EFFECT OF DIFFERENT SAWDUST AND EFFECT OF DIFFERENT SUBSTRATE ON THE GROWTH, YIELD AND PROXIMATE COMPOSITION OF SHIITAKE MUSHROOM (*Lentinus edudes*)

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DEDICATED TO MY BELOVED PARENTS AND ELDER BROTHER

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CERTIFICATE

This is to certify that the thesis entitled **"Effect of Different Sawdust on the Growth, Yield and Proximate Composition of shiitake mushroom (*Lentinus edudes*)."** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN BIOCHEMISTRY,** embodies the result of a piece of bonafide research work carried out by **MD. IQBAL HOSSAIN,** Registration No. **15-06884** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Dated:
Dhaka, Bangladesh

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EFFECT OF DIFFERENT SAWDUST AND EFFECT OF DIFFERENT SUBSTRATE ON THE GROWTH, YIELD AND PROXIMATE COMPOSITION OF SHIITAKE MUSHROOM (*Lentinus edudes*)

ABSTRUCT

Five different sawdust viz. Teak tree (*Tectona grandis*), Mango tree (*Mangifera indica*), White siris (*Albizia procer),* Jackfruit (*Artocarpus heterophyllus*), Timber tree (*Anogeissus latifolia*) sawdust supplemented with 12% wheat bran and 1% as basal substrate were selected for studies their performance on growth, yield and proximate composition of shiitake mushroom (*Lentinus edudes*). The highest mycelium growth rate (4.23 mm/day) was recorded in Le 11 with 1000g of spawn packet The highest time (114.80 days) required to completion of mycelium running was obtained from the treatment completion of strain Le 12 with 1000g spawn packet. The highest time (149.30 days) required for bump formation was obtained from the treatment combination of strain Le 11 with 1000g spawn packet. The highest time (16.50 days) required from opening to first harvest was obtained from the treatment combination of strain Le 12 with 1000g spawn packet. The highest diameter (10.38 cm) of pileus was recorded from the treatment combination of Le 8 with 1000g weight of spawn packet. The highest average number of primordial per packet was recorded from MT (66.45), The highest average length of stripe was recorded from MT (1.60). The highest average thickness of pileus was recorded from KT (0.70), The highest amount of nitrogen content was recorded in MT (6.78%). The highest amount of potassium was attained from MT (1.98%), The highest amount of iron was recorded from MT (625.23ppm). The highest amount of zinc was obtained from KT (9.67%). Therefore, it can be concluded that mango tree sawdust supplemented with 12% wheat bran can be further used as a better substrate for shiitake mushroom (*Lentinus edudes*) production reducing cost and increasing the yield and nutritional quality.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF PLATES

LIST OF ABBREVIATED TERMS

ABBREVIATION	FULL NAME
AEZ	Agro-Ecological Zone
et al.	and others
BBS	Bangladesh Bureau of Statistics
cm	Centimeter
^{0}C	Degree Celsius
etc	Etcetera
FAO	Food and Agriculture Organization
LSD	Least Significant Difference
CV	Coefficient of Variation
m^2	Square meter
SAU	Sher-e-Bangla Agricultural University
ppm	Parts per million
BARI	Bangladesh Agricultural Research Institute
NAMDEC	National Mushroom Development and Extension Center
BMD	Bangladesh Metrological Department

BCR	Benefit Cost Ratio
BE	Biological Efficiency
w/v	Weight per Volume
w/w	Weight per Weight
MCC	Mushroom Culture Centre
MRR	Mycelium Running Rate

CHAPTER I

INTRODUCTION

Mushroom is fleshy, spore-bearing fruiting body of a fungus, typically produced above ground on soil or on its food source. Most mushroom-producing fungi are members of the phylum Basidiomycota or Ascomycota. The vegetative part of mushroom consists of thread like long thin mycelium which under suitable condition forms fruiting body or sporocarps. This fruiting body is used as edible mushroom. Mushrooms had long been used for medicinal and food purposes since decades(Suzuki and Oshima,1979)**..** Some have medicinal properties such as complex carbohydrates that strengthen the immune system. There is a common saying that "Medicines and foods have a common origin" (Kaul, 2001). Out of 2000 species of prime edible mushrooms about 80 have been grown experimentally, 20 cultivated commercially and 4-5 produced on Mushrooms are low in calories, high in fiber, and contain many important vitamins and minerals industrial scale throughout the world (Chang and Miles, 1988). Mushroom a food of high quality, flavour and nutrition value have high content of protein, low content of fat (4%), vitamins (B1, B2, C, niacin, biotin etc), minerals (P, Na, K, Ca) and high content of fibers and carbohydrates (Peter, 1991). The Greeks believed that mushroom provided strength for warriors in battle and Romans regarded mushroom as the "Food of the Gods". Which was served only on festive occasions. The Chinese treasured mushrooms as a health food, the "Elixir of life." The Mexican Indians used mushrooms as hallucinogens in religious ceremonies and in witchcraft as well as for therapeutic purposes (Chang & Miles, 1988). Iman Bukhari (Ra) quoted from holly verse of Prophet Mohammed (S) that "Mushrooms originated from the extract of Manna (the holy Devine food) and it curse eye diseases". A mushroom (or toadstool) is the fleshy, spore-bearing fruiting body of a fungus, typically produced above ground on soil or on its food source. The standard for the name "mushroom" is the cultivated white button mushroom, *Agaricus bisporus*; hence the word "mushroom" is most often applied to those fungi (Basidiomycota, Agaricomycetes) that have a stem (stipe), a cap (pileus), and gills (lamellae, sing. lamella) on the underside of the cap. These gills produce microscopic spores that help the fungus spread across the ground or its occupant surface. "Mushroom" describes a variety of gilled fungi, with or without stems, and the term is used even more generally, to describe both the fleshy fruiting bodies of some Ascomycota and the woody or leathery fruiting bodies of some Basidiomycota, depending upon the context of the word. Forms deviating from the standard morphology usually have more specific names, such as "bolete", "puffball", "stinkhorn", and "morel", and gilled mushrooms themselves are often called "agarics" in reference to their similarity to *Agaricus* or their order Agaricales. By extension, the term "mushroom" can also designate the entire fungus when in culture; the thallus (called a mycelium) of species forming the fruiting bodies called mushrooms; or the species itself.

Many species of mushrooms seemingly appear overnight, growing or expanding rapidly. This phenomenon is the source of several common expressions in the English language including "to mushroom" or "mushrooming" (expanding rapidly in size or scope) and "to pop up like a mushroom" (to appear unexpectedly and quickly). In reality all species of mushrooms take several days to form primordial mushroom fruit bodies, though they do expand rapidly by the absorption of fluids. The cultivated mushroom as well as the common field mushroom initially form a minute fruiting body, referred to as the pin stage because of their small size. Slightly expanded they are called buttons, once again because of the relative size and shape. Once such stages are formed, the mushroom can rapidly pull in water from its mycelium and expand, mainly by inflating preformed cells that took several days to form in the primordia. Similarly, there are even more ephemeral mushrooms, like *Parasola plicatilis* (formerly *Coprinus plicatlis*), that literally appear overnight and may disappear by late afternoon on a hot day after rainfall. The primordia form at ground level in lawns in humid spaces under the thatch and after heavy rainfall or in dewy conditions balloon to full size in a few hours, release spores, and then collapse. They "mushroom" to full size.

The shiitake *Lentinula edodes*) is an edible mushroom native to East Asia, which is cultivated and consumed in many Asian countries. It is considered a medicinal mushroom in some forms of traditional medicine. The earliest written record of shiitake cultivation is seen in the *Records of Longquan County* compiled by He Zhan in 1209 during the Southern Song dynasty. The 185-word description of shiitake cultivation from that literature was later crossed-referenced many times and eventually adapted in a book by a Japanese horticulturist in 1796, the first book on shiitake cultivation in Japan. The Japanese cultivated the mushroom by cutting shii trees with axes and placing the logs by trees that were already growing shiitake or contained shiitake spores. Before 1982, the Japan Islands' variety of these mushrooms could only be grown in traditional locations using ancient methods. A 1982 report on the budding and growth of the Japanese variety revealed opportunities for commercial cultivation in the United States. Shiitake are now widely cultivated all over the world, and contribute about 25% of total yearly production of mushrooms. Commercially, shiitake mushrooms are typically grown in conditions similar to their natural environment on either artificial substrate or hardwood logs, such as oak.

In a 100 gram amount, raw shiitake mushrooms provide 34 calories and are 90% water, 7% carbohydrates, 2% protein and less than 1% fat (table for raw mushrooms). Raw shiitake mushrooms are rich sources (20% or more of the Daily Value, DV) of B vitamins and contain moderate levels of some dietary minerals (table). When dried to about 10% water, the contents of numerous nutrients increase substantially.Like all mushrooms, shiitakes produce vitamin D2 upon exposure of their internal ergosterol to ultraviolet B (UVB) rays from sunlight or broadband UVB fluorescent tubes.

Fresh and dried shiitake have many uses in the cuisines of East Asia. In Japan, they are served in miso soup, used as the basis for a kind of vegetarian *dashi*, and as an ingredient in many steamed and simmered dishes. In Chinese cuisine, they are often sautéed in vegetarian dishes such as Buddha's delight. One type of high-grade shiitake is called *donko* in Japanese and in Chinese, literally "winter mushroom". Another high-grade of mushroom is called in Chinese, literally "flower mushroom", which has a flower-like cracking pattern on the mushroom's upper surface. Both of these are produced at lower temperatures.

Basic research is ongoing to assess whether consumption of shiitake mushrooms affects disease properties, although no effect has been proven with sufficient human research to date. Rarely, consumption of raw or slightly cooked shiitake mushrooms may cause an allergic reaction called "shiitake dermatitis", including an erythematous, micro-papular, streaky pruriginous rash that occurs all over the body including face and scalp, appearing about 24 hours after consumption, possibly worsening by sun exposure and disappearing after 3 to 21 days. This effect – presumably caused by the polysaccharide, lentinan – is more common in Asia but may be growing in occurrence in Europe as shiitake consumption increases. Thorough cooking may eliminate the allergenicity.

Objectives of the research work:

- To improve the cultivation of shiitake mushroom (*Lentinus edudes*).
- To improve the yield of shiitake mushroom (*Lentinus edudes*).
- To know the characteristics of different sawdust in the cultivation of shiitake mushroom (*Lentinus edudes*).
- To develop method for farmers of shiitake mushroom.

CHAPTER II
REVIEW OF LITERATURE

Mushrooms are fruiting bodies of Fungi especially of Ascomycetes or Basidiomycetes, which are macro fungi with distinctive fruiting bodies large enough to be seen with the naked eye and to be picked up by hand (Chang and Miles, 1992). In history, human beings used to collect only wild mushrooms at the beginning (Imtiaj and Rahman, 2008). Mushrooms have been considered as a special kind of food since the earliest time. The Chinese were the first to grow mushrooms for the human consumption. In the last 25 years, worldwide mushroom production had increased over 300%, reaching approximately 2,961,491 tons in 2002 (USDA 2003). Presently, mushrooms have become popular throughout the world since they have wonderful food and medicinal values. The local demand for mushrooms is also progressively increasing (Nita, 2002). Tewari (1986) reported that the fresh mushroom contains about 85-90% moisture, 3% protein, 4% carbohydrates, 0.3-0.4% fats and 1% minerals and vitamins. The review of literature given below was based on the present information about the performance of shiitake mushroom (*Lentinus edudes*) and the effect of different kinds of substrate on mushroom cultivation.

Shiitake mushroom is the first medicinal macrofungus to enter the realm of modern biotechnology. It is the second most popular edible mushroom in the global market which is attributed not only to its nutritional value but also to possible potential for therapeutic applications. Shiitake mushroom is used medicinally for diseases involving depressed immune function (including AIDS), cancer, environmental allergies, fungal infection, frequent flu and colds, bronchial inflammation, heart disease, hyperlipidemia (including high blood cholesterol), hypertension, infectious disease, diabetes, hepatitis and regulating urinary inconsistancies. It is the source of several well-studied preparations with proven pharmacological properties, especially the polysaccharide lentinan, eritadenine, shiitake mushroom mycelium, and culture media extracts (LEM, LAP and KS-2). Antibiotic, anti-carcinogenic and antiviral compounds have been isolated intracellularly (fruiting body and mycelia) and extracellularly (culture media). Some of these substances were lentinan, lectins and eritadenine. The aim of this review is to discuss the therapeutic applications of this macrofungus. The potential of this macrofungus is unquestionable in the most important areas of applied biotechnology.

Several mushroom species have been pointed out as sources of antioxidant compounds, in addition to their important nutritional value. *Agaricus blazei* and shiitake mushroom are among the most studied species all over the world, but those studies focused on their fruiting bodies instead of other presentations, such as powdered preparations, used as supplements. In the present work the chemical composition (nutrients and bioactive compounds) and antioxidant activity (free radical scavenging activity, reducing power and lipid peroxidation inhibition) of dried powder formulations of the mentioned mushroom species (APF and LPF, respectively) were evaluated. Powder formulations of both species revealed the presence of essential nutrients, such as proteins, carbohydrates and unsaturated fatty acids. Furthermore, they present a low fat content (<2 g/100 g) and can be used in low-calorie diets, just like the mushrooms fruiting bodies. APF showed higher antioxidant activity and higher content of tocopherols and phenolic compounds (124 and 770 μg/100 g, respectively) than LPF (32 and 690 μg/100 g). Both formulations could be used as antioxidant sources to prevent diseases related to oxidative stress.

