixed together according to treatment combinations. Water was added to adjust moisture content at 60% and

$CaCO_3$ was mixed at the rate of 0.2% of the mixture. Polypropylene bags of 25 cm × 18 cm size were filled with 500 g of substrate mixture and packets were tied, plugged and covered as mentioned above. The packets were sterilized in an autoclave for 2 hour at 121 ^{0}C under 1.5 kg/cm^2 pressure. After sterilization the packets were cooled and transferred into an inoculation chamber. The packets were inoculated separately with the mother culture of the strains to be tested at the rate of two teaspoonfuls per packet. The inoculated packets were incubated at 22 ± 2^{0}C.

4.2.2.3 Mycelial colonization, bump formation and cultivation condition:

Mycelial colonization and bump formation as well as cultivation conditions for fruiting were same described earlier in chapter III.

4.2.2.4 Data collection and analysis:

The experiment was laid out in Completely Randomized Design (CRD) with four (4) replications. Data were recorded on mycelium growth rate (MGR), time required for mycelium running (TRMR), time required for bump formation (TRBF), time required from opening to first harvest (TROFH), time required for harvesting (TRH), number of fruiting body (NFB), number of effective fruiting body (NEFB), length of stalk (LS), diameter of stalk (DS), diameter of pileus (DP), thickness of pileus (TP), yield (g), biological efficiency (BE%).

Data were analyzed following MSTAT-C computer program. Means were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

4. 2. 3 RESULTS AND DISCUSSION

4. 2. 3. 1 Main effect of strains on various parameters:

Main effect of the factor strain of shiitake mushroom on various growth, yield and yield attributes is shown in Table 14 and Table 15.

4.2.3.1.1 Mycelium growth rate (MGR):

Mycelium growth rate was observed in two strains of shiitake mushroom was non significant (Appendix VI). The highest mycelium growth rate (0.40 cm/day) was recorded in Le 11 while the lowest mycelium growth rate (0.39 cm/day) was recorded in Le 8 (Table 14).

4.2.3.1.2 Time required to completion of mycelium running (TRMR):

Time required to completion of mycelium running was significantly influenced by two strains of shiitake mushroom (Appendix VI). The lowest time (38.79 days) required to completion of mycelium running was found in Le 8. The highest time (40.58 days) required to completion of mycelium running was recorded in Le 11 (Table 14).

4.2.3.1.3 Time required for bump formation (TRBF):

Time required for bump formation was significantly influenced by two strains of shiitake mushroom (Appendix VI). The highest time required for bump formation (93.12 days) was recorded in Le 11. The lowest time required for bump formation (90.04 days) was found in Le 8 (Table 14).

4.2.3.1.4 Time required from opening to first harvest (TROFH):

Time required from opening to first harvest was significantly influenced by two strains of shiitake mushroom (Appendix VI). The highest time required from opening to first harvest (11.83 days) was recorded in Le 8. The lowest time required from opening to first harvest (8.75 days) was found in Le 11 (Table 14).

4.2.3 .1.5 Time required for harvest (TRH):

Time required for harvest was observed in two strains of shiitake mushroom non significant (Appendix VI). The time required for harvest was recorded in Le 8 of 101.96 days and Le 11 of 101.88days (Table 14).

4.2.3.1.6 Number of fruiting body (NFB):

Number of fruiting body observed in two strains of shiitake mushroom was non significant (Appendix VI). The number of fruiting body was recorded in Le 8 (28.08) and Le 11 was (28.25). The highest NFB (24.42 days) was recorded in Le 8 and the lowest number of fruiting body (NFB) 22.21 in Le 11 (Table 15).

4.2.3.1.7 Number of effective fruiting body (NFEB):

Number of effective fruiting body observed in two strains of shiitake mushroom was non significant (Appendix VI). The highest Number of effective fruiting body (24.42

days) was recorded in Le 8 and the lowest number of effective fruiting body (22.21) was recorded in Le 11 (Table 15).

4.2.3.1.8 Length of stalk (LS):

The length of stalk was found non significant by the effect of two strains of shiitake mushroom (Appendix VI). The highest length of stalk (5.58 cm) was found in Le 8 and lowest length of stalk (5.43 cm) was recorded in Le 11 (Table 15).

4.2.3.1.9 Diameter of stalk (DS):

The diameter of stalk was also found non significant by the effect of two strains of shiitake mushroom (Appendix VI). The highest diameter of stalk (1.35 cm) was observed in Le 8 and the lowest diameter of stalk (1.22 cm) was observed in Le11 (Table 15).

4.2.3.1.10 Diameter of pileus (DP):

The diameter of pileus was influenced by two strains of shiitake mushroom at 1% level of significant (Appendix VI). The highest diameter of pileus (6.76 cm) was recorded in Le 8 and the lowest diameter of pileus (6.09 cm) was observed in Le 11 (Table 15).

4.2.3.1.11 Thickness of pileus (TP):

The thickness of pileus was not significantly influenced by two strains of shiitake mushroom (Appendix VI). The highest thickness of pileus (1.33 cm) as recorded in Le 8 and the lowest thickness of pileus (1.28 cm) was recorded in Le 11 (Table 15).

4.2.3.1.12 Yield:

The yield was not significantly influenced by two strains of shiitake mushroom (Appendix VI). The highest yield (134.21g) was recorded in Le 8 and the lowest yield (125.13 g) was recorded in Le 11 (Table 15).

4.2.3.1.13 Biological efficiency (BE %):

Remarkable variation was observed in biological efficiency of two strains of shiitake mushroom (Appendix VI). The highest biological efficiency (90.85%) was obtained

from Le 8 and the lowest biological efficiency (61.85%) was recorded in Le 11 (Fig. 3).

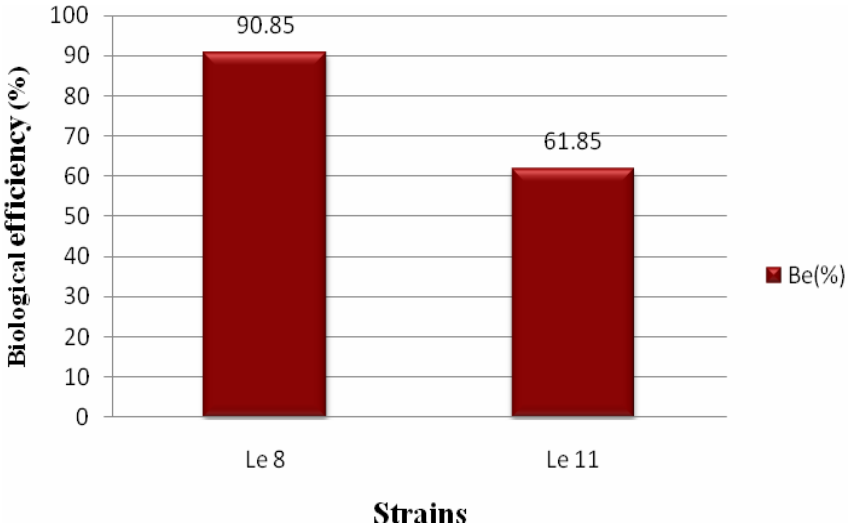


Fig. 3: Effect of strain of shiitake mushroom on biological efficiency.

4.2.3.2 Main effect of different supplements

4.2.3.2.1 Mycelium growth rate (MGR):

Mycelium growth rate was observed highly significant by the effect of different level of supplements (Appendix VI). The highest mycelium growth rate (0.50 cm/day) was recorded when rice bran used as supplements. The lowest mycelium growth rate (0.35 cm/day) was recorded in wheat bran supplement which was statistically similar to the mixture of wheat bran + maize powder (1:1) (Table14).

4.2.3.2.2 Time required to completion of mycelium running (TRMR):

Time required to completion of mycelium running was highly significant by the influenced of different supplements (Appendix VI). The time required to completion of mycelium running in spawn ranged from 33.63 to 43.88 (days). The lowest time required to completion of mycelium running (33.63 days) was found in wheat bran and the highest time required to completion of mycelium running (43.88 days) was recorded from the mixture of wheat bran + rice bran (1:1) (Table 14).

4.2.3.2.3 Time required for bump formation (TRBF):

Time required for bump formation was highly significant influenced by different supplements (Appendix VI). The highest time required for bump formation (104.40 days) was recorded in wheat bran which was statistically similar to rice bran. The lowest time required for bump formation (83.00 days) was found in maize powder which was statistically similar to mixture of wheat bran+ rice bran+ maize powder (1:1:1) (Table 14).

4.2.3.2.4 Time required from opening to first harvest (TROFH):

High significant variations were observed in time required from opening to first harvest by different supplements (Appendix VI). The highest time required from opening to first harvest (13.38 days) was recorded in wheat + rice bran (1:1) which was statistically similar to wheat bran+ rice bran+ maize powder (1:1:1). The lowest time required from opening to first harvest (7.20 days) was found when maize powder used as supplements (Table 14).

4.2.3.2.5 Time required for harvest (TRH):

Time required for harvest was highly significant by the influence of different supplements (Appendix VI). The highest time required for harvest (113.90 days) when wheat bran used as supplement and it was statistically similar to rice bran. The lowest time required for harvest (90.50 days) was found from maize powder supplement (Table 14).

4.2.3.2.6 Number of fruiting body (NFB):

The number of fruiting body was found highly significant by the effect of different supplements (Appendix VI). The number of fruiting bodies ranged from 12.50 to 52.63. The highest number of fruiting body (52.63) was recorded in wheat bran supplements. The lowest number of fruiting body (12.50) was recorded in rice bran (Table 15).

4.2.3.2.7 Number of effective fruiting body (NFEB):

The number of fruiting body was also found highly significant by the effect of different supplements (Appendix VI). The highest number of effective fruiting body

(44.00) was recorded in wheat bran supplements. The lowest number of effective fruiting body (10.38) was observed rice bran supplement which was statistically similar to wheat bran+ rice bran+ maize powder (1:1:1) (Table 15).

4.2.3.2.8 Length of stalk (LS):

The length of stalk was highly significant by the effect different supplements (Appendix VI). The highest length of stalk (6.55 cm) was found in from the mixture of wheat bran+ rice bran+ maize powder (1:1:1) which was statistically similar to wheat bran+ maize powder (1:1). The lowest length of stalk (4.23 cm) was recorded in wheat bran supplement which was statistically similar to wheat bran + rice bran (1:1) (Table 15).

4.2.3.2.9 Diameter of stalk (DS):

The diameter of stalk was not significantly influenced by supplements (Appendix VI). The highest diameter of stalk (1.41 cm) was found from wheat bran + maize powder (1:1) supplement which was statistically similar to others supplements (Table 15).

4.2.3.2.10 Diameter of pileus (DP):

The diameter of pileus in different types of supplement was highly significant (Appendix VI). The highest diameter of pileus (7.36 cm) was recorded from wheat bran + maize powder (1:1) supplements were statistically similar to rice bran, wheat bran + rice bran (1:1) and rice bran + wheat bran + maize powder (1:1:1). The lowest diameter of pileus (4.70 cm) was observed in maize powder supplement which was statistically similar to the treatments of wheat bran (Table 15).

4.2.3.2.11 Thickness of pileus (TP):

The thickness of pileus in different types of supplements was also highly significant (Appendix VI). The highest thickness of pileus (1.55 cm) was recorded from wheat bran + rice bran + maize powder (1:1:1) supplements which was statistically similar to wheat bran, rice bran and wheat bran + maize powder (1:1). The lowest thickness of pileus (0.87 cm) was recorded from maize powder supplements (Table 15).

4.2.3.2.12 Yield:

Appreciable variation was found in the yield of two strains of shiitake mushroom on three different types of supplement (Appendix VI). The highest yield (155.30g) was recorded in wheat bran which was higher than all other treatments. The lowest yield (103.30 g) was recorded from rice bran supplement (Table 15).

4.2.3.2.13 Biological efficiency (BE %):

The effect of three different types of supplement on biological efficiency was highly significant (Appendix VI). The highest biological efficiency (90.85%) was obtained from wheat bran treatment and the lowest biological efficiency (61.85%) was recorded in rice bran treatment (Fig. 4).

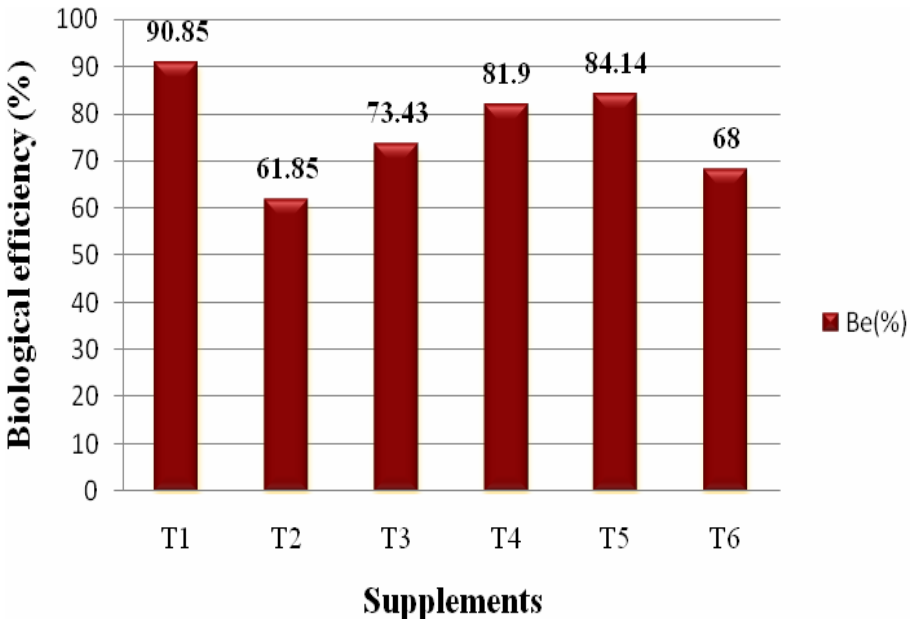


Fig. 4: Effect of different level of supplements on biological efficiency.
T_1 = wheat bran, T_2 = Rice bran, T_3 = Maize powder, T_4 = Rice bran + Wheat bran (1:1), T_5 = Wheat bran + Maize powder (1:1), T_6 = Rice bran + Wheat bran + Maize powder (1:1:1).

Table 14: Effect of strain and different level of supplement on growth of shiitake mushroom

Strains	Mycelium growth rate (cm/day)	Time required for mycelium running (days)	Time required for bump formation (days)	Time required from opening to first harvest (days)	Time required for harvest (days)
Le 8	0.39	38.79	90.04	11.83	101.96
Le 11	0.40	40.58	93.12	8.75	101.88
Supplements					
Wheat bran (WB)	0.35d	33.63d	104.40a	9.50bc	113.90a
Rice bran (RB)	0.50a	36.88c	102.00a	9.30bc	111.40a
Maize powder (MP)	0.37cd	40.00b	83.00d	7.20c	90.50d
WB + RB	0.42b	43.88a	88.50b	13.38a	101.90b
WB+ MP	0.35d	42.50ab	86.88bc	10.50b	97.38c
WB+ RB +MP	0.41bc	41.25b	84.75cd	11.75ab	96.50c
LSD (0.05)	**0.05**	**2.41**	**3.21**	**2.57**	**2.60**
CV (%)	**7.06**	**4.24**	**2.44**	**17.44**	**1.78**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Table 15: Effect of strain and different level of supplements on yield attributes and yield of shiitake mushroom

Strain	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of Pileus (cm)	Thickness of pileus (cm)	Yield (g)
Le 8	28.08	24.42	5.58	1.35	6.76	1.33	134.21
Le 11	28.25	22.21	5.43	1.22	6.09	1.28	125.13
Supplements							
Wheat bran (WB)	52.63a	44.00a	4.23c	1.18a	5.68bc	1.28ab	155.30a
Rice bran (RB)	12.50d	10.38c	5.66b	1.33a	6.69ab	1.18b	103.30c
Maize powder (MP)	26.25c	24.00b	5.78b	1.20a	4.70c	0.87c	124.80bc
WB + RB	35.00b	23.63b	4.89c	1.30a	6.81ab	1.35ab	136.50ab
WB+ MP	28.50c	24.88b	5.93ab	1.41a	7.36a	1.41ab	141.30ab
WB+ RB + MP	14.13d	13.00c	6.55a	1.28a	7.31a	1.55a	117.00bc
LSD (0.05)	**5.35**	**4.95**	**0.68**	**0.33**	**1.14**	**0.30**	**22.76**
CV (%)	**13.25**	**14.80**	**8.64**	**17.87**	**12.37**	**16.48**	**12.24**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.2.3.3 Combined effect of strain and different supplements on growth and yield of shiitake mushroom

4.2.3.3.1. Mycelium growth rate (MGR):

The effect of strains and different supplements was found non significant (Appendix VI). The highest mycelium growth rate (0.51cm) was found in strain Le 8 and rice bran which was statistically similar to Le 11 and rice bran (0.50 cm) treatment combination. The second highest mycelium growth rate (0.43 cm) was found in Le 8 and wheat bran + rice bran (1:1) treatment combination which was statistically similar to Le 11 with rice bran + wheat bran + maize powder (1:1:1) treatment combination. The lowest mycelium growth rate (0.34 cm) was found in Le 8 and wheat bran treatment combination which was statistically similar to the treatment combination of Le 8 with wheat bran + maize powder (1:1) (Table 16).

4.2.3.3.2 Time required to completion of mycelium running (TRMR):

The combined effect of strain and different supplements on time required to completion of mycelium running was highly significant (Appendix VI). The highest time (45 days) required to completion of mycelium running was obtained from the strain Le 11 and wheat bran + rice bran (1:1) treatment combination which was statistically similar to Le 11 and wheat bran + maize powder as well as Le 8 and rice bran + wheat bran (1:1) treatment combination. The seconds highest time (41.75 days) required to completion of mycelium running was obtained from the strain Le 11 and wheat bran + rice bran + maize powder (1:1) treatment combination. The lowest time (33.25 days) required to completion of mycelium running was obtained from the strain Le 8 with 27.27% wheat bran treatment combination (Table 16).

4.2.3.3.3 Time required for bump formation (TRBF):

The combined effect of strains and different supplements showed highly significant in respect of time required for bump formation (Appendix VI). The maximum time (105 days) required for bump formation was recorded from the treatment combination of strain Le 11 with wheat bran and the minimum time (81 days) was found from the strain Le 8 with maize powder treatment combination (Table 16).

4.2.3.3 .4 Time required from opening to first harvest (TROFH):

Time required from opening to first harvest was highly significant by the combined effect of strains and different supplements (Appendix VI). The maximum time required from opening to first harvest (18.50 days) was obtained from the treatment combination strain Le 8 with wheat bran + rice bran (1:1) and the minimum time required from opening to first harvest (5.50 days) was found from the strain Le 11 with maize powder treatment combination (Table 16).

4.2.3.3 .5 Time required for harvest (TRH):

Time required for harvest was non significant by the combined effect of strains and different supplements (Appendix VI). The maximum time (114.30 days) required for

Table 16: Growth of two strains of shiitake mushroom on different supplements

Supplements and their combinations	Mycelium growth rate (cm/day)	Time required for mycelium running (days)	Time required for bump formation (days)	Time required from opening to first harvest (days)	Time required for harvest (days)
Strains of shiitake mushroom (Le 8)					
Wheat bran (WB)	0.34d	33.25e	103.80a	10.50b-e	114.30.a
Rice bran (RB)	0.51a	35.50e	101.80a	10.25b-e	112.00ab
Maize powder (MP)	0.37cd	38.75cd	81.00e	9.00cde	90.50e
WB + RB	0.43b	42.75ab	83.50de	18.50a	102.00c
WB+ MP	0.34d	41.75b	86.00cd	11.50bc	97.50d
WB+ RB +MP	0.41bc	40.75bcd	84.25cde	11.25bcd	95.50d
Strains of shiitake mushroom (Le 11)					
Wheat bran (WB)	0.36cd	34.00e	105.00a	8.50de	113.50a
Rice bran (RB)	0.50a	38.25d	102.30a	8.50de	110.80b
Maize powder (MP)	0.37cd	41.25bc	85.00cd	5.50f	90.50e
WB + RB	0.41bc	45.00a	93.50b	8.25e	101.80c
WB+ MP	0.35d	43.25ab	87.75c	9.50b-e	97.25d
WB+ RB +MP	0.43b	41.75b	85.25cd	12.25b	97.50d
LSD (0.05)	**0.05**	**2.41**	**3.21**	**2.57**	**2.61**
CV (%)	**7.06**	**4.24**	**2.44**	**17.44**	**1.78**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

harvest was obtained from the treatment combination of strain Le 8 with wheat bran and the minimum (90.50 days) was from strain Le 11 with maize powder and strain Le 8 with maize powder treatment combination (Table 16).

4.2.3.3 .6 Number of fruiting body (NFB):

The number of fruiting body was non significant by the combined effect of strains and different supplements (Appendix VI). The maximum number of fruiting body (53.00) was obtained from the treatment combination of strain Le 11 with wheat bran which was statistically similar to Le 8 and wheat bran treatment combination. The minimum number of fruiting body **(**11.00) was found from strain Le 11 with rice bran treatment combination (Table 17).

4.2.3.3 .7 Number of effective fruiting body (NFEB):

The number of effective fruiting body was highly significant by the combined effect of strains and different supplements (Appendix VI). The maximum number (45.50) of effective fruit body was obtained from the treatment combination of strain Le 11 with wheat bran which was statistically similar to Le 8 and wheat bran treatment combination. The minimum number of fruit body **(**9.25) was obtained from strain Le 11 with rice bran treatment combination (Table 17).

4.2.3.3 .8 Length of stalk (LS):

The length of stalk was highly significant by the combined effect of strains and different supplements (Appendix VI). The maximum length (6.73 cm) of stalk was obtained from the treatment combination of strain Le 11 with wheat bran + rice bran + maize powder (1:1:1) which was statistically similar to strain Le 8 with wheat bran + rice bran + maize powder (1:1:1) treatment combination and Le 8 with maize powder respectively. The minimum length (4.15 cm) of stalk was obtained from the treatment combination of strain Le 8 with wheat bran (Table 17).

4.2.3.3 .9 Diameter of stalk (DS):

The diameter of stalk was non significant by the combined effect of strains and different supplements (Appendix VI). The maximum diameter (1.58 cm) of stalk was

obtained from the treatment combination of strain Le 8 with wheat bran + maize powder (1:1) which was statistically similar to strain Le 8 with wheat bran+ rice bran (1:1) treatment combination and Le 11 with rice bran respectively. The minimum diameter (1 cm) of stalk was obtained from the treatment combination of strain Le 11 with wheat bran (Table 17).

4.2.3.3 .10 Diameter of pileus (DP):

The diameter of pileus was non significant by the combined effect of strains and different supplements (Appendix VI). The maximum diameter of pileus (7.98 cm) was obtained from the treatment combination of strain Le 8 with wheat bran+ maize powder (1:1) which was statistically similar to Le 8 with wheat bran + rice bran + maize powder (1:1:1) treatment combination. The minimum diameter (3.85 cm) of pileus was obtained from the treatment combination of strain Le 11 with maize powder (Table 17).

4.2.3.3 .11 Thickness of pileus (TP):

The thickness of pileus was non significant by the combined effect of strains and different supplements (Appendix VI). The maximum thickness (1.65 cm) of pileus was obtained from the treatment combination of strain Le 8 with wheat bran + maize powder (1:1) and strain Le 8 with wheat bran + rice bran + maize powder (1:1:1) treatment combination which was statistically similar to Le 8 and wheat bran + maize powder treatment combination. The minimum thickness (0.83 cm) of pileus was obtained from the treatment combination of strain Le 11 with maize powder (Table 17).

4.2.3.3.12 Yield:

Appreciable variation was found in the yield of two strains of shiitake mushroom on three different types of supplement and their combinations (Appendix VI). The highest yield (159.00 g) was recorded in Le 8 with wheat bran treatment combination which was statistically similar to the treatment combinations of Le 11 supplemented with wheat bran and Le 8 with wheat bran + maize powder (1:10 treatment combination. The lowest yield (98.25 g) was recorded from strain Le 11 with rice bran treatment combination (Table 17).

