DEVELOPMENT OF PRODUCTION TECHNOLOGIES FOR MILKY WHITE MUSHROOM (*Calocybe indica*) CULTIVATION IN BANGLADESH

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DEVELOPMENT OF PRODUCTION TECHNOLOGIES FOR MILKY WHITE MUSHROOM (*Calocybe indica*) CULTIVATION IN BANGLADESH

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HORTICULTURE

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

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January, 2020 *The Author*

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Dated: June 2020
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ABSTRACT

The present research work was conducted to identify suitable strains and develop appropriate production technologies for commercial cultivation of milky white mushroom in Bangladesh. To satisfy the objectives, eight different experiments were conducted in Mushroom Development Institute, Savar, Dhaka, Bangladesh during 2018 to 2020. In the first experiment, performance of four strains of milky white mushroom Cid-1, Cid-A, Cid-In and Cid-S were evaluated during three growing seasons in a year. Among the strains Cid-1 performed better during summer and rainy season and Cid-A during autumn season. Whereas, performance of strain Cid-S was worst during all the growing season. DNA finger print showed that the four strains were genetically different from each other. In the second experiment nine different combination of substrates were evaluated to identify suitable substrate for milky white mushroom cultivation. Results revealed that, economic yield (427.33 g/packet) was highest in rice straw + sawdust (1:1) substrate followed by rice straw along (352.00 g/packet) on the other hand it was lowest in sawdust + wheat bran (2:1) substrate (264.05 g/packet). Wide variation was observed in nutrient content of fruiting body grown on different substrates. Eleven different combination of casing materials were evaluated in the third experiment. Results indicated that, coconut coir dust + decomposed cow dung (1:1) was the best and coconut coir dust alone was the worst performing casing material. Among different sterilization and spawning methods, hot water treated substrate spawning both in three layers and thoroughly performed better. Different moisture levels of rice straw substrate viz; 35, 40, 45, 50, 55, 60, 65, and 70 percent were used to determine appropriate moisture level of the substrate in the fifth experiment. It was observed that mycelium colonization was faster (14.5 days) at 70% moisture and no mycelium colonization at 35% moisture level. No substrate contamination was observed at 65% and 70% moisture level. Highest yield (361.1g) was recorded at 70% moisture level which was similar to 60% (315.2 g/packet) and 65% (303.8 g/packet) moisture level. In the sixth experiment five different techniques of casing material management were practiced and observed that, removal of dried non effective fruiting bodies after each harvest produced highest number of effective fruiting bodies (8.83) and number of flushes (2.81) but the economic yield and biological efficiency was not insignificantly affected by casing material management technique. To determine appropriate spawn density, 10, 20, 30, 40 and 50 percent rice grain spawn (dry weight basis) was used to inoculate rice straw substrate in the seventh

experiment. Results revealed that partial mycelium colonization in spawn packets problem was completely disappeared with the increase of spawn density. Shortest time was required to complete spawn run (12.90 days) and primordia initiation (9.58 days), highest number of effective fruiting body (9.10) and number flushes (3.10), highest economic yield (454.88 g/packet) and biological efficiency (109.61%) were recorded at 50% spawn density but benefit cost ratio (3.83) was highest at 40% spawn density. To determine appropriate harvesting age of fruiting body for getting maximum yield and longer shelf life of milky white mushroom, fruiting bodies were harvested at 5 to 14 days old and stored in refrigerator and ambient condition- in open tray, cellophane wrapped tray and polypropylene bag. Results revealed that the highest number of effective fruiting bodies (9.15) were recoded from five days aged fruiting body harvest but average weight of fruiting body (63.35 g) and economic yield (483.13 g/packet) were highest at eight days harvest. The appearance and odor score of fruiting body decreased after nine days aged and lost its acceptability for consumption after thirteen days aged. The mushrooms lost its acceptability rapidly when it was stored in an open tray than cellophane paper wrapped tray and polypropylene bag both in refrigerator and in ambient condition. Within six days of storage at ambient condition milky mushroom lost its acceptability irrespective of fruiting body age and storage method. In refrigerator six to nine days aged fruiting body stored in cellophane paper wrapped tray and polypropylene bag was remained in good condition for consumption even after 15 days of storage.

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CHAPTER – I

INTRODUCTION

The word mushroom has been used in a variety of ways at different times and in different countries. Broad use of the term mushroom embraces all larger fungi, or all fungi with stalks and caps, or all large fleshy fungi. The term mushroom is broadly defined as "a mushroom is a macro fungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand" (Chang and Miles, 1992). Currently, it is known about 14,000 mushroom species, which would account for 10% of the estimated mushroom species. Of these, about 50%, or 7000 species, are considered to possess varying degrees of edibility, and more than 3000 species from 31 genera are regarded as prime edible mushrooms (Chang and Miles, 2004). About 2000 species are medicinal mushrooms with a variety of health attributes (Chang and Mshigeni, 2001). Less than 25 species are largely used as foods, being produced in commercial scale. For around 300 million years, mushrooms have been a part of the fungal diversity (Chang and Miles, 2004). Prehistoric humans probably used mushrooms collected in the wild as food and possibly for medicinal purposes. But, cultivation technology of mushrooms did not come into existence being until 600 A.D. when *Auricularia auricular* was first cultivated in China on wood logs. But the biggest advance in mushroom cultivation came in French about 1600 A.D. when *Agaricus bisporus* was cultivated upon a composted substrate. Evolution of food culture has brought mushroom in the limelight. Its unique nutritional value and taste derived human understanding in civilized societies and compulsion/ethnic food (as wild and farm-grown), the mushroom is becoming popular (Bokaria *et al.*, 2014).

Since ancient time mushrooms have been used as food, especially in the eastern countries and recognized as natural and healthy foods. As time progress, there was an increase in awareness about mushrooms as they are rich in proteins, fibres and contain low amount of calories and fat (Khan *et al.*, 2011). Mushrooms not only provide nutritious, protein-rich food, but some species also produce medicinally effective products. Mushrooms are

rich sources of nutraceuticals and their bioactive properties are already reported (Lindequist *et al*., 2005 and Çaglarirmak, 2007). Many studies demonstrated that mushroom has antioxidant potential and antitumor, antibacterial, antiviral and haematological activity (Yang *et al*., 2002 and Ribeiro *et al*., 2006). It also provides an economically acceptable alternative for the production of food of superior taste and quality, as well as high value-added secondary metabolites such as enzymes or polysaccharides (Suman *et al*., 2018).

According to world population prospect (2019), the world population is currently 7.7 billion and it is increasing at a faster rate. By the year 2050, the global population is expected to reach 9.7 billion and during 2100 it could be 10.9 billion. Shortage of food and diminishing quality of human health will be growing concerns because of the population increase and urbanization, with a concomitant reduction in arable land. Mushroom is one of the most economically viable and sustainable biotechnology processes to address world food demand, especially protein demand. Consumption of edible fungi to fulfil human nutritional needs has been a common denominator in the history of mankind (Chang, 2006 and Wakchaure, 2010). Cultivated mushrooms are now an important agricultural product worldwide. Fortune Business Insights said in their market research report (2019), the total world production of edible and medicinal mushrooms was estimated to exceed 12.74 million metric tons, with a value of about U.S. $38.13 billion.

One major problem and irony of the planet is, there is a concern for food safety and security on one hand and huge loss from agricultural waste on the other hand. Though the major part of the crop residues is being used as fodder, rest is wasted in different ways. The agricultural waste constitutes mainly of cellulose, hemicellulose and lignin. Lignin fraction which is generally considered as recalcitrant, but in mushroom this fraction has remained as the material of choice as mushroom possesses a group of complex extracellular enzymes which can degrade and utilize the lignocellulosic wastes for fruit body production (Bokaria *et al*., 2014) and play an important role to reduce environmental pollution. Recently it has been revealed that mushroom mycelia can play a significant role in the restoration of damaged environments.

Historically, mushrooms were classified among the so-called lower plants in the Division Thallophyta by Linnaeus. This was largely due to the relatively simple, anatomically uncomplicated structural attributes (lack of true roots, true stems, true leaves, true flowers, and true seeds). The presence of a cell wall-related them to plants rather than to animals. Modern studies have established that mushroom biota, together with other fungi, have features of their own, which are sufficiently and significantly distinct to place them in a separate fungal kingdom, the Kingdom Myceteae. The fungi differ from the plant and animal kingdoms by their possession of a cell wall that is different in composition from that of plants and a mode of nutrition that is heterotrophic but, unlike animals, is absorptive (osmotrophic) rather than digestive.

The milky mushroom (*Calocybe indica* P & C) is relatively new to the world of mushroom industry and third most important in production in India. This mushroom was first reported in India by Purkayastha and Chandra in 1974. *Calocybe* is a small genus of about 40 species of mushroom, which is edible and distributed in the tropical parts of the world (Shukla and Jaitly, 2013). It belongs to the kingdom Fungi, phylum Basidiomycota, class Agaricomycetes, order Agaricales and family Lyophyllaceae (Purkayastha and Chandra, 1974). This is a tropical mushroom, suitable for cultivation during summer and rainy season. It naturally grows on the humus rich soil under road side tree, in garden and forest during rainy season. This mushroom is also known as dudh chatta because of its attractive milky whitish appearance with excellent shelf life and large sized basidiocarp with fleshy stipe and broadly adnate to decurrent gills. It is rich in protein, lipids, fibers, minerals, carbohydrate and contains an abundant amount of essential amino acids. It is an excellent source of thiamine, riboflavin, nicotinic acid, pyridoxine and ascorbic acid. Nutritive value of milky mushroom is comparable with other edible mushrooms (Zahid *et al*., 2010). Since most of the edible mushrooms have favourable growth conditions at lower temperatures (< 25°C), creation of infrastructure for commercial cultivation, especially in warm humid tropical regions, is always expensive (Thakur *et al*., 2014). Identification and cultivation of warm weather (30-38°C) varieties of edible mushrooms have been scientifically challenging. Milky white is one of such mushroom varieties. This mushroom requires a temperature of 30-35^{o}C and relative humidity of 70-80% for cultivation which is conducive to the environmental conditions

of Bangladesh (Krishnamoorthy *et al.,* 2000). Its cultivation can be best fitted in early cropping when no other mushroom can grow except *Volvariella spp*. at such a higher temperature.

A substrate is an important substance for growing mushrooms. A good substrate for mushroom growth must be suitable both chemically and physically, as well as having the proper condition for microbial activities. Usually, a wide range of diverse cellulosic substrates are used for cultivating mushrooms. In Bangladesh, rice straw is widely used as the substrate for cultivating milky mushroom and is also considered the best substrate for yield (Mangat *et al*., 2008). Huge quantities of lignocellulosic residues other than rice straw, such as wheat straw, mustard straw, maize straw, waste cotton, water hyacinth, sugarcane bagasse, coconut coir, and kash are generated annually through the activities of agricultural, forest, and food processing industries in Bangladesh which might prove suitable for this mushroom cultivation. There is no need to compost the substrate for its cultivation as the mycelium can degrade the cellulose, hemicelluloses and lignin by secretion of various extracellular enzymes. Milky mushroom grows well on uncomposed substrate under artificial indoor condition (Vijaykumar *et al*., 2014). But for the better mycelial colonization and yield it is necessary to sterilize or pasteurize the substrate. Khan *et al*. (2011) stated that substrate sterilization is much more appropriate method for effective and smooth cultivation of mushroom to remove the existence of a number of microorganisms. Substrates are pasteurized by steam or soaking in hot water to kill many of the potential competitive microorganisms. Pathan *et al*. (2009) stated that maximum percent yield (61.75%) was in case of soaking and boiling of substrate for 75 minutes. Spawning method may have influence on evenly mycelium running in the bulk substrate. It can be spawning by different methods viz. thorough mixing, layer spawning, top spawning etc. Moisture content in substrate is very important factor for the growth, development and yield of mushroom. Optimum mycelial growth and mushroom production is dependent upon adequate moisture and gas exchange within the substrate (Shen *et al*., 2008). Different moisture levels in different substrates under different environmental conditions have been tested by researchers. It is generally recognized that most fungi require high moisture levels. Casing is another important cultural practice of milky white mushroom cultivation. Casing the surface of substrate fully colonized by

mycelium of mushroom is an essential function in stimulation and promoting the development of fruit bodies (Farsi *et al*., 2011). Although different materials may adequately function as a casing layer, peat is commonly used and recommended as a good casing in mushroom cultivation (Gulser and Peksen, 2003). But, in Bangladesh peat is not available. Farmers use the mixture of soil and sand as casing material. The size, shape, colour, taste, yield and shelf life of the mushroom depends on the maturity of the fruiting body. Premature harvesting of the mushroom may reduce the yield. On the other hand, if it is harvested at over age, the mushroom shades spores, loses its attractive colour, robust in size and sporophore become fibrous, leathery and ultimately tasteless (Sharker *et al*., 2011). Therefore, proper time harvest of mushroom is very important. After harvest mushrooms shelf life can end up due to high rate of respiration, high rate of dehydration, browning and texture changes. So, storage condition had a significant effect on mushroom's quality and shelf life.

In Bangladesh milky mushroom is getting popularity day by day due to its high biological efficiency (100%), better keeping quality, white attractive colour, suitable for pickle and chutney, sustainable yield, delicious taste, long shelf life and unique texture. Moreover, because of suitable condition, high local demand and export potential of this mushroom, many private entrepreneurs are interested in its commercial cultivation (Amin *et al*., 2010). But the producers are facing the problems due to lack of suitable strains and production technologies. The strains of milky white mushroom in Bangladesh are very limited. The performance of all the strains preserved in Mushroom Development Institute is not yet evaluated and research work for appropriate production technology is very limited. Utilization of various lignocellulosic wastes and casing material for commercial cultivation, selection of appropriate pre-harvest and post-harvest techniques of *Calocybe indica* are the demand of the hour.

Therefore, the current research was conducted to achieve the following objectives-

1. To identify the suitable strains of milky white mushroom;
2. To identify suitable substrates, appropriate sterilization techniques and spawning methods;
3. To identify suitable casing materials and its management practices;
4. To assess sufficient moisture content of the substrate and
5. To identify the appropriate age of harvesting for milky white mushroom.

CHAPTER- II

REVIEW OF LITERATURE

Strain performance

Kumar *et al.* (2011) conducted an experiment to explore the role of different temperatures, pH levels and media on growth of milky mushroom and yield attributes for identification of a potential strain of milky mushroom. They observed significant variation among the strain in respect of yield attributes. Among 5 different strain CI-6 took a minimum period for spawn run (16 d), pinhead formation (16 d) and for the first harvest (24 d). More pinheads were observed in strain CI-6 (64/bag) with its highest yield (620 g/kg of dry substrate with 62% BE). Average weight per sporophore (32 g) was also highest in strain CI-6.

Dhakad *et al.* (2015) carried out an experiment in the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India with five different strains of *Calocybe indica* viz. CI-4, CI-13, CI-14, CI-15 and CI-18 for growth behaviour and yield potential. They observed significant difference in yield performance among the strains. The strain CI-18 showed better performance compare to other strain.

Kerketta, *et al.* (2017) conducted an experiment to see the effect of different media, temperature range on mycelial growth and yield performance of five different strains of *Calocybe indica* (CI-1, CI-4, CI-522, CI-524 and CI-530). They observed that mycelial growth of each strain differed significantly on all tested media and temperatures.

Kerketta *et al.* (2018) conducted an experiment to see the biological efficiency of five strains of *Calocybe indica*, collected from different region and cultivated in Raipur district of Chhattisgarh state, India. They reported that the strain CI-524 was found to be superior in yield attributing characters and yield performance with highest biological efficiency compare to all tested strains.

Shrikhandia and Sumbali (2019) carried out an experiment to screen some strains of *Calocybe indica* viz., DMRO-309, DMRO-319, CI-6, CI-9 and APK-2 procured from

Directorate of Mushroom Research, Solan, India for their growth behaviour, morphometric characters, yield and biological efficiency on wheat and paddy straw for cultivation in Jammu district. They observed significant differences in the sporophore yield of all the tested strains of *Calocybe indica* on both the agro wastes that were utilized.

Effects of growing season

Sarmah *et al.* (2006) conducted an experiment to find out whether milky white mushroom can be grown under the natural conditions of Assam or not. The results revealed that this mushroom can be successfully grown from May to August under the prevailing climatic conditions of Assam. The mean temperature, mean relative humidity and mean light intensity during that period ranged between 26.1 to 32.5 ºC, 79.5 to 85 percent and 4925-5900 lux, respectively. The highest yield was scored in the month of June.

Raja and Ganesh (2012) carried out an experiment to find the most favourable month for the cultivation of *Calocybe indica* and the influence of temperature and relative humidity on mushroom yield. They reported that significant variation in yield and other parameters were observed when *Calocybe indica* was grown during different periods of study the year of 2011 to 2012. In summer months (March to July), a general increase in mushroom yield was observed but variation of temperature during other months did not affect the yield. The maximum yield was recorded in the month of May and June 2012. Mushroom attained harvesting maturity almost one day earlier during monsoon months.

Shukla *et al.* (2013) studied on the effect of temperature on mycelia growth of six strains of milky mushroom viz. CI- 4, CI- 6, CI- 7, C1- 8, CI- 9 and CI- 10. They reported that the most suitable temperature for fast and full mycelial impregnation and growth was 30°C.

Kumar *et al*. (2015) conducted an experiment to find the suitable time for milky mushroom cultivation and reported that minimum time was required for spawn run (19 and 18 days) during June - August, while for pin head formation (12 and 11 days) and first harvesting (20.0 and 19.7 days) were noticed during July - September for strain CI-4

and CI-6, respectively. Maximum number of pinhead initiation was recorded during April - June. However, maximum number of fruiting bodies were harvested during September - November in CI-4 and CI-6 strains. Whereas, maximum yield per kg substrate and average weight per fruit body were recorded during July - September from strain CI-4 and CI-6, respectively.

Singh *et al*. (2015) studied on five strains (i.e., APK-2, CI-6, CI-8, CI-9, CI-10) of *Calocybe indica* and incubated at five different temperatures viz., 21, 24, 27, 30, and 33°C. The results revealed that all strains showed maximum mycelial growth at 30°C followed by 27°C and minimum at 21°C on 3rd, 5th, 7th and 9th day's observations. However, the mycelial growth of each strain varied significantly at all the temperature tested. At 30°C temperature on 9th day's strain APK-2 showed maximum radial growth (full growth) of mycelium (9.0 cm).

ALsowadi and ALhomam (2019) conducted a study in India to observe the effect of different temperature on the biomass of milky mushroom. The results revealed that all strains showed maximum mycelial growth at 30°C followed by 28°C and minimum at 20°C on 3rd, 5th, 10th and 15th day's observations.

Effects of growing substrate

Amin *et al.* (2010) conducted an investigation to determine a suitable substrate and the appropriate thickness of casing materials for the cultivation of *Calocybe indica* and reported that rice straw was the best substrate for the commercial cultivation of *Calocybe indica*. The highest biological and economic yield (448.5 g/packet), and biological efficiency (91.75%) were obtained in the rice straw substrate.

Lakshmipathy *et al.* (2012) conducted a study to enhance the yield performance of *Calocybe indica* through optimization of the cultivation parameters by utilizing cheaper substrates that are available in Tamil Nadu, India. They observed that the shortest time was required to complete mycelial growth in the paddy straw substrate (20 ± 2 days), followed by the Vettivera leaves (26 ± 2 days) and sugarcane bagasse (27 ± 2 days) substrates. The longest time (30 ± 2 days) required to complete mycelial growth was

observed in the coconut coir substrate. The minimum time for first flush was observed in paddy straw substrate (8 ± 1 days) and longest time was recorded in sugarcane leaf substrate (14 ± 1 day). The maximum amount of nutritive value was obtained from the paddy straw and the lowest from sugarcane leaves as substrate.

Elaiya Raja and Ganesh (2013) undertaken a study to utilize ragi straw, black gram empty pod, green gram empty pod, bamboo dry leaf and *Casuarina* dry leaf as supplement on paddy straw substrate to study their effect on the sporophore production of *Calocybe indica*. They recorded maximum yield and biological efficiency in paddy straw + radi straw (1:2).

In order to study the effect of different substrates on the growth and yield of *Calocybe indica* Kumar and Chandra (2013) carried out an experiment with four different substrates viz., wheat straw, paddy straw, chopped leaf of sorghum and saw dust. They reported that substrate of paddy straw was found significantly superior than other substrates as it provided highest number of fruit body and maximum yield.

Vijoykumar *et al.* (2013) conducted an experiment to find out the efficacy of different substrates such as paddy straw, wheat straw, soybean straw, coconut coir pith, cotton waste and sugarcane baggage for the cultivation of milky mushroom. They observed that among the six different substrates, wheat straw substrate was superior which recorded minimum days for spawn run (15.67 days), pinhead formation (28.67 days), for first harvest (33.67 days) with maximum yield and highest biological efficiency (146.3%). Paddy straw was the next best substrate for milky mushroom cultivation.

In an experiment Jadhav *et al.* (2014) evaluated the wheat and soybean straws and their mixture for optimization of fruit body yield of milky mushroom and reported that the mixture of wheat and soybean straw (1:1) as a substrate performed better than individual, reflected in higher fruit body yields of milky mushroom.

Kumar *et al*. (2014) conducted two experiment to exploit locally available substrates with high biological efficiency and to explore suitable substrate combination for quality spawn production of milky mushroom. They reported that in one experiment highest yield (653.3 g kg-1 dry substrate) with highest biological efficiency (65.3%) was

recorded in paddy straw substrate. In another experiment among the 13 different combinations of substrate sorghum grains at 60% moisture resulted in excellent and fast spawn run followed by paddy straw in combination with sorghum grain in the ratio of 1:1, 2:2 and 3:1 and the lowest yield was obtained in coconut husk alone.

Navathe *et al*. (2014) cultivated *Calocybe indica* with locally available substrates viz. paddy straw, horse gram waste, wild grass (*Themeda quadrivolvis*), bamboo leaves and different casing materials and reported that among the four substrates evaluated for cultivation of milky mushroom, paddy straw was the best with 81.05 per cent biological efficiency.

Rawal and Doshi (2014), tested three different combinations of substrates viz., wheat straw + paddy straw (1:1), wheat straw + paddy straw (1:2), wheat straw + paddy straw (2:1), along with wheat straw and paddy straw alone for yield performance of milky mushroom. They reported that, the wheat straw alone substrate significantly took minimum days for spawn run, pinhead formation as well as 1st harvest. In terms of average production, wheat straw alone substrate was found significantly superior than others by giving the maximum yield. The significant lower yield was obtained from paddy straw alone substrate. However significantly maximum number and average weight of fruit bodies were obtained from the substrate wheat straw + paddy straw (2:1). *Calocybe indica* strain APK-2 recorded maximum average biological efficiency and average mushroom size in the same substrate.

Chiwan and Sumbali (2016) conducted an experiment to compare the yield and biological efficiency of *Calocybe indica* on different locally available substrates (agro-wastes, garden wastes and forest wastes) and reported that maximum biological efficiency was achieved on paddy straw (87.4%). Garden and forest wastes have not been used much in the past and incidentally the ones that have been used did not show good productive potential.

Chakraborty *et al.* (2016) Cultivated *Calocybe indica* with locally available substrates viz. paddy straw, maize stalk waste, bamboo leaves and young coconut fibre alone and various combinations with paddy straw + maize stalk (1:1 v/v), paddy straw + saw dust

(1:1 v/v), and paddy straw + saw dust (1:2 v/v) and different casing materials. They reported that among the substrates, paddy straw was the best with 196.12 % biological efficiency (BE) followed by different substrate combinations but the bamboo leaves alone were recorded as substrate with lowest potential (84%) for cultivation.

Patel and Trivedi (2016) cultivated *Calocybe Indica* on different agricultural substrate viz., paddy straw, wheat straw, sugarcane trace and mango dry leaves. They observed that minimum days required for completion of spawn run (18.4 days), primordial formation (25.2 days) & days for first harvest (32.4 days) and maximum yield and biological efficiency (134.86 %) was with the paddy straw substrate.

Kudada, *et al.* (2017) conducted an experiment to find out the effect of spawn produced on different grains and their effects on sporophore development and yield of milky mushroom. They reported that among seven spawn substrates viz., wheat, jowar, ragi, maize, paddy and bajra, jowar grain-based spawn was found to be the best spawn substrate as compared to other substrates studied with to spawn run period, size (lenghth of stipe, diameter of stipe, pileus, weight of sporophore, yield and biological efficiency. The significantly higher yield (1586.21 g/bed) with maximum biological efficiency (105.74%) was obtained by the jowar grain-based spawn.

To test the feasibility of utilizing maize stalks for oyster mushroom cultivation Lakshmipathy *et al*. (2017) cultivated milky mushroom (*Calocybe indica*) using maize stalks, paddy straw and with different ratios of these two substrates for three years. This study indicated that yield and bio-efficiency was more in 100% paddy straw substrate. This study clearly elucidated the possibility of utilization of maize stalks for milky mushroom cultivation instead of burning.

Singh *et al.* (2017) evaluated five locally available substrates in pure form and in combinations with wheat straw for production of wildly collected *Calocybe sp*. (DMRO-600) and reported that wheat straw substrate gave highest yield (1052.50 g), maximum number of fruiting bodies (40.75), early spawn run (21.50 days) along with early first harvest (33.25 days).

Dakshayini and Mallesha (20018) carried out an investigation to find the effect of different substrates such as paddy straw, sugarcane trash and their combination on growth and yield of different mushroom species. The results revealed that early bud initiation was found in the paddy straw among all the substrate used. In milky mushroom (*Calocybe indica*) early bud initiation was observed in Paddy straw (31.50 days) alone as substrate, which is on par with the combination of paddy straw + sugarcane trash (32.66 days). The highest yield was observed in the first flush and it was decreased in the second and third flush on all the substrates. In milky mushroom (*Calocybe indica*) paddy straw + Sugarcane trash combination recorded higher bio-efficiency of 111.63% and found to be the best substrate for cultivation of milky mushroom.

Kerketta *et al.* (2018) conducted an experiment to know the effect of alone and combination of different growing straw substrates and casing materials on yield of *Calocybe indica*. The combination of wheat + Lathyrus straw gave significantly maximum yield (765g) with BE (76.5%) followed by alone wheat straw (696.67g) with BE (69.66%), whereas the minimum fresh yield (390g) with BE (39%) was obtained from the combination of wheat + mustard straw.

Mohit *et al.* (2018) carried out an experiment to evaluate cheap locally available substrates for production milky mushroom (CI.-16-02 and CI.-16-03) and reported that maximum yield, minimum days for spawn run (19.50days), minimum days for pin head formation (21.75 days), minimum days for first harvesting (27.00 days), maximum number of pinhead initiation (62.25), maximum numbers of fruiting bodies (24.75) and maximum average weight of fruiting bodies (27.42 gm) were observed in sugarcane leaf + wheat straw (2:1).

Suman *et al.* (2018) carried out an experiment to evaluate the effect of three different agrocellulosic wastes using plastic bag technology for production of the *Calocybe indica*. They reported that among the three different substrates, wheat straw substrate was superior which recorded minimum days for spawn run (15.3 days), pinhead formation (27 days) and for first harvest (33 days) with highest number of fruit bodies (24). Maximum yield (1283.6 g/kg dry substrate) and highest biological efficiency (128.36%) was also obtained in the wheat straw substrate.

Prameela and Uma Devi (2019) evaluated performance of milky mushroom (*Calocybe indica*) on different agricultural wastes like paddy straw, castor stalks, sunflower stalks, coconut coir pith, oil palm waste and groundnut shells and reported that paddy straw substrate was best compare to the other substrates.

Effects of supplement

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 *Calocybe indica*. They observed that 30% maize powder supplement was effective for producing viable fruiting bodies. The highest biological and economic yield and biological efficiency were also obtained with 30% maize powder as a supplement.

Kumar *et al.* (2012) designed a study to assess the effects of eleven organic supplements as substrate and casing as well as casing mixtures on yield of two strains (CI-6&CI-4) of milky mushroom. Results revealed that minimum time 17.3 and 17.7 days was recorded for spawn run in the mustard cake supplemented substrate as well as 9.7 and 10.0 days for pinhead formation, 17.3 and 18.0 days for first harvesting from strains CI-6 and CI-4, respectively. Maximum yield was obtained from soybean flour supplemented substrate 648.3 and 599.7 g/kg of dry substrate.

Krishnamoorthy *et al*., (2015) reported that the addition of different supplements with the substrates influenced the spawn run, days for pinning, number of pinhead initiation, flushing pattern and overall mushroom yield.

Katiyar *et al.* (2018) conducted an experiment to examine the effect of different types of sugars viz. Sucrose (1.5%), Sucrose (1.0%), Maltose (1.5%), Maltose (1.0%), Glucose (1.5%), Glucose (1.0%), Lactose (1.5%) and Lactose (1.0%) were used as supplements in spawn production of milky mushroom strain (CI-16-04). Results

revealed that maximum spawn growth was observed at 20 days observations in glucose @ 1.5% and sucrose @ 1.5% followed by glucose @ 1%. The minimum spawn growth in lactose @ 1% followed by lactose @ 1.5%, which is significantly, at par while in control as observed. Regarding spawn growth rate (mm/day) in strain CI-1604, maximum spawn growth rate was observed in two treatments in glucose @ 1.5% and sucrose @ 1.5% followed by glucose @ 1%. The minimum spawn growth rate was found in lactose @ 1%.

Singh *et al.* (2018) carried out an experiment to study the effect of different chemical supplements on two strains of milky mushroom (APK-2 and CI-6). Completely impregnated mycelial growth substrate was sprayed with chemical solution of ferrous sulphate (1%), manganese sulphate (1%), potassium sulphate (1%), magnesium sulphate (1%), zinc sulphate (1%) and calcium carbonate (1%) before casing. Results revealed that the highest numbers of fruiting bodies were plucked from strain APK-2 (25.00/bag) with calcium carbonate supplemented treatment and from strain CI-6 (23.33/bag), in control (without supplementation). Maximum yield was harvested significantly well in magnesium sulphate treatment from strains APK-2 and CI-6 (650.00 and 648.33 g kg-1 of dry substrate with 65.00 and 64.83% B.E.) followed by calcium carbonate (635.00 and 623.33 g kg-1 of dry substrate with 63.50 and 62.33% B.E.), respectively. These chemical additives are also very cost effective and having no any residual effect on the quality and taste of mushroom.

Effects of casing material

Kumar *et al.* (2012) designed a study to assess the effects of eleven organic supplements as substrate and casing as well as casing mixtures on yield of two strains (CI-6&CI-4) of milky mushroom. They reported that the combination of casing mixture FYM + VC + PM (1:1:1) took less period for pinhead formation 12.0 and 12.3 days and 20.0 and 20.7 days for first harvesting, while maximum yield 607.0 and 591.7g/kg of dry substrate was harvested with casing mixture FYM + VC + GS (1:1:1) from strain CI-6 and CI4, respectively

Sharma *et al.* (2013) evaluated different casing materials formulation on yield related parameters of *Calocybe indica*. Casing soil formulations were prepared from farm yard manure, garden soil and sand with different combinations. They reported that, out of various casing material combinations FYM: Garden soil : Sand (2:1:1) having pH (8) and 3 cm width were found suitable and showed best result in yield as well as biological efficiency.

Jadhav *et al*. (2014) carried out an experiment to assess the effect of nitrogen fixing and phosphate solubilizing biofertilizers and different substrates for improvement of casing quality and fruit body yield in milky mushroom. They observed that inoculation of bacterial inoculants either in alone or in different combinations resulted in an increase in mycelial growth of *Calocybe indica* compared to un-inoculated control under in vitro conditions.

Navathe *et al*. (2014) cultivated *Calocybe indica* with locally available substrates and different casing materials and reported that the biological efficiency of *Calocybe indica* was doubled by using a combination of sand + soil + dried biogas spent slurry (BE 180.32%) or vermicompost (BE 176.28 %) as casing material. Use of dried biogas spent slurry alone also recorded 130 percent biological efficiency but combination of sand + soil (BE 79.94 %) was inferior.

Chakraborty *et al.* (2016) conducted an experiment with locally available substrates and different casing materials such as vermicompost, soil + sand (1:1v/v), dried saw dust, hard paper (wet condition) and combination of tea waste + soil + sand, saw dust + sand (1:1 v/v), tea waste + sand (1:1 v/v) in paddy straw cultivating condition and reported that as casing material the spent mushroom compost (SMC) of *Agaricus bisporus* resulted in the highest biological efficiency (207%) followed by soil + sand (196%), sand + saw dust (163%) but combination of tea waste + soil + sand was inferior (151%). Saw dust gave the lowest (96.8%) biological efficiency.

Ashrafi *et al*. (2017) conducted an experiment to reuse composted spent mushroom substrate (SMS) as casing material for the production of milky white mushroom. They used five casing mixture viz. Loam soil + Sand (3:1) (farmer's practice), SMS compost

+ Sand (3:1), Loam soil + SMS compost + Sand (2:1:1), Loam soil + SMS compost + FYM (1:1:1) and SMS compost + FYM (1:1) and reported that casing treatments containing SMC alone or combination with sand (3:1) and soil + FYM (1:1:1) produced statistically similar yield which were statistically higher than farmer's practice.

Kerketta *et al.* (2018) conducted an experiment to know the effect of alone and combination of different growing straw substrates and casing materials on yield of *Calocybe indica* and observed that fresh yield of strain CI-524 of *Calocybe indica* differed significantly with different casing materials. Compost + Vermicompost gave significantly higher yield (813.33g) with BE (81.33%) followed by vermicompost (748.33g) with BE (74.83%) and compost [Garden soil + FYM (1:1)] gave minimum yield (690g) with BE (69.0%).

Yadav and Kumari (2018) conducted a trial to show the effect of casing and supplementation in casing at the time of its application on productivity of milky mushroom (*Calocybe indica*). Before casing application, soil was chemically sterilized. Six casing materials viz., SC (spent compost) + FYM (Farm Yard Manure) + Sand + GS (Garden soil) (1:1:1:1), SC + FYM (2:1), SC alone, SC + FYM (1:1), SC + FYM (1:2) and FYM alone were taken. The casing materials using SC + FYM + Sand and GS in the ratio of 1:1:1:1 gave the highest yield as well as maximum number of fruit bodies. In another trial casing material of SC + FYM + Sand + GS (1:1:1:1) was supplemented with grain spawn @ 2 percent and soybean meal @ 4 percent, sweet pea meal @ 4 percent and Gram meal @ 4 percent. Supplementation of soybean meal @ 4 percent produced higher yield as well as number of sporophores than other treatments.

Sardar *et al*. (2020) conducted an experiment to evaluate the influence of various casing materials viz. peat moss, spent mushroom substrate and loam soil on the yield and biological efficiency of milky mushroom. Results revealed that among the casing materials used, the highest yield and biological efficiency were observed on peat moss.

Effects of substrate moisture content

Siwulski *et al*. (2007) carried out an experiment to determine the influence of moisture of the substrate from rye straw on *Pleurotus* yielding. Two oyster mushroom strains HK-35 and K-22 were used in that experiment. The substrate was pasteurized at the temperature of 95^{o} C for 5 hours and then tap water was added to the moisture of 60, 65, 70, 75 and 80 percent. The result revealed that optimum moisture of substrate was 75 percent, when the total and marketable yields were the biggest. The moisture content of 80 percent influenced negatively the yielding because of the high share of misshaped carpophores.

Shen *et al.* (2008) carried out an experiment to evaluate effects of three substrate moisture contents (50%, 55% and 60%) two log weight and three porosities of bag filter on Shiitake mushroom (*Lentinula edodes*) yield and biological efficiency. They reported that 55% substrate moisture content gave the highest yield and biological efficiency.

Pani *et al*. (2017) conducted an experiment to determine the effect of substrate moisture on the production of straw mushroom (*Volvariella volvacea*). Varied moisture content *viz.* 0 (dry straw without soaking), 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 % were maintained in the substrate prior to spawning. It was revealed that soaked paddy straw with 60 % moisture sustained the highest yield of mushroom (1214.7 g, 12.1 % BE) compared to other treatments. Dry straw failed to produce any fruiting body. The increase in moisture content from 10 to 60 % is directly proportional to the increase in yield. There was a gradual decline in yield as the moisture content was increased from 60 to 90 %. There was no fruiting in saturated moisture (100 %) condition.

Singh *et al*. (2017) evaluated different substrates and moisture regimes for mycelial growth optimization of *Ganoderma lucidum*. The fungal mycelium was grown on three substrates, viz. wheat straw, saw dust and wheat straw + saw dust (1:1) with four moisture levels, viz., 60 ± 2 percent, 70 ± 2 percent, 80 ± 2 percent and 90 ± 2 percent and radial growth was recorded at an interval of 3, 6, 9 and 12 days after inoculation. They observed that, maximum mycelial growth of *G. lucidum* was obtained in substrate, wheat straw + saw dust (24.78 mm) followed by saw dust (18.75 mm) and wheat straw (13.33 mm) at 70 ± 2 percent moisture content. On the other hand, minimum mycelial

growth was recorded at 90 ± 2 percent moisture content of substrates in wheat straw + saw dust (13.73mm), followed by saw dust (9.30 mm) and wheat straw (7.13 mm) alone. Among the evaluated substrates having different moisture content, maximum mycelial growth was obtained at a moisture level of 70 ± 2 percent followed by 80 ± 2 percent, 60 ± 2 percent and 90 ± 2 percent irrespective of substrate used.

Suganthi and Krishnakumari (2018) carried out an experiment to evaluate the effect of four different substrates and their six different combinations on the growth and yield of *Pleurotus cornucopiae* with five moisture levels (50 ± 2% to 90 ± 2%) and radial growth. Highest mycelial growth rate was recorded in substrate sugarcane bagasse and combination of banana leaf + sugarcane bagasse (1:1) at 70 ± 2% moisture.

Substrate sterilization

Pathan *et al*. (2009) cultivated *Pleurotus sajor-caju* on fresh, dried and chopped sugarcane leaves and reported that the pinheads were noted first (11.25 days after spawning) in case of substrate soaking till it starts boiling followed by soaking and boiling for 15 and 75 minutes. Minimum period for maturation of fruiting bodies (5.25 days) was in case of soaking and boiling for 30 minutes and soaking and boiling for 75 minutes. The minimum period between flushes (4.25 days) was recorded in soaking and boiling for 30 minutes, followed by soaking and boiling for 75 and 90 minutes. The maximum number of flushes (2.75) was recorded in case of soaking and boiling for 75 minutes followed by 90 and 60 minutes. The maximum fresh yield (61.75%) was obtained in case of soaking and boiling for 75 minutes, followed by soaking and boiling for 90 and 60 minutes. Soaking and boiling of the substrate for 75 minutes results in earlier formation of pinheads, maximum number of flushes and the maximum yield percentage.

Kumari *et al*. (2018) carried out an experiment to study the effect of substrates pre-treatment on growth and production of *Calocybe indica*. They reported that the substrates pre-treated by autoclaving (T_1) recorded significantly shortest spawn run (20.25 days), pin head initiation after casing (6.50 days), maximum number of pin head (23.50),

number of sporophores (15.00), yield (1546.35g per bed) and biological efficiency (103.09%) in comparison to other treatments. The substrates pre- treated by autoclaving recorded maximum length of stipe (14.76 cm), diameter of stipe (7.90 cm) & diameter of pileus (11.74 cm), weight of sporophore (103.09g) followed by substrates pre-treated by hot water and by Formaldehyde @100 ml/100 liters water plus Bavistin (Carbendazim) @10 gm/100 litre of water.

Ziombra and Nowak (2013) investigated the yield of *Pleurotus cornucopiae* grown on substrates of wheat and rye straw. They reported that highest yields were obtained on the rye straw pasteurized for 72h and on wheat straw pasteurized for 48-72h. The optimal pasteurization period at 60°C for wheat straw was 48 h and for rye straw –72 h.

Saritha and Pandey (2015) evaluated three traditional substrate pasteurization methods (chemical, steam, and hot water) and seven alternate methods (solarization, botanicals, and sanitizers) for the cultivation of culinary-medicinal white oyster mushroom *Pleurotus ostreatus* var. *florida*. Results revealed that steam pasteurization (80°C, 2 h) was found to be the most efficient method and showed the highest biological efficiency (BE) at 82.8%, followed by the hot water method (80°C, 60 min) with BE at 77.6%. The chemical sterilization technique (500 ppm formaldehyde + 75 ppm carbendazim-Bavistin) showed 49.6% BE and solarization 34.7% BE. All other treatments showed low BE. The hot-water, chemical sterilization, and solarization techniques were the most effective in preventing competitor molds.

Akhter *et al*. (2017) conducted an experiment to evaluate the influence of hot water treatment of rice straw on yield, yield attributes and contamination of oyster mushroom (*Pleurotus ostreatus*), rice straw subjected to hot-water treatment at 60^{o}C, 80^{o}C and 100^{o}C temperature. Each temperature was maintained for 1hr, 2 hour and 3hour, which were compared with untreated (without hot water treatment). They observed that significant effects of substrate pretreatment methods on contamination, yield attributes and biological efficiency. Different dominant contaminants were found such as, *Trichoderma harzianum*, *Coprinus spp*., *Apergillus niger* and *penicillium sp*. were obtained during the incubation and cultivation period of the spawn. The contamination of

rice straw was higher at non-treated spawn packets and from 60^{o}C treated packets. Better performance, such as BE was 57.44%, the economic yield (280g), the highest average diameter of pileus (5.0 cm) and the highest average length of stipe (3.70 cm) were obtained from the treatment of 80^{o}C for 3 hours.

Roksana *et al*. (2018) investigated the effect of chemically disinfected wheat straw on the growth and yield of oyster mushroom (*Pleurotus ostreatus*). Various levels of treatments combined with different time (12, 18 and 24 hours) and dose of Formalin (250, 500 and 750ppm) and Bavistin (75ppm) were used, and the results were compared with the control. They reported that compared to the control, almost all the treatments showed increased values, and among them significantly higher mycelium running rate, the lowest time from stimulation to primordial initiation and to harvest, number of primordial per packet, number of fruiting body per packet were found in treatment consists of 750ppm of Formalin with 75ppm of Bavistin for 18 hours. Length and diameter of the stipe, diameter and thickness of pileus, biological yield, economic yield, dry yield and biological efficiency were also significantly increased in this treatment. However, weight of individual fruiting body was significantly higher when treated with 250ppm Formalin with 75ppm of Bavistin for 24 hours.

Kerketta *et al.* (2019) conducted an experiment to know the effect of different methods of substrate treatment (carbendazim + formaldehyde, hot water, lime 2% and plane water) on spawn run, pin head initiation and yield of *Pleurotus spp*. on wheat straw substrate. Results revealed that among the evaluated different methods of substrate treatment, the average period recorded for spawn run was statistically differed and it was significantly less (9.94 days) recorded in carbendazim + formaldehyde followed by hot water and lime 2% (10 days), faster pinhead initiation was found in hot water treated substrate (4.55 days) while it was took maximum (5.36 days) time in carbendazim + formaldehyde and maximum yield (398.75 gm) was recorded in hot water treatment method and minimum yield (174.44 gm) in plane water.

Alam and Singha (2020) carried out a research work to investigate the efficacy of different mother culture media and substrate sterilization techniques viz., sun dried for 8 hrs covering with transparent polythene (A-1), black polythene (A-2), blue polythene (A-

3) sheet, autoclave for two hrs at 121°C (A-4), and hot water for one hr (A-5) for the commercial cultivation of *Volvariella volvacea* (Bull.) Singer. They reported that considering the experimental results on the sterilization techniques it may be suggested that hot water sterilization of rice straw substrate was the best sterilization technique for the commercial production and yield improvement of *Volvariella volvacea*.

Effects of spawn density

Dahmardeh (2012) studied the effect of polythene color container and spawn rates on mushroom (*Pleurotus ostreatus*) at College of Agriculture, University of Zabol during 2011 growing season and reported that there was significant difference in the yields from different spawn and color of container. Maximum yield (weight of fresh mushrooms harvested at maturity) was obtained in 5% spawn rate (1248 g/2 kg wet substrate). The least yield of mushrooms was obtained from 2.5 % spawn rate and cultivation of mushroom in blue polythene container. It was concluded that the mushroom should be cultivated in green polythene bags under 5% spawn rate to achieve higher productivity.

Aziz *et al.* (2016) conducted an experiment to study the effect of different spawn densities on spawn run of *Calocybe indica.* 2, 6 and 10 percent wheat grain-based spawn were used on lignocellulosic wastes like agricultural wastes, garden wastes and forest wastes for its cultivation. Results revealed that 6% spawn density proved the best for complete spawn run in least number of days on most of the tested substrates. Results obtained by using different agricultural wastes, garden wastes and forest wastes indicated that an increase in the rate of spawning from 2 to 6% enables fast mycelial spread, whereas beyond it the growth was slow or the difference was insignificant.

Idowu *et al*. (2016) carried out an experiment to determine the effect of spawn quantity and spawning techniques on the growth and yield of *P. ostreatus*. They investigated the use of different spawning methods (on-spot, top and bottom, mixing and layering) and spawn levels (3, 5, 7, 9, 11, and 13%) on the mushroom. The results obtained showed that as the spawn level increased, growth and yield parameters also increased. The highest number of fruits (11.33), fruit weight (65.69g), widest pileus (657cm.) and longest stipe

(5.53cm) were observed at 13% spawn level and least in others. The densest mycelia were obtained as from 9% spawn levels; the mean fruit weight was highest (7.56g) at 9%. Significantly shortest days to substrate colonization and primodia initiation were observed at 13% spawn level and the longest at 3%. The findings implied that when sufficient amount of spawn is added to a fruiting substrate and applied bi-directionally, the mycelium grows faster and has more energy available for fruiting body formation, hence the increased yield and better biological efficiency.

A study was conducted by Pal *et al.* (2017) to evaluate the effect of different spawn rates and substrate supplementation on yield of *Pleurotus pulmonarius* (Fr.) Quel. Among six spawn rates viz., 0.5%, 1%, 2%, 4%, 6% and 8%, respectively tried on wheat straw substrate, the spawn run was fastest (10.50 days) when spawn dose was 8%, followed by 6%, 4%, 2%, 1% and 0.5%, respectively. The pinheads appeared in 12.27 days by using spawn @ 8%, which proved to be the best spawn dose followed by 6%, 4%, 2%, 1% and 0.5%, respectively. Highest yield of 168.7g per 200g dry substrate was achieved @ 8% spawn rate. Lesser yields were recorded when spawn rate was reduced. The results also revealed the significantly highest biological efficiency of 84.33% at 8% spawn rate followed by 6%, 4%, 2%, 1% and 0.5%, respectively. It was concluded that spawn run was rapid at higher spawn rate but there was not much difference in yield when spawn dose was increased from 4 to 8%. Considering spawn cost and performance shown by different doses, 2 - 4% was found optimum dose for its cultivation.

Sutha Raja Kumar *et.al*. (2019) conducted an experiment to assess the effect of spawn density, no. of holes on the polybags and the epidemiological factors on the production of *Hypsizygus ulmarius*. Among the different spawn densities viz., 5, 10, 15, 20, 25 & 30 %, spawn density at 20 percent level completely colonized the beds within 17.6 days and the same treatment recorded comparatively lesser number of days for pin head formation (3.6 days).