Shiitake mushroom contains several therapeutic actions such as antioxidant and antimicrobial properties, carried by the diversity of its components. In the present work, extracts from shiitake mushroom were obtained using different extraction techniques: high-pressure operations and low-pressure methods. The high-pressure technique was applied to obtain shiitake extracts using pure CO_2 and CO_2 with co-solvent in pressures up to 30 MPa. Organic solvents such as *n*-hexane, ethyl acetate and dichloromethane were furthermore used to produce the shiitake extracts in low-pressure extraction process. The different extraction procedures were evaluated for antioxidant activity by 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) essays and the results compared with data from Folin–Denis method, used to measure the total phenolic content. Antimicrobial activities of the extracts were also subjected to preliminary screening against four strains of bacteria and one fungal strain using agar dilution method. The results indicate that the fractions obtained with CO_2 using ethanol as co-solvent, at 40 °C, 20 MPa and 15% EtOH, and for dichloromethane in low-pressure technique had similar antioxidant activities. Furthermore, only the supercritical fluid extracts had antimicrobial activity against *Micrococcus luteus* and *Bacillus cereus*. The shiitake extraction yields were up to 3.81% w/w and up to 1.01%

w/w for supercritical fluid extraction with ethanol as co-solvent and with pure CO_2, respectively, while the low-pressure extraction indicates yields up to 1.25% w/w for *n*-hexane as solvent.

Ingestion of raw or undercooked shiitake mushrooms is associated with a distinctive flagellate erythema. We describe a 61-year-old Caucasian man who presented with a pruritic, erythematous eruption of multiple linear streaks on the trunk and extremities starting 1 day after eating raw shiitake mushrooms. His symptoms and skin lesions resolved with minimal hyperpigmentation within approximately 1 week after treating with topical steroids and oral antihistamines. Skin biopsy showed non-specific findings, including a sparse perivascular and interstitial dermatitis as well as focal vacuolar interface changes. Our case illustrates that this condition is a visibly striking dermatitis with a self-limited course. The pathomechanism of the skin eruption remains unclear.

A 72-year-old man, a Shiitake mushroom grower over fifty years, was admitted to our hospital because of bilateral chest interstitial shadow with chronic cough and breathlessness. Chest computed tomography showed traction bronchiectasis, subpleural micro-cystic changes and partial ground-glass opacities in both lungs, and mild mediastinal lymphadenopathy. A diagnosis of chronic hypersensitivity pneumonitis induced by Shiitake mushrooms was comprehensively confirmed by occupational history, radiological findings, and positive findings of an incidental environmental provocation test and lymphocyte stimulation test for Shiitake mushroom extracts. We reviewed the clinical features in five patients with chronic hypersensitivity pneumonitis induced by Shiitake mushrooms reported in Japan. There was a tendency toward increasing lymphocytes and high CD4/CD8 ratio in bronchoalveolar lavage fluids. Treatment with steroids seems to have a limited effect, while avoidance of the antigen is important.

The supplementation of sawdust with cow dung had remarkable effect on biological yield, economic yield, the dry yield, biological efficiency and cost benefit ratio. The highest biological yield (217.7 g), economic yield (213 g), dry yield (21.27 g) biological efficiency (75.06%) and cost benefit ratio (8.41) were observed due to sawdust supplemented with cow dung @ 10%. Among the chemical characteristics highest contest of protein (31.30%), ash (8.41%), crube fiber (24.07%), the lowest lipid (3.44%) and carbohydrate (32.85%) were observed due to sawdust supplemented with cow dung @ 10%. Among the minerals the highest amount of nitrogen (5.01%), potassium (1.39%), calcium (22.15%), magnesium (20.21%), sulfur (0.043%), iron

(43.4%), and the lowest phosphorus (0.92) were observed due to sawdust supplemented with cow dung @ 10%.

Shiitake mushroom dermatitis (also known as "Flagellate mushroom dermatitis, "Mushroom worker's disease, and "Shiitake-induced toxicoderma, is an intensely pruritic dermatitis characterized by disseminated 1mm erythematous micropapules seen in a linear grouped arrangement secondary to Koebnerization due to patient scratching. It is caused by the ingestion of shiitake mushrooms and was first described in 1977 by Nakamura. Although it is rarely seen outside of China and Japan due to a lower incidence of shiitake consumption outside these regions, there is a well-established association between flagellate dermatitis and shiitake mushroom (Lentinula edodes) ingestion. Bleomycin ingestion may also cause similar findings. On physical exam, one key difference between the two is that post-inflammatory hyperpigmentation changes are usually seen with bleomycin-induced flagellate dermatitis and are not typically present with mushroom induced flagellate dermatitis. The median time of onset from ingestion of shiitake mushrooms is typically 24 hours, ranging from 12 hours to 5 days. Most patients completely recover by 3 weeks, with or without treatment. Although the pathogenesis of shiitake induced flagellate dermatitis is not clear, the theory most argued for is a toxic reaction to lentinan, a polysaccharide isolated from shiitake mushrooms. However, Type I and Type IV allergic hypersensitivities have also been supported by the 24-hour median time of onset, clearance in 3–21 days, severe pruritus, benefit of steroids and antihistamines, and lack of grouped outbreaks in people exposed to shared meals containing shiitake mushrooms. Most cases reported shortly after its discovery were due to consumption of raw shiitake mushrooms, but several cases have since been reported after consumption of fully cooked mushrooms.

Auricularia species has been reported to exert strong antioxidant effects (Mau et al., 2001; Fan et al., 2006). Mau et al., (2001) reported as, all methanolic extracts from red, jin, and snow ears showed excellent antioxidant activities in the conjugated diene method at 5.0 mg/ml. It is not good scavengers for hydroxyl free radicals but were good chelators for ferrous ions (Mau et al., 2001). Meanwhile the polysaccharides in the fruit bodies of Auricularia auricula (black woody ear) was extracted and tested by formulating into bread. The result showed that up to 9% of AAP (Auricularia auricula polysaccharides) flour could be included (IC50 =100mg/ml) in bread formulation without altering the sensory acceptance of the blended bread. The incorporation of

AAP in bread markedly increases the antioxidant property of the bread as tested by DPPH free radical scavenging method (Fan et al., 2006).

Baysal (2003) investigated paper waste supplemented with rice husk, chicken manure and peat for Pleurotus ostreatus cultivation. Highest yield for fresh weight was recorded as 350.2 grams in the substrate containing 20% rice husk. The values of commercial cultivation of mushrooms, especially in a developing economy like Nigeria, is the availability of large quantities of several agro–industrial wastes which can serve as substrates for the cultivation of mushrooms .(Banjo et al., 2004) has been reported that mushrooms can grow on chopped cocoa pods, cotton waste, dried chopped maize straw, oil palm (fibre and bunch) wastes, tobacco straw, used tea leaves, rice straw, sugarcane bagasse, newsprint, old rags and sawdust Silva et al. (2005) reported that mycelium extension is related to bio availability of nitrogen when they found that eucalyptus residues supplemented with cereal bran supported fast growth . However, the low amount of available nitrogen (N) in the lignocellulosic substrate of wood components is often considered as a limitation to its use as mushroom substrate. Pleurotus species are popular and widely cultivated throughout the world mostly in Asia and Europe owing to their simple and low cost production technology and higher biological efficiency (Mane et al., 2007). Moonmoon et al., (2010) studied king shiitake mushroom on saw dust and rice straw in Bangladesh and found that saw dust showed the highest biological efficiency (73.5%) than other strains. He has also reported on saw dust, the yield and efficiency were better than those cultivated on rice straw, however, on straw; the mushroom fruiting bodies were larger in size. This study shows the prospects of cultivation in Bangladesh and suggests further study in controlled environment for higher yield and production.

Stanley et al., 2011 has evaluated the effect of supplementing corn cob substrate with rice bran on yield of Pleurotus pulmonarius (Fr) Quel. Un-supplemented corn cob (0% supplementation) gave the best yield in terms of the mean diameter of pileus 5.50cm, mean fresh weight of fruiting bodies 53.2g, mean height of stipe 3.64cm and number of healthy fruiting bodies as 12. The least yield was recorded with 30% supplementation as follows: mean diameter 3.20cm, mean fresh weight of fruiting bodies 30.0g, mean height of stipe 1.65cm and number of healthy fruiting bodies as 5.in terms of quantity and quality, the un-supplemented substrate produced better edible mushrooms.

Mushrooms can be considered as a functional food or dietary supplements for their great nutritional and medicinal values (Chang & Miles, 2004). The bioactivities of mushrooms have been confirmed by extensive studies. In 1957, Lucas discovered the bioactivity of Basidiomycetes mushrooms for the first time by isolating a substance from Boletus edulis which demonstrated a significant inhibitory effect against Sarcoma 180 tumor cells (Lucas, 1957). Since then, numerous antitumor mushroom polysaccharides have been extracted from a variety of mushrooms. Recently, a large number of compounds isolated from mushrooms have been greatly highlighted for their sound pharmaceutical applications. These compounds, including lectins, polysaccharides, polysaccharide-peptides, and polysaccharide-protein complexes, have proven to possess effective functions such as: immunomodulatory, anticancer (Moradali, Mostafavi, Ghods, & Hedjaroude, 2007), anti-inflammatory (Lu, Cheng, Lin, & Chang, 2010), and antioxidant (Rout, & Banerjee, 2007; Mau, Chao, & Wu, 2001) effects, and lowering blood cholesterol levels (Cheung, 1998). In particular, the commercialization of several polysaccharides and polysaccharide conjugates has allowed patients to benefit from such anticancer therapy. They are schizophyllan, lentinan, grifolan, PSP (polysaccharide-peptide complex) and krestin (polysaccharide-protein complex) (Zhang et al., 2007).

Shiitake mushrooms have long been used medicinally in Asia. Some studies have found the fungi to hold antitumor properties. But many shiitake mushrooms you'll find at the supermarket are grown in sawdust, not on logs, leaving them with fewer nutrients. And shiitakes grown in the sun can have much higher doses of vitamin D. While log-grown shiitakes go for up to $40 a pound in Japan, you can enjoy their superior flavor and increase your nutrient intake by growing your own at home.

Shiitake logs can be obtained both from the stems of healthy young trees selected for thinning and from healthy branch-wood taken from the tops of trees felled for saw-timber. In either case, only logs with intact bark, free of hear trot, and with as much sapwood as possible, should be selected. Logs already dead or with hear trot will be infected with other decay fungi and must be avoided. If you do not have access to forested areas, purchase logs from a public land management agency or a contract logger. If you contract for logs, be sure to specify undamaged bark and appropriate diameter. The ideal time to fell trees is mid- to late-winter, for early spring inoculation. This is especially true for sugar maple, which begins sap flow earlier than oaks. For spring inoculation, it is best to harvest trees in February for inoculation in April or early May. Felled logs should be protected from desiccation (wind and sun) to maintain an internal moisture content above 35 percent.

For more detailed information on this method, please refer to our catalog or web page titled "Cultivating Mushrooms in Natural Logs". Shiitake grows best in hardwood logs with spawn inoculated into small drilled holes in oaks or other dense hardwoods. Oyster grows best in softer hardwoods like cottonwood, poplar, and willow. Select healthy hardwood trees having a medium-thick bark, and cut while the tree is dormant in the Fall or Winter to provide a richer sap, less contamination, and minimize damage to the forest and the critters in the trees. Since the bark is a barrier against contamination of the semi-sterile sapwood, the logs should be handled gently and kept fairly clean. Reishi logs should be partially buried horizontally in a moist, shady location. Maitake and Lions Mane require either sterile media methods, or the natural methods described below. Please note that Shiitake is far more reliable and prolific grower than Reishi, Maitake, or Lions Mane.

Bhuyan (2008) conducted an experiment to study the effect the various supplements at different levels with sawdust showed significant effort on mycelium running rate and reduced the required days to complete mycelium running in the spawn packet. The supplementation of sawdust found to be significant in yield and yield contributing characters of shiitake mushroom with some content. The highest biological yield, economic yield, dry yield, biological efficiency (BE) and benefit cost ratio (BCR) of 270.5 g, 266.5 g, 26.34 g, 93.29 g, 9.57%, respectively was observed in sawdust supplemented with NPK mixed fertilizer (N=0.6%, P=0.3%, K=0.3%). Sawdust supplemented with different levels has a profound effect on chemical composition of shiitake mushroom. Sawdust supplemented at different substrate found to be significant with mineral content of the fruiting body. Considering all parameters in five experiments, NPK mixed fertilizer (N=0.6%, P=0.3%, K=0.3%) supplemented with sawdust is found promising for lowering the cost of production as well as increasing the yield and quality of fruiting body. Cow dung (11.5%) and starch (5.5%) as supplement with substrate may be the fair choice.

Shiitake Dermatitis is often presented as papules and erythemato-violaceous linear streaks. It can be associated with bleomycin treatment, dermatomyositis and shiitake mushroom (Lentinus edodes). There is not any previous report concerning this rare etiology in our country. Shiitake is the second most consumed mushroom worldwide and it can cause flagellate erythema when ingested raw or half cooked. It has a higher incidence in Oriental countries because of their eating habits, this is the first case reported in Brazil, in a male patient that presented a cutaneous rash after consuming this raw mushroom.

Shiitake mushroom (*Lentinus edodes*) is the second most consumed mushroom in the world. It has long been known in Asian medicine for its anticarcinogenic, antihypertensive and serum cholesterol level reduction properties. Nevertheless, the consumption of raw or not well-cooked mushrooms may cause skin eruptions which usually occur 24 to 48 hours after ingestion and are characterized by linearly arranged pruritic erythematous papules and plaques. We present a 36-year-old patient that developed typical symptoms 24 hours after consumption of shiitake mushrooms and summarize therapeutic options and particularities of this disease.

There are myriad uses for shiitake mushrooms, both in anti-aging medicine and nutritional potential. For many years, shiitake has been a primary focus of research at the Mushroom Research Institute in Tokyo. This is because of ever-mounting evidence demonstrating its powerful ability to fight cancer cells, which is good news in a world increasingly riddled with cancer. Multiple studies have shown that populations living in close proximity to where shiitake mushrooms grow (mostly in Japan), **experience significantly lower rates of cancer compared to other people groups.** This correlation is quickly proving to be causative. More advanced research into the nutritional constituents of shiitake reveal the presence of demonstratively anti-cancer compounds such as lentinan, which directly up-regulates the body's overall immune response, thus helping it to better fend off cancer cells.therapeutics. But the area I want to focus on for the purpose of this article is shiitake's anti-cancer.