Table 17: Yield attributes and yields of shiitake mushroom on different supplements

Supplements and their combinations	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of Pileus (cm)	Thickness of pileus (cm)	Yield (g)
Strains of shiitake mushroom (Le 8)							
Wheat bran (WB)	52.25a	42.50a	4.15f	1.35abc	6.00cde	1.35abc	159.00a
Rice bran (RB)	14.00e	11.50e	5.30cd	1.20abc	6.45b-e	1.20cd	108.30ef
Maize powder (MP)	27.50cd	24.75c	6.48ab	1.25abc	5.55be	0.91de	128.50b-e
WB + RB	31.75c	30.00b	5.35cd	1.43ab	7.03abc	1.28bc	143.30abc
WB+ MP	27.75cd	23.75c	5.83bc	1.58a	7.98a	1.58ab	147.30ab
WB+ RB + MP	15.25e	14.00de	6.38ab	1.28abc	7.55ab	1.65a	119.00c-f
Strains of shiitake mushroom (Le 11)							
Wheat bran (WB)	53.00a	45.50a	4.30f	1.00c	5.35e	1.20cd	151.50ab
Rice bran (RB)	11.00e	9.25e	6.02abc	1.45ab	6.93abc	1.15cd	98.25f
Maize powder (MP)	25.00d	23.25c	5.08de	1.15bc	3.85f	0.83e	120.00c-f
WB + RB	38.25b	17.25d	4.43ef	1.18bc	6.60b-e	1.43abc	129.80b-e
WB+ MP	29.25cd	26.00bc	6.03abc	1.25abc	6.75a-d	1.25bc	135.30a-d
WB+ RB + MP	13.00e	12.00e	6.73a	1.28abc	7.08abc	1.45abc	115.00def
LSD (0.05)	**5.35**	**4.95**	**0.68**	**0.33**	**1.14**	**0.30**	**22.76**
CV (%)	**13.25**	**14.80**	**8.64**	**17.87**	**12.37**	**16.48**	**12.24**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.2.3.3.13 Biological efficiency (BE %):

Remarkable variation was observed in biological efficiency of two strains of shiitake mushroom on six different types of supplement (Appendix VI). The highest biological efficiency (90.85%) was obtained from Le 8 with wheat bran treatment combination. The second highest biological efficiency (86.57%) was obtained from Le 11 with wheat bran treatment combination. The lowest biological efficiency (56.10%) was recorded from the strain Le 11 with rice bran treatment combination (Fig. 5).

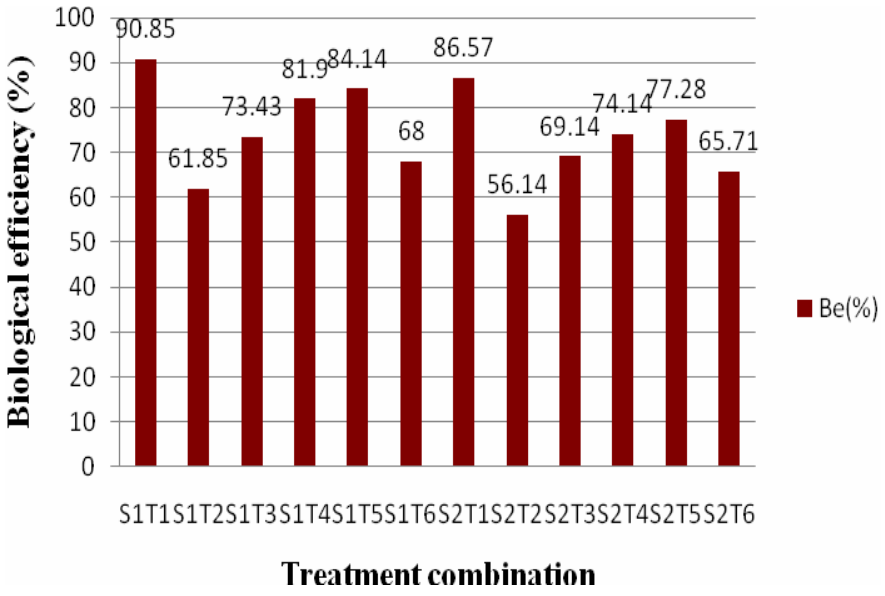


Fig. 5: Combined effect of strain and different supplements on biological efficiency of shiitake mushroom.

S_1 = Le 8, S_2 = Le 11, T_1 = wheat bran, T_2 = Rice bran, T_3 = Maize powder, T_4 = Rice bran + Wheat bran (1:1), T_5 = Wheat bran + Maize powder (1:1), T_6 = Rice bran + Wheat bran + Maize powder (1:1:1).

Results of the present experiment reveal that there are appreciable variations in growth, yield and yield contributing attributes with the variation of supplements and their ratio as well as strains of shiitake mushroom. In terms of yield and yield attributes, performance of the strain Le 8 was better than Le 11. Among the supplements wheat bran can give maximum yield and yield contributing characters of both the strains. Rice bran is not suitable as supplement to cultivate shiitake mushroom. Many other investigators also found variations in effect of different supplements on growth, yield and yield contributing characters of mushroom.

Sawdust was supplemented with wheat bran, rice bran, maize powder and their combinations at different ratio for higher growth and yield of mushroom fruiting bodies. According to Han *et al.* (1981), there is an optimum concentration of different supplements, which can enhance or stimulate mycelial growth of shiitake mushroom. Extreme use of supplements may reduce the effects of substrates on mushroom production. In this study, the highest MGR was observed when sawdust supplemented

with 27.27% rice bran (RB). This result is partially similar with Moonmoon *et al.* (2011) who observed the highest MGR (40 cm/day) was 20% in RB and 15–25% in the composition of WB+RB+MP**.** Kapoor *et al*. (2009) observed the maximum mycelial extension rate with 10% RB and 20% WB additive. Moonmoon *et al.* (2011) also reported that time required to harvest ranged from 104.3 to 147days by supplementation.

Moonmoon *et al*. (2011) who reported that 25% of wheat bran supplement produced a higher number of fruit bodies in comparison to rice bran and maize powder. Rossi *et al*. (2003) reported that any amount of rice bran added to the substrate increased the number of fruit body up to 25% and 30%.

Moonmoon *et al.* (2011) also found more yield on wheat bran (WB) than rice bran (RB). According to Fasidi and Kadiri (1993), the increased productivity of shiitake mushroom supplemented with 30% rice bran can be attributed to the carbohydrates, amino acids and mineral elements present in this supplement. Han *et al.* (1981) reported that shiitake produces higher yield when supplements were added to the substrates at different concentrations. Rossi *et al.* (2003) also obtained higher productivity of *Lentinus edodes* by the addition of 25% and 30% rice bran. Moonmoon *et al.* (2011) who found more biological efficiency on wheat bran than rice bran.

4.3 PERFORMANCE OF DIFFERENT SUBSTRATES ON GROWTH AND YIELD OF SHIITAKE (*Lentinus edodes*) MUSHROOM

4.3.1 INTRODUCTION

Shiitake mushroom, also called black oak mushroom [(*Lentinula edodes* (Berk.) Pegler)] is one of the most widely grown species of mushrooms and a very efficient bio-degrader (Royse, 1985). Substrate is an important input for growing mushroom. Various species of trees have been used for the cultivation of shiitake mushroom. One of the primary species used in one area of Japan in recent past years was the shii tree thus the derivation of the name shiitake. The mushroom may also grow on sawdust of various species of oak (*Quercus* spp.) trees (Harris, 1986; Przybylowicz and Donoghue, 1988). In India, shiitake mushroom is commonly grown on sawdust, wheat straw and rice or wheat bran (Thakur and Sharma, 1992; Shukla, 1995; Sharma *et al.*, 2006). For mushroom spawn production, generally sterilized grains, sawdust and wood chips are used (Stamets, 2005). Spawn production using sawdust is a very popular and economical method.

Mushrooms depend on substrates for nutrition and the substrate is normally a source of lignocelluloses material which supports growth, development and fruiting of mushroom (Chang and Miles, 2004). Sawdust is the most popular basal ingredient used in substrates to grow shiitake (Miller and Jong, 1987; Grodzinskaya *et al*., 2003). Other basal ingredients can include straw and corn cobs, or their mixes. Yield of mushroom might vary due to use of heterogeneous mixes of sawdust. It is a kind of medium, which supports good growth of mushroom mycelium but does not encourage growth of competitor. Mushroom substrate may be defined as a kind of lignocellulose material which supports the growth, development and fruiting of mushroom (Chang and Miles, 1988). Large quantity of freely available sawdust of different trees offers a potential substrate source for mushroom cultivation in the tropics.

Mushroom cultivation has a special relevance to Bangladesh, because sawdust and other materials are available to the farmers. The quality and quantity of the spawn used in the cultivation of mushrooms directly influence the quality and yield. Shiitake strains vary widely, particularly in fruiting temperature and mycelial maturation (early

or late; shorter or longer production time). Substrate selectivity, growth rate (some fast strains may produce pre-mature fruiting), quality (shape, size, thickness, color, flavor and aroma etc.), yield and ecological adaptability to extreme temperature (usually cold tolerance) are also strain-related. The present experiment was undertaken to evaluate influence of locally available substrates containing sawdust of different trees and agricultural waste on growth and yield of shiitake mushroom.

4.3.2 MATERIALS AND METHODS

Eight substrates were prepared. Sawdust of teak chambul (*Michelia campaca*), ipil-ipil (*Leucaena leucocephala*), teak (*Tectona grandis*), gamari (*Gmelina arborea*), rain tree (*Albizia saman*), mahagony (*Swietenia mahagoni*), mango (*Mangifera indica*) trees and mixed sawdust were tested in the study as basic materials. The sawdust was supplemented with wheat bran, rice husk and $CaCO_3$ @ 27.27, 4.56 and 0.2% (w/w). Two additional substrates containing mixed wood chips and rice straw were included in the experiment without any supplement. So, there were ten treatments in the experiment. The rice straw were chopped into 4-5 cm long and soaked in warm water at 60^0C for one hour. After soaking excess water was drained off and the straw was air dried to fix the moisture content at 65%. To estimate the moisture content, air dried straw pieces were pressed between palms. If no water drop was released from the straw the moisture content was considered to be appropriate. Woodchips were cut in small pieces (4-5 cm) and soaked in water overnight. Then drained out the water, was used without any supplementation. The experiment was carried out at a mushroom culture house of the National Mushroom Development and Extension Centre, Savar, Dhaka, during the period from October 2011 to March 2012. Two strains of shiitake mushroom (*Lentinus edodes*), namely Le 8 and Le 12 were used as test materials.

4.3.2.1 Preparation of pure and mother culture:

Pure cultures of two strains were grown on potato dextrose agar (PDA) following hyphal tip method described in chapter III**.** Procedure for preparation of mother culture also described in chapter III**.**

4.3.2.2 Preparation of spawn packets:

The spawn packets were prepared separately using each type of substrate according individual treatment. The substrate mixture was poured into 18 cm × 25 cm polypropylene bags at 500 g/bag. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the bag was made for space to introduce the inocula. The neck of each poly bags was plugged with cotton, covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for 2 h at 121^0C under 1.1 kg/cm^2 pressures. After sterilization, the packets were cooled and transferred to an inoculation chamber. The packets were inoculated separately with the mother culture of the two strains at the rate of two tea spoonful per packet. The inoculated packets were incubated at 22 ± 2^0C.

4.3.2.3 Mycelial colonization and bump formation:

Same as described in chapter III.

4.3.2.4 Cultivation for fruiting body:

Same as described in chapter III.

4.3.2.5 Collection and analysis of data:

The packets were arranged in culture house following completely randomized design with 4 replications. Data on mycelium growth rate, time required to completion of mycelium running, time required for bump formation, time required from opening to first harvest, time required for harvest, number of fruiting body, number of effective fruiting body, length and diameter of stalk, diameter and thickness of pileus, yield (g/packet) and biological efficiency were recorded. Weight of fruiting body was recorded after removing the lower hard and dirty portion of stipe.

The biological efficiency was determined using the following formula:

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

Data were analyzed using MSTAT-C computer program. Means were compared following Duncan's multiple range test using the same computer program.

4.3.3 RESULTS AND DISCUSSION

4.3.3.1 Main effect of strain on growth and yield of shiitake mushroom

4.3.3.1.1 Mycelium growth rate (MGR):

Mycelium growth rate was found highly significant by two strains of shiitake (Appendix VII). The highest mycelium growth rate (2.53 cm/day) was recorded in Le 8. The lowest mycelium growth rate (2.04 cm/day) was recorded in Le 12 (Table 18).

4.3.3.1.2 Time required to completion of mycelium running (TRMR):

Time required to completion of mycelium running was significantly influenced by two strains of shiitake mushroom (Appendix VII). The lowest time required to completion of mycelium running (47.55 days) was found in Le 8. The highest time required to completion of mycelium running (58.05 days) was recorded in Le 12 (Table 18).

4.3.3.1.3 Time required for bump formation (TRBF):

Time required for bump formation was not significantly influenced by two strains of shiitake mushroom (Appendix VII). The time required for bump formation (103.92 days) was recorded in Le 12. The time required for bump formation (103.71 days) was found in Le 8 (Table 18).

4.3.3.1.4 Time required from opening to first harvest (TROFH):

Time required from opening to first harvest (days) was observed highly significant by two strains of shiitake mushroom (Appendix VII). The highest time (9.29 days) required from opening to first harvest was recorded in Le 8. The lowest time (8.20 days) required from opening to first harvest was found in Le 12 (Table 18).

4.3.3.1.5 Time required for harvest (TRH):

The response of strain on time required for harvest was not significant (Appendix VII). The time required for harvest was recorded in Le 8 (113.04 days) and in Le 12 was (112.33 days) (Table 18).

4.3.3.1.6 Length of stalk (LS):

The length of stalk was significantly influenced by two strains of shiitake mushroom

(Appendix VII). The highest length of stalk (4.68 cm) was found in Le 12. The lowest length of stalk (4.33 cm) was recorded in Le 8 (Table 19).

4.3.3.1.7 Diameter of stalk (DS):

The diameter of stalk was significantly influenced by two strains of shiitake mushroom (Appendix VII). The highest diameter of stalk (1.45 cm) was observed in Le 12 and the lowest diameter of stalk (1.40 cm) was observed in Le 8 (Table 19).

4.3.3.1.8 Diameter of pileus (DP):

The diameter of pileus was found to be highly significant influenced by two strains of shiitake mushroom (Appendix VII). The highest diameter of pileus (6.59 cm) was recorded in Le 12 and the lowest diameter of pileus (5.67 cm) was observed in Le 8 (Table 19).

4.3.3.1.9 Thickness of pileus (TP):

The thickness of pileus was significantly influenced by two strains of shiitake mushroom (Appendix VII). The highest thickness of pileus (1.44 cm) was recorded in Le 12 and the lowest thickness of pileus (1.31 cm) was recorded in Le 8 (Table 19).

4.3.3.1.10 Number of fruiting body (NFB):

The strain showed significant variation in case of number of fruiting body by two strains of shiitake mushroom (Appendix VII). The highest number (28.54) of fruiting body was recorded in Le 8 and the lowest number (11.29) of fruiting body was recorded in Le 12 (Table 20).

4.3.3.1.11 Number of effective fruiting body (NEFB):

Two strains of shiitake mushroom showed significant variation in case of number of effective fruiting body (Appendix VII). The highest number of effective fruiting body (17.67) was recorded in Le 8. The lowest number of effective fruiting body (5.13) was recorded in Le 12 (Table 20).

4.3.3.1.112 Yield:

The yield was found to be highly significant between the strains of shiitake mushroom (Appendix VII). The highest yield (112.58 g) was recorded in Le 8 and the lowest yield (65.50 g) was recorded in Le 12 (Fig. 6).

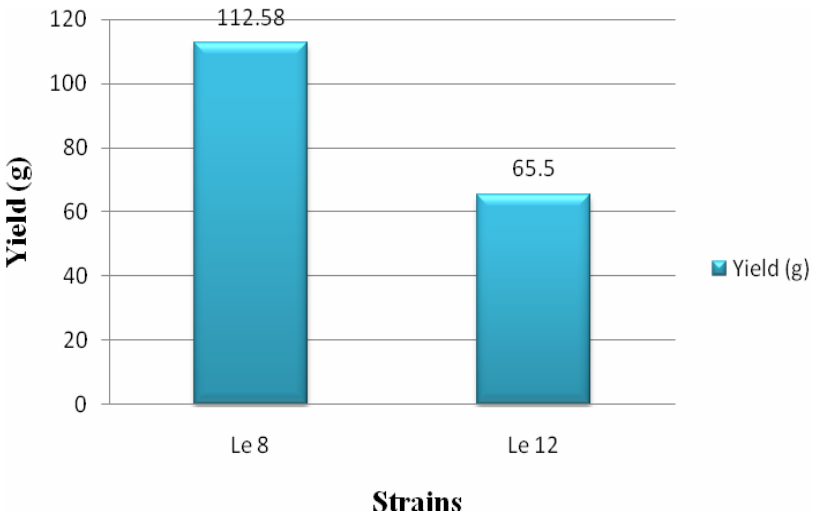


Fig. 6: Yield of two strains of shiitake mushroom.

Plate 7: Yield performance of two strains of shiitake mushroom

4.3.3.1.13 Biological efficiency (BE %):

Remarkable variation was observed in biological efficiency of two strains of shiitake mushroom (Appendix VII). The highest biological efficiency (64.33 %) was obtained from Le 8. The lowest biological efficiency (37.43%) was recorded in Le 12 (Table 20).

4.3.3.2 Effect of different substrates on growth and yield of shiitake mushroom

4.3.3.2.1 Mycelium growth rate (MGR):

Significant variation in mycelium growth rate was observed in different substrate (Appendix VII). The highest mycelium growth rate (3.23 mm/day) was recorded in mango sawdust. The lowest mycelium growth rate (1.15 mm/day) was recorded in wood chips (Table18).

4.3.3.2.2 Time required to completion of mycelium running (TRMR):

Time required to completion of mycelium running was significantly influenced by different substrate (Appendix VII). The time required to completion of mycelium running in spawn ranged from 36.25 to 94.25 (days). The lowest time required to completion of mycelium running (36.25 days) was found in gamari sawdust. The highest time required to completion of mycelium running (94.25 days) was recorded in wood chips (Table 18).

4.3.3.2.3 Time required for bump formation (TRBF):

Time required for bump formation was significantly influenced by different substrate (Appendix VII). The highest time required for bump formation (120.00 days) was recorded in teak chambul and the lowest time required for bump formation (93.60 days) was found in gamari sawdust (Table 18).

4.3.3.2.4 Time required from opening to first harvest (TROFH):

Time required from opening to first harvest was found to be highly significant by the influenced of different substrates (Appendix VII). The highest time required from opening to first harvest (13.50 days) was recorded in teak chambul. The lowest time required from opening to first harvest (6.63 days) was found in mixed saw dust (Table 18).

4.3.3.2.5 Time required for harvest (TRH):

Time required for harvest was significantly influenced by different type of substrates (Appendix VII). The highest time (133.50 days) required for harvest was recorded in teak chambul. The lowest time required for harvest (100.88 days) was found in gamari sawdust (Table 18).

4.3.3.2.6 Length of stalk (LS):

The length of stalk was significantly influenced (Appendix VII) by different strains of *Lentinus edodes* when cultured on different types of substrate and ranged from 3.75 to 5.54 cm. The highest length of stalk (5.54 cm) was found in teak chambul which was statistically similar to ipil-ipil sawdust. The lowest length of stalk (3.75 cm) was recorded in mango sawdust which was statistically similar to gamari and mixed sawdust (Table 19).

4.3.3.2.7 Diameter of stalk (DS):

The diameter of stalk was significantly influenced (Appendix VII) by different strains of *Lentinus edodes* when cultured on different types of substrate and ranged 1.20 to 1.59 cm respectively. In case of diameter of stalk, the highest diameter of stalk (1.59 cm) was observed in rain tree which was statistically similar to teak chambul and mango sawdust. The lowest diameter of stalk (1.20 cm) was observed in mixed sawdust (Table 19).

4.3.3.2.8 Diameter of pileus (DP):

The diameter of pileus influenced by different type of substrates was highly significant (Appendix VII). The highest diameter of pileus (7.06 cm) was recorded in mango sawdust which was statistically similar to rain tree and mixed sawdust. The lowest diameter of pileus (4.81 cm) was observed in the treatments of gamari sawdust (Table 19).

4.3.3.2.9 Thickness of pileus (TP):

The thickness of pileus influenced by different type of substrates was highly significant (Appendix VII). The highest thickness of pileus (1.64 cm) was recorded in rain tree sawdust which was statistically similar to mango sawdust. The lowest

thickness of pileus (1.16 cm) was recorded in mixed sawdust which was statistically similar to gamari sawdust (Table 19).

4.3.3.2.10 Number of fruiting body (NFB):

Substrate showed significant variation in case of number of fruiting body (Appendix VII). The number of fruiting bodies ranged from 12.25 to 29.27. The highest number of fruiting bodies (29.27) was recorded in mixed sawdust. The lowest number of fruiting bodies (12.25) was recorded in teak chambul (Table 20).

4.3.3.2.11 Number of effective fruiting body (NEFB):

Significant variations were observed in number of effective fruiting body by different substrates (Appendix VII). The highest number (20.88) of effective fruiting bodies was recorded from mixed sawdust. The lowest number (5.00) of effective fruiting body was recorded in rain tree sawdust (Table 20).

4.3.3.2.12 Yield:

Appreciable variation was found in the yield of two strains of shiitake mushroom on six different types of substrate (Appendix VII). The highest yield (128.63g) was recorded in mixed sawdust which was significantly higher than all the treatments. The lowest yield (48.88 g) was recorded in teak chambul (Fig. 7).

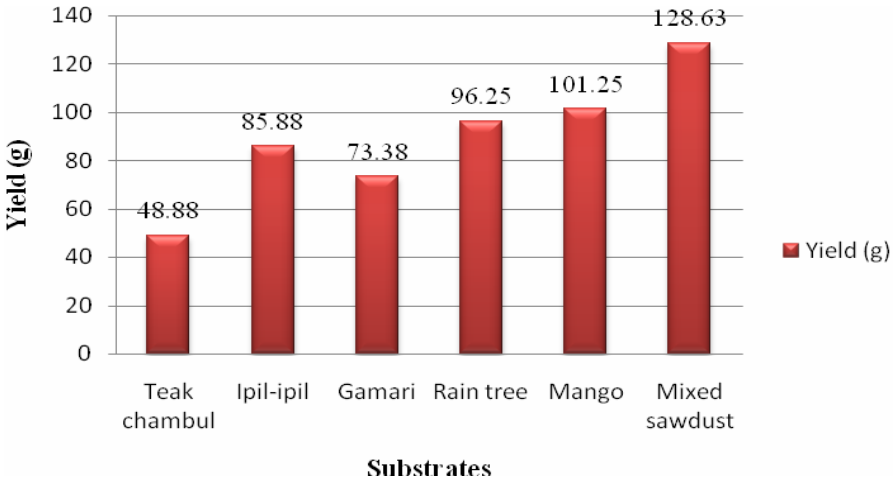


Fig. 7: Yield performance of shiitake mushroom on six different types of substrate

4.3.3.2.13 Biological efficiency (BE %):

Remarkable variation was observed in biological efficiency of two strains of shiitake mushroom on six different types of substrate (Appendix VII). The highest biological efficiency (73.50%) was obtained from the treatment of mixed sawdust and the lowest biological efficiency (27.93%) was recorded in teak chambul (Table 20).

Table18: Effect of strain and different substrates on growth of shiitake mushroom

Strain	Mycelium growth rate (mm/day)	Time required to completion of mycelium running (days)	Time required for bump formation (days)	Time required from opening to first harvest (days)	Time required for harvest (days)
Le 8	2.53	47.55	103.71	9.29	113.04
Le 12	2.04	58.05	103.92	8.20	112.33
Substrates					
Teak chambul	1.96g	54.13c	120.00a	13.50a	133.50a
Ipil-ipil	2.18f	48.25de	102.50c	6.88d	110.13c
Segun	2.46de	43.00ef	------	---	------
Gamari	2.94b	36.25g	93.60d	7.00cd	100.88e
Rain tree	2.69c	39.88fg	95.38d	10.00b	105.38d
Mahagony	2.33ef	45.75de	------	-----	------
Mango	3.23a	39.13fg	106.63b	8.50bc	115.13b
Mixed sawdust	2.54cd	50.88cd	104.50c	6.63d	111.13c
Wood chips	1.15i	94.25a	------	---	---
Rice straw	1.40h	76.50b	------	----	----
LSD (0.05)	**0.02**	**2.48**	**2.02**	**1.52**	**2.07**
CV (%)	**6.10**	**6.76**	**1.36**	**12.12**	**1.28**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

----- = Not formed bump and fruit body.