Kannan (2019) conducted a study to find out the optimum spawn density and suitability of various commonly available agro and agro-industrial wastes as substrates for shiitake cultivation. Data pertaining to evaluation of optimum spawn density indicated that the spawn density of 20 per cent level completely colonized the beds within 31.3 days.

Shelf life of mushroom

Kamal *et al*. (2015) carried out a study to evaluate the effects of respiratory gases on shelf life of fresh Oyster mushrooms. After sorting of collected cultivated mushrooms were packed in different polymeric packaging materials-polystyrene trays over wrapped with polyvinyl chloride (PVC) microfilm and polypropylene (PP) at refrigerated and ambient temperature condition for 12 days. Gas composition as CO_2, O_2, N_2, concentration at 3 days intervals of the total 12 days duration also including sensorial quality were evaluated. The results revealed that CO_2 contents were found to be increased but O_2 contents was found to be reduced for both packaging materials within 3 days storage at ambient temperature. In refrigerator, oxygen content in both of trays increased sharply within 3 days of storage. Off flavor appeared strongly and started to spoil from third days after Oyster mushrooms packed in ambient temperature, which on the contrary was not detected in mushroom packed and stored until 12 days in refrigerator. Shortest storage period for a single day at ambient condition and extended period of 12 days self-life was determined when mushrooms were stored in refrigerator in respect of sensorial quality in sealed polypropylene bag or in polystyrene trays.

Prerna *et al.* (2016) conducted an investigation to overcome the transpiration loss of mushroom. They used different desiccants (sorbitol, silica gel, citric acid and calcium chloride) at different concentrations under saturated condition within the packages and reported that highest total sugars, mannitol, total phenols, antioxidant potential and overall acceptability were found in mushroom packed with sorbitol (5g/100g of mushroom). Similar treatment also resulted in lowest browning index, enzyme activity and microbial growth as compared to the others. It was further observed that, by the use of all the desiccants, a delay in veil opening and maturity index was observed as compared to control. As a whole, it was concluded that the shelf life of the fresh button mushrooms can be prolonged using sorbitol as a desiccant.

Fu *et al.* (2019) conducted an experiment to study the quality characteristics of fresh *Tricoloma matsutake* during its shelf life period in modified atmosphere packaging (MAP) conditions and establish its remaining shelf life prediction models in a cold chain. In this study, they measured and analyzed quality indicators of fresh *T. matsutake*,

including hardness (cap, stipe), color, odor of sensory characteristics, pH, soluble solids content (SSC), and moisture content (MC) of physical and chemical characteristics under the temperature condition of 4^{o}C and relative humidity (RH) of 90%. The sensory evaluation results showed that the odor indicator in sensory characteristics was more sensitive to the freshness of *T. matsutake*. The changes of pH, SSC, and MC were divided into three periods to analyze the physiological changes of *T. matsutake*. The cap spread process could affect the changes of pH, SSC, and MC in period S1, and they changed gradually in period S2. In the period S3, they changed complicatedly because of deterioration.

CHAPTER-III

GENERAL MATERIALS AND METHODS

Eight different experiments were conducted in the Laboratory and Mushroom Culture House (MCH) of Mushroom Development Institute (MDI), Savar, Dhaka, Bangladesh during February 2018 to October 2020. Different common methods were involved in the experiments are described below and other methods are described in the respective experiment.

Environmental condition

The temperature of mushroom microbiology laboratory was maintained within the range of 20-22^{o}C by air cooler. The temperature and relative humidity of mushroom culture house was ambient, not controlled. The temperature of the culture house during the experimental period was within 28 to 33^{o}C. The environmental data during that period are given in Appendix-I.

Mushroom strain collection

Four strains of milky white mushroom (*Calocybe indica*) such as Cid-1, Cid-A, Cid-In and Cid-S were collected from MDI Laboratory, Savar, Dhaka, Bangladesh.

Preparation of pure culture

Pure culture of selected four strains of milky white mushroom fungi were prepared on Potato Dextrose Agar (PDA) media (Plate-1). To prepare PDA media 250 g of peeled sliced potato were boiled for 30 minutes and the extract was collected. The extract was mixed with 20 g of dextrose, 20 g of agar and 250 mg aspergin. The mixture was boiled on a burner until the agar dissolved. The medium was poured into per glass petri dish (90 mm diameter) at 15 ml/petri dish. The medium in petri dish was sterilized in an autoclave

for 30 minutes at 121°C under 1.5 PSI pressure. After sterilization and solidification, the plates were inoculated with the inoculum of the fungus. Pieces of inner tissues of stalks were used as inoculum. A fresh and full grown milky white mushroom was surface sterilized with 70% ethanol by rubbing cotton soaked in the alcohol. The stalk was peeled from outside. Tissues were collected from inner region of stalk of the sporophore. The tissues were cut into small pieces and placed on the solidified petri dish containing PDA. After inoculation the PDA plates were covered with cellophane paper. All operations were done under sterile condition in a clean bench. The inoculated petri dishes were transferred to a growth chamber maintaining temperature 27°C and incubated for 8 days. This eight days old PDA culture was used for inoculation of mother culture.

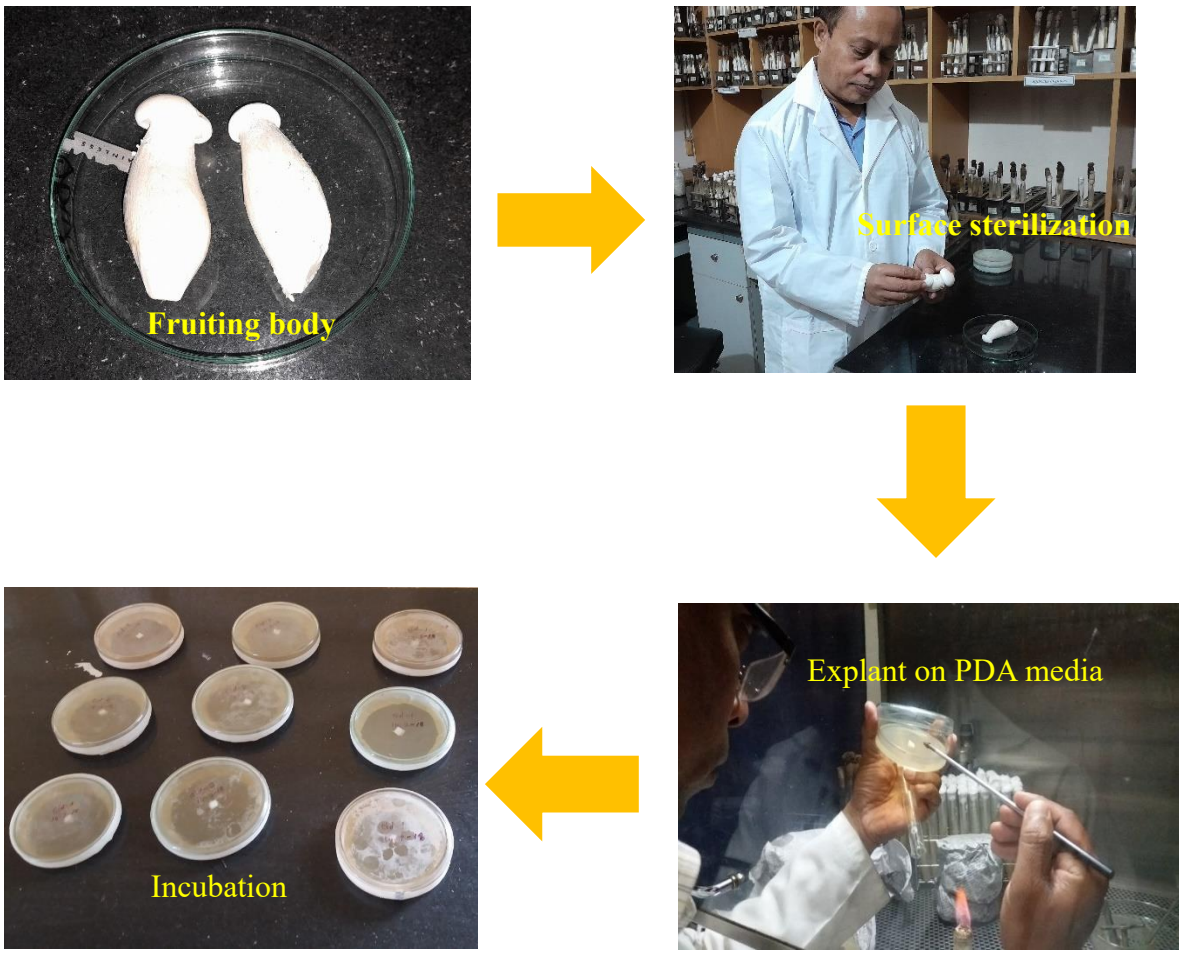

Plate-1: Preparation of milky white mushroom pure culture

Preparation of mother culture

Mother culture was prepared by mixing sawdust and wheat bran at the ratio of 2:1 (Plate-2). Calcium carbonate was used at the rate of 0.2% of the mixture. The moisture level of the mixture was maintained at 65% by adding tap water. Polypropylene bags of 18 x 25 cm size were filled with 200 g of substrate mixture and packed tightly. The necks of the bags were prepared by using heat resistant plastic tube. A hole of about two third deep of the volume of the bag was made at the center with a sharp end stick for space to put inoculums. The neck was plugged with cotton and covered with brown paper and tied with placing a rubber band. The packets were sterilized in an autoclave for one hour at 121^{o}C 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 milky white mushroom was placed aseptically in the hole of mother culture packet and again plugged the packet as mentioned before. Then the inoculated packets were placed on an iron rake in the MDI laboratory at 25 ± 2^{o}C temperature for incubation. The medium of the mother culture was colonized by the fungus as manifested by white colony growth mycelium within 20-25 days of inoculation. The fully colonized packets were used for spawning.

Preparation of sub mother culture

The sub mother culture was prepared by mixing sawdust and wheat bran following the same procedure as mother culture. After preparing and autoclaving the cooled media was inoculated by the fully colonized mother culture through the neck in a clean bench. Then the inoculated packets were placed on an iron rake in the MDI laboratory at 25 ± 2^{o}C temperature for incubation.

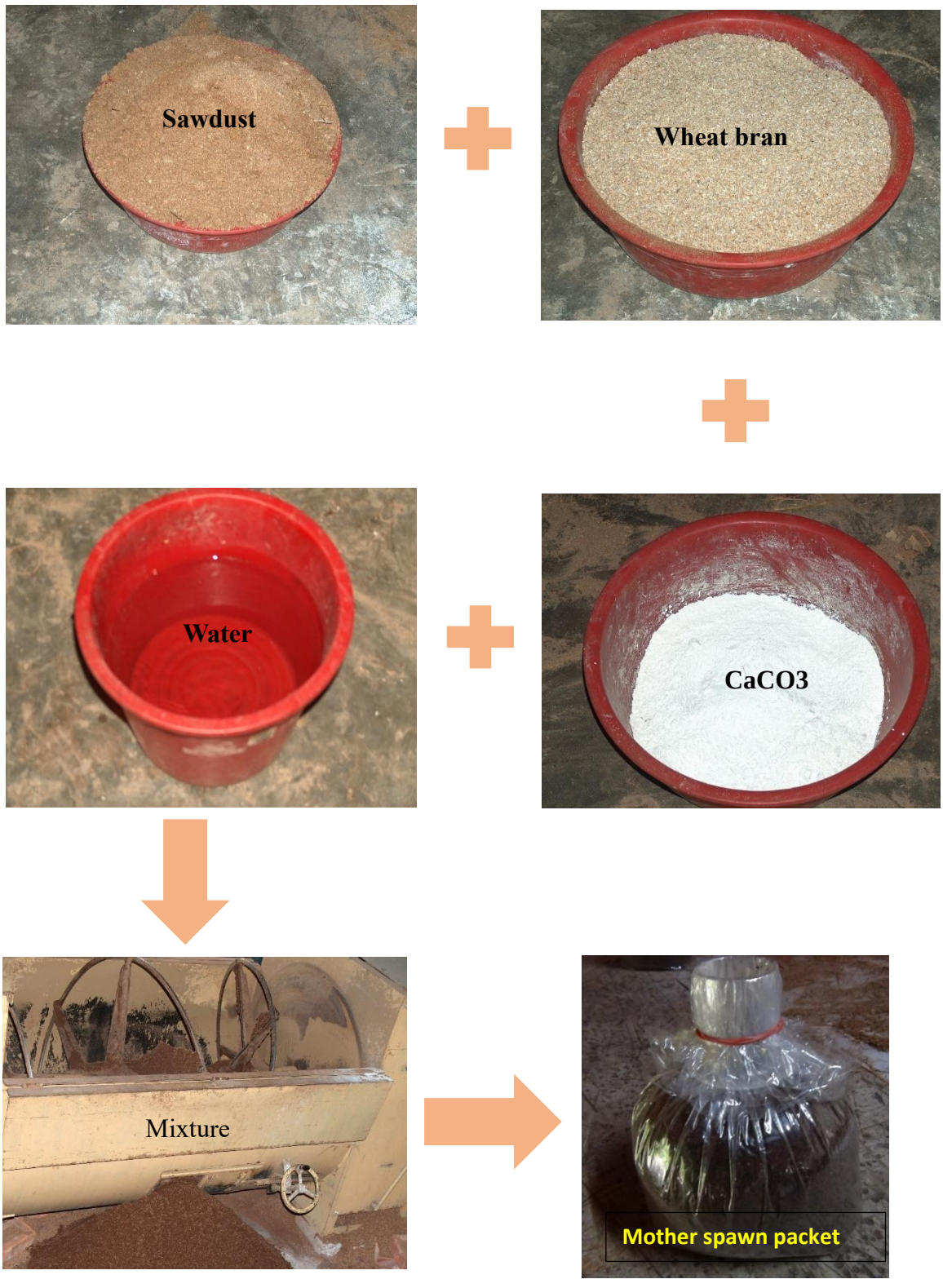

Plate-2: Preparation of mother culture

Preparation of spawn packet

Rice straw was used as substrate in all the treatments except evaluation of substrate for the cultivation of milky white mushroom. The straw was chopped to convenient length of 2.5 to 5 cm. The substrate was mixed with appropriate amount of water and then filled in net bag. The net bags filled with substrate were placed in the sterilization cum inoculation chamber. Door of the chamber was closed and tightened with the help of screws. Water heater was turned on to produce steam that flows in to the chamber. When the temperature of the chamber rises to 60^{o}C, the steam flow was adjusted to maintain a constant temperature of 70^{o}C – 80^{o}C up to 90 minutes. After 90 minutes water heater was turned off and kept it for about 20 hours. After 20 hours substrate was taken out and used for preparation of spawn packet. Pasteurized substrate was filled into the polypropylene bags (12”x18”) and inoculated with sawdust-based mother culture by thorough mixing (Plate-3). Then the spawn packets were transferred to the culture house for mycelium run. After 16-25 days the substrate was completely colonized by the mycelium.

Plae-3: Preparation of spawn packet

Culture house activities

After completion of spawn run rubber band, cotton plug and plastic neck of spawn packets were removed and the mouths of polypropylene bag were opened. Upper portion of the spawn packets were covered with 2.5 to 4 cm thick sterilized loamy soil casing material (Plate-4). After casing the packets were placed in the floor of culture house according to treatments. Culture house was well ventilated and there was scope of sufficient light diffusion in to the house. Relative humidity and temperature was not controlled. The casing surface of the packets were gently soaked with tap water using a spray gun twice a day to maintain moist environment. The mushroom fruiting bodies were harvested at 7 to 8 days before starting the pollen shade.

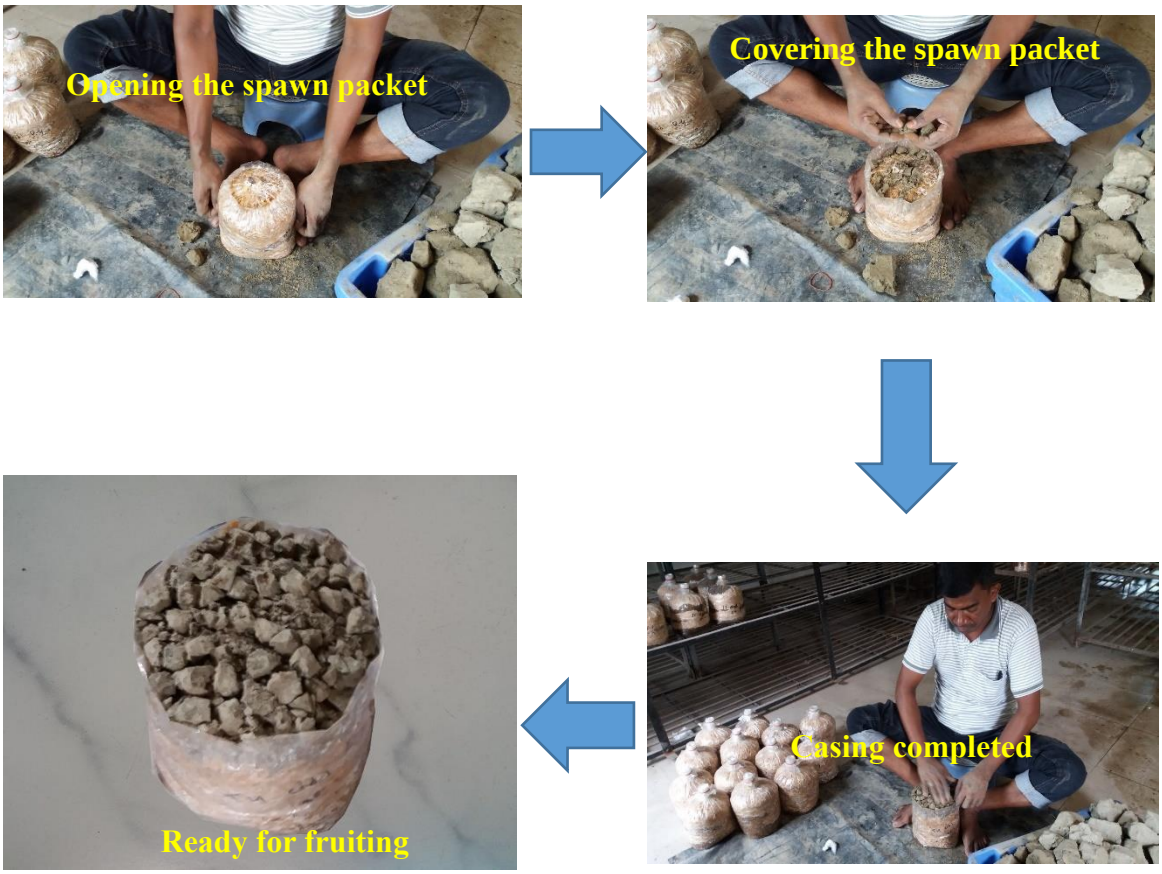

Plate-4: Casing of spawn packet

Data collection

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

Mycelium run rate

Mycelium running rate (MRR) on PDA media was measured radially and, in the mother, and sub mother culture it was measured vertically. In the mother and sub mother culture MRR measurement was started after the mycelium colony crossed the shoulder of the packet.

The linear length was measured at four different places of packet.

$$MRR = \frac{L}{N} \text{ cm/day} \quad \text{---------- (i)}$$

Where, L = Average length of mycelium running of four different places (cm) and N = Number of days

Time required for completion of mycelium running (day)

Days required from inoculation to completion of mycelium running in the spawn packets, days required from casing to primordia initiation and days required from primordia initiation to complete harvest were recorded.

Number of fruiting body

Number of effective fruiting bodies (NEFB) were recorded. Small sized dried and non-edible fruiting bodies were discarded.

Weight of fruiting body (g)

Average weight of individual fruiting body (WFB) was calculated as total weight of fruiting body per packet divided by total number of effective fruiting body per packet:

$$WFB = \frac{\text{Total weight of fruiting body per packet (g)}}{\text{Total number of effective fruiting body per packet}} \quad \text{---------- (ii)}$$

Diameter of pileus and stalk

Thickness of the pileus, diameter of pileus, diameter of stalk and length of stalk of all the effective fruiting bodies were recorded using a slide caliper and a centimeter scale.

Economic yield

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

Biological efficiency

Biological efficiency (BE) was determined by the following formula (Kerketta *et al.*, 2018):

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrate used (g)}} \times 100 \quad \text{---------- (iii)}$$

Benefit Cost Ratio

The Benefit Cost Ratios (BCR) for different spawn density were computed based on present market price of mushroom and cost of different inputs used in the study. 500 g of dried rice straw substrate was used for each spawn packet preparation. Rice grain mother culture prepared from 50g, 100g, 150g, 200g and 250g dried grain were used for inoculation incase of 10%, 20%, 30%, 40% and 50% spawn density respectively. Rice

straw @ 25.0 taka and grain @ 30.0 taka per Kg were used for calculation. Approximate common cost (labor, electricity, water, gas etc.) was 10.0 taka per packet. Total cost of production per packet was calculated by adding cost of substrate, cost of spawn and common cost. Total benefit per packet was calculated as total production multiplied by 250 (250.0 Tk./kg mushroom). Benefit cost ratio was calculated as-

$$BCR = \frac{\text{Total price of mushroom (Tk./packet)}}{\text{Total cost of production (Tk./packet)}} \quad \text{(iv)}$$

Shelf life (day)

The harvested mushroom was stored in open tray, cellophane wrapped tray and polypropylene (0.02 mm thick) bag at ambient temperature and in refrigerator (4^{o}C) and the shelf life was determined by counting the days from harvesting to off odor development and deformed appearance up to a stage when the mushrooms were not suitable for consumption. Appearance and odor of mushroom was determined by sensorial evaluation of selected five member's panel using nine-point hedonic rating scale. When the fruiting bodies especially pileus was started to shrink, deform, discolour and unpleasant scent evolved then it was considered as unfit for consumption.

Protein content estimation

Five grams of dried of each sample was taken and mixed with 50ml 1.25 N NaOH in a beaker and boiled for 30 minutes on a burner. The volume of solution maintained 50 ml by adding extra NaOH. The solution was cooled at room temperature and filtered through a Double Ring filter paper no. 102. The extract was collected and total protein content was measured according to the Biuret method (Burtis and Ashwood, 2006) with a diagnostic kit (Total protein Colorimetric test- Biuret method/Crescent Diagnostics, Saudi Arabia). Three test tube was marked as Blank, Standard and Test. 1 ml biuret reagent was taken in each test tube. Then 10 µL standard solution (4gm/DL protein) was taken in "Standard" marked test tube and 10 µL sample solution in "Test" marked test

tube. After 10 minutes reading of solution recorded using Biochemichel analyzer (Model-3000 Evolution, Code: RM 4030, Made in Itali). Amount of protein was calculated by using the following formula:

$$\text{Protein (g/100g sample)} = \frac{\text{Reading of sample x Amount of extract (ml)}}{\text{Wt. of sample x 100}} \times 100 \quad \text{-------- (v)}$$

Determination of total lipid

Total lipid content of mushroom was determined following the methods of Folch *et al*. (1957) with slight modification. Five grams of grinding mushroom powder was suspended in 50 ml of chloroform: methanol (2:1 v/v) mixture in a beaker. The content was mixed thoroughly, mouth of the beaker was sealed with parafilm paper and let stand for 3 days. A test tube was weighed (We) and the solution was filtered through a Double Ring filter paper No.102 in the test tube. The test tube was placed in an oven at 50^{o}C until the chloroform and methanol was evaporated. Then the test tube was again weighed (Ws). The difference between the two weights of the test tube was the amount of total lipid.

$$\text{Amount of lipid (g/100g sample)} = \frac{\text{Ws} - \text{We}}{\text{Wt. of sample}} \times 100 \quad \text{---------- (vi)}$$

Determination of crude fiber

Five grams of moisture and fat free sample was taken in a beaker and 200 ml 1.25% H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by frequent adding of water at regular intervals. The mixture was then filtered through a muslin cloth and the residue was washed with methyl alcohol till free from acid. The material was transferred to the same beaker and 200 ml of 1.25% NaOH was added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue was washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was transferred to a crucible, dried

overnight at 50-55^{o}C and weighted (We) in an electric balance (KEYI: JY-2003; China). The crucible was heated in a muffle furnace (Nebertherm: Mod-L9/11/c6; Germany) at 600^{o}C for 5-6 hours, cooled and weighted again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber (Raghuramulu *et al*., 2003).

$$\text{Crude fiber (g/100g sample)} = \frac{[100 - (\text{Moisture} + \text{fat})] \times (We - Wa)}{\text{Wt. of sample}} \quad \text{(vii)}$$

Determination of total ash

One gram of sample was weighted accurately in a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5-6 hours at 600^{o}C. It was then cooled in a desiccator and weighted. This was repeated till two consecutive weights were the same and the ash was almost white or grayish white in color. Total ash was calculated following the equation shown below (Raghuramulu *et al*., 2003):

$$\text{Ash content (g/100g sample)} = \frac{\text{Wt. of ash} \times 100}{\text{Wt. of sample taken}} \quad \text{(viii)}$$

Mineral content

Content of Calcium (Ca), Copper (Cu), Iron (Fe), Zinc (Zn), Selenium (Se) and Cobalt (Co) was determined following flame method of atomic absorption spectrophotometer (AAS 240, Varian) and the Molybdenum (Mo) was determined following graphite furnace method (GTA 120, Varian).

Total ash as determined earlier was taken in a beaker. Two milliliters of concentrated HNO_3 were added to the ash and heated for 2 minutes. One drop of hydrogen peroxide was added in to the solution to remove turbidity. The solution was transferred in to a volumetric flask and total volume was made 100 ml by adding de-ionized water.

For each mineral, one milliliter of the primary standard solution was taken in a 100 ml volumetric flask and the volume was adjusted to 100 ml with de-ionized water and mixed properly. The solution was considered as the secondary stock solution of the respective mineral. Standard solution of the mineral was prepared as per the instruction of the AAS for the particular mineral (Fluka Analytical SIGMA-ALDRICH product of Switzerland).

CHAPTER – IV

RESULTS AND DISSCUSSION

EXPT. NO. 01: PERFORMANCE EVALUATION OF FOUR DIFFERENT STRAINS OF MILKY WHITE MUSHROOM (*Calocybe indica*) AVAILABLE IN MUSHROOM DEVELOPMENT INSTITUTE, SAVAR, DHAKA, BANGLADESH

INTRODUCTION

Calocybe indica is an edible white summer mushroom also known as milky mushroom. It can be easily grown in the temperature range from 25 to 30°C. The major advantage is that it can be best fitted in relay cropping when no other mushroom can be grown at higher temperature. It has a very good scope of cultivation and can replace the other tropical mushrooms like *Pleurotus spp*. and *Volvariella spp*. (Kerketta *et al*., 2018).

The milky mushroom is relatively new to the world of mushroom industry and third most important in production in India and that can be cultivated on lignin rich agricultural wastes. This mushroom was first reported by Purakayasha and Chandra (1974) from West Bengal India. The production and marketing potential of the milky white mushroom in Bangladesh is promising because this mushroom requires a temperature of 30-35^{o}C and a relative humidity of 70-80% (Senthilnambi *et al.*, 2011) for cultivation, which is conducive to the environmental conditions of Bangladesh. The advantages of this mushroom over other mushrooms are easy method of cultivation, less investment, very attractive fruiting body, pleasing milk white color, long shelf life, more nutritious and less time to grow (Bokaria *et al*., 2014).

The choice of the farmers for growing of any crop's variety depends upon its yielding ability. Scientists are suggesting to farmers for growing the high yielding mushroom strains. The number of cultivated species is increasing, and this will have the effect on augmenting the total world production in the future because new species are unlikely to

be substituted for others by the consumer, but they will be added to the ones that are already popular. Production of the existing cultivated species has shown a steady increase for decades, and more countries are engaging in mushroom cultivation as an agricultural technology. These factors argue against total mushroom production reaching a plateau in the near future (Chang and Miles, 2004). Different *Calocybe indica* strains are reported which show much diversity in their yield potential depending on the substrates used.

Because of suitable condition, high local demand and export potential of this mushroom, many private entrepreneurs of Bangladesh are interested in its commercial cultivation (Amin *et al*., 2010). But the producers are facing the problems of lack of suitable strains. The strains of milky white mushroom in Bangladesh are very limited. The performance of all the strains preserved in Mushroom Development Institute is not yet evaluated. Therefore, the present study was undertaken to evaluate the performance of all the strains of milky white mushroom preserved in MDI and identify suitable strain for commercial cultivation.

MATERIALS AND METHODS

The experiment was conducted at Mushroom Development Institute, Department of Agricultural Extension, Savar, Dhaka, Bangladesh from mid-February 2018 to mid-October 2018. Performance of four different milky white mushroom strains viz.; Cid-1, Cid-A, Cid-In and Cid-S (Plate-5) were evaluated in three growing season- summer, rainy season and autumn.

Plate -5: Fruiting body of four different strains of milky white mushroom

Preparation of pure and mother culture

Pure mycelium culture of four milky white mushroom strains were prepared on potato dextrose agar (PDA) media as described in chapter III. The pure culture was prepared in petri plates. PDA media containing petri plates were inoculated with mycelium in a clean bench and then transferred to the laboratory room for mycelium growth at ambient

temperature. Mother and sub mother culture was prepared in sawdust and wheat bran mixed media as described in chapter III.

Mycelium growth measurement

Mycelium growth in pure culture was measured radially (Plate-6) by centimeter scale after every 3 days and in the mother culture and sub mother culture it was measured vertically (Plate-7) by centimeter scale after every 7 days.

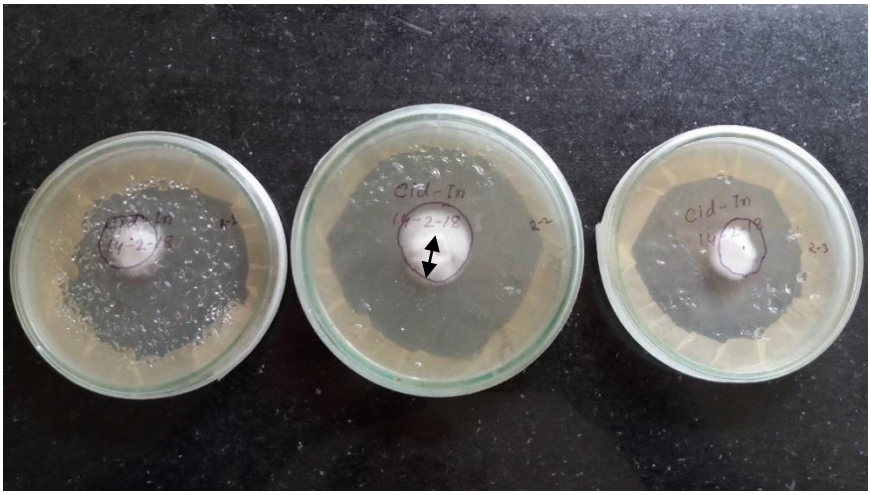

Plate-6: Mycelium growth in PDA media measured radially

Plate-7: Mycelium growth in sawdust-based mother culture measured vertically

Preparation of spawn packet, casing and after care

Rice straw was used as substrate for the cultivation of milky white mushroom strains. Spawn packets were prepared as described in chapter III. The spawn packets were transferred to the culture house for mycelium run. Within 16-25 days of inoculation the substrate was completely colonized by the mycelium. Then cotton, brown paper and neck were removed from the packets and the mouth of the plastic bags were folded 4-5 cm above the spawn. Previously sterilized loamy soil casing materials were used to cover over the mycelium on the substrate up to 4 cm thickness. Watering was done at regular interval to maintain moisture at 60 to 70%. Fruiting body primordia initiated within 12 - 24 days and developed in to fruiting bodies.

Harvesting and data collection

The fruiting bodies were harvested at 7-8 days of primordial initiation and data were collected on days to spawn run, days to primordial initiation, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, number of effective fruiting body (NEFB), number of flushes, economic yield and biological efficiency (BE). The BE was measured by the formula described in chapter III.

Nutrient analysis

Nutrient content of fruiting body of the four strains were analyzed following standard methods as described in chapter III.

DNA finger print-

DNA finger printing of the four strains was performed to know whether the strains were collected from the same source or not. Milky white mushroom strain Cid-1 (M-1), Cid-A (M-2), Cid-In (M-3) and (Cid-S) were collected from MDI, Savar, Dhaka.

DNA extraction: Filamentous fungi have strong cell walls which are often difficult to rupture in traditional method. In the present study modified method of Aljanabi *et al*. (1999) has been used to isolate the total genomic DNA from Mushroom. DNA of four different strains were extracted from 0.2–0.3g fruiting body of each strain. It was grinded in extraction buffer (200 mM Tris-HCl, pH-8.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) with a morter pestle. The lysates were incubated at 65^{o}C for 40 min in water bath and centrifuge 30 mins at 10000 rpm. DNA was precipitated from the supernatant by adding equal volumes of isopropanol and resultant pallet was washed with 70% ethanol. The DNA pellet was air dried and dissolved in 50 µL TE buffer. DNA quantification was performed and a dilution of 50ng/µL was used in downstream application.

RAPD analysis: Genomic DNA was amplified by the RAPD technique (Williams *et al*., 1990) in which two sorts of arbitrary 10-base oligonucleotide primers (Operon technologies Inc.) such as OPA-03, AGTCAGCCAC; OPA-04, AATCGGGCTG) were used to produce amplified fragments. RAPD-PCR reaction was performed using a thermal cycler within an initial denaturation stage of 5 min at 94^{o}C, followed by 40 cycles of denaturation for 1 min at 94^{o}C, annealing for 1 min at 36^{o}C, extension for 2 min at 72^{o}C and a final extension for 10 min at 72^{o}C.

Gel electrophoresis and RAPD data scoring: RAPD product were electrophoresed on 1.4% agarose gels 1X TBE buffer for 1.15 hr at 100v with 1kb DNA ladder as a size maker and stained while agitating in an EtBr solution (0.5% µg/ml). The stained gels were visualized under a UV trans illuminator and photographed using Bio-Rad gel documentation system. The amplification product generated by each RAPD primer were scored as ‘1’ or ‘0’ for presence or absence of specific allele, respectively. To estimate the similarity and genetic distance among different strains, cluster analysis based on Nei’s unweighted pair-group with arithmetic average (UPGMA) was performed using the ‘statistica’ software and dendrogram were constructed.

Experimental design and statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. The data were statistically analyzed following SPSS (version 26.0) computer program. Difference among the treatment means were determined by Tukey's Test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Mycelium growth in PDA media

Mycelium growth of different strains of milky white mushroom in PDA media was significantly varied during the summer season but it was insignificant both in rainy and autumn season (Table-1). Mycelium growth rate of strain Cid-S in PDA media was highest (0.37cm/day) during the summer season which was similar to Cid-1 (0.35 cm/day) but was different from other two strains. During the rainy season mycelium growth rate of strain Cid-A and Cid-In was highest (0.39 cm/day) and growth rate of strain Cid-1 & Cid-S was lowest (0.35 cm/day). Mycelium growth rate of strain Cid-A (0.38 cm/day) was highest during the autumn season whereas it was lowest in strain Cid-S (0.27 cm/day) during the same growing season. Kerketta *et al.* (2017) reported that mycelium growth of five milky white mushroom strains was ranges from 6.61 cm to 9.00 cm per day which differed from this study. It may be due to the variation in strain and growing condition.

Table-1: Mycelium growth of different strains of milky white mushroom in PDA media during different growing season

Strains	Mycelium growth in PDA media (cm/day)		
	Summer season	Rainy season	Autumn season
Cid-1	0.35ab	0.35a	0.31a
Cid-A	0.33bc	0.39a	0.38a
Cid-In	0.32c	0.39a	0.30a
Cid-S	0.37a	0.35a	0.27a
P	0.001	0.017	0.109

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Mycelium growth in mother culture

Mycelium growth of different strains of milky white mushroom in sawdust base mother culture was varied significantly during all the three-growing season (Table-2). Mycelium growth rate of strain Cid-1 was highest (0.42 cm/day) in mother culture during summer season which was statistically similar to strain Cid-A (0.40 cm/day) but was different from other two strains. Mycelium growth rate of strain Cid-S was lowest (0.27 cm/day) in mother culture during the same growing season.

Mycelium growth rate of strain Cid-In was highest (0.48 cm/day) during rainy season in mother culture which was similar to Cid-A (0.43 cm/day) and Cid-1(0.42 cm/day). Mycelium growth rate of Cid-S was lowest (0.32cm/day) in mother culture during the same growing season.

During the autumn season mycelium growth of strain Cid-In in mother culture was highest (0.54 cm/day) which was statistically similar to strain Cid-A (0.49 cm/day). Growth rate of mycelium in mother culture of strain Cid-1 and Cid-S was lowest (0.39 cm/day) in autumn season.

Table-2: Mycelium growth of different strains of milky white mushroom in sawdust base mother culture during different growing season

Strains	Mycelium growth in mother culture (cm/day)		
	Summer season	Rainy season	Autumn season
Cid-1	0.42a	0.42a	0.39b
Cid-A	0.40a	0.43a	0.49a
Cid-In	0.35b	0.48a	0.54a
Cid-S	0.27c	0.32b	0.39b
P	< 0.001	0.001	< 0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Mycelium growth in sub mother culture

Mycelium growth of different strains of milky white mushroom in sawdust base sub mother culture was significantly varied during all the growing season (Table-3). During the summer season growth of strain Cid-1was highest (0.38 cm/day) which was similar to strain Cid-In (0.37cm/day). Mycelium growth rate of strain Cid-S was lowest (0.32 cm/day) and was similar to strain Cid-A (0.34 cm/day) during summer season.

During the rainy season mycelium growth rate of strain Cid-1 in sub mother culture was highest (0.48 cm/day) which was different from other three strains. Growth rate of Cid-S was lowest (0.44cm/day) during the same growing season in the same media.

Mycelium growth of strain Cid-1in sub mother culture was highest (0.39cm/day) during the autumn season which was significantly higher than other three strains. Slowest

growth of mycelium in sub mother culture during autumn season was observed with strain Cid-S (0.31 cm/day).

Table-3: Mycelium growth of different strains of milky white mushroom in sawdust base sub mother culture during different growing season

Strains	Mycelium growth in sub mother culture (cm/day)		
	Summer season	Rainy season	Autumn season
Cid-1	0.38a	0.48a	0.39a
Cid-A	0.34b	0.46b	0.35c
Cid-In	0.37a	0.45b	0.37b
Cid-S	0.32b	0.44b	0.31d
P	< 0.001	0.001	< 0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Comparative growth rate of mycelium among the culture media and season are shown in Figure-1. Among the three different culture, mycelium growth rate was maximum in sawdust base mother culture followed by sub mother culture during summer and autumn season but during rainy season maximum growth rate was observed in sub mother culture. Mycelium growth rate was minimum in PDA media during all the growing season. During summer season mycelium growth rate of strain Cid-1 (0.42 cm/day) and Cid-A (0.40 cm/day) was highest in mother culture and lowest in PDA media (0.35 & 0.33 cm/day respectively). Growth rate of Cid-In (0.37 cm/day) was maximum in sub mother culture and minimum in PDA media (0.32 cm/day). Mycelium growth rate of Cid-S was maximum in PDA media (0.37 cm/day) and minimum in mother culture (0.27

cm/day) in the same season. Mycelium growth rate of Cid-1 (0.48 cm/day), Cid-A (0.46 cm/day) and Cid-S (0.44cm/day) was maximum in sub mother culture and minimum in PDA media (0.35, 0.39 & 0.35 cm/day) during rainy season. Mycelium growth rate of Cid-In (0.48 cm/day) was maximum in mother culture and minimum in PDA media (0.39 cm/day). During autumn season mycelium growth rate of Cid-A (0.49 cm/day) and Cid-In (0.54 cm/day) was maximum in mother culture. Growth rate of Cid-1 was same (0.39 cm/day) in mother and sub mother culture and minimum in PDA media (0.31cm/day). Growth rate of Cid-S was maximum in mother culture (0.39 cm/day) and minimum in PDA media (0.27 cm/day) during the same season. Mycelium growth rate in PDA media, saw dust mother and sub mother culture was varied among the strains during different growing season might be due to the variation in culture media and environmental condition. These results were in accordance with the findings of Kerketta *et al*. (2017) who also reported that mycelium growth of five *Calocybe indica* strains differed significantly on all tested media and temperature.

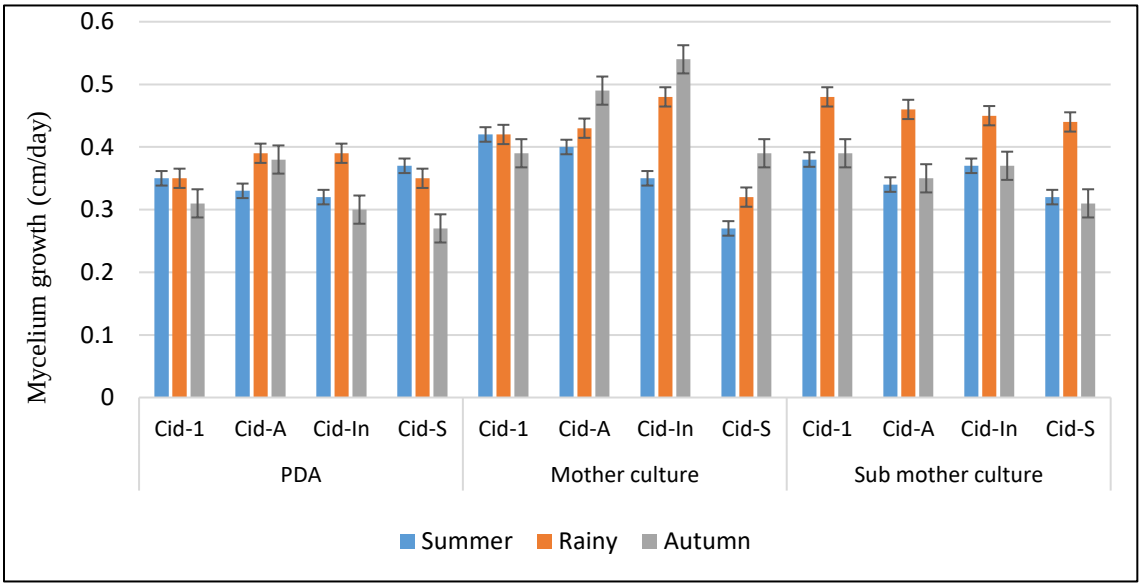

Figure-1: Mycelium growth of different strains in different culture media and growing season (I = Standard Error)

Days to spawn run, pinhead formation, first harvest and to complete total harvest

Days required for spawn run, pinhead formation and first harvest were significantly varied but variation in days to total harvest among the four milky white mushroom strains was insignificant during summer season (Table-4). Among the four milky white mushroom strains Cid-1 takes shortest time (20.45 days) to complete spawn run in rice straw substrate which was similar to strain Cid-A (20.78 days) during summer season. Strain Cid-S required longest time (25.58days) to complete spawn run in the same substrate and growing season.

Pinhead of fruiting body was appeared earlier (10.40 days) in strain Cid-A during summer season which was similar to strain Cid-In (11.03 days). Strain Cid-S takes more time (12.63 days) to produce fruiting body pinhead during summer season.

Strain Cid-S takes maximum time (56.20 days) from spawning to first harvest during summer season which was similar to strain Cid-In (54.70 days). Minimum time (50.13 days) was required for Strain Cid-1 from spawning to first harvest. To complete total harvest, strain Cid-In required maximum time (65.95 days) and strain Cid-A required minimum time (64.58 days) during the same growing season.

Table-4: Time to spawn run, pinhead formation and harvest of different strains of milky white mushroom during summer season

Strains	Days to spawn run	Days to pinhead formation	Days to 1st harvest	Time to complete total harvest (days)
Cid-1	20.45b	11.65ab	50.13b	64.98a
Cid-A	20.78b	10.40c	49.33b	64.58a
Cid-In	25.53a	11.03bc	54.70a	65.95a
Cid-S	25.58a	12.63a	56.20a	65.23a
P	< 0.001	0.001	< 0.001	0.883

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Days required for spawn run, pinhead formation, first harvest and last harvest were significantly varied among the four milky white mushroom strains during the rainy season (Table-5). To complete spawn run in the rice straw substrate strain Cid-A required minimum time (17.28 days) during rainy season which was significantly different from other strains. Strain Cid-S required maximum time (24.20 days) to complete span run in the same substrate and season. Days required from casing to fruiting body pinhead formation was also minimum in case of strain Cid-A (11.45 days) which was similar to strain Cid-1(11.98 days). Strain Cid-S took maximum time (14.28 days) to produce fruiting body pinhead after casing which was similar to strain Cid-In (13.53 days) during rainy season. Time to first harvest from spawning was maximum (56.40 days) in case of strain Cid-S which was significantly higher than other three strains during rainy season. Minimum time (46.73 days) was required for strain Cid-A during the same season which was significantly lower than other three strains. Strain Cid-S took maximum time to complete total harvest from spawning (67.73 days) which was similar to strain Cid-In

(66.58 days). Strain Cid-A also took minimum time (62.53 days) to complete total harvest which was similar to strain Cid-1(62.58 days).

Table-5: Time to complete spawn run, pinhead formation and harvest of different strains of milky white mushroom during rainy season

Strains	Days to spawn run	Days to pinhead formation	Days to 1st harvest	Time to complete total harvest (days)
Cid-1	19.48c	11.98b	49.35c	62.58b
Cid-A	17.28d	11.45b	46.73d	62.53b
Cid-In	20.83b	13.53a	51.33b	66.58a
Cid-S	24.20a	14.28a	56.40a	67.73a
P	< 0.001	< 0.001	< 0.001	0.004

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Days required for spawn run, pinhead formation, first harvest and last harvest of four milky white mushroom strains were significantly varied during autumn season (Table-6). To complete spawn run in the rice straw substrate during autumn season strain Cid-1 required minimum time (19.18 days) which was similar to strain Cid-A (19.78 days). Strain Cid-S took maximum time (24.58 days) to complete spawn run during the same season. Among the four milky white mushroom strains earlier fruiting body pinhead formation was observed in strain Cid-A and delay in strain Cid-S during autumn season. Strain Cid-A took 12.20 days for fruiting body pinhead formation after casing which was similar to strain Cid-1(13.20 days). Strain Cid-S required 16.10 days for fruiting body pinhead formation which was similar to strain Cid-In (15.08 days). Strain Cid-A took shortest time (46.95 days) to first harvest from spawning during autumn season which

was similar to strain Cid-1 (47.35 days). Strain Cid-S required longest time (55.65 days) to first harvest which was significantly higher than other three strains. To complete total harvest strain Cid-A required highest time (59.03 days) which was similar to strain Cid-S (58.65 days). Strain Cid-1 required lowest time (56.35 days) to complete total harvest which was similar to strain Cid-In (56.60 days).