Multiple studies have shown that populations living in close proximity to where shiitake mushrooms grow (mostly in Japan), experience signicantly lower rates of cancer compared to other people groups. Deeper research into the nutritional constituents of shiitake reveal of presence of demonstratively anti-cancer compounds such as lentinan, which directly up-regulates the body's overall immune response, thus helping it to better fend off cancer cells. Lentinan has so helpful has been immune system "wingman" that it's an officially approved cancer treatment in Japan since 1985! Many clinicians there use it intravenously as an adjunct chemotherapy. But research suggests that it may work in its own to support enhanced immune function in the prevention and treatment of cancer.

Native to China, Shiitake mushrooms (non-GMO) have been eaten for nearly 6000 years. The mushrooms gained prominence during the Ming Dynasty (1368-1644), where they were considered the "elixir of life" and reserved to be enjoyed only by the emperor and his family. Aside from being a rich source of protein, shiitake mushrooms supply niacin, riboflavin, thiamin, potassium and iron, and are a good source of vitamins A, B and C. When used in their sun-dried form they provide a valuable dose of Vitamin D, found in very few foods. They contain more than 50 different enzymes, including pepsin that supports digestion and asparaginase, which is a substance that supports a healthy immune response. Recent studies have traced shiitakes' legendary benefits to an active compound contained in these mushrooms called lentinan. Among lentinan's supportive benefits is its, ability to support the immune system, strengthening the body's ability to fight infection and disease. Against influenza and other viruses, lentinan has been shown to be even very effective; it even may possibly improve the immune status of individuals infected with HIV, the virus that can cause AIDS. A large number of animal studies conducted over the last ten years have shown that another active component in shiitake mushrooms called eritadenine may support healthy lipid levels. Even when lab animals are given dietary protein rich in methionine (an amino acid researchers have found causes an increase in cholesterol formation), eritadenine still lowers plasma cholesterol levels in a dose-dependent manner. In other words, the more eritadenine given, the better the support for healthy lipid levels are.

The most famous immune-supportive components in shiitake mushrooms are its polysaccharides. (Polysaccharides are large-sized carbohydrate molecules composed of many different sugars arranged in chains and branches.) Although many fungi are well-known for their polysaccharides, no single fungus has been more carefully studied than the shiitake mushroom. We know that this fungus is unique in its variety of polysaccharides, and especially its polysaccharide glucans. (Glucans are polysaccharides in which all of the sugar components involve the simple sugar glucose.) Among the glucans contained in shiitake mushroom are alpha-1,6 glucan, alpha-1,4 glucan, beta-1,3 glucan, beta-1,6 glucan, 1,4-D-glucans, 1,6-D-glucans, glucan phosphate, laminarin, and lentinan. Shiitake mushrooms also contain some important non-glucan polysaccharides, including fucoidans and galactomannins. The immune-related effects of polysaccharides in shiitake mushrooms have been studied on laboratory animals under a wide variety of circumstances, including exercise stress, exposure to inflammation-producing toxins, radiation exposure, and immunodeficiency. Under all of these circumstances, the polysaccharides in shiitake mushrooms have been shown to lessen problems. There is also some evidence that shiitake mushrooms' polysaccharides can help lower total cholesterol levels.

The process of drying the shiitake mushroom improves its overall nutritional value. Just one example is vitamin D, which increases from 4 IU in one serving of raw mushrooms to 26 IU when dried. Some sources state that shiitakes provide 19 percent of the daily value (DV) of iron and 10 percent of the DV of vitamin C, protein and fiber. Although those values are accurate, they're based on 226 grams of mushrooms. A very generous serving size of four shiitake mushrooms only weighs 15 grams. Because this article includes nutrition information for one serving size, the values will be much smaller. One serving has 44 calories, 3 percent DV of protein, 4 percent DV of total carbohydrates and an insignificant amount of fats and sugars. Shiitakes are a great source of dietary fiber; one serving provides 7 percent of your daily value.

Manzi et al. (2001) analyzed fresh and processed mushrooms (*Agaricus bisporus, Lentinus edudes, and Boletus group*). Result shows that botanical variety, processing and cooking are all effective determinants of mushroom proximate composition. Dietary fiber, chitin and beta glucans, all functional constituents of mushroom are present in variable amounts. Chitin level ranges from 0.3 to 3.9 g/100 g, while beta glucans which are negligible in a Agaricus, range from 139 to 666 mg/100 g in *Lentinus edudes* and Boletus group. On an average, a serving (100 g) of mushroom will supply 9 to 40% of the recommended of dietary fiber.

Habib (2005) tested different substrates such as sawdust, sugarcane biogasse, rice straw, wheat straw, wheat straw and waste paper for the production of shiitake mushroom in polypropylene bag. Different substrates significantly affected the number of primordial, number of fruiting bodies and amount of fresh weight or yield. This experiment revealed that the highest number of primordial and fruiting bodies were found in waste paper 43.75 and 31.00 respectively. The highest amount of fresh weight was also found in waste paper 94.25 g.

Kulsum et al. (2009) conducted an experiment to determine the effect of five different levels of cowdung (0%, 5%, 10%, 15% and 20%) as supplement with sawdust on the performance of shiitake mushroom. Among the chemical characteristics highest content of protein (31.30%), ash (8.41%) crude fiber (24.07%), the lowest lipid (3.44%) and carbohydrate (32.85%) were observed due to sawdust supplemented with cowdung @ 10%. Among the minerals the highest amount of nitrogen (5.01%), potassium (1.39%), calcium (22.15%), magnesium (20.21%), sulfur (0.043%), iron (43.4%), and the lowest phosphorus (0.92) were observed due to sawdust supplemented with cowdung @ 10%.

Khlood and Ahmed (2005) conducted an experiment to study the ability of shiitake mushroom (*Lentinus edudese)* P015 strain to grow on live cake mixed with wheat straw. The treatments comprised: 90% straw + 5% wheat bran + 5% gypsum (control); 80% straw + 10% olive cake + 5% wheat bran + 5% gypsum (T_1); 70% straw + 20% olive cake + 5% wheat bran + 5% gypsum (T_2); 60% straw + 30% olive cake + 5% wheat bran + 5% gypsum (T_3); 50% straw + 40% olive cake + 5% wheat bran + 5% gypsum (T_4); and 90% olive cake + 5% wheat bran + 5% gypsum (T_5). Carbohydrate, protein and fiber contents were high in the *L. edudes* basidiomete, ash contents were moderate, while fat content was low. For mineral contents were high compared to the other minerals in all treatments, sodium was moderate while both Mg and Ca were found at low concentrations (Mg was relatively higher than Ca). Fe and Zn were relatively high compared to Cu and Mn which that very low concentrations.

Anaconda-Mendex et al. (2005) conducted an experiment to grow shiitake mushroom (*Lentinus edudes*) in either maize or pumpkin straw. Samples were taken for each one of the three harvests and analyzed for total nitrogen (N) and amino acid profile. The substrate had no effect on N contents and amino acid profile of the fruits. However, N (g/100 g DM) increased from 4.13 g in the first harvest to 5.74 g in the third harvest. In general, the amino acids tended to be higher on the first harvest samples, but no changes were found in the amino acid profile due to substrate or harvest, except for valine decreasing from 3.96 to 3.15 g/ 16 g N. Changes in the N content of the fruit could be explained by changes in the stipe and pileus proportions as they had different N content (3.15 and 5.48 + or 0.031 g N/100 g DM respectively). The amino acid profile of the mushroom was adequate according to the FAO/WHO/UNU adult human amino acid requirements.

Upamanya and Rathaiah (2000) conducted an experiment to test the effect of fortification of rice straw with rice bran on the yield and quality of shiitake mushroom (*Lentinus edudes*) in Jorhat, Assam, India. Treatment comprised: (i) addition of rice bran at 5% w/w (weight of rice bran/weight of dry substrate) at the time of spawning and (ii) control (without rice bran). They reported that rice bran application had no effect on the crude protein content of mushroom.

Nuruddin et al. (2010) carried out an experiment to investigate the effect of different levels cowdung (0, 5, 10, 15 and 20%) on yield and proximate composition of *Lentinus edudes*. The highest number of primordia (70.63) and fruiting body (51.92) were observed in rice straw supplemented with 5% level of cowdung. The highest weight of individual fruiting body (4.71 g), biological yield (234.24 g), economic yield (227.72 g), dry yield (22.83 g) biological efficiency (140.26%) and benefit cost ratio (5.69) were observed in rice straw supplemented with 10% level of cowdung.

Amin et al. (2007) carried out an experiment to find out the primordia and fruiting body formation and yield of shiitake mushroom (*Lentinus edudes*) on paddy straw supplemented with wheat bran (WB), wheat flour (WF), maize powder (MP), rice bran (RB) their three combination (WB + MP, 1:1), WB + MP + RB, 1:1:1) and wheat braken (WBr) at six different levels namely 0, 10, 20, 30, 40 and 50% were studied. The minimum time (4.5 days) for primordial initiation was observed in the MP at 20% level and the highest number of effective fruiting bodies (60.75) was obtained in WF at 50% level. The highest biological yield (247.3 g/packet) was recorded at 10% level of (WBr).

Obodai et al. (2003) evaluated eight lignocellulosic by-products as substrate, for cultivation of the shiitake mushroom (*Lentinus edudes*). The yields of mushroom on different substrates were 183.1, 151.8, 111.5, 87.5, 49.5, 23.3, 13.0 and 0.0 g for composted sawdust of *Triplochiton scleroxylon*, rice straw, banana leaves, maize stover, corn husk, rice husk, fresh sawdust and elephant grass respectively. The biological efficiency (BE) followed the same pattern and ranged from 61.0%, for composted sawdust to 50.0% for elephant grass.

Baysal et al. (2003) conducted an experiment to spawn running, pin head and fruit body formation and mushroom yield of shiitake mushroom (*Lentinus edudes*) on waste paper supplemented with peat, chicken manure and rice husk (90 + 10; 80 + 20 W:W). The fastest spawn running (mycelia development) (15.8 days), pin head formation (21.4 days) and fruit body formation (25.6 days) and the highest yield (350.2 g) were realized with the substrate composed of 20% rice husk in weight. In general, increasing the ratio of rice husk within the substrate accelerated spawn running, pin head and fruit body formation resulted increase mushroom yields, while more peat and chicken manure had a negative effect on growing.

Silva et al. (2005) reported that mycelium extension is related to bio availability of nitrogen when they found that eucalyptus residues supplemented with cereal bran supported fast growth. However, the low amount of available nitrogen (N) in the lignocellulosic substrate of wood components is often considered as a limitation to its use as mushroom substrate. Lentinus species are popular and widely cultivated throughout the world mostly in Asia and Europe owing to their simple and low cost production technology and higher biological efficiency (Mane et al., 2007).

Mushroom possesses a vital portion of fungal kingdom creating a huge diversity. As a food major portion of these are unfit but as a whole these play an important role to maintain a sound health. Among them some are used as food and some as medicine. Many unknown mysterious components, very much needed for homeostasis of human body, are present in mushroom, which are now recognized as important area for biomedical researches. Shiitake mushroom (*Lentinus edudes*) is such a mushroom which is used both as food and medicine to ensure the fitness of body. It contains protein, carbohydrates, fat, fiber, water, different kinds of vitamins and minerals as well as secondary metabolites. Its statins are outstanding in decreasing the harmful plasma lipids and in reducing blood pressure thereby reducing the risk of cardiovascular diseases. The beta-glucan component of shiitake stimulates the immune system of the body. This mushroom is also found to be effective and beneficial in diabetes, cancer, microbial infections and so on. However, there are many other life saving components in shiitake mushroom whose nature, effectiveness and mode of actions are yet to be characterized and defined.These valuable unknown information are now subjects of researches. Bangladesh, like other countries of the world is conducting such type of researches but still has to go a long way in this field. At this very moment, it can be said beyond any doubt that shiitake mushroom is very good as an alternative food and as a medicine.

A study was conducted to examine the effect of different types of spawns on shiitake mushroom (*Lentinus edudes*) production using sawdust. Locally available grains of kurakkan (Eleusine coracana), maize (broken) (Zea mays), sorghum (Sorghum bicolor), and paddy (Oryza sativa) were used for spawn production. Sawdust spawned with different types *L. edudes* spawns were examined for spawn running (mycelia development), pinhead formation and fruit body formation, mean yield, and biological efficiency. The experiment was setup as a complete randomized design with three replicates. The kurakkan spawn produced an acceleration of spawn running, pinhead formation, fruit body formation and increased yield, compared with other types of spawn viz.; maize, sorghum, and paddy. The fastest spawn running of 21 ± 1 days, pinhead formation of 35 ± 1 days, highest mean yield of 55.37 ± 0.67 g and maximum fresh mushroom yield percentage of 30.76 ± 0.01 were realized for kurakkan spawn.