Table 19: Effect of strain and different substrates on size of fruit body of shiitake mushroom

Strain	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Le 8	4.33	1.40	5.67	1.31
Le 12	4.68	1.45	6.59	1.44
Substrates				
Teak chambul	5.54a	1.49a	5.73c	1.32cd
Ipil-ipil	5.18a	1.39ab	6.15bc	1.43bc
Gamari	4.11c	1.35ab	4.81d	1.18d
Rain tree	4.63b	1.59a	6.59ab	1.64a
Mango	3.75c	1.53a	7.06a	1.54ab
Mixed sawdust	3.81c	1.20b	6.44ab	1.16d
LSD (0.05)	**0.44**	**0.25**	**0.62**	**0.16**
CV (%)	**6.83**	**12.27**	**7.05**	**8.14**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Table 20: Effect of strain and different substrates on yield attributes of shiitake mushroom

Strain	Number of fruiting body	Number of effective fruiting body	Biological efficiency (%)
Le 8	28.54	17.67	64.33
Le 12	11.29	5.13	37.43
Substrates			
Teak chambul	12.25e	8.13d	27.93e
Ipil-ipil	15.13d	6.25de	49.07c
Gamari	15.75d	11.25c	41.93d
Rain tree	21.25c	5.00e	55.00b
Mango	25.75b	16.88b	57.86b
Mixed sawdust	29.37a	20.88a	73.50a
LSD(0.05)	**2.43**	**2.07**	**4.38**
CV (%)	**8.49**	**12.64**	**6.00**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.3.3.3 Combined effect of strain and different types of substrate on growth and yield of shiitake mushroom

4.3.3.3.1 Mycelial growth rate (MGR):

Remarkable differences were observed in mycelium growth rate by the combined effect of spawn packets of different substrates and strains used (Appendix VII). It ranged from 1.0 to 4.50 mm/day. Among different substrates, Le 8 showed the highest growth rate (4.50 mm/day) on mango sawdust followed by mixed sawdust. Least growth (1.0 mm/day) was observed from the treatment combination Le 12 and woodchips. Both the strains gave the lowest mycelium growth rate as recorded on woodchips and rice straw (Table 21).

4.3.3.3.2 Time required to completion of mycelium running (TRMR):

Time required to completion of mycelium running was highly significant by the influenced of different substrate and two stains of shiitake mushroom (Appendix VII). The maximum time required (106.50 days) for completion of mycelium running was recorded from the treatment combination of substrate woodchips and strain Le 12 followed by rice straw. The minimum time required (23.75 days) for completion of mycelium running was on mango sawdust and Le 8 followed by mixed sawdust (Table 21).

4.3.3.3.3 Time required for bump formation (TRBF):

Two strains and different substrates showed highly significant in respect of time required for bump formation (Appendix VII). The maximum time (120.80 days) required for bump formation from the combination of strain Le 8 with teak chambul which was statistically similar to Le 12 and teak chambul followed by Le and mango saw dust. The minimum time (90.75 days) was found from strain Le 8 with rain tree treatment combination (Table 21).

4.3.3.3.4 Time required from opening to first harvest (TROFH):

Highly significant variation was found in time required from opening to first harvest of two strains on ten different substrates (Appendix VII). The duration from opening of spawn packet to first harvest on different substrates ranged from 2.50 to 15.50

days. The maximum time (15.50 days) required from opening to first harvest was recorded from the treatment combination Le 8 and teak chambul followed by ipil-ipil which was statistically similar to Le 12 and teak chambul. The minimum days (2.50 days) required from opening to first harvest was recorded from the treatment combination of strain Le 12 and ipil-ipil (Table 21).

4.3.3.3.5 Time required for harvest (TRH): The combined effect of two strains with ten different substrates on time required for harvest was highly significant (Appendix VII). Rice straw, woodchips, segun, mahagony did not produce any primodia and

Table 21: Effect of different substrates containing sawdust and agricultural waste on growth of two strains of shiitake mushroom

Substrates	Mycelium growth rate (mm/day)	Time required to completion of mycelium running (days)	Time required for bump formation (days)	Time required from opening to first harvest (days)	Time required for harvest (days)
Strain (Le 8)					
Teak Chambul	1.95j	54.50d	120.80a	15.50a	136.30a
Ipil-ipil	2.15ij	49.00ef	103.80de	11.25b	115.30c
Segun	2.58ef	41.00hi	---	---	---
Gamari	2.68de	39.50hi	93.00h	6.00fg	99.00h
Rain tree	2.85d	37.50ij	90.75i	11.00b	101.80g
Mahagony	2.23hi	47.75fg	---	----	---
Mango	4.50a	23.75l	108.00b	7.00ef	115.00c
Mixed sawdust	3.60b	29.50k	106.00bc	5.00g	111.00de
Wood chips	1.30k	82.00b	---	---	---
Rice straw	1.50k	71.00c	---	---	---
Strain (Le 12)					
Teak Chambul	1.98j	53.75de	119.30a	11.50b	130.80b
Ipil-ipil	2.23hi	47.50fg	101.30fg	2.50h	105.00f
Segun	2.35ghi	45.00fgh	---	---	---
Gamari	3.20c	33.00jk	94.75h	8.00de	102.80g
Rain tree	2.53efg	42.25ghi	100.00g	9.00cd	109.00e
Mahagony	2.43fgh	43.75fgh	---	--	---
Mango	1.95j	54.50d	105.30cd	10.00bc	115.30c
Mixed sawdust	1.48k	72.25c	103.00ef	8.25de	111.30d
Woodchips	1.00l	106.50a	---	---	---
Rice straw	1.30k	82.00b	---	---	---
LSD (0.05)	**0.02**	**2.48**	**2.02**	**1.52**	**2.07**
CV (%)	**6.10**	**6.76**	**1.36**	**12.12**	**1.28**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

there was no data for yield. Substrate affected the duration from spawning to harvesting of two strains. The shortest time required (99 days) to harvesting of fruit body was on the Le 8 and gamari substrate followed by rain tree. The longest time required (136.30 days) to harvesting was on Le 8 and teak chambul followed by Le 12 and teak chambul (Table 21).

4.3.3.3.6 Length of stalk (LS):

The combined effect of different substrate with different strain of shiitake mushroom on length of stalk was statistically significant (Appendix VII). Strain Le 12 produced the longest stalk (5.58 cm) on teak chambul sawdust followed by ipil-ipli, rain tree and gamari sawdust. The shortest stalk (3.03 cm) was found from the strain Le 8 with mixed sawdust which was statistically similar to Le 8 with mango sawdust (Table 22).

4.3.3.3.7 Diameter of stalk (DS):

The combined effect of different substrate with two strains of shiitake mushroom on diameter of stalk was statistically significant (Appendix VII). The maximum diameter of stalk (1.60 cm) was found the treatment combination Le 8 and sawdust of rain tree which was statistically similar to Le 12 with mango, teak chambul and rain tree sawdust. The lowest diameter **(**1.10 cm) of stalk was recorded from strain Le 8 with mixed sawdust (Table 22).

4.3.3.3.8 Diameter of pileus (DP):

Diameter of pileus was highly significant by the combined effect of strain with six different types of substrates (Appendix VII). The maximum diameter of pileus (8.45 cm) was obtained when Le 12 culture on mango sawdust and followed by mixed sawdust.The lowest diameter of pileus (4.53 cm) was found from the treatment combination Le 12 and gamari (Table 22).

4.3.3.3.9 Thickness of pileus (TP):

The thickness of pileus was also highly significant by the combined effect of strain with different type of substrates (Appendix VII). The thickest pileus (1.70 cm) was produced when Le 12 culture on the mango sawdust which was statistically similar to Le 12 with rain tree. The thinnest pileus (1.03 cm) was obtained from the treatment

combination Le 8 and mixed sawdust. The thickness of pileus on the substrates of chambul, ipil-ipil, mango, gamari and Le 8 as well as Le 12 and chambul, mixed sawdust did not vary significantly (Table 22).

Table 22: Effect of different substrates containing sawdust and agricultural waste on size of stalk and pileus of two strains of shiitake mushroom

Substrates	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Strain (Le 8)				
Teak Chambul	5.50a	1.45abc	5.58fg	1.25d
Ipil-ipil	5.00bc	1.30bcd	5.80ef	1.35cd
Gamari	4.25de	1.48abc	5.10gh	1.28d
Rain tree	4.95bc	1.60a	6.30cde	1.60ab
Mango	3.23f	1.45abc	5.68efg	1.38cd
Mixed sawdust	3.03f	1.10d	5.58fg	1.03e
Strains (Le 12)				
Teak Chambul	5.58a	1.53ab	5.88def	1.39cd
Ipil-ipil	5.35ab	1.48abc	6.50cd	1.50bc
Gamari	3.98e	1.23cd	4.53h	1.08e
Rain tree	4.30de	1.58ab	6.88bc	1.68a
Mango	4.28de	1.60a	8.45a	1.70a
Mixed sawdust	4.60cd	1.30bcd	7.30b	1.30d
LSD (0.05)	**0.44**	**0.25**	**0.62**	**0.16**
CV (%)	**6.83**	**12.27**	**7.05**	**8.14**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.3.3.3.10 Number of fruiting body (NFB):

The combined effect of strain with different type of substrates on number of fruiting body was statistically highly significant (Appendix VII). The highest number (44.50) of fruit body was obtained from the treatment combination Le 8 and mango sawdust which was statistically similar to mixed sawdust and followed by rain tree. The lowest number (7.00) of fruit body was obtained from Le 12 and mango sawdust. Rice straw, woodchips, segun, mahagony did not produce any fruit body (Table 23).

4.3.3.3.11 Number of effective fruiting body (NFEB):

The number of effective fruiting body varied highly significant on different substrates (Appendix VII). Number of well-developed fruiting body was recorded and presented in Table 23. Dry, pin headed, twisted fruiting body was discarded but tiny fruiting body was included during counting. Substrate affected number of effective fruiting bodies formed. The highest number (33.75) of effective fruiting bodies was obtained from the treatment combination Le 8 and mixed sawdust followed by mango substrate. It may be mixed sawdust usually mixture of mango, jackfruit, rain tree etc. in the local sawmill. The lowest (2.75) was from Le 12 and rain tree which was statistically similar to Ipil- ipil and mango.

4.3.3.3.12 Yield:

Highly Significant variation was found in yield of shiitake mushroom grown on different substrates (Appendix VII). The maximum yield (189.80 g) was recorded from the treatment combination mixed sawdust and Le 8 followed by mango sawdust because number of effective fruit body highest in this treatment. The lowest (38.75g) yield was observed in Le 12 and teak chambul sawdust (Table 23).

4.3.3.3.13 Biological efficiency (%):

The combined effect of strain with different type of substrates on biological efficiency was statistically highly significant (Appendix VII). The highest biological efficiency (108.40%) was obtained from Le 8 and mixed sawdust followed by mango sawdust. The lowest biological efficiency (22.14%) was recorded in Le 12 and teak chambul sawdust (Table 23).

Table 23: Effect of different substrates containing sawdust and agricultural waste on yield, yield attributes and biological efficiency of two strains of shiitake mushroom

Substrates	Number of fruiting body	Number of effective fruiting body	Yield (g)	Biological efficiency (%)
Strain (Le 8)				
Teak Chambul	13.25de	8.75d	59.00g	33.71g
Ipil-ipil	18.75c	9.50d	81.75e	46.72e
Gamari	21.00c	17.00c	88.75de	50.72de
Rain tree	30.00b	7.25de	120.80c	69.00c
Mango	44.50a	29.75b	135.50b	77.43b
Mixed sawdust	43.75a	33.75a	189.80a	108.40a
Strain (Le 12)				
Teak Chambul	11.25ef	7.50de	38.75h	22.14h
Ipil-ipil	11.50ef	3.00g	90.00d	51.43d
Gamari	10.50f	5.50ef	58.00g	33.14g
Rain tree	12.50def	2.75g	71.75f	41.00f
Mango	7.0g	4.00fg	67.00f	38.28f
Mixed sawdust	15.00d	8.00d	67.50f	38.57f
LSD (0.05)	**2.43**	**2.07**	**7.66**	**4.38**
CV (%)	**8.49**	**12.64**	**6.00**	**6.00**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Results of the present experiment reveal that there are appreciable variations in growth, yield and yield contributing attributes with the variation of substrates and strains of shiitake mushroom. In terms of yield and yield attributes, performance of the strain Le 8 was better than Le 12. Among the substrates mango sawdust and mixed sawdust can give maximum yield and yield contributing characters of both the strains. The presence of the right proportion of alpha-cellulose, hemi-cellulose, pectin and lignin was the probable cause of higher rate of mycelium running in mango sawdust. Rice straw and wood chips are not suitable as substrates to cultivate shiitake

mushroom. Many other investigators also found variations in effect of different substrates on growth, yield and yield contributing characters of mushroom.

Ashrafuzzaman *et al*. (2009) reported the highest mycelial growth on jackfruit sawdust followed by mango sawdust and least growth on rice straw. He found the maximum pileus diameter (7.11 cm) on mango sawdust. The maximum number of fruit body was obtained by him from the jackfruit sawdust substrate. He did not find any primordia and fruiting bodies on paddy straw substrate. Ashrafuzzaman (2009) also recorded lowest number of fruiting bodies from mango, shimul sawdust and mixture of all sawdust.

It is likely that mango sawdust has a high C: N ratio which resulted in the enhanced growth of the pileus (Veena *et al*., 1998). Pegler (2003) recorded the diameter of pileus 5-15 cm as well as Gaitan and Mata (2004) recorded the pileus diameter ranging from 5 to 20 cm. Suitable C: N ratio might be responsible for the higher mycelial growth. The capacity of mushroom to grow on ligno-cellulosic substrates is related to the vigor of its mycelium (Permana *et al*., 2004). Variation in DMR on different substrates might be due to variations in the chemical composition and C: N ratio of substrates as reported by Bhatti *et al*. (1987).

Kitamoto *et al*. (1975) reported that substrates containing glucose, fructose and trehalose produced the highest number of primordia while those containing glycerol, xylose, sucrose and fructose produced abnormal fruiting bodies. The best fruiting body production was found on glucose and fructose containing substrates.

Chaudhary *et al.* (1985) explained the process of break-down of lignin. There is an apparent correlation between the ability to degrade lignin and the production of phenolases, which oxidize phenolic compounds to simple aromatic compounds that can be absorbed by mushroom mycelium and is used for its growth. The product of cellulolytic action in simple and soluble carbohydrates and the end products being glucose was absorbed by the fungal mycelium for growth and energy. Therefore, cellulose rich organic substrates are good for the cultivation of mushroom (Gerrits and Muller, 1965; Quimio, 1987). High cellulose content in wood results in enhanced cellulose enzyme production and increased yield of mushroom (Ramasamy and

Kandaswamy, 1976). *Lentinus edodes* is white rot fungus that produces a set of lignocellulolytic enzymes, which allow it to grow on lignocellulosic substances rich in lignin (Leatham, 1986). The substrates with high lignin and phenolic content decreased the activity of the enzyme, hence slow growth and low yield.

Based on findings of the study it may be concluded performance of Le 8 was better than Le 12 in terms of growth rated, mushroom yield and biological efficiency of substrate. Le 8 gave highest yield on mango sawdust followed by mixed sawdust. Considering yield and yield attributes, strain Le 8 and substrate prepared from mango sawdust and mixed sawdust may be recommended to grow shiitake mushroom in Bangladesh.

4.4 EFFECT OF OPENING PATTERN AND PLACEMENT OF SPAWN PACKET ON BUMP INITIATION AND YIELD OF SHIITAKE (*Lentinus edodes*) MUSHROOM

4.4.1 INTRODUCTION

Shiitake (*Lentinus edodes)* is an edible mushroom commonly used as food in Asian countries, and also a traditional Chinese medicine (Lin *et al*., 2008) which is cultivated on a large scale in many countries (Poppe and Hofte, 1995; Chang and Miles, 2004). It can be produced commercially in Latin America for the world market. Its cultivation in Latin America started during the early 1980's, and several attempts for its commercial cultivation have been carried out in Guatemala, Colombia, Mexico, Argentina and Brazil (Martínez-Carrera, 2002).

It is the second most popular edible mushroom (Chang, 1999 and Chiu *et al*., 1999). The production system of this mushroom is quite different from other edible mushrooms. Many strains of shiitake mushroom are available in the world which is extensively cultivated. The strains of this valuable mushroom vary widely, particularly in the time required for mycelium colonization, bump formation and fruiting body development. The mycelium growth in the vegetative phase involves producing quality fruiting bodies in the reproductive phase. A spawn run of different strains is of ultimate importance for adjusting the reproductive phase. To shift the mycelium growth stage to reproductive stage for the formation of bump as well as fruiting body generally some kinds of stimuli are needed. These stimuli can be initiated by some management practices like opening and placement of spawn packets during incubation. Opening is a process to remove cotton plug or total polypropylene bag of the sawdust bags with a sharp blade. On the other hand placement means the spawn packets put in different locations like rack, floor etc. These process stimulates the formation of blister like bumps. Among these conditions, opening of spawn packet is the most important aspect for early bump initiation and fructification. For bump initiation stage low temperature and high CO_2 concentration is required. Time of bump formation varies with strains, substrate and temperature. Usually bumps form 10 days faster at 25^0C than at 15^0C (Miles and Chang, 1989). Fluctuation of temperature and high CO_2 concentration encourage bump formation. Lower the CO_2

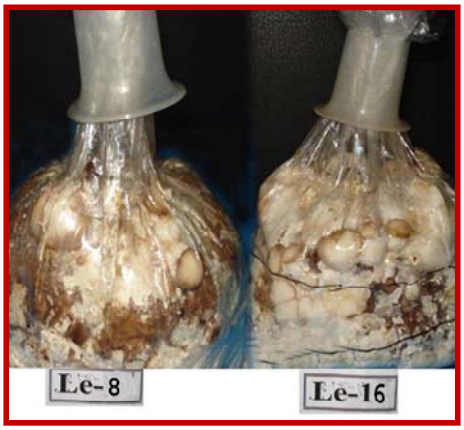
in the bag, when bumps become too numerous by cutting slits on the bag. In any case, some aeration should be provided when bumps are formed. In many countries different opening system has been followed for mushroom production. After completion of the spawn run in the substrate, the polythene or gunny bags are cut open and the coverings are removed from the trays (Chadha & Sharma, 1998). If the bag opening is too early or too late, the crop may be failed. It is reported that a time period of 60 to 90 days is necessary for incubation of spawn packet (Kawai *et al.*, 1997 and Iizuka, 1997). The production of shiitake mushroom varies depending on the opening pattern of spawn packet. Many growers produce shiitake mushroom with the opening of the bag partially or fully. Fan *et al.* (2005) suggested opening the bag at the places where primordia have formed. It will give higher yield of quality mushroom; but it time consuming and laborious task. However, Ramkumar *et al.* (2010) suggested cutting the top portion of polypropylene bag after browning of shiitake packet.

Many studies have been carried out in the world to improve the quality and increase the production of *Lentinus edodes.* But, the production of this mushroom is fairly new in Bangladesh. Considering the above facts the present study was under taken to determine the suitable opening pattern and placement of spawn packet on early bump initiation and yield of shiitake mushroom.

4.4.2 MATERIALS AND METHODS

The experiment was conducted at the tissue culture laboratory and culture house of National Mushroom Development and Extension Center, Savar, Dhaka during the period from September 2012 to February 2013. Eleven different types of opening pattern such as top open place on floor (T_1), top open place on rack (T_2), total open and covered with polypropylene bag place on floor (T_3), Total open and covered with polypropylene bag place on rack (T_4), Only cotton plug open and place on floor (T_5), No open and place on floor (T_6), Only cotton plug open and place on rack (T_7), No open and place on rack (control) (T_8), Total open and place on rack (T_9), Total open and place on floor (T_{10}), No open and place on culture house floor (T_{11}). These opening were done in incubation period for early bump initiation. Two strains of

shiitake mushroom (*Lentinus edodes*), namely Le 8 and Le 16 were used as test materials.



T_1

T_2

T_3

T_4

T_5

T_6

T_7

T_8 **(Control)**

T_9

T_{10}

T_{11}

T_{11}

Plate 8: Different types of opening pattern (T_1-T_{11}) during incubation for early bump formation.

4.4.2.1 Mycelial colonization and bump formation:

During incubation period, whitish mycelium started to grow in the inoculated substrate. Both the strains showed optimal mycelial growth at 22 ± 2^0C and 60-70% relative humidity under controlled condition. After full colonization of the spawn

packets, a thick mycelial coat formed on the outer surface of colonized substrate. Clumps of mycelia appeared as blister like bumps of various sizes on the surface of the mycelial coat in each packet. Bumping usually started when color of the colonized white mycelia became brown.

4.4.2.2 Opening of spawn packets:

After completion of mycelium running spawn packets were opened and placed according to the treatments to determine the right opening pattern for early bump initiation.

4.4.2.3 Cultivation for fruiting body:

After bump formation, all the packets were fully opened, and placed separately on the rack in the culture house. Temperature, relative humidity and light intensity of the culture house were maintained at 18-22^{0}C, 60-70% and 10-20 lux, respectively. Sufficient water was sprayed every day and proper aeration was maintained in culture house for the release excess CO_2 and supply of sufficient O_2 as required for the development of primordia and fruiting bodies.

4.4.2.4 Collection and analysis of data:

The packets were arranged in culture house following completely randomized design with 4 replications. Data on time required for bump formation, time required for bump formation after treatment, time required from opening to first harvest, time required for harvest, number of fruiting body, number of effective fruiting body, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, yield (g/packet) and biological efficiency were recorded. Weight of fruiting body was recorded after removing the lower hard and dirty portion of stipe.

The biological efficiency was determined using the following formula:

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

Data were analyzed using MSTAT-C computer program. Means were compared following Duncan's multiple range test using the same computer program.

4.4.3 RESULTS AND DISCUSSION

4.4.3.1 Effect of strain on growth and yield of shiitake mushroom

4.4.3.1.1 Time required for bump formation (TRBF):

Time required for bump formation was significantly influenced by two strains of shiitake mushroom (Appendix VIII). The highest time (116.84 days) required for bump formation was recorded in Le 8 while the lowest time (107.16 days) required for bump formation was found in Le 16 (Table 24).

4.4.3.1.2 Time required for bump formation after treatment (TRBFAT):

Time required for bump formation after treatment was highly significant influenced by two strains of shiitake mushroom (Appendix VIII). The highest time (8.84 days) required for bump formation after treatment was recorded in Le 8. The lowest time (5.16 days) required for bump formation after treatment was found in Le 16 (Table 24).

4.4.3.1.3 Time required from opening to first harvest (TROFH):

Time required from opening to first harvest was highly significant influenced by two strains of shiitake mushroom (Appendix VIII). The highest time (8.30 days) required from opening to first harvest was recorded in Le 8. The lowest time (7.34 days) required from opening to first harvest was found in Le 16 (Table 24).

4.4.3.1.4 Time required for harvest (TRH):

Time required for harvest was highly significant influenced by two strains of shiitake mushroom (Appendix VIII). The highest time (125.18 days) required for harvest was recorded in Le 8 and the lowest time (114.46 days) were found in Le 16 (Table 24).

4.4.3.1.5 Number of fruiting body (NFB):

The number of fruiting body was highly significant influenced by two strains of shiitake mushroom (Appendix VIII). The highest number (36.96) of fruiting body was recorded in Le 16 and the lowest number (13.91) of fruiting body was recorded in Le 8 (Table 25).

4.4.3.1.6 Number of effective fruiting body (NEFB):

The number of effective fruiting body was also highly significant influenced by two strains of shiitake mushroom (Appendix VIII). The highest number (22.36) of effective fruiting body was recorded in Le 16. The lowest number (11.36) of effective fruiting body was in Le 8 (Table 25).