Table-6: Time to spawn run, pinhead formation and harvest of different strains of milky white mushroom during autumn season

Strains	Days to spawn run	Days to pinhead formation	Days to 1st harvest	Time to complete total harvest (days)
Cid-1	19.18c	13.20b	47.35c	56.35b
Cid-A	19.78c	12.20b	46.95c	59.03a
Cid-In	22.15b	15.08a	52.23b	56.60b
Cid-S	24.58a	16.10a	55.65a	58.65a
P	< 0.001	< 0.001	< 0.001	0.007

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Comparative duration of spawn run, fruiting body pinhead formation, first harvest and total harvest among the three growing seasons are shown in Figure-2. During summer season all the four strains took more time to complete spawn run than other two growing season. Fruiting body pinhead formation was earlier during summer season followed by rainy and autumn season in case of all the four strains. Days to first harvest also took slightly more time during summer season than other two growing season. To complete total harvest, it was required less time during autumn season compare to other two

growing season for all the strains. Strain Cid-1 took shortest time to complete spawn run during autumn season (19.18 days) and longest time during summer season (20.45 days). Strain Cid-A (17.28 days), Cid-In (20.83days) and Cid-S (24.20 days) took shortest time during rainy season and longest time during summer season (20.78, 25.53 & 25.58 days) to complete spawn run. Strain Cid-1 required heist time (50.13 days) to first harvest during summer season and lowest time (47.35 days) during third growing season. Strain Cid-A and Cid-In took maximum time (49.33 & 54.70 days) during summer season and minimum time (46.73 & 51.33 days) during rainy season to first harvest. In case of strain Cid-S it was required highest time (56.40 days) during rainy season and lowest time (55.65 days) during autumn season to first harvest. To complete total harvest strain Cid-1 and Cid-A required highest time (64.98 & 64.58days) during summer season and lowest time (56.35 & 59.03 days) during autumn season. Strain Cid-In and Cid-S required highest time (66.58 & 67.73 days) during rainy season and lowest time (56.60 & 58.65 days) during autumn season to complete total harvest.

These results were an agreement with the findings of Dhakad *et al*. (2015) who also reported that different strains of milky white mushroom took 58.33 to 70.67 days for total harvest. These results were also supported by the findings of Kumar *et al*. (2011) who observed significant variation among the strain in respect of yield attributes and among five strains of milky mushroom CI-6 took at least 16 days for spawn run and 16 days for pinhead formation.

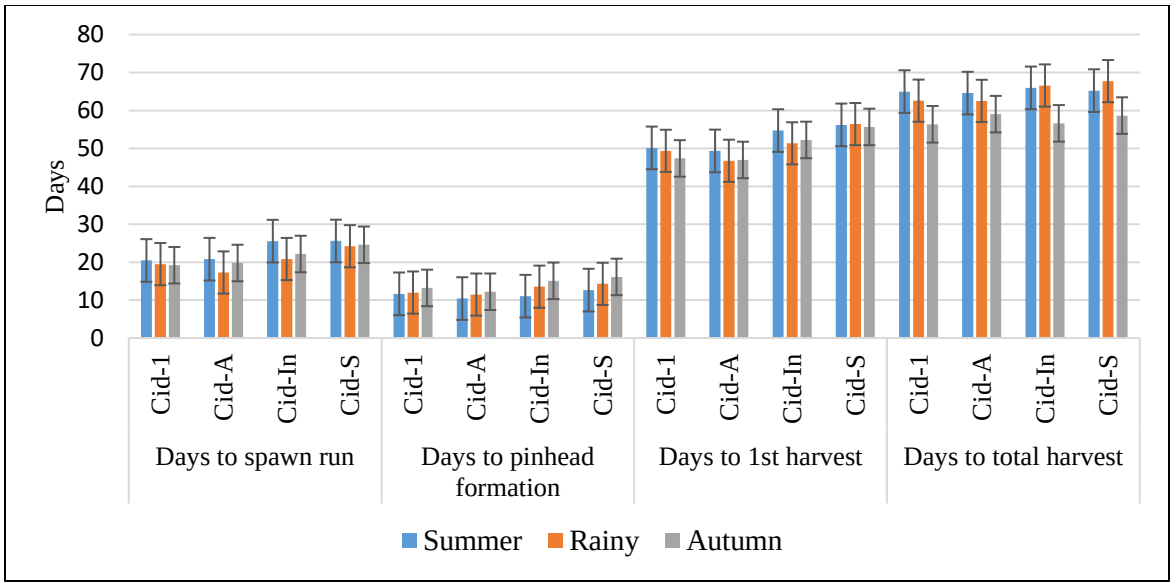

Figure-2: Time to spawn run, fruiting body pinhead formation and harvesting of different strains of milky white mushroom in different growing season (I = Standard Error)

Number and size of fruiting body

Variation in number of fruiting body and length of stalk among the four strains of milky white mushroom was insignificant during summer season but there was significant variation among the strains considering diameter of stalk, diameter of pileus and thickness of pileus (Table-7). Highest number of effective fruiting body (6.23) was recorded from strain Cid-A which was statistically similar to other three strains. Number of effective fruiting body was lowest in strain Cid-S (5.48). Length of stalk (8.40 cm) was highest in strain Cid-1 which was similar to other three strains. Lowest stalk length (7.75 cm) was recorded from the strain Cid-In. Stalk diameter was highest (2.65 cm) in strain Cid-A which was similar to Strain Cid-1(2.55 cm). Diameter of stalk was lowest in both the strain Cid-In and Cid-S (92.35 cm). Diameter of pileus was highest (6.98 cm) in strain Cid-1 which was statistically similar to strain Cid-A (6.68 cm) but different from other two strains. Pileus diameter was lowest (5.90 cm) in strain Cid-S which was similar to strain Cid-In (6.20 cm). Pileus thickness was also highest (2.98 cm) in strain Cid-1 followed by strain Cid-A (2.68 cm). Lowest diameter of pileus was recorded from strain Cid-S and Cid-In (2.58 cm).

Table-7: Number and size of fruiting body of different strains of milky white mushroom during summer season

Strains	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Cid-1	6.20a	8.40a	2.55a	6.98a	2.98a
Cid-A	6.23a	8.35a	2.65a	6.68ab	2.68ab
Cid-In	5.58a	7.75a	2.35b	6.20bc	2.58b
Cid-S	5.48a	8.05a	2.35b	5.90c	2.58b
P	0.111	0.046	0.002	0.001	0.030

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

During rainy season, number of effective fruiting body, diameter of stalk, diameter of pileus and thickness of pileus was significantly varied among the four strains of milky white mushroom but variation in stalk length was insignificant (Table-8). Highest number of effective fruiting body (6.98) was recorded from strain Cid-1 which was similar to strain Cid-A (6.20). Strain Cid-S produces lowest number of effective fruiting body (5.53) which was similar to strain Cid-In (5.60) and Cid-A (6.20). Stalk length of strain Cid-A was highest (6.63 cm) and stalk length of strain Cid-In was lowest (7.83 cm). Highest diameter of stalk (2.88 cm) was recorded from strain Cid-A which was similar to strain Cid-1 (2.83 cm) and strain Cid-In (2.73 cm). Lowest diameter of stalk (2.60 cm) was recorded from strain Cid-S which was similar to strain Cid-In (2.73 cm). Diameter of pileus was highest (5.85cm) in strain Cid-1 which was similar to strain Cid-A (5.83 cm) and strain Cid-In (5.43 cm). Lowest diameter of pileus (4.90 cm) was recorded from strain Cid-S which was significantly lower than other three strains. Thickness of pileus was highest (2.33 cm) in strain Cid-A which was similar to strain Cid-1 (2.30cm) and

Cid-In (2.23 cm). Pileus thickness was lowest in strain Cid-S (2.05 cm) which was significantly lower than other three strains.

Table-8: Number and size of fruiting body of different milky white mushroom strains during rainy season

Strains	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Cid-1	6.98a	8.05a	2.83a	5.85a	2.30a
Cid-A	6.20ab	8.63a	2.88a	5.83a	2.33a
Cid-In	5.60b	7.83a	2.73ab	5.43a	2.23a
Cid-S	5.53b	7.88a	2.60b	4.90b	2.05b
P	0.022	0.192	0.011	< 0.001	0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Number of effective fruiting body and stalk length was significantly varied among the four strains of milky white mushroom during autumn season but diameter of stalk, diameter of pileus and thickness of pileus among the strains were insignificant (Table-9). Highest number of effective fruiting body (6.95) was recorded from strain Cid-1which was similar to strain Cid-A (6.35) and Cid-S (5.90). Strain Cid-In produces lowest number of effective fruiting body (5.30). Stalk length of strain Cid-A was highest (8.85 cm) which was similar to strain Cid-1 (8.68 cm). Shortest length of stalk (7.35 cm) was recorded from strain Cid-S which was significantly different from other strains. Diameter of stalk was highest in strain Cid-1 & Cid-A (2.73 cm) and lowest in strain Cid-S (2.63 cm). Both diameter of pileus (5.80 cm) and thickness of pileus (2.30 cm) was highest in

strain Cid-In on the other hand diameter of pileus (5.20 cm) and thickness of pileus (2.15 cm) was lowest in strain Cid-S.

Table-9: Number and size of fruiting body of different strains of milky white mushroom during autumn season

Strains	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Cid-1	6.95a	8.68ab	2.73a	5.45a	2.28a
Cid-A	6.35ab	8.85a	2.73a	5.78a	2.23a
Cid-In	5.30b	8.13b	2.68a	5.80a	2.30a
Cid-S	5.90ab	7.35c	2.63a	5.20a	2.15a
P	0.016	< 0.001	0.815	0.110	0.112

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Comparative number of effective fruiting body, length & diameter of stalk and diameter and thickness of pileus among the growing season and strains are shown in Figure-3. Highest number of effective fruiting bodies were recorded from strain Cid-1 during rainy season (6.98) and lowest number from summer season (6.20). Number of effective fruiting body produced by strain Cid-A was maximum during autumn season (6.35) and minimum during rainy season (6.20). Strain Cid-In produces highest number of effective fruiting body (5.60) during rainy season and lowest during autumn season (5.30). Number of effective fruiting body was highest (5.90) during autumn season and lowest (5.48) during summer season in case of strain Cid-S. Stalk length of fruiting body of strain Cid-1 was highest during autumn season (8.68cm) and lowest during rainy season (8.05cm). Strain Cid-A and Cid-In produces longest (8.85cm & 8.13cm) number of

fruiting body during autumn season and shortest (8.35cm & 7.75cm) during summer season. Fruiting body stalk length of strain Cid-S was highest (8.05cm) during summer season and decreased in the subsequent growing season. Among the four strains stalk diameter of Cid-1 (2.83cm), Cid-A (2.88cm) and Cid-In (2.73cm) was highest during rainy season and lowest (2.55cm, 2.65cm & 2.35cm) during summer season. In case of strain Cid-S stalk diameter was highest (2.63cm) during autumn season and lowest (2.35cm) during summer season. Pileus diameter of strain Cid-1(6.98cm) and Cid-A (6.68cm) was maximum during summer season and minimum (5.45cm & 5.78cm) during autumn season. In case of strain Cid-In (6.20cm) and Cid-S (5.90cm) it was also maximum during summer season but minimum (5.43cm & 4.90cm) during rainy season. Similar trend was observed in case of all the strains considering pileus thickness. This result was an agreement of the findings of Dhakad *et al.* (2015) who reported that average stalk length of five strains of milky white mushroom was ranges from 4.74 cm to 7.43 cm, stalk diameter 2.39 cm to 3.17 cm and diameter of pileus 6.72 cm to 8.50 cm.

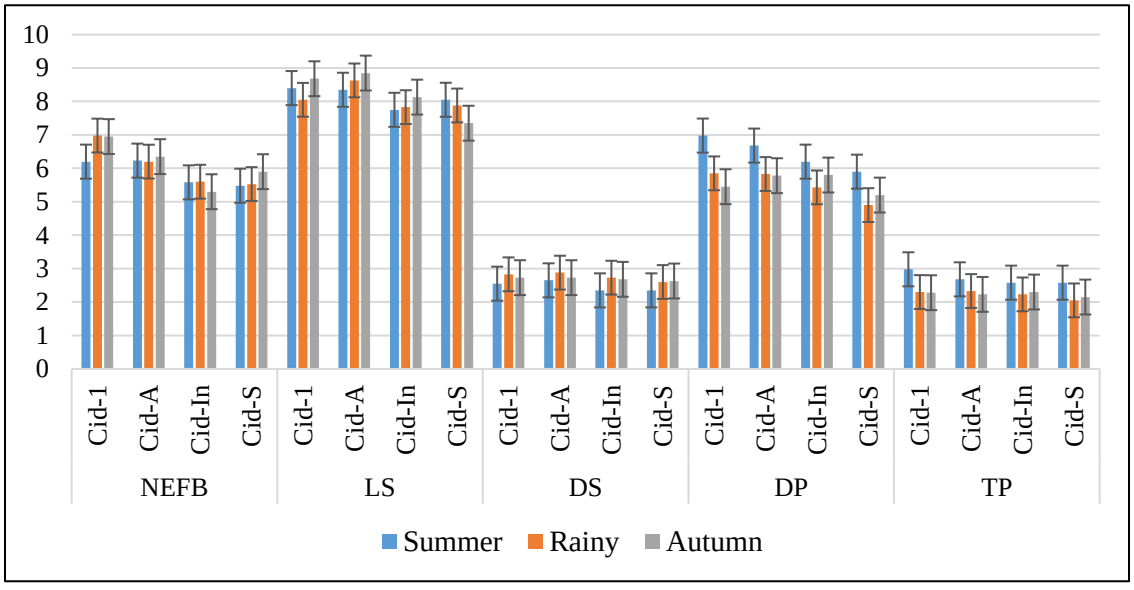

Figure-3: Number of effective fruiting body, length & diameter of stalk and diameter & thickness of pileus of milky white mushroom in different growing season (I = Standard Error)

Weight of fruiting body, economic yield and biological efficiency

Weight of fruiting body, economic yield and biological efficiency of four milky white mushroom strains were significantly varied during summer season (Table-10). Strain Cid-1 produces largest size and strain Cid-S produces smallest size fruiting body. Average weight of fruiting body (55.93 g) was highest in strain Cid-1which was similar to strain Cid-A (52.80 g). Fruiting body weight was lowest (45.95 g) in strain Cid-S which was similar to strain Cid-In (47.68 g). Highest economic yield (345.75 g/packet) was recorded from strain Cid-1 which was similar to strain Cid-A (328.70 g/packet). Economic yield was lowest (250.35 g/packet) in strain Cid-S which was similar to strain Cid-In (264.60 g/packet). Biological efficiency was also highest (83.30%) in strain Cid-1 which was similar to strain Cid-A (79.20%). Lowest biological efficiency (60.33%) was recorded from strain Cid-S which was similar to strain Cid-In (63.78%).

Table-10: Weight of fruiting body, economic yield and biological efficiency of different strains of milky white mushroom during summer season

Strains	Weight of fruiting body (g)	Economic yield (g/packet)	Biological efficiency (%)
Cid-1	55.93a	345.75a	83.30a
Cid-A	52.80ab	328.70a	79.20a
Cid-In	47.68bc	264.60b	63.78b
Cid-S	45.95c	250.35b	60.33b
P	0.004	< 0.001	< 0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Average weight of fruiting body, economic yield and biological efficiency was significantly varied among the four milky white mushroom strains during rainy season (Table-11). Highest average weight of fruiting body (52.98 g) was recorded from the strain Cid-A during that growing season which was similar to strain Cid-1 (48.15 g). Average weight of fruiting was lowest (40.93 g) in strain Cid-S which was similar to strain Cid-In (43.18 g). Strain Cid-1 produces highest economic yield (332.23 g/packet) which was similar to strain Cid-A (327.48 g/packet). Strain Cid-S produces lowest economic yield (267.03 g/packet) among the four strain which was similar to strain Cid-In (273.48 g/packet). Biological efficiency (80.05 %) was highest in strain Cid-1which was similar to strain Cid-A (78.85). Lowest biological efficiency (64.33 %) was recorded from strain Cid-S which was similar to strain Cid-In (65.88 %).

Table-11: Weight of fruiting body, economic yield and biological efficiency of different strains of milky white mushroom during rainy season

Strains	Weight of fruiting body (g)	Economic yield (g/packet)	Biological efficiency (%)
Cid-1	48.15ab	332.23a	80.05a
Cid-A	52.98a	327.15a	78.85a
Cid-In	43.18b	273.48b	65.88b
Cid-S	40.93b	267.03b	64.33b
P	0.008	< 0.001	< 0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Variation in average weight of fruiting body among the four strains of milky white mushroom was insignificant during autumn season whereas the economic yield and biological efficiency varied significantly among the strains during the same season (Table-12). Average weight of fruiting body was highest (50.53 g) in strain Cid-In and

lowest fruiting body weight (43.68 g) was recorded from strain Cid-1. Highest economic yield (314 g/packet) was produced by strain Cid-A during autumn season which was similar to strain Cid-1 (304.00 g/packet). Lowest economic yield (260.83 g/packet) was produced by strain Cid-S which was similar to strain Cid-In (264.70 g/packet). As economic yield biological efficiency was also highest in strain Cid-A (75.78 %) and similar to strain Cid-1(73.25 %). Biological efficiency was lowest (62.83 %) in strain Cid-S which was similar to strain Cid-In (63.78 %).

Table-12: Weight of fruiting body, economic yield and biological efficiency of different strains of milky white mushroom during autumn season

Strains	Weight of fruiting body (g)	Economic yield (g/packet)	Biological efficiency (%)
Cid-1	43.68a	304.00ab	73.25ab
Cid-A	49.73a	314.50a	75.78a
Cid-In	50.53a	264.70b	63.78b
Cid-S	43.88a	260.83b	62.83b
P	0.079	0.010	0.010

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Comparative weight of fruiting body, economic yield and biological efficiency of four milky white mushroom strains among different growing season are shown in Figure 4 to 6. Average weight of fruiting body of strain Cid-1 was highest (55.93 g) in summer season and lowest (43.68 g) in autumn season. Fruiting body weight of strain Cid-A was highest (52.98 g) in rainy season and lowest (49.73 g) in autumn season. Average weight of fruiting body of Cid-In was highest (50.53 g) in autumn season and lowest (43.18 g) in rainy season. In case of strain Cid-S fruiting body weight was highest (45.95 g) in

summer season and lowest (40.93 g) in rainy season. Economic yield of strain Cid-1 and Cid-A was highest during summer season and lowest in autumn season. Strain Cid-In and Cid-S gave highest economic yield (273.48 g & 267.03 g)) during rainy season and lowest yield (264.60 g & 250.35 g) during summer season. Similar trend was observed in case of biological efficiency.

The results are in accordance with the findings of Kaur *et al*., (2011) in which nine strains of *Calocybe indica* were evaluated for the yield grown on wheat straw. The biological efficiency, estimated from the harvested yield, was maximum in strain Ci-3 (81.28%). Kerketta *et al*. (2018) also reported that average fruiting body weight of five different strains of milky white mushroom varied from 54.0 g to 82.0 g and biological efficiency from 27.33% to 95.16%. Dhakad *et al*. (2015) reported biological efficiency of five strains of milky white mushroom was ranges from 60.80% to 81.16%.

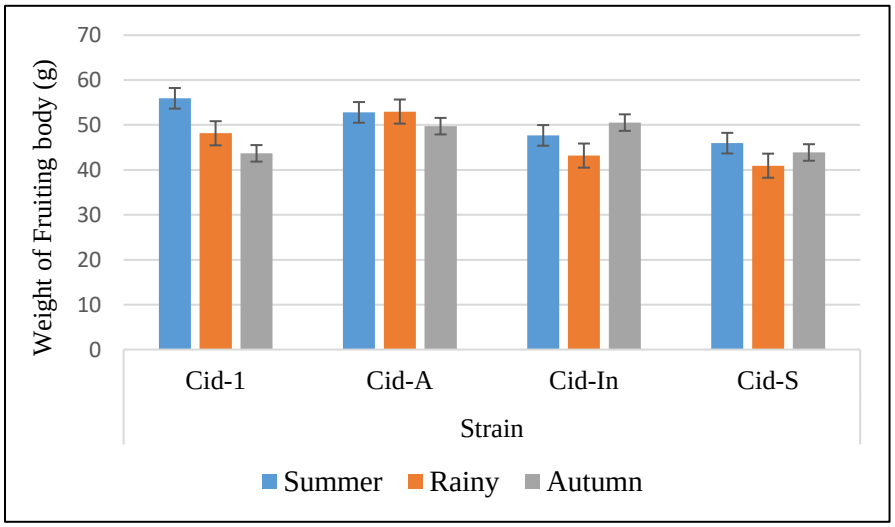

Figure-4: Weight of fruiting body of four milky mushroom strains in different growing season (I = Standard Error)

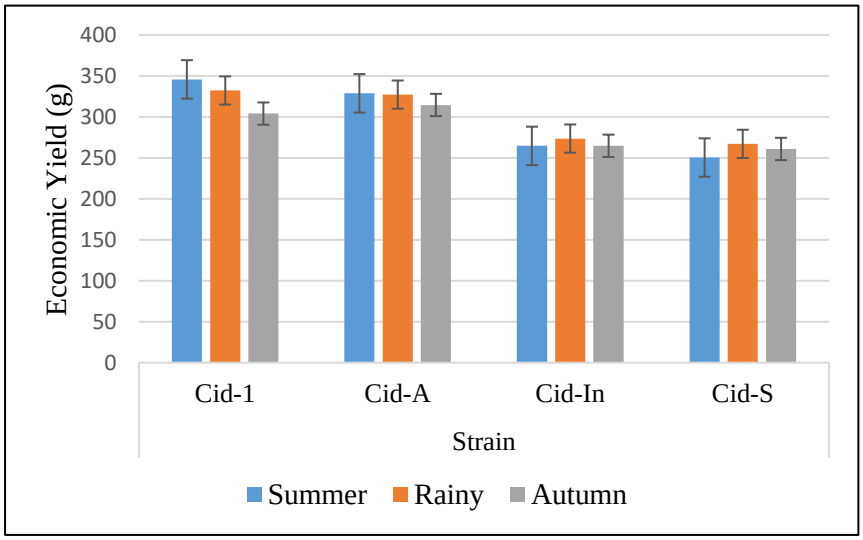

Figure-5: Economic yield of four milky mushroom strains in different growing season (I = Standard Error)

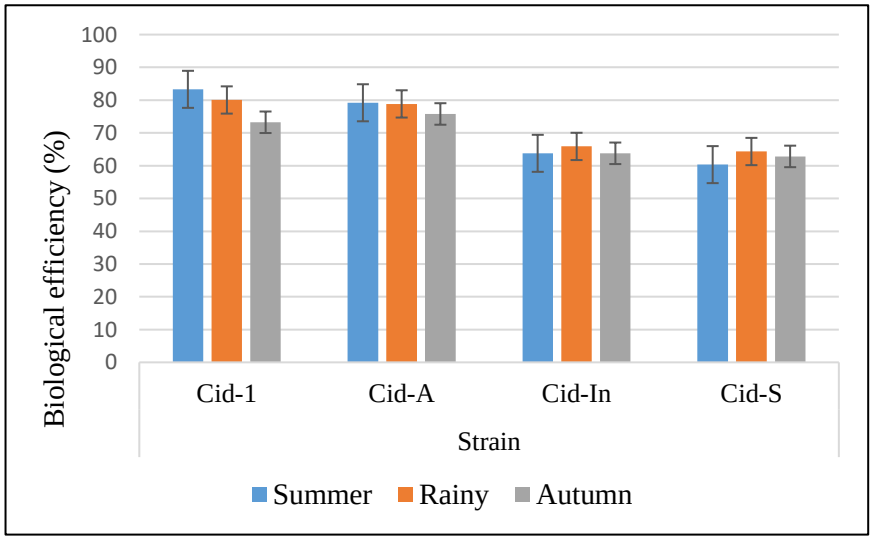

Figure-6: Biological efficiency of four milky mushroom strains in different growing season (I = Standard Error)

Nutrient contents

Nutrient content of four milky white mushroom strains is shown in Table-13. Amount of moisture, carbohydrate, protein, lipid, fiber and ash were more or less similar among the strains. Moisture content of fresh mushroom ranges from 89.87 to 92.14%, carbohydrate from 61.20 to 62.40 g, protein from 11.60 to 14.60 g, lipid from 2.38 to 2.96 g, fiber from 12.30 to 13.80 g and ash from 9.23 to 9.52 g per 100 g dry weight of mushroom. Strain Cid-1 contained highest amount of protein, Cid-In contained highest amount of carbohydrate and lowest amount of lipid, Cid-A contain highest amount of fibre and strain Cid-S contained highest amount of ash.

Table-13: Nutrient content of different strains of milky white mushroom (g/100g dry weight)

Strains	Moisture (%)	Carbohydrate (g)	Protein (g)	Lipid (g)	Fiber (g)	Ash (g)
Cid-1	92.14	61.20	14.60	2.38	12.3	9.52
Cid-A	91.30	62.40	11.60	2.96	13.8	9.23
Cid-In	89.87	63.10	13.00	1.89	12.8	10.21
Cid-S	90.96	62.40	12.10	3.10	12.03	10.40

Molecular Result

Band size

The sizes of the amplified bands in the four mushroom strains ranged from 250 to 3000bp (Table-14). Among the two RAPD primers, OPA-03 revealed band sizes that ranged from 400 bp to 3000 bp and primer OPA-04 ranged from 250bp-1500bp.

Number of bands

All the five RAPD primer amplified a total of 120 bands from the four strains of mushroom using the Thermal Cycler (Finzymes) and 1% Agarose gel (Table-14). The primer OPA-03 amplified the highest number of bands (17) with four monomorphic bands and primer OPA-04 amplified a total of 15 band. Primer OPA-04 amplified a specific monomorphic band and showed 93.33 % polymorphism.

Table-14: RAPD primers with corresponding DNA bands scored and their size ranges in four different varieties of Oyster mushroom which were given below

Primer	Size ranges (bp)	Total number of bands scored	Number of polymorphic bands	Number of monomorphic bands	Polymorphism (%)
OPA-03	400-3000	17	13	4	76.47
OPA-04	250-1500	15	14	1	93.33
Total		32	27	05	

Genetic distances

The values of pair-wise comparisons of genetic distances analyzed by using computer software “Statistica” between strains were computed from combined data for the two primers, ranged from 1.0 to 13.0 (Table- 15). The highest linkage distance (13.00) was recorded between strain pairs Cid-1 and Cid-A; it was also found between Cid-S and Cid-

In. The linkage distance (7.00) was recorded between strain Cid-S and Cid1. The lowest linkage distance (1.00) was recorded between strain pairs Cid-A and Cid-In.

Table-15: Summary of linkage distances for different pairs of four mushroom strains for RAPD markers

Strains	Cid-1	Cid-S	Cid-A	Cid-In
Cid-1	0.0	7.0	13.0	12.0
Cid-S	7.0	0.0	12.0	13.0
Cid-A	13.0	12.0	0.0	1.0
Cid-In	12.0	13.0	1.0	0.0

Cluster analysis (Tree Diagram)

Genetic relationships among the strains at the average distance of 13.00, showed two major clusters C_1 and C_2 presented in the Figure-7. Major cluster C_1 comprised Cid1 and Cid-S. At the linkage distance of 7.00, Cid-1 and Cid-S strains were subdivided from each other. On the other hand, major cluster C_2 belonged to Cid-A and Cid-In. These strains were also differentiating from each other at the linkage distance of 1.00.

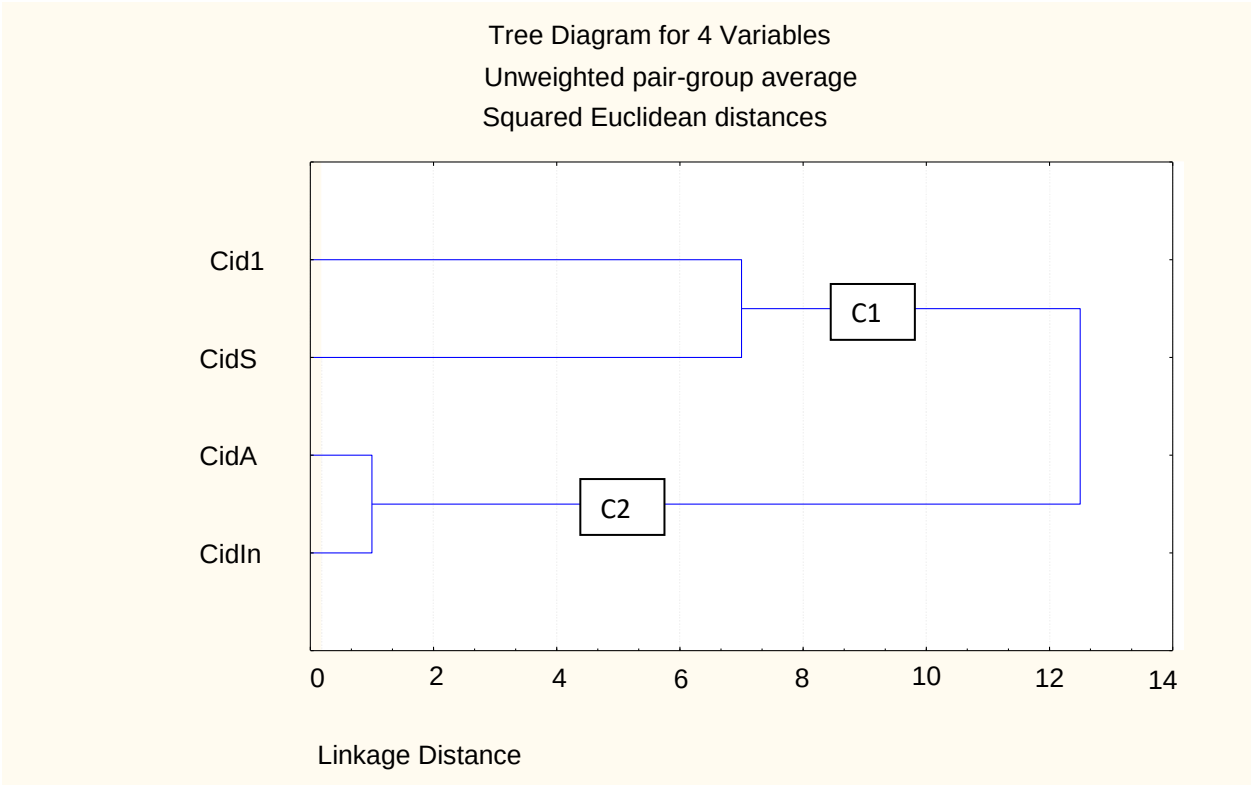

Figure.7: Cluster analysis by Unweighted pair group method of arithmetic means average (UPGMA) of four mushroom strains based on two RAPD markers.

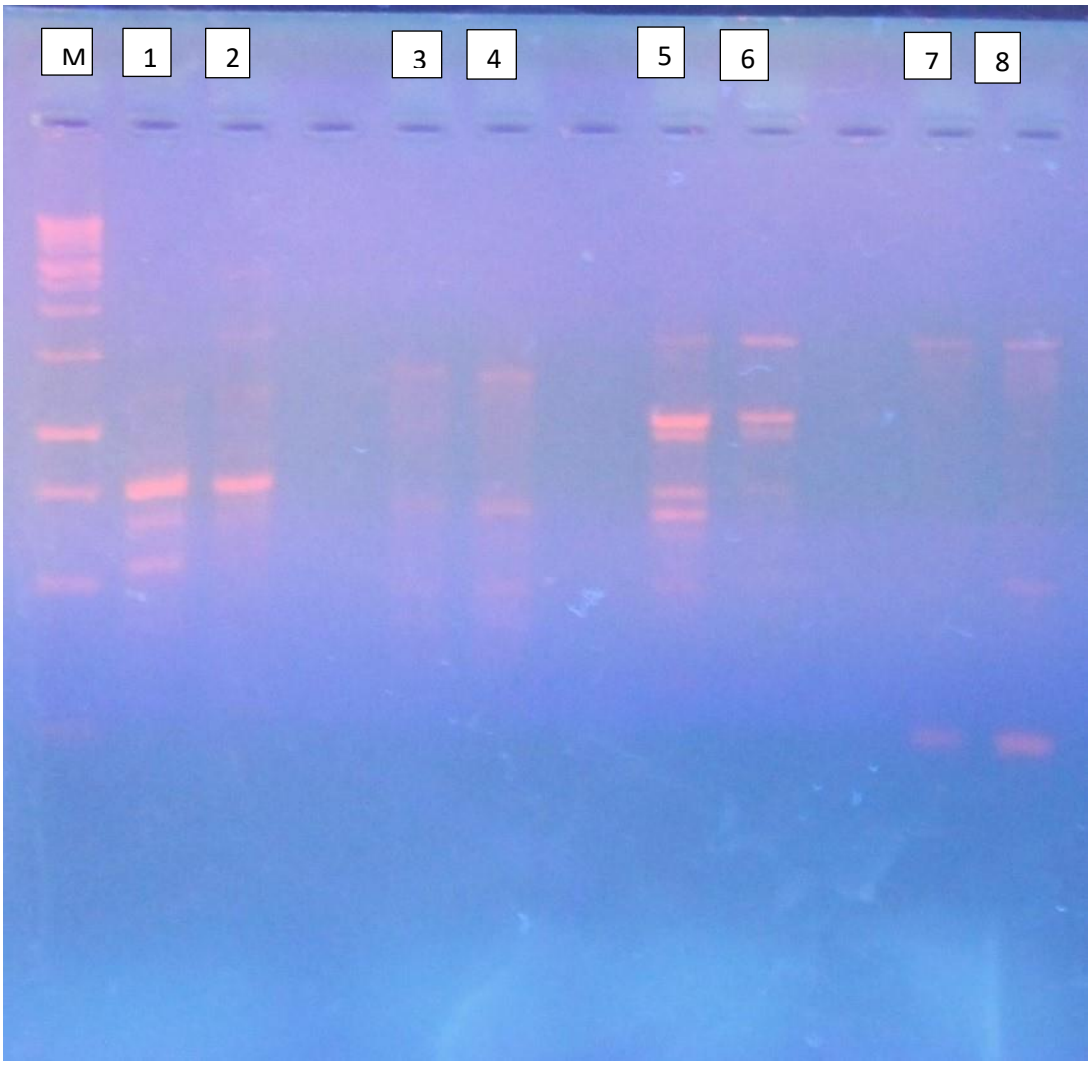

Figure-8: RAPD profiles in different strains of *Calocybe indica*. Primer OPA-3 and OPA-4 showing polymorphism among the strains. M- Marker (1 kb DNA ladder); Lane 1, 2 = Cid-1; Lane 3, 4 = Cid-S; Lane 5,6 = Cid-A; Lane 7, 8 = Cid-In.

Conclusion

From the above study it was concluded that considering all the yield contributing characters among the four strains of milky white mushroom preserved at Mushroom Development Institute, Cid-1 performed better. Performance of strain Cid-A was similar to Cid-1 during all the three growing seasons whereas strain Cid-S performed worse during all the growing season. There was no significant variation in yield among the three seasons. The results indicated that Strain Cid-A of milky white mushroom could also be successfully cultivated from March to September in a year besides Strain Cid-1. There was genetically variation among the strains.

EXPT. NO. 2: SCREENING OF SUITABLE SUBSTRATES FOR COMMERCIAL CULTIVATION OF MILKY WHITE MUSHROOM

INTRODUCTION

Mushroom substrate may be simply defined as a kind of lignocellulosic material that supports the growth, development, and fruiting of mushroom mycelium. Mushroom fruiting bodies depend on the substrates on which they grow for all their nutritional requirements like carbon, water, nitrogen and minerals (Rajarathnam *et al*., 1997). Waksman and co-workers conducted studies from which they concluded that most of the nutrients required for mycelial growth and mushroom development were obtained from lignin, cellulose, hemicellulose, and protein (Chang and Miles, 2004). The mycelium of mushrooms, like all fungal cells, lacks chlorophyll and consequently is unable to utilize carbon dioxide, mineral ions, and water for photosynthesis as do green plants. Nutritionally, mushrooms are heterotrophs and obtain their nutrients by absorbing soluble inorganic and organic materials from substrates such as wood logs, manure composts, or other organic synthetic composts. These soluble compounds can move through the fungal cell wall and cell membrane into the cytoplasm where they become metabolized. Once organic compounds have entered the fungal cell, they can be converted to the various sugars, polysaccharides, proteins, lipids, purines, pyrimidines, vitamins, etc. required for the vital activities and structural needs of the fungus.

Different types of mushrooms require different types of substrates. The optimum C: N ratio for the mushroom is about 17. A wide range of diverse cellulosic substrates are used for cultivating mushrooms. Milky mushroom can be cultivated on varieties of cellulosic substrates like, paddy straw, wheat straw, maize stalks, sorghum stalks, pearl millet stalks, sugarcane trace, sugarcane baggase, soya bean straw, cotton waste, coconut coir pith, groundnut haulms etc. Lignocellulosic materials are generally low in protein, which is insufficient for commercially cultivating mushrooms. These materials require different supplements or additives with sufficient amounts of nitrogen, phosphate, potassium, and vitamins for better growth and yield of mushrooms (Mangat *et. al.*, 2008).

Rice straw is the most common lignocellulosic substrate, whose major component is cellulose, and it is also considered the best substrate for cultivating milky white mushroom (Amin *et. al.*, 2010). But, huge quantities of other lignocellulosic residues such as wheat straw, mustard straw, maize straw, waste cotton, water hyacinth, sugarcane bagasse, coconut coir, and kash are generated annually through the activities of agricultural, forest, and food processing industries in Bangladesh which might prove suitable for this mushroom cultivation. Utilization of these various lignocellulosic wastes for commercial cultivation and value addition of milky white mushroom are the demand of the hour. Therefore, the present study was conducted to screening out the suitable substrate for commercial cultivation of milky white mushroom in Bangladesh.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Mushroom Development Institute, Department of Agricultural Extension, Savar, Dhaka, Bangladesh during May 2019 to July 2019.

Treatments

Nine different combinations of substrates were used in this experiment as treatments for growing milky white mushroom. Treatments were: T_1 = Rice straw; T_2 = Wheat straw; T_3 = Barley straw; T_4 = Waste paper + wheat bran (2:1); T_5 = Sugarcane baggage + wheat bran (2:1); T_6 = Maize cob + wheat bran (2:1); T_7 = Sawdust + wheat bran (2:1); T_8 = Rice straw + Sawdust (1:1) and T_9 =Maize straw.

Preparation of substrate and spawn packets

Substrates such as rice straw, wheat straw, barley straw, maize straw, sugarcane baggage and maize cob were chopped to convenient length of 2.5 to 5 cm and waste paper was tore by hand. The substrates were mixed with appropriate amount of water and then filled in net bags separately and pasteurized following the procedure described in chapter III. Pasteurized substrates were mixed according to treatment and filled into the polypropylene bags (12″x18″) and inoculated with 10% rice grain mother culture by thorough mixing. Then the spawn packets were transferred to the culture house for mycelium run. After 16-30 days the substrates were completely colonized by the mycelium.

Casing and after care

After completing mycelium colonization in the spawn packets, cotton, brown paper and neck were removed from the packets and the mouth of the plastic bags were folded 4-5 cm above the spawn. Loamy soil was used as casing material and was sterilized at 65^{o}C for 4 hours. Cooled casing material was covered over the mycelium on the substrate up to

4 cm thickness. Watering was done at regular interval to maintain moisture at 60 to 70%. Primordia initiated within 8-14 days and developed in to fruiting bodies.

Harvesting and data collection

The fruiting bodies were harvested at 7 - 8 days of primordia initiation and data were collected on days to complete spawn run, days to primordia initiation, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, number of effective fruiting body (NEFB), days to final harvest, yield and biological efficiency (BE). The BE was measured by the formula described in chapter III.

Experimental design and statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. The data were statistically analyzed following SPSS (version 26.0) computer program. Difference among the treatment means were determined by Tukey's Test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Days to mycelium run in the spawn packet, primordia initiation and complete total harvest

Days to complete mycelium run in the spawn packet, primordia initiation and total harvest was significantly varied among different substrates (Table-16). Mycelium running was faster in the wheat and barley straw substrate (14 days) which was statistically similar to maize straw (15 days) but was significantly different from all other substrates used in this experiment. Mycelium running was slowest in the mixture of saw dust and wheat bran (2:1) substrate (30days). This result supported by the findings of Suman *et al*. (2018) who also reported that minimum time was required for spawn run of milky white mushroom in wheat straw substrate. Rawal and Doshi (2014) and Bhatt *et al*. (2007) showed that among the tested substrates for cultivation of *Calocybe indica,* wheat straw took minimum period for spawn run (14 days). Kumar *et al*. (2017) reported that time required for spawn run in different substrate was between 21.50 to 27.50 days. Kumar and Chandra (2013) reported that spawn run time of milky mushroom in different substrate was varied from 23.0 to 27.0 days and highest time was required in sawdust + wheat straw substrate. Fruiting body primordia initiation after casing was earlier (9.3 days) in waste paper mixed with wheat bran (2:1) substrate which was statistically similar to T_6, T_3, T_2, T_5 and T_7. Highest time (16 days) was required for primordia initiation after casing in rice straw mixed with sawdust (1:1) substrate. Amin *et al.* (2010) also reported that primordia initiation with different substrates and casing materials was observed between the 13th and 19th days. Navathe *et al.* (2014) observed that time to pinhead formation of milky mushroom on different substrate was 14 to 23 days. Days required for complete total harvest from spawning of milky white mushroom was highest (76.2 days) in rice straw mixed with sawdust (1:1) substrate which was statistically similar to T_7, T_6, T_1 and T_4 but was different from other treatments. Lowest time (46 days) was required for spawning to last harvest in sugar cane baggage mixed with wheat bran (2:1) substrate.

Table-16: Effects of substrate on mycelium run, primordia initiation and time to complete total harvest of milky white mushroom

Treatments	Days to spawn run	Days to primordia initiation	Time to complete total harvest (days)
T_1- Rice straw	18.0e	13.5b	61.1abc
T_2- Wheat straw	14.0f	9.9c	58.6bc
T_3- Barley straw	14.0f	9.9c	49.7bc
T_4- Waste paper + wheat bran (2:1)	25.0c	9.3c	60.8abc
T_5- Sugarcane baggage + wheat bran (2:1)	17.0e	11.0c	46.0c
T_6- Maize cob + wheat bran (2:1)	27.0b	9.6c	64.5ab
T_7- Sawdust + wheat bran (2:1)	30.0a	11.0c	74.5a
T_8- Rice straw + Sawdust (1:1)	22.0d	16.0a	76.2a
T_9- Maize straw	15.0f	13.0b	49.7bc
P	< 0.001	< 0.001	< 0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Number and size of fruiting body

Size and number of effective fruiting body of milky white mushroom grown on different substrate varied significantly (Table-17). Highest number of effective fruiting bodies (7.5) were recorded from rice straw mixed with sawdust (1:1) substrate which was similar to waste paper + wheat bran (2:1) (6.7), sugarcane baggage + wheat bran (2:1) (6.5), maize cob + wheat bran (2:1) (6.4) and barley straw (6.0) substrate. Number of effective fruiting bodies (5.2) were lowest in maize straw substrate. Stalk length of fruiting body

was highest (9.1 cm) in rice straw and wheat straw substrate which was statistically similar to maize straw (8.9 cm) and rice straw + sawdust (1:1) (8.5cm). Lowest stalk length (7.1 cm) was recorded from sugar cane baggage mixed with wheat bran (2:1) substrate. Diameter of stalk was highest (2.5 cm) in maize and rice straw substrate which was similar to Maize cob + wheat bran (2:1) (2.4 cm), Rice straw + Sawdust (1:1) (2.48), Sugarcane baggage + wheat bran (2:1) (2.3 cm) and Waste paper + wheat bran (2:1) (2.3 cm). Stalk diameter was lowest (2.1cm) in wheat straw substrate. Diameter of pileus was highest (7.8 cm) in rice straw substrate whereas lowest (6.7 cm) in sawdust mixed with wheat bran (2:1) substrate. Thickness of pileus was highest (2.4 cm) in rice straw mixed with sawdust (1:1) substrate and lowest (2.0 cm) in barley straw substrate. These results were supported by the findings of Amin *et al*. (2010) and Chakraborty *et al.* (2016) who also reported that sporophore size (i.e., length of stalk x diameter of pileus) was significantly higher in paddy straw substrate.

Table-17: Effects of substrate on number and size of fruiting body of milky white mushroom

Treatments	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1- Rice straw	5.8b	9.1a	2.5a	7.8a	2.35abc
T_2- Wheat straw	5.6b	9.1a	2.1d	7.0ab	2.18bcd
T_3- Barley straw	6.0ab	7.9bc	2.1cd	7.0ab	2.03d
T_4- Waste paper + wheat bran (2:1)	6.7ab	7.9bc	2.3abc	7.3ab	2.30abc
T_5- Sugarcane baggage + wheat bran (2:1)	6.5ab	7.1c	2.3ab	6.8ab	2.25abc
T_6- Maize cob + wheat bran (2:1)	6.4ab	7.8bc	2.4a	7.1ab	2.35abc
T_7- Sawdust + wheat bran (2:1)	5.8b	7.3c	2.1bcd	6.7b	2.13cd
T_8- Rice straw + Sawdust (1:1)	7.5a	8.5ab	2.48a	7.2ab	2.43a
T_9- Maize straw	5.2b	8.9a	2.5a	7.2ab	2.40ab
P	0.007	< 0.001	< 0.001	0.108	< 0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Yield and biological efficiency

Both economic yield and biological efficiency of milky white mushroom was significantly influenced by different substrate materials (Figure-9). Highest yield and biological efficiency (427.3g & 103.5%) were recorded from rice straw mixed with sawdust (1:1) substrate which was statistically similar to rice straw (352.0g & 85.2%), waste paper + wheat bran (2:1) (347.4g & 84.1%) and Maize straw (347.2g & 84.1%) substrate. Yield and biological efficiency were lowest (264.1g & 62.9%) in sawdust mixed with wheat bran (2:1) substrate. This result was similar to the findings of Navathe

et al. (2014) who also reported that among the four substrates evaluated for cultivation of milky mushroom, paddy straw was the best with 81.05 percent biological efficiency. Kerketta *et al*. (2018) observed biological efficiency of milky mushroom in different substrate was 40.50 to 69.66 percent.

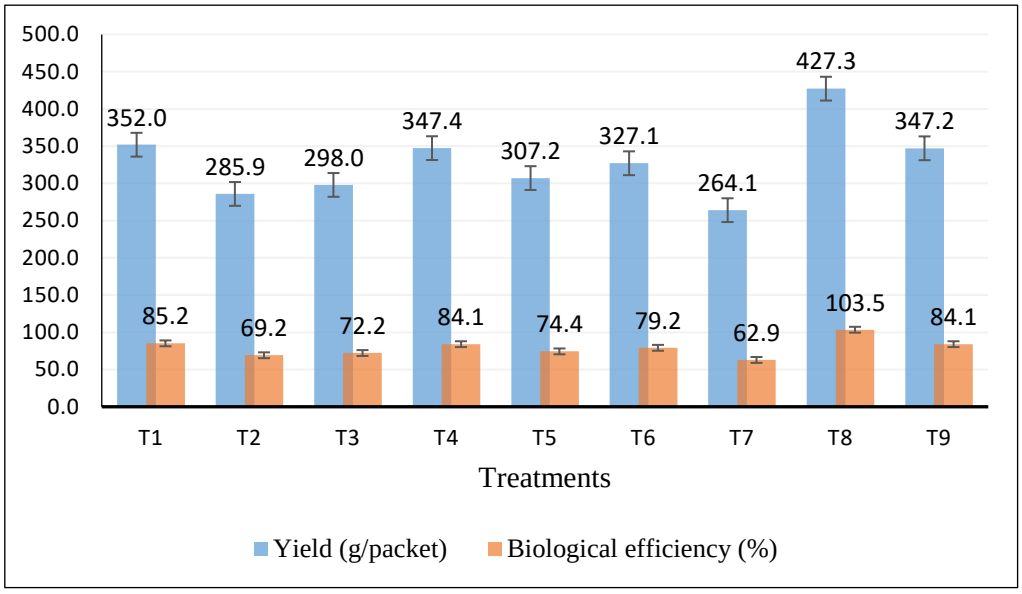

Figure-9: Effects of substrates on the yield and biological efficiency of milky white mushroom

Nutrient content

Nutrient content of milky white mushroom grown in different substrates are shown in Table-18. Moisture content of fresh mushroom was ranges from 88.10 to 91.24%. Carbohydrate, protein, lipid, fibre and ash content per 100 g dry mushroom were 56.70 to 65.30g, 9.90 to 15.70g, 2.01 to 3.78g, 11.90 to 14.0g and 9.26 to 13.86g respectively. Amount of carbohydrate was lowest (56.70g) and protein was highest (15.70g) when mushroom grown in rice straw + sawdust (1:1) substrate. Lipid content was lowest (2.01g) and ash content was highest (13.86g) when mushroom grown in sawdust + wheat bran (2:1) substrate. Amount of fibre was highest (14.0g) in mushroom grown in maize straw substrate but amount of moisture was highest (91.24%)) in mushroom grown in rice straw substrate.