So many agricultural wastes such as coconut coir and bean straw can be turned into useful products to increase their biomass use in Ghana. Proper composting and effective management of these wastes can render them useful in mushroom production. The project was carried out to compare the response of *Lentinus edudes* on three types of substrates at three different composting periods. The substrate types used included sole bean straw, bean straw mixed with coconut coir at 2:3 ratio and sole coconut coir substrate. These were composted at different time periods and different parameters such as rate of mycelia formation; number of bags fully colonized; time of first flush; length, width and perimeter of fruits; yield of mushroom; dry matter content of the mushroom; biological efficiency and cost benefit analysis of using the different substrate types were determined. The sole coconut coir substrates gave the highest C:N ratios ranging from 97. 75 for the sole coir composted for 21 days to 106. 85 for the same substrate composted for a day. The sole bean straw and the mixed substrates produced similar C:N ratios of between 33.70 – 53.48. The substrates with the least C:N ratio gave the highest yield of mushrooms more especially in the sole coir and in the mixed substrates. Composting period did not affect the rate of mycelia formation, however, it affected the yield of the mushrooms. Substrates composted for longer periods in the sole coir gave the highest yield while the substrate composted for just a day in the mixed substrates produced the highest yield with the highest profit margin. The mixed substrates gave the greatest biological efficiencies ranging between 11.0 to 22.90 % as well as the highest returns. The results indicated that the effectiveness of substrates to produce higher returns depends on the initial C:N ratio of the substrates. As a result, substrates with high C:N ratio need longer periods of composting, while substrates with initial C:N ratio equal or below 55 do not need any composting.

A study to determine the winter mushroom production of the fruiting bodies on *Lentnus edudes* and to utilize ragi straw, black gram empty pod, green gram empty pod, bamboo dry leaf and casuarina dry leaf as supplements on paddy straw substrate and their effect on the sporophore production mushroom *Lentnus edudes*. The ragi straw(RS), black gram empty pod(BGP) and green gram empty pod(GGP) either alone or in combination with paddy straw (1:1, 1:2, 1:3 and 1:0 v/v) were used for sporophore production of *Lentnus edudes*. However, the biological efficiency of the fungus could be improved when these were used in various combinations with paddy straw. There was also faster substrate colonization and primordial initiation and higher number of fruiting bodies. The higher yield produced on by GGP + paddy straw (2:1) with total yield 905.30, B.E 90.53%, moisture 90.90%. folled GGP + paddy straw (1:1) having total yield 893.00, B.E 89.30%, moisture 92.12%. This study suggests using these three agricultural wastes to make good yield, income and eco friendly nature.

This review shows the biotechnological potential of shiitake mushrooms with lignocellulosic biomass. The bioprocessing of plant byproducts using *Lentinus* species provides numerous value-added products, such as basidiocarps, animal feed, enzymes, and other useful materials. The biodegradation and bioconversion of agro wastes (lignin, cellulose and hemicellulose) could have vital implication in cleaning our environment. The bioprocessing of lignin depends on the potent lignocellulolytic enzymes such as phenol oxidases (laccase) or heme peroxidases (lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase) produced by the organism. The cellulose-hydrolysing enzymes (that is, cellulases) basically divided into endo-β-1,4-glucanase , exo-β-1,4-glucanase I and II, and β-glucosidase, they attack cellulose to release glucose, a monomers units from the cellobiose, while several enzymes acted on hemicellulose to give D-xylose from xylobiose. These enzymes have been produced by species of *Lentinus* from lignocellulose and can also be used in several biotechnological applications, including detoxification, bioconversion, and bioremediation of resistant pollutants.

Tokyo Sanki Kohmuten, which installs solar power generation facilities, announced that it will harvest shiitake mushrooms grown by “solar sharing.” The company placed about 5,500 logs to grow shiitake mushrooms under about 290kW of solar panels. In 2015, Sanki Kohmuten started to grow shiitake mushrooms in Komaki City, Aichi Prefecture by using a solar sharing method. In the fall of 2016, the shiitake mushrooms grown under the solar panels became ready for harvest, and the company decided to harvest them with local concerned parties. The project site was formerly an abandoned agricultural land on which there were deserted houses. Sanki Kohmuten obtained the land, tore down the houses and reclaimed the land. On wisteria trellis-like mounting systems, 1,125 rectangular solar panels (output: 225W each) were installed in five areas. The company used its proprietary pile-based foundations and steel-frame mounting systems. Under the panels, Colocasia esculenta was also grown.

Cultivation of shiitake mushrooms is a fairly simply process. This guise will help you plan for an effective cultivation experience and give you some insight before you have the logs out and ready to grow mushrooms. Keep in mind that this process is slightly different for each grower an will benefit from personal adjustments as your become a more accomplished grower. Here is how we do it and some advice we learned along the way. The origins of Shiitake cultivation have been traced back thousands of years to Japan. People often found these mushrooms growing on downed 'shii' trees (this is where the mushroom got its name). People would take the mushroom-clad logs, place them next to logs without mushrooms and simply wait for the wind to disperse the spores. Thankfully since then, allot of research in Japan and China has gone into Shiitake-growing techniques, and we don't have to wait for the wind anymore. In fact, Shiitake is the second most produced mushroom in the world, following the common button mushroom.

The mushroom is a fungus and is quite finicky about its food source. Mushrooms lack the ability to use energy from the sun. They are not green plants because they do not have chlorophyll. Mushrooms extract their carbohydrates and proteins from a rich medium of decaying, organicmatter vegetation. This rich organic matter must be prepared into nutrient-rich substrate composts that the mushroom can consume. When correctly made, this food may become available exclusively to the mushroom and would not support the growth of much else. At a certain stage in the decomposition, the mushroom grower stops the process and plants the mushroom so it becomes the dominant organism in that environment.

Shiitake Mushrooms (Lentinus edodes), also known as Shitake Mushrooms, Black Oak Mushrooms, Black Forest Mushrooms, Golden Oak Mushrooms, Oakwood Mushrooms, and Japanese Mushrooms are an edible fungus and the second most consumed Mushroom in the world (behind Button Mushrooms). Shitake Mushrooms are native to Japan, Korea and China and naturally grow in forests on fallen oak logs. It is probably not surprising then, that the name "Shiitake" comes from two Japanese words, "Shii" (oak) and "Take" (Mushroom). In China, Shiitake Mushrooms are called "Hsaing Ku" which is Chinese for "Fragrant Mushroom." Shiitake Mushrooms are large and brown Mushrooms with a very fleshy, broad, flat cap that is an inch or two in diameter and curls inwards. Shiitakes vary from golden brownish to very dark brown because they get browner as they age. The cap looks something like an umbrella and the underside and stem are ivory or white. Shiitake Mushrooms fit the model, of the typical Mushroom (sporophore) and consist of a Cap (Pileus) and a Stem or Stalk (Stipe). Shiitake Mushrooms have been used in Chinese Herbal Medicine since prehistoric times. During the Ming Dynasty (AD 1368–1644), physician Wu Juei wrote that the mushroom could be used as a food and as a medicinal mushroom: taken as a remedy for upper respiratory diseases, poor blood circulation, liver trouble, exhaustion and weakness, and to boost qi or life energy, and it was also believed to prevent premature aging. The mushrooms have been widely consumed as a food for thousands of years in the East and more recently in the West. Today, shiitake mushrooms are very popular in the United States as well for their flavor as well as their medical properties. Research into the anticancer properties of shiitake mushrooms has been going on since at least the 1960s. Though Smart Kitchen does not advise it, Shiitake Mushrooms are widely foraged in Asia, even by novice mushroom hunters who take a risk. Unless you are an expert, or foraging with an expert, we vote against guide book foragin.

CHAPTER III
MATERIALS AND METHODS

The experiment was carried out to find out on the growth, yield and proximate composition of shitake mushroom (*Lentinum edodes*) grown on supplement sawdust. This chapter deals with a brief description on location and design of experiment, experiments and treatments, preparation of substrates, preparation of packets, cultivation of spawn packet, collection of produced mashrooms, proximate analysis of the mashrooms, data recording and their analysis under the following headings and sub-headings:

3.1 Location of experiment

The experiment was carried out at the Biochemistry laboratory and Mashroom Culture House (MCH) of the Department of a Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, during the period from October 2016 to March 2017.

3.2 Experimental materials

Mother culture of shitake mushroom was collected from National Mashroom Development and Extension Center (NAMDEC), Savar, Dhaka, Bangladesh.

3.3 Varietal characteristics of Shitake Mashroom

Shitake mushrooms (*Lentinus edodes*) are characterized by the rapidity of the mycelia growth and high saprophytic colonization activity on cellulosic substrates. If the temperature increases above 32^0C, its production markedly decreases.

3.4 Experiments and treatments

The experiment consists of five different type of sawdust with 12% wheat bran was taken as basal substrates. The experiment consists of the following treatments:

MT: Mango tree (*Mangifera indica*) sawdust supplemented with 12% wheat bran and 1% lime

ST: Teak tree (*Tectona grandis*) sawdust supplemented with 12% wheat bran and 1% lime

JFT: Jackfruit (*Artocarpus heterophyllus*) sawdust supplemented with 12% wheat bran and 1% lime

KT: White siris (*Albizia procer)* sawdust supplemented with 12% wheat bran and 1%

GT: Timber tree (*Anogeissus latifolia*) sawdust supplemented with 12% wheat bran and 1%

3.5. Medium preparation

A medium was prepared using different sawdust. With spawn preparing substrate; different supplements(at the different rate on dry weight basis) and $CaCO_3$(1%) Was added. The measured materials were taken in a plastic bowl and mixed thoroughly by hand and moisture was increased by adding water.

Moisture was measured by using the moisture meter and adjusted the moisture content at 50%. The mixed substrates were filled into 9x12 inch polypropylene bag @ 200g. The filled polypropylene bags were prepared by using plastic neck and plugged the neck with cotton and covered with brown paper placing rubber band to hold it tightly in place.

3.6. Sterilization, inoculation and mycelium running in spawn packets

Determination of spawn run

The growth of mycelium (linear length) in each bag was measured by a measuring tape at 6 day intervals. Using this data, spawn run rate (cm/day) was determined for every spawn type. When the mycelium fully covered the substrate bag(spawn run completed), bag were kept open in the growing house. The days required for the completion of spawn running in the substrate bag were recorded. Days for pinhead formation, fruit body (flush) formation and for harvest was recorded. Therefore the packets were about 2 hours and then these were kept for cooling. After cooling, 5g mother spawn were inoculated into the laminar airflow cabinet and were kept at 20-22^{0}C temperature until the packets become white with the mushroom mycelium. After completion of the mycelium running the rubber band, brown paper, cotton plug and plastic neck of the mouth of spawn packet were removed and the mouth was wrapped tightly with rubber band. Than this spawn packets were transferred to the culture house.

3.7 Cultivation of spawn packet

Two ends, opposite to each other of the upper position of plastic bag were cut in "D" shape with a blade and opened surface of substrate was scraped slightly with a tea spoon for removing the thin whitish mycelia layer. Then the spawn packets were soaked in water for 15 minutes and invested to remove excess water for another 15 minutes. The packets of each type were placed separately on the floor of culture room and covered with news paper. The moisture of the culture around 300-500 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22-25^{0}C. The first primordia appeared 2-4 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

3.8 Collection of produced mushroom

Shiitake mushroom matured within 5-7 days after primordial initiation. The mature fruiting body was identified by curial margin of the cap, as described by Hossain (2010). Mushroom were harvested by twisted to uproot from the base.

3.9 Data collection

3.9.1 Mycelium growth (%): Mycelium growth was counted by taking the full packet as a full unit and generally the data was taken at every two days intervals.

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

MRR= $\frac{L}{N}$ cm/day

Where,

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

N= Number of days

3.9.3 Time required to completion of mycelium running: Days required from inoculation to completion of mycelium running were recorded.

3.9.4 Time required for bump formation: Time required for bump formation were recorded.

3.9.5 Time required for opening to first harvest: Time required for opening to first harvest were recorded.

3.9.6 Length of stalk: Length of stalk was significantly influenced by different amount of substrate and different strain of shiitake mushroom. Measurements were recorded.

3.9.7 Diameter of stalk: Diameter of stalk was significantly influenced by different amount of substrates and different strains of shiitake mushroom. Measurements were recorded.

3.9.8 Diameter of pileus: Diameter of pileus was also highly significant influenced by the combined effect of different strains and different amount of substrates. Data were recorded.

3.9.9 Thickness of pileus: Thickness of pileus was also highly significant influenced by the combined effect of different strains and different amount of substrates. Data were recorded.

3.9.10 Number of effective fruiting body per packet: Number of well-developed fruiting body was recorded. Tiny fruiting body were discarded in counting.

3.9.11 Weight of individual fruiting body per packet: Average weight of individual fruiting body was calculated by dividing the total weight of fruiting body per packet by the total number of fruiting body per packet.

3.9.12 Biological yield

Biological yield per 300 g, 500g, 750g and 1000g packet was measured by weighing the whole cluster of fruiting body without removing the lower hard and dirty portion.

3.9.13 Economic yield

Economic yield per 300g, 500g, 750g and 1000g packet was recorded by weighing all the fruiting bodies in a packet after removing the lower hard and dirty portion.

3.9.14 Dry yield

About 50 g of randomly selected mushroom sample was taken in a paper envelop and was weighed correctly. The mushroom was oven dried at 72^0C temperature for 24 hours and weighed again. The weight of blank envelop was subtracted from both the initial weight. The dry yield was calculated using the following formula (Sarker, 2004):

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

3.9.15 Biological efficiency

Biological efficiency was determined by the following formula (Ahmed, 1998):

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

3.9.16 Benefit cost ratio:

The benefit cost ratio for different low cost substrates were computed based on present market price of mushroom and cost of different inputs in the markets (Hossain, 2010). Market price of raw mushroom is 300-400tk/kg in Bangladesh.

3.10 Proximate analysis of the mushrooms

3.10.1 Collection of the samples

Mushrooms grown from the spawn were collected packet wise and all the wastes and dusts were removed from the fruiting body. Therefore they are ready to be analyzed.

3.10.2 Moisture

Determination of Moisture was done by conventional method. About 10-20 g of each sample were weighed into separated and weighed petridishes and dried in an oven at 100^0C to 105^0C till the weight of the petridishes with their contents was constant. The moisture content was expressed as percent of the fresh fruiting bodies.

$$\text{Moisture\%} = \frac{\text{Initial weight - Final weight}}{\text{weight of the sample}} \times 100$$

3.10.3 dry matter

The dry matter content of the mushroom sample was calculated by subtracting of the percent moisture of each sample from 100. The process was repeat 3-4 times for achieving constant weight of the sample used. The constant weight of the dry sample was termed as dry matter.