4.4.3.1.7 Length of stalk (LS):

The Length of stalk was significant at 1% level influenced by two strains of shiitake mushroom (Appendix VIII). The highest length of stalk (1.42 cm) was found in Le 8. The lowest length of stalk (1.35 cm) was recorded in Le 16 (Table 25).

4.4.3.1.7 Diameter of stalk (DS):

The diameter of stalk was non significant influenced by two strains of shiitake mushroom (Appendix VIII). The diameter of stalk (4.08 cm) was observed in Le 8 and the diameter of stalk (4.06 cm) was observed in Le 16 (Table 25).

4.4.3.1.8 Diameter of pileus (DP):

The diameter of pileus was highly significant influenced by two strains of shiitake mushroom (Appendix VIII). The highest diameter of pileus (6.70 cm) was recorded in Le 8 and the lowest diameter of pileus (6.56 cm) was observed in Le 16 (Table 25).

4.4.3.1.9 Thickness of pileus (TP):

The thickness of pileus was highly significant influenced by two strains of shiitake mushroom (Appendix VIII). The highest thickness of pileus (1.54 cm) as recorded in Le 16 and the lowest thickness of pileus (1.34 cm) was recorded in Le 8 (Table 25).

4.4.3.1.9 Yield:

The yield was highly influenced by two strains of shiitake mushroom (Appendix VIII). The highest yield (137.96 g) was recorded in Le 16 and the lowest yield (98.91 g) was recorded in Le 8 (Fig. 8).

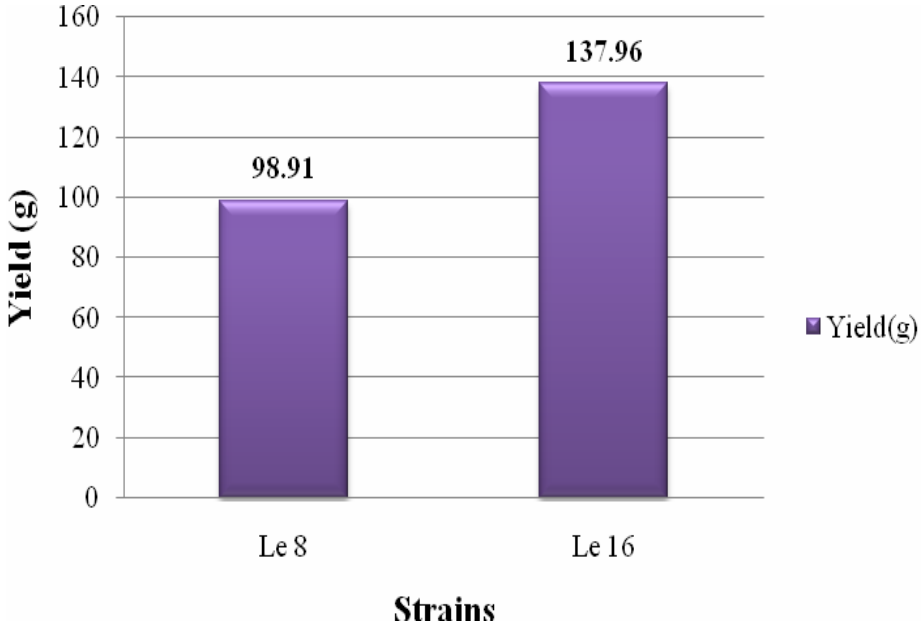


Fig. 8: Yield performance of two strains of shiitake mushroom.

4.4.3.1.10 Biological efficiency (BE %):

Remarkable variation was observed in biological efficiency of two strains of shiitake mushroom (Appendix VIII). The highest biological efficiency (78.93 %) was obtained from Le 16 and the lowest biological efficiency (56.61%) was recorded in Le 8 (Table 24).

4.4.3.2 Effect of different opening patterns on growth, yield attributes and yield of shiitake mushroom

4.4.3.2.1 Time required for bump formation (TRBF):

The time required for bump formation was significantly influenced by different opening patterns (Appendix VIII). The highest time (115.40 days) required for bump formation was recorded in the treatment T_8, where packets were no open and place on rack i.e. control treatment. The lowest time (109.50 days) required for bump formation was found in T_5, where only cotton plug open and place on floor (Table 24).

4.4.3.2.2 Time required for bump formation after treatment (TRBFAT):

Time required for bump formation after treatment was highly significant by the effect

of different substrates (Appendix VIII).The highest time (10.38 days) required for bump formation after treatment was recorded in $T_{8,}$ where the packets were no open and place on rack i.e. control. The lowest time (4.50 days) required for bump formation after treatment was found in the treatment of T_5 (Only cotton plug open and place on floor) (Table 24).

4.4.3.2.3 Time required from opening to first harvest (TRFOH):

The time required from opening to first harvest was highly significant by the effect of different substrates (Appendix VIII). The highest time (9.75 days) required from opening to first harvest was recorded in $T_{9,}$ where packets were total open and place on rack. The lowest time (7.00 days) required from opening to first harvest was found in treatment $T_{5,}$ where only cotton plug open and place on floor which was statistically similar to $T_{11,}$ where packets were no open and place on culture house floor (Table 24).

4.4.3.2.4 Time required for harvest (TRH):

The time required for harvest was highly significant by the effect of different substrates (Appendix VIII). The highest time (124.30 days) required for harvest was recorded in the treatment $T_{9,}$ where packets were total open and place on rack which was statistically similar to the treatment T_8, where packets were no open and place on rack i.e. control treatment. The lowest time (116.50 days) was found in the treatment $T_{5,}$ where only cotton plug open and place on floor (Table 24).

4.4.3.2.5 Number of fruiting body (NFB):

The number of fruiting body was highly significant by the effect of different substrates (Appendix VIII). The highest number (39.25) of fruiting body was recorded in the treatment T_{11} (No open and place on culture house floor) which was statistically similar to the treatment $T_{1,}$ where the packets were top open and place rack. The lowest number (2.00) of fruiting body was recorded in the treatment T_{10} (Total open and place on floor) which was statistically similar to the treatment $T_{9,}$ where packets were total open and place on rack (Table 25).

4.4.3.2.6 Number of effective fruiting body (NEFB):

The number of effective fruiting body was also highly significant by the effect of different substrates (Appendix VIII). The highest number (26.38) of effective fruiting body was recorded in the treatment $T_{11,}$ where the packets were no open and place on culture house floor which was statistically similar to the treatment $T_{1,}$ where the packets were top open and place rack. The lowest number (1.38) of effective fruiting body was recorded in the treatment $T_{10,}$ where the packets were total open and place on floor which was statistically similar to the treatment $T_{9,}$ where the packets were total open and place on rack (Table 25).

4.4.3.2.7 Length of stalk (LS):

The length of stalk was statistically highly significant by the effect of different substrates (Appendix VIII). The highest length of stalk (1.61 cm) was found in the treatment $T_{9,}$ where the packets were total open and place on rack. The lowest length of stalk (1.21 cm) was recorded in the treatment $T_{8,}$ where the packets were no open and place on floor (control) (Table 25).

4.4.3.2.8 Diameter of stalk (DS):

The diameter of stalk was also statistically highly significant influenced by different substrate (Appendix VIII). The highest diameter of stalk (4.85 cm) was observed in the treatment $T_{5,}$ where the packets were only cotton plug open and place on floor and the lowest diameter of stalk (3.69 cm) was observed in the treatment $T_{9,}$ where the packets were total open and place on rack (Table 25).

4.4.3.2.9 Diameter of pileus (DP):

The diameter of pileus was statistically highly significant by the effect of different substrates (Appendix VIII). The highest diameter of pileus (9.80 cm) was recorded in the treatment $T_{9,}$ where the spawn packets were total open and place on rack followed by the treatment T_{6}, where the spawn packets were no open and place on floor. The lowest diameter of pileus (5.23 cm) was observed in T_{4}, where the spawn packets were total open and covered with polypropylene bag place on rack which was

statistically similar to the treatment of T_8 where the spawn packets were no open and place on floor i.e. control (Table 25).

4.4.3.2.10 Thickness of pileus (TP):

The thickness of pileus was also statistically highly significant by the effect of different substrates (Appendix VIII). The highest thickness of pileus (1.91 cm) was recorded in the treatment of $T_{9,}$ where the spawn packets were total open and place on rack while the lowest thickness of pileus (1.16 cm) was recorded in the treatment of $T_{3,}$ where the spawn packets were total open and covered with polypropylene bag place on floor (Table 25).

4.4.3.2.9 Yield:

The yield was highly significant influenced by different substrates (Appendix VIII). The highest yield (173.00g) was obtained from the treatment of $T_{5,}$ where the spawn packets were only cotton plug open and place on floor and the lowest yield (34.00g) was obtained from the treatment of $T_{10,}$ where the spawn packets were total open and place on floor (Fig. 9).

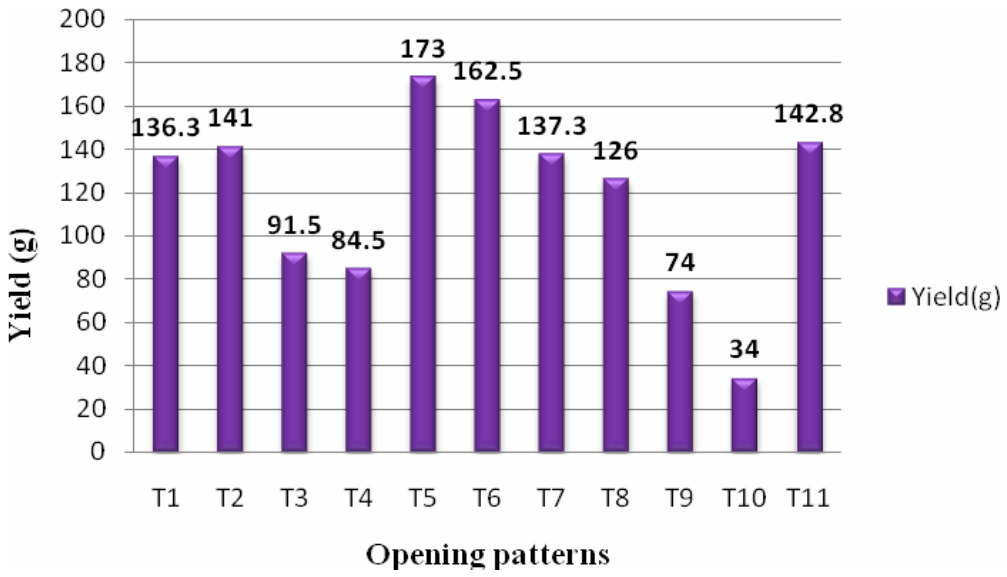


Fig. 9: Yield performance of shiitake mushroom on different types of opening pattern.

T_1= Top open place on floor, T_2= Top open place on rack, T_3= Total open and covered with polypropylene bag place on floor, T_4= Total open and covered with polypropylene bag place on rack, T_5= Only cotton plug open and place on floor, T_6= No open and place on floor, T_7= Only cotton plug open and place on rack, T_8= No open and place on rack (control), T_9= Total open and place on rack, T_{10}= Total open and place on floor, T_{11}= No open and place on culture house floor.

4.4.3.2.10 Biological efficiency (BE %):

Remarkable variation was observed in biological efficiency of different opening pattern (Appendix VIII). The highest biological efficiency (99.11 %) was obtained from the opening pattern T_5 (Only cotton plug open and place on floor) and the lowest biological efficiency (19.43 %) was recorded from the treatment of T_{10} (Total open and place on floor) (Table 25).

Table 24: Effect of strain and opening pattern on growth of shiitake mushroom

Strain	Time required for bump formation (days)	Time required for bump formation after treatment (days)	Time required from opening to first harvest (days)	Time required for harvest (days)
Le 8	116.84	8.84	8.30	125.18
Le 16	107.16	5.16	7.34	114.46
Opening patterns				
T_1	111.00de	6.00de	7.50d	118.50cde
T_2	112.00cd	7.00cd	7.25d	119.50cd
T_3	110.50de	5.50de	9.00ab	119.50cd
T_4	110.50de	5.50de	8.50bc	119.00cde
T_5	109.50e	4.50e	7.00d	116.50e
T_6	112.00cd	7.00cd	7.50d	119.50cd
T_7	110.30de	5.25de	7.13d	117.40de
T_8	115.40a	10.38a	7.38d	122.8ab
T_9	114.50ab	9.50ab	9.75a	124.30a
T_{10}	113.40bc	8.38bc	8.00cd	121.10bc
T_{11}	113.00bc	8.00bc	7.00d	120.00cd
LSD(0.05)	**1.68**	**1.68**	**0.91**	**2.39**
CV (%)	**1.06**	**17.00**	**8.26**	**1.41**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

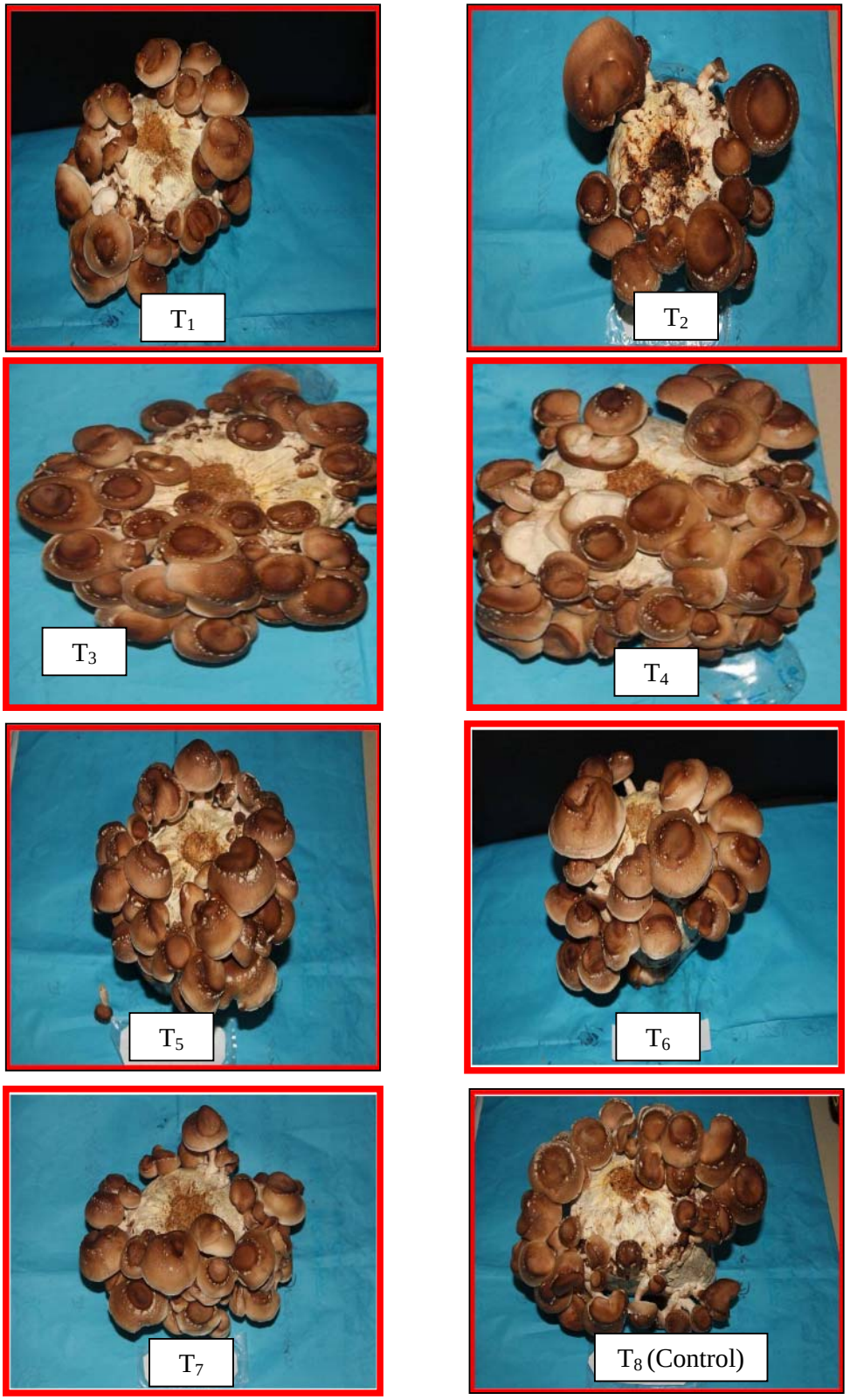
T_1= Top open place on floor, T_2= Top open place on rack, T_3= Total open and covered with polypropylene bag place on floor, T_4= Total open and covered with polypropylene bag place on rack, T_5= Only cotton plug open and place on floor, T_6= No open and place on floor, T_7= Only cotton plug open and place on rack, T_8= No open and place on rack (control), T_9= Total open and place on rack, T_{10}= Total open and place on floor, T_{11}= No open and place on culture house floor.

Table 25: Effect of strain and different opening patterns on yield attributes and yield of shiitake mushroom

Strain	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Biological efficiency (%)
Le 8	13.91	11.36	1.42	4.08	6.70	1.34	56.61
Le 16	36.96	22.36	1.35	4.06	6.56	1.54	78.93
Opening patterns							
T_1	38.00ab	25.00ab	1.30bc	3.19cd	6.56f	1.33cde	77.86d
T_2	25.50cde	17.13d	1.54ab	3.98cd	7.49de	1.68ab	80.57cd
T_3	23.50de	16.50d	1.23c	3.86cd	5.76g	1.16e	52.29f
T_4	22.00e	13.63d	1.25c	3.90cd	5.23g	1.31cde	48.29g
T_5	35.50b	22.63bc	1.45abc	4.85a	7.79cd	1.48bcd	99.11a
T_6	27.00cd	20.50c	1.40abc	4.08cd	8.45b	1.65ab	92.73b
T_7	28.00c	16.38d	1.34bc	3.69d	6.98e	1.25de	78.68cd
T_8	34.50b	23.00abc	1.21c	4.06cd	5.58g	1.21de	72.68e
T_9	4.50f	3.00e	1.61a	3.69d	9.80a	1.91a	42.41h
T_{10}	2.00f	1.38e	1.45abc	4.54ab	8.25bc	1.30cde	19.43i
T_{11}	39.25a	26.38a	1.44abc	4.23bc	6.48c	1.58bc	81.44c
LSD (0.05)	**3.39**	**3.24**	**0.22**	**0.41**	**0.53**	**0.25**	**2.67**
CV (%)	**9.45**	**13.61**	**11.42**	**7.14**	**5.31**	**12.37**	**2.79**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

T_1= Top open place on floor, T_2= Top open place on rack, T_3= Total open and covered with polypropylene bag place on floor, T_4= Total open and covered with polypropylene bag place on rack, T_5= Only cotton plug open and place on floor, T_6= No open and place on floor, T_7= Only cotton plug open and place on rack, T_8= No open and place on rack(control), T_9= Total open and place on rack, T_{10}= Total open and place on floor, T_{11}= No open and place on culture house floor.


T1
T2
T3
T4
T5
T6
T7
T8 (Control)

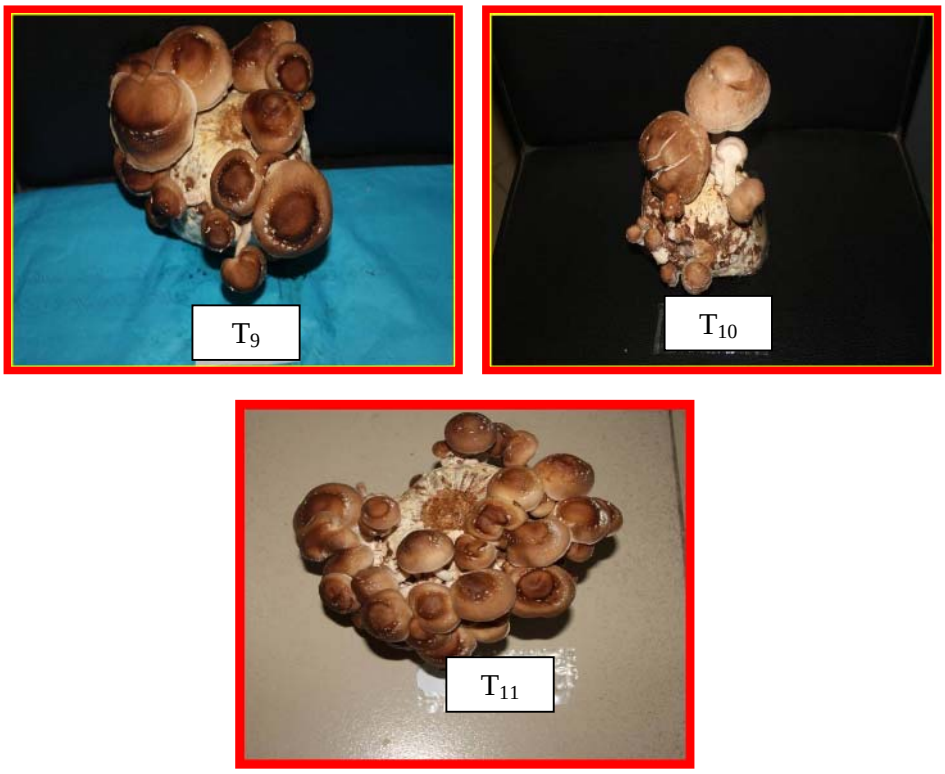


Plate 9: Yield performance of shiitake mushroom on eleven different types of opening pattern.

4.4.3.3 Combined effect of strain and different opening patterns on yield attributes and yield of shiitake mushroom

4.4.3.3.1 Time required for bump formation (days):

The time required for bump formation was non significant influenced by different opening pattern with strain (Appendix VIII). The highest time (120.80 days) required for bump formation was recorded in the treatment combination of Le 8 with the treatment T_8 where spawn packets were no open and place on rack i.e. control which was statistically similar (120.00) to the treatment combination of Le 8 with the treatment of T_9 where spawn packets were total open and place on rack. The lowest days (105.00) required for bump formation was found in the treatment combination of Le 16 with T_5 where spawn packets were only cotton plug open and place on floor (Table 26).

4.4.3.3.2 Time required for bump formation after treatment (days):

The time required for bump formation after treatment was also non significant influenced by different opening pattern with strain (Appendix VIII). The highest time (12.75 days) required for bump formation after treatment was found in the treatment combination of $T_{8,}$ where spawn packets were no open and place on rack i.e. control with the strain Le 8. The lowest time (3.00 days) required for bump formation after treatment was found in the treatment combination $T_{5,}$ where spawn packets were only cotton plug open and place on floor with Le 16 (Table 26).

4.4.3.3.3 Time required from opening to harvest (TRFOH):

The time required from opening to harvest was highly significant influenced by different opening pattern with two strains of shiitake mushroom (Appendix VIII). Significantly the highest time (11.00 days) required from opening to harvest was recorded in the treatment combination of $T_{9,}$ where spawn packets were total open and place on rack with strain Le 16. On the other hand, the lowest time (6.00) required from opening to harvest was found in the treatment combination of $T_{5,}$ where spawn packets were only cotton plug open and place on floor with strain Le 16 which was statistically similar to $T_{8,}$ where spawn packets were no open and place on rack i.e. control with Le 16 and the in the treatment from T_7, where spawn packets were only cotton plug open and place on rack with the same strain (Table 26).

4.4.3.3.4 Time required for harvest (TRH):

The combined effect of two strains and different opening pattern on time required for harvest was non significant (Appendix VII). The highest time (129.50 days) required for harvest was found in the treatment combination of $T_{8,}$ where the spawn packets were no open and place on rack i.e. control with the strain Le 8 which was statistically similar (128.50) to T_9 where the spawn packets were total open and place on rack with Le 8. The lowest time (111.00 days) required for harvest was found in the treatment combination of $T_{5,}$ where the spawn packets were only cotton plug open and place on floor with Le 16 which was statistically similar (111.80) to T_7 where the spawn packets were only cotton plug open and place on rack with the same strain (Table 26).