Table-18: Nutrient contents of milky white mushroom grown on different substrates

Substrates	Moisture (%)	Carbohydrate (g)	Protein (g)	Lipid (g)	Fiber (g)	Ash (g)
T_1- Rice straw	91.24a	63.60ab	11.80c	2.16ef	13.10ab	9.26c
T_2- Wheat straw	90.80a	63. 80ab	10.35de	2.56cde	12.20b	10.15ab
T_3- Barley straw	89.50bc	64.6ab	9.90e	2.98bc	12.40b	10.04ab
T_4- Waste paper + wheat bran (2:1)	91.00a	65.30a	10.20de	2.66cd	11.90b	9.88ab
T_5- Sugarcane baggage + wheat bran (2:1)	88.90bc	63.42b	10.25de	2.10ef	12.50b	10.75ab
T_6- Maize cob + wheat bran (2:1)	89.75bc	63.30b	11.30cd	2.31def	12.80ab	10.28ab
T_7- Sawdust + wheat bran (2:1)	88.10c	59.00c	12.30c	2.01f	12.80ab	13.86a
T_8- Rice straw + Sawdust (1:1)	90.36ab	56.70d	15.70a	3.78a	13.80a	9.99ab
T_9- Maize straw	90.10ab	58.00d	13.80b	3.28b	14.00a	10.90ab
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Conclusion

From the above study it was concluded that rice straw mixed with sawdust (1:1) substrate was best for milky white mushroom cultivation as it gave highest yield. But the results indicated that rice straw alone, waste paper mixed with wheat bran (2:1) and maize straw could also be used for commercial cultivation of milky white mushroom.

EXPT. NO. 3: EFFECTS OF CASING MATERIALS ON THE GROWTH AND YIELD OF MILKY WHITE MUSHROOM

INTRODUCTION

Casing is an important cultural practice of milky white mushroom cultivation. Casing means covering the cultivation substrate with a layer of soil or soil like material after spawn run which enhances the transformation of vegetative stage to reproductive stage (Pani, 2012; Suess and Curtis, 2009). Casing the surface of composted substrate fully colonized by mycelium of mushroom is an essential function in stimulation and promoting the development of fruit bodies (Farsi *et al*., 2011). Recent studies on the constraints in the cultivation of milky mushroom indicated casing is the most important factor affecting the yield. The production of *Calocybe indica* depends on top dressing after the substrate has been fully colonized with mycelium. After complete mycelia formation casing is done to provide a reservoir of water for the developing fruiting body. The composition of casing mixture determines its quality (texture, structure, pH, water holding capacity, C:N ratio etc.), which directly affect the mycelia growth in casing layer and initiation of fruiting bodies (Tewari, 2005).

Before the early 1950s, sterilized soil or subsoil was used for casing, and this is still used by some farms. The characteristics of a good casing medium are that it should have an open texture, good water-holding capacity, freedom from pests and diseases, and a pH between 6.5 and 8.0. A peat moss mixture with the pH adjusted by lime, chalk, or ground limestone fulfills the requirements of a good casing soil and is now widely used in many advanced mushroom industries. With the help of modern technology, understanding the specific relationship between water tension in the casing layer and mushroom yield could add a great deal to the future success of mushroom crop management.

Although different materials may adequately function as a casing layer, peat is commonly used and recommended as a good casing in mushroom cultivation (Gulser and Peksen, 2003). But peat is not so available in many mushroom growing areas. It is a costly and

nonrenewable input. Not only the import cost of peat but also the depletion of its available resources worldwide discourages the investors to use peat as casing layer (Sassine *et al*., 2005). After that many materials, alone or in combination, have been used as casing both commercially and experimentally (Gimenez and Gonzalez, 2008). Different materials are used in the shape of casing throughout the world, but a few casing substances have been developed and suggested for use. Mantel (1973) recommended the use of compost with slaked lime and sand (4:1:1). It was stated that farmyard manure and loamy soil in 1:1 ratio in the production of *Agaricus bisporus* (Hayes and Shandilya, 1977) and the mixture of soil and sand 1:1 ratio in the production of *Calocybe indica* (Purkayastha and Chandra, 1985) had supported better fructification compared to other substrates. Sharma *et al*. (1997) evaluated 16 combinations of casing material and reported that 2 years old cow dung patties were excellent casing material, using 1 inch thick in milky mushroom cultivation.

Beside physical, chemical and biological factors of the suitable casing material, cost and availability are more important factors in successful application and acceptance by the mushroom growers. Therefore, the present study was conducted to evaluate different casing materials for successful production of milky white mushroom.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Mushroom Development Institute (MDI), Department of Agricultural Extension, Savar, Dhaka, Bangladesh from May 2019 to July 2019.

Treatments

Eleven different combination of casing materials were used in this experiment as treatments for growing milky white mushroom. Treatments were: T_1= coconut coir dust (CC); T_2 = coconut coir dust (CC) + decomposed cow dung (CD) (1:1); T_3 = coconut coir dust (CC) + loamy soil (LS) (1:1); T_4 = ash (AS); T_5 = ash (AS) + loamy soil (LS) (1:1); T_6 = loamy soil (LS) + sand (S) (3:1); T_7 = decomposed spent mushroom substrate (SMS); T_8 = decomposed spent mushroom substrate (SMS) + loamy soil (LS) (1:1); T_9 = decomposed spent mushroom substrate (SMS) + decomposed cow dung (CD) (1:1); T_{10} = decomposed spent mushroom substrate (SMS) + ash (AS) (1:1) and T_{11} = loamy soil (LS) (control).

Preparation of spawn packets

Rice straw was used as substrate for the cultivation of milky white mushroom. Spawn packets were prepared as described in chapter III. The substrate of spawn packets were completely colonized by the mycelium within 16-25 days. After completing mycelium colonization in the spawn packets, cotton, brown paper and neck were removed from the packets and the mouth of the plastic bags were folded 4 - 5 cm above the spawn.

Preparation of casing materials, casing and after care

Different casing materials such as loamy soil, coconut coir dust, ash, sand, well decomposed cow dung and well decomposed spent mushroom substrates were collected locally. Collected materials were sterilized at 65^{o}C for 4 hours separately. Casing

materials were mixed as per treatments. In case of treatment T_{11} (control) sterilized loamy soil was made small clods and used for covering the completely colonized spawn packets. Sterilized casing materials were used to cover over the mycelium on the substrate (Plate-8) up to 4 cm thickness. Watering was done at regular interval to maintain moisture at 60 to 70%. Fruiting body primordia initiated within 12 - 24 days and developed in to fruiting bodies.

Plate-8: Different casing materials used for covering the spawn packets

Harvesting and Data collection

The fruiting bodies were harvested at 7 - 8 days of primordial initiation and data were collected on days to primordial initiation, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, number of effective fruiting body (NEFB), number of flashes, yield and biological efficiency (BE). The BE was measured by the formula described in chapter III.

Statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. The data were statistically analyzed following SPSS (version 26.0) computer program. Difference among the treatment means were determined by Tukey's Test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Days to primordia initiation, Number of flush and Number of effective fruiting body

Casing materials had significant influence on primordial initiation, number of flash and number of effective fruiting body of milky white mushroom (Table-19). Spawn packets covered with loamy soil + sand at 3:1 ratio (T_6) produced earlier (12 days) fruiting body primordia than all other casing materials which was statistically similar to Coconut coir dust + Loamy soil (1:1) (13.0 days), Ash + Loamy soil (1:1) (13.0 days) and Ash alone (13.1 days). Whereas Spawn packet covered with decomposed spent mushroom substrate (SMS) + ash (AS) at 1:1 ratio (T_{10}) required highest time (24 days) for fruiting body primordia initiation which was significantly higher than all other casing materials. Time required for fruiting body primordia initiation in T_2, T_{11}, T_7, T_1, T_9 and T_8 was between 15.0 to 15.9 days which were statistically similar. T_3, T_5 and T_4 required similar time (13.0-13.1days) for fruiting body primordia initiation. This result supports the findings of Ashrafi *et al*. (2017) who also observed earlier primordia initiation after casing with soil + sand (3:1). Amin *et al.* (2010) also reported that primordia initiation with different substrates and casing materials was between the 13th and 19th day.

Number of flushes and number of effective fruiting bodies were also affected by casing materials (Table-17). Highest number of flushes (3.1) and effective fruiting bodies (7.6) per packet were recorded from spawn packets covered with coconut coir dust mixed with well decomposed cow dung at 1:1 ratio (T_2) whereas it was lowest (1.9) in spawn packet covered with mixture of decomposed spent mushroom substrate and cow dung at 1:1 ratio (T_9). This result was similar to that of Ashrafi *et al*. (2017) who also reported that number of effective fruiting body significantly affected by different casing materials. But was different from that of Amin *et al*. (2010) who reported that number of effective fruiting bodies were statistically similar in all of the casing materials used.

Table-19**:** Effects of casing materials on fruiting body primordia initiation, number of flush and number of effective fruiting body of milky white mushroom

Treatments	Days to primordia initiation	Number of flushes	Number of effective fruiting bodies
T_1- Coconut coir dust	15.8b	2.6ab	6.1ab
T_2- Coconut coir dust + Cow dung (1:1)	15.0bc	3.1a	7.6a
T_3- Coconut coir dust + Loamy soil (1:1)	13.0cd	2.8ab	6.8ab
T_4- Ash	13.1cd	2.4ab	6.8ab
T_5- Ash + Loamy soil (1:1)	13.0cd	2.5ab	7.1ab
T_6- Loamy soil + Sand (3:1)	12.0d	2.4ab	6.5ab
T_7- Decomposed spent mushroom substrate	15.6b	2.6ab	6.5ab
T_8- Decomposed spent mushroom substrate + Loamy soil (1:1)	15.9b	2.3ab	6.5ab
T_9- Decomposed spent mushroom substrate + Cow dung (1:1)	15.8b	1.9b	5.9b
T_{10}- Decomposed spent mushroom substrate + Ash (1:1)	24.0a	2.5ab	6.3ab
T_{11}- Loamy soil (control)	15.2b	2.3ab	6.8ab
P	< 0.001	= 0.070	= 0.048

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Length and diameter of stalk, diameter and thickness of pileus

Length of stalk and diameter & thickness of pileus were significantly affected by casing materials (Table-20). Highest stalk length (11.0 cm) was recorded from spawn packets covered with decomposed spent mushroom substrate mixed with decomposed cow dung at 1:1 ratio (T_9) which was statistically similar to spawn packet covered with only decomposed spent mushroom substrate (T_7). Stalk length was lowest (7.1 cm) when spawn packets were covered with mixture of loamy soil and sand at 3:1 ratio (T_6). Diameter of stalk was highest (2.9 cm) when spawn packets were covered with loamy soil + sand (3:1). Stalk diameter was significantly smaller (1.8 cm) when spawn packets were covered with only coconut coir dust (T_1) than all other treatments. Diameter of pileus was highest (7.6 cm) when spawn packets were covered with mixture of coconut coir dust and well decomposed cow dung at 1:1 ratio (T_2) (Plate-9) which was statistically similar to spawn packet covered with mixture of decomposed spent mushroom substrate and decomposed cow dung at 1:1 ratio (T_9). Pileus thickness was highest (2.5 cm) when spawn packets were covered with mixture of decomposed spent mushroom substrate and ash at 1:1 ratio (T_{10}) which was statistically similar to all other treatments except T_1. Pileus thickness was lowest (1.8 cm) when spawn packets were covered with only coconut coir dust (T_1). This result was similar to that of Amin *et al.* (2010) and Ashrafi *et al.* (2017) who also observed greatest stalk length with spent mushroom substrate as casing material.

Plate-9: Fruiting bodies from substrate covered with coconut coir dust + cow dung (1:1)

Table-20**:** Effects of casing materials on length & diameter of stalk and diameter & thickness of pileus of milky white mushroom

Treatments	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1- Coconut coir dust	7.4c	1.8b	4.5d	1.8b
T_2- Coconut coir dust + Cow dung (1:1)	7.7c	2.5a	7.6a	2.3ab
T_3- Coconut coir dust + Loamy soil (1:1)	7.2c	2.4a	6.2bcd	2.2a
T_4- Ash	7.8c	2.8a	6.0cd	2.1ab
T_5- Ash + Loamy soil (1:1)	7.6c	2.4a	6.5bcd	2.1ab
T_6- Loamy soil + Sand (3:1)	7.1c	2.9a	6.0cd	2.2ab
T_7-Decomposed spent mushroom substrate	10.2ab	2.7a	6.4bcd	2.4a
T_8- Decomposed spent mushroom substrate + Loamy soil (1:1)	9.8b	2.6a	6.3bcd	2.1ab
T_9- Decomposed spent mushroom substrate + Cow dung (1:1)	11.0a	2.6a	7.2ab	2.3a
T_{10}- Decomposed spent mushroom substrate + Ash (1:1)	9.4b	2.7a	6.6bc	2.5a
T_{11}- Loamy soil (control)	8.3c	2.5a	6.6bc	2.3a
P	<0.001	<0.001	<0.001	=0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Economic Yield

Economic yield of milky white mushroom was significantly affected by different casing materials used (Table-21). Yield was highest (374.1g/packet) when spawn packets were covered with mixture of coconut coir dust + well decomposed cow dung at 1:1 ratio (T_2) which was significantly different from coconut coir dust alone (126.4g/packet), loamy soil + sand (3:1) (279.4g/packet) and coconut coir dust + loamy soil (1:1) (289.0g/packet) but was similar to other treatments. Lowest yield (126.4g/packet) was recorded from spawn packet covered with only coconut coir dust (T_1). Among the flushes maximum mushroom were harvested from 1st flush (73.88%) and minimum from 4th flush (0.61%) (Figure-10). This result was supported by the findings of Ashrafi *et al.* (2017) who also reported that biological yield of milky mushroom was significantly affected by different casing materials. But, Amin *et al*. (2010) reported that the biological and economic yields, and biological efficiency were statistically similar in all of the casing materials tested.

Considering all the yield contributing parameters only coconut coir dust as a casing material (T_1) performed worse than all other treatments. This might be due to reduced aeration and access water retention on the top layer of spawn packet. Cocopeat has very high water holding capacity which causes poor aeration in the root zone. Fazilah and Ahmad (2017) obtained water holding capacities of coconut coir dust samples 912.54% of dry weight. This will later affect the oxygen diffusion to the roots. Depending on the handling and processing technique, the physical properties of cocopeat can easily affect the air capacity and water retention (Abad *et al.* 2002). Incorporation of coarser material into cocopeat media can solve this problem and improve aeration (Yahya *et al*., 2009). For this reason, coconut coir dust mixed with decomposed cow dung (T_2) performed better than only coconut coir dust (T_1).

Table-21: Effects of casing materials on economic yield of milky white mushroom

Treatments	Yield of 1st flush (g)	Yield of 2nd flush (g)	Yield of 3rd flush (g)	Yield of 4th flush (g)	Total yield (g)
T_1- Coconut coir dust	85.6d	29.3b	8.8a	0.0b	126.4c
T_2- Coconut coir dust + Cow dung (1:1)	239.0abc	77.9a	41.4a	10.3a	374.1a
T_3- Coconut coir dust + Loamy soil (1:1)	187.8c	71.6a	31.5a	2.4ab	289.0b
T_4- Ash (AS)	229.0abc	70.5a	12.3a	0.0b	315.5ab
T_5- Ash + Loamy soil (1:1)	226.0abc	69.6a	19.1a	3.0ab	316.5ab
T_6- Loamy soil + Sand (3:1)	208.6bc	59.1a	11.6a	0.0b	279.4b
T_7- Decomposed spent mushroom substrate	242.0abc	57.6a	17.5a	3.4ab	316.0ab
T_8- Decomposed spent mushroom substrate + Loamy soil (1:1)	235.4abc	52.6ab	7.0a	0.0b	297.5ab
T_9- Decomposed spent mushroom substrate + Cow dung (1:1)	272.8a	51.5ab	4.6a	0.0b	328.8ab
T_{10}- Decomposed spent mushroom substrate + Ash (1:1)	235.8abc	59.0a	20.5a	0.0b	309.3ab
T_{11}- Loamy soil (control)	262.4ab	49.4ab	14.5a	1.2b	324.2ab
P	<0.001	<0.001	0.058	0.022	<0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

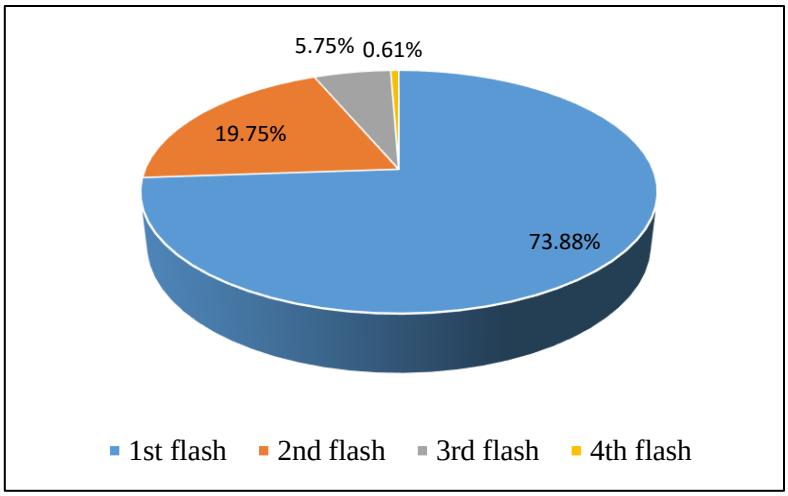

Figure-10: Flush wise yield of milky white mushroom

Biological efficiency

Different casing materials has significant effect on biological efficiency of milky white mushroom (Figure-11). It was highest (90.6%) when spawn packets were covered with coconut coir dust in combination with well decomposed cow dung at 1:1 ratio (T_2) which was significantly higher than coconut coir dust alone (30.6%), loamy soil + sand (3:1) (67.7%) and coconut coir dust + loamy soil (1:1) (70.0%) but was similar to other treatments. Biological efficiency was lowest (30.6%) when spawn packets were covered with only coconut coir dust (T_1). In this study economic yield and biological efficiency of milky mushroom were better when casing materials either coconut coir dust, loamy soil or spent mushroom substrate mixed with decomposed cow dung. This might be due to mushroom mycelium takes sum nutrient from the cow dung. This result supports the findings of Kerketta *et al.* (2018) who also reported that biological efficiency of *Calocybe indica* strain CI-524 significantly higher (81.33%) with compost + vermicompost casing materials. Chakraborty *et al.* (2016) also reported that casing materials significantly influenced the biological efficiency of milky mushroom.

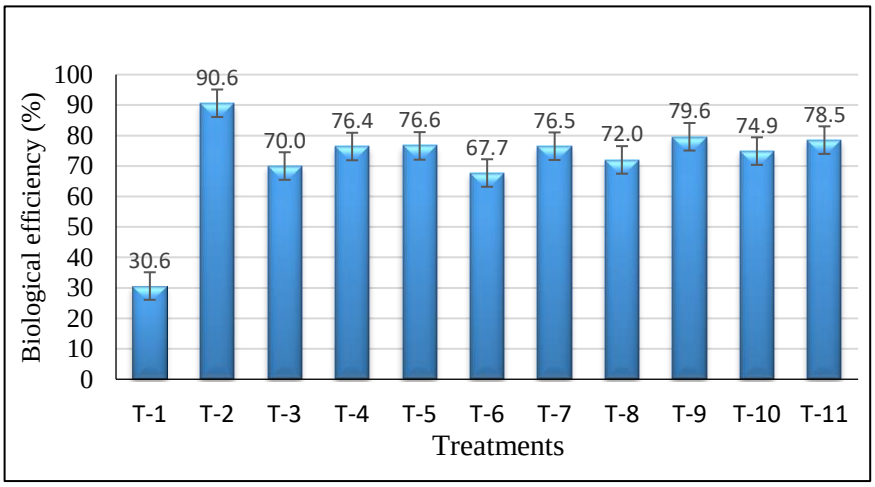

Figure-11**:** Effects of casing materials on biological efficiency of milky white mushroom

Conclusion

From the above study it could be concluded that coconut coir dust in combination with decomposed cow dung (1:1) was the best casing material for successful cultivation of milky white mushroom. The results revealed that decomposed spent mushroom substrate mixed with decomposed cow dung (1:1), loamy soil, decomposed spent mushroom substrate alone, ash mixed with loamy soil (1:1) and ash alone were also good as casing material. Use of spent mushroom substrate would be a great relief from the environmental pollution.

EXPT. NO. 4: EFFECTS OF SUBSTRATE STERILIZATION AND SPAWNING METHOD ON THE YIELD AND YIELD ATTRIBUTES OF MILKY WHITE MUSHROOM

INTRODUCTION

Milky white mushroom (*Calocybe indica*) is a tropical edible mushroom of Indian origin and can be cultivated indoor in high temperature and humid areas (Purkayastha and Chandra, 1974). A substrate is an important substance for making spawn and growing mushrooms (Singh *et. al*., 2017). Milky white mushroom can be cultivated on varieties of cellulosic substrates like, paddy straw, wheat straw, maize stalks, sorghum stalks, pearl millet stalks, sugarcane trace, sugarcane baggase, soya bean straw, cotton waste, coconut coir pith, groundnut haulms etc. (Patel and Trivedi, 2016). But paddy straw is the principal substrate used for its cultivation in Bangladesh. Pani (2010) also stated that paddy straw was best among the substrates as it produced the maximum yield and biological efficiency (71.2% BE) of *Calocybe indica*.

Milky white mushroom grew well on uncomposed substrate under artificial indoor condition (Vijaykumar *et al*., 2014.). There is no need to compost the substrate for its cultivation as the mycelium can degrade the cellulose, hemicelluloses and lignin by secretion of various extracellular enzymes (Singh *et al*., 2017). But for the better mycelial colonization and yield it is necessary to sterilize or pasteurize the substrate. Khan *et al*. (2011) stated that substrate sterilization is much more appropriate method for effective and smooth cultivation of mushroom to remove the existence of a number of microorganisms. While the composition of substrates for different mushrooms varies greatly and there is variation in the preparation of substrates used for every cultivated species, most substrates are treated with moist heat and then, said to be pasteurized. The process is intended to kill all seeds, nematodes, insects and other organisms that flourish at the temperatures used to grow the mushrooms. Substrates are pasteurized by steam to kill off potential competitive microorganisms. When we pasteurize, we kill many organisms, but we expect those that remain to easily controlled, poor competitors and beneficial to the organism we intend to grow. Substrate pre-treatment namely; steaming

for 30 minutes or soaking in hot water (80° C) for 60 minutes are recommended for commercial purpose (Bokaria, *et al*., 2014). Pani (2010) stated that 6 hours of soaking the substrate and steaming for 60 minutes yielded the maximum production with 0.6% contamination which was found to be economically viable. Pathan (2009) also stated that the maximum percent yield (61.75%) was in case of soaking and boiling of substrate for 75 minutes. The pasteurized substrate is allowed to cool for the next 16 - 20 hours (Kurtzman, 2010).

In mushroom growing technology the inoculum is known as the "spawn". Spawn is a medium that is impregnated with mycelium made from a pure culture of the chosen mushroom strain. The purpose of the spawn is to boost the mycelium to a state of vigor such that it will rapidly colonize the selected bulk growing substrate. Substrate can be spawning by different ways such as thorough mixing, layer spawning, top spawning etc. Spawning method may have influence on evenly mycelium running in the bulk substrate. But no research work has been conducted on the effect of substrate sterilization and spawning method on milky white mushroom cultivation in Bangladesh.

Therefore, the present study was conducted to find out appropriate substrate sterilization technique and spawning method for better mycelium run in the substrate and achieve a good yield.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Mushroom Development Institute (MDI), Department of Agricultural Extension, Savar, Dhaka, Bangladesh from May 2018 to July 2018.

Treatments

Seven different treatments of substrate sterilization technique in combination with spawning methods namely T_1 = Steam treatment of substrate and spawning in 3 layers, T_2 = Steam treatment of substrate and spawning thoroughly, T_3 =Autoclaving of substrate and top spawning, T_4 = Autoclaving of substrate and spawning in 3 layers, T_5 = Autoclaving of substrate and spawning thoroughly, T_6 = Hot water treatment of substrate and spawning in 3 layers and T_7 = Hot water treatment of substrate and spawning thoroughly were used in this experiment.

Steam treatment

Rice straw was used for the cultivation of milky white mushroom. The straw was chopped to convenient length of 2.5 to 5 cm. For treatment T_1 and T_2 the substrate was mixed with appropriate amount of water and then filled in net bag. The net bag filled with substrate were placed in the sterilization cum inoculation chamber. Door of the chamber was closed and tightened with the help of screws. Water heater was turned on to produce steam that flows in to the chamber. When the temperature of the chamber rises to 60^oC, the steam flow was adjusted to maintain a constant temperature of 70^oC – 80^oC up to 90 minutes. After 90 minutes water heater was turned off and kept it for about 20 hours. After 20 hours substrate was taken out and used for preparation of spawn packet.

Autoclaving

In case of treatment T_3 substrate was mixed with appropriate amount of water and then filled into the polypropylene bags (12″x18″) and autoclaved in an autoclave machine for 1 hours at 121°C and 1.5 kg/cm^2 pressure. For treatment T_4 and T_5 water mixed substrate was filled into net bag and autoclaved in the same way. Upon cooling substrate was used for spawn packet preparation.

Hot water treatment

Rice straw substrate chopped to convenient length of 2.5 to 5 cm was poured into a net bag and treated with hot water at 60°C in a drum for 60 minutes and allowed to drain out the excess water by hanging the bag for 20 hours. The moisture content of the hot water treated substrate was allowed to leave by spreading them on plastic sheet so that excess moisture was evaporated to obtain 60 to 65 percent moisture.

Spawning

For treatment T_1, T_4 and T_6 pasteurized or sterilized substrate was filled into the polypropylene bags (12″x18″) and inoculated with 10% sawdust mother culture in three layers (Plate-10). For treatment T_3 autoclave sterilized substrate bags were inoculated with sawdust mother culture on the top of the substrate through the neck (Plate-11). For treatment T_2, T_5 and T_7 pasteurized/sterilized substrate was filled into the polypropylene bags (12″x16″) and inoculated with 10% sawdust mother culture by thorough mixing (Plate-12). Then the spawn packets were transfer to the culture house for mycelium run. After 16 to 25 days the substrate was completely colonized by the mycelium and polypropylene covered was opened.

Plate-10: Substrate spawning in three layers

Plate -11: Substrate spawning through the neck (Top spawning)

Plate-12: Substrate spawning thoroughly

Casing and after care

Loamy soil was used as casing material and was sterilized at 65^{o}C for 4 hours. Casing material was covered over the mycelium on the substrate up to 4 cm thickness. Watering was done at regular interval to maintain moisture at 60 to 70%. Primordia initiated at 8-14 days and developed in to fruiting bodies.

Harvesting and data collection

The fruiting bodies were harvested at 7 to 8 days of primordia initiation and data were collected on days to complete spawn run, rate of spawn packet contamination, days to primordia initiation, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, number of effective fruiting body (NEFB), days to final harvest, yield and biological efficiency (BE). The BE was measured by the formula described in chapter III.

Experimental design and statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. The data were statistically analyzed following SPSS (version 26.0) computer program. Difference among the treatment means were determined by Tukey's Test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Days to complete mycelium run in the spawn packet and rate of substrate contamination

Mycelium run in the spawn packet are shown in Table-22 and percent substrate contamination in Figure -11. Mycelium colonization was faster (16.50 days) in substrate treated with hot water and inoculated by thorough spawning (T_7) which was statistically similar (18.25 days) to substrate treated with hot water and inoculated in three layers (T_6). Slowest colonization (24.25 days) was observed in steam treated substrate inoculated in 3 layers (T_1) and thorough spawning (T_2). Mycelium run was almost zero and substrate contamination was very high (90%) in substrate sterilized by autoclaving and spawning through the neck (T_3) which was significantly differed from other treatments. The rate of substrate contamination was zero in hot water treated substrate inoculated thoroughly (T_7) and hot water treated substrate inoculated in 3 layers (T_6) which was statistically similar to autoclaved substrate spawning in 3 layers T_4 (25%) and steam treated substrate spawning in 3 layers (T_1) (35%). Result suggested that hot water treatment of rice straw substrate was the best substrate sterilization technique for milky white mushroom cultivation. Sarker *et al*. (2012) also reported similar result in case of *Volverilla volvacea*. Highest rate of contamination of spawn packet in T_3 might be due to lack of proper aeration and less amount of mother culture used. Akhtar *et al*. (2017) reported that hot water treatment of rice straw substrate at 80^oC for 3 hrs gave better results and prevalence of contaminant were low.

Days to primordia initiation and time to complete total harvest

Days required for fruiting body primordia initiation (Table-22) after casing was minimum (8.63 days) in steam treated substrate inoculated in 3 layer (T_1) which was statistically similar to hot water treated substrate spawning thoroughly (T_7), hot water treated substrate spawning in 3 layers (T_6) and autoclaved substrate spawning in 3 layers (T_4). Maximum time was required (14.28 days) in steam treated substrate inoculated thoroughly (T_2) which was similar to autoclaved substrate spawning thoroughly T_5 (13.0

days). As the maximum spawn packet was contaminated, it was not possible to analyze data from T_3. Highest time (51.0 days) was required from casing to complete harvest in hot water treated substrate inoculated in 3 layers (T_6) which was significantly higher than other treatments. Lowest time (33.0 days) was required in autoclaved substrate inoculated in 3 layers (T_4) which was similar to T_1, T_2 and T_7. Highest time required for crop harvest might be due to better mycelium colonization leading to a greater number of flushes and fruiting body production in T_6. Patra and Pani (1995) reported that time required for primordia initiation of *Calocybe indica* was 13-16 days. Sarker *et al.* (2012) also reported that days required for primordia initiation of *Volverilla volvacea* in all substrate sterilization treatments was statistically similar. This might be difference of substrate sterilization technique.

Table-22: Effects of substrate sterilization technique and spawning methods on spawn run, primordia initiation and harvesting time of milky white Mushroom

Treatment	Days to Spawn run	Days to primordia initiation	Time to complete total harvest (days)
T_1	24.25a	8.63b	33.25c
T_2	24.25a	14.28a	35.50bc
T_3	-	-	-
T_4	20.50c	10.13b	33.00c
T_5	22.25b	13.00a	39.00b
T_6	18.25cd	9.15b	51.00a
T_7	16.50d	8.85b	35.75bc
P	<0.001	<0.001	<0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance. '-'indicates no data was recorded as all the packets were contaminated

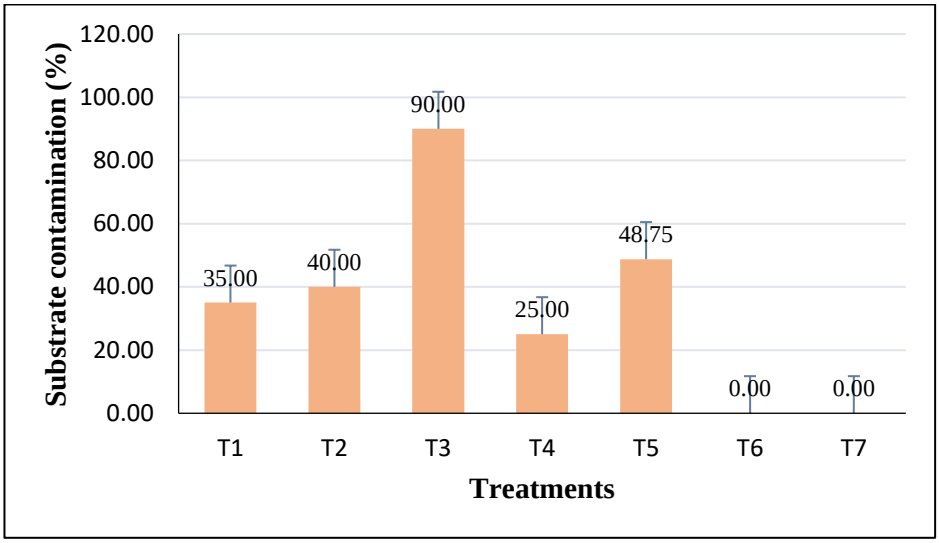

Figure-12: Effects of substrate sterilization and spawning method on substrate contamination.

Number and size of effective fruiting body

Number and size of effective fruiting body are presented in Table-23. Highest number of effective fruiting body (NEFB) per packet (6.83) was observed in hot water treated substrate inoculated in 3 layers (T_6) (Plate-13) which was similar to hot water treated substrate spawning thoroughly (T_7) (6.70) and autoclaved substrate spawning in 3 layers (T_4) (6.33). Lowest NEFB (3.90) was observed in steam treated substrate inoculated thoroughly (T_2). Length of stalk was slightly influenced by substrate sterilization and spawning method but there was no significant effect was observed on stalk diameter. Highest stalk length (8.83 cm) was observed in autoclaved substrate inoculated in 3 layers (T_4) which was statistically similar to all other treatments except T_7 (6.58 cm). Highest stalk diameter was observed in steam treated substrate spawning in 3 layers (T_1) (2.85 cm) and lowest in autoclaved substrate spawning in 3 layers (T_4) (2.45 cm) but there was no significant difference among the treatments. Pileus dimeter was maximum in T_1 (6.95cm) and minimum in T_2 (5.65 cm). T_2 was significantly different from T_1 but was similar to other treatments. Highest thickness of pileus (2.80 cm) was observed in T_1

which was significantly different from T_6 but was similar to all other treatments. These results were supported by Akhter et al. (2017) who also reported that highest number of effective fruiting bodies, highest length of stipe and diameter of pileus of oyster mushroom were in rice straw substrate treated with hot water at 80^{o}C. Sarker *et.al*. (2012) also reported that highest number of effective fruiting body of *Volvariella volvacea* was recorded with hot water sterilization of rice straw substrate.

Plate-13: Fruiting body from substrate treated with hot water and inoculated in three layers (T_6)

Table-23: Effects of substrate sterilization technique and spawning methods on size fruiting body of milky white mushroom

Treatment	NEFB	Length of Stalk (cm)	Diameter of Stalk (cm)	Diameter of Pileus (cm)	Thickness of Pileus (cm)
T_1	4.30c	8.33a	2.85a	6.95a	2.80a
T_2	3.90c	8.33a	2.65a	5.65b	2.35ab
T_3	-	-	-	-	-
T_4	6.33ab	8.83a	2.45a	5.83ab	2.63ab
T_5	4.96bc	8.18a	2.50a	5.75b	2.50ab
T_6	6.83a	7.58ab	2.48a	5.78ab	2.28b
T_7	6.70a	6.58b	2.65a	6.20ab	2.45ab
P	< 0.001	0.002	0.230	0.025	0.046

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. NEFB = Number of Effective Fruiting Body. '-'indicates no data was recorded as all the packets were contaminated. P represents the level of significance.

Yield and Biological Efficiency

Yield and Biological efficiency were significantly influenced by spawning method and substrate sterilization technique (Figure-13). Maximum yield (296.31g/packet) and biological efficiency (73.16%) were recorded in hot water treated substrate spawning in 3 layers (T_6) which was similar to hot water treated substrate spawning thoroughly (T_7) (69.3%) and autoclaved substrate inoculated in 3 layers (T_4) (66.96%). Lowest yield (165.06 g/packet) and biological efficiency (38.54%) were in steam treated substrate inoculated thoroughly (T_2) which was significantly lower than all other treatments. No yield was recorded from T_3 because of 90% spawn packets were contaminated. Result suggested that hot water treated rice straw substrate spawning in 3 layers was the best substrate sterilization technique and spawning method of milky white mushroom

cultivation. Sarker *et.al*. (2012) also reported similar result in case of *Volvariella volvacea.* Suman *et.al*. (2018) reported that biological efficiency of *Calocybe indica* was 61.9% in rice straw substrate. Akhter *et al*. (2017) reported that highest economic yield and biological efficiency of oyster mushroom were recorded when rice straw substrate was treated for 3 hrs. at 80^{o}C.

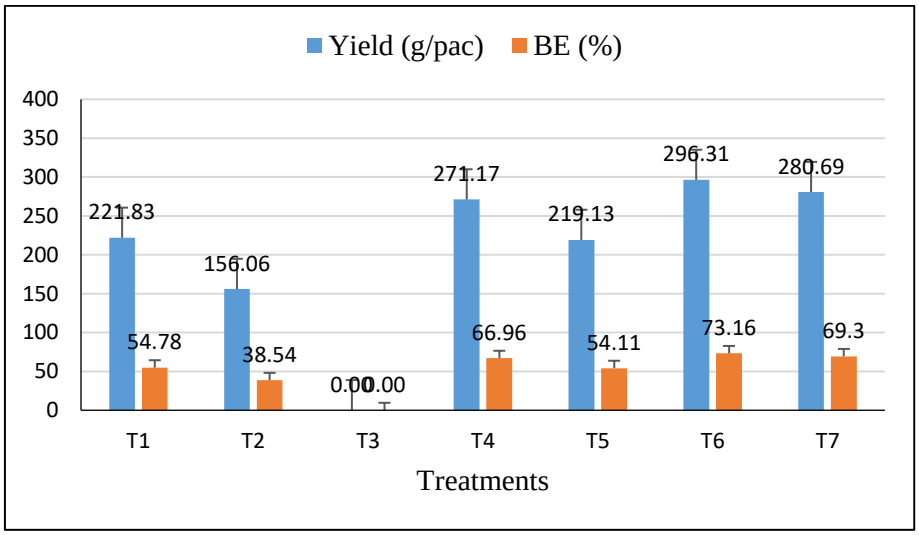

Figure-13: Effects of substrate sterilization and spawning method on yield and biological efficiency of milky white mushroom

Conclusion

In conclusion, the values obtained for mycelium growth, substrate contamination, yield contributing parameters and biological efficiency of milky white mushroom for treatment T_6 were better than those obtained from other treatments. Thus, substrate treated with hot water and inoculated by three-layer spawning (T_6) can be used for milky white mushroom cultivation as it provides no contamination and highest biological efficiency.

EXPT. NO. 5: EFFECTS OF SUBSTRATE MOISTURE CONTENT ON GROWTH AND YIELD OF MILKY WHITE MUSHROOM

INTRODUCTION

A good substrate for mushroom growth must be suitable both chemically and physically, as well as having the proper condition for microbial activities (Chang and Miles, 2004). Moisture content in substrate is very important factor for the growth, development and yield of oyster mushroom (Sarker *et al*., 2007). Optimum mycelium growth and mushroom production is dependent upon adequate moisture and gas exchange within the substrate (Shen *et al*., 2008). Various environmental conditions must be regulated to be optimal for the cultivation procedure or state of development of the mushroom (Chang and Miles, 2004). Different moisture levels in different substrates under different environmental conditions have been tested by researchers. It is generally recognized that most fungi require high moisture levels. Mushrooms grow well at substrate moisture levels of 50 to 75% (Bratkovich and Stephen, 2004). Akiyama *et al*. (1974) reported that for natural logs optimum moisture content for hyphal elongation of shiitake mushroom was in the range of 55 to 70%. Yoshida *et al.* (1993) adjusted the moisture content between 65-70% to either chopped straw or sawdust for *Pleurotus ostreatus* production. Optimum moisture of substrate was 75 percent, when the total and marketable yields were highest of two oyster mushroom strains HK-35 and K-22. The moisture content of 80 percent influenced negatively the yielding because of the high share of misshaped carpophores (Siwulski *et al*., 2007). The correct amount of water should be available everywhere in the substrate. After mixing, the moisture content should be 60 – 65%. Oei (2005) reported that moisture content of the grain substrate, after boiling, should be around 50%. If it is higher, mycelial growth may be faster, but the danger of wet spot bacteria is also greater. If it is drier than 35% mycelial growth will be rather slow.

Rice straw is the most common lignocellulosic substrate whose major component is cellulose and it is also the best substrate for cultivating milky white mushrooms (Mangat *et al.*, 2008 and Amin *et al*., 2010). But suitable moisture level for the production of milky white mushroom on rice straw substrate is yet to be standardized.

Therefore, the present experiment was undertaken to determine the appropriate substrate moisture level for production of milky white mushrooms.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Mushroom Development Institute (MDI), Department of Agricultural Extension, Savar, Dhaka, Bangladesh from May 2019 to July 2019.

Treatments

Eight different moisture levels of the substrate were tested in this experiment as treatments. Such as 35, 40, 45, 50, 55, 60, 65, and 70 percent moisture of the dry substrate.

Preparation of spawn packets

Rice straw and Cid-1 were used as substrate and milky white mushroom strain respectively in this experiment. Rice straw substrate was chopped to convenient length of 2.5 to 5 cm. Existing moisture content of the substrate was measured by using moisture analyzer 'weighed moisture box (A & D company Ltd. N-92; P1011656, Japan)'. Three random sample was taken from different location of the bulk substrate for moisture analysis. At first the analyzer was turned and reading was adjusted to zero. 0.18gm dry rice straw was placed on the tray of analyzer and lead was closed. After pressing the start button waited for few minutes until the reading was stable. First reading was discarded as on test and the second to subsequent reading was recorded for respective sample. Then the required amount of water was added to the substrate according to the treatment and mixed thoroughly and filled in net bags. Spawn packets were prepared as described in chapter III. Substrate was completely colonized by the mycelium within 14 -19 days. After completing mycelium colonization in the spawn packets, cotton, brown paper and neck were removed from the packets and the mouth of the plastic bags were folded 4 - 5 cm above the spawn.

Casing and after care

Loamy soil was used as casing material and was sterilized at 65ºC for 4 hours. Cooled casing material was covered over the mycelium on the substrate up to 4 cm thickness. Watering was done at regular interval to maintain moisture at 60 to 70%. Primordia initiated within 12-19 days and developed in to fruiting bodies.

Harvesting and data collection

The fruiting bodies were harvested at 7-8 days of primordia initiation and data were collected on days to complete spawn run, rate of spawn packet contamination, days to primordia initiation, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, number of effective fruiting body (NEFB), days to final harvest, yield and biological efficiency (BE). The BE and nutrient contents of fruiting body grown at different moisture level of substrate were determined following the standard procedure described in chapter III.

Experimental design and statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. The data were statistically analyzed following SPSS (version 26.0) computer program. Difference among the treatment means were determined by Tukey's Test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Days to complete mycelium run in the spawn packet, fruiting body primordia initiation and harvesting time

Mycelium run in the spawn packet and fruiting body primordia initiation was significantly influenced by substrate moisture content (Table-24). Mycelium colonization was faster (14.5 days) in the substrate containing 70% moisture which was statistically similar to substrate containing 60% (16.0 days) and 65% (15.7days) moisture (Plate-14). Mycelium colonization was slowest (19.0 days) in the substrate containing 45% moisture. No mycelium colonization was observed in the substrate containing 35% moisture and only 12.5% mycelium colonization was observed in the substrate containing 40% moisture. Fruiting body primordia initiation was also significantly influenced by substrate moisture level. Lowest time (12.2 days) was required for fruiting body primordia initiation in substrate containing 65% moisture which was statistically similar to substrate containing 60% (12.3days) and 70% (12.6 days) moisture. This result was supported by the findings of Singh *et al*. (2017) who also reported that, maximum mycelium growth of Ganoderma lucidum was recorded at 70 ± 2% moisture of wheat straw + saw dust substrate. Suganthi and Krishnakumari (2018) also reported that maximum mycelium growth of *Pleurotus cornucopiae* was observed in sugarcane bagasse at 70 ± 2% moisture. Sarker *et al*. (2007) reported that duration of mycelium running in spawn packet of *Pleurotus ostreatus* decreased with the increase in substrate moisture level up to 70%.

Substrate moisture content had a significant influence on harvesting time of milky white mushroom (Table-22). Lowest time was required from spawning to first harvest (44.0 days) and spawning to last harvest (50.0 days) in substrate containing 70% moisture which was statistically similar to substrate containing moisture level 65% and 60% respectively. Highest time was required both for spawning to first (62.2 days) and last (77.0 days) harvest of milky white mushroom in substrate containing 45% moisture. This might be due to faster colonization of mycelium in the substrate at 70% moisture. These results are an agreement with the findings of Sarker *et al.* (2007). They reported that time

required to first harvest and total harvest were significantly influenced by the moisture levels in substrate of *Pleurotus ostreatus.*

Plate-14: Fully mycelium colonized substrate and fruiting body of T_8 (70% moisture)

Table- 24: Effects of substrate moisture content on spawn run, fruiting body primordia initiation, and harvesting time of milky white mushroom

Substrate moisture level	Days to spawn run	Days to primordial initiation	Days to first harvest	Time to complete total harvest (days)
35%	-	-	-	-
40%	-	-	-	-
45%	19.0a	19.0a	62.2a	77.0a
50%	17,0b	14.9b	47.7b	53.8bc
55%	17.0b	16.5b	51.6bc	54.5b
60%	16.0bc	12.3c	44.4bc	46.6bc
65%	15.7bc	12.2c	44.4bc	45.0c
70%	14.5c	12.6c	44.0c	50.0bc
P	< 0.001	<0.001	<0.001	<0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. '–' indicate no data were recorded due to maximum spawn packets were contaminated. P represents the level of significance.

Substrate contamination

Rate of substrate contamination was significantly influenced by substrate moisture level (Figure-14). The rate of substrate contamination was highest (100%) in substrate with 35% moisture level which was statistically similar (12.5%) to substrate with 40% moisture level and the contamination rate was gradually decreased with the increase of moisture level in the substrate. No substrate contamination was recorded in 65% and 70% moisture level.

The rate of contamination in substrate containing 35% and 40% moisture was very high, this might be due to uneven pasteurization of the substrate. Dry rice straw is a good

thermal insulating material and have low conductivity. Ashour *et al*. (2011) and Goodhew *et al*. (2004) reported that rice straw has been used as a building insulation material due to their low density and high heat insulation. Therefore, when adequate water added to the substrate, its thermal conductivity become high and temperature can easily reach inside the substrate filled net bag. In case of 35 and 40% moisture content, substrate remain relatively drier and temperature cannot reach inside the substrate properly, resulting uneven pasteurization of the substrate.