% Dry matter = 100- % moisture content

3.10.4 Determination of protein

The Protein contents of the fruiting bodies of the mushrooms were determined by the standard Micro-kjeldhal procedure. According to this method total nitrogen contents of the samples were estimated and protein contents were finding out by multiplying by 6.25 to the total nitrogen values. The total nitrogen was determined by the Kjeldahl methods, which depends upon the conversion of protein nitrogen into ammonium sulfate, by digestion ammonia liberated from the ammonium sulfate by making the solution alkaline were distilled into known volume of a standard acid, which was then back titrated.

3.10.5 Carbohydrate estimation

The content of the available carbohydrate was determined by the following equation:

Carbohydrate (g/l00 g sample) = 100 - [(Moisture + Fat + Protein + Ash + Crude Fiber) g/100 g]

3.10.6 Total ash estimation

One gram of the sample was weighed accurately into 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°C. It was then cooled in a desiccator and weighed. To ensure completion of ashing, the crucible was then heated in the muffle furnace for 1h, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or grayish white in color. Then total ash was calculated as following equation:

Ash content (g/l00 g sample) = Wt of ash × 100/Wt of sample taken

3.11 Elementary composition analysis

3.11.1 Equipments

Electric balance, grinding machine, desiccators, Atomic Absorption Spectrometer (AAS), Spectrophotometer, Porcelain crucible, Muffle furnace, Oven, Beaker and Flame Photometer.

3.11.2 Nitrogen estimation

Total nitrogen was determined by a micro kjaldhal apparatus in the traditional method and calculated using the following formula.

$$\% \text{ N in the supplied fiber sample} = \frac{(a \times M_{HCl} - b \times M_{NaOH}) \times 1.401}{c}$$

Where,

a = ml HCl measured into the conical task in the distill (usually 20.00 ml)

b= ml NaOH used for titration of the content in the conical flask

M_{HCl} = Molarity of the HCl measured into the conical flask

M_{NaOH}= Molarity of the NaOH used for titration

c= g of mushroom powder used for the analysis

3.11.3 Phosphorus estimation

5 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette. 30 ml water was added, mixed and then 10 ml ammonium molybdate-ascorbic acid solution was added to volume with water and mixed. After 15 minutes, the absorbance was measured on a spectrophotometer at 890 nm.

3.11.4 Calcium and magnesium estimation

20 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette. 5 ml $LaC1_3$-solution was added and the volume was made with water and mixed. Then the content of Ca and Mg was measured by atomic absorption spectrophotometer (AAS).

3.11.5 Estimation of Fe, Zn and Co

The content of Fe, Zn and S elements were measured by atomic absorption spectrophotometer (AAS) directly in the undiluted filtrate.

3.11.6 Calculations

For Ca, Mg, K

$$\text{mg per kg sample} = \frac{a \times 25000}{b \times c}$$

Where, a= mg/L Ca, Mg, K or P measured on atomic absorption spectrophotometer, flame photometer or spectrophotometer

b= ml diluted filtrate transferred into the 50 ml volumetric flask for determination of Ca, Mg, K or P

c = g sample weighed into the digestion tube

If an additional dilution is made before the transfer to the 50 ml volumetric flask, the result is multiplied with the dilution factor. But the above elements were in trace. So addition of dilution was not to be performed.

For Fe, Zn, S

$$\text{mg per kg sample} = \frac{d \times 100}{c}$$

Zn and Fe measured on atomic absorption spectrophotometer

c = g sample weighed into the digestion tube

3.12 Statistical analysis of data

The data obtained for different parameters were statistically analyzed to find out the significance of the difference among the treatment. All the data collected on different parameters were statistically analyzed by Duncan's Multiple Range Test (DMRT). The mean values of all the characters were evaluated and analysis of variance was performing by the 'F' test. The significance of the difference among the treatments means was estimated by the least significant difference (LSD) test at 5% level of probability (Chan, 2001).

CHAPTER IV

RESULTS AND DISCUSSION

The study was conducted to find out the effect of different sawdust on the production and proximate composition of *Lentinus edudes*. The results have been presented and discussed with the help of table and graphs and possible interpretations given under the following headings:

Experiment 1: Effect of different sawdust substrates on yield and yield contributing character of *Lentinus edudes*

4. 1. Effect on mycelium growth

4.1.1.1 Effect of different sawdust substrates on mycelium running rate in spawn (mm)

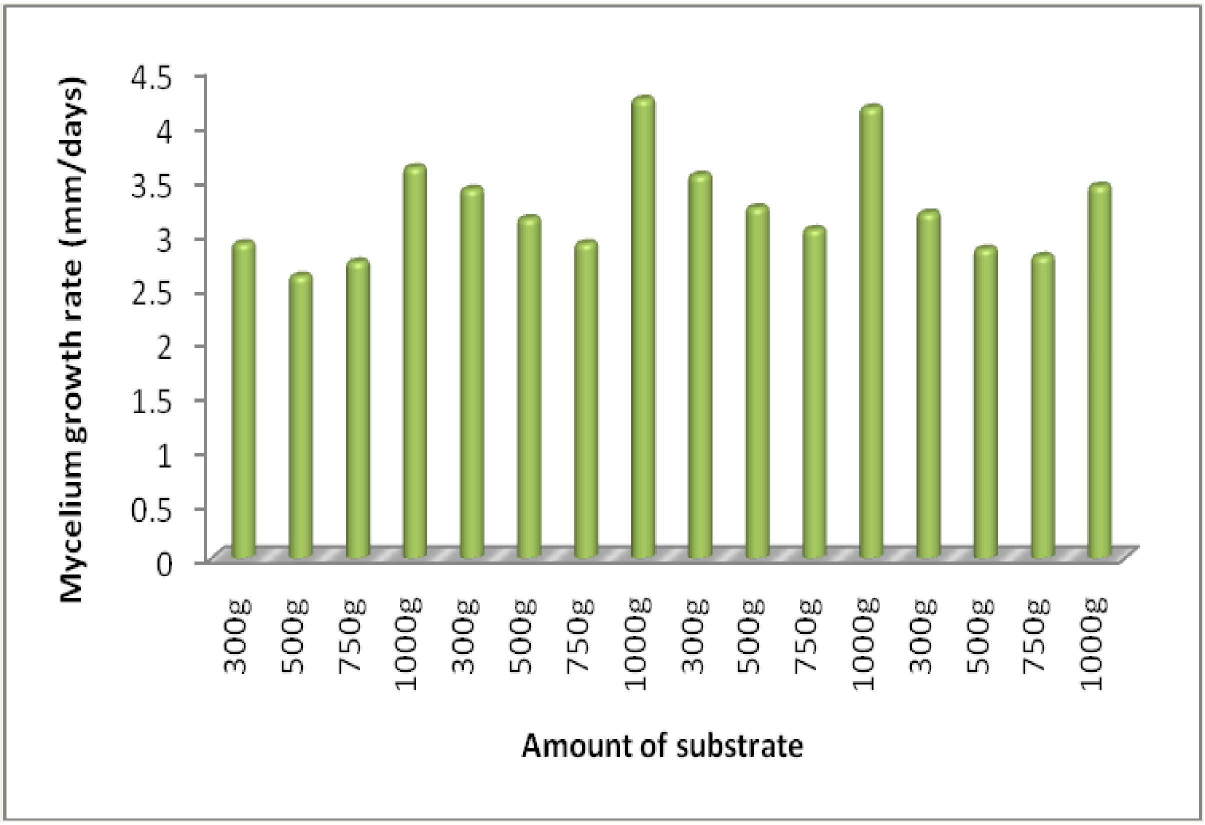

Fig.1 Effect of mycelium growth rate

Mycelium running rate per day (MMR) for each type of substrates was measured after the mycelium colony crossed the shoulder of the packet. The linear length was measured at different places of packet. Statistically significant variation was recorded in terms of mycelium running rate per day (MMR) of *Lentinus edudes* due to different sawdust substrate used (Table 1). The highest mycelium growth rate (4.23 mm/day) was recorded in Le 11 with 1000g of spawn packet which was statistically similar to the combined effect of Le 12 with 1000g of spawn packet. The lowest mycelium growth rate (2.60 mm/day) was recorded in Le 8 with 500g weight of spawn packet. Different sawdust showed different mycelium running because of different carbohydrate based on availability and the environment of the spawn. The present findings found more or less similar with the previous workers. Hossain *et al.* (2010) reported that sawdust amended with different organic supplement like wheat chaff, wheat bran, paddy straw, cotton waste etc. provided suitable condition for spawn running. Chen (2001) found that the mycelium running rate of shiitake mushroom greatly influenced with the supplement of wheat barns in different levels. Bhuyan (2008) also found similar result as found in the present experiment.

4.1.1.2 Effect of different sawdust substrates on time required to completion of mycelium running (TRMR)

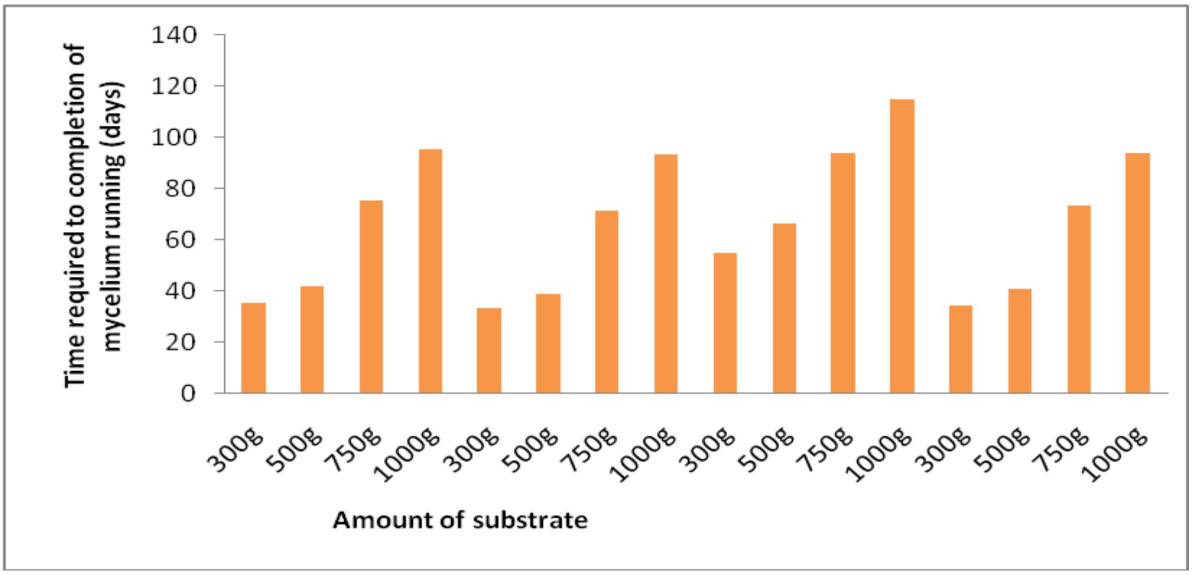

Fig.2 Effect of different sawdust substrates on time required to completion of mycelium running.

Time from stimulation to primordial initiation varied significantly due to different sawdust used (Table 1). The highest time (114.80 days) required to completion of mycelium running was obtained from the treatment completion of strain Le 12 with 1000g spawn packet. The lowest time (33.25 days) required to completion of mycelium running was obtained from the strain Le 11 with 300g spawn packet which was statistically similar to the treatment combination of Le 8 with 300g spawn packet and Le 16 with 300g spawn packet. The result of present findings keeps in with the findings of previous scientists (Hossain 2010, Sarkar 2004, Ruhul Amin, 2007, Bhuyan, 2008). Hossain (2010) found that the fruiting bodies appeared 15-20 days after the bags were removed and the first crop was harvested 3-5 days later on wheat straw and *Lentinus edudes* can be successfully cultivated in winter season. Sarkar, (2004) observed that duration from primordial initiation to first harvest of winter mushroom was significantly lower as compared to control where no supplement was used and the duration required for total harvest of winter mushroom increased with the level of supplement used. In the present study, the time required for total harvest also decreased with the level of supplements increased compared to rice straw alone. Ruhul Amin *et al.* (2007) found significant differences among the level of supplements used for preparing the substrates. Bhuyan (2008) also found similar effect as found in the present study.