Table 26: Combined effect of strain and opening pattern on growth of shiitake mushroom

Opening pattern	Time required for bump formation (days)	Time required for bump formation after treatment (days)	Time required from opening to first harvest (days)	Time required for harvest (days)
Strains of shiitake mushroom (Le 8)				
T1	116.00cd	8.00cde	8.00cde	124.00bcd
T2	117.00bc	9.00bcd	8.00cde	125.50bc
T3	115.00de	7.00ef	10.00b	125.0bc
T4	115.00de	7.00ef	8.00cde	123.00cd
T5	114.00e	6.00fg	8.00cde	122.00de
T6	117.00bc	9.00bcd	8.00cde	125.00bc
T7	115.00de	7.00ef	8.00cde	123.00cd
T8	120.80a	12.75a	8.75cd	129.50a
T9	120.00a	12.00a	8.50cd	128.50a
T10	117.50bc	9.50bc	8.00cde	125.50bc
T11	118.00b	10.00b	8.00cde	126.00b
Strains of shiitake mushroom (Le 16)				
T1	106.00ij	4.00hi	7.00ef	113.00hij
T2	107.00hi	5.00gh	6.50f	113.50ghij
T3	106.00ij	4.00hi	8.00cde	114.00ghi
T4	106.00ij	4.00hi	9.00c	115.00fgh
T5	105.00j	3.00i	6.00f	111.00j
T6	107.00hi	5.00gh	7.00ef	114.00ghi
T7	105.00j	3.50hi	6.25f	111.80ij
T8	110.00f	8.00cde	6.00f	116.00fg
T9	109.00fg	7.00ef	11.00a	120.00e
T10	109.30fg	7.25def	8.00cde	116.80f
T11	108.00gh	6.00fg	6.00f	114.00ghi
LSD (0.05)	**1.68**	**1.68**	**0.91**	**2.39**
CV (%)	**1.06**	**17.00**	**8.62**	**1.41**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

T_1= Top open place on floor, T_2= Top open place on rack, T_3= Total open and covered with polypropylene bag place on floor, T_4= Total open and covered with polypropylene bag place on rack, T_5= Only cotton plug open and place on floor, T_6= No open and place on floor, T_7= Only cotton plug open and place on rack, T_8= No open and place on rack (control), T_9= Total open and place on rack, T_{10}= Total open and place on floor, T_{11}= No open and place on culture house floor.

4.4.3.3.5 Length of stalk (LS):

The combined effect of strain and different types of opening pattern on length of stalk was highly significant (Appendix VII). The highest length of stalk (1.88 cm) was found from the treatment combination $T_{2,}$ where the spawn packets were top open place on rack with Le 16. The lowest length of stalk (1.10 cm) was recorded in the treatment $T_{4,}$ where the spawn packets were total open and covered with polypropylene bag place on rack with the strain Le 16 (Table 27).

4.4.3.3.5 Diameter of stalk (DS):

The combined effect of strain and different types of opening pattern on diameter of stalk was statistically significant (Appendix VII). The highest diameter of stalk (5.10 cm) was observed in the treatment T_5 where the spawn packets were only cotton plug open and place on floor with Le 8 and the lowest diameter of stalk (3.10 cm) was observed in the treatment T_9 where the spawn packets were total open and place on rack with Le 16 (Table 27).

4.4.3.3.6 Diameter of pileus (DP):

The combined effect of strain and different types of opening pattern was statistically significant on diameter of pileus (Appendix VIII). The highest diameter (11.50 cm) of pileus was recorded in the treatment of $T_{9,}$ where the spawn packets were total open and place on rack with the strain Le 16 and the lowest diameter of pileus (3.90 cm) was observed in $T_{4,}$ where the spawn packets were total open and covered with polypropylene bag place on rack which was statistically similar to the treatment of $T_{3,}$ where the spawn packets were total open and covered with polypropylene bag place on floor with the strain Le 16 (Table 27).

4.4.3.3.6 Thickness of pileus (TP):

The thickness of pileus was also highly significant by the combined effect of strain and different types of opening pattern. (Appendix VIII). The highest thickness of pileus (2.50 cm) was recorded from the treatment combination of $T_{9,}$ where the spawn packets were total open and place on rack with Le 16 while the lowest thickness of pileus (1.10 cm) was recorded in the treatment combination of T_1 where the spawn packets were top open place on floor with Le 8 (Table 27).

Table 27: Combined effect of strain and different opening patterns on size of fruit body of shiitake mushroom

Opening pattern	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Strains of shiitake mushroom (Le 8)				
T_1	1.40cdef	4.10cde	6.60g	1.10f
T_2	1.20fg	3.50fgh	7.45e	1.30def
T_3	1.25efg	3.90def	7.48e	1.10f
T_4	1.40cdef	4.30bcd	6.55g	1.50cde
T_5	1.60bc	5.10a	9.00c	1.55cd
T_6	1.60bc	4.05cde	10.40b	1.70c
T_7	1.33defg	3.30gh	7.20ef	1.15f
T_8	1.20fg	4.17bcde	7.15ef	1.22ef
T_9	1.70ab	4.28bcd	8.10d	1.32def
T_{10}	1.40cdef	4.02cde	7.50e	1.20f
T_{11}	1.55bcd	4.20bcde	7.10ef	1.60cd
Strains of shiitake mushroom (Le 16)				
T_1	1.20fg	3.73efg	6.53g	1.55cd
T_2	1.88a	4.45bc	7.53e	2.05b
T_3	1.20fg	3.83def	4.05i	1.21ef
T_4	1.10g	3.50fgh	3.90i	1.12f
T_5	1.30d-g	4.60b	6.58g	1.40def
T_6	1.20fg	4.10cde	6.50g	1.60cd
T_7	1.35c-g	4.08cde	6.75fg	1.35def
T_8	1.23fg	3.95def	4.00i	1.20f
T_9	1.53bcd	3.10h	11.50a	2.50a
T_{10}	1.50b-e	5.05a	8.90c	1.40def
T_{11}	1.33d-g	4.25bcd	5.88h	1.55cd
LSD (0.05)	**0.22**	**0.41**	**0.53**	**0.25**
CV (%)	**11.42**	**7.41**	**5.31**	**12.37**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

T_1= Top open place on floor, T_2= Top open place on rack, T_3= Total open and covered with polypropylene bag place on floor, T_4= Total open and covered with polypropylene bag place on rack, T_5= Only cotton plug open and place on floor, T_6= No open and place on floor, T_7= Only cotton plug open and place on rack, T_8= No open and place on rack (control), T_9= Total open and place on rack, T_{10}= Total open and place on floor, T_{11}= No open and place on culture house floor.

4.4.3.3.7 Number of fruiting body (NFB):

The number of fruiting body was significantly influenced by different opening pattern with two strains of shiitake mushroom. (Appendix VIII) The highest number of fruiting body (62.00) was recorded in the treatment combination of $T_{5,}$ where spawn packets were only cotton plug open and place on floor with Le 16. The lowest number (2.00) of fruiting body was recorded in the treatment combination of $T_{10,}$ where spawn packets were total open and place on floor with both the strain (Table 28).

4.4.3.3.8 Number of effective fruiting body (NEFB):

The number of effective fruiting body was significantly influenced by different opening pattern with two strains of shitake mushroom (Appendix VIII). The highest number of effective fruiting body (37.25) was recorded in the treatment combination of $T_{5,}$ where spawn packets were only cotton plug open and place on floor with Le 16. The lowest number (1.25) of effective fruiting body was recorded in the treatment combination of $T_{10,}$ where the spawn packets were total open and place on floor with Le 8 (Table 28).

4.4.3.3.9 Yield:

The yield was highly significant on the effect of two strain of shiitake mushroom with different opening patterns (Appendix VIII). The highest yield (193.00 g) was obtained from the treatment combination of $T_{5,}$ where the spawn packets were only cotton plug open and place on floor with Le 16 and the lowest yield (29.00g) was obtained from the treatment combination of $T_{10,}$ where the spawn packets were total open and place on floor with Le 8 (Table 28).

4.4.3.3.10 Biological efficiency (BE %):

The biological efficiency was also highly significant by the combined effect of two strains of shiitake mushroom with different opening pattern (Appendix VIII). The highest biological efficiency (110.30%) was obtained from the treatment combination of $T_{5,}$ where the spawn packets were only cotton plug open and place on floor with Le 16 and the lowest yield (16.57%) was obtained from the treatment combination of $T_{10,}$ where the spawn packets were total open and place on floor with Le 8 (Table 28).

Table 28: Combined effect of strain and different opening patterns on yield attributes and yield of shiitake mushroom

Opening pattern	Number of fruiting body	Number of effective fruiting body	Yield (g)	Biological efficiency (%)
Strains of shiitake mushroom (Le 8)				
T_1	23.00f	18.50def	114.00g	65.14h
T_2	18.00h	14.00gh	126.00f	72.00g
T_3	17.00hi	15.00fg	98.00hi	56.00j
T_4	19.00gh	12.25gh	89.00j	50.86kl
T_5	9.00k	8.00i	153.00d	87.93e
T_6	14.00ij	13.00gh	146.00e	83.18f
T_7	12.00jk	11.00hi	87.00j	50.22kl
T_8	13.00j	11.00hi	92.00ij	52.57k
T_9	4.00l	3.00j	50.00l	28.82n
T_{10}	2.00l	1.25j	29.00n	16.57p
T_{11}	22.00fg	18.00ef	104.00h	59.43i
Strains of shiitake mushroom (Le 16)				
T_1	53.00b	31.50b	158.50d	90.57de
T_2	33.00e	20.25de	156.00d	89.14e
T_3	30.00e	18.00ef	85.00jk	48.57l
T_4	25.00f	15.00fg	80.00k	45.72m
T_5	62.00a	37.25a	193.00a	110.30a
T_6	40.00d	28.00c	179.00c	102.30c
T_7	44.00c	21.75d	187.50ab	107.10b
T_8	56.00b	35.00a	160.00d	92.79d
T_9	5.00l	3.00j	98.00hi	56.00j
T_{10}	2.00l	1.50j	39.00m	22.28o
T_{11}	56.50b	34.75	181.50bc	103.50c
LSD (0.05)	**3.39**	**3.24**	**6.50**	**2.67**
CV (%)	**9.45**	**13.61**	**3.89**	2.79

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

T_1= Top open place on floor, T_2= Top open place on rack, T_3= Total open and covered with polypropylene bag place on floor, T_4= Total open and covered with polypropylene bag place on rack, T_5= Only cotton plug open and place on floor, T_6= No open and place on floor, T_7= Only cotton plug open and place on rack, T_8= No open and place on rack (control), T_9= Total open and place on rack, T_{10}= Total open and place on floor, T_{11}= No open and place on culture house floor.

4.5 EFFECT OF AMOUNT OF SUBSTRATE ON THE GROWTH AND YIELD OF SHIITAKE (*Lentinus edodes*) MUSHROOM

4.5.1 INTRODUCTION

Shiitake is the most popular and important edible medicinal mushroom in many countries (Chen, 2001 and Royse, 2001). It is the second most cultivated edible mushroom, comprising 25.4% of the world production (Chang, 1999). The commercial cultivation of shiitake mushroom on artificial substrates, based on enriched sawdust, has increased in the last few years (Donoghue and Denison, 1995). The productivity of this mushroom depends on growing techniques, amount of supplementation, types of spawn and temperature ranges. One common technique involves heat-sealed larger bags which filled with 2-3 kg or more substrate for shiitake cultivation and produce more flushes of mushroom. Larger bag size may be suitable for reducing the labour cost and increasing the flushes and yield. The mushroom cultivation literature revealed that its production depends on many factors. Amount of substrate i.e. bag size is an important factor. The latest development in the U. S. i.e. the use of much larger sawdust-substrate blocks in sealed polypropylene bags. This methodology leads itself to faster and greater productivity by mixing the spawn thoroughly with the substrate, which produces more flushes of mushroom in much shorter growing cycles. European growers use larger bags and more substrate. Each bag contains 15kg of substrate in a flat slate shaped. The growing cycle in Europe is usually longer than in the United States (Oei, 2003). Today, a common cylindrical bag method is used in Southeast Asia. Longer cylindrical bags seem to produce better than the same weight of substrate closely packed together. Hence, it is essential for growers to identify the suitable size of spawn packet. Thus, the present study is aimed to determine the appropriate size of spawn packet in which shiitake mushroom could be grown better.

4.5.2 MATERIALS AND METHODS

The experiment was conducted at National Mushroom Development and Extension Centre, Savar, Dhaka during September 2011 to January 2012 to determine the appropriate size of spawn packets and better strain of shiitake mushroom. Four strains

of shiitake (Le 8, Le 11, Le 12 and Le 16) combination with four amount of substrate *viz*. 300g, 500g, 750g and 1000g were used as treatments.

300 g

500 g

700 g

1000 g

Plate 10: Different amount of substrates

4.5.2.1 Spawn packet preparation:

The substrate of spawn packets were prepared by using sawdust and wheat bran at the ratio of 3:1 (dry basis). Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the total mixture. Different sizes of polypropylene bags were filled with substrate mixture as above treatments. After filling the bags, the mouths of the packet were plugged by inserting absorbent cotton with the help of plastic neck and autoclaved at 121^0C and 1.5 kg/cm^2 pressure for 2 hours. After autoclaving and cooling, the bags were inoculated separately with the

mother culture of Le 8, 11, 12 and 16 strain. Then, the packets were incubated in the laboratory at about 22 ± 2^0C temperatures.

4.5.2.2 Mycelial colonization and bump formation as well as culture condition for fruiting were same described earlier in chapter III.

4.5.2.3 Experimental design, data collection and analysis:

The experiment was laid out in a completely randomized design (CRD) with 4 replications. Data were collected on mycelium growth rate, time required for mycelium running, time required for bump formation, time required from opening to first harvest, time required for harvest (days), number of fruiting body & number of effective fruiting body/ packet, length & diameter of stalk, diameter of pileus, thickness of pileus, yield (g/ packet) and biological efficiency. The yield was recorded by removing the lower hard and dirty portion of the fruiting bodies. The biological efficiency (%) was determined by the following formula:

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

The data were analyzed following MSTAT-C computer program and means were computed and separated following Duncan's multiple range test (DMRT) using the same computer program.

4.5.3 RESULTS AND DISCUSSION

4.5.3.1 Effect of different strains on various parameters

4.5.3.1.1 Mycelium growth rate (MGR):

Significant variation in mycelium growth rate was observed in four strains of shiitake mushroom (Appendix IX). The highest mycelium growth rate (3.48 mm/day) was recorded in strain Le 12 which was statistically similar to the strain Le 11. The lowest mycelium growth rate (2.95 mm/day) was recorded in Le 8 which was statistically similar to the strain Le 16 (Table 29).

4.5.3.1.2 Time required to completion of mycelium running (TRMR):

Time required to completion of mycelium running was significantly influenced by

different strains of shiitake mushroom (Appendix IX). The lowest time (59.25 days) required to completion of mycelium running was found in Le 11. The highest time (82.38 days) required to completion of mycelium running was recorded in the strain Le 8 which was statistically similar to Le 16 (Table 29).

4.5.3.1.3 Time required for bump formation (TRBF):

Time required for bump formation was significantly influenced by different strains of shiitake mushroom (Appendix IX). The highest time (125.40 days) required for bump formation was recorded in Le 11 while the lowest time (99.31 days) required for bump formation was found in Le 8 (Table 29).

4.5.3.1.4 Time required from opening to first harvest (TROFH):

Significant variation on time required from opening to first harvest was observed in four strains of shiitake mushroom (Appendix IX). The highest time (9.81 days) required from opening to first harvest was recorded in Le 12 and the lowest time (5.31 days) required from opening to first harvest was found in Le 8 (Table 29).

4.5.3.1.5 Time required for harvest (TRH):

The response of strains to time required for harvest was significant (Appendix IX). The highest time (132.80 days) required for harvest was recorded in Le 11 which was statistically similar (131.20 days) to Le 12. The lowest time (104.50 days) required for harvest was recorded in Le 8 (Table 29).

4.5.3.1.6 Number of fruiting body (NFB):

The strain showed significant variation in case of number of fruiting body (Appendix IX). The highest number (27.44) of fruiting body was recorded in Le 12 and the lowest number (11.31) of fruiting body was recorded in the strain Le 8 (Table 30).

4.5.3.1.7 Number of effective fruiting body (NEFB):

In case of number of effective fruiting body in different strains showed significant variation (Appendix IX). The highest number (17.94) of effective fruiting body was recorded in Le 12 and the lowest number (9.25) of effective fruiting body was

observed in the strain Le 8 which was statistically similar to the other strains (Table 30).

4.5.3.1.8 Length of stalk (LS):

The length of stalk significantly affected by different strains of shiitake mushroom (Appendix IX). The highest length of stalk (5.16 cm) was found in Le 16 and statistically different to other strains. The lowest length of stalk (4.33cm) was recorded in Le 12 which was statistically similar to other strains (Table 30).

4.5.3.1.8 Diameter of stalk (DS):

The diameter of stalk significantly affected by different strains of shiitake mushroom (Appendix IX). In case of diameter of stalk, the highest diameter of stalk (3.61 cm) was observed in Le 12 and the lowest diameter of stalk (0.95 cm) was observed in Le 11 (Table 30).

4.5.3.1.9 Diameter of pileus (DP):

Diameter of pileus was also significantly affected by different strains of shiitake mushroom (Appendix IX). The highest diameter of pileus (7.50 cm) was recorded in Le 8 and the lowest diameter of pileus (3.55 cm) was observed in Le 12 (Table 30).

4.5.3.1.9 Thickness of pileus (TP):

Thickness of pileus was also significantly affected by different strains of shiitake mushroom (Appendix IX). The highest thickness of pileus (1.56cm) was recorded in Le 8 and the lowest thickness of pileus (0.94 cm) was recorded in Le 11 (Table 30).

4.5.3.1.10 Yield:

The data obtained from the experiment showed significant variation in yield among the strains (Appendix IX). The highest yield (81.13g) was recorded in Le 8 and the lowest yield (62.81 g) was recorded in Le 11 (Fig. 10).

4.5.3.1.11 Biological efficiency (BE %):

Remarkable variation was observed in biological efficiency of different strains of shiitake mushroom (Appendix IX). The highest biological efficiency (46.36%) was obtained from Le 8 and the lowest biological efficiency (35.89%) was recorded in Le 11 (Fig. 10).

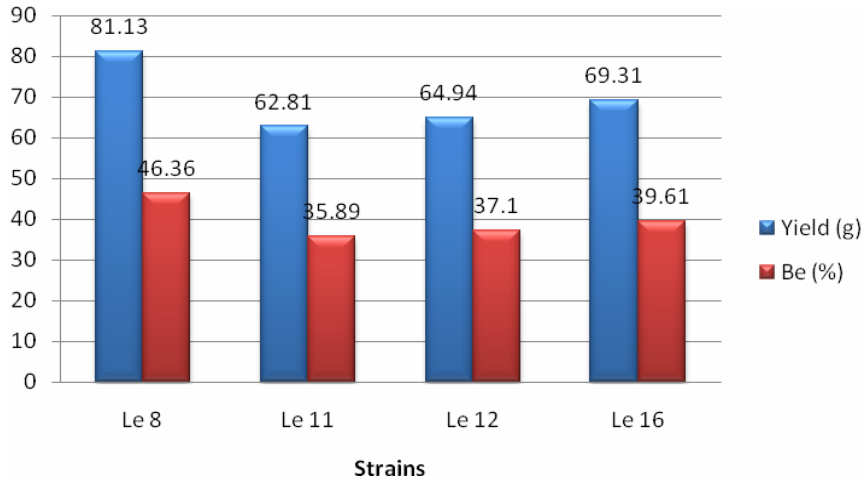


Fig. 10: Effect of strains of shiitake mushroom on yield and biological efficiency

4.5.3.2 Effect of different amount of substrate on various parameters

4.5.3.2.1 Mycelium growth rate (MGR):

Significant variation in mycelium growth rate was observed in different amount of substrate (Appendix IX). The highest mycelium growth rate (3.85 mm/day) was recorded in 1000 g weight of spawn packet. The lowest mycelium growth rate (2.86 cm/day) was recorded in 750 g of spawn packet which was statistically similar to 500 g spawn packet (Table 29).

4.5.3.2.2 Time required to completion of mycelium running (TRMR):

Time required to completion of mycelium running were significantly influenced by different amount of substrate (Appendix IX). The time required to completion of mycelium running in different amount of substrate ranged from 39.31 to 99.38 (days). The lowest time (39.31 days) required for mycelium running was found in 300g spawn packet and the highest time (99.38 days) required for mycelium running was recorded in 1000 g weight of spawn packet (Table 29).

4.5.3.2.3 Time required for bump formation (TRBF):

Time required for bump formation was significantly influenced by different amount of substrate (Appendix IX). The highest time (135.60 days) required for bump formation was recorded in 1000 g spawn packet and the lowest time (90.00 days) required for bump formation was found in 300 g span packet (Table 29).

4.5.3.2.4 Time required from opening to first harvest (TROFH):

Significant variations were observed in time required from opening to first harvest by different amount of substrates (Appendix IX). The highest time (10.94 days) required from opening to first harvest was recorded in 1000 g weight of spawn packet. The lowest time (4.69 days) required from opening to first harvest was found in 300 g weight of spawn packet (Table 29).

4.5.3.2.5 Time required for harvest (TRH):

Time required for harvest was significantly influenced by different amount of substrates (Appendix IX). The highest time (147.00 days) required for harvest was recorded in 1000 g weight of spawn packet. The lowest time (94.69 days) required for harvest was found in 300 g weight of spawn packet (Table 29).

Table 29: Effect of strain and different amount of substrates on growth of shiitake mushroom

Strain	Mycelium growth rate (mm/day)	Time required for completion of mycelium running (days)	Time required for bump formation (days)	Time required from opening to first harvest (days)	Time required for harvest (days)
Le 8	2.95b	82.38a	99.31d	5.31c	104.50c
Le 11	3.41a	59.25c	125.40a	7.38b	132.80a
Le 12	3.48a	61.88b	120.80b	9.81a	131.20a
Le 16	3.05b	60.56bc	109.7c	6.56bc	116.30b
Amount of Substrates					
300 g	3.25b	39.31d	90.00d	4.69c	94.69d
500 g	2.95c	46.81c	107.70c	5.56c	113.30c
750 g	2.86c	78.56b	121.90b	7.88b	129.80b
1000g	3.85a	99.38a	135.60a	10.94a	147.00a
LSD(0.05)	**0.25**	**2.19**	**4.54**	**1.34**	**4.39**
CV (%)	**5.42**	**2.33**	**2.81**	**12.99**	**2.55**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.5.3.2.6 Number of fruiting body (NFB):

The number of fruiting body was significantly influenced by different amount of substrates (Appendix IX). The highest number (38.81) of fruiting body was recorded in 500 g weight of spawn packet and the lowest number (2.56) of fruiting body was recorded in 1000 g weight of spawn packet (Table 30).

4.5.3.2.7 Number of effective fruiting body (NFEB):

The number of effective fruiting body was also significantly influenced by different amount of substrates (Appendix IX). The highest number (26.25) of effective fruiting body was recorded in 500 g weight of spawn packet and the lowest number (1.81) of effective fruiting body was observed in 1000 g weight of spawn packet (Table 30).

4.5.3.2.8 Length of stalk (LS):

The length of stalk significantly influenced by different amount of substrates (Appendix IX). The highest length (5.29 cm) of stalk was found in 750 g weight of spawn packet. The lowest length (4.17 cm) of stalk was recorded in 1000 g weight of spawn packet (Table 30).

4.5.3.2.9 Diameter of stalk (DS):

The diameter of stalk significantly influenced by different amount of substrates (Appendix IX). The highest diameter (3.71 cm) of stalk was observed in 1000 g weight of spawn packet and the lowest diameter (1.16 cm) of stalk was observed in 300 g weight of spawn packet which was statistically similar to 500 g weight of spawn packet (Table 30).

4.5.3.2.10 Diameter of pileus (DP):

Diameter of pileus was also significantly affected by different amount of substrate (Appendix IX). The highest diameter (6.74 cm) of pileus was recorded in 1000 g weight of spawn packet and the lowest diameter (5.29 cm) of pileus was recorded in 500 g weight of spawn packet which was statistically similar to 300 g and 750 g weight of spawn packet (Table 30).