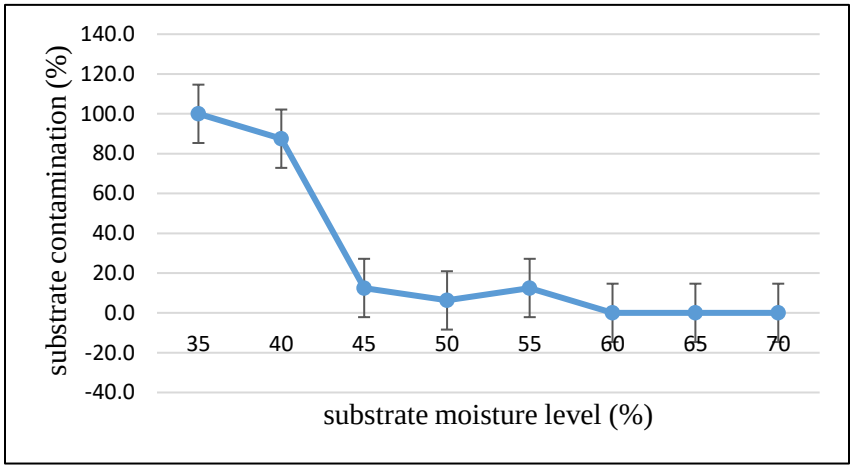

Figure-14: Effects of substrate moisture content on the rate of substrate contamination

Number and size of fruiting body

Number of effective fruiting body was not affected by substrate moisture level but the size of fruiting body was significantly affected by substrate moisture level (Table-25). Stalk length of fruiting body was highest (9.5cm) but stalk diameter was lowest (2.3 cm) in substrate having 45% moisture. That means substrate containing 45% moisture produces thinner fruiting body. Big size fruiting body was produced in substrate containing 70% moisture. Stalk diameter was highest (2.6 cm) in substrate containing 70% moisture which was significantly higher than other moisture level. Both diameter (7.2 cm) and thickness (2.8 cm) of pileus was highest in substrate containing 70%

moisture and lowest (6.1 cm and 2.1 cm) in substrate containing 55% moisture. Higher number and big size fruiting bodies were produced at 70% moisture level might be due to appropriate moisture content in the substrate facilitate mushroom mycelium to easy uptake of their nutrient from the substrate. This result supports the findings of Sarker *et al*. (2007) who also reported that fruiting body weight of oyster mushroom was highest at 70% moisture level of the substrate.

Table-25: Effects of substrate moisture content on number and size of fruiting body of milky white mushroom

Substrate moisture level	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
35%	-	-	-	-	-
40%	-	-	-	-	-
45%	5.8a	9.5a	2.3c	6.7ab	2.3bc
50%	5.6a	8.3b	2.3c	6.2b	2.2bc
55%	5.4a	8.4b	2.4bc	6.1b	2.1c
60%	6.1a	8.3b	2.4bc	6.6ab	2.4bc
65%	6.0a	8.0b	2.5b	6.5ab	2.4bc
70%	6.4a	8.2b	2.6a	7.2a	2.8a
P	<0.001	<0.001	<0.001	<0.001	<0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. '–' indicate no data were recorded due to maximum spawn packets were contaminated. P represents the level of significance.

Yield and biological efficiency

Yield and biological efficiency (BE) of milky white mushroom were significantly affected by substrate moisture level (Figure-15). Highest yield (361.1g) and biological efficiency (87.4%) were recorded from substrate containing 70% moisture which was statistically similar to substrate containing 60% (315.2g & 76.3%) and 65% (303.8g & 73.6%) moisture. Yield and biological efficiency were lowest (271.3g & 65.7%) in substrate containing 45% moisture. No yield was recorded from substrate containing 35% and 40% moisture because no mycelium colonization was observed in substrate containing 35% moisture and only12.5% mycelium colonization was observed in substrate containing 40% moisture. Appropriate moisture content is the prerequisite for proper physical and chemical properties of mushroom substrate. Increased yield and biological efficiency at 70% moisture might be due to this moisture level could ensure suitable physical and chemical condition of rice straw substrate for better growth and yield of milky white mushroom. This result was similar to that of Sarker *et al.* (2007) who also reported wide variation of biological and economic yield among the moisture levels in substrate of oyster mushroom. Siwulski *et al*. (2007) reported that optimum moisture of substrate for oyster mushroom strain HK-35 and K-22 was 75% at which they gave highest yield and biological efficiency. Shen *et al.* (2008) reported that yield and biological efficiency of shiitake mushroom (*Lentinula edodes*) were highest on wood log substrate containing 55% moisture. This might be due to the difference of substrate and mushroom species. Pani *et al*. (2017) reported that soaked paddy straw with 60 % moisture sustained the highest yield of straw mushroom compared to other treatments.

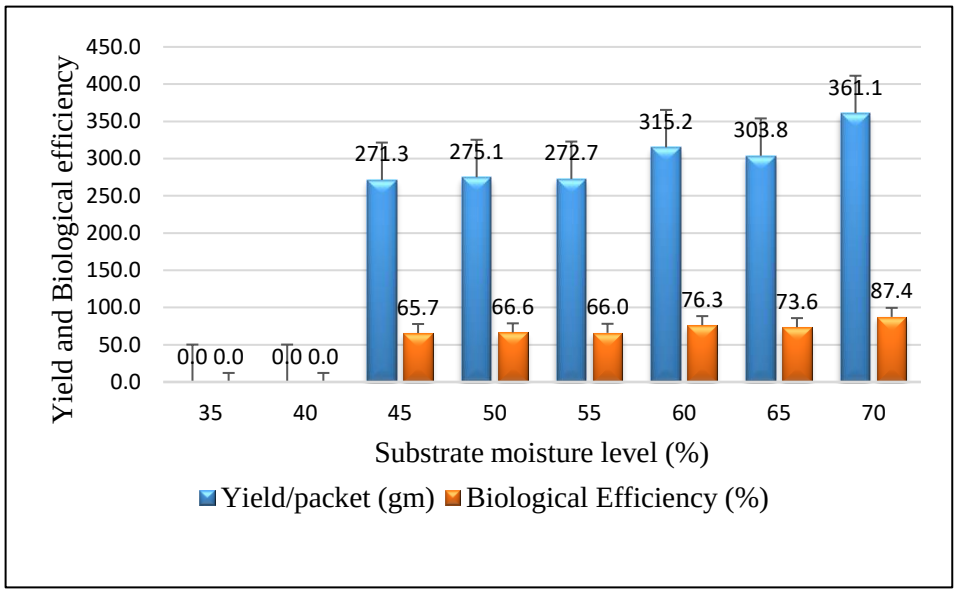

Figure-15: Effects of substrate moisture content on yield and biological efficiency of milky white mushroom

Relationship between substrate moisture content and the rate of substrate contamination

Significant negative correlation (r = - 0.803) was observed between substrate moisture content and the rate of substrate contamination. The relationship between the two variables were linear and could be expressed by the equation y = 173.44 - 2.78x (R^2 = 0.645*) where y = rate of substrate contamination, x = substrate moisture content. The R^2 value indicated that 64.5% substrate contamination was attributed to the substrate moisture content and the rate of substrate contamination was gradually decreased with the increase of substrate moisture content (Figure-16).

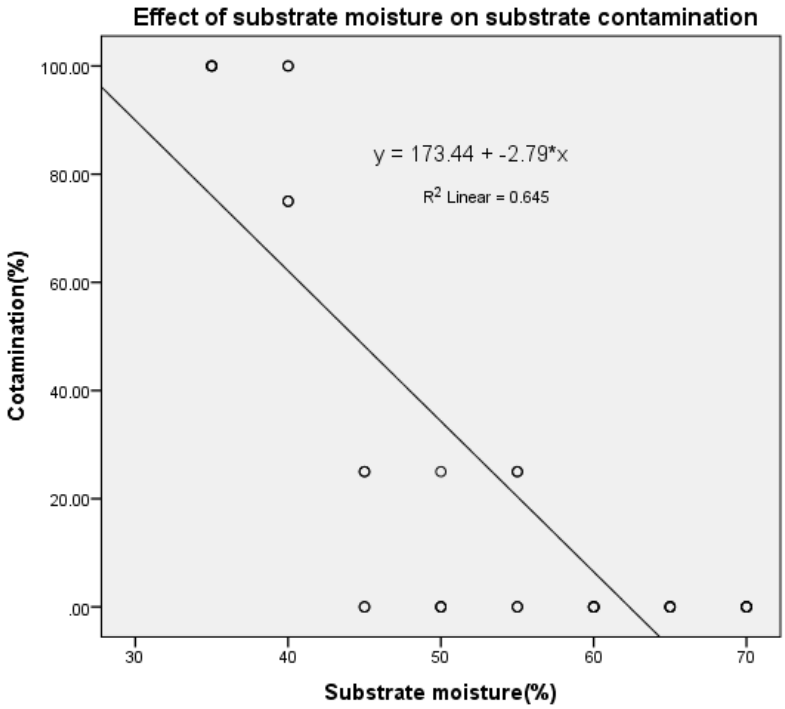

Figure-16: Relationship between substrate moisture content and the rate of substrate contamination

Nutrient content

Protein and dietary fiber content of whole fruiting body of milky white mushroom was significantly affected by moisture level of the substrate but ash and lipid content were not affected (Table-26). Protein and dietary fiber are important compounds of mushroom. Amount of protein was ranges from 11.6g to 19.3g and fiber from 10.85 to 11.48g per 100g dried mushroom. Highest amount of protein (19.3g) was observed at 65% moisture level of the substrate and lowest (19.6g) at 50% moisture level. Fiber content was highest (11.48g) at 45% moisture level and lowest (10.85g) at 65% moisture level. Ash and lipid content of milky mushroom was observed 10.17 to 11.95g and 4.52 to 5.58g respectively in this study. This result supports the findings of Alam *et al*. (2008), Breene (1990), Sumathy *et al*. (2015) and Zahid *et al*. (2010).

Calocybe indica have a mix of minerals and their fruiting bodies are characterized by high level of assimilable mineral constituents. In the present study it was observed that

among the minerals Ca, Cu, Fe and Zn content was not affected by moisture level of the substrate but amount of Co, Mo and Se were affected by substrate moisture level (Table-23). Ca content was varied from 6.71 to 8.02 mg, Cu from 7.43 to 8.19 mg, Fe from 23.17 to 29.83 mg and Zn from 6.82 to 7.48mg per 100g dry weight of milky mushroom. Co content was maximum (0.74mg) at 60% moisture level of the substrate and minimum at 50% moisture level (0.65mg). Mo content was ranges from 232 to 275µg. Maximum Mo content was observed at 70% moisture level and minimum at 45% moisture level of the substrate. Se content was highest (25.0µg) at 65% moisture level and lowest (18.5µg) at 45% moisture level. It was reported that the individual chemical composition of the mushroom largely varies with species and also depends on the age of the fruiting body, composition of the compost and substrate. It was also reported that the nutritional property changes by flush-to-flush Sumathy *et al*. (2015). In some cases, result of the present study differed with the findings of Alam *et al*. (2008) might be due to variations of environmental condition, water, soil, substrate etc. which influences the quality of mushrooms.

Table-26: Nutrient content of milky white mushroom grown on rice straw substrate at different moisture level (per 100g dry weight)

Substrate moisture level	Protein (g)	Fibre (g)	Ash (g)	Lipid (g)	Ca (mg)	Cu (mg)	Fe (mg)	Co (mg)	Mo (µg)	Zn (mg)	Se (µg)
35%	-	-	-	-	-	-	-	-	-	-	-
40%	-	-	-	-	-	-	-	-	-	-	-
45%	12.2c	11.48a	11.36a	4.98a	7.22a	8.19a	24.10a	0.69ab	232c	7.48a	18.5d
50%	11.6c	11.32a	10.77a	5.58a	7.51a	7.61a	23.17a	0.65b	245bc	7.42a	20.5cd
55%	13.1bc	10.96b	11.95a	4.92a	8.02a	7.43a	24.17a	0.67ab	260ab	6.82a	21.6bc
60%	17.2ab	10.92b	10.41a	4.52a	7.82a	7.79a	26.17a	0.74a	241bc	7.21a	23.1ab
65%	19.3a	10.85b	10.43a	4.91a	6.93a	7.99a	29.83a	0.66ab	272a	7.28a	25.0a
70%	14.1c	10.90b	10.17a	4.67a	6.71a	7.52a	28.23a	0.67ab	275a	7.38a	19.5cd
P	<0.001	0.002	0.061	0.108	0.041	0.365	0.107	0.038	<0.001	0.278	<0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. '–' indicate no data were recorded due to maximum spawn packets were contaminated. P represents the level of significance.

Conclusion

From the above experiment it could be concluded that there is a positive relationship between yield and moisture content and negative relationship between substrate contamination and moisture content of rice straw substrate up to 70% moisture level. Protein, dietary fiber, Mo, Co and Se content of the whole fruiting body varied with substrate moisture content though some other mineral content does not vary. Therefore 70% moisture of rice straw substrate can be used for milky white mushroom cultivation as it provides faster mycelium colonization, no substrate contamination and highest yield, whereas below 60% moisture level mycelium colonization become slower, yield decreases and rate of substrate contamination increases gradually.

EXPT. NO. 6: EFFECTS OF CASING MATERIAL MANAGEMENT ON YIELD AND YIELD ATTRIBUTES OF MILKY WHITE MUSHROOM

INTRODUCTION

Casing is an important cultural practice of milky white mushroom cultivation. Casing means covering the cultivation substrate with a layer of soil or soil like material after spawn run which enhances the transformation of vegetative stage to reproductive stage (Pani, 2012; Suess and Curtis, 2009). Casing the surface of composted substrate fully colonized by mycelium of mushroom is an essential function in stimulation and promoting the development of fruit bodies (Farsi *et al*., 2011). Recent studies on the constraints in the cultivation of milky mushroom indicated casing is the most important factor affecting the yield. The production of *Calocybe indica* depends on top dressing after the substrate has been fully colonized with mycelium. After complete mycelium formation casing is done to provide a reservoir of water for the developing fruiting bodies.

Milky white mushroom produces numerous fruiting body primordia during first flush and decreases in the following flushes. Among the fruiting body primordia some are grown vigorously to produce effective fruiting body and rest of the primordia remain as non-effective fruiting body which ultimately become dry or rotten. General practice in our country is, after harvest producers leave the non-effective fruiting body in the spawn packet as it is. But every fruiting body primordium takes some nutrient from the substrate, and the dried and rotten primordia may encourage other competitive fungi or harmful microorganism to grow on the upper surface of the substrate which may affect the fruiting body formation and yield in the subsequent flushes. Therefore, the present study was under taken to know the effect of casing material management on the yield and biological efficiency of milky white mushroom.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Mushroom Development Institute, Department of Agricultural Extension, Savar, Dhaka, Bangladesh from May 2020 to July 2020.

Treatments

Five different casing material management techniques were practiced in this experiment such as T_1= removal of dried non effective fruiting bodies after each harvest; T_2= removal of dried non effective fruiting bodies and filling the casing hole with fresh casing material after each harvest; T_3= scraping the upper surface of the substrate after each harvest; T_4= scraping the upper surface of the substrate and adding 10% fresh casing material after each harvest; and T_5= no disturbance of the casing material (control).

In T_1, non-effective fruiting bodies were removed by hand picking after each harvest; in T_2, non-effective fruiting bodies were removed and the holes of casing material were filled with fresh casing material after each harvest; in T_3, after each harvest upper surface of the substrate was scraped gently with finger; in case of T_4, after each harvest upper surface of the substrate was scraped gently with finger and added 10% fresh casing material; and in T_5, casing material was allowed to remain undisturbed.

Preparation of spawn packets

Rice straw substrate was used for the cultivation of milky white mushroom. Spawn packets were prepared as described in chapter III. Within 16-25 days mycelium colonization was completed in the substrate. After completing mycelium colonization in the spawn packets, cotton, brown paper and neck were removed from the packets and the mouth of the polypropylene bags were folded 4 - 5 cm above the substrate. Loamy soil was used as casing material and was sterilized at 65^{o}C for 4 hours. Casing material was covered over the mycelium on the substrate up to 4 cm thickness. Watering was done at

regular interval to maintain moisture at 60 to 70%. Primordia initiated within 7-14 days and developed in to fruiting bodies.

Harvesting and data collection

The fruiting bodies were harvested at 7-8 days of primordia initiation and data were collected on days to primordia initiation, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, number of effective fruiting body (NEFB), number of flushes, days to total harvest, weight of fruiting body, economic yield and biological efficiency (BE). The BE was measured by the formula described in chapter III.

Experimental design and statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. The data were statistically analyzed following SPSS (version 26.0) computer program. Difference among the treatment means were determined by Tukey's Test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Number of effective fruiting body, number of flush and time to complete total harvest

Number of effective fruiting bodies (NEFB), number of flushes and time to complete total harvest of milky white mushroom were significantly affected by casing material management technique (Table-27). Both number of effective fruiting bodies (8.83) and number of flushes (2.81) were highest when non effective dried fruiting bodies were removed after each harvest (T_1) (Plate-15) and were lowest (4.65 & 1.63) when upper surface of the substrate was scrapped with hand and 10% fresh casing material was added after each harvest (T_4). Highest time (65.08 days) was required to complete total harvest (spawning to last harvest) of milky white mushroom when upper surface of the substrate was scrapped after each harvest (T_3) and lowest time (53.15 days) was required when the casing material was allowed to remain undisturbed (T_5). Days to spawn run and days to fruiting body primordia initiation were also significantly varied in this study (Table-25). Minimum time (15.15 days) was required for completing spawn run and maximum time (11.15 days) was required for fruiting body primordia initiation in T_4 but maximum time (23.95 days) was required for completing spawn run in T_3 and minimum time (7.73 days) was required for fruiting body primordia initiation in T_1. This result supports the findings of Amin *et al*. (2010) who reported that number of effective fruiting bodies of milky white mushroom varied from 1.57 to 7.75 and time to complete total harvest varied from 44.0 to 64.75 days as affected by casing material thickness.

Before 1st harvest

After 1st harvest

2nd flush

Plate-15: Removal of non-effective dried fruiting body after each harvest (T_1)

Table-27: Effects of casing material management technique on number of effective fruiting body, number of flush and time to complete total harvest of milky white mushroom

Treatments	Days to spawn run	Days to pin head formation	Number of effective fruiting body	Number of flush	Time to complete total harvest (days)
T_1	20.03b	7.73d	8.83a	2.81a	62.95a
T_2	20.08b	8.15cd	8.08ab	2.44ab	60.70a
T_3	23.95a	9.28b	5.65bc	1.94bc	65.08a
T_4	15.15d	11.15a	4.65c	1.63c	55.15b
T_5	17.15c	8.60c	7.28abc	2.44ab	53.15b
P	<0.001	<0.001	0.002	0.001	<0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Size of fruiting body, yield and biological efficiency

Length of stalk, diameter of pileus and thickness of pileus were significantly influenced by casing material management technique but diameter of stalk was not affected (Table-28). Length of stalk (11.18 cm), diameter of pileus (7.48 cm) and thickness of pileus (2.75 cm) were highest when upper surface of the substrate was scrapped and 10% fresh casing material was added after each harvest (T_4), but diameter of stalk (2.95 cm) was highest when upper surface of the substrate was scrapped after each harvest (T_3) which was similar to other treatments. Stalk length (8.63 cm), pileus diameter (5.23 cm) and pileus thickness (2.15 cm) were lowest when non effective dried fruiting bodies were removed after each harvest (T_1), but stalk diameter (2.65 cm) was lowest when the casing material was allowed to remain undisturbed (T_5). Similar result was reported by Amin *et al*. (2010) who recorded stalk length ranges from 2.68 to 9.51cm, stalk diameter from

2.39 to 3.05 cm and pileus diameter from 5.05 to 7.75 cm as affected by casing material thickness.

Average weight of fruiting body was significantly affected by casing material management technique but variation in yield and biological efficiency among the treatments were insignificant (Table-28). Average weight per fruiting body was highest (85.93g) when upper surface of the substrate was scrapped and 10% fresh casing material was added after each harvest (T_4) and was lowest (41.65g) when non effective dried fruiting bodies were removed after each harvest (T_1). Highest yield (400.75 g) and biological efficiency (96.85%) were recorded in treatment T_2 and lowest (321.90 g & 77.58%) in T_5. This result is comparable with the findings of Kerketta *et al*. (2018) who recorded average weight of sporophore of different *Calocybe indica* strain was 54 to 82g.

Table-28: Effects of casing material management technique on size of fruiting body, economic yield and biological efficiency of milky white mushroom

Treatments	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Weight of fruiting body (g)	Economic yield (g/packet)	Biological efficiency (%)
T_1	8.63c	2.75a	5.23c	2.15b	41.65c	340.25a	82.00a
T_2	10.13ab	2.85a	6.18abc	2.35b	52.40ab	400.75a	96.58a
T_3	9.85abc	2.95a	7.00a	2.73a	67.28ab	341.33a	82.28a
T_4	11.18a	2.85a	7.48a	2.75a	85.93a	327.13a	76.58a
T_5	9.40bc	2.65a	5.55bc	2.23b	47.23c	321.90a	77.58a
P	0.002	0.406	0.003	0.001	0.017	0.225	0.136

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Number of effective fruiting body and yield per flush

Number of effective fruiting body (NEFB) was significantly varied among the treatments in 2nd flush but in 1st, 3rd and 4th flush but variation in NEFB among the treatments was insignificant (Table-29). Highest NEFB were recorded in T_1 both in 1st and 2nd flush (4.31 & 3.56) but it was highest in T_2 (1.06) and T_5 (0.38) in 3rd flush and 4th flush respectively.

Significant variation in economic yield among the treatments were observed in 1st, 2nd and 3rd flush but in 4th flush it was insignificant (Table-30). T_4 gave the highest yield in 1st flush (294.75g) but it was lowest in 2nd flush (33.63g). No fruiting body was recorded in 3rd and 4th flush in T_4. T_1 produces highest yield in 2nd flush (115g) but T_5 in 3rd (38.94g) and 4th flush (14.13g). Economic yield per flush was varied significantly and it was decreased gradually from 1st flush to the subsequent flushes. This result was comparable with the findings of Patel and Trivedi (2016) who also recorded gradual decrease in yield from 1st harvest to the subsequent harvest of *Calocybe indica*.

Table-29: Flush wise number of effective fruiting body, weight of fruiting body and economic yield as affected by casing material management technique

Treatments	Number of fruiting body per flush				Economic yield (g) per flush			
	1st flush	2nd flush	3rd flush	4th flush	1st flush	2nd flush	3rd flush	4th flush
T_1	4.31a	3.56a	1.06a	0.06a	202.13b	115.0a	22.63ab	1.63a
T_2	3.75a	2.94ab	1.06a	0.25a	278.38a	92.38ab	24.00ab	6.00a
T_3	3.44a	2.06bc	0.13a	0.00a	284.88a	53.19c	3.38ab	0.00a
T_4	3.38a	1.19c	0.00a	0.00a	294.75a	33.63c	0.00b	0.00a
T_5	3.56a	2.13bc	0.81a	0.38a	199.63b	64.94bc	38.94a	14.13a
P	0.580	<0.001	0.037	0.377	0.005	<0.001	0.038	0.246

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Variation in average weight of fruiting body among the treatments were insignificant during all the flushes (Figure-17). But fruiting body weight was significantly varied among the flushes and it was gradually decreased from 1st flush to the subsequent flushes. Fruiting body weight was highest in T_4 during 1st flush (94.98g), in T_1 during 2nd flush (32.46g) and in T_5 during 3rd (25.29g) and 4th (19.0g) flush. This result is comparable with the findings of Patel and Trivedi (2016) who also recorded gradual decrease in yield from 1st harvest to the subsequent flushes.

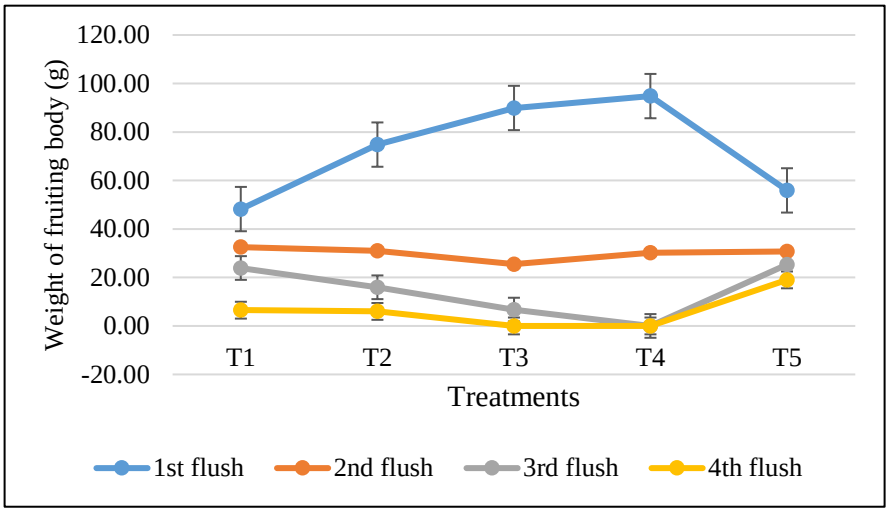

Figure-17: Flush wise weight of fruiting body as affected by casing material management technique

Conclusion

From the above study it can be concluded that casing material management technique influences the yield and yield attributes of milky white mushroom after 1st harvest. Among different technique, removal of dried non effective fruiting bodies after each harvest was the best technique for casing material management as it helps to produce highest number and average weight of effective fruiting body and economic yield of milky white mushroom from the 2nd flush to the subsequent flush.

EXPT. NO.7: EFFECTS OF SPAWN DENSITY ON MYCELIUM RUNNING, YIELD AND YIELD ATTRIBUTES OF MILKY WHITE MUSHROOM

INTRODUCTION

Spawn is the mushroom mycelium that has fully colonized a steam sterilized substrate that is used to 'seed' the final fruiting substrate. It serves as the planting material in mushroom cultivation (Romaine *et al.*, 2007). It provides the backbone to any mushroom growing operation. It is as the equivalent of seeds for a mushroom farm. The spawn is used to transfer mycelium onto any material from which mushrooms will grow, called a substrate. Bulk substrates are rarely directly inoculated with spores or tissue culture. They are almost always inoculated with a spawn substrate in a process commonly referred to as "spawning". During this process, the bulk substrate is hydrated to field capacity and pasteurized before a spawn substrate is broken up and mixed with the material.

When moving a large amount of fully colonized spawn substrate to a mildly nutritious bulk substrate, the mycelium has a great advantage over any other contaminants due to its full colonization of the highly nutritious substrate and its many points of inoculation throughout the bulk substrate. Because of this resistance to contamination, many cultivators perform bulk inoculation in open air environments that have merely been cleaned carefully beforehand.

The quantity of spawn used does not directly affect the yield of mushrooms (Quimio *et al.*, 1990). However, the use of more spawn has been found to influence mushroom growth, development and yield. Growers have sought, in the past, to optimize the amount of spawn used to inoculate their substrate. Increasing the amount of spawn used (up to 5 per cent of the wet weight of the substrate) has resulted in increased yields (Royse, 2002). Increasing spawn rates from 1.25 to 5 per cent may result in increases of nearly 50 per cent. Dahmardeh *et al*. (2010) found out that the maximum average yield (1810 g/2 kg wet barely substrate) of mushrooms was estimated from the barley substrate at 150 g

spawn level. Fan *et al*., (2000) carried out the studies with 2.5-25% spawn rates, 25% spawn rate appeared superior, but recommended 10% spawn rate in view of the process economics. In our country rice straw substrates are generally inoculated with 10% saw dust spawn for milky white mushroom cultivation which creates partial coverage of substrate with mycelium but fully mycelium colonized substrate is prerequisite for better yield and biological efficiency. Therefore, the present study has been carried out to evaluate the effect of spawn quantities on the growth and yield of milky white mushroom.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Mushroom Development Institute, Department of Agricultural Extension, Savar, Dhaka, Bangladesh from July 2020 to September 2020.

Treatments

Five different spawn density were used to inoculate the substrate, considered as treatments in this experiment viz. T_1 = 10%, T_2 = 20%, T_3 = 30%, T_4 = 40% and T_5 = 50% of the substrate (dry weight basis).

Preparation of rice grain mother culture

Rice grain spawn was used as spawn. Fresh dry rice grain was soaked for overnight and the soaked grain was boiled for about 30 minutes so that the grain hull began to crake. After 30 minutes boiling was stopped and excess water was drained out from the grain and the grains were air dried. The grain was treated with calcium carbonate @ 5g/kg grain. 250g of calcium carbonate treated grain was filled in 7″x10″ sized polypropylene bag and a plastic neck was fitted with rubber band and sealed with cotton roll covered with brown paper. Then the grain filled bags were autoclaved in an autoclave machine for an hour at 121^{o}C and 15 PSI pressure. After cooling the grain filled bags were inoculated with fully colonized pure culture of milky white mushroom in a clean bench. The inoculated grain bags were kept in an inoculation room at 25^{o}C temperature for two weeks. After completion of mycelium running the rice grain mother culture was used as spawn for bulk substrate.

Preparation of spawn packets

Rice straw was used as substrate. The substrate was prepared as described in chapter III. Pasteurized substrate was inoculated by thorough mixing the rice grain spawn according

to the treatments. Then the spawn packets were transferred to the culture house for mycelium run. After 10-25 days the substrate was completely colonized by the mycelium.

Casing and after care

After completion of mycelium colonization, cotton, brown paper and neck were removed from the packets and the mouth of the polypropylene bags were folded 4-5 cm above the spawn. Previously sterilized loamy soil casing material was used to cover over the mycelium on the substrate up to 4 cm thickness. Watering was done at regular interval to maintain moisture at 60 to 70%. Fruiting body primordia initiated within 9-15 days and developed in to fruiting bodies.

Harvesting and data collection

The fruiting bodies were harvested at 7-8 days of primordial initiation and data were collected on days to primordial initiation, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, number of effective fruiting body (NEFB), number of flushes, yield and biological efficiency (BE). The BE was measured by the formula described in chapter III.

Experimental design and statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. The data were statistically analyzed following SPSS (version 26.0) computer program. Difference among the treatment means were determined by Tukey's Test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Days to spawn run, pinhead formation and time to complete total harvest

Days to complete spawn run and pinhead formation was significantly influenced by the spawn density used for commercial spawn production of milky white mushroom but time to complete total harvest was insignificant (Table-30). Maximum time (23.83 days) was required to complete spawn run in the rice straw substrate when 10% rice grain mother culture was used for inoculation and minimum time (12.90 days) was required to complete spawn run when 50% mother culture was used. This might be due to higher spawn density prevented other unwanted micro-organisms from becoming established in the substrate. Earlier pinhead of fruiting body formation (9.58 days) was observed in case of 50% spawn density and longest time (14.53 days) was required in 10% spawn density. From the study it was observed that spawn run time and fruiting body pinhead formation was gradually decreased with the increase of amount of spawn used. To complete total harvest, spawn packets inoculated with 40% mother culture takes longest time (65.23 days) and spawn packets inoculated with 10% mother culture takes shortest time (59.98 days).

In our country rice straw substrates are generally inoculated with 10% saw dust or rice grain spawn for milky white mushroom cultivation which creates partial coverage of substrate with mycelium but fully mycelium colonized substrate is prerequisite for better yield and biological efficiency. These results revealed that partial mycelium colonization problem was gradually disappeared with the increase of spawn density (Plate-16-20). These results are in accordance with the findings of Pani (2011) who also observed that there was quicker substrate colonization, earlier pinhead appearance and higher number of sporophores as the amount of spawn increased in the cultivation substrate. Kuforiji and Fasidi (2009) similarly observed that high spawning rate led to more rapid colonization of substrate.

Table-30: Effects of spawn density on spawn run, pinhead formation and harvesting time of milky white mushroom

Spawn density (%)	Days to spawn run	Days to pinhead formation	Number of effective fruiting body	Number of flush	Time complete to total harvest (days)
10	23.83a	14.53a	6.15c	1.65c	59.98a
20	20.40b	13.25b	7.08bc	1.90c	62.65a
30	16.78c	12.88b	8.05ab	2.35b	62.08a
40	15.45d	10.43c	8.53ab	2.85a	65.23a
50	12.90e	9.58d	9.10a	3.10a	65.13a
P	<0.001	<0.001	0.001	<0.001	0.079

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Plate-16: Ten percent rice grain spawn used for inoculation

Plate-17: Twenty percent rice grain spawn used for inoculation

Plate-18: Thirty percent rice grain spawn used for inoculation

Plate-19: Forty percent rice grain spawn used for inoculation

Plate-20: Fifty percent rice grain spawn used for inoculation

Number of effective fruiting body and number of flush

Density of spawn used to commercial spawn packet production of milky white mushroom had significant influence on number of effective fruiting body and number of flush (Table-30). Highest number of effective fruiting bodies (9.10) and flushes (3.10) were recorded from the spawn packets which were inoculated with 50% rice grain spawn (Plate-21). Number of effective fruiting bodies and flushes were gradually decreased with the decrease of amount of spawn used. Lowest number of effective fruiting bodies and flushes were recorded from the spawn packet which were inoculated with 10% rice grain spawn. Highest number of fruiting body and number of flushes produced at 50% spawn density might be due to evenly and densely colonized mycelium and more amount of grain spawn acted as a supplement for mycelium. This result was similar to the findings of Pani (2011) who also reported that number of sporophore was increased with the increase of spawn density in paddy straw substrate and the highest number of sporophore (8.0) was recorded at 500 g spawn/kg substrate. Idowu et al. (2016) also reported that as the psawn density increased number of fruiting body increased.

Plate- 21: Fruiting body from 50 percent spawn density

Relationship between spawn density, time to complete spawn run and fruiting body pinhead formation

There was a strong negative correlation (r = - 0.986**) between the spawn density used and time to complete spawn run. It was observed that the equation y = -2.680x + 25.910 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.971**) showed that the fitted regressing line had a significant regression co-efficient (Figure-18). The R^2 value indicated that 97.10% time to complete spawn run was attributed to the amount of spawn used to prepare commercial spawn packet and the spawn run time was gradually decreased with the increase of amount of spawn used.

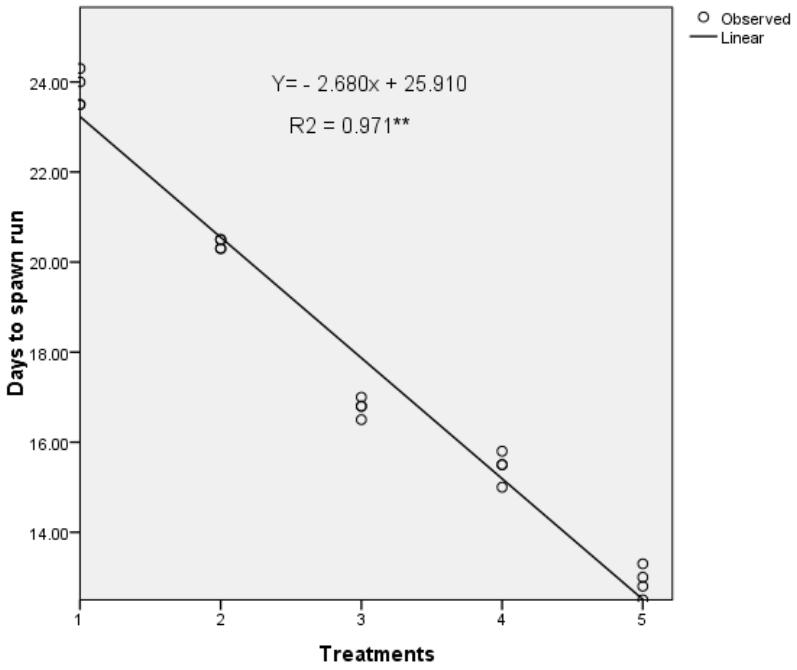

Figure-18: Relationship between spawn density and time to complete spawn run

There was also a strong negative correlation (r = - 0.960**) between the amount of spawn used and time to fruiting body pinhead formation of milky white mushroom. A significant linear relationship was observed between the two variables and the relationship could be described by the equation y = -1.273x + 15.948 (Figure-19). The value of co-efficient of determination (R^2 = 0.922**) indicated that 92.20% time to pinhead formation was attributed to the spawn used to prepare spawn packet. Higher

grain spawn supplies more energy to the mushroom mycelium, so it grows faster and reduce the time to complete spawn run and fruiting body pinhead formation. Idowu *et al.* (2016) and Pal *et al*. (2017) also reported that time to complete spawn run and fruiting body pinhead formation was decreased with the increase of spawn density.

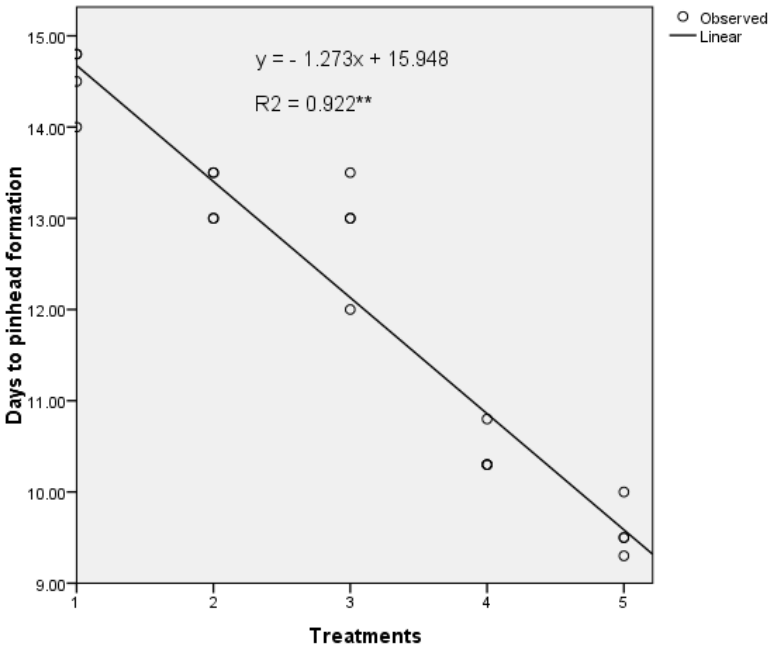

Figure-19: Relationship between the spawn density and days to pinhead formation

Relationship between the spawn density, number of effective fruiting body and number of flush

There was a significant positive correlation (r = 0.824**) between the spawn density and number of effective fruiting body. It was observed that the equation y = 0.735x + 5.575 gave a good fit to the data and the value of co-efficient of determination (R^2 = 0.679**) showed that the fitted regression line had a significant regression co-efficient (Figure-20). The R^2 value indicated that 67.90% number of effective fruiting body was attributed to the spawn density and the number of effective fruiting bodies were increased with the increase of amount of spawn used.

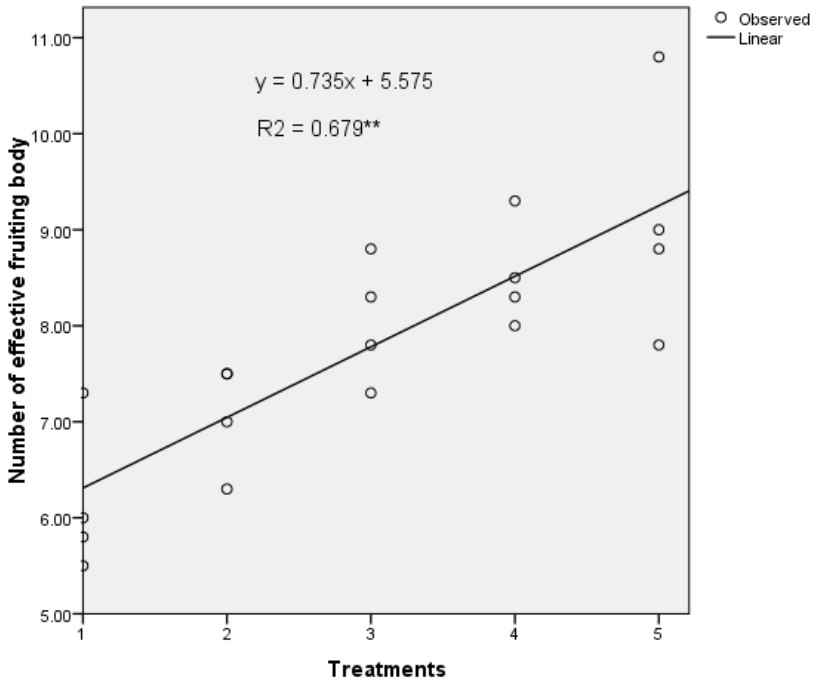

Figure-20: Relationship between the spawn density and number of effective fruiting body

As like number of effective fruiting body, there was also a significant positive correlation (r = 0.964**) between the amount of spawn and number of flushes. The relationship between the two variable was linear and could be expressed by the equation y = 0.385x + 1.215 (R^2 = 0.929**) (Figure-21). The R^2 value indicated that 92.90% number of flushes were attributed to the amount of spawn used for commercial spawn production of milky white mushroom and it was also indicated that the number of flushes were increased with the increase of amount of spawn used. These results are an agreement with the findings of Idowu *et al.* (2016) and Pal *et al*. (2017) also reported that number of effective fruiting body and number of flushes increased with the increase of spawn density.

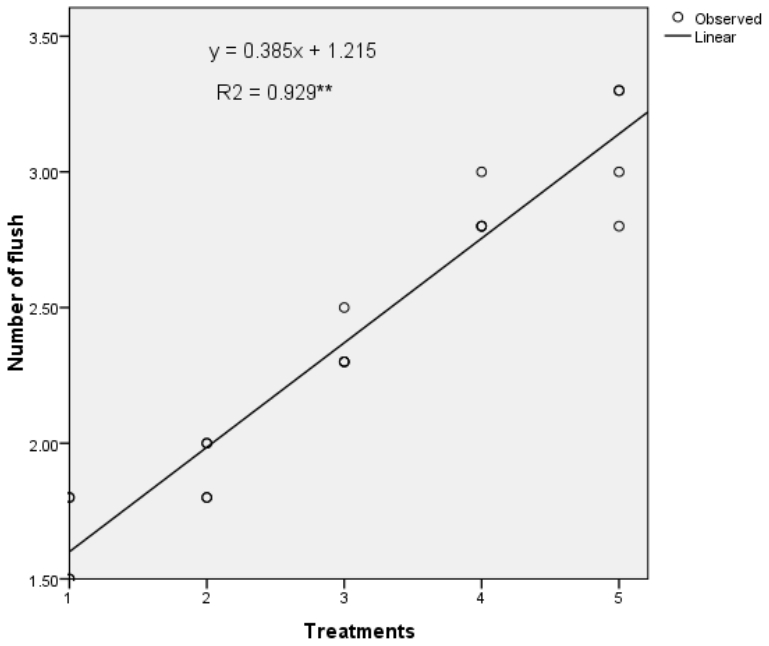

Figure-21: Relationship between the spawn density and number of flushes

Length & diameter of stalk and diameter & thickness of pileus

Stalk length and stalk diameter was significantly influenced by the amount of spawn used for preparation of spawn packets but the effect of spawn density on diameter and thickness of pileus was insignificant (Table-31). Highest stalk length (9.55 cm) was recorded from the spawn packet inoculated with 50% rice grain spawn which was significantly higher than other treatments. Stalk length was lowest (8.38 cm) in spawn packet inoculated with 10% mother culture. Stalk diameter was also highest (2.83 cm) when spawn packets were inoculated with 50% rice grain mother culture which was significantly higher than other treatments but stalk diameter was lowest (2.38cm) when spawn packets were inoculated with 30% mother culture.

Diameter of pileus was highest (6.90 cm) when spawn packets were inoculated with 40% mother culture which was similar to all other treatments. Pileus diameter was lowest (5.85 cm) when spawn packets were inoculated with 10% mother culture. Thickness of pileus was highest (2.35 cm) when spawn packets were inoculated with 50% mother culture which was similar to all other treatments. Pileus thickness was lowest (2.23 cm)

when spawn packets were inoculated with 10% mother culture. These results were partially supported by the findings of Idowu *et al.* (2016) who also reported that longest stipe and widest pileus of *Pleurotus ostreatus* was observed at highest spawn density they used. This might be the difference of mushroom species studied.

Table-31: Effects of spawn density on length & diameter of stalk and diameter & thickness of pileus

Spawn density (%)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
10	8.38b	2.40b	5.85a	2.23a
20	8.63b	2.40b	5.93a	2.28a
30	8.48b	2.38b	6.70a	2.28a
40	8.75b	2.45b	6.90a	2.33a
50	9.55a	2.83a	6.65a	2.35a
P	<0.001	<0.001	0.114	0.792

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Weight of fruiting body, economic yield, biological efficiency and benefit cost ration

Economic yield per packet and biological efficiency of milky white mushroom was significantly influenced by the spawn density used for commercial spawn production but effect on average weight of fruiting body was insignificant (Table-32). Highest average weight (51.55 g) of fruiting body was recorded from the spawn packets which were inoculated with 40% rice grain mother culture and lowest fruiting body weight (45.28 g) was recorded from spawn packets inoculated with 10% mother culture. Economic yield (454.88 g) and biological efficiency (109.61%) was highest when the spawn packets were

inoculated with 50% rice grain spawn which was similar to 40% spawn density (436.40 g & 105.23%). Economic yield and biological efficiency were lowest (273.08 g & 65.80%) when the spawn packets were inoculated with 10% rice grain mother culture. Benefit Cost Ratio (BCR) was highest when 40% spawn density was used. This finding implied that sufficient amount of spawn added to the fruiting substrate, the mycelium grows faster and has more energy available for fruiting body formation, hence the increased yield and better biological efficiency. This result supports the findings of Pani (2011), Idowu *et al*. (2016) and Pal *et al*. (2017) who also reported that yield and biological efficiency were increased with the increase in spawn doses of *Clocybe indica* and *Pleurotus pulmonarius* respectively.

Table-32: Effects of spawn density on weight of fruiting body, economic yield, biological efficiency and benefit cost ratio

Spawn density (%)	Weight of fruiting body (g)	Economic yield per packet (g)	Biological efficiency (%)	Benefit Cost Ratio (BCR)
10	45.28a	273.08d	65.80d	2.84
20	47.29a	332.28c	80.07c	3.26
30	49.88a	397.28b	95.73b	3.68
40	51.55a	436.40a	105.23a	3.83
50	50.86a	454.88a	109.61a	3.79
P	0.555	<0.001	<0.001	

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Relationship between spawn density and economic yield

There was a strong positive correlation (r = 0.960**) between the spawn density and economic yield of milky white mushroom. Relationship between the two variables were quadratic and could be expressed by the equation $y = - 7.688x^2 + 92.928x + 184.620$ (Figure-22). The value of co-efficient of determination (R^2=0.956**) showed that the fitted regression line had a significant regression co-efficient. The graph also indicated that spawn density had a diminishing increase effect on economic yield of milky white mushroom. These results are in accordance with the findings of Idowu *et al.* (2016) and Pal *et al*. (2017) who also observed that as the spawn level increased, the yield of *Pleurotus ostreatus* increased.