4.1.1.3 Time required for bump formation (TRBF)

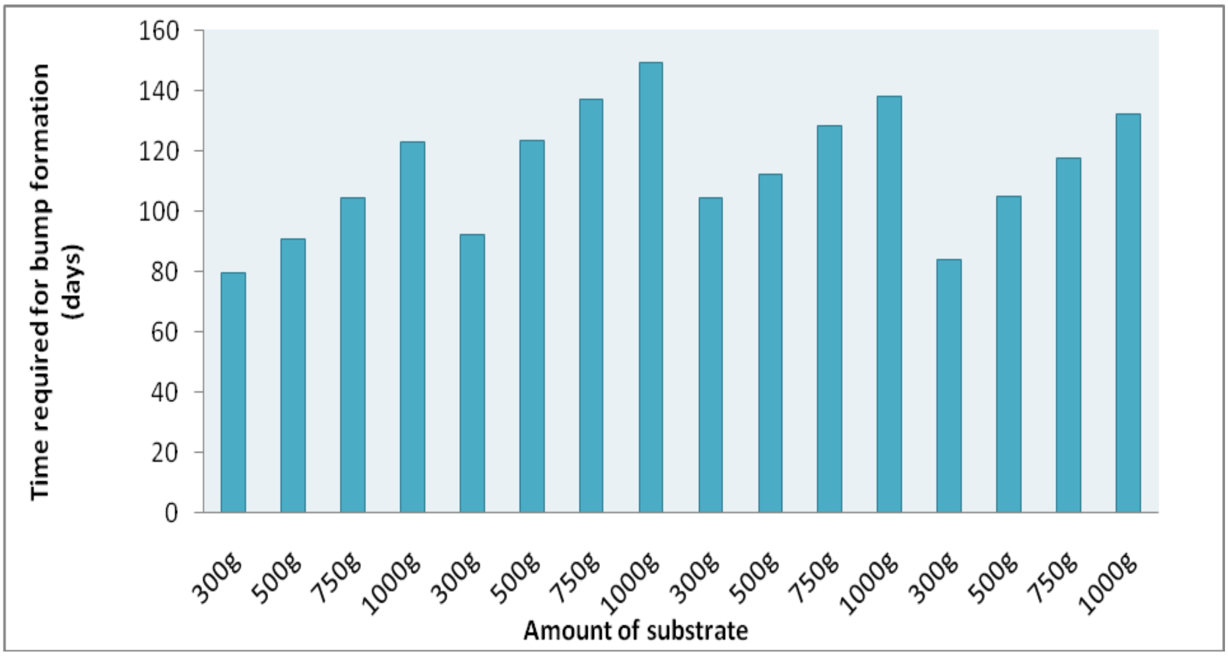

Fig.3 Time required for bump formation.

Significant variation was found on time required for bump formation by the combined effect of strain and different amount of substrates. The highest time (149.30 days) required for bump formation was obtained from the treatment combination of strain Le 11 with 1000g spawn packet. The lowest time (79.50 days) required for bump formation was obtained from the strain Le 8 with 300g spawn packet which was statistically similar to the treatment combination of Le 16 with 300g spawn packet (Table 1).The result of present findings keeps in with the findings of previous scientists (Khan *et al.* 2001, Dhoke *et al.* 2001, Royse, 2001). Khan *et al*. (2001) reported that after spwan running pinhead formation took 7-8 days and fruting body formed after 3-5 days, sporocarps may be harvested after 10-12 days. Dhoke *et al.* (2001) found significant effect of different agro-waste on the yield of mushroom. The days required for first picking varied from 11.25-12.00 days and the final picking varied from 42.25-43.50 days depending on different substrates. Royse (2001) found as the spwan rate increased the number of days to production decreased.

Table 1. Effect of strains and different amount of substrates on growth and development of shiitake mushroom

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

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

Table 2: Effect of strains and different amount of substrates on yield attributes and yield of shiitake mushroom

Amount of substrates	Number of fruiting body	Number of effective fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield (g)
Strain of shiitake (Le 8) mushroom							
300g	19.00 d	14.50 c	3.95 fg	1.78 cd	6.15 c	1.05 efg	85.75 e
500g	21.00 cd	17.50 bc	4.90 bcd	1.25 efg	5.93 c	1.10 ef	112.30 b
750g	4.00 cd	3.75 de	7.18 a	1.40 de	7.55 b	1.78 b	56.50 h
1000g	1.25 g	1.25 e	3.95 fg	2.45 b	10.38 a	2.33 a	70.00 f
Strain of shiitake (Le 11) mushroom							
300g	24.00 c	17.75 bc	5.25 bc	0.83 fg	5.88 c	0.90 gh	89.75 de
500g	29.00 b	18.75 b	5.33 bc	1.20 efg	6.00 c	1.15 ef	94.00 d
750g	11.00 e	6.25 d	3.38 g	0.98 efg	4.43 def	0.88 h	29.50 k
1000g	6.00 f	3.75 de	3.88 fg	0.80 g	5.70 cd	0.83 h	38.00 j
Strain of shiitake (Le 12) mushroom							
300g	23.75 c	16.00 bc	3.85 fg	0.98 efg	5.05 c-f	1.05 fg	63.50 g
500g	81.50 a	53.00 a	4.28 def	0.88 fg	3.75 f	0.80 h	135.80 a
750g	2.75 fg	1.75 a	5.05 bc	2.33 b	4.13 ef	1.38 cd	24.50 l
1000g	1.75 g	1.00 e	4.13 efg	10.25 a	1.28 g	1.43 c	34.00 j
Strain of shiitake (Le 16) mushroom							
300g	23.50 c	17.50 bc	5.50 b	1.05 efg	4.33 def	1.20 ef	72.75 f
500g	23.75 c	15.75 bc	4.85 b-e	1.43 de	5.50 cde	1.23 de	104.50 c
750g	4.25 fg	3.50 de	5.55 b	2.00 bc	5.20 cde	1.38 cd	43.00 i
1000g	1.25 g	1.25 e	4.73 cde	1.33 def	9.60 a	1.50 c	57.00 h
LSD(0.05)	**3.30**	**3.08**	**0.67**	**0.45**	**1.24**	**0.16**	**4.41**
CV(%)	**13.39**	**17.92**	**10.01**	**16.31**	**15.30**	**8.89**	**4.45**

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

4.1.1.4 Time required from opening to first harvest (TROFH)

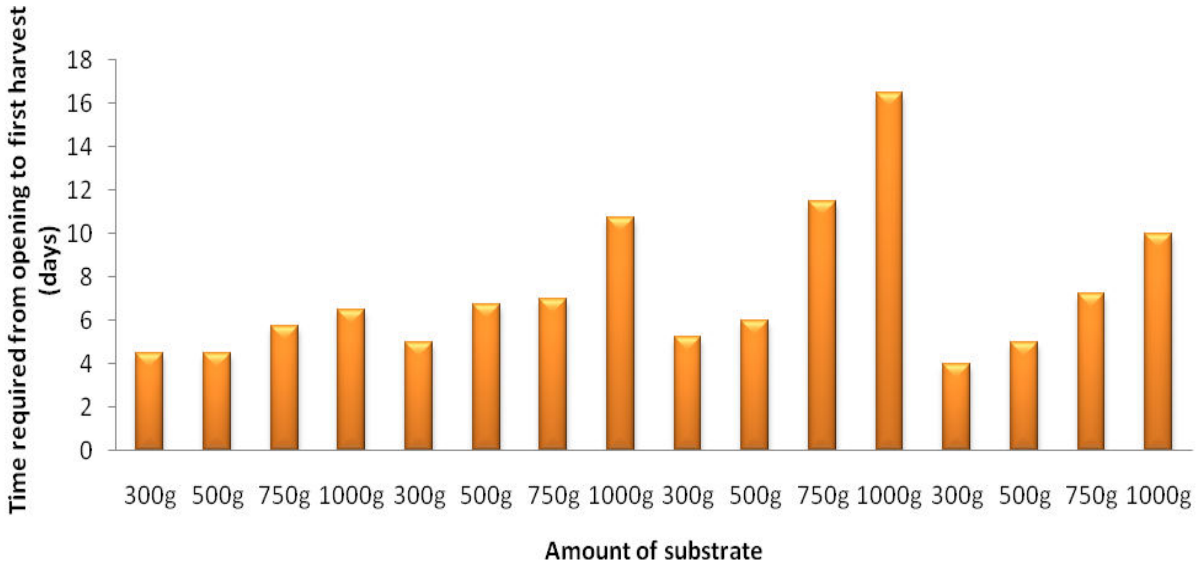

Fig.4 Time required from opening to first harvest.

Statistically significant variation was found in terms of average number of primordia per packet of *Lentinus edudes* due to diferent sawdust used. Significant variations were found on time required from opening to first harvest by the combined effect of strain and different amount of substrates. The highest time (16.50 days) required from opening to first harvest was obtained from the treatment combination of strain Le 12 with 1000g spawn packet. The lowest time (4.00 days) required from opening to first harvest was obtained from the strain Le 16 with 300g spawn packet (Table 1). The result of the present study supported with the previous findings (Amin, 2004; Sarker, 2004 and Dey, 2006). Amin (2004) in his experiment found that the highest number of primordia of shiitake mushroom was found in sterilized paddy straw but lowest was found in saw dust. Dey (2006) found that the number of primordia and the average yield of shiitake mushroom give the lowest value with sawdust. Ahmed (1998) reported significantly different number of primordia on different substrates. Bhuyan (2008) found similar findings when he growing shiitake mushroom on saw dust supplemented with different levels of cow dung.

4.1.1.5 Time required for harvest (TRH)

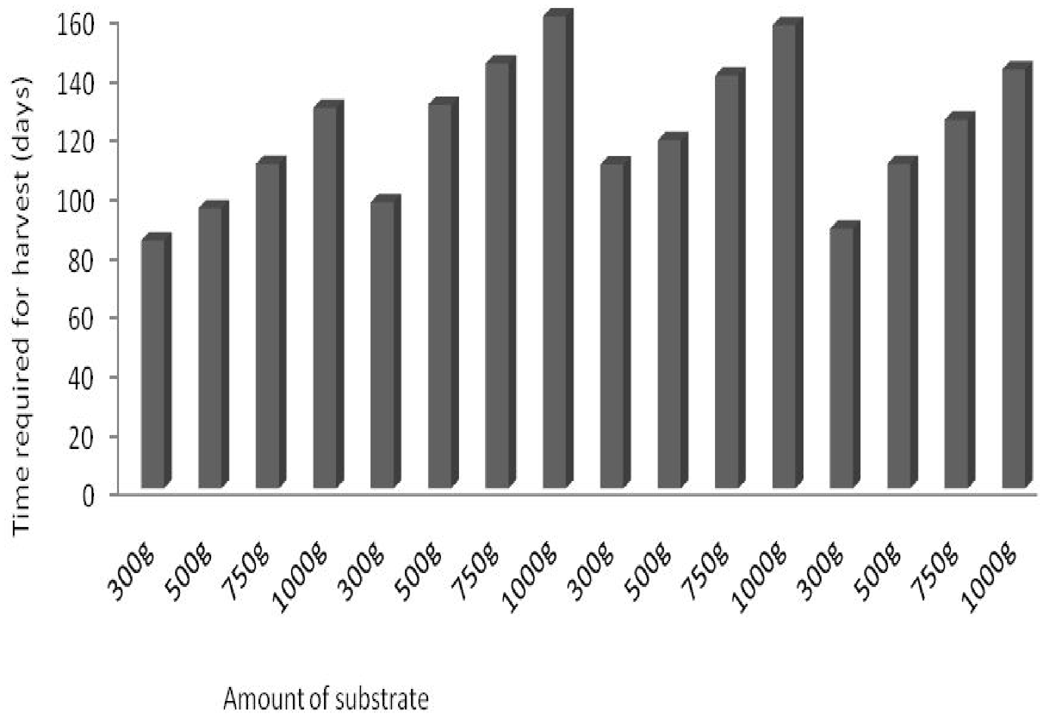

Fig.5 Time required for harvest.

Statistically significant variation was found in terms of average number of effective fruiting body per packet of *Lentinus edudes* due to different sawdust used (Table 1). The highest time (160.00 days) required for harvest was recorded from the treatment combination of Le 11 with 1000g weight of spawn packet which was statistically similar to the treatment combination Le 12 with 1000g weight of spawn packet. The lowest time (84.00 days) required for harvest was found from the treatment combination of Le 8 with 300g weight of spawn packet which was statistically similar to the treatment combination of Le 16 with 300g spawn packet. The findings of the present study matches with the study of Hossain *et al.* (1993) who reported that the number of effective fruting body were lowest, but increase when the substrates was mixed with different supplements. The comparative similar findings were also found by Adamovie *et al.* (1996), Ruhul Amin (2007) and Ahmed (1998).

4.1.2 Effect of strains and different amount of substrates

4.1.2.1 Length of stalk (LS)

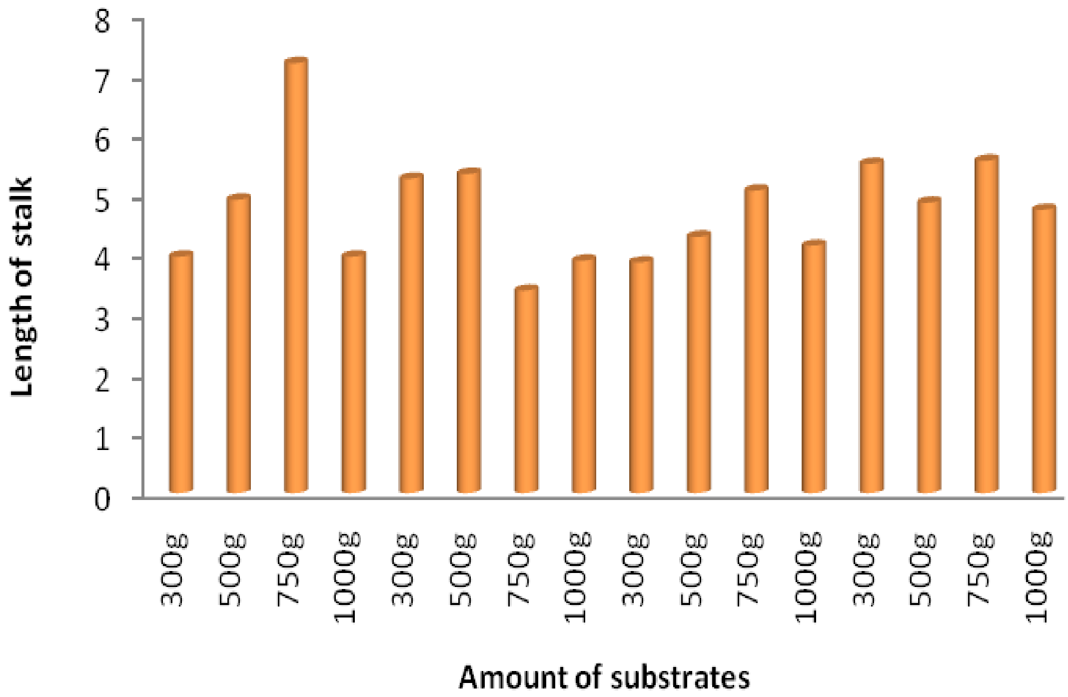

Fig.6 Length of stalk

Statistically significant variation was found in terms of average weight of individual fruiting body of *Lentinus edudes* due to different sawdust used (Table 2). Different sawdust substrates had great effect on average weight of individual fruiting body. The highest length (7.18 cm) of stalk was found from the treatment combination of Le 8 with 750g weight of spawn packet followed by Le 16 with 750g weight of spawn packet. The lowest length (3.38 cm) of stalk was recorded from the treatment combination of Le 11 with 750g weight of spawn packet. The findings of this experiment were also supported by the findings of Hossain *et al*. (2010) and Bhuyan (2008). Sarker (2004) found significant increase in weigh of fruiting body in gram per sporocarps over control in spawn packet containing different supplement in compared with sawdust alone. Bhuyan (2008) found comparatively higher weigh of individual fruiting body ranged from (5.02g to 7.01g), which may be due to environmental conditions or growing season.