4.5.3.2.11 Thickness of pileus (TP):

Thickness of pileus was also significantly affected by different amount of substrates

(Appendix IX). The highest thickness (1.52 cm) of pileus was recorded in 1000 g weight of spawn packet and the lowest thickness (1.05 cm) of pileus was recorded in 300 g weight of spawn packet which was statistically similar to 750 g weight of spawn packet (Table 30).

Table 30: Effect of strain and different amount of substrate on yield attributes of shiitake mushroom

Strain	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Le 8	11.31c	9.25b	4.99ab	1.72b	7.50a	1.56a
Le 11	17.50b	11.63b	4.45ab	0.95c	5.50b	0.94d
Le 12	27.44a	17.94a	4.33b	3.61a	3.55c	1.16c
Le 16	13.19c	9.50b	5.16a	1.45b	6.16b	1.33b
Amount of Substrates						
300 g	22.56b	16.44b	4.64ab	1.16c	5.35b	1.05c
500 g	38.81a	26.25a	4.84ab	1.19c	5.29b	1.07c
750g	5.50c	3.81c	5.29a	1.68b	5.33b	1.35b
1000 g	2.56c	1.81c	4.17b	3.71a	6.74a	1.52a
LSD(0.05)	**3.304**	**3.076**	**0.674**	**0.447**	**1.241**	**0.156**
CV (%)	**13.39**	**17.92**	**10.01**	**16.31**	**15.30**	**8.89**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.5.3.2.12 Yield:

The yield was significantly influenced by different amount of substrate (Appendix IX). The highest yield (111.60 g) was recorded in 500 g weight of spawn packet and the lowest yield (38.38 g) was recorded in 750 g weight of spawn packet (Fig. 11).

4.5.3.2.13 Biological efficiency (BE %):

The biological efficiency was also significantly influenced by different amount of substrates (Appendix IX). The highest biological efficiency (74.43%) was obtained from 300 g substrate and the lowest biological efficiency (14.35%) was recorded in 1000 g substrate (Fig. 11).

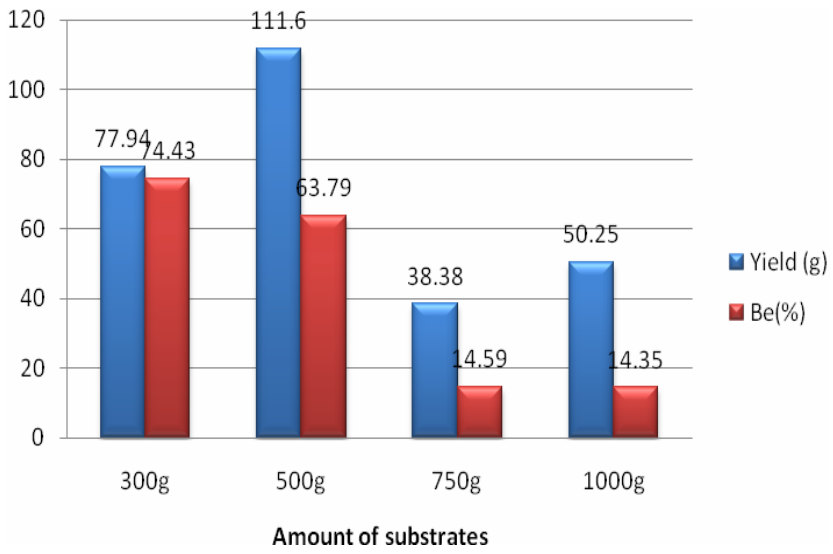


Fig. 11: Effect of amount of substrates on yield and biological efficiency.

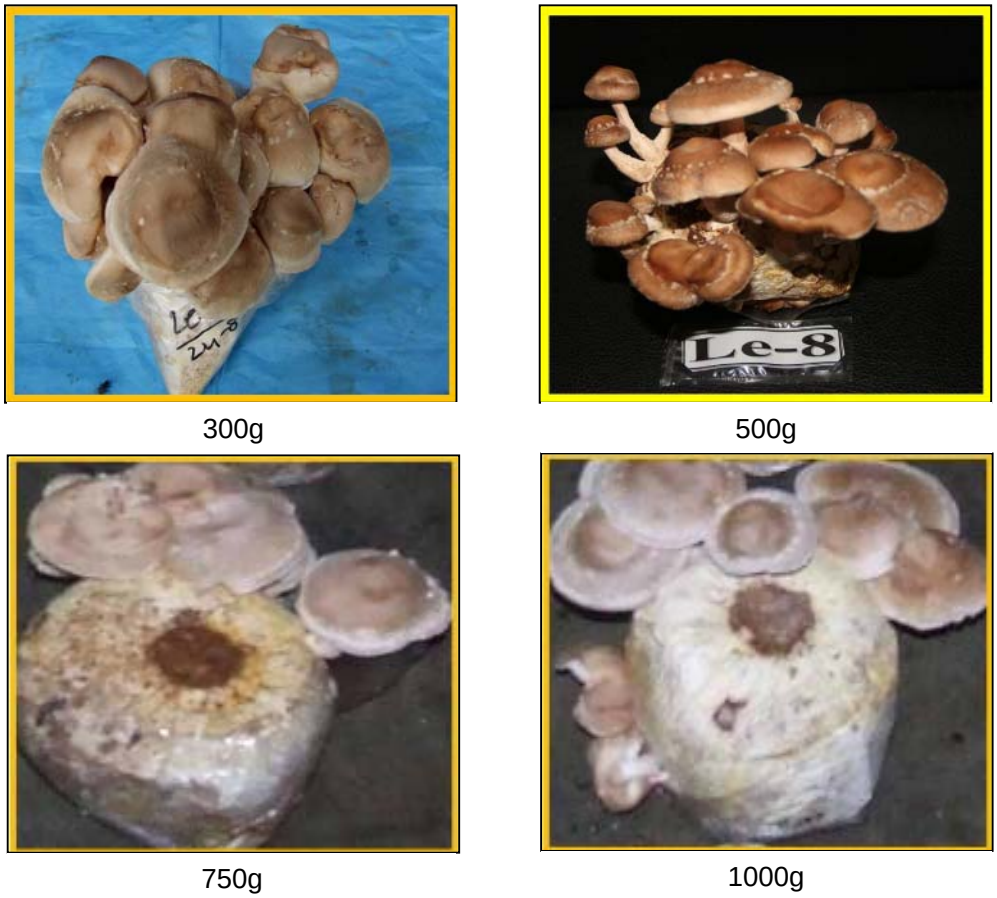


Plate 11: Yield influenced by different amount of substrates

4.5.3.3 Combined effect of strains and different amount of substrates on growth and yield of shiitake mushroom

4.5.3.3.1 Mycelium growth rate (MGR):

Significant variation in mycelium growth rate was observed in different strains of shiitake mushroom with different amount of substrates (Appendix IX). The highest mycelium growth rate (4.23 mm/day) was recorded in Le 11 with 1000 g of spawn packet which was statistically similar to the combined effect of Le 12 with 1000 g of spawn packet. The lowest mycelium growth rate (2.60 mm/day) was recorded in Le 8 with 500 g weight of spawn packet (Table 31).

4.5.3.3.2 Time required to completion of mycelium running (TRMR):

Significant variations were found between the strain and different amount of substrates (Appendix IX). The highest time (114.80 days) required to completion of mycelium running was obtained from the treatment combination of strain Le 12 with 1000 g spawn packet. The lowest time (33.25 days) required to completion of mycelium running was obtained from the strain Le 11 with 300 g spawn packet which was statistically similar to the treatment combination of Le 8 with 300 g spawn packet and Le 16 with 300 g spawn packet (Table 31).

4.5.3.3.3 Time required for bump formation (TRBF):

Significant variations were found on time required for bump formation between the strain and different amount of substrate (Appendix IX). The highest time (149.30 days) required for bump formation was obtained from the treatment combination of strain Le 11 with 1000 g spawn packet. The lowest time (79.50 days) required for bump formation was obtained from the strain Le 8 with 300 g spawn packet which was statistically similar to the treatment combination of Le 16 with 300 g spawn packet (Table 31).

4.5.3.3.4 Time required from opening to first harvest (TROFH):

Significant variations were found on time required from opening to first harvest between the strain and different amount of substrates (Appendix IX). The highest time (16.50 days) required from opening to first harvest was obtained from the treatment combination of strain Le 12 with 1000 g spawn packet. The lowest time (4.00 days) required from opening to first harvest was obtained from the strain Le 16 with 300 g spawn packet (Table 31).

4.5.3.3.5 Time required for harvest (TRH):

Time required for harvest was significantly influenced by different amount of substrates and different strains of shiitake mushroom (Appendix IX).

Table 31: Combined effect of strains and different amount of substrates on growth and development of shiitake mushroom

Strains	Mycelium growth rate (mm/day)	Time required for mycelium running (days)	Time required for bump formation (days)	Time required from opening to first harvest (days)	Time required for harvest (days)
Strain of shiitake (Le 8) mushroom					
300 g	2.90fgh	35.25i	79.50i	4.50fg	84.00h
500 g	2.60i	41.50g	90.50h	4.50fg	95.00g
750g	2.73hi	75.50c	104.30g	5.75def	110.00f
1000 g	3.60b	95.25b	123.00d	6.50de	129.00cd
Strain of shiitake (Le 11) mushroom					
300 g	3.40bc	33.25i	92.00h	5.00efg	97.00g
500 g	3.13def	38.75h	123.30d	6.75d	130.00c
750g	2.90efgh	71.50d	137.00b	7.00d	144.00b
1000 g	4.23a	93.50b	149.30a	10.75bc	160.00a
Strain of shiitake (Le 12) mushroom					
300 g	3.53b	54.75f	104.50g	5.25efg	109.80f
500 g	3.23cd	66.25e	112.00f	6.00def	118.00e
750g	3.03defg	93.75b	128.50c	11.50b	140.00b
1000 g	4.15a	114.80a	138.00b	16.50a	157.00a
Strain of shiitake (Le 16) mushroom					
300 g	3.18cde	34.00i	84.00i	4.00g	88.00h
500 g	2.85fghi	40.75gh	105.00g	5.00fg	110.00f
750g	2.78ghi	73.50cd	117.80e	7.25d	125.00d
1000 g	3.43bc	94.00b	132.00c	10.00c	142.00b
LSD (0.05)	**0.25**	**2.19**	**4.54**	**1.34**	**4.39**
CV (%)	**5.42**	**2.33**	**2.81**	**12.99**	**2.55**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

The highest time (160.00 days) required for harvest was recorded from the treatment combination of Le 11 with 1000 g weight of spawn packet which was statistically similar to the treatment combination of Le 12 with 1000 g weight of spawn packet. The lowest time required for harvest (84.00 days) was found from the treatment combination of Le 8 with 300 g weight of spawn packet which was statistically similar to the treatment combination of Le 16 with 300 g spawn packet (Table 31).

4.5.3.3.6 Number of fruiting body (NFB):

The combined effect of different strain with different amount of substrates on number of fruiting body was highly significant (Appendix IX). The highest number (81.50) of fruiting body was recorded from the treatment combination Le 12 with 500 g weight of spawn packet and the lowest number (1.25) of fruiting body was recorded from the treatment combination of Le 8 with 1000 g weight of spawn packet which was statistically similar to the treatment combination of Le 16 with 1000 g weight of spawn packet (Table 32).

4.5.3.3.7 Number of effective fruiting body (NEFB):

The number of effective fruiting body was also highly significant by the combined effect of different strain and different amount of substrates (Appendix IX). The highest number (53.00) of effective fruiting body was recorded from the treatment combination Le 12 with 500 g weight of spawn packet. The lowest number (1.00) of effective fruiting body was found from the treatment combination of Le 12 with 1000 g weight of spawn packet which was statistically similar to the treatment combination of Le 8 with 1000 g spawn packet, Le 12 with 750 g spawn packet and Le 16 with 1000 g spawn packet (Table 32).

4.5.3.3.8 Length of stalk (LS):

Length of stalk was significantly influenced by different amount of substrate and different strain of shiitake mushroom (Appendix IX). The highest length (7.18 cm) of stalk was found from the treatment combination of Le 8 with 750 g weight of spawn packet which was followed by Le 16 with 750 g amount of substrate but statistically different. The lowest length (3.38 cm) of stalk was recorded from the treatment combination of Le 11 with 750 g weight of spawn packet (Table 32).

4.5.3.3.9 Diameter of stalk (DS):

Diameter of stalk was significantly influenced by different amount of substrate and different strain of shiitake mushroom (Appendix IX). The highest diameter (10.25 cm) of stalk was observed from the treatment combination of Le 12 with 1000 g weight of spawn packet. The lowest diameter (0.80 cm) of stalk was observed from the treatment combination of Le 11 with 1000 g weight of spawn packet (Table 32).

4.5.3.3.9 Diameter of pileus (DP):

Diameter of pileus was also highly significant by the combined effect of different strains and different amount of substrates (Appendix IX). The highest diameter (10.38 cm) of pileus was recorded from the treatment combination of Le 8 with 1000 g weight of spawn packet which was statistically similar to the treatment combination of Le 16 with 1000 g spawn packet and the lowest diameter (1.28 cm) of pileus was recorded from the treatment combination of Le 12 with 1000 g weight of spawn packet (Table 32).

4.5.3.3.10 Thickness of pileus (TP):

Thickness of pileus was also highly significant by the combined effect of different strains and different amount of substrates (Appendix IX). The highest thickness (2.33 cm) of pileus was recorded from the treatment combination of Le 8 with 1000 g weight of spawn packet and the lowest diameter (0.80 cm) of pileus was recorded from the treatment combination of Le 12 with 500 g weight of spawn packet which was statistically similar to the treatment combination of Le 11 with 750 g and 1000 g weight of spawn packet (Table 32).

4.5.3.3.11 Yield:

The yield was significantly influenced by the combined effect of different strains and different amount of substrates (Appendix IX). The highest yield (135.80g) was recorded from the treatment combination of Le 12 with 500 g weight of spawn packet which was followed by Le 8 with 500g and Le 16 with 500g substrates. The lowest yield (24.50 g) was recorded from the treatment combination of Le 12 with 750 g weight of spawn packet (Table 32).

Table 32: Combined effect of strains and different amount of substrates on yield attributes and yield of shiitake mushroom

Strain	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield(g)
Strain of shiitake (Le 8)							
300 g	19.00d	14.50c	3.95fg	1.78cd	6.15c	1.05efg	85.75e
500 g	21.00cd	17.50bc	4.90bcd	1.25efg	5.93c	1.10ef	112.30b
750g	4.00fg	3.75de	7.18a	1.400de	7.55b	1.78b	56.50h
1000 g	1.25g	1.25e	3.95fg	2.45b	10.38a	2.33a	70.00f
Strain of shiitake (Le 11)							
300 g	24.00c	17.75bc	5.25bc	0.83fg	5.88c	0.90gh	89.75de
500 g	29.00b	18.75b	5.33bc	1.20efg	6.00c	1.15ef	94.00d
750 g	11.00e	6.25d	3.38g	0.98efg	4.43def	0.88h	29.50k
1000 g	6.00f	3.75de	3.88fg	0.80g	5.70cd	0.83h	38.00j
Strain of shiitake (Le 12)							
300 g	23.75c	16.00bc	3.85fg	0.98efg	5.05c-f	1.05fg	63.50g
500 g	81.50a	53.00a	4.28def	0.88fg	3.75f	0.80h	135.80a
750 g	2.75fg	1.75e	5.05bc	2.33b	4.13ef	1.38cd	24.50l
1000 g	1.75g	1.00e	4.13efg	10.25a	1.28g	1.43c	36.00j
Strain of shiitake (Le 16)							
300 g	23.50c	17.50bc	5.50b	1.05efg	4.33def	1.20ef	72.75f
500 g	23.75c	15.75bc	4.85b-e	1.43de	5.50cde	1.23de	104.50c
750g	4.25fg	3.50de	5.55b	2.00bc	5.20cde	1.38cd	43.00i
1000 g	1.25g	1.25e	4.73cde	1.33def	9.60a	1.50c	57.00h
LSD(0.05)	**3.30**	**3.08**	**0.67**	**0.45**	**1.24**	**0.16**	**4.41**
CV (%)	**13.39**	**17.92**	**10.01**	**16.31**	**15.30**	**8.89**	**4.45**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.5.3.3.11 Biological efficiency (BE %):

The biological efficiency was also significantly influenced the combined effect of different strain and different amount of substrate (Appendix IX). The highest biological efficiency (85.47%) was obtained from the treatment combination Le 11 and 300 g substrate. The second highest biological efficiency (81.66%) was obtained from the treatment combination Le 8 and 300 g substrate. The lowest biological efficiency (9.31%) was recorded in Le 12 and 750 g substrate which was statistically similar to Le 11 and Le 16 with 750 g substrate as well as also similar to Le 11, Le 12 and Le 16 with 1000 g substrate (Fig. 12).

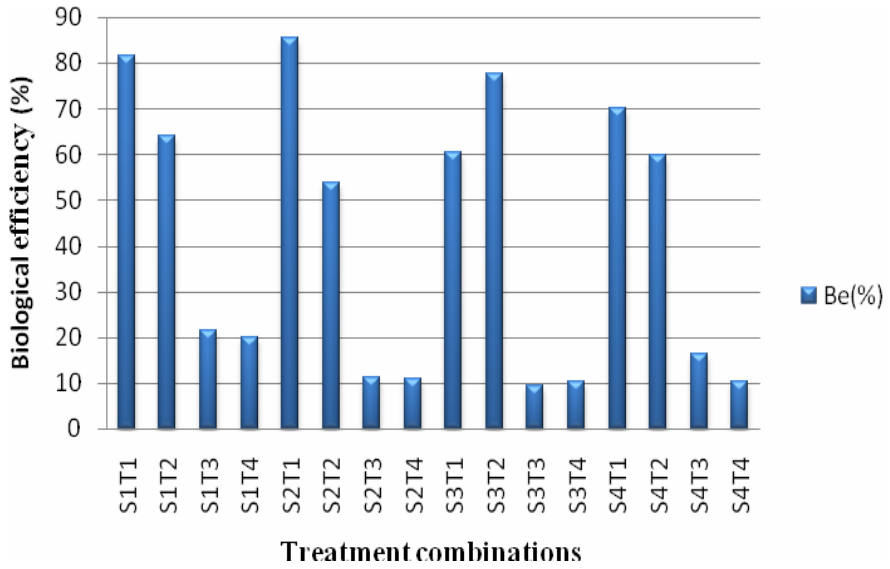


Fig.12. Combined effect of strain and amount of substrates on biological efficiency.

S_1T_1 = Le 8 × 300g, S_1T_2 = Le 8 × 500g, S_1T_3 = Le 8 × 750g, S_1T_4 = Le 8 × 1000g, S_2T_1 = Le 11×300g, S_2T_2 = Le 11 ×500g S_2T_3 = Le 11 × 750g S_2T_4= Le 11 × 1000g S_3T_1 = Le 12×300g S_3T_2= Le 12× 500g S_3T_3 = Le 12× 750g S_3T_4 = Le 12× 1000g S_4T_1 = Le 16 ×300g S_4T_2= Le 16 × 500g S_4T_3=Le16×750g, S_4T_4=Le16×1000g.

Results of the present experiment reveal that there are appreciable variations in yield and yield contributing attributes with the variation of amount of substrates and strain of shiitake mushroom. In terms of yield and yield attributes performance of the strain Le 8 was best among the strains. In case of different amount of substrates 500g weight spawn packet gave the highest yield while the highest biological efficiency obtained when spawns packets prepared with 300g substrates. The yield decreased with the

increase of amount of substrates. On the other hands the larger spawn packets delay the harvesting period. Many other investigators also found variations in effect of amount of substrates on growth, yield and yield contributing characters of shiitake mushroom.

Hossain *et al.* (2010) reported that minimum days required when minimum amount of substrate were used for spawn packet preparation. He observed that minimum days required when the packets were cylindrical shape with 500 g substrate and maximum days required where packets were cylindrical 1500g. He also (2010) reported that larger packets took more time than small packets for harvest, the lowest number of fruiting body was recorded from 500 g cylindrical spawn packet and the lowest yield was obtained from 500 g cylindrical spawn packet while highest yield was recorded 1500g cylindrical spawn packets. It was evident from Hossain *et al.* (2010) who studied that mushroom yield was positively correlated to the substrate amount and the results of Hossain study corroborated with the findings of Chen (2001).

Hossain *et al.* (2010) reported that biological efficiency decreased with the increase of size of spawn packet. He also reported that highest biological efficiency recorded in 500 g size square block shape packet and lowest biological efficiency from 1500 g block shape packet. The block shape of spawn packet performed better in small size of spawn packet whereas cylindrical shape is better in larger size of spawn packet.

4.6 EFFECT OF AGE OF SPAWN PACKET ON THE GROWTH AND YIELD OF SHIITAKE (*Lentinus edodes*) MUSHROOM

4.6.1 INTRODUCTION

Shiitake mushroom (*Lentinus edodes*) is commonly used in China and Japan as high valued food and medicine. It is produced on different kinds of lignocelluloses substrates. The production system of shiitake mushroom is quite different from other edible mushrooms. There are two period of mushroom cultivation, the incubation period and the harvesting period. During the incubation period, the mycelium colonizes on the substrates with some distinct stages, such as, mycelium running, thickening, pigmentation, hardening and bumping for growth improvement (Stamets, 1993). It is gaining popularity among the potential mushroom growers as well as perspective consumers owing to attractive shape and size, simple growing technique, low capital investment, wide substrate range, sustainable yield, long shelf-life and ability to thrive in a wide range of climatic conditions. The incubation period of shiitake mushroom is very crucial which can affect the yield. Quality and quantity of spawn play an important role in the successful production of any mushroom species. Spawn age is an important factor for growth and development of shiitake mushroom. Short days/ age of spawn packet and so many older days of spawn packet give lower yield. But an appropriate days the spawn packet give the better performance. Mushroom yield decreased with increase in spawn age after a certain age. Considering the facts the present work was undertaken to determine the right age of spawn packet for shiitake mushroom cultivation to get higher yield and better quality.

4.6.2 MATERIALS AND METHODS

The experiment was conducted at National Mushroom Development and Extension centre, Savar, Dhaka during October 2012 to March 2013. Ten different age (40, 50, 60, 70 80, 90, 100, 110, 120 and 130 days) of spawn packets and two strain (Le 8 and Le 16) of shiitake mushroom were used in the experiment.

4.6.2.1 **Preparation of pure culture, mother culture and spawn packets**:

The procedures for preparation of pure culture, mother culture and spawn packets were same as described in chapter III.

4.6.2.2 Opening of spawn packet:

The inoculated packets were placed on a still rack at 22 ± 2^0C temperature for incubation. Whitish mycelia began to grow on the substrate and after full colonization a thick mycelial coat forms on the outer surface of colonized substrate. To determine the right age of spawn, the packets were fully opened at 40, 50, 60, 70 80, 90, 100, 110, 120 and 130 days after inoculation.

4.6.2.3 Cultivation conditions for fruiting:

Treatment wise, the packets were moved to culture house and placed on racks. The temperature, relative humidity and light were maintained at 18-22 ^{0}C, 70-80% and 10-20 lux respectively. Watering was done 3 to 4 times per day to maintain temperature and relative humidity. Excess CO_2 was removed by exhaust fan.

4.6.2.4 Data collection and analysis:

The experiment was laid out in a completely randomized design (CRD) with 4 replications. Data on time required from opening to first harvest (days), time required for harvest (days), number of fruiting body, number of effective fruiting body, length of stalk (cm), diameter of stalk (cm), diameter of pileus (cm), thickness of pileus (cm), yield (g) and biological efficiency were recorded. Yield in g/packet was recorded by the removing the dirty portion of fruiting bodies. Biological efficiency was determined by following formula:

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

The data were analyzed following MSTAT-C computer program me and means were computed and separated following Duncan's Multiple Range Test (DMRT) using the same computer program.

4.6.3 RESULTS AND DISCUSSION

4.6.3.1 Effect of strain on various growth and yield parameters

4.6.3.1.1 Time required from opening to first harvest (TROFH):

Significant variation on time required from opening to first harvest (days) was observed in two strains of shiitake (Appendix X). The highest time (13.75 days)

required from opening to first harvest was recorded in Le 8 and the lowest time (10.00days) required from opening to first harvest was recorded in Le 16 (Table 33).