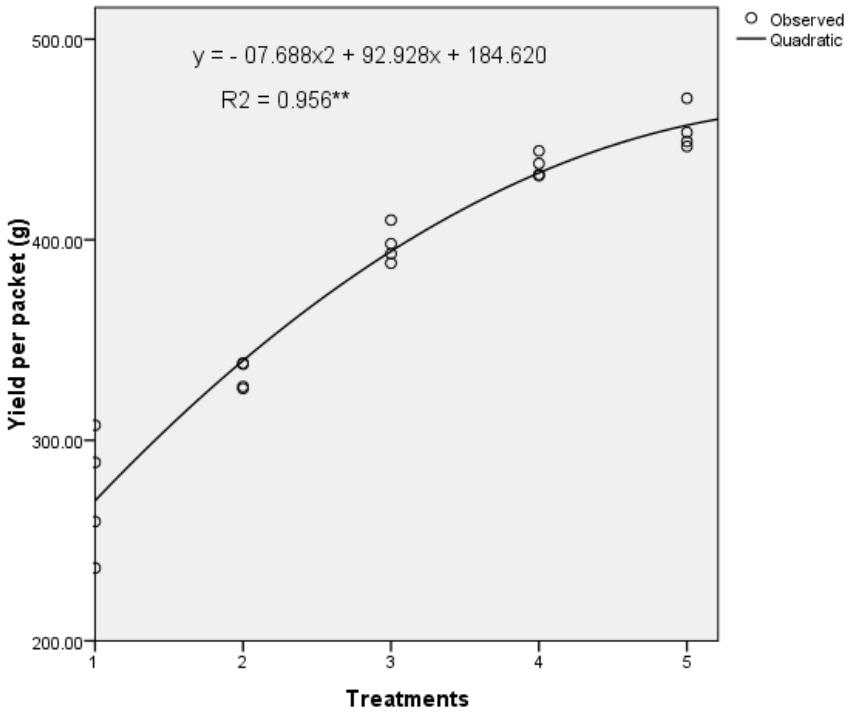

Figure-22: Relationship between the spawn density and economic yield per packet

Relationship between spawn density and biological efficiency

There was also a significant correlation was observed between spawn density and biological efficiency of milky white mushroom. The relationship between the two variable was also quadratic and could be described by the equation $y = - 1.853x^2 + 22.394x + 44.487$ (Figure-23). The value of co-efficient of determination (R^2= 0.956**) indicated that 95.60% biological efficiency was attributed to the spawn density. The equation also indicated that spawn density had a diminishing increase effect on the biological efficiency of milky white mushroom. This result supports the findings of Pani (2011), Idowu *et al*. (2016) and Pal *et al*. (2017) who also reported that biological efficiency was increased with the increase in spawn doses of *Clocybe indica* and *Pleurotus pulmonarius* respectively.

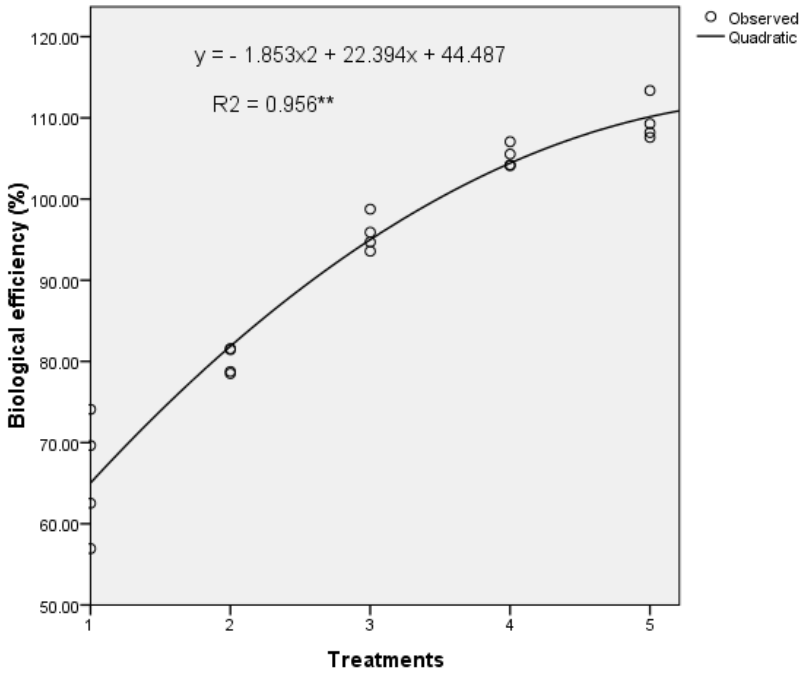

Figure-23: Relationship between the spawn density and biological efficiency of milky white mushroom

Conclusion

From the above experiment it could be concluded that fully mycelium colonization in the substrate is very important for a good harvest. Partial colonization of mycelium in spawn packets was completely disappeared with the increase of spawn density. The relationship between spawn density and economic yield and biological efficiency was significant and quadratic. Maximum growth and yield contributing parameters of milky white mushroom were increased with the increase of spawn density and 50% spawn density gave the highest yield but benefit cost ratio was highest at 40% spawn density.

EXPT. NO. 8: EFFECTS OF FRUITING BODY AGE ON YIELD AND SHELF LIFE OF MILKY WHITE MUSHROOM

INTRODUCTION

Mmilky white mushroom is also known as dudh chatta because of its attractive milky whitish appearance with excellent shelf life and large sized basidiocarp with fleshy stipe and broadly adnate to decurrent gills. This mushroom is a great choice to the local consumer of Bangladesh due to its milky white color, delicate in texture, robust in size and delicious taste. Because of suitable condition, high local demand and export potential of this mushroom, many private entrepreneurs are interested in its commercial cultivation (Amin *et al*., 2010). The size, shape, color, taste, yield and shelf life of the mushroom depends on the maturity of the fruiting body. Premature harvesting of the mushroom may reduce the yield. On the other hand, if it is harvested at over age, the mushroom shades spores, loses its attractive color, robust in size and sporophore become fibrous, leathery and ultimately tasteless (Sharker *et al.*, 2011). Mushroom quality and consumer acceptability of fresh mushrooms is strongly influenced by color, texture and appearance. Therefore, proper time harvest of mushroom is very important.

Compare to other vegetables mushrooms have a shorter shelf life. In mushrooms, post-harvest biological changes are particularly fast. Mushrooms have high respiration rate, tends to lose moisture rapidly and gets discolored at a very fast rate. Immediately after harvest, fresh mushroom starts to soften and its color turned into brown due to enzymatic degradation of cells and losing moisture through respiration (Brennan *et al*., 2000; Lespindrad *et al*., 2009). Mushrooms shelf life can end up due to high rate of respiration, high rate of dehydration, browning and texture changes. Storage temperature had a significant effect in mushrooms quality. A gradual yellowness and high mass losses were also found throughout storage life, with an increase rate as storage temperature increases. Low temperatures and high humidity decrease mass losses over storage time and therefore transpiration rate. Therefore, the present study was carried out to determine the appropriate harvesting age of milky mushroom to get maximum yield and longer shelf life.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Mushroom Development Institute, Department of Agricultural Extension, Savar, Dhaka, Bangladesh from June 2019 to September 2019.

Treatments

Ten different harvesting age of fruiting body was considered as treatments. Mushrooms were harvested at 5^{th}, 6^{th}, 7^{th}, 8^{th}, 9^{th}, 10^{th}, 11^{th}, 12^{th}, 13^{th} and 14^{th} days of primordial initiation. Harvested mushroom was stored in refrigerator and ambient condition in open tray, cellophane wrapped tray and polypropylene bag.

Preparation of spawn packets

Rice straw substrate was used for the cultivation of milky white mushroom. Spawn packets were prepared following the procedure described in chapter III. Mycelium colonization in spawn packets was completed within 16-25 days of spawning.

Casing and after care

After completion of mycelia colonization, cotton, brown paper and neck were removed from the packets and the mouth of the plastic bags were folded 4-5 cm above the spawn. Previously sterilized casing material (loamy soil) were used to cover over the mycelium on the substrate up to 4 cm thickness. Watering was done at regular interval to maintain moisture at 60 to 70%. Fruiting body primordia initiated within 10-12 days and developed in to fruiting bodies.

Harvesting and data collection

The fruiting bodies were harvested at 5^{th}, 6^{th}, 7^{th}, 8^{th}, 9^{th}, 10^{th}, 11^{th}, 12^{th}, 13^{th} and 14^{th} days of primordia initiation and stored at ambient temperature and at $4^{o}C$ (in refrigerator) in

open tray, polypropylene bag and cellophane wrapped tray. Data were collected on days to primordial initiation, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, number of effective fruiting body (NEFB), number of flushes, time to total harvest, shelf life, yield and biological efficiency (BE). The biological efficiency was measured by the formula described in chapter III.

Determination of Shelf life

To determine the shelf life, color, texture, appearance and odor of fruiting body were measured. Color, texture, appearance and odor of fruiting body were measured at the time of storage, 3rd, 6th, 9th, 12th and 15th days of storage. Color of the fruiting body was measured by Chroma Meter (Model FLDO 712, China) and texture with texture profile analyzer (Texvol, TVT-300XP, Sweden) in the laboratory of MDI. Mushroom color was commonly measured using the L value of the hunter scale (Brennan, *et al*, 2000; Cliffe-Byrnes and O'Beirne, 2007). However, some studies indicated that changes in other parameters of the hunter scale (A and B) related to browning (Aguirre *et al*., 2008).

Appearance and odor were also evaluated by the sensorial quality (Xiao *et al*., 2011) to determine the shelf life of mushroom considering 1-9 points hedonic rating scale. Five members panel was recruited from the officer and staff of MDI. Hedonic appearance values are based on a nine-point scale (1 = "dislike extremely," 5 = "neither dislike nor like," 9 = "like extremely"). Odor description values are based on a nine-point scale (1 = "Serious peculiar smell," 5 = "Normal, no peculiar smell," 9 = "strong fragrance").

Experimental design and statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with 4 replications. The data were statistically analyzed following SPSS (version 26.0) computer program. Difference among the treatment means were determined by Tukey's Test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Days to spawn run, fruiting body pinhead formation, number of effective fruiting body, number of flush and time to complete total harvest

Number of effective fruiting body, number of flush and days to total harvest was significantly influenced by fruiting body age of milky white mushroom (Table-33). Highest number of effective fruiting bodies (9.15) were recoded when it was harvested at five days aged. Number of effective fruiting bodies were gradually decreased with the increase of harvesting age. Number of effective fruiting bodies were lowest (6.60) when it was harvested at fourteen days old. Number of flushes was highest (3.98) when fruiting bodies were harvested at 6 days old which was statistically similar to fruiting body harvested at five (3.68), seven (3.55) and eight (3.40) days old. Number of flushes were lowest (1.50) when it was harvested at fourteen days old. To complete total harvest, highest time (94.33 days) was required when the fruiting bodies were harvested at six days old which was similar to fruiting bodies harvested at seven (81.63 days) and eight (85.08 days) days old. Significant variation was observed to complete spawn run and fruiting body pinhead formation in this experiment. Shortest time (15.40 days) was required to complete spawn run when fruiting bodies were harvested at twelve days old and highest time (22.40 days) was required when fruiting bodies were harvested at fourteen days old. Fruiting body pinhead formation was earlier (9.08 days) in case of fruiting body harvested at five days old and it was delay (10.45 days) in case of twelve days old harvest. Highest number of fruiting body at five days old harvest might be due to earlier harvest facilitate more fruiting body production in the subsequent flushes. Days to complete spawn run and pinhead formation was not affected by the fruiting body age because effect of fruiting body age starts after the first harvest. This result was similar to the findings of Sarker *et al.* (2011) who also reported that highest number of fruiting bodies of milky white mushroom was at four days old harvest which was similar to five days old harvest. Number of flushes were highest at four days old and did not differ significantly up to 6 days old.

Table-33: Effects of fruiting body age on number of effective fruiting body, number of flush and time to complete total harvest of milky white mushroom

Fruiting body age (days)	Days to spawn run	Days to pinhead formation	Number of effective fruiting body	Number of flush	Time to complete total harvest (days)
5	16.40c	9.08c	9.15a	3.68a	60.48b
6	18.80b	9.90abc	8.18ab	3.98a	94.33a
7	16.60c	9.45bc	7.30ab	3.55a	81.63a
8	18.53b	9.25c	7.75ab	3.40ab	85.08a
9	18.73b	9.85abc	7.50ab	2.43bc	55.43b
10	16.45c	9.50bc	7.33ab	2.30c	58.90b
11	16.45c	10.20ab	6.98b	2.08c	54.68b
12	15.40d	10.45a	6.60b	2.00c	52.50b
13	16.08cd	10.28ab	6.43b	1.63c	52.83b
14	22.40a	9.68abc	6.60b	1.50c	50.08b
P	< 0.001	< 0.001	0.007	< 0.001	< 0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Relationship between fruiting body age and number of effective fruiting body

There was a significant negative correlation (r = - 0.640**) between fruiting body age and number of effective fruiting body per packet. The relationship between the two variable was linear (Figure-24). It was observed that the equation y = 9.75 – 0.25x gave a good fit to the data and the value of co-efficient of determination (R^2 = 0.410) showed that the fitted regression line had a significant regression co-efficient. The graph indicated that the number of effective fruiting bodies were decreased with the increase of fruiting body age. This result supports the findings of Sarker *et al.* (2011) who also

reported that number of fruiting body of milky mushroom decreased with the increase of fruiting body age.

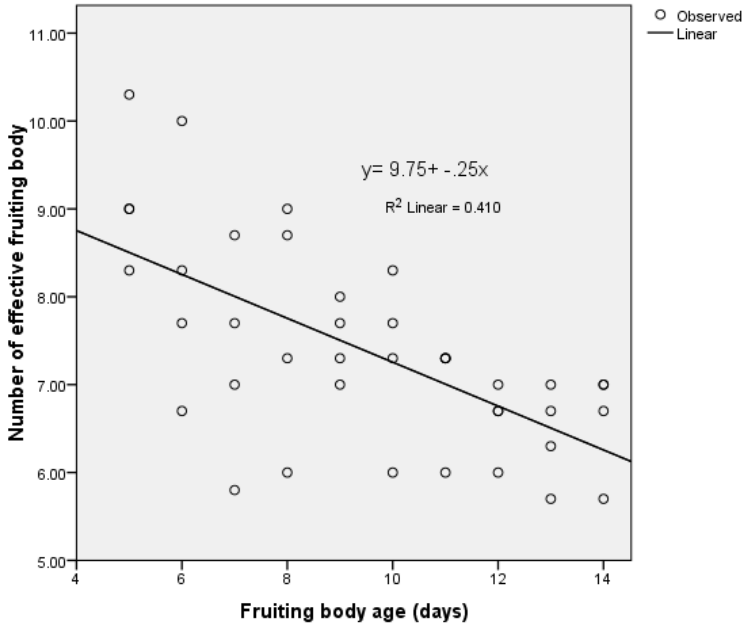

Figure-24: Relationship between fruiting body age and number of effective fruiting body per packet of milky white mushroom

Relationship between fruiting body age and number of flush

A significant negative correlation (r = - 0.857**) was also observed between age of fruiting body and number of flushes of milky white mushroom. It was observed that the equation y = 5.41– 0.29x gave a good fit to the data and the value of co-efficient of determination (R^2 = 0.734**) showed that the fitted regression line had a significant regression co-efficient (Figure-25). The R^2 value indicated that 73.4% number of flushes was attributed to the harvesting age of fruiting body and the number of flushes were gradually decreased with the increase of harvesting age of fruiting body. Sarker *et al.* (2011) also reported that number of flushes of milky mushroom was decreased with the increase of fruiting body age.

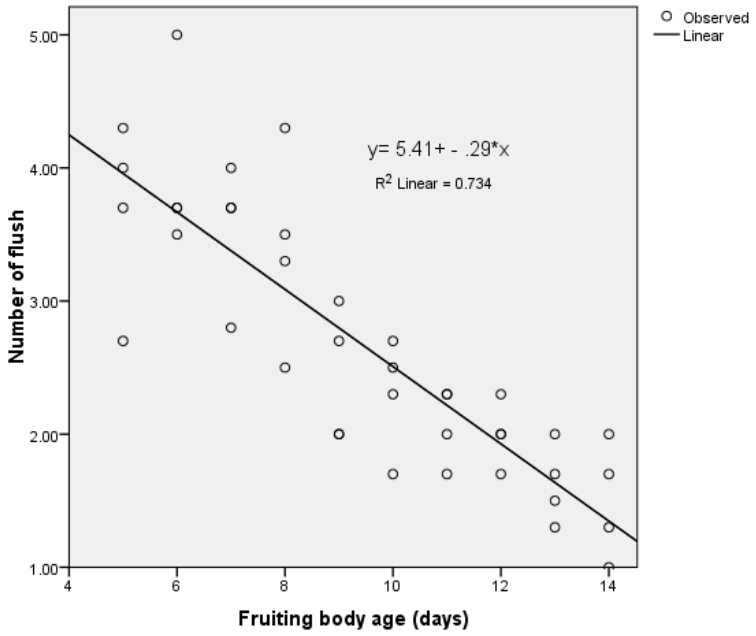

Figure-25: Relationship between fruiting body age and number of flush per packet of milky white mushroom

Length of stalk, diameter of stalk, diameter of pileus and thickness of pileus

Length of stalk, diameter of stalk, diameter of pileus and thickness of pileus was significantly influenced by harvesting age of fruiting (Table-34). Stalk length was highest (9.18 cm) when the fruiting bodies were harvested at seven days old which was similar to fruiting bodies harvested at up to fourteen days old. Stalk length was lowest (7.98 cm) when the fruiting bodies were harvested at five days old which was similar to six days old (8.13 days). Highest diameter of stalk (2.65 cm) was recorded when the fruiting bodies were harvested at eight days old. Diameter of stalk was lowest (2.38 cm) when the fruiting bodies were harvested at five days old. Diameter of pileus was also highest (6.95 cm) when the fruiting bodies were harvested at eight days old which was similar to other fruiting body age except five days and six days old fruiting body. Pileus diameter was lowest (4.83 cm) when the fruiting bodies were harvested at five days old which was similar to six days old fruiting bodies (4.98 cm). Thickness of pileus was highest (2.85 cm) when fruiting bodies were harvested at nine days old which was similar to other

harvesting age except five days and six days old fruiting body harvest. Fruiting body thickness of pileus was lowest (1.69 cm) when it was harvested at five days old which was significantly lower than all other harvesting age. Length of stalk, diameter of stalk and pileus were gradually increased up to a certain age of fruiting body and again decreased with the increase of age might be due to fruiting body attained its full size at this age and there after it started to shrinking due to over aged and respiratory loss. These results were similar to the findings of Sarker *et al.* (2011) who also reported that the size of fruiting body increased with the increase of age up to a certain limit. They observed stipe length was increased up to 12 days, diameter of stipe up to 10 days, diameter and thickness of pileus up to 11 and 12 days respectively.

Table-34: Effects of fruiting body age on length & diameter of stalk and diameter & thickness of pileus of milky white mushroom

Fruiting body age (days)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
5	7.98b	2.38c	4.83c	1.69c
6	8.13b	2.53abc	4.98c	2.14b
7	9.18a	2.58abc	6.54ab	2.41ab
8	9.08a	2.65a	6.95a	2.60a
9	9.08a	2.58abc	6.57ab	2.85a
10	9.10a	2.63ab	6.77ab	2.62a
11	9.00a	2.63ab	6.61ab	2.61a
12	8.88a	2.47abc	6.94a	2.61a
13	8.53ab	2.43bc	6.73ab	2.51ab
14	8.85a	2.56abc	6.36b	2.54ab
P	< 0.001	0.002	< 0.001	< 0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

The relationship between fruiting body age and stalk length

The relationship between fruiting body age and stalk length of fruiting body was found significant (R^2 = 0.487*) at 5% level of probability. The quadratic response curve was fitted to the observed stalk length against different harvesting age of fruiting body (Figure-26). From the response curve it was observed that harvesting age of fruiting body had an increasing effect on stalk length up to ten days. At this age stalk length was highest (9.10 cm). Further increase in fruiting body age had decreasing effect on stalk length. The relationship could be mathematically expressed as $y = - 0.041x^2 + 0.826x + 4.930$.

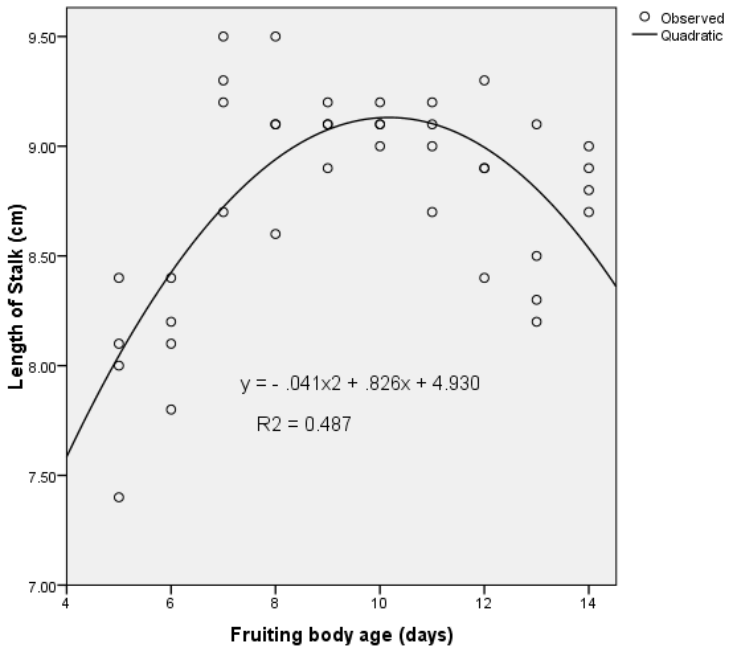

Figure-26: Relationship between fruiting body age and stalk length of milky white mushroom

The relationship between fruiting body age and diameter & thickness of pileus

There was a quadratic relationship between fruiting body age and diameter of pileus (Figure-27) as y= - $0.062x^2$ + 1.388x – 0.517 (R^2 = 0.771**). The R^2 value of the relationship was moderate and significant. The value indicated that 77.10% of the pileus diameter was attributed due to the harvesting age of fruiting body. From the response curve it was observed that harvesting age of fruiting body had an increasing effect on pileus diameter of milky white mushroom up to eight days. At this fruiting body age pileus diameter was highest (6.95 cm). Further increase in the fruiting body age had decreasing effect on pileus diameter. The relationship between fruiting body age and pileus thickness was also quadratic (Figure-38). The equation y = - 0.030x2 + 0.628x – 0.599 gave a good fit to the data and the value of co-efficient of determination (R2 = 0.677**) showed that the fitted regression line had a significant regression co-efficient. The graph also indicated that the highest thickness of pileus (2.85 cm) was observed at nine days and it decreased with the increase of fruiting body age.

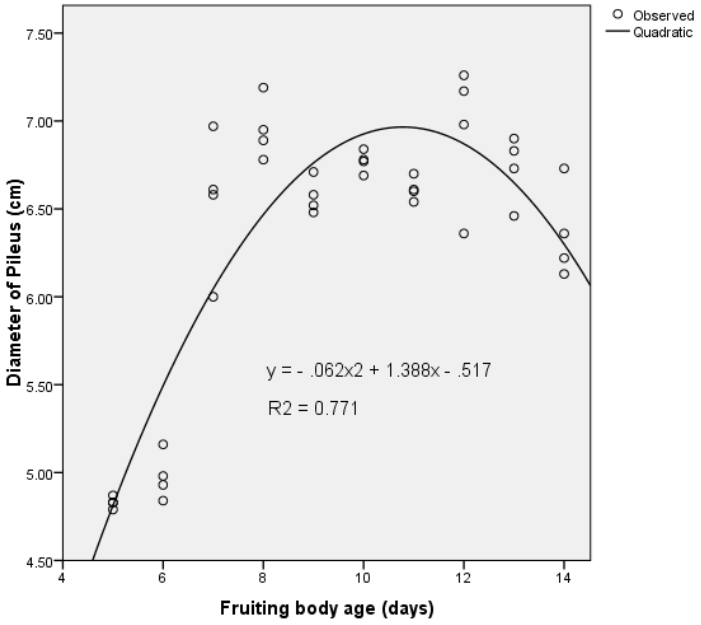

Figure-27: Relationship between fruiting body age and diameter of pileus of milky white mushroom

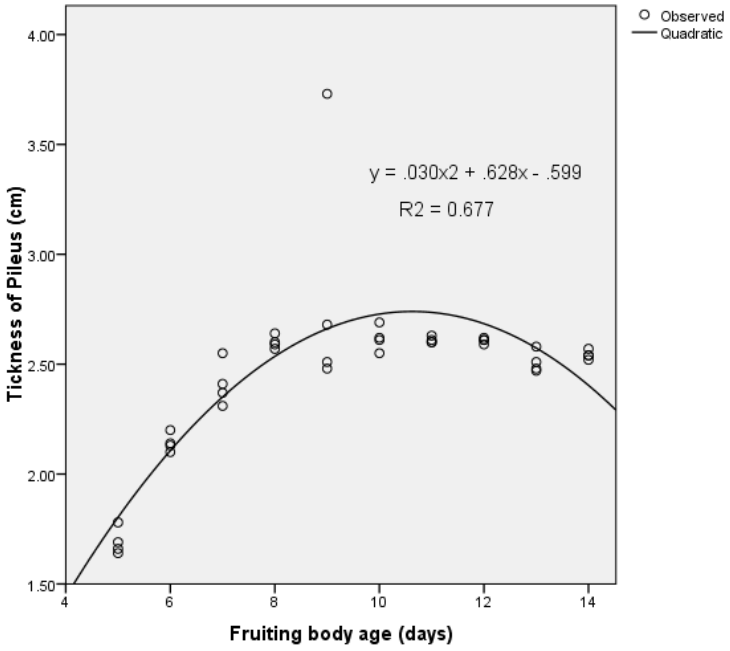

Figure-28: Relationship between fruiting body age and thickness of pileus of milky white mushroom

Weight of fruiting body, economic yield and biological efficiency

Weight of fruiting body, economic yield and biological efficiency of milky white mushroom was significantly affected by harvesting age (Table-35). Fruiting body weight was highest (63.35 g) when it was harvested at eight days old and it was similar to other harvesting age except five days and six days old fruiting bodies. Weight of fruiting bodies were lowest (27.63 g) when it was harvested at five days old which was significantly lower than other harvesting age. Highest economic yield (483.13 g) was produced when it was harvested at eight days old which was significantly higher than all other harvesting age. Economic yield was lowest (253.13 g) when it was harvested at five days old which was statistically similar to fruiting bodies harvested at fourteen days old (320.33 g). Biological efficiency was also highest (116.43 %) when fruiting bodies were harvested at eight days old lowest (60.98 %) when fruiting bodies were harvested at five days old. The average weight of fruiting body, economic yield and biological efficiency were lowest at five days old might be due to premature harvest of fruiting body and after eight days these parameters were decreased gradually due to over age, staying long time on the bed delays the next crop and loss of reserve food for respiration. Similar result was reported by Sarker *et al.* (2011) who also observed highest yield and biological efficiency at 10 days old which did not differ significantly with 6 days and 7 days old harvest.

Table-35: Effects of fruiting body age on weight of fruiting body, economic yield and biological efficiency of milky white mushroom

Fruiting body age (days)	Weight of fruiting body (g)	Economic yield (g)	Biological efficiency (%)
5	27.63c	253.13c	60.98c
6	43.60b	357.58b	86.23b
7	53.03ab	380.85b	91.78b
8	63.35a	483.13a	116.43a
9	47.38ab	373.33b	89.98b
10	50.96ab	368.23b	88.73b
11	51.92ab	360.68b	86.90b
12	54.69ab	358.58b	86.40b
13	53.60ab	342.03b	82.40b
14	48.91ab	320.33bc	77.20bc
P	< 0.001	< 0.001	< 0.001

In column figures having same letters do not differ significantly at 5% level according to Tukey's test. P represents the level of significance.

Relationship between fruiting body age and weight of fruiting body, economic yield and biological efficiency

There was a significant correlation (r = 0.394*) between fruiting body age and weight of fruiting body of milky white mushroom at 5% level of probability. The relationship between fruiting body age and weight of fruiting body showed a quadratic relation and could be described by the equation $y = -0.732x^2 + 15.359x - 24.293$ ($R^2 = 0.408$*) (Figure-29). The R^2 value indicated that 40.80% weight of fruiting body was attributed due to the fruiting body age. The equation also indicated that fruiting body weight was increased with the increase of fruiting body age up to eight days. At this age fruiting body

weight was highest (63.35 g). Further increase in harvesting age had decreasing effect on the weight of fruiting body.

The relationship between fruiting body age and economic yield was quadratic and it could be expressed by the equation y = 5.291x2 + 100.587x - 74.646 (R^2 = 0.346^{NS}) (Figure-30). The R^2 value indicated that 34.60% economic yield was attributed due to the fruiting body age of fruiting body of milky white mushroom. From the response curve it was observed that harvesting age had an increasing effect on economic yield up to eight days. At this fruiting body age economic yield was highest (483.13 g/packet). Further increase in fruiting body age had decreasing effect on the economic yield.

The relationship between fruiting body age and biological efficiency was also quadratic like economic yield. The relationship could be described by the equation y = 1.275x2 + 24.244x + 18.012 (R2 = 0.346^{NS}). The co-efficient of regression values showing the influence of fruiting body age on biological efficiency was comprehensible (Figure-31). Sarker *et al*. (2011) also reported that there was a highly significant correlation between biological efficiency and age of fruiting body of milky mushroom and the relationship was quadratic.

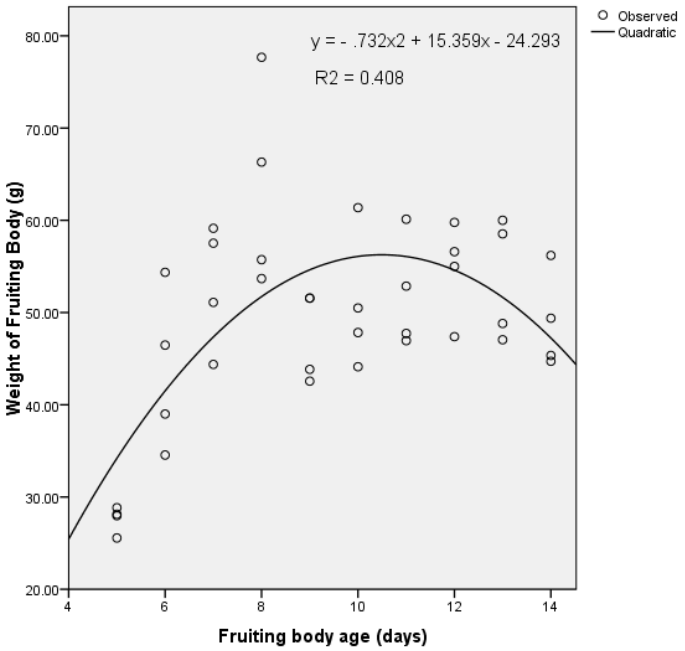

Figure-29: Relationship between fruiting body age and weight of fruiting body of milky white mushroom

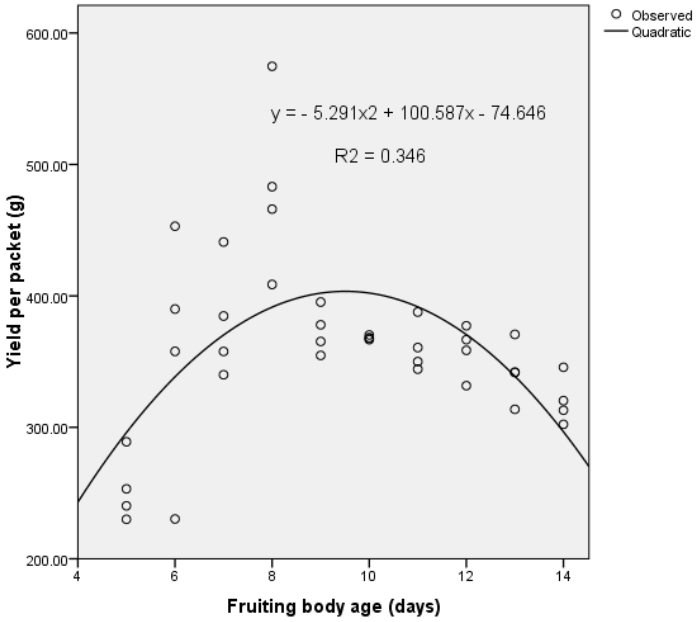

Figure-30: Relationship between fruiting body age and economic yield of milky white mushroom

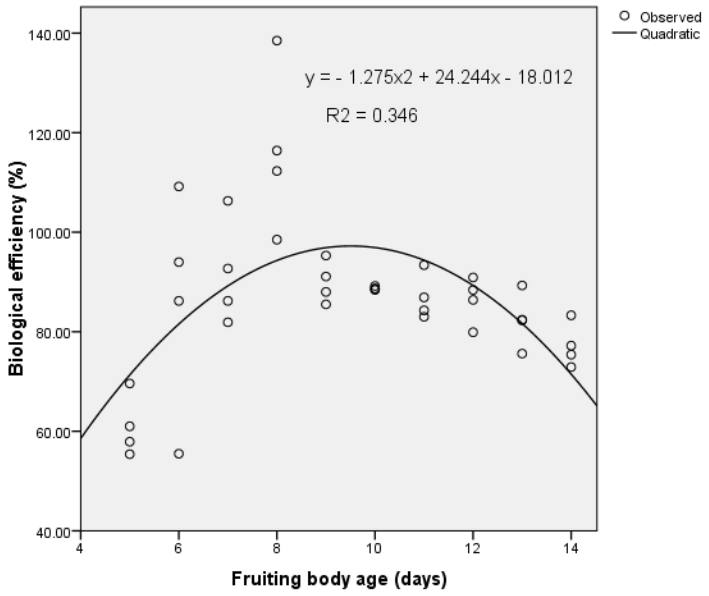

Figure-31: Relationship between fruiting body age and biological efficiency of milky white mushroom

Nutrient content

Nutrient content of milky white mushroom harvested at different age are shown in Table-36. Moisture content of fresh mushroom harvested at different age was ranges from 86.12 to 94.41%. Per 100g dry mushroom contained 57.10 to 64.40g carbohydrate, 11.30 to 14.20g protein, 1.78 to 3.30g lipid, 11.70 to 14.00g fibre and 9.69 to 11.80g ash. Amount of protein (14.20g), fibre (14.00g) and ash (11.80g) were highest in fourteen days old fruiting body. Amount of carbohydrate (64.40g) was highest in nine days and lipid (3.30g) in five days old fruiting body. Lowest amount of carbohydrate (57.10g) in fourteen days, protein (11.30g), lipid (1.78g) in nine days, fibre (11.70g) in ten days and ash (9.69g) in seven days old fruiting body.

Table-36: Nutrient content of milky white mushroom harvested at different age (per 100g dry weight)

Age of fruiting body (Days)	Moisture (%)	Carbohydrate (g)	Protein (g)	Lipid (g)	Fiber (g)	Ash (g)
5	86.12	58.10	13.00	3.30	13.90	11.70
6	91.73	61.20	14.10	2.14	12.70	9.81
7	93.12	62.10	13.40	1.93	12.80	9.69
8	91.34	60.20	13.70	2.27	13.30	10.50
9	90.87	64.40	11.30	2.45	11.90	9.88
10	94.41	63.10	12.80	2.30	11.70	10.30
11	94.15	60.40	12.90	1.78	13.80	11.10
12	93.37	62.40	11.80	2.01	13.30	10.46
13	90.45	62.10	12.00	2.16	13.60	10.13
14	87.20	57.10	14.20	2.87	14.00	11.80

In column figures having same letters do not differ significantly at 5% level according to Tukey's test.

Shelf life

Fresh mushrooms' shelf life is limited to 1-3 days at ambient temperature and 4-7 days at 4^{o}C (Lopeze-Briones, 1992). The main process responsible for mushrooms' sensory quality loss are browning and texture change (Lopeze-Briones, 1992, Ares *et al*., 2006). Mushrooms' quick deterioration is mainly caused by their high metabolic activities, respiration rate and dehydration (Burton *et al*., 1995). To determine the effect of harvesting age on shelf life of milky white mushroom appearance, odor, color and texture had taken in consideration in this experiment.

Appearance

Initially fresh milky white mushroom had excellent overall whiteness, good aroma and texture characteristics at optimum maturity. In this study there was no significant difference in initial appearance score up to nine days old fruiting bodies (Table-37). After nine days, initial appearance score was decreased gradually (Plate 22-31) and after 13 days it became less than 5.0. That means milky white mushroom loses its acceptability for consumption if it is harvested after 13 days of pinhead formation. In ambient condition the mushroom lost its acceptability rapidly when it is stored in an open tray than cellophane paper wrapped tray and polypropylene bag. Appearance score of mushrooms stored in open tray was ≥ 5.0 after 3 days of harvest when it was harvested at 6 to 10 days but the score was below 5.0 when mushroom was harvested at 5 and 11 to 14 days old. 3 days after harvest appearance of mushroom was remained acceptable when stored in cellophane paper wrapped tray and polypropylene bag which was harvested at 5 to 10 days of pinhead formation. 11 to 14 days aged mushroom had lost its acceptability within 3 days of harvest even stored in cellophane paper wrapped tray and polypropylene bag. Before 6 days of storage at ambient condition milky mushroom lost its acceptability irrespective of harvesting age and storage method.

Appearance score of milky white mushroom was at acceptable level for long time in refrigerator than ambient (Table-35). Appearance score was at least 5.0 up to 6 days stored in open tray in case of mushroom harvested at 7 and 8 days but it was below 5.0 in case of other harvesting age. Mushroom harvested at 5 to 9 days old had acceptable appearance score (>5.0) after 15 days of storage in cellophane paper wrapped tray and polypropylene bag kept in refrigerator (4^{o}C) but the score was below 5.0 after 9 days aged harvest. Kamal *et al*. (2015) observed similar results in case of oyster mushroom. They reported that shortest storage period for a single day at ambient condition and extended period of 12 days self-life was determined when mushrooms were stored in refrigerator in respect of sensorial quality in sealed polypropylene bag or in polystyrene trays.

Plate-22: Five days old fruiting body

Plate-23: Six days old fruiting body

Plate-24: Seven days old fruiting body

Plate-25: Eight days old fruiting body

Plate-26: Nine days old fruiting body

Plate-27: Ten days old fruiting body

Plate-28: Eleven days old fruiting body

Plate-29: Twelve days old fruiting body

Plate-30: Thirteen days old fruiting body

Plate-31: Fourteen days old fruiting body

Table - 37: Effects of fruiting body age on appearance of milky white mushroom stored in open tray, cellophane paper wrapped tray and polypropylene bag

Fruiting body age (Days)	Rating score for appearance of fruiting body stored at ambient temperature														
	During harvest			3rd day after harvest			6th day after harvest			9th day after harvest			15th day after harvest		
	OT	CWT	PB	OT	CWT	PB	OT	CWT	PB	OT	CWT	PB	OT	CWT	PB
5	8.6	8.8	8.8	2.8	6.4	6.2	1.4	3.0	3.8	-	-	-	-	-	-
6	8.8	8.6	8.8	6.2	7.2	6.0	4.0	3.0	1.4	-	-	-	-	-	-
7	8.0	8.8	8.8	7.4	7.8	7.2	4.0	4.8	2.0	-	-	-	-	-	-
8	7.5	8.8	8.8	7.6	8.6	7.0	4.0	6.0	4.6	-	-	-	-	-	-
9	8.0	8.6	6.8	4.0	2.2	4.6	3.0	1.4	4.0	-	-	-	-	-	-
10	7.0	8.0	7.2	5.0	6.7	6.0	1.6	2.0	1.8	-	-	-	-	-	-
11	7.0	8.0	7.0	1.8	7.0	3.8	0.0	1.6	1.4	-	-	-	-	-	-
12	6.0	7.0	6.6	2.0	2.0	3.8	1.4	0.0	1.2	-	-	-	-	-	-
13	5.0	7.2	4.0	1.4	4.4	2.0	2.0	1.8	1.2	-	-	-	-	-	-
14	3.0	4.0	2.6	1.4	1.4	2.0	-	-	-	-	-	-	-	-	-
	Rating score for appearance of fruiting body stored in refrigerator (4^0C)														
5	8.8	8.6	8.6	6.8	7.6	8.2	3.6	7.0	7.2	3.6	6.8	5.6	-	-	-
6	8.8	8.8	8.6	5.8	7.4	7.2	3.8	7.4	7.2	1.2	7.4	7.2	1.2	7.2	7.0
7	8.6	8.8	8.6	7.0	8.6	8.6	5.0	7.6	7.2	3.0	7.4	7.0	2.0	7.2	7.0
8	8.8	8.8	7.8	7.0	8.2	7.4	5.0	8.0	7.2	1.4	8.0	7.0	1.4	7.2	6.0
9	7.0	8.0	8.0	4.0	8.0	6.2	3.6	7.6	6.2	1.6	7.6	5.0	0.0	6.6	4.8
10	8.0	8.0	6.0	6.8	7.0	5.8	3.2	6.8	5.2	4.0	6.0	4.6	-	-	-
11	6.6	7.6	7.0	4.6	7.6	6.0	1.2	7.2	5.0	-	-	-	-	-	-
12	6.0	6.6	7.0	3.8	6.0	3.8	2.8	4.6	2.0	-	-	-	-	-	-
13	5.0	5.8	4.2	3.8	5.6	4.0	3.0	5.6	3.4	1.4	5.4	2.0	-	-	-
14	3.2	3.6	3.4	2.6	3.4	2.0	-	-	-	-	-	-			

OT = Open tray, CWT = Cellophane wrapped tray, PB = Polypropylene bag. '–` indicates data were not recorded due to rotten

Odor

Initial rating score for odor of milky white mushroom was above 5.0 (Normal, no peculiar smell) when the mushrooms were harvested at 5 to 13 days after pinhead formation but it was below 5.0 when the mushrooms were harvested after 13 days of pinhead formation (Table-38). In ambient condition, 6 days after harvest odor score was 5.0 – 6.8 stored in open tray when mushrooms were harvested at 7-8 days old but odor score of mushrooms harvested at 5 and 9 to 14 days was below 5.0 after 3 days of harvest. Odor score of mushrooms stored in cellophane wrapped tray and polypropylene bag was ≥ 5.0 up to 6 days after harvest. Odor score was above 5.0 up to 3 days after harvest when mushroom stored in polypropylene bag & ambient condition harvested within 12 days of pinhead formation but the score was below 5.0 in case of 13 and 14 days of harvest.

In case of refrigerator storage, odor score of mushrooms kept in open tray was above 5.0 up to 6 days of harvest when mushrooms were harvested at 6 to 8 days aged but the score was below 5.0 when the mushrooms were harvested at 5 and 9 to 14 days aged. Mushroom harvested at 6 to 9 days after pinhead formation and stored in cellophane paper wrapped tray & polypropylene bag was remained in good condition for consumption after 15 days of storage (scored >5.0) but mushroom harvested at 10 to 13 days aged lost its edibility after 6 days and 5 days aged lost edibility after 9 days of storage.

Table-38: Effects of fruiting body age on odor of milky white mushroom stored in open tray, cellophane paper wrapped tray and polypropylene bag

Fruiting body age (Days)	Rating score for odor of fruiting body stored at ambient temperature														
	During harvest			3rd day after harvest			6th day after harvest			9th day after harvest			15th day after harvest		
	OT	CWT	PB	OT	CWT	PB	OT	CWT	PB	OT	CWT	PB	OT	CWT	PB
5	8.6	8.6	8.6	4.6	6.8	6.6	1.4	1.2	2.4	-	-	-	-	-	-
6	8.4	8.8	8.6	6.4	7.4	7.2	4.8	4.0	1.6				-	-	-
7	8.6	8.8	8.6	7.0	6.0	5.4	5.0	5.6	4.0	-	-	-	-	-	-
8	8.6	8.8	8.4	7.8	6.8	6.6	6.8	6.0	4.6	-	-	-	-	-	-
9	8.0	8.4	7.0	4.6	2.0	5.6	4.0	1.2	2.8	-	-	-	-	-	-
10	7.0	7.4	7.0	4.6	5.6	5.8	3.8	4.8	4.0	-	-	-	-	-	-
11	7.6	8.2	7.6	4.6	6.8	6.2	0.0	1.2	1.4	-	-	-	-	-	-
12	7.0	7.4	7.6	5.0	4.0	5.2	1.5	0.0	1.2	-	-	-	-	-	-
13	6.0	7.2	5.2	1.2	4.8	2.0	1.0	1.0	1.0	-	-	-	-	-	-
14	4.0	4.6	3.8	1.4	1.2	1.2	-	-	-	-	-	-	-	-	-
	Rating score for odor of fruiting body stored in refrigerator (4^0C)														
5	8.6	8.6	8.8	7.8	7.8	7.6	4.0	6.6	6.2	5.0	6.2	5.6	-	-	-
6	8.8	8.6	8.8	7.2	8.2	8.2	6.8	7.4	7.2	1.2	7.2	7.2	1.2	7.0	7.0
7	8.4	8.6	8.8	6.8	7.2	7.6	5.2	7.0	7.2	5.0	6.8	6.0	4.8	6.6	5.8
8	8.6	8.6	8.6	7.2	7.4	7.0	6.8	7.2	7.0	1.4	6.8	6.4	1.4	6.4	6.0
9	7.8	8.4	7.6	5.2	7.0	6.2	2.8	6.8	6.0	1.6	6.6	5.2	0.0	6.0	5.0
10	7.6	7.6	7.0	5.4	6.6	5.2	5.2	6.1	5.0	3.0	6.0	4.0	-	-	-
11	7.0	7.8	7.0	5.2	7.4	5.4	1.4	7.0	5.0	-	-	-	-	-	-
12	6.0	7.0	7.6	5.0	6.2	5.6	2.0	4.0	2.0	-	-	-	-	-	-
13	6.0	6.8	5.0	3.8	6.0	3.0	3.0	5.6	2.6	1.2	4.8	2.0	-	-	-
14	3.8	4.8	3.8	1.2	2.0	1.2	-	-	-	-	-	-			

OT = Open tray, CWT = Cellophane wrapped tray, PP = Polypropylene, '-' indicates data were not recorded due to rotten

Color

Color is an important parameter of mushroom for its acceptability to the consumers. Color and appearance attract the consumer to a product and can help in impulse purchase. Consumers have a preferred color for a specific item (Crisosto *et al*., 2003). Color of mushroom may be determined using nondestructive methods founded on visual or physical measurements. These methods are based on evaluation of either the light reflected from the surface of a product or transmitted through it. There are three components necessary to the perception of color. 1. A source of light, 2. An object that modifies light by reflection or transmission and 3. Eye/brain combination of an observer (Leggett, 4004). Color space may be divided into a three-dimensional (L, a and b) rectangular area (Plate-32) such that 'L' (lightness) axis goes vertically from 0 (perfect black) to 100 (perfect white) in reflectance of perfect clear in transmission (Hunerlab, 1996; Leggett, 4004). The 'a' axis (red to green) considers the positive values as red and negative values as green; 0 is neutral. The 'b' axis (blue to yellow) expresses positive values as yellow and negative values as blue; 0 is neutral. Fruits and vegetables are often described in terms of their L, 'a' and 'b' values.