4.1.2.2 Diameter of stalk (DS)

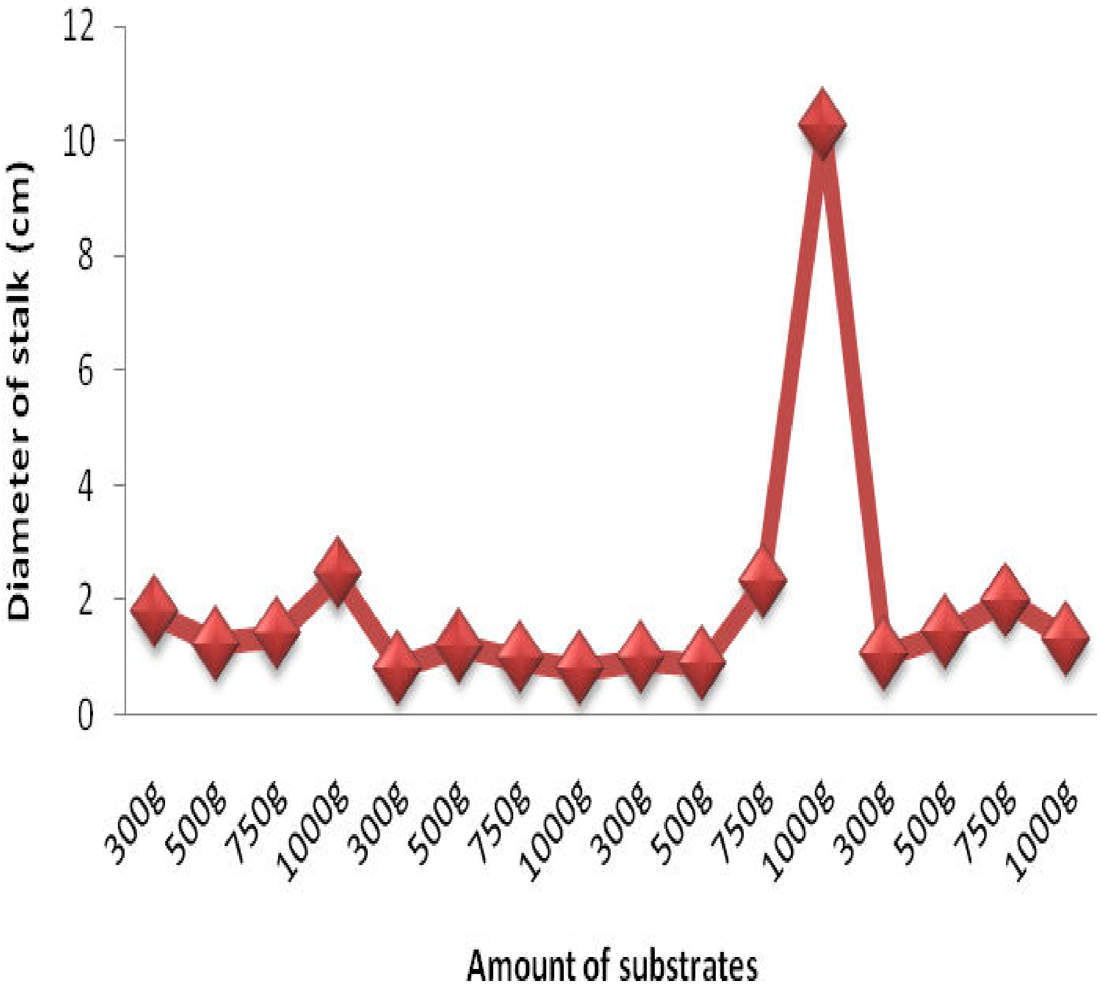

Fig.7 Diameter of stalk

Statistically significant variation was found in terms of Diameter of stalk of *Lentinus edudes* due to different sawdust used (Table 2). The highest diameter (10.25 cm) of stalk was observed from the treatment combination of Le 12 with 1000g weight of spawn packet. The lowest diameter (0.80 cm) of stalk was observed from the treatment combination of Le 11 with 1000g weight of spawn packet**.** The findings of the present study matches with the study of Habib (2005) and Hossain *et al.* (2010). Both of two mentioned that the stalk diameter of *Lentinus edudes.* On different substrate varied from 1.93 cm to 2.97cm and diameter range from 0.74cm to 1.05cm.

4.1.2.3 Diameter of pileus (DP)

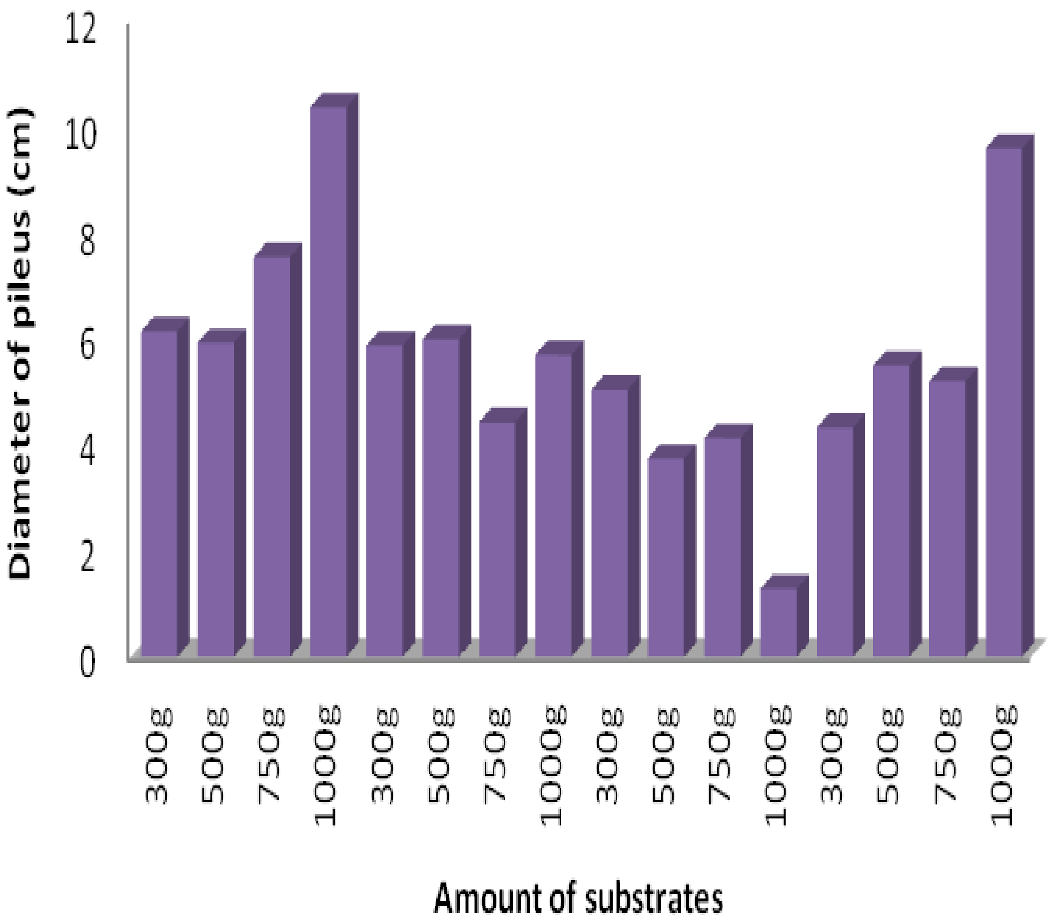

Fig.8 Diameter of pileus

Different sawdust showed statistically significant differences in terms of diameter of pileus. All the treatments were statistically similar (Table 2). The highest diameter (10.38 cm) of pileus was recorded from the treatment combination of Le 8 with 1000g weight of spawn packet which was statistically similar to the treatment combination of Le 16 with 1000g spawn packet. The lowest diameter (1.28 cm) of pileus was recorded from the treatment combination of Le 12 with 1000g weight of spawn packet. The findings of present study matches with the study of Habib (2005) who found that the thickness of pileus ranged from 0.45cm to 0.70cm due to different substrates and Hossain *et al.* (2010) reported that the diameter of pileus ranged from 0.05cm to 0.80 cm in case of shiitake mushroom.

4.1.2.4 Thickness of pileus (TP)

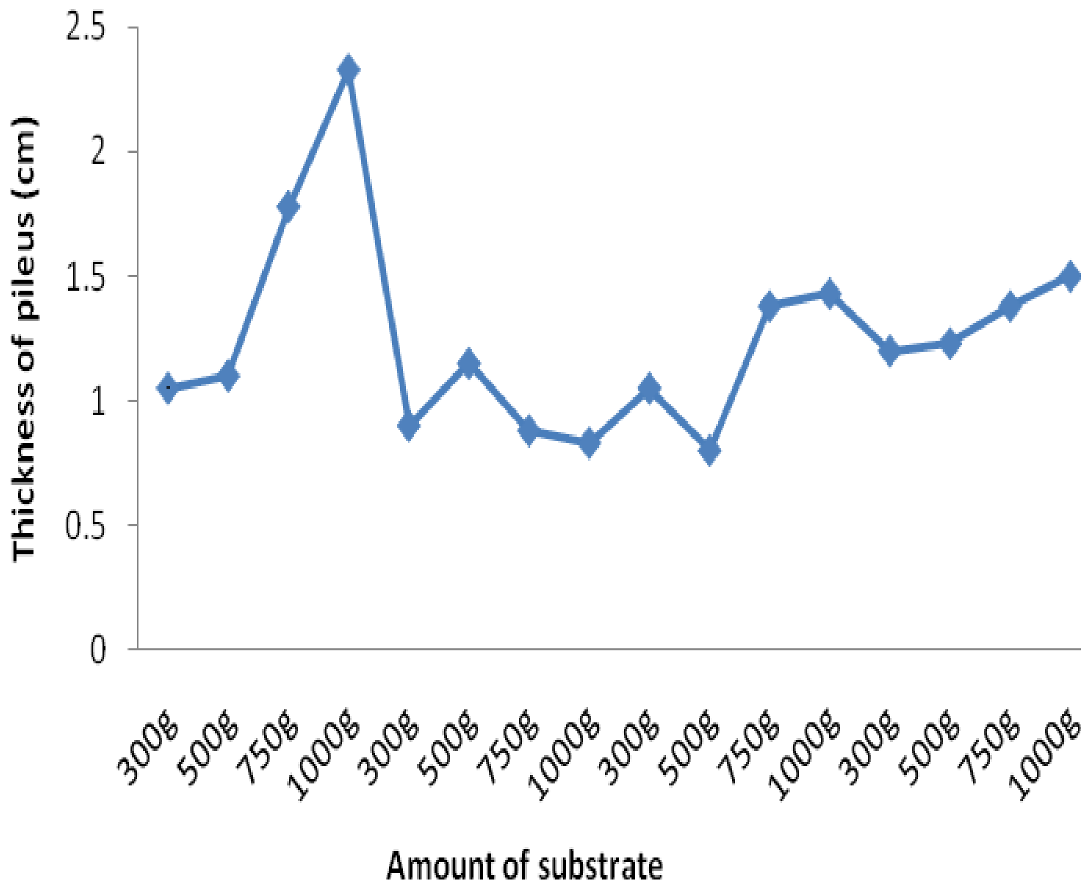

Fig.9 Thickness of pileus

Thickness of pileus was also highly significant influenced by the combined effect of different strains and different amount of substrates. The highest thickness (2.33 cm) of pileus was recorded from the treatment combination of Le 8 with 1000g weight of spawn packet. The lowest thickness (0.80 cm) of pileus was recorded from the treatment combination of Le 12 with 500g weight of spawn packet which was statistically similar to the treatment combination of Le 11 with 750g and 1000g weight of spawn packet (Table 2). The findings of present study matches with the study of Habib (2005) who found that the thickness of pileus ranged from 0.45cm to 0.70cm due to different substrates and Hossain *et al.* (2010) reported that the diameter of pileus ranged from 0.05cm to 0.80 cm in case of shiitake mushroom.