4.6.3.1.2 Time required for harvest (TRH):

The response of strain to time required for harvest was significant (Appendix X). The highest time required for harvest was recorded (108.75 days) in Le 8 and the lowest time (105.09 days) required for harvest was recorded in Le 16 (Table 33).

4.6.3.1.3 Number of fruiting body (NFB):

The strain showed significant variation in case of number of fruiting body (Appendix X). The highest number (20.50) of fruiting body was recorded in Le 16 and the lowest number (17.81) of fruiting body was recorded in Le 8 (Table 33).

4.6.3.1.4 Number of effective fruiting body (NEFB):

In case of number of effective fruiting body the strain showed significant variation (Appendix X). The highest number (14.50) of effective fruiting body was recorded in Le 16 and the lowest number (14.00) of fruiting body was recorded in Le 8 (Table 33).

4.6.3.1.5 Length of stalk (LS):

The length of stalk significantly affected by the two strain of shiitake mushroom (Appendix X).The highest length (4.49 cm) of stalk was found in Le 8 and lowest length (4.40 cm) of stalk was recorded in Le 16 (Table 34).

4.6.3.1.6 Diameter of stalk (DS):

The diameter of stalk significantly affected by the two strain of shiitake mushroom (Appendix X). In case of diameter of stalk, the highest diameter (1.42 cm) of stalk was observed in Le 16 and the lowest diameter (1.38cm) of stalk was observed in Le 8 (Table 34).

4.6.3.1.7 Diameter of pileus (DP):

Diameter of pileus was also significantly affected by the two strain of shiitake mushroom (Appendix X). The highest diameter (7.48 cm) of pileus was recorded in Le 16 and the lowest diameter (6.68 cm) of pileus was observed in Le 8 (Table 34).

4.6.3.1.8 Thickness of pileus (TP):

Thickness of pileus was also significantly affected by the two strain of shiitake mushroom (Appendix X). The highest thickness (1.48cm) of pileus was recorded in Le 16 and the lowest thickness (1.29 cm) of pileus was recorded in Le 8 (Table 34).

4.6.3.1.9 Yield:

The data obtained from the experiment showed significant variation in yield between the strain of shiitake mushroom (Appendix X). Significantly the highest yield (111.25 g) was recorded in Le 16 and the lowest yield (98.09g) was recorded in Le 8 (Fig. 13).

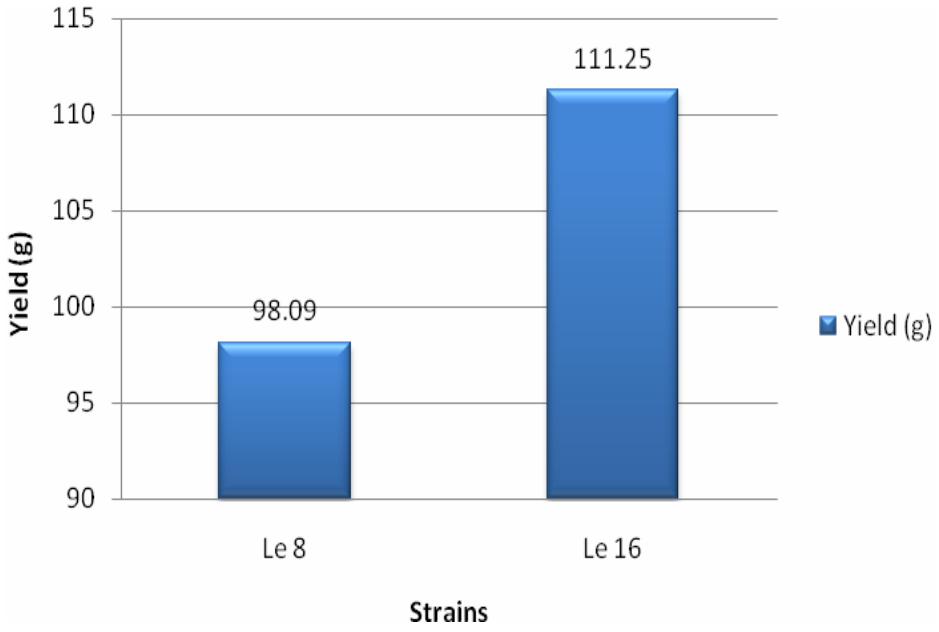


Fig. 13: Effect of two strains of shiitake mushroom on yield.

4.6.3.1.8.10 Biological efficiency (BE %):

Remarkable variation was observed in biological efficiency of two strains of shiitake mushroom (Appendix X). The highest biological efficiency (63.57 %) was obtained from Le 16 and the lowest biological efficiency (56.05 %) was recorded in Le 8 (Table 34).

4.6.3.2 Effect of different age of spawn packets on various parameters

4.6.3.2.1 Time required from opening to first harvest (TROFH):

Significant variations were observed in time required from opening to first harvest by different age of spawn packets (Appendix X). The highest time (26.38 days) required from opening to first harvest was recorded in 60 days and the lowest time (5.38 days) required from opening to first harvest was found in 120 days (Table 33).

4.6.3.2.2 Time required for harvest (TRH):

Time required for harvest was significantly influenced by different age of spawn packet (Appendix X). The highest time (136.00 days) required for harvest was recorded in 130 age of spawn packet and the lowest time (86.38 days) required for harvest was found in 60 days age of spawn packet (Table 33).

4.6.3.2.3 Number of fruiting body (NFB):

In respect of number of fruiting bodies influenced by different age of spawn packet showed significant variation (Appendix X). The number of fruiting bodies ranged from 3.63 to 37.13 (Table 33). The highest number (37.13) of fruiting body was recorded in 100 days spawn packet and the lowest number (3.63) of fruiting body was recorded in 60 days age of spawn packet.

4.6.3.2.4 Number of effective fruiting body (NEFB):

Significant variations were observed in number of effective fruiting body by different age of spawn packet (Appendix X). The highest number (27.63) of effective fruiting body was recorded in 100 days age of spawn packet and the lowest number (2.25) of effective fruiting body was recorded in 60 days old age of spawn packet (Table 33).

4.6.3.2.5 Length of stalk (LS):

The length of stalk was significantly different (Appendix X) by two strains of *Lentinus edodes* when cultured on different age of spawn packet ranged from 3.61 to 5.56 cm. The highest length (5.56 cm) of stalk was found in 60 days old of spawn packet. The lowest length (3.61 cm) of stalk was recorded in 100 days old of spawn packet (Table 34).

4.6.3.2.6 Diameter of stalk (DS):

The diameter of stalk was highly significant influenced by different age of spawn packet. The highest diameter (1.85 cm) of stalk was observed in 80 days old of spawn **packet and the lowest diameter (1.14 cm) of stalk was observed in 90 days old of** spawn packet (Table 34).

Table 33: Effect of strain and age of spawn packet on growth and yield attributes of shiitake mushroom

Strain	Time required from opening to first harvest (days)	Time required for harvest (days)	Number of fruiting body	Number of effective fruiting body
Le 8	13.75	108.75	17.81	14.00
Le 16	10.00	105.09	20.50	14.50
Age of spawn (Days)				
40	--	-----	----	----
50	---	---	----	---
60	26.38a	86.38h	3.63g	2.25e
70	22.38b	92.38g	6.50g	4.25e
80	14.50c	94.50f	10.00f	7.75d
90	7.63d	97.63e	32.75b	24.63a
100	6.50d	106.50d	37.13a	27.63a
110	6.25d	116.30c	26.38c	20.13b
120	5.38d	125.80b	21.38d	16.63c
130	6.00d	136.00a	15.50e	10.75d
LSD(0.05)	**2.17**	**2.07**	**3.35**	**3.14**
CV (%)	**12.83**	**1.36**	**12.29**	**15.51**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

--- = Not produce fruit body.

4.6.3.2.7 Diameter of pileus (DP):

The diameter of pileus of ten different age of spawn packet was varied significantly (Appendix X). The highest diameter (8.50 cm) of pileus was recorded in 70 days old of spawn packet which was statistically significant to 60 and 80 days old of spawn

packet. The lowest diameter (5.70 cm) of pileus was observed in130 days old of spawn packet (Table 34).

4.6.3.2.8 Thickness of pileus (TP):

Thickness of pileus of ten different age of spawn packet were varied significantly (Appendix X).The highest thickness (1.79 cm) of pileus was recorded in 60 days old of spawn packet which was statistically similar to 80 days old of spawn packet. The lowest thickness (1.11 cm) of pileus was recorded in 100 days old of spawn packet (Table 34).

4.6.3.2.9 Yield:

Appreciable variation was found in the yield of ten different age of spawn packet (Appendix X). The highest yield (146.90g) was recorded from 100 days old of spawn packet which was statistically similar to 90 days and 110 days old of spawn packet. The lowest yield (47.50 g) was recorded in 60 days old of spawn packet. No yield was obtained from 40 and 50 days old of spawn packet (Fig. 14). The study revealed that yields were increased with the increases of age of spawn packet at certain level and yield decreased when cultured over age spawn packet.

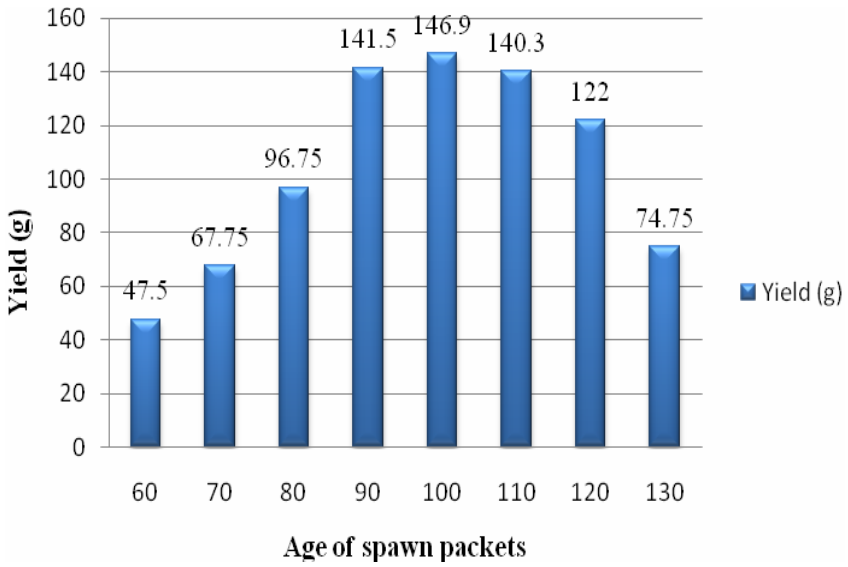


Fig. 14: Effect of age of spawn packets on yield.

4.6.3.2.10 Biological efficiency (BE %):

Remarkable variation was observed in biological efficiency of different days old of spawn packet (Appendix X). The highest biological efficiency (83.93%) was obtained from 100 days old of spawn packet which was statistically similar to 90 days and 110 days old of spawn packet. The lowest biological efficiency (27.14%) was recorded in 60 days old of spawn packet (Table 34).

Table 34: Effect of strain and age of spawn on yield attributes of shiitake mushroom

Strain	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Biological efficiency (%)
Le 8	4.49	1.38	6.68	1.29	56.05
Le 16	4.40	1.42	7.48	1.48	63.57
Age of spawn (Days)					
40	---	----	---	---	----
50	---	----	----	----	----
60	5.56a	1.36bcd	7.98a	1.79a	27.14e
70	4.99b	1.36bcd	8.50a	1.40bcd	38.72de
80	5.21ab	1.85a	8.48a	1.63ab	55.28c
90	3.83de	1.14d	6.39bc	1.33cde	80.85ab
100	3.61e	1.49b	5.98cd	1.11e	83.93a
110	4.31c	1.45bc	6.74b	1.44bc	80.14ab
120	4.16cd	1.21cd	6.89b	1.16de	69.71b
130	3.89de	1.30bcd	5.70d	1.23cde	42.71d
LSD(0.05)	**0.40**	**0.234**	**0.504**	**0.234**	**12.09**
CV (%)	**6.30**	**11.85**	**5.02**	**11.91**	**14.22**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.
---- = Not produce fruit body.

60 days

70 days

80 days

90 days

100 days

110 days

120 days

130 days

Plate: 12 Yield performance of different age of spawn packets.

4.6.3.3 Combined effect of strain and different age of spawn packet on growth, yield and yield attributes of shiitake mushroom

The data relating to the combined effect of strain and different age of spawn packet on growth, yield and yield attributes of shiitake mushroom have been presented in Table(s), Plate(s) and the data of combined effect have been presented in Appendices.

4.6.3.3.1 Time required from opening to first harvest (TROFH):

The combined effect of strain and different age of spawn packet on time required from opening to first harvest was found to be significant (Appendix X). The highest time (30.00 days) required from opening to first harvest was found from the strain Le 8 with 60 days old spawn packet treatment combination and the lowest time (4.00 days) required from opening to first harvest was found from the strain Le 16 with 90 days old spawn packet treatment combination (Table 35).

4.6.3.3.2 Time required for harvest (TRH):

The combined effect of strain and different age of spawn packet on time required for harvest was found to be significant (Appendix X). The highest time (137.00 days) required for harvest was found from the strain Le 8 with 130 days old of spawn packet treatment combination which was statistically similar to the treatment combination of Le 16 with 130 days. The lowest time (82.75 days) required for harvest was found from the strain Le 16 with 60 days old spawn packet treatment combination (Table 35).

4.6.3.3.3Number of fruiting body (NFB):

Significant variations were observed in number of fruiting body by the combined effect of strain of shiitake mushroom with different age of spawn packet (Appendix X). The number of fruiting bodies ranged from 2.50 to 45.25. The highest number (45.25) of fruiting body was recorded from the strain Le 16 with 100 days old spawn packet which was statistically similar to the same strain with 90 days old spawn packet. The lowest number (2.50) of fruiting body was recorded in 60 days age of spawn packet with the strain Le 8 (Table 35).

4.6.3.3.4 Number of effective fruiting body (NEFB):

The combined effect of strain and different age of spawn packet on number of effective fruiting body was found to be significant (Appendix X). The highest number (32.75) of effective fruiting body was recorded from the strain Le 16 with 100 days old spawn packet which was statistically similar to the same strain with 90 days old spawn packet. The lowest number (1.50) of effective fruiting body was recorded in 60 days age of spawn packet with the strain Le 8 (Table 35).

Table 35: Combined effect of strain and different age of spawn on growth and yield attributes of shiitake mushroom

Age of spawn (Days)	Time required from opening to first harvest (days)	Total days for harvest	Number of fruiting body	Number of effective fruiting body
Strain of shiitake mushroom (Le 8)				
40	-----	-----	-----	-----
50	-----	-----	-----	-----
60	30.00a	90.00i	2.50i	1.50g
70	25.00b	95.00h	5.75hi	3.50fg
80	17.25e	97.25g	15.25f	12.50e
90	11.25f	101.30f	21.00de	17.00cd
100	7.75g	107.80d	29.00b	22.50b
110	5.75ghi	115.80c	29.75b	25.00b
120	6.00ghi	126.00b	17.75ef	14.25de
130	7.00gh	137.00a	21.50cd	15.75cde
Strain of shiitake mushroom (Le 16)				
40	-----	-----	-----	-----
50	-----	-----	------	-----
60	22.75c	82.75j	4.75hi	3.00fg
70	19.75d	89.75i	7.25gh	5.00f
80	11.75f	91.75i	4.75hi	3.00fg
90	4.00i	94.00h	44.50a	32.25a
100	5.25hi	105.30e	45.25a	32.75a
110	6.75gh	116.80c	23.00cd	15.25de
120	4.75hi	125.50b	25.00c	19.00c
130	5.00	135.00a	9.50g	5.75f
LSD (0.05)	**2.167**	**2.070**	**3.347**	**3.142**
CV (%)	**12.83**	**1.36**	**12.29**	**15.51**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

4.6.3.3.5 Length of stalk (LS):

The length of stalk was significantly different (Appendix X) in two strains of *Lentinus edodes* when cultured on different age of spawn packet ranged from 3.15 to 5.75 cm. The highest length (5.75cm) of stalk was found from the treatment combination of strain Le 8 with 80 days old of spawn packet which was statistically similar to the treatment combination of Le 8 with 60 days old of spawn packet. The lowest length (3.15 cm) of stalk was recorded from the treatment combination of strain Le 16 with 130 days old of spawn packet (Table 36).

4.6.3.3.5 Diameter of stalk (DS):

The diameter of stalk was highly significant by the combined effect of strain and different age of spawn (Appendix X) The highest diameter (1.95 cm) of stalk was observed from the treatment combination of strain Le 16 with 80 days old of spawn packet and the lowest diameter (1.10 cm) of stalk was observed from the treatment combination of strain Le 16 with 90 days old of spawn packet (Table 36).

4.6.3.3.6 Diameter of pileus (DP):

The combined effect of two strains and different age of spawn packet on diameter of pileus were found significant (Appendix X). The highest diameter (10.95 cm) of pileus was recorded from the treatment combination of strain Le 16 with 80 days old of spawn packet. The lowest diameter (4.40 cm) of pileus was observed from the treatment combination of strain Le 16 with 130 days old of spawn packet (Table 36).

4.6.3.3.7 Thickness of pileus (TP):

The combined effect of two strains and different age of spawn packet on thickness of pileus were found significant (Appendix X). The highest thickness (2.15cm) of pileus was recorded from the treatment combination of strain Le 16 with 80 days old of spawn packet and the lowest thickness (1.02 cm) of pileus was observed from the treatment combination of strain Le 16 with 130 days old of spawn packet (Table 36).

4.6.3.3.8 Yield:

Appreciable variation was found in the yield from the treatment combination of two strains of shiitake mushroom with different age of spawn packet (Appendix X). The highest yield (179.50 g) was recorded from the treatment combination of strain Le 16 with 90 days old of spawn packet which was statistically similar to the same strain with 100 days old of spawn packet. The lowest yield (36.75 g) was recorded from the treatment combination of strain Le 16 with 130 days old of spawn packet. The yield decreased with the increase of age of spawn packet because more age of spawn packet becomes dry and aborted. 40 and 50 days old spawn packet did not produce any fruit body (Fig. 15).

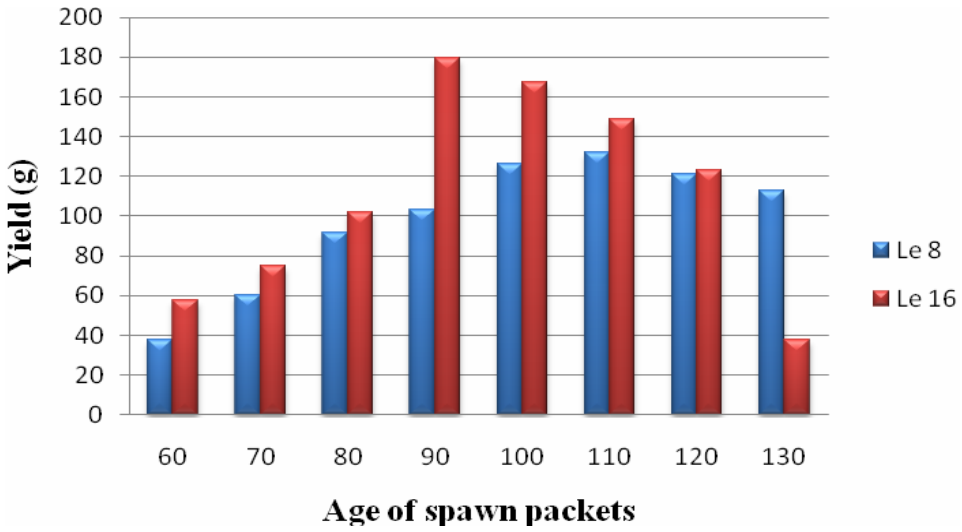


Fig. 15: Effect of strain and age of spawn packets on yield of shiitake mushroom.

4.6.3.3.9 Biological efficiency (BE %):

Remarkable variation was observed in biological efficiency from the treatment combination of different strain of shiitake mushroom with different age of spawn packet (Appendix X). The highest biological efficiency (102.60%) was obtained from the treatment combination of Le 16 with 90 days old of spawn packet which was statistically similar to the same strain with 100 days old of spawn packet. The lowest

biological efficiency (21.00%) was recorded from the treatment combination of Le 16 with 130 days old of spawn packet (Table 36).

Table 36: Combined effect of strain and different age of spawn packet on yield attributes of shiitake mushroom

Age of spawn (Days)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Biological efficiency (%)
Strain of shiitake mushroom (Le 8)					
40	-----	-----	-----	-----	-----
50	-----	-----	-----	-----	-----
60	5.58a	1.35c-f	6.68efg	1.70b	21.43j
70	4.95b	1.25def	7.00def	1.38cd	34.57i
80	5.75a	1.75ab	6.00h	1.10e	52.28gh
90	3.83d	1.18ef	6.53fgh	1.38cd	59.14efg
100	3.60d	1.55bc	6.58fg	1.10e	72.14de
110	3.75d	1.48cd	6.30gh	1.18cde	75.29cd
120	3.85d	1.15ef	7.35d	1.10e	69.14def
130	4.63bc	1.30cdef	7.00def	1.43c	64.43d-g
Strain of shiitake mushroom (Le 16)					
40	-----	-----	-----	-----	-----
50	-----	-----	-----	-----	-----
60	5.55a	1.38cde	9.28c	1.88b	32.86ij
70	5.02b	1.48cd	10.00b	1.43c	42.86hi
80	4.68bc	1.95a	10.95a	2.15a	58.28fg
90	3.83d	1.10f	6.25gh	1.28cde	102.60a
100	3.63d	1.43cde	5.38i	1.13de	95.71ab
110	4.88bc	1.43cde	7.18de	1.70b	85.00bc
120	4.48c	1.28c-f	6.43gh	1.23cde	70.28def
130	3.15e	1.30c-f	4.40j	1.02e	21.00j
LSD (0.05)	**0.40**	**0.23**	**0.50**	**0.23**	**12.09**
CV (%)	**6.30**	**11.85**	**5.02**	**11.91**	**14.22**

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

---- = Not produce fruit body.

Results of the present experiment reveal that there are appreciable variations in yield and yield contributing attributes with the variation of age of spawn packets and strain of shiitake mushroom. In terms of yield and yield attributes performance of the strain Le 16 was better than Le 8. In case of different age of spawn packets 100 days old spawn packet gave the highest yield while 40 and 50 days old spawn packet did not produce any fruit body. The yield decreased with the increase of age of spawn packets. On the other hands the spawn opens too early the crop may be failed. Many other investigators also found variations in effect of age of spawn packets on growth, yield and yield contributing characters of shiitake mushroom.

Ahmed *et al.* 2010 reported that the lowest days required from opening to first harvest was observed in spawn packets opened after 130 days of spawning which was statistically similar to all the spawn age except 80 and 90 days. The highest days required from opening to first harvest was recorded in 80 days spawn age.

Leatham (1985) reported that shorter period of spawn run gave limited fruiting bodies and longer period 105 to 150 days gave more fruiting bodies. Ahmed *et al.*, 2010 also reported that highest number of fruiting body was recorded in 110 days old spawn packet which was statistically similar to 120 days old spawn packet whereas the lowest number of fruiting body was recorded in 80 days old spawn.

Ahmed *et al.*, 2010 described that yields were increased with the increase of age of spawn packets. Similar result was reported by Royse (1985). Ahmed *et al*., 2010 also reported the highest yield was obtained from 110 days old spawn packet which was statistically similar to 120 and 130 days old spawn and the lowest yield was recorded in 80 days old spawn.

Ahmed *et al.*, 2010 reported highest biological efficiency was recorded in 110 days old spawn which was statistically similar to 120 days and 130 days and lowest in 80 days. Almost similar result was reported by Royse and Bahler (1986) stating that biological efficiency increased with the increase of incubation time.

CHAPTER V
SUMMARY AND CONCLUSION

Six experiments were conducted at the Plant Tissue Culture (PTC) laboratory and the Mushroom culture house (MCH) of National Mushroom Development and Extension Centre, Savar, Dhaka during the period from July 2011 to March 2014 to study the effect of different strains of shiitake mushroom under four seasons, effect of level of supplement and their combination, effect of different substrates, effect of different opening patterns, effect of amount of substrate, effect of age of spawn packets on growth and yield of shiitake in Bangladesh. The experiment was laid out in the Completely Randomized Design (CRD) with 4 replications. All the experiments were two factors except first experiment. The first experiment was single factor.