Color measurement of different aged milky white mushroom stored in different storage method at different interval are shown in Table 39-41. From the recorded data it was observed that initial whiteness ('L' value) of milky white mushroom was more or less similar up to 13 days aged. After 13 days initial whiteness was reduced and browning ('a' and 'b' value) was increased. After harvest whiteness ('L' value) was also gradually reduced and browning ('a' and 'b' value) was increased with time irrespective of harvesting age and storage method.

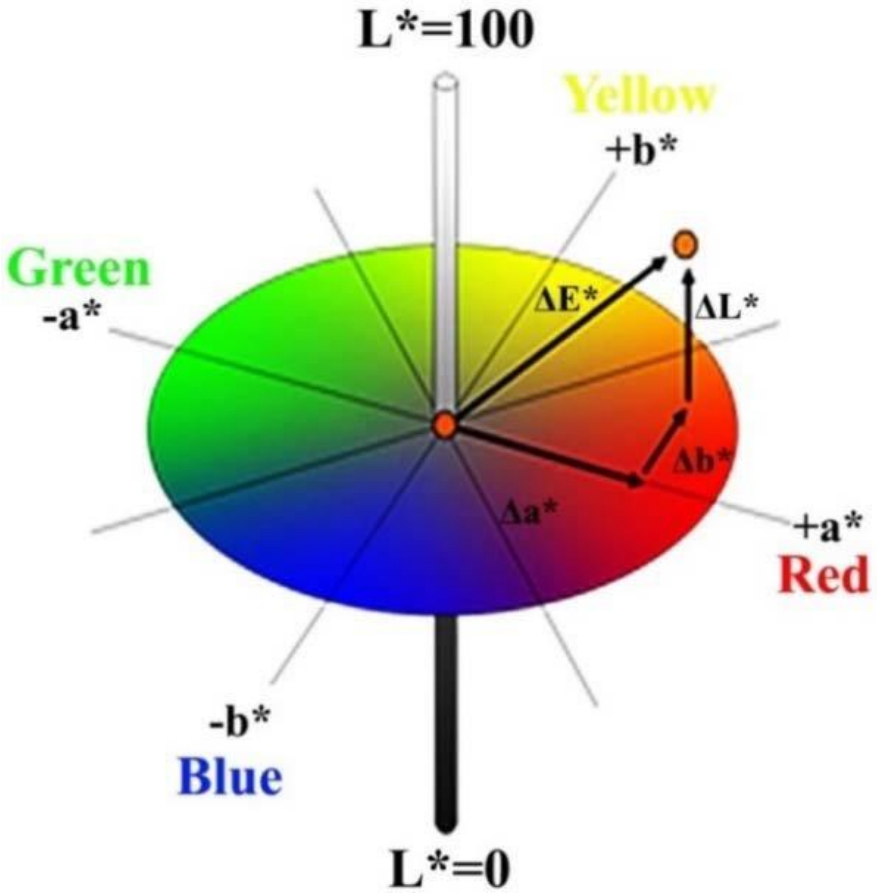

Plate-32: Diagram depicting three-dimensional L, a and b color space

Table -39: Effects of fruiting body age on color of milky white mushroom stored in open tray

Fruiting body age (Days)	Color of fruiting body stored at ambient temperature in open tray											
	During harvest			3rd day after harvest			6th day after harvest			9th day after harvest		
	L	a	b	L	a	b	L	a	b	L	a	b
5	99.83	0.75	1.01	96.58	1.76	4.92	95.32	2.02	5.77	-	-	-
6	96.98	2.35	1.73	97.12	2.03	6.18	94.18	3.03	9.81	-	-	-
7	97.57	1.35	4.28	95.71	2.07	6.67	95.84	1.95	6.81	-	-	-
8	98.73	0.6	1.90	97.52	1.24	5.78	82.91	5.54	16.94	-	-	-
9	96.47	1.8	6.62	95.69	2.6	9.55	91.56	3.47	10.89	-	-	-
10	98.03	0.58	2.62	96.23	1.65	7.62	-	-	-	-	-	-
11	99.63	0.18	2.63	92.51	2.91	10.36	-	-	-	-	-	-
12	97.57	0.79	6.29	86.21	6.417	16.56	-	-	-	-	-	-
13	90.03	4.97	17.26	91.33	4.193	4.193	95.05	2.49	7.00	-	-	-
14	7.00	9.43	25.52	58.71	14.60	23.69	-	-	-	-	-	-
	Color of fruiting body stored in refrigerator (4^0C) in open tray											
5	99.48	0.85	1.11	96.98	1.58	4.05	94.00	2.44	8.67	92.36	2.48	7.92
6	89.45	2.2	3.14	92.35	3.36	6.68	97.12	1.33	4.18	86.61	1.63	8.53
7	98.88	0.88	3.00	97.4	1.46	4.88	94.83	2.12	6.88	91.87	2.59	9.65
8	96.19	2.51	9.49	89.27	5.13	16.45	83.62	6.29	19.97	71.62	9.29	20.98
9	99.21	0.59	4.36	96.45	1.74	8.66	91.11	8.25	10.85	87.88	4.73	14.96
10	100.0	-0.2	1.11	100	0.04	3.62	96.09	1.61	7.03	92.73	2.80	8.74
11	97.40	0.73	8.19	96.19	1.26	10.62	90.54	3.51	12.79	-	-	-
12	97.96	0.59	3.5	98.68	0.20	3.78	93.01	2.00	7.27	-	-	-
13	99.02	0.27	2.93	97.49	1.23	7.61	94.99	2.09	10.43	-	-	-
14	78.84	9.43	23.33	69.8	13.00	25.58	-	-	-	-	-	-

OT = Open tray, CWT = Cellophane wrapped tray, PP = Polypropylene, '-' indicates data were not recorded due to rotten

Table -40: Effects of fruiting body age on color of milky white mushroom stored in cellophane wrapped tray

Fruiting body age (Days)	Color of fruiting body stored at ambient temperature in cellophane wrapped tray											
	During harvest			3rd day after harvest			6th day after harvest			9th day after harvest		
	L	a	b	L	a	b	L	a	b	L	a	b
5	98.68	0.95	1.93	99.65	0.65	1.67	98.74	0.90	2.73	-	-	-
6	97.13	1.72	4.62	97.82	1.60	7.19	97.74	1.77	6.56	-	-	-
7	99.37	0.69	3.13	99.93	0.12	1.99	99.94	0.29	2.22	99.92	0.23	1.82
8	99.22	1.04	3.37	99.84	0.78	2.85	99.84	0.30	1.89	-	-	-
9	98.08	1.48	5.91	98.91	0.78	3.45	-	-	-	-	-	-
10	99.74	0.57	0.99	99.79	0.39	0.69	99.91	0.36	1.97	-	-	-
11	100.0	0.09	1.56	99.88	0.23	1.76	-	-	-	-	-	-
12	98.62	0.34	4.34	98.11	0.62	3.27	-	-	-	-	-	-
13	99.87	0.07	0.51	99.91	0.07	0.71	-	-	-	-	-	-
14	89.91	4.50	14.01	-	-	-	-	-	-	-	-	-
	Color of fruiting body stored in refrigerator (4^0C) in cellophane wrapped tray											
5	99.18	0.82	1.67	100	0.85	1.56	99.48	1.02	3.17	98.78	0.55	2.11
6	97.66	1.88	3.41	98.48	1.83	2.79	100	0.8	1.57	100	0.2	2.27
7	99.26	0.98	3.07	100	0.52	1.22	99.72	0.9	2.81	96.91	1.7	5.81
8	100.0	0.35	1.72	97.42	2.12	3.15	98.28	0.91	7.22	100	0.18	1.82
9	98.76	0.71	3.69	96.8	2.23	9.1	91.06	4.79	15.9	97.21	1.88	8.01
10	100.0	-0.28	1.34	100	0.0	0.92	100	-0.3	1.24	100	0.19	2.6
11	99.36	0.07	0.77	100	-0.22	0.14	100	0.02	0.62	100	-0.46	-0.07
12	98.67	0.41	5.34	97.89	0.92	5.64	99.04	0.35	4.01	99.42	0.03	3.52
13	98.67	0.41	5.34	97.89	0.92	5.64	99.04	0.35	4.01	99.42	0.03	3.52
14	76.08	9.5	21.68	78.82	9.31	22.29	-	-	-	-	-	-

OT = Open tray, CWT = Cellophane wrapped tray, PP = Polypropylene, '-' indicates data were not recorded due to rotten

Table -41: Effects of fruiting body age on color of milky white mushroom stored in polybag

Fruiting body age (Days)	Color of fruiting body stored at ambient temperature in polybag											
	During harvest			3rd day after harvest			6th day after harvest			9th day after harvest		
	L	a	b	L	a	b	L	a	b	L	a	b
5	98.60	0.88	1.71	99.36	0.68	1.86	96.34	0.86	5.1	-	-	-
6	100.0	0.70	1.14	100.0	0.8	1.3	98.49	0.3	2.66	-	-	-
7	96.66	1.77	4.98	98.89	0.67	2.91	96.23	1.47	6.57	93.13	2.85	9.03
8	90.93	4.40	13.62	92.60	4.06	16.5	91.99	3.05	12.6	-	-	-
9	97.76	1.34	6.60	97.73	1.02	6.39	93.15	2.93	10.9	91.86	3.48	12.8
10	100.0	-0.16	0.98	100.0	0.11	0.69	98.5	0.28	2.88	100	-0.16	0.98
11	100.0	0.01	0.20	98.24	0.56	3.65	-	-	-	-	-	-
12	95.50	1.97	11.17	92.64	2.97	11.5	93.44	2.72	9.97	-	-	-
13	80.25	9.89	23.85	75.15	12.13	23.8	80	9.8	18.7	-	-	-
14	91.74	3.36	10.94	80.64	5.91	15.6	-	-	-	-	-	-
	Color of fruiting body stored in refrigerator (4^0C) in polybag											
5	99.17	1.02	2.11	98.8	1.45	2.03	99.55	0.79	2.03	99.96	1.01	1.94
6	98.94	1.02	2.95	95.13	1.21	3.6	100	0.52	2.68	91.43	4.29	15.8
7	97.00	1.53	4.66	98.33	0.57	4.97	99.3	1.18	4.37	98.03	1.59	5.95
8	94.50	2.81	11.54	93.73	2.82	13.4	94.42	2.65	11.5	92.73	3.31	14.5
9	98.83	0.59	3.78	100.0	0.29	2.18	100	0.32	1.71	100	0.38	2.46
10	100.0	0.03	1.23	99.99	0.08	0.39	97.58	0.58	2.47	96.28	0.6	3.55
11	95.04	2.17	11.0	95.83	1.75	9.51	95.83	1.76	8.85	96.23	1.5	8.25
12	95.53	1.58	8.46	96.81	1.54	7.12	95.92	1.53	9.44	95.53	1.58	8.46
13	92.02	4.11	14.92	91.02	4.62	15.6	95.01	2.7	11.1	92.97	3.88	14.1
14	71.04	11.12	27.38	74.29	10.11	23.2	-	-	-	-	-	-

OT = Open tray, CWT = Cellophane wrapped tray, PP= Polypropylene, '-' indicates data were not recorded due to rotten

Texture

Texture is an important quality parameter for fresh mushrooms (Lopez-Briones *et al*., 1992). One of the main changes associated with mushrooms deterioration are changes in their texture (Lopez-Briones *et al*., 1992; Ares *et al*., 2006). Mushroom softening or loss of firmness during postharvest storage has been ascribed to changes in membrane (Beelman *et al*., 1987). Texture analysis data are presented in Table 42-44. From the texture data it was revealed that hardness of milky white mushroom was more or less similar from 6 to 13 days aged but before 6 days and after 13 days hardness was reduced. It might be due to immature soft tissue before 6 days and over mature senescence tissue lost its firmness after 13 days. After harvest mushroom lost its hardness gradually with increasing the storage time irrespective of harvesting age and storage method. Sarker *et al*. (2014) also reported that after 9 days of fruiting body formation acceptability of milky white mushroom were reduced due to deterioration of their color and texture.

Table -42: Effects of fruiting body age on texture of milky white mushroom stored in open tray

Fruiting body age (Days)	Texture of fruiting body stored at ambient temperature in open tray															
	During harvest				3rd day after harvest				6th day after harvest				9th day after harvest			
	Height	Weight	Hardness	Resilience	Height	Weight	Hardness	Resilience	Height	Weight	Hardness	Resilience	Height	Weight	Hardness	Resilience
5	22	4	643	0.26	12	-13	565	0.65	15	-22	698	0.17	-	-	-	-
6	28	51	759	0.51	24	34	700	0.63	19	12	338	0.45	-	-	-	-
7	36	156	1031	0.66	24	60	330	0.64	17	34	387	0.45	-	-	-	-
8	39	156	749	0.53	43	107	768	0.67	32	47	369	0.63	-	-	-	-
9	32	37	806	0.62	24	4	237	0.54	16	-20	449	0.39	-	-	-	-
10	30	129	1043	0.46	23	48	640	0.61	-	-	-	-	-	-	-	-
11	30	50	565	0.56	20	7	113	0.57	-	-	-	-	-	-	-	-
12	22	41	492	0.55	18	4	573	0.51	-	-	-	-	-	-	-	-
13	23	28	717	0.66	16	-8	471	0.52	16	7	750	0.55	-	-	-	-
14	15	-3	687	0.5	9	-20	780	0.28	-	-	-	-	-	-	-	-
	Texture of fruiting body stored in refrigerator (4^0C) in open tray															
5	33	29	602	0.51	29	17	678	0.68	27	8	536	0.66	26	0	513	0.59
6	32	44	874	0.43	29	32	926	0.57	31	20	753	0.59	24	5	569	0.55
7	38	155	846	0.61	37	107	690	0.68	32	83	905	0.69	29	68	740	0.65
8	39	135	603	0.45	33	104	935	0.55	34	79	690	0.61	30	46	605	0.59
9	23	39	844	0.61	21	8	445	0.56	19	-6	367	0.48	16	-7	332	0.37
10	38	101	695	0.5	36	60	598	0.49	29	34	568	0.57	27	22	484	0.55
11	37	44	550	0.63	30	18	398	0.55	18	-1	185	0.45	-	-	-	-
12	25	75	361	0.62	22	56	401	0.61	19	16	414	0.58	-	-	-	-
13	26	52	941	0.58	24	15	1247	0.65	21	1	1074	0.64	18	-12	746	0.57
14	12	-10	696	0.49	12	-20	255	0.27	-	-	-	-	-	-	-	-

'-' Indicates data were not recorded due to rotten

Table - 43: Effect of fruiting body age on texture of milky white mushroom stored in cellophane wrapped tray

Fruiting body age (Days)	Texture of fruiting body stored at ambient temperature in cellophane wrapped tray															
	During harvest				3rd day after harvest				6th day after harvest				9th day after harvest			
	Height	Weight	Hardness	Resilience	Height	Weight	Hardness	Resilience	Height	Weight	Hardness	Resilience	Height	Weight	Hardness	Resilience
5	29	22	564	0.34	24	20	362	0.51	24	16	219	0.43	-	-	-	-
6	29	65	885	0.52	26	63	576	0.59	23	59	236	0.51	-	-	-	-
7	27	85	949	0.56	23	76	234	0.59	19	72	311	0.54	18	70	359	0.55
8	39	140	970	0.59	37	136	677	0.64	31	124	322	0.62	-	-	-	-
9	36	37	625	0.54	24	46	423	0.58	-	-	-	-	-	-	-	-
10	29	61	773	0.67	25	48	289	0.65	21	44	329	0.62	-	-	-	-
11	39	73	781	0.59	35	61	461	0.65	-	-	-	-	-	-	-	-
12	38	90	590	0.62	36	78	190	0.57	-	-	-	-	-	-	-	-
13	23	54	1770	0.58	20	42	704	0.58	-	-	-	-	-	-	-	-
14	15	-4	576	0.43	-	-	-	-	-	-	-	-	-	-	-	-
Texture of fruiting body stored in refrigerator (4^0C) in cellophane wrapped tray																
5	24	14	517	0.44	25	13	476	0.4	25	10	439	0.44	25	8	358	0.39
6	27	27	585	0.44	23	29	610	0.43	23	26	704	0.46	23	21	555	0.49
7	36	73	993	0.7	36	71	834	0.56	38	65	846	0.62	37	64	717	0.61
8	31	99	1276	0.55	34	98	1256	0.55	35	95	1135	0.45	34	89	1003	0.58
9	29	50	1318	0.61	36	33	668	0.52	35	26	647	0.57	35	27	715	0.61
10	28	80	877	0.64	26	70	1201	0.64	27	67	1116	0.66	26	64	979	0.67
11	36	78	752	0.6	35	71	1037	0.55	34	68	812	0.6	33	59	639	0.61
12	29	36	403	0.61	28	28	344	0.53	26	26	551	0.64	24	16	408	0.66
13	34	43	726	0.56	31	35	736	0.6	38	29	877	0.61	30	26	569	0.62
14	21	2	267	0.6	18	1	316	0.57	-	-	-	-	-	-	-	-

'-' Indicates data were not recorded due to rotten

Table - 44: Effects of fruiting body age on texture of milky white mushroom stored in polypropylene bag

Fruiting body age (Days)	Texture of fruiting body stored at ambient temperature in polypropylene bag															
	During harvest				3rd day after harvest				6th day after harvest				9th day after harvest			
	Height	Weight	Hardness	Resilience	Height	Weight	Hardness	Resilience	Height	Weight	Hardness	Resilience	Height	Weight	Hardness	Resilience
5	21	21	466	0.46	19	-1	545	0.39	19	-3	271	0.48	-	-	-	-
6	42	67	683	0.46	38	67	207	0.49	26	65	75	0.24	-	-	-	-
7	37	86	677	0.52	32	82	150	0.43	15	79	233	0.47	21	77	155	0.46
8	35	74	971	0.53	36	74	611	0.64	32	70	328	0.59	-	-	-	-
9	27	38	937	0.68	27	37	576	0.69	30	28	216	0.53	27	30	186	0.53
10	28	89	780	0.45	26	76	555	0.54	17	75	144	0.27	-	-	-	-
11	36	68	542	0.73	25	60	247	0.46	-	-	-	-	-	-	-	-
12	26	26	623	0.6	27	20	500	0.59	17	18	314	0.37	-	-	-	-
13	22	28	915	0.48	21	20	322	0.53	21	16	310	0.59	-	-	-	-
14	8	-21	1524	0.33	11	-20	569	0.47	-	-	-	-	-	-	-	-
	Texture of fruiting body stored in refrigerator (4^0C) in polypropylene bag															
5	19	3	587	0.46	19	2	604	0.4	18	0	656	0.42	19	-1	562	0.38
6	28	21	692	0.54	27	24	587	0.48	29	21	743	0.49	27	18	706	0.47
7	27	80	1613	0.61	27	78	1713	0.63	28	75	1517	0.56	27	74	1636	0.68
8	32	99	1128	0.64	34	99	1165	0.57	32	97	978	0.67	32	93	506	0.65
9	30	60	844	0.66	27	57	1146	0.57	30	50	611	0.65	29	50	696	0.67
10	21	59	1081	0.55	20	51	1006	0.51	20	51	697	0.5	18	49	443	0.56
11	24	52	567	0.58	21	45	935	0.6	21	43	784	0.61	19	35	432	0.6
12	21	25	422	0.62	27	19	350	0.48	27	17	188	0.31	-	-	-	-
13	19	0	656	0.58	18	-6	690	0.55	18	-8	577	0.54	17	-9	481	0.56
14	15	-5	393	0.47	15	-6	271	0.45	-	-	-	-	-	-	-	-

‘-‘ Indicates data were not recorded due to rotten

Relation between fruiting body age, appearance and odor of milky white mushroom

There was significant negative correlation between fruiting body age, appearance and odor of milky white mushroom. The relationship between fruiting body age and appearance may be described by the equation y = 9.873 – 0.542x (Figure-32). The R^2 (0.833**) value indicated that 83.3% appearance score may be attributed due to the harvesting age of milky white mushroom. The equation also indicated that appearance of milky white mushroom was deteriorated with the increase of fruiting body age.

The relationship between fruiting body age and odor of milky white mushroom was linear (Figure-33). The equation y = 9.720 – 0.425x gave a good fit to the data and the value of co-efficient of determination (R^2 = 0.753**) showed that the fitted regression line had a significant regression co-efficient. The graph also indicated that odor of milky white mushroom lost its acceptability with the increase of fruiting body age.

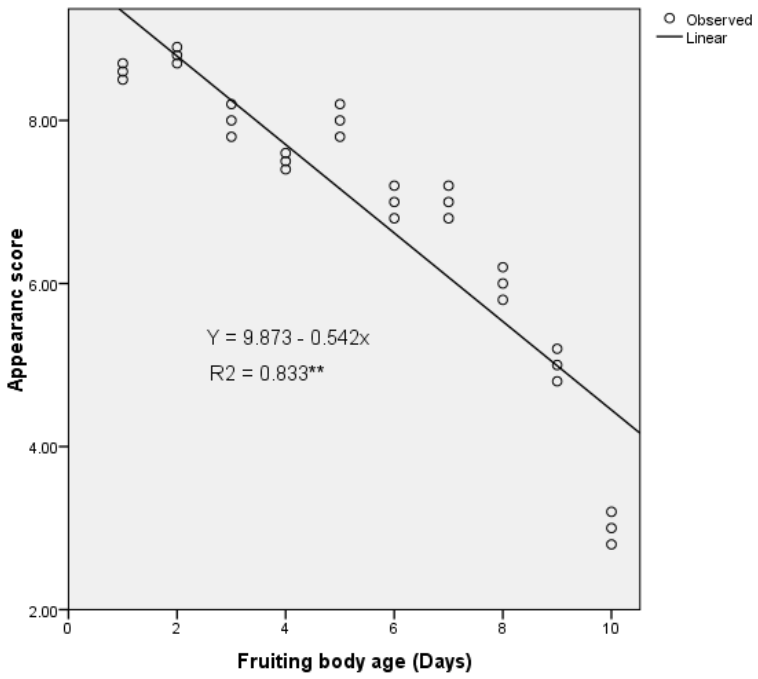

Figure-32: Relationship between fruiting body age and appearance of milky white mushroom

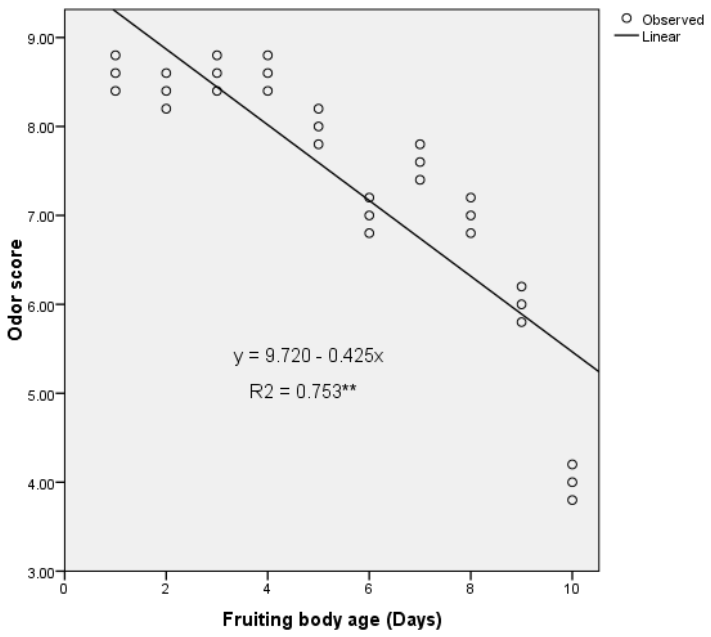

Figure-33: Relationship between fruiting body age and odor of milky white mushroom

Conclusion

From the above study it could be concluded that harvesting age of fruiting body significantly affected yield, biological efficiency, appearance, color and shelf life of milky white mushroom. Eight days aged fruiting body harvest gave highest economic yield and biological efficiency. Shelf life of seven to eight days old fruiting body was highest both in refrigerator and in ambient condition. Five to nine days aged fruiting bodies kept in cellophane paper wrapped tray and polypropylene bag remained edible for more than 15 days when it was stored in refrigerator (4^{o}C).

CHAPTER – V

SUMMERY

Eight different experiments were carried out during 2018-2020 at culture house and laboratory of Mushroom Development Institute (MDI), Department of Agricultural Extension, Savar Dhaka, Bangladesh to standardize the production technologies of milky white mushroom using locally available materials. All the experiments were carried out under ambient and semi-controlled environment and were arranged in completely randomized design with four replications.

The first experiment in the series carried out during February 2018 to September 2018 to evaluate the performance of four different strains of milky white mushroom preserved in Mushroom Development Institute, Savar, Dhaka, Bangladesh. The strains were namely Cid-1, Cid-A, Cid-In and Cid-S. Among them Cid-1 is already released as variety but performance of other strains was not evaluated. The four strains were cultivated in three different growing seasons such as summer, rainy season and autumn of the same year. The mycelium run rate of four strains were significantly varied in summer season but it was insignificant in rainy and autumn season on PDA media. Mycelium run rate of Cid-S was highest (0.37 cm/day) in summer season whereas it was highest in strain Cid-A both in rainy season (0.39 cm/day) and in autumn season (0.38 cm/day). In saw dust base mother culture mycelium run rate of four strains were significantly varied during all the three seasons. Run rate of strain Cid-1 was highest in summer but it was highest in case of strain Cid-In during rainy and autumn season. Mycelium run rate of four strain varied significantly and it was highest in case of strain Cid-1 and lowest in case of Cid-S in sub mother culture during all the three seasons.

Time required for mycelium colonization in the spawn packet and fruiting body pinhead formation was varied significantly among the strains in all the three seasons. Strain Cid-1 takes lowest time in summer and autumn season but Cid-A takes lowest time in rainy season to complete spawn run. Strain Cid-S required highest time to complete mycelium colonization in spawn packet during all the three seasons. Among the four strains Cid-A

takes shortest time and strain Cid-S takes longest time to produces fruiting body pinhead during all the three seasons. There was also a significant variation in time to first harvest and total harvest among the milky white mushroom strains. Time required for first harvest was lowest in case of strain Cid-A and highest in case of strain Cid-S during all the three seasons. For total harvest strain Cid-A takes shortest time during summer and rainy season and Cidi-1 takes shortest time during autumn season. Variation in number of effective fruiting body among the strains was insignificant during summer and significant during rainy and autumn season. Strain Cid-1produces highest number of effective fruiting body during all the three growing season and strain Cid-S produces lowest number of fruiting body during summer and rainy season. Average weight of fruiting body was highest in strain Cid-1 during summer season, Cid-A during rainy season and Cid-In during autumn season.

There was a significant variation in economic yield and biological efficiency among the four strains of milky white mushroom studied in this experiment. Highest economic yield and biological efficiency was recorded from strain Cid-1 during summer and rainy season and from Cid-A during autumn season. Strain Cid-S gave lowest economic yield and biological efficiency during all the growing season. Nutrient contents of four strains were analyzed. Every 100 g of dry mushroom contains 61.20 - 63.10 g carbohydrate, 11.60 - 14.60 g protein, 1.89 - 3.10 g lipid, 12.03 - 12.80 g fiber and 9.23 - 10.40 g ash. Moisture content of four strains was 89.87 to 92.14 %. For the confirmation of genetic variability among the strains DNA finger printing was done. From the DNA finger print it was concluded that the strains were genetically different from each other.

The second experiment was conducted during May 2019 to July 2019 to screening out the potential cheap agricultural and household by-products available in Bangladesh as substrate for milky white mushroom cultivation. Nine different substrates and their combinations such as rice straw, wheat straw, barley straw, waste paper + wheat bran (2:1), sugarcane baggage + wheat bran (2:1), Maize cob + wheat ban (2:1), sawdust + wheat bran (2:1), rice straw + sawdust (1:1) and maize straw were evaluated. Time required to complete mycelium running in the spawn packet varied significantly in different substrates. Minimum time required to complete spawn running was 14.0 days in

wheat and barley straw which differed significantly from all other substrates. Sawdust in combination with wheat bran @ 2:1 ratio required maximum time (30.0 days) to complete spawn run. Time to fruiting body pinhead formation and total harvest varied significantly among the substrates. Fruiting body pinhead formation was earlier (9.30 days) in waste paper in combination with wheat bran @ 2:1 ratio and rice straw in combination with sawdust @ 1:1 ration required maximum time (16.0 days) to pinhead formation. Crop duration was highest (76.2 days) in rice straw in combination with sawdust @ 1:1 ratio and lowest (46.0 days) in sugar cane baggage in combination with wheat bran @ 2:1.

The number of effective fruiting body grown on different substrates differed significantly and the highest number of fruiting body per packet (7.5) was recorded on rice straw in combination with sawdust @ 1:1 ratio which was significantly higher compare to all other substrate. Lowest number of fruiting body (5.2) was recorded in maize straw. Significant variation in economic yield and biological efficiency of milky white mushroom were observed in different substrates. Highest yield and biological efficiency was recorded from rice straw mixed with sawdust (1:1) substrate (427.3g & 103.5%) and lowest from sawdust mixed with wheat bran (2:1) substrate (264.1g & 62.9%). Nutrient contents of milky white mushroom grown on different substrates were analyzed. Every 100 g of dry mushroom contains 56.70-65.30 g carbohydrate, 9.90-15.70 g protein, 2.10-3.78 g lipid, 11.90-14.00 g fiber and 9.88-13.86 g ash. Mushroom grown on waste paper in combination with wheat bran @ 2:1 ratio contained highest amount of carbohydrate (65.30 g), rice straw in combination with wheat bran @ 1:1 ration contained highest amount of protein (15.70) and lipid (3.78 g), maize straw contained fiber (14.0 g), and sawdust in combination with wheat bran @ 2:1 contained highest amount of ash. Moisture content of fresh mushroom grown on different substrates was 88.10 to 91.24 %.

The third experiment was carried out during May 2019 to July 2019 to find out a suitable casing material for successful cultivation of milky white mushroom. Eleven different combination of casing materials were used in this experiment like- coconut coir dust (CC), CC + decomposed cow dung (CD) (1:1), CC + loamy soil (LS) (1:1), ash (AS), AS + LS (1:1), LS + sand (S) (3:1), decomposed spent mushroom substrate (SMS), SMS +

LS (1:1), SMS + CD (1:1), SMS + AS (1:1) and LS (control). Fruiting body pinhead formation, number of flush and number of effective fruiting body were significantly influenced by different casing materials used. Shortest time to pinhead formation was 12 days in case of spawn packets covered with LS + sand (S) (3:1) and spent mushroom substrate mixed with ash (1:1) takes longest time (24.0 days) to fruiting body pinhead formation. Both number of flush (3.1) and number of effective fruiting body (7.6) was highest when spawn packets were covered with coconut coir dust mixed with decomposed cow dung @ 1:1 ratio. Length, diameter and thickness of stalk and pileus of fruiting body were significantly influenced by the casing materials but the influence was greater on stalk length. Highest stalk length (11.0 cm) was observed when spawn packets were covered with decomposed spent mushroom substrate mixed with decomposed cow dung @ 1:1 ratio whereas it was lowest (7.1 cm) when spawn packets were covered with loamy soil mixed with sand @ 3:1 ratio. Economic yield and biological efficiency of milky white mushroom was significantly influenced in every flush by the casing materials used. Total economic yield and biological efficiency were highest (374.1 g & 90.6%) when the spawn packets were covered with coconut coir dust mixed with decomposed cow dung @ 1:1 ratio and the economic yield and biological efficiency were lowest (126.4 g & 30.6%) when spawn packets were covered with only coconut coir dust. 73.88% economic yield was recorded from the first lush and only 0.61% economic yield was recorded from the 4^{th} flush.

The fourth experiment was conducted during May 2018 to July 2018 to find out the appropriate substrate sterilization technique and spawning method for better mycelium running in the substrate and achieve a good yield. Seven different treatments of substrate sterilization technique in combination with spawning methods like- steam treatment of substrate and spawning in 3 layers, steam treatment of substrate and spawning thoroughly, autoclaving of substrate and top spawning, autoclaving of substrate and spawning in 3 layers, autoclaving of substrate and spawning thoroughly, hot water treatment of substrate and spawning in 3 layers and hot water treatment of substrate and spawning thoroughly were practiced in this experiment. From the study it was observed that mycelium colonization was significantly affected by substrate sterilization and spawning method of rice straw substrate. Mycelium colonization was faster (16.50 days)

when hot water treated substrate spawning thoroughly and slowest (24.25 days) when steam treated substrate spawning thoroughly and steam treated substrate spawning in 3 layers. Mycelium colonization was not completed due to 90% of the spawn packets were contaminated when autoclaved substrate spawning through the neck. Substrate sterilization technique and spawning method had a significant influence on fruiting body primordial initiation and time to complete total harvest of milky white mushroom. Steam treated substrate spawning thoroughly takes minimum time (8.63 days) and steam treated substrate spawning thoroughly takes maximum (14.28 days) time to initiate fruiting body primordia. Highest time to complete total harvest after casing was 51.0 days when hot water treated substrate inoculated in 3 layers and lowest time was 33.0 days when autoclaved substrate spawning thoroughly. Maximum spawn packets (90%) were contaminated when autoclaved substrate inoculated through the neck and no spawn packets were contaminated when hot water treated substrate inoculated in 3 layers and thoroughly.

Number of effective fruiting body was significantly influenced by different substrate sterilization techniques and spawning methods. Highest number of effective fruiting bodies (6.83) were recorded from the spawn packets prepared from hot water treated substrate inoculated in three layers. Substrate sterilization technique and spawning method significantly influenced the economic yield and biological efficiency of milky white mushroom. Hot water treated substrate inoculated in three layers gave highest economic (296.31 g/packet) yield and biological (73.16%).

The fifth experiment was carried out during May 2019 to July 2019 to determine the substrate moisture level suitable for production of milky white mushrooms. Eight different moisture levels of rice straw substrate were tested in this experiment- such as 35, 40, 45, 50, 55, 60, 65, and 70 percent moisture of the substrate. Mycelium runing in the spawn packet and fruiting body primordial initiation was significantly influenced by substrate moisture content. Mycelium colonization was faster (14.5 days) in the substrate containing 70% moisture and no mycelium colonization was observed in the substrate containing 35% moisture. Lowest time (12.2 days) was required for fruiting body

primordial initiation in substrate containing 65% moisture and highest (19.0 days) in substrate containing 45% moisture level.

Days to first harvest and last harvest was significantly influenced by the substrate moisture level. Time required for both first harvest and last harvest was highest (62.2 & 77.0 days) in substrate containing 45% moisture level and lowest in substrate containing 65% moisture level. Moisture level of the substrate was also affected the contamination rate of substrate. Highest contamination (100%) and no mycelium colonization was observed at 35% moisture level of the substrate. No substrate contamination was observed at 65% and 70% moisture level. There was significant negative correlation between substrate moisture level and rate of substrate contamination. Rate of contamination was decreased with the increase in substrate moisture level.

Influence of substrate moisture level was insignificant on number of effective fruiting body and it was highest (6.4) at 70% moisture level. There was a significant positive correlation between substrate moisture content, economic yield per packet and biological efficiency. Yield and biological efficiency were significantly affected by substrate moisture level. Highest yield (361.1g) and biological efficiency (87.4%) was recorded in substrate containing 70% moisture which was statistically similar to substrate containing 60% and 65% moisture.

The sixth experiment was conducted during May 2020 to July 2020 to identify the appropriate management technique of casing material for successful cultivation of milky white mushroom. Five different casing material management techniques such as removal of dried non effective fruiting bodies after each harvest, removal of dried non effective fruiting bodies and filling the casing hole with fresh casing material after each harvest, scraping the upper surface of the substrate after each harvest, scraping the upper surface of the substrate and adding 10% fresh casing material after each harvest, and no disturbance of the casing material (control). Number of effective fruiting body (NEFB), number of flushes and days to total harvest of milky white mushroom was significantly affected by casing material management technique. Both number of effective fruiting body (8.83) and number of flushes (2.81) were highest when non effective dried fruiting bodies were removed after each harvest and were lowest (4.65 & 1.63) when upper

surface of the substrate was scrapped with hand and 10% fresh casing material was added after each harvest. Highest time (65.08 days) was required for total harvest (spawning to last harvest) when upper surface of the substrate was scrapped after each harvest.

Average weight of fruiting body was significantly affected by casing material management technique but variation in economic yield and biological efficiency among the treatments were insignificant. Highest yield (400.75 g) and biological efficiency (96.85%) were recorded when dried non effective fruiting bodies were removed and casing holes were filled with fresh casing material after each harvest and lowest (321.90 g & 77.58%) when casing material was allowed to remain undisturbed. Variation in average weight of fruiting body among the treatments were insignificant during all the flushes but it was significantly varied among the flushes and gradually decreased from 1st flush to the subsequent flushes.

The seventh experiment was carried out during July 2020 to September 2020 to identify appropriate spawn density to overcome the partial colonization of mycelium in spawn packets and to get maximum benefit from milky white mushroom cultivation. Days to complete spawn run, fruiting body pinhead formation, number of effective fruiting body and number of flushes were significantly influenced by spawn density but days to total harvest was not affected. Partial colonization of mycelium in spawn packets was completely disappeared with the increase of spawn density. Shortest time was required to complete spawn run (12.90 days) and pinhead formation (9.58 days) in spawn packets inoculated with 50% rice grain mother culture. Highest number of effective fruiting body (9.10) and number of flush (3.10) were recorded from the spawn packet inoculated with 50% mother culture. There was significant negative correlation between spawn density and time to spawn run, and spawn density and fruiting body pinhead formation. Significant positive correlation was observed between spawn density and number of effective fruiting body and spawn density and number of flushes.

Effect of spawn density on weight of fruiting body was insignificant but it was significant on economic yield and biological efficiency. Economic yield (454.88 g/packet) and biological efficiency (109.61%) was highest when the spawn packets were inoculated

with 50% rice grain mother culture. The relationship between spawn density and economic yield and biological efficiency was significant and quadratic.

The eighth experiment was conducted during June 2019 to September 2019 to find out appropriate harvesting age of fruiting body for getting maximum yield and longer shelf life of milky white mushroom. Mushrooms were harvested at ten different ages viz; 5 to 14 days old sporophore and stored in refrigerator and ambient condition in open tray, cellophane wrapped tray and poly bag. Number of effective fruiting body, number of flush and days to total harvest was significantly influenced by harvesting age of milky white mushroom. Highest number of effective fruiting body (9.15) was recoded when it was harvested at five days aged but number of flushes was highest (3.98) when harvested at 6 days old. Six days old fruiting body harvest was also required highest time (94.33 days) to complete total harvest. There was significant negative correlation between harvesting age, number of fruiting body and number of flushes. Length, diameter and thickness of stalk and pileus were significantly influenced by the harvesting age of fruiting body. Highest stalk length (9.18 cm) was at 7 days and diameter of stalk (2.65 cm) and pileus (6.95 cm) was at 8 days aged harvest. Thickness of pileus was highest (2.85 days) at 9 days aged. The relationship between fruiting body age and length, diameter and thickness of stalk and pileus was quadratic.

Weight of fruiting body, economic yield and biological efficiency of milky white mushroom was significantly affected by fruiting body age. Fruiting body weight was highest (63.35 g) when it was harvested at eight days old but weight was lowest (27.63 g) when harvested at five days old. Highest economic yield (483.13 g) and biological efficiency (116.43%) was recorded from eight days old fruiting body harvest. Relationship between harvesting age, fruiting body weight, economic yield and biological efficiency were quadratic.

To determine the shelf life of milky white mushroom, appearance, odor, color and texture were taken in consideration. Appearance and odor were evaluated through sensory evaluation using 9-pint hedonic rating scale and color and texture were measured by Chroma Meter (Model FLDO 712, China) and texture profile analyzer (Texvol, TVT-300XP, Sweden) respectively. There was no significant difference in initial appearance

score up to nine days old fruiting bodies but it was decreased gradually and after 13 days it became less than 5.0 therefore lost acceptability for consumption. In ambient condition the mushroom lost its acceptability rapidly when it is stored in an open tray than cellophane paper wrapped tray and polypropylene bag. Appearance score of mushrooms stored in open tray was ≥ 5.0 after 3 days of harvest when it was harvested at 6 to 10 days but the score was below 5.0 when mushroom was harvested at 5 and 11 to 14 days old. Before 6 days of storage at ambient condition milky mushroom lost its acceptability irrespective of harvesting age and storage method.

Appearance score of milky white mushroom was at acceptable level for long time in refrigerator than ambient. Appearance score was at least 5.0 up to 6 days stored in open tray in case of mushroom harvested at 7 and 8 days but it was below 5.0 in case of other harvesting age. Mushroom harvested at 5 to 9 days old had acceptable appearance score (>5.0) more than 15 days of storage in cellophane paper wrapped tray and polypropylene bag.

In ambient condition, odor score was above 5.0 up to 3 days after harvest irrespective of storage method and harvested within 12 days of pinhead formation. In case of refrigerator storage, odor score of mushrooms kept in open tray was above 5.0 up to 6 days after harvest when mushrooms were harvested at 6 to 8 days aged. Mushroom harvested at 6 to 9 days after pinhead formation and stored in cellophane paper wrapped tray & polypropylene bag was remained in good condition for consumption more than 15 days of storage (scored >5.0).

Whiteness (‘L’ value) of milky white mushroom was more or less similar up to 13 days aged. After 13 days whiteness was reduced and browning (‘a’ and ‘b’ value) was increased. After harvest whiteness (‘L’ value) was also gradually reduced and browning (‘a’ and ‘b’ value) was increased with time irrespective of harvesting age and storage method. Hardness of milky white mushroom was more or less similar from 6 to 13 days age but before 6 days and after 13 days hardness was reduced. After harvest mushroom lost its hardness gradually with increasing the storage time irrespective of harvesting age and storage method.

CHAPTER –VI

CONCLUSION AND RECOMMENDATION

Conclusion

From the above study it was concluded that, considering the yield performance of milky white mushroom, strain Cid-A could be successfully cultivated from March to October in Bangladesh beside the recommended variety Cid-1. There was genetically variation among the strains. Rice straw mixed with sawdust (1:1) was a good substrate for milky white mushroom cultivation. Maize straw could be a good alternate of rice straw substrate for commercial cultivation of this mushroom. Coconut coir dust in combination with decomposed cow dung (1:1) was the best casing material. Decomposed spent mushroom substrate mixed with decomposed cow dung (1:1), loamy soil, decomposed spent mushroom substrate alone and ash mixed with loamy soil (1:1) were also good as casing material. Use of spent mushroom substrate would be great relief from the environmental pollution. Substrate treated with hot water and inoculated both in three layers and thorough mixing were appropriate method of substrate sterilization and spawning of milky white mushroom as it provides no contamination and highest biological efficiency. Sixty-five to seventy percent moisture of rice straw substrate could be used for milky white mushroom cultivation as it provides faster mycelium colonization, no substrate contamination and highest yield. Casing material management techniques had no significant influences on yield but had positive impact on other qualitative parameters of milky white mushroom. Therefore, removal of dried non effective fruiting bodies after each harvest may be practiced for its commercial cultivation.

Complete mycelium colonization in the substrate is very important factor for a good harvest. Partial colonization of mycelium in the spawn packets might be solved by using increased spawn density. To get maximum yield and biological efficiency with higher benefit cost ratio, 40% spawn density was proved suitable for cultivation of this

mushroom. Age of fruiting body significantly affected the yield, biological efficiency and shelf life of milky white mushroom. For maximum yield, eight days aged fruiting body might be harvested but five to six days fruiting body might be a good alternate of button mushroom. For longer Shelf-life mushroom might be stored in cellophane paper wrapped tray or polypropylene bag in refrigerator (4^{o}C).

Recommendations

The following recommendations may be taken in consideration on the basis of the results of the study.

1. Strain Cid-A may be recommended for commercial cultivation in Bangladesh beside the recommended strain Cid-1.
2. Rice straw mixed with sawdust (1:1), rice straw alone, and maize straw treated with hot water may be recommended for commercial cultivation of milky white mushroom.
3. Coconut coir dust in combination with decomposed cow dung (1:1) may be recommended as casing material.
4. Sixty-five to seventy percent moisture level of rice straw substrate may be recommended for milky white mushroom cultivation.
5. Removal of dried non effective fruiting bodies after each harvest may be practiced for successful cultivation of this mushroom.
6. To get maximum yield and biological efficiency with higher benefit cost ratio forty percent spawn density may be recommended.
7. Seven to eight days aged fruiting body may be recommended for harvest to get maximum yield, biological efficiency and shelf life.
8. For longer shelf life, mushroom may be stored in cellophane paper wrapped tray or polypropylene bag in refrigerator.