The objectives of the first experiment to find out the suitable strain under four seasons for successful cultivation of shiitake mushroom. The experiment was carried out during June 2011 to April 2012. Twenty three (23) strains of shiitake mushroom were cultivated in four different seasons like autumn (August to October), late autumn (October to December), winter (December to February) and spring (February to April). The highest mycelium growth rate (7.95 mm/day) and the lowest time (12.25 days) required to completion of mycelium running was found in autumn season by the strain Le 19. From all the strain and all seasons, the highest number of effective fruiting body (39.50) was recorded from the strain Le 12 in autumn and the lowest number of effective fruiting body (1.00) was recorded from the strain Le 20 in spring season. Among the strains, maximum number of strain produces fruit body in winter and spring season and minimum in autumn season. Out of 23 strains, only five strains produce fruit body in autumn season, sixteen in late autumn season and twenty two in winter as well as spring season. Most of the strains gave higher yield in winter season compare to other seasons while yield performance was poor in autumn season. Yield of different strains of shiitake mushroom in autumn, late autumn, winter and spring season ranged from 45.25-145.80 g, 18.75-175.50 g, 40.50-191.00 g and 39.50-163.50 g, respectively. The highest yield (191.00 g) was obtained from the strain Le 8 in winter season and the lowest yield (18.75g) gave the strain Le 18 in late autumn season. Each strain gave different yield on season based. Le 8 gave the highest yield

(191.00 g) and highest biological efficiency (109.10 %) in winter season. The strain Le 8 also gave highest yield (145.00 g) and highest biological efficiency (83.29 %) in autumn season. Le 16 gave the highest yield (175.00 g) and highest biological efficiency (100.30%) in late autumn season which was statistically similar to the strain Le 8 and second highest yield (171.80 g) in winter season. Le 12 gave second highest yield (99.00 g) and biological efficiency (56.57 %) in autumn season which was statistically similar to Le 11. Le 1 gave the highest yield (163.50 g) and highest biological efficiency (93.43 %) in spring season but no yield was obtained in autumn season. Le 13 did not produce any fruit body, so no yield was found from the strain in any season. Considering the strain and season, it can be concluded that four strains like Le 8, Le 11, Le 12 and Le 16 can be commercially cultivated in autumn, late autumn and winter season in Bangladesh condition. Of them, Le 8 was the best strain and winter was the best season in Bangladesh context.

The second experiment was conducted during August 2011 to March 2012 and the objective of the study to determine the optimum level of supplement. Two strains of shiitake mushroom namely Le 8 and Le 11 as well as three different supplements and their combination viz. wheat bran, rice bran, maize powder, wheat bran + rice bran (1:1), wheat bran + maize powder (1:1) and wheat bran + rice bran + maize powder (1:1:1) were used in the experiment as treatments. The highest mycelium growth rate (0.51cm/day) was found in strain Le 8 and rice bran treatment combination while the lowest (0.34 cm/day) mycelium growth rate was found in Le 8 and wheat bran treatment combination. The highest (45 days) time required to completion of mycelium running was obtained from the strain Le 11 and rice bran + wheat bran (1:1) treatment combination. The maximum time required for bump formation (105 days) from the combination of strain Le 11 with wheat bran and minimum time was found (81 days) from strain Le 8 with maize powder treatment combination. The highest yield (159.00 g) was recorded in Le 8 with wheat bran treatment combination. The lowest yield (98.25 g) was recorded from strain Le 11 with rice bran treatment combination. The number of fruiting body, biological efficiency, biological yield and economic yield of shiitake mushroom increased gradually with the increased levels of supplements up to a certain level and started to decrease thereafter. Among the six

supplements used in the experiments wheat bran was better than other supplements and Le 8 performs better than Le 11.

The third experiment in the series carried out during October 2011 to March 2012 to identify the suitable strain and best substrate for cultivation of shiitake mushroom available in Bangladesh. Ten different substrates like sawdust of teak chambul (*Michelia campaca*), ipil-ipil (*Leucaena leucocephala*), teak (*Tectona grandis*), gamari (*Gmelina arborea*), rain tree (*Albizia saman*), mahagony (*Swietenia mahagoni*) and mango (*Mangifera indica*), mixed sawdust, mixed woodchips and rice straw on growth, yield and yield attributes of two strains (Le 8, Le 12) of shiitake mushroom (*Lentinus edodes*). Mycelium growth rate, time required to completion of mycelium running, time required for bump formation, time required from opening to harvest, time required for harvest in spawn packet varied significantly with different substrates. Yield attributes of two strains such as stalk diameter, pileus diameter and pileus thickness were significantly higher when culture on mango sawdust. The highest number of effective fruiting body (33.75), yield (189.8g/packet) and biological efficiency (108.4%) were recorded from the strain Le 8 grown on mixed sawdust followed by mango sawdust. In general, performance of Le 8 was better than Le 12 in terms of yield and yield contributing characters. Considering yield and yield attributes, strain Le 8 and substrate prepared from mango sawdust and mixed sawdust may be recommended to grow shiitake mushroom in Bangladesh.

The fourth experiment was conducted during October 2011 to March 2012 to find out the appropriate opening pattern and suitable strain. The experiments consists of eleven types of opening pattern such as top open place on floor, top open place on rack, total open and covered with polypropylene bag place on floor, total open and covered with polypropylene bag place on rack, only cotton plug open and place on floor, no open and place on floor, only cotton plug open and place on rack, no open and place on rack (control), total open and place on rack, total open and place on floor, no open and place on culture house floor were used on growth, yield and yield attributes of two strains (Le 8, Le 16) of shiitake mushroom. Time required for bump formation, time required for bump formation after treatment, time required from opening to harvest, time required for harvest was found lowest from the treatment combination Le 16

with T_5 (only cotton plug open and place on floor) and those parameters were highest except time required from opening to harvest in the treatment T_8 (where spawn packets were no open and place on rack i.e. control) with the strain Le 8. Yield attributes of two strains such as diameter and thickness of pileus were significantly higher when packets were total open and place on rack with Le 16 and diameter of stalk was higher in treatment where only cotton plug open and place on floor with Le 8. The highest length of stalk (1.88 cm) was found from the treatment combination of Le 16 with T_2 (Top open place on rack.). The highest number of fruiting body (62.00), the highest number of effective fruiting body (37.25, highest yield (193.00 g) and highest biological efficiency (110.30%) were recorded from the strain Le 16 with treatment T_5 (Only cotton plug open and place on floor) and those parameters were lowest from the treatment combination of Le 8 with T_{10} (Total open and place on floor). In general, performance of Le 16 was better than Le 8 in terms of yield and yield contributing characters. Strain Le 16 and treatment T_5 i.e. only cotton plug open and place on floor may be recommended to grow shiitake mushroom under Bangladesh conditions.

The fifth experiment in the series carried out during October 2011 to March 2012 to identify the suitable strain and appropriate amount of substrate for successful cultivation of shiitake mushroom available in Bangladesh. The experiment consists of four different amount of substrates (300g, 500g, 750g and 1000g) and four strains (Le 8, Le 11, Le 12 and Le 16) of shiitake mushroom. The growth, yield and yield contributing characters of shiitake mushroom were significantly influenced by the different amount of substrate. The time required for mycelium running and total harvest were increased with the increases of amount of substrate. The number of fruiting body was highest (81.50) in 500g size of spawn packet and it was lowest (1.25) in 1000g size of spawn packet. The maximum yield (135.80g) was recorded from Le 12 when cultured on 500g size of spawn packet and the lowest yield (24.50 g) was obtained from 750g size of spawn for similar strain. The highest biological efficiency (85.47%) was observed from the treatment combination of 300 g spawn packet with Le 11. The biological efficiency decreased with the increase of size of spawn packet. Among the strain Le 8 perform better than other variety and 500 g spawn packet gave the highest yield.

The sixth experiment was carried out during September 2011 to January 2012 to determine the right age of spawn packet for shiitake mushroom cultivation. In the experiment ten different age (40, 50, 60, 70 80, 90, 100, 110, 120 and 130 days) of spawn packets and two different strain (Le 8 and Le 16) of shiitake mushroom were used. A wide variation was observed in yield and biological efficiency in different ages. The highest yield (146.90 g) and biological efficiency (83.93%) were recorded in 100 days old spawn packet followed by 90 and 110 days old spawn packets and the lowest yield (47.50 g) and biological efficiency (27.14%) were found in 60 days old spawn packet. No yield was obtained from the 40 and 50 days spawn packets. Among the strain Le 16 perform better than other strain and 90, 100 and 110 days old spawn packet gave better performance than other days old of spawn packet in all aspects.

From the above discussion, it can be concluded that the production technology of shiitake mushroom depends on several factors such as: strains or varietals characteristics, environmental factors such as co_2 concentration, low temperature, substrate quality and composition, level of supplement as well as composition, bump initiation and bump quality, amount of substrate or bag size, age of spawn packet, opening pattern and placement of spawn packet. Strain Le 8, Le 11, Le 12, Le 16 can be commercially cultivated in Bangladesh. In case of season, autumn suitable for mycelium running, late autumn and winter suitable for cultivation of shiitake mushroom. Mango and mixed sawdust can be used as best substrate. Wheat bran (6kg/100 packets) can be used as best supplement. Only cotton plug open and place on floor perform better than other opening pattern. 500g bag size give maximum yield and 90-100 days old spawn packet suitable for cultivation of shiitake mushroom. However, the experiments may be repeated to confirm the present findings.

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APPENDICES

Appendix I: Weather data during the period of experimental site (July 2011 to March 2014)

Year	Month	Max. (^{0}C)	Min. (^{0}C)	Av. (^{0}C)	Humidity (%)	Rainfall (mm)
2011	July	32.3	26.7	29.5	86.50	305.00
	August	31.1	26.5	28.8	83.05	258.00
	September	32.4	26.4	29.4	84.00	308.00
	October	32.7	24.7	28.7	82.35	127.00
	November	29.7	19.2	24.4	73.10	0.00
	December	25.0	15.0	20.0	77.20	0.00
	Average	**30.5**	**23.0**	**26.8**	**81.03**	**166.33**
2012	January	23.8	12.8	18.3	72.50	5.00
	February	28.9	16.2	22.5	78.45	0.00
	March	34.1	23.3	28.7	79.55	88.00
	April	35.5	26.4	30.9	68.50	26.80
	May	34.3	25.9	30.1	76.06	107.80
	June	33.1	26.7	29.9	76.70	91.20
	July	33.0	27.4	30.2	87.25	493.40
	August	33.1	27.1	30.1	85.93	161.30
	September	32.5	26.6	29.5	85.03	130.80
	October	32.4	25.1	28.7	86.90	272.00
	November	30.1	20.9	25.5	82.16	0.00
	December	26.1	15.5	20.8	75.03	0.80
	Average	**31.4**	**22.8**	**27.1**	**79.50**	**114.75**
2013	January	25.4	12.7	19.0	75.61	0.00
	February	28.1	15.5	21.8	71.32	0.00
	March	32.5	20.4	26.4	59.38	7.40
	April	33.7	23.6	28.7	69.43	36.80
	May	32.9	24.5	28.7	77.22	182.90
	June	32.1	26.1	29,1	84.96	188.90
	July	31.4	26.2	28.8	85.87	127.20
	August	31.6	26.3	28.9	86.06	248.40
	September	31.8	25.9	28.8	86.83	303.40
	October	31.6	23.8	27.7	82.87	36.40
	November	29.6	19.2	24.4	80.53	10.40
	December	26.4	14.1	20.2	79.38	0.00
	Average	**30.6**	**21.5**	**26.0**	**78.28**	**95.15**
2014	January	25.7	11.2	18.4	77.47	0.00
	February	27.4	17.5	22.4	70.31	0.00
	March	33.1	22.7	27.9	57.82	7.40
	Average	**28.7**	**17.1**	**22.9**	**68.53**	**2.47**

Appendix II: Analysis of variance of the data in respect of time required from opening to first harvest (days), time required for harvest (days), number of fruiting body, number of effective fruiting body, length of stalk (cm), diameter of stalk (cm), diameter of pileus (cm), thickness of pileus (cm), yield (g) and biological efficiency (%) due to the effect of different strains of shiitake mushroom in autumn season

Source of variation	Degrees of freedom	Mean of Sum of Square				
		Time required from opening to first harvest (days)	Time required for harvest (days	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)
Factor A	**4**	1.050NS	329.675***	1700.200***	1121.125***	6.372***
Error	**15**	1.517	3.367	3.133	4.483	0.101

Contd.

Source of variation	Degrees of freedom	Mean of Sum of Square				
		Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)	Biological efficiency (%)
Factor A	4	0.119**	3.362***	0.213***	5468.200***	1785.419***
Error	15	0.019	0.074	0.016	10.867	3.540

Appendix III: Analysis of variance of the data in respect of time required from opening to first harvest (days), time required for harvest (days), number of fruiting body, number of effective fruiting body, length of stalk (cm), diameter of stalk (cm), diameter of pileus (cm), thickness of pileus (cm), yield and biological efficiency (%) due to the effect of different strains of shiitake mushroom in late autumn season

Source of variation	Degrees of freedom	Mean of Sum of Square				
		Time required from opening tofirst harvest (days)	Time required for harvest (days	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)
Factor A	**4**	19.034***	378.886***	1152.211***	614.592***	10.096***
Error	**15**	2.044	10.018	6.215	5.096	0.246

Contd.

Source of variation	Degrees of freedom	Mean of Sum of Square				
		Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)	Biological efficiency (%)
Factor A	4	0.624***	10.178***	0.723***	6549.627***	2138.521***
Error	15	0.051	0.315	0.047	24.430	7.975

= Highly significant* = Significant at 1% level* = Significant at 5% level NS= Non significant

Appendix IV: Analysis of variance of the data in respect of time required from opening to first harvest (days), time required for harvest (days), number of fruiting body, number of effective fruiting body, length of stalk (cm), diameter of stalk (cm), diameter of pileus (cm), thickness of pileus (cm), yield and biological efficiency (%) due to the effect of different strains of shiitake mushroom in winter season

Source of variation	Degrees of freedom	Mean of Sum of Square				
		Time required from opening to first harvest (days)	Time required for harvest (days	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)
Factor A	**22**	20.736***	781.444***	1088.397***	635.212***	2.387***
Error	**69**	2.068	18.345	8.875	4.242	0.286

Contd.

Source of variation	Degrees of freedom	Mean of Sum of Square				
		Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)	Biological efficiency (%)
Factor A	22	0.931***	10.219***	0.680***	7446.927***	2441.428***
Error	69	0.082	0.535	0.041	66.110	20.644

Appendix V: Analysis of variance of the data in respect of time required from opening to first harvest (days), time required for harvest (days), number of fruiting body, number of effective fruiting body, length of stalk (cm), diameter of stalk (cm), diameter of pileus (cm), thickness of pileus (cm), yield and biological efficiency (%) due to the effect of different strains of shiitake mushroom in spring season

Source of variation	Degrees of freedom	Mean of Sum of Square				
		Time required from opening to first harvest (days)	Time required for harvest (days	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)
Factor A	**22**	20.736***	1686.422***	492.438***	336.379***	5.846***
Error	**69**	1.490	4.072	1.981	1.282	0.050

Contd.

Source of variation	Degrees of freedom	Mean of Sum of Square				
		Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)	Biological efficiency (%)
Factor A	22	1.251***	8.351***	0.831***	4388.892***	1433.048***
Error	69	0.018	0.056	0.021	6.670	2.180

***= Highly significant ** = Significant at 1% level * = Significant at 5% level NS= Non significant

Appendix VI: Analysis of variance of the data in respect of mycelium growth rate (mm/day), time required for mycelium running (days) , time required for bump formation (days), time required from opening to first harvest (days), time required for harvest (days), number of fruiting body, number of effective fruiting body, length of stalk (cm), diameter of stalk (cm), diameter of pileus (cm), thickness of pileus (cm), yield and biological efficiency (%) due to the effect of two strains and different level of supplements on growth and yield of shiitake mushroom

Source of variation	Degrees of freedom	Mean of Sum of Square					
		Mycelium growth rate (mm/day)	Time required for mycelium running (days)	Time required for bump formation (days)	Time required from opening to first harvest (days)	Time required for harvest (days)	Number of fruiting body
Treatment	11	0.002	14.194	74.427	16.259	663.000	161.136
Factor A	5	0.028***	116.238***	678.683***	35.833***	660.433***	1746.083***
Factor B	1	0.000NS	38.521***	114.083***	114.083***	0.083NS	0.333NS
AB	5	0.001NS	1.371NS	25.933***	28.933***	2.483NS	26.083NS
Error	36	0.001	2.826	5.014	3.222	3.306	13.931

Contd

Source of variation	Degrees of freedom	Mean of Sum of Square						
		Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)	Biological efficiency (%)
Treatment	11	113.593	0.640	0.033	1.374	0.057	342.045	111.813
Factor A	5	1127.538***	5.417***	0.060NS	8.678***	0.440***	2748.533***	897.840***
Factor B	1	58.521*	0.270NS	0.200NS	5.333**	0.146NS	990.083NS	324.220NS
AB	5	63.471***	1.356***	0.105NS	1.111NS	0.051NS	23.883NS	7.890NS
Error	36	11.910	0.226	0.052	0.632	0.044	251.903	82.238

*****=** Highly significant

** = Significant at 1% level

* = Significant at 5% level

NS= Non significant

Appendix VII: Analysis of variance of the data in respect of mycelium growth rate (mm/day), time required for mycelium running (days), time required for bump formation (days), time required from opening to first harvest (days), time required for harvest (days), number of fruiting body, number of effective fruiting body, length of stalk (cm), diameter of stalk (cm), diameter of pileus (cm), thickness of pileus (cm), yield and biological efficiency (%) due to the effect of two strains and different substrates on growth and yield of shiitake mushroom

Source of variation	Degrees of freedom	Mean of Sum of Square					
		Mycelium growth rate (mm/day)	Time required for mycelium running (days)	Time required for bump formation (days)	Time required from opening to first harvest (days)	Time required for harvest (days)	Number of fruiting body
Treatment	19	0.536	289.270	39.646	6.093	58.496	226.207
Factor A	9	3.336***	2737.478***	707.338***	56.450***	1025.788***	358.983***
Factor B	1	4.802***	2205.000***	0.521NS	14.083***	6.021NS	3570.750***
AB	9	2.050***	553.667***	45.371***	45.233****	79.621***	368.200***
Error	60	0.020	12.725	1.979	1.125	2.090	2.861

Contd.

Source of variation	Degrees of freedom	Mean of Sum of Square						
		Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)	Biological efficiency (%)
Treatment	19	128.198	0.382	0.013	0.934	0.030	1920.271	627.041
Factor A	9	316.771***	4.373***	0.157***	4.918***	0.298***	5818.683***	1900.123***
Factor B	1	1887.521***	1.505***	0.035NS	10.083***	0.194***	26602.083***	8686.548***
AB	9	231.471***	1.383***	0.058NS	2.750***	0.069***	4064.383***	1327.119***
Error	60	2.076	0.095	0.030	0.187	0.013	28.514	9.308

*****=** Highly significant

** = Significant at 1% level

* = Significant at 5% level

NS= Non significant

Appendix VIII: Analysis of variance of the data in respect of time required for bump formation (days), time required for bump formation after treatment (days), time required from opening to first harvest (days), time required for harvest (days), number of fruiting body, number of effective fruiting body, length of stalk (cm), diameter of stalk (cm), diameter of pileus (cm), thickness of pileus (cm), yield and biological efficiency (%) due to the effect of different strains and different types of opening pattern on growth and yield of shiitake mushroom

Source of variation	Degrees of freedom	Mean of Sum of Square					
		Time required for bump formation (days)	Time required for bump formation after treatment (days)	Time required from opening to first harvest (days)	Time required for harvest (days)	Number of fruiting body	Number of effective fruiting body
Treatment	21	99.612	15.612	1.495	122.711	644.138	161.823
Factor A	10	28.275***	28.275***	6.534***	39.734***	1235.409***	547.011***
Factor B	1	2062.227***	298.227***	20.045***	2531.636***	11684.045***	2662.000***
AB	10	1.352NS	1.352NS	4.820***	5.561NS	607.445***	189.275***
Error	66	1.417	1.417	0.417	2.856	5.773	5.265

Contd.

Source of variation	Degrees of freedom	Mean of Sum of Square					
		Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)	Biological efficiency (%)
Treatment	21	0.021	0.096	2.594	0.082	2388.567	780.770
Factor A	10	0.136***	1.000***	15.272***	0.448***	14183.109***	4640.319***
Factor B	1	0.124*	0.016NS	28.751***	0.870***	33540.045***	10960.905***
AB	10	0.174***	1.004***	10.455***	0.396***	2436.745***	794.951***
Error	66	0.025	0.084	0.143	0.032	21.227	3.583

***= Highly significant

** = Significant at 1% level

* = Significant at 5% level

NS= Non significant

Appendix IX: Analysis of variance of the data in respect of mycelium growth rate (mm/day), time required for mycelium running (days), time required for bump formation (days), time required from opening to first harvest (days), time required for harvest (days), number of fruiting body, number of effective fruiting body, length of stalk (cm), diameter of stalk (cm), diameter of pileus (cm), thickness of pileus (cm), yield and biological efficiency (%) due to the effect of different strain and different amount of substrates on growth and yield of shiitake mushroom

Source of variation	Degrees of freedom	Mean of Sum of Square					
		Mycelium growth rate (mm/day)	Time required for mycelium running (days)	Time required for bump formation (days)	Time required from opening to first harvest (days)	Time required for harvest (days)	Number of fruiting body
Treatment	15	0.003	964.957	559.172	13.085	732.660	413.171
Factor A	3	0.032***	12544.474***	6093.938***	124.807***	8025.891***	4516.807***
Factor B	3	0.012***	1921.516***	2181.771***	57.641***	2861.557***	829.641***
AB	9	0.001**	8.363**	111.868***	13.821***	102.446***	851.127***
Error	48	0.000	2.370	10.188	0.891	9.516	5.401

Contd.

Source of variation	Degrees of freedom	Mean of Sum of Square						
		Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)	Biological efficiency (%)
Treatment	15	215.620	0.658	4.300	4.380	0.154	1262.685	1115.134
Factor A	3	2098.932***	3.445***	23.307***	8.013***	0.827***	16986.807***	16225.924***
Factor B	3	786.922***	2.614***	21.575***	43.245***	1.112***	1070.432***	184.828***
AB	9	348.460***	3.822***	19.610***	14.445***	0.384***	883.043***	316.268***
Error	48	4.682	0.225	0.099	0.754	0.012	9.599	5.270

***= Highly significant\
** = Significant at 1% level
* = Significant at 5% level
NS= Non significant

Appendix X: Analysis of variance of the data in respect of time required from opening to first harvest (days), time required for harvest (days), number of fruiting body, number of effective fruiting body, length of stalk (cm), diameter of stalk (cm), diameter of pileus (cm), thickness of pileus (cm), yield and biological efficiency (%) due to the effect of strain and age of spawn packet on growth and yield of shiitake mushroom

Source of variation	Degrees of freedom	Mean of Sum of Square				
		Time required from opening to first harvest (days)	Time required for harvest (days)	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)
Treatment	15	52.985	180.250	110.017	60.242	0.941
Factor A	7	551.714***	2470.569***	1215.205***	714.500***	4.135***
Factor B	1	225.000***	213.891***	115.563***	4.000NS	0.131NS
AB	7	18.071***	19.283***	319.491***	185.143***	9.857***
Error	48	2.323	2.120	5.542	4.885	3.767

Contd.

Source of variation	Degrees of freedom	Mean of Sum of Square				
		Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)	Biological efficiency (%)
Treatment	15	0.029	2.194	0.089	1214.263	396.484
Factor A	**7**	0.376***	9.740***	0.429***	11763.891***	3841.082***
Factor B	1	0.026NS	10.320***	0.526***	2769.391***	904.355***
AB	**7**	0.034***	12.857***	0.381***	3680.676***	1201.827***
Error	48	0.027	0.126	0.027	221.516	72.336

***= Highly significant
** = Significant at 1% level
* = Significant at 5% level
NS= Non significant.