CHAPTER- VII

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CHAPTER- VIII

APPENDICES

Appendix-I: Environmental data during growing period of Milky white mushroom

Month	Decals	Temperature (°C)		Relative humidity (%)	
		Maximum	Minimum	Maximum	Minimum
February ’18	1*	26.9	17.1	90.2	64.1
	2	29.2	15.2	87.3	45.0
	3	31.8	18.9	90.1	47.6
March ’18	1	32.6	19.6	89.3	49.2
	2	33.2	21.2	91.5	50.8
	3	33.3	21.7	90.5	53.2
April ’18	1	32.3	23.1	83.0	56.8
	2	33.5	24.1	73.6	57.3
	3	31.9	22.0	86.9	66.1
May ’18	1	31.7	22.7	91.3	74.9
	2	30.9	22.2	94.4	72.9
	3	32.4	24.7	92.6	74.3
June ’18	1	33.7	25.6	94.6	71.9
	2	31.5	25.9	92.2	81.8
	3	32.5	26.3	93.9	74.3
July ’18	1	32.5	26.2	93.8	76.7
	2	34.6	27.1	88.4	69.4
	3	31.3	26.1	95.0	81.0
August ’18	1	32.5	26.3	92.3	74.6
	2	34.4	27.7	87.6	69.6
	3	32.6	26.6	91.7	74.5

1* stands for day 01 to 10, 2 for day 11 to 20 and 3 for day 21 to rest of the month

Contd. Appendix-I

Month	Decals	Temperature (^{0}C)		Relative humidity (%)	
		Maximum	Minimum	Maximum	Minimum
September ’18	1*	33.2	26.6	90.5	72.1
	2	33.5	26.4	93.5	70.1
	3	34.3	26.1	91.8	68.1
October ’18	1	31.7	25.1	90.9	62.1
	2	30.0	22.3	94.3	69.5
	3	30.7	21.2	91.5	66.2
February ’19	1	28.4	14.0	82.3	43.8
	2	28.2	14.5	87.6	51.4
	3	27.6	18.5	88.8	62.3
March ’19	1	28.0	17.1	85.2	53.0
	2	32.6	22.1	88.4	57.4
	3	33.3	20.5	88.8	51.1
April ’19	1	30.5	21.2	90.2	65.2
	2	34.4	24.6	84.8	57.5
	3	34.8	25.9	83.4	52.4
May ’19	1	34.6	26.6	89.1	66.8
	2	35.3	24.8	87.0	67.2
	3	34.5	25.4	90.5	69.4
June ’19	1	31.9	25.2	92.3	77.5
	2	34.4	27.4	92.0	71.4
	3	34.9	27.7	91.6	72.3
July ’19	1	32.2	27.2	85.8	77.0
	2	32.2	25.8	94.5	80.6
	3	33.5	26.7	91.3	75.1

1* stands for day 01 to 10, 2 for day 11 to 20 and 3 for day 21 to rest of the month

Contd. Appendix-I

Month	Decals	Temperature (^{0}C)		Relative humidity (%)	
		Maximum	Minimum	Maximum	Minimum
August ’19	1*	34.0	27.0	86.7	71.2
	2	33.0	26.6	94.8	77.0
	3	34.5	27.5	90.8	70.4
September ’19	1	34.2	27.3	89.5	70.8
	2	34.0	26.5	93.2	71.6
	3	32.4	25.1	95.1	75.9
October ’19	1	31.8	25.2	95.0	77.2
	2	32.5	24.2	95.8	69.1
	3	30.2	24.4	93.3	74.8
February ’20	1	25.4	12.3	91.6	50.0
	2	28.1	14.1	94.2	55.9
	3	29.2	15.2	90.2	51.3
March ’20	1	30.0	18.6	86.2	51.6
	2	32.9	19.9	80.4	53.5
	3	34.3	24.7	66.1	37.5
April ’20	1	34.1	29.4	66.6	55.1
	2	32.7	26.6	77.8	62.0
	3	31.3	25.2	83.4	62.8
May ’20	1	32.2	26.6	74.8	60.9
	2	33.4	29.4	72.5	62.7
	3	31.7	25.9	88.9	71.1
June ’20	1	33.5	25.4	93.9	74.7
	2	32.2	26.4	95.3	79.1
	3	33.8	27.3	89.8	74.1

1* stands for day 01 to 10, 2 for day 11 to 20 and 3 for day 21 to rest of the month

Contd. Appendix-I

Month	Decals	Temperature (^{0}C)		Relative humidity (%)	
		Maximum	Minimum	Maximum	Minimum
July ’20	1*	33.8	28.1	86.0	75.2
	2	32.6	26.7	93.1	80.2
	3	33.1	26.2	94.6	78.5
August ’20	1	35.0	27.6	91.1	70.4
	2	32.8	26.3	92.1	74.0
	3	33.8	26.8	89.2	72.6
September ’20	1	34.7	27.3	90.2	69.9
	2	34.1	26.4	94.2	74.6
	3	31.3	25.9	96.8	83.6
October ’20	1	33.7	26.8	92.4	75.0
	2	34.2	26.9	90.4	70.5
	3	30.0	24.9	93.1	79.1

1* stands for day 01 to 10, 2 for day 11 to 20 and 3 for day 21 to rest of the month

Appendix II- Mean squire for days to spawn run, days to pinhead formation, number of flush, number of effective fruiting body, days to total harvest and weight of fruiting body (Ref. 2nd Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Days to spawn run	Days to pinhead formation	Number of flush	Number of effective fruiting body	Days to total harvest	Weight of fruiting body
Between	8	143.778**	20.364**	0.543^{NS}	1.933**	451.331**	208.141**
Within	27	0.889	0.894	0.252	0.557	48.954	50.134
Total	35						

** = Significant at 1% level, NS = Not significant

Appendix III - Mean squire for length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, economic yield and biological efficiency (Ref. 2nd Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Length of stalk	Diameter of stalk	Diameter of pileus	Thickness of pileus	Economic yield	Biological efficiency
Between	8	2.316**	0.109**	0.382^{NS}	0.077**	9187.426**	554.729**
Within	27	0.197	0.010	0.205	0.011	1471.393	89.299
Total	35						

** = Significant at 1% level, NS = Not significant

Appendix IV - Mean squire for days to spawn run, days to pinhead formation, number of flush, number of effective fruiting body, days to total harvest and weight of fruiting body (Ref. 3rd Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Days to spawn run	Days to pinhead formation	Number of flush	Number of effective fruiting body	Days to total harvest	Weight of fruiting body
Between	10	2.469^{NS}	41.133**	0.410^{NS}	0.921*	382.158**	312.981**
Within	33	2.327	0.919	0.208	0.428	129.091	18.377
Total	43						

** = Significant at 1% level, * = Significant at 5% level, NS = Not significant

Appendix V - Mean squire for length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, economic yield and biological efficiency (Ref. 3rd Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Length of stalk	Diameter of stalk	Diameter of pileus	Thickness of pileus	Economic yield	Biological efficiency
Between	10	7.403**	0.364**	1.370**	0.165**	15363.145**	899.924**
Within	33	0.280	0.044	0.226	0.042	1236.855	72.558
Total	43						

** = Significant at 1% level

Appendix VI - Mean squire for days to spawn run, days to pinhead formation, number of flush, number of effective fruiting body, days to total harvest and weight of fruiting body (Ref. 4th Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Days to spawn run	Days to pinhead formation	Rate of substrate contamination	Number of effective fruiting body	Days to total harvest	Weight of fruiting body
Between	5	40.600**	22.821**	3855.655**	6.543**	183.067**	
Within	18	1.167	1.598	281.845	0.536	3.250	
Total	23						

** = Significant at 1% level

Appendix VII - Mean squire for length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, economic yield and biological efficiency (Ref. 4th Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Length of stalk	Diameter of stalk	Diameter of pileus	Thickness of pileus	Economic yield	Biological efficiency
Between	5	2.502**	0.092^{NS}	0.964*	0.145*	10883.861**	663.470**
Within	18	0.404	0.060	0.284	0.051	581.322	35.442
Total	23						

** = Significant at 1% level, * = Significant at 5% level, NS = Not significant

Appendix VIII- Mean squire for days to spawn run, days to pinhead formation, days to first harvest, days to last harvest and rate of substrate contamination (Ref. 5th Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires				
		Days to spawn run	Days to pinhead formation	Days to first harvest	Days to last harvest	Rate of substrate contamination
Between	7	241.138**	203.938**	2205.088**	2932.294**	6872.210**
Within	24	0.656	1.405	11.308	17.980	97.656
Total	31					

** = Significant at 1% level

Appendix IX- Mean squire for number of effective fruiting body, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, economic yield and biological efficiency (Ref. 5th Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires						
		Number of effective fruiting body	Length of stalk	Diameter of stalk	Diameter of pileus	Thickness of pileus	Economic yield	Biological efficiency
Between	7	29.908**	61.813**	4.972**	37.037**	4.925**	80588.962**	4723.564**
Within	24	0.196	0.189	0.005	0.151	0.011	844.994	49.508
Total	31							

** = Significant at 1% level

Appendix X - Mean squire for days to spawn run, days to pinhead formation, number of flushes, number of effective fruiting body, days to total harvest and weight of fruiting body (Ref. 6th Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Days to spawn run	Days to pinhead formation	Number of flushes	Number of effective fruiting body	Days to total harvest	Weight of fruiting body
Between	4	44.589	7.204**	0.875**	11.852**	103.623**	1276.609*
Within	15	0.047	0.077	0.100	1.743	8.217	299.481
Total	19						

** = Significant at 1% level

Appendix XI - Mean squire for length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, economic yield and biological efficiency (Ref. 6th Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Length of stalk	Diameter of stalk	Diameter of pileus	Thickness of pileus	Economic yield	Biological efficiency
Between	4	3.533**	0.052^{NS}	3.603**	0.316**	3989.192^{NS}	256.517^{NS}
Within	15	0.479	0.049	0.550	0.038	2486.700	124.227
Total	19						

** = Significant at 1% level, NS = Not significant

Appendix XII: Benefit cost ratio of milky white mushroom as affected by spawn density (Ref. 7th Expt., Chapter IV)

Treatments (spawn density)	Amount of substrate per packet (g)	Unit price of substrate (Tk./Kg)	Price of substrate (Tk./packet)	Amount of spawn (rice grain) per packet (g)	Unit price of rice grain (Tk./Kg)	Price of spawn (Tk./packet)	Approximate common cost (Electricity, Labor, water etc.)	Total cost of production (Tk./packet)	Economic yield of milky mushroom per packet (g/packet)	Unit price of milky mushroom (Tk./packet)	Total price of milky mushroom (Tk./packet)	Benefit Cost Ratio (Price of mushroom per packet/Total production cost per packet)
10%	500	25.0	12.50	50.0	30.0	1.50	10.0	24.0	273.08	250.0	68.27	2.84
20%	500	25.0	12.50	100.0	30.0	3.00	10.0	25.50	332.28	250.0	83.07	3.26
30%	500	25.0	12.50	150.0	30.0	4.50	10.0	27.00	397.28	250.0	99.32	3.68
40%	500	25.0	12.50	200.0	30.0	6.00	10.0	28.50	436.40	250.0	109.10	3.83
50%	500	25.0	12.50	250.0	30.0	7.50	10.0	30.00	454.88	250.0	113.72	3.79

Appendix XIII - Mean squire for days to spawn run, days to pinhead formation, number of flushes, number of effective fruiting body, days to total harvest and weight of fruiting body (Ref. 7th Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Days to spawn run	Days to pinhead formation	Number of flushes	Number of effective fruiting body	Days to total harvest	Weight of fruiting body
Between	4	73.619**	16.980**	1.503**	5.524**	19.595^{NS}	27.459^{NS}
Within	15	0.087	0.155	0.025	0.648	7.572	35.185
Total	19						

** = Significant at 1% level, NS = Not significant

Appendix XIV - Mean squire for length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, economic yield and biological efficiency (Ref. 7th Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Length of stalk	Diameter of stalk	Diameter of pileus	Thickness of pileus	Economic yield	Biological efficiency
Between	4	0.872**	0.143**	0.930^{NS}	0.010^{NS}	22823.485**	1325.069**
Within	15	0.085	0.010	0.417	0.023	254.993	14.808
Total	19						

** = Significant at 1% level, NS = Not significant

Appendix XV - Mean squire for days to spawn run, days to pinhead formation, number of flushes, number of effective fruiting body, days to total harvest and weight of fruiting body (Ref. 8th Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Days to spawn run	Days to pinhead formation	Number of flushes	Number of effective fruiting body	Days to total harvest	Weight of fruiting body
Between	9	17.266**	0.828**	3.324**	2.764**	1031.502**	344.049**
Within	30	0.167	0.142	0.264	0.845	59.164	45.946
Total	39						

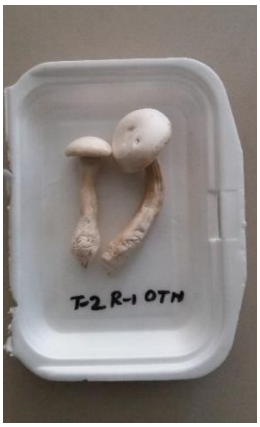
** = Significant at 1% level

Appendix XVI - Mean squire for length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, economic yield and biological efficiency (Ref. 8th Expt., Chapter IV)

Source of variation	Degrees of freedom	Mean squires					
		Length of stalk	Diameter of stalk	Diameter of pileus	Thickness of pileus	Economic yield	Biological efficiency
Between	9	0.727**	0.033**	2.391**	0.419**	12962.761**	753.346**
Within	30	0.087	0.008	0.050	0.038	1807.321	105.024
Total	39						

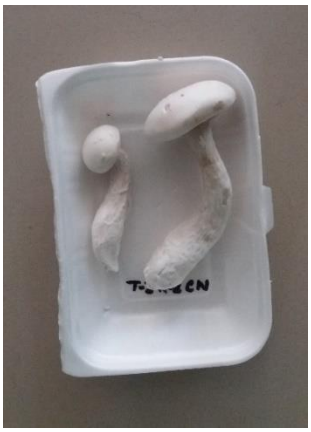
** = Significant at 1% level

Appendix XVII- Photo of milky white mushroom fruiting body harvested at different date and stored in different methods (Ref. 8th Expt., Chapter IV)



During harvest Three days after harvest Six days after harvest

Fruiting body harvested at five days old and stored in open tray at ambient temperature

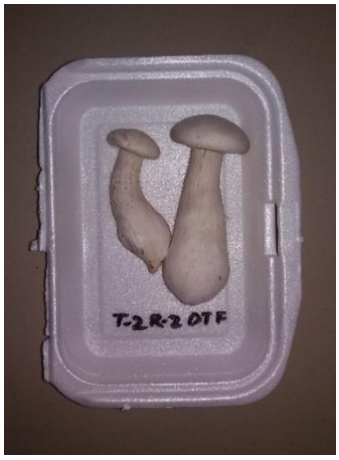


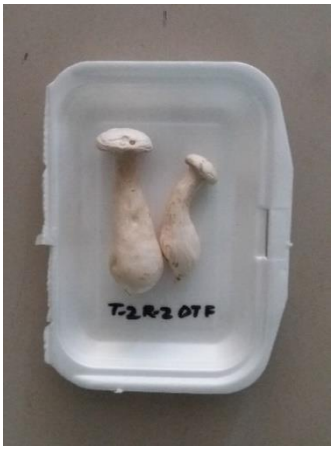
During harvest Three days after harvest Six days after harvest

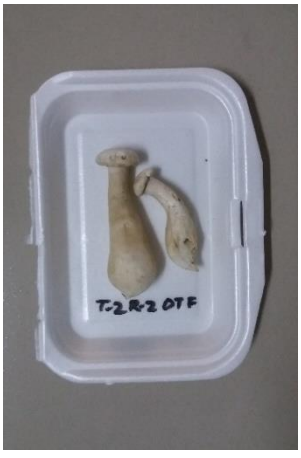
Fruiting body harvested at five days old and stored in cellophane wrapped tray at ambient temperature

During harvest Three days after harvest Six days after harvest

Fruiting body harvested at five days old and stored in polypropylene bag at ambient temperature

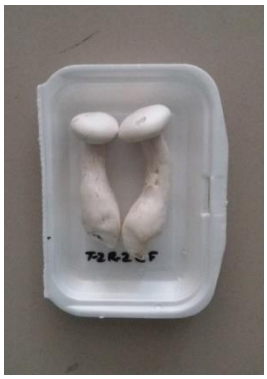






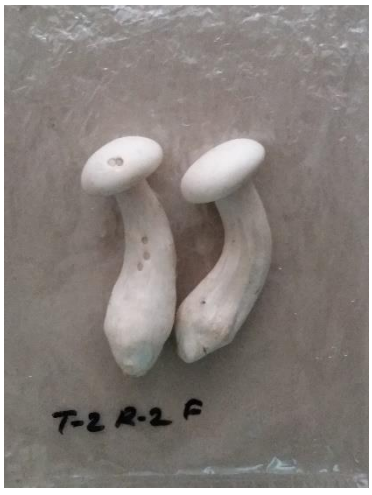
During harvest Six days after harvest Nine days after harvest

Fruiting body harvested at five days old and stored in open tray kept in refrigerator


During harvest Six days after harvest Fifteen days after harvest

Fruiting body harvested at five days old and stored in cellophane wrapped tray kept in refrigerator



During harvest Six days after harvest Fifteen days after harvest

Fruiting body harvested at five days old and stored in polypropylene bag kept in refrigerator

During harvest Five days after harvest

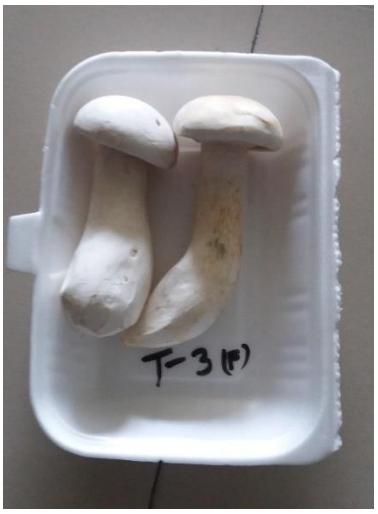
Fruiting body harvested at six days old and stored in open tray at ambient temperature

During harvest Five days after harvest

Fruiting body harvested at six days old and stored in cellophane wrapped tray at ambient temperature

During harvest Five days after harvest

Fruiting body harvested at six days old and stored in polypropylene bag at ambient temperature



During harvest Five days after harvest Twelve days after harvest

Fruiting body harvested at six days old and stored in open tray kept in refrigerator



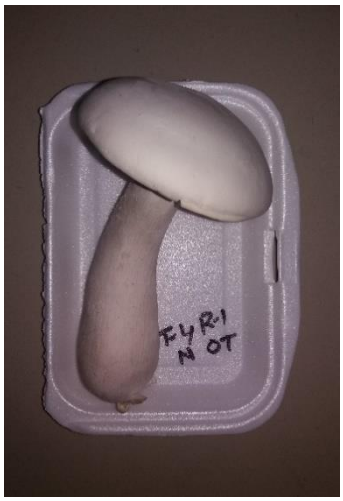
During harvest Twelve days after harvest Fifteen days after harvest

Fruiting body harvested at six days old and stored in cellophane wrapped tray kept in refrigerator



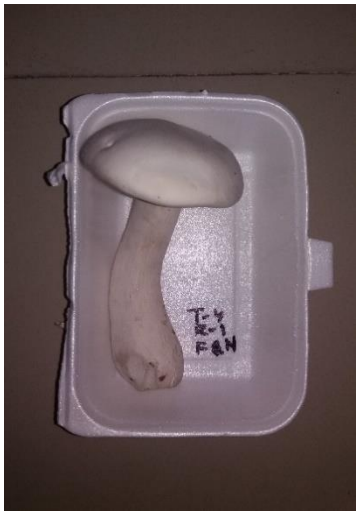
During harvest Twelve days after harvest Fifteen days after harvest

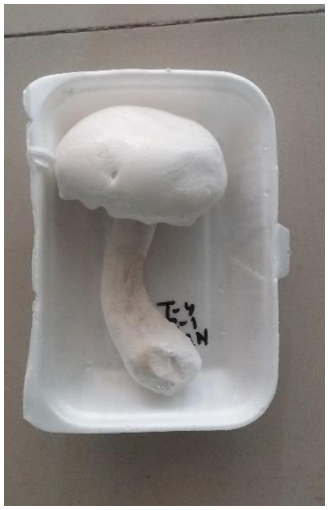
Fruiting body harvested at six days old and stored in polypropylene bag kept in refrigerator



During harvest Three days after harvest Six days after harvest

Fruiting body harvested at seven days old and stored in open tray at ambient temperature



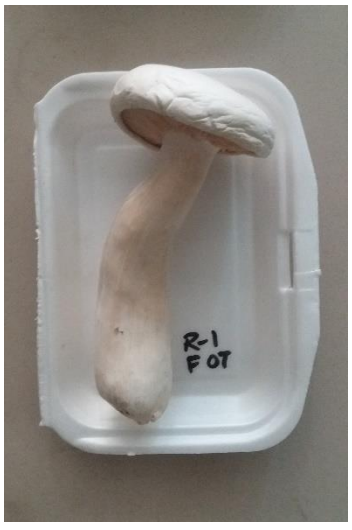


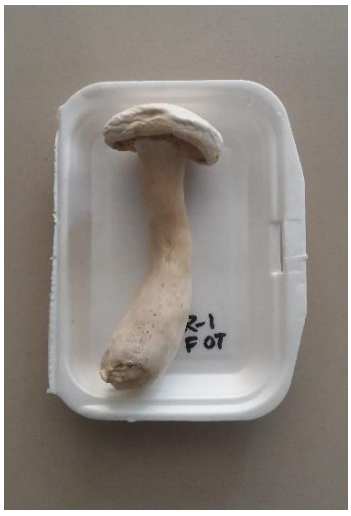
During harvest Three days after harvest Six days after harvest

Fruiting body harvested at seven days old and stored in cellophane wrapped tray at ambient temperature

During harvest Three days after harvest Six days after harvest

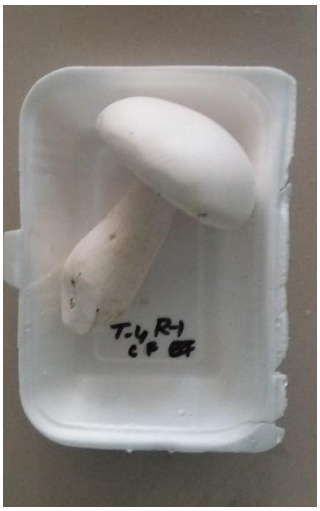
Fruiting body harvested at seven days old and stored in polypropylene bag at ambient temperature





During harvest Seven days after harvest Fifteen days after harvest

Fruiting body harvested at seven days old and stored in open tray kept in refrigerator

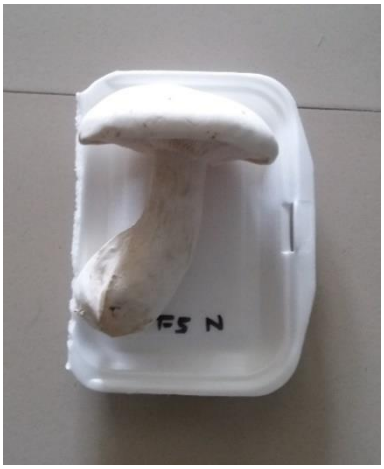



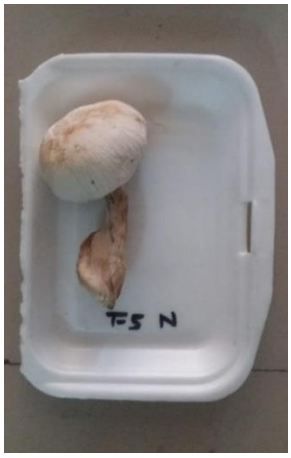

During harvest Seven days after harvest Fifteen days after harvest

Fruiting body harvested at seven days old and stored in cellophane wrapped tray kept in refrigerator

During harvest Seven days after harvest Fifteen days after harvest

Fruiting body harvested at seven days old and stored in polypropylene bag kept in refrigerator



During harvest Five days after harvest Twelve days after harvest

Fruiting body harvested at eight days old and stored in open tray at ambient temperature



During harvest Five days after harvest Twelve days after harvest

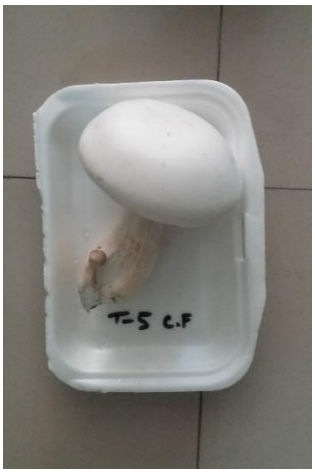
Fruiting body harvested at eight days old and stored in cellophane wrapped tray at ambient temperature

During harvest Five days after harvest Twelve days after harvest

Fruiting body harvested at eight days old and stored in polypropylene bag at ambient temperature

During harvest Five days after harvest Twelve days after harvest

Fruiting body harvested at eight days old and stored in open tray kept in refrigerator

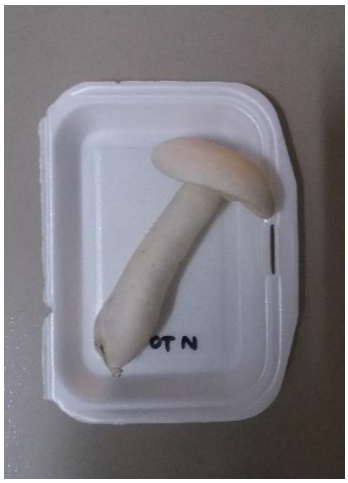
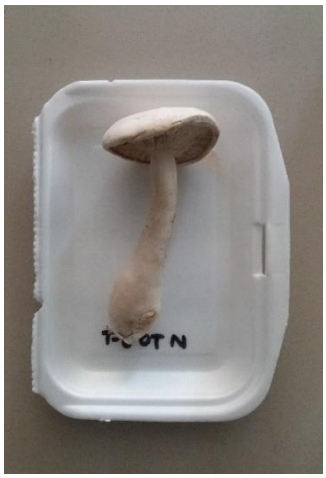
During harvest Twelve days after harvest Fifteen days after harvest

Fruiting body harvested at eight days old and stored in cellophane wrapped tray kept in refrigerator



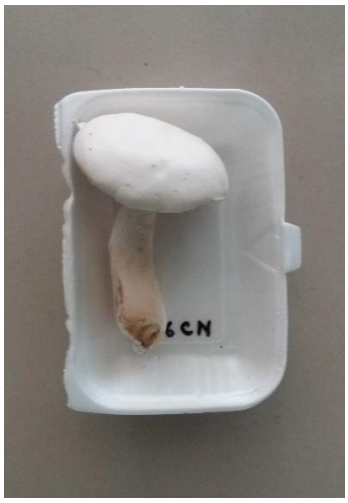
During harvest Twelve days after harvest Fifteen days after harvest

Fruiting body harvested at eight days old and stored in polypropylene bag kept in refrigerator


During harvest Three days after harvest Six days after harvest

Fruiting body harvested at nine days old and stored in open tray at ambient temperature


During harvest Three days after harvest Six days after harvest

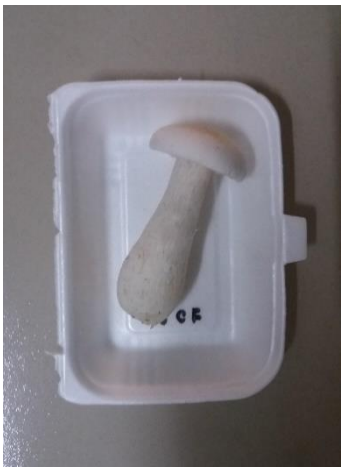
Fruiting body harvested at nine days old and stored in cellophane wrapped tray at ambient temperature

During harvest Three days after harvest Six days after harvest

Fruiting body harvested at nine days old and stored in polypropylene bag at ambient temperature

During harvest Three days after harvest Six days after harvest

Fruiting body harvested at nine days old and stored in open tray kept in refrigerator

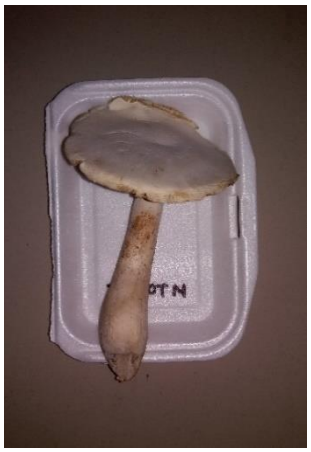
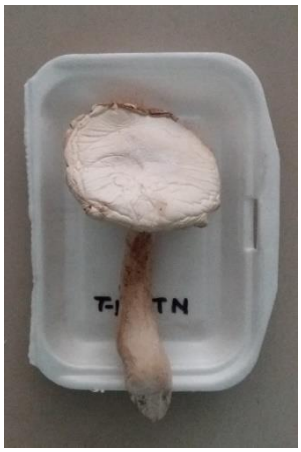


During harvest Nine days after harvest Fifteen days after harvest

Fruiting body harvested at nine days old and stored in cellophane wrapped tray kept in refrigerator

During harvest Nine days after harvest Fifteen days after harvest

Fruiting body harvested at nine days old and stored in polypropylene bag kept in refrigerator

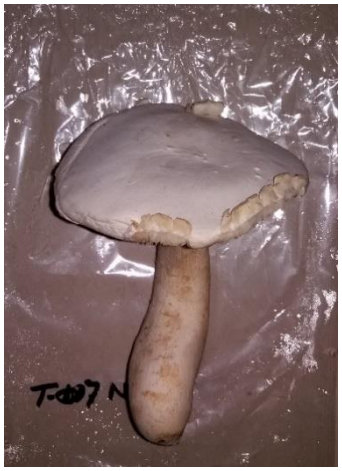
During harvest Three days after harvest Six days after harvest

Fruiting body harvested at ten days old and stored in open tray at ambient temperature



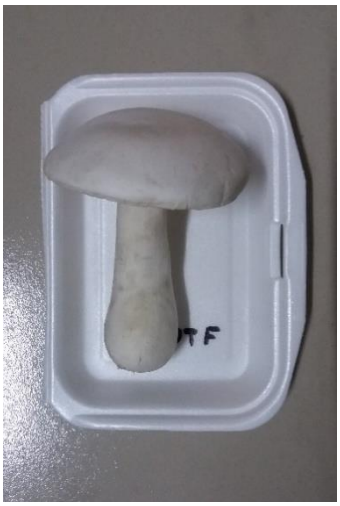
During harvest Three days after harvest Six days after harvest

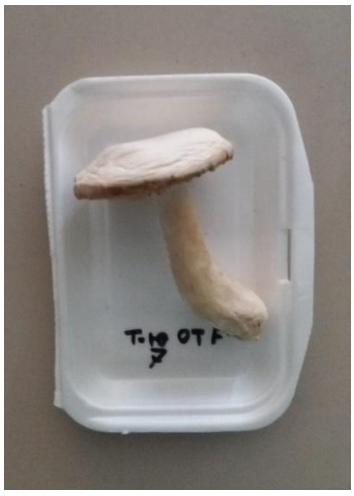
Fruiting body harvested at ten days old and stored in cellophane wrapped tray at ambient temperature



During harvest Three days after harvest Six days after harvest

Fruiting body harvested at ten days old and stored in polypropylene bag at ambient temperature





During harvest Six days after harvest Nine days after harvest

Fruiting body harvested at ten days old and stored in open tray kept in refrigerator

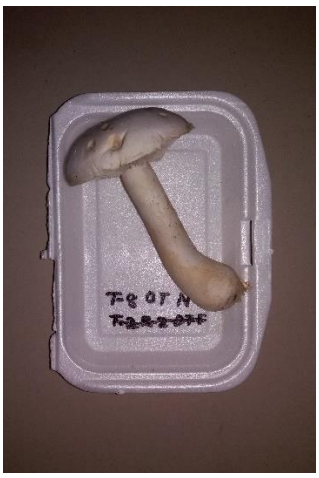


During harvest Nine days after harvest Fifteen days after harvest

Fruiting body harvested at ten days old and stored in cellophane wrapped tray kept in refrigerator

During harvest Six days after harvest Nine days after harvest

Fruiting body harvested at ten days old and stored in polypropylene bag kept in refrigerator



During harvest Three days after harvest

Fruiting body harvested at eleven days old and stored in open tray at ambient temperature

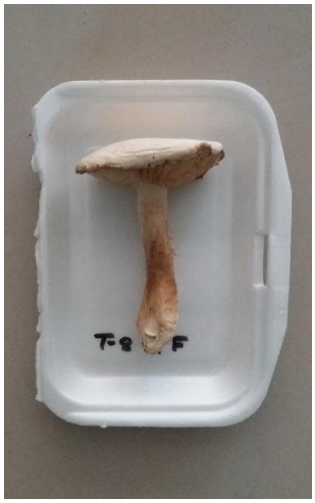
During harvest Three days after harvest Six days after harvest

Fruiting body harvested at eleven days old and stored in cellophane wrapped tray at ambient temperature

During harvest Three days after harvest Six days after harvest

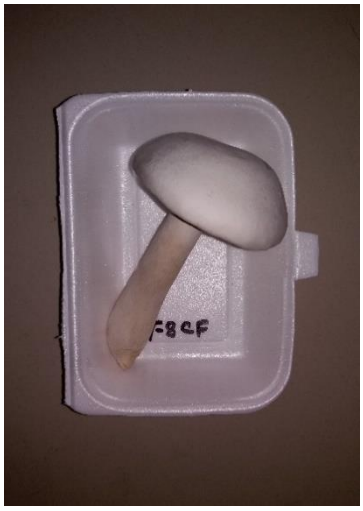
Fruiting body harvested at eleven days old and stored in polypropylene bag at ambient temperature





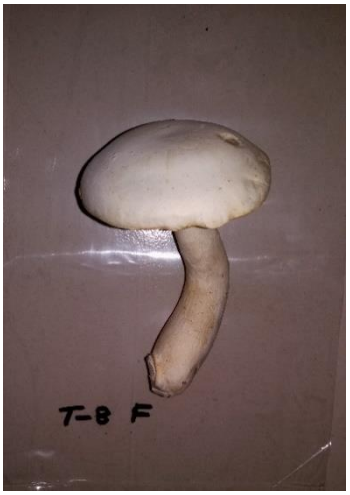
During harvest Three days after harvest Six days after harvest

Fruiting body harvested at ten days old and stored in open tray kept in refrigerator



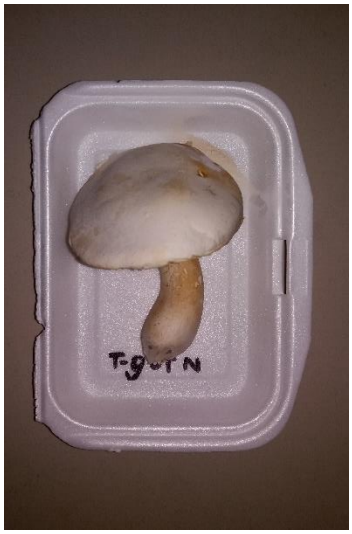
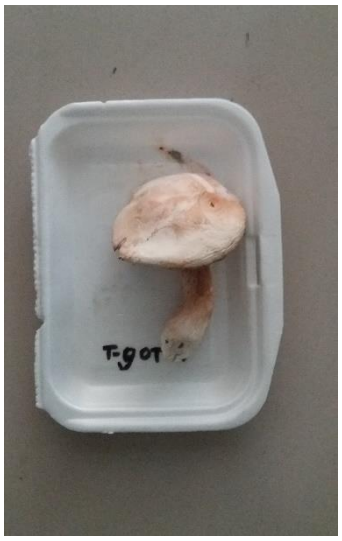
During harvest Six days after harvest Fifteen days after harvest

Fruiting body harvested at eleven days old and stored in cellophane wrapped tray kept in refrigerator



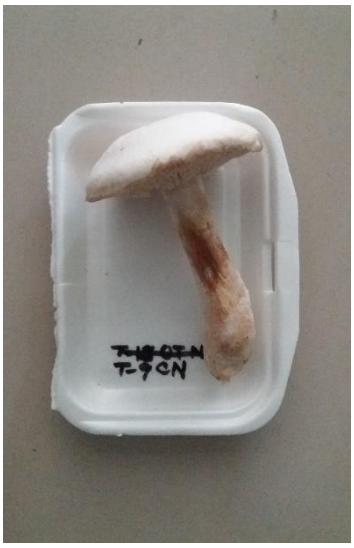
During harvest Six days after harvest Fifteen days after harvest

Fruiting body harvested at eleven days old and stored in polypropylene bag kept in refrigerator

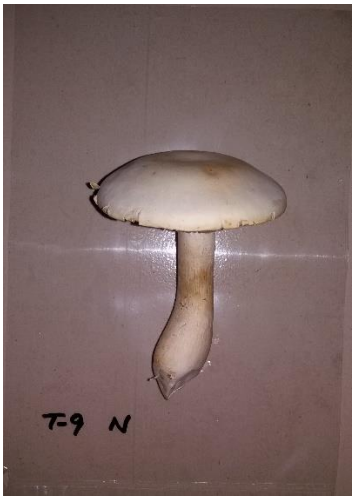

During harvest Three days after harvest Six days after harvest

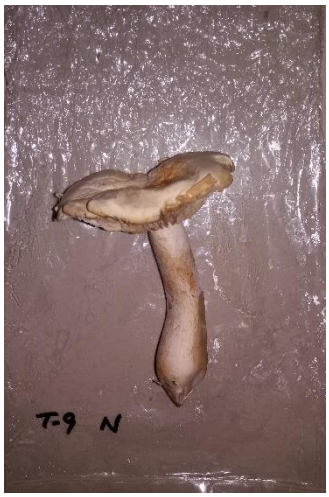
Fruiting body harvested at twelve days old and stored in open tray at ambient temperature

During harvest Three days after harvest

Fruiting body harvested at twelve days old and stored in cellophane wrapped tray at ambient temperature

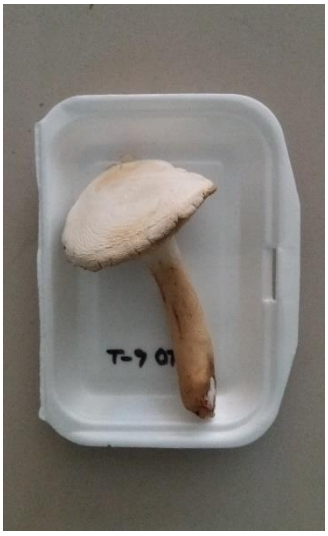




During harvest Three days after harvest Six days after harvest

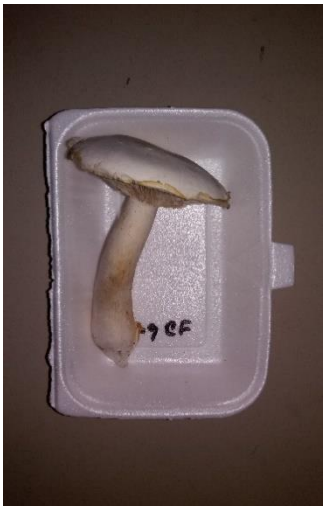
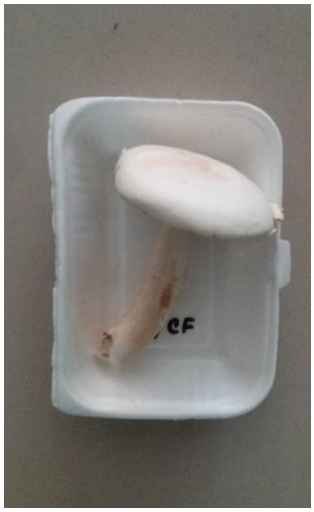
Fruiting body harvested at twelve days old and stored in polypropylene bag at ambient temperature





During harvest Three days after harvest Six days after harvest

Fruiting body harvested at twelve days old and stored in open tray kept in refrigerator

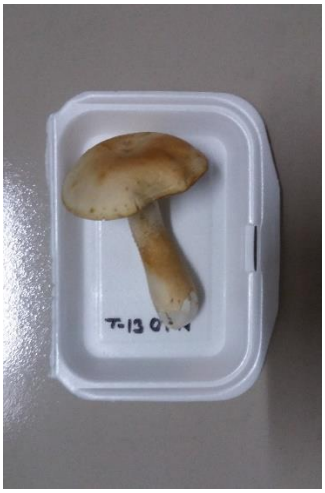

During harvest Six days after harvest Fifteen days after harvest

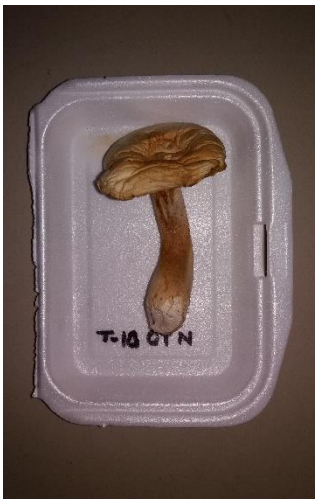
Fruiting body harvested at twelve days old and stored in cellophane wrapped tray kept in refrigerator



During harvest Three days after harvest Six days after harvest

Fruiting body harvested at twelve days old and stored in polypropylene bag kept in refrigerator



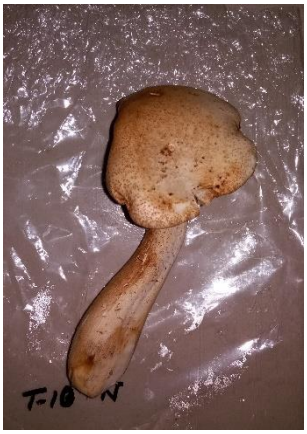


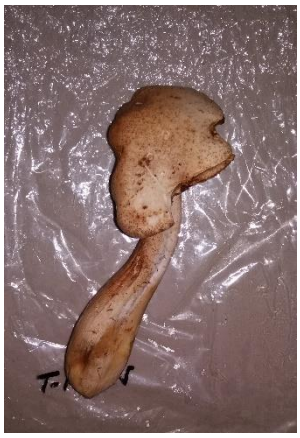
During harvest Three days after harvest

Fruiting body harvested at thirteen days old and stored in open tray at ambient temperature

During harvest Three days after harvest Six days after harvest

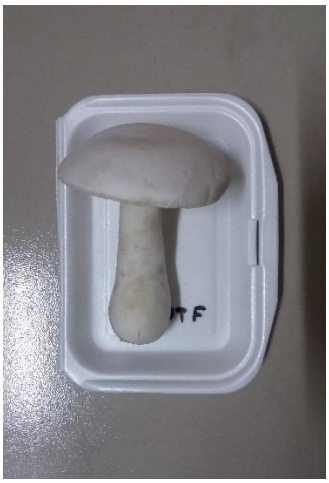
Fruiting body harvested at thirteen days old and stored in cellophane wrapped tray at ambient temperature

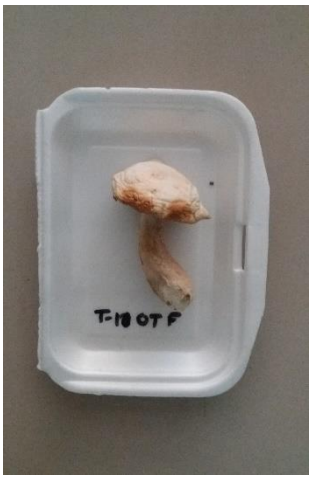




During harvest Three days after harvest Six days after harvest

Fruiting body harvested at thirteen days old and stored in polypropylene bag at ambient temperature





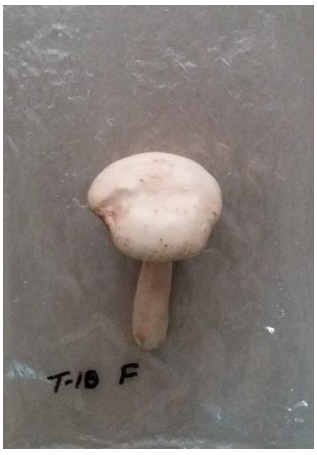
During harvest Three days after harvest Six days after harvest

Fruiting body harvested at thirteen days old and stored in open tray kept in refrigerator



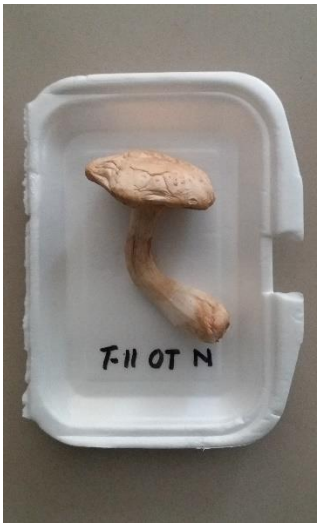
During harvest Nine days after harvest Fifteen days after harvest

Fruiting body harvested at thirteen days old and stored in cellophane wrapped tray kept in refrigerator



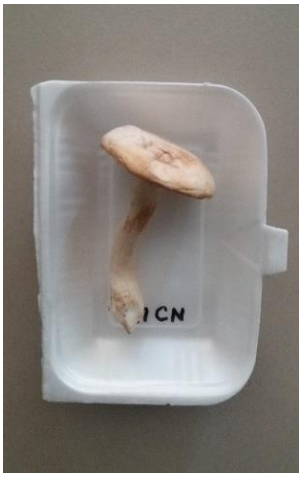
During harvest Nine days after harvest Fifteen days after harvest

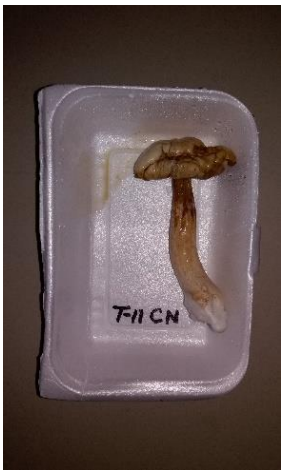
Fruiting body harvested at thirteen days old and stored in polypropylene bag kept in refrigerator



During harvest Three days after harvest

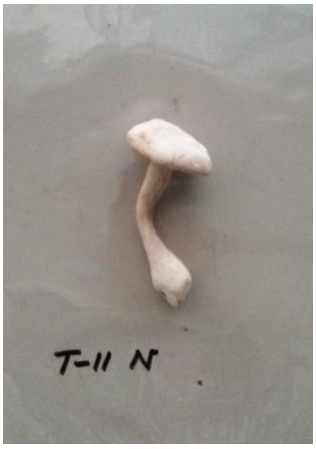
Fruiting body harvested at fourteen days old and stored in open tray at ambient temperature





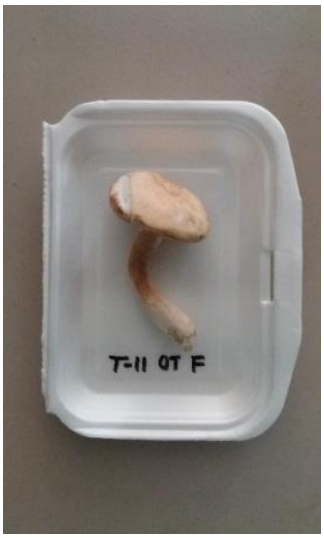
During harvest Three days after harvest

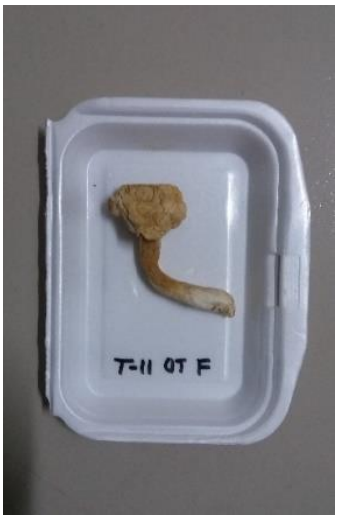
Fruiting body harvested at fourteen days old and stored in cellophane wrapped tray at ambient temperature



During harvest Three days after harvest

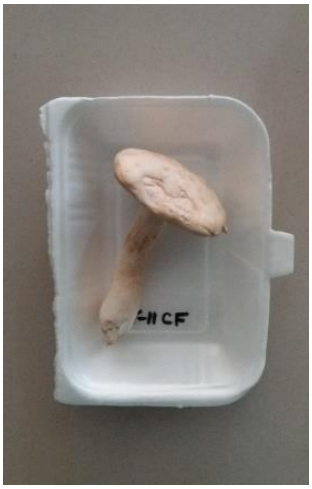
Fruiting body harvested at fourteen days old and stored in polypropylene bag at ambient temperature

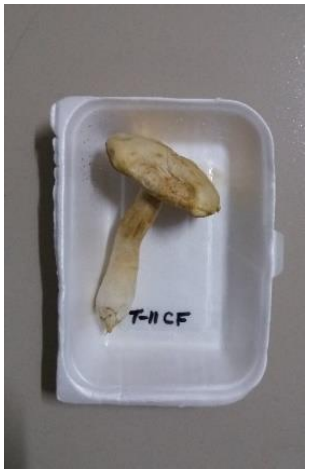




During harvest Three days after harvest

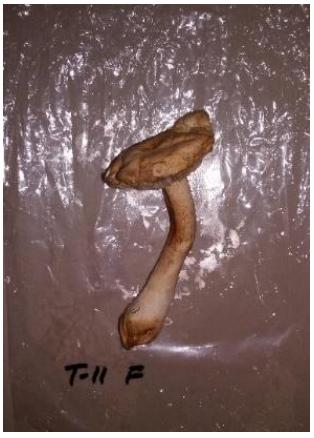
Fruiting body harvested at fourteen days old and stored in open tray kept in refrigerator





During harvest Three days after harvest

Fruiting body harvested at fourteen days old and stored in cellophane wrapped tray kept in refrigerator



During harvest Three days after harvest

Fruiting body harvested at fourteen days old and stored in polypropylene bag kept in refrigerator