4.1.2.5 Yield (g)

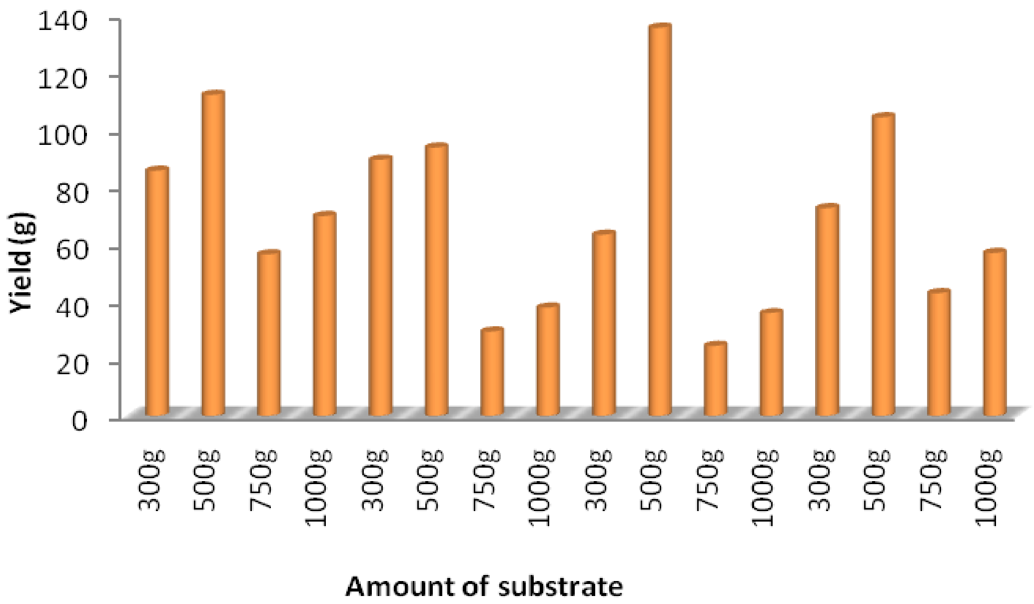

Fig.10 Yield

Yield of *Lentinus edudes* mushroom varied significantly due to different sawdust used under the present trial (Table 2). Different sawdust substrates had great effect on yield**.** The yield was significantly influenced by the combined effect of different strains and different amount of substrates. The highest yield (135.80g) was recorded from the treatment combination of Le 12 with 500g weight of spawn packet followed by Le 8 with 500g weight of spawn packet. The lowest yield (24.50g) was recorded from the treatment combination of Le 12 with 750g weight of spawn packet. The result of the present study found similar with the of previous studies (Hossain *et al.,* 2010; Chen *et al.,* 2001 and Dhoke *et al.*, 2001). Amin *et al.* (2004) found the highest yield 141.3 g/packet. Hossain *et al.* (2010) examined the effects of adding different supplements to substrates for growing shiitake mushrooms (*Lentinus edudes*) and found adding 5% supplements gave the highest yield of shiitake mushroom. Dhoke *et al.* (2001) found significant effect of different agro-wastes on yield of shiitake mushroom. Baysal *et al.* (2003) found the highest yield of shiitake mushroom (*Lentinus edudes*) with the substrate composed of 20% wheat brand in weight.

Table 3. Effect of different sawdust on yield attributes of *Lentinus edudes*

Treatments	Average no. of primordial/packet	Average no. of Fruiting body/packet	Average weight of individual fruiting body(g)	Average length of stripe (cm)	Average thickness of pileus (cm)
ST	57.00 b	46.37 b	3.61 b	1.02 b	0.67 b
MT	66.45 a	70.23 a	3.94 a	1.60 b	0.69 a
KT	62.59 b	48.67 a	3.35 c	1.58 a	0.70 a
JFT	57.23 b	44.17 c	3.58 b	1.03 c	0.61 b
GT	53.24 c	42.89 d	3.28 c	0.96 c	0.55 c
CV (%)	0.52%	0.42%	0.56%	1.29%	5.35%
LSD(0.05)	0.681	0.411	0.025	0.056	0.046

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

ST: Teak tree (*Tectona grandis*) sawdust supplemented with 12% wheat bran and 1% lime

MT: Mango tree (*Mangifera indica*) sawdust supplemented with 12% wheat bran and 1% lime

KT: White siris (*Albizia procer)* sawdust supplemented with 12% wheat bran and 1%

JFT: Jackfruit (*Artocarpus heterophyllus*) sawdust supplemented with 12% wheat bran and 1% lime

GT: Timber tree (*Anogeissus latifolia*) sawdust supplemented with 12% wheat bran and 1%

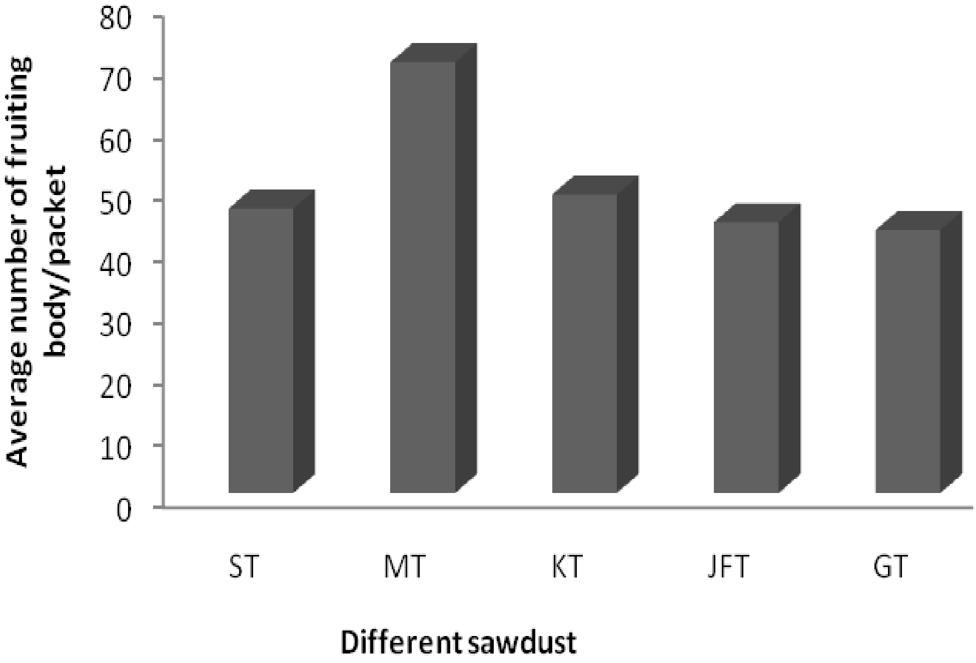

Fig.11 Performance of sawdust substrate on the average number of fruiting body/ packet of shiitake mushroom

Statistically significantly variation of fruiting body/packet of shiitake mushroom (*Lentinus edudes*) showed due to different sawdust under the present trial (Figure 2). The highest average number of fruiting body per packet was recorded from ST (70.23) while the lowest average number of fruiting body per packet was observed from GT (42.89) where JFT (48.67), MT (46.37), KT (44.17) are statistically similar. The result of the present findings keeps in with the findings of previous scientists (Yoshida et al., 1993; Al Amin, 2004; Sarker, 2004, Bhuyan, 2008). Yoshida et al. (1993) reported that the number of fruiting bodies was lower, but increased when the substrates was mixed with different supplements. Al Amin (2004) reported that the number of the primordial grown on different substrates differed significantly. Sarker, (2004) found that the number of primordial increased with the levels of supplement and continued up to a certain range and decline thereafter. In the present study the average number of fruiting body in creased up to 10% of cow dung used as supplement and decreased thereafter. Bhuyan (2008) in a same type of experiment found similar results.

4.1.3 Effect of different sawdust on the yield

4.1.3.1 Effect of different sawdust substrates on biological yield

Biological yield of *Lentinus edudes* mushroom varied significantly due to different sawdust used under the present trial (Table 4). Different sawdust substrates had great effect on biological yield. The highest biological yield was recorded from MT (211.78 g), which was statistically similar with KT (201.23 g) followed by ST (194.35 g), while the lowest biological yield was recorded in GT (169.76 g) followed by JFT (180.70 g). The result of the present study found similar with the of previous studies Hossain *et al.,* 2010; Chen *et al.,* 2001 and Dhoke *et al.*, 2001). Amin *et al.* (2004) found the highest biological yield 247.3 g/packet. Hossain *et al.* (2010) examined the effects of adding different supplements to substrates for growing shiitake mushrooms (*Lentinus edudes*) and found adding 5% supplements gave the highest yield of shiitake mushroom. Dhoke *et al.* (2001) found significant effect of different agro-wastes on yield of shiitake mushroom. Baysal *et al.* (2003) found the highest yield of shiitake mushroom (*Lentinus edudes*) with the substrate composed of 12% wheat bran in weight.

4.1.3.2 Effect of different sawdust substrates on economical yield

Economic yield of *Lentinus edudes* grown on different sawdust showed statistically significant variation (Table 4). The highest economic yield was recorded from MT (245.38 g), followed by KT (200.21 g), whereas the lowest economic yield was observed in GT (172.32 g) which was followed by ST (198.67 g). The findings of this experiment also supported by the earlier findings of Hossain *et al.* (2010) and Chen *et al.* (2001). Chen *et al.* (2001) found that the trend of economic yield corresponded with different supplements at different level. Hossain *et al.* (2010) found the highest yield of shiitake mushroom (*Lentinus edudes*) with the substrate composed of 12% wheat bran in weight. Appreciable variations in economic yield also observed at different levels of supplements under different substrate-supplement combinations. Payapanon *et al.* (1994) mentioned that suitable amount of supplements added to sawdust medium maximized economic yield of shiitake mushroom at optimum production cost. Hossain et al. (2010) found appreciable variations in economic yield also observed at different levels of supplements under different substrate-supplement combinations. Bhuyan (2008) observed that the yield of *Lentinus edudes* responded with the levels of supplements used with sawdust and increased with the level of supplementation and declined thereafter.

4.1.3.3 Effect of different sawdust substrates on Dry yield

Different sawdust showed statistically significant variation in terms of dry yield of *Lentinus edudes* mushroom (Table 4). The highest dry yield was observed from MT (25.11 g), followed by KT (22.21 g) .On the other hand, the lowest dry yield was attained in GT (15.91 g) which was statistically similar with ST (19.79 g). The result of the present study was supported by the study of previous researcher Hossain *et al*. (2010) who found the range of dry yield ranged from 24.28 to 29.98 g/packet of *Lentinus* grown on different substrate. Kulsum *et al.* (2009) found that the highest dry yield was 21.27 g due to sawdust. Ahmed (1998) observed that the diameter of pileus increased the quality and yield mushroom and highest dry yield from mango sawdust.

4.1.3.4 Effect of different sawdust substrates on Biological efficiency

Different sawdust showed statistically significant variation for biological efficiency of shiitake mushroom under the present trial (Table 4). The maximum biological efficiency was recorded from MT (123.21%) again the lowest biological efficiency was observed in GT (92.97%), which was statistically similar with KT (111.46%) and ST (101.13%). Karim et al. (1997); Chen and Royse (2001); Obodai et al. (2003); and many other researchers reported earlier similar findings from their experiment. Kalita et al., (1997) observed biological efficiency for different substrates ranged 35.2 gto 60.9%. Obodai et al. (2003) found biological efficiency (BE) followed a pattern and range from 61.0% to 80.0%. But Biswas et al. (1997) found supplementation of promoted biological efficiency (125.75%). Shen and Royse (2001) found supplements combined with basal ingredient results better mushroom quality as well as biological efficiency.

4.1.3.5 Effect of different sawdust substrates on Benefit cost ratio

Different sawdust showed statistically significant variation in terms of benefit cost ratio of *Lentinus edudes* mushroom (Table 4). The highest benefit cost ratio was found from MT (5.91), which was statistically similar with KT (5.38) and followed by ST (5.23) and JFT (4.67). On the other hand, the lowest benefit cost ratio was recorded in GT (3.93). The present findings found similar with the findings of previous research. Lim *et al.* (1997) analyzed the cost and return of *Lentinus* mushroom production and found the BCR of 8.9 and 5.1, respectively. Ahmed (1998) also observed the benefit cost ratio of 7.32, 23.78 and 16.23 in case of *Lentinus edudes*. The cause of these variations between the results of this study might be due to consideration of other costs involved in the production of shiitake mushroom or might be due to measuring system Hossain *et al*, (2010) mentioned the performances of substrates were significantly differed based on benefit cost ratio. They reported the highest cost benefit Ratio of 6.50 with wheat straw

Table 4. Effect of different sawdust on the yield of (*Lentinus edudes*)

Treatments	Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)	Benefit cost ratio
ST	194.35 b	198.67 b	19.79 b	101.13 b	5.23 b
MT	211.78 a	245.38 a	25.11 a	123.21 a	5.91 a
KT	201.23 b	200.21 b	22.21 b	111.46 b	5.38 a
JFT	180.70 c	187.43 c	16.32 c	98.56 c	4.67 b
GT	169.76 d	172.32 d	15.91 d	92.97 c	3.93 c
CV (%)	0.02%	0.01%	0.10%	0.15%	1.43%
LSD (0.05)	0.143	0.084	0.081	0.113	0.101

In a column means having similar letter(s) are statistically similar and those having dissimilar Letter (s) differ significantly at 0.05 level of probability

ST: Teak tree (*Tectona grandis*) sawdust supplemented with 12% wheat bran and 1% lime

MT: Mango tree (*Mangifera indica*) sawdust supplemented with 12% wheat bran and 1% lime

KT: White siris (*Albizia procer)* sawdust supplemented with 12% wheat bran and 1%

JFT: Jackfruit (*Artocarpus heterophyllus*) sawdust supplemented with 12% wheat bran and 1% lime

GT: Timber tree (*Anogeissus latifolia*) sawdust supplemented with 12% wheat bran and 1%

4.2 Experiment 1: Effect of different sawdust substrates on proximate analysis of *Lentinus edudes*

4.2.1 Effect on proximate composition of *Lentinus edudes* mushroom that produced in different treatment of this experiment.

4.2.1.1 Moisture content

Moisture content showed statistically significant variation in different treatment (Table 5). The highest moisture content was observed from MT (98.76%), which was statistically similar to KT (87.46%) and followed by ST(83.34%), while the lowest moisture content was found in GT (67.76%) which was statistically similar with JFT (76.21%). The result of the present study found more or less similar with the study of previous researchers (Hossain *et al*., 2010; Chen *et al.,* 2001 and Rahman, 1994).

Hossain *et al*. (2010) cultivated the shiitake mushroom (*Lentinus edudes*) on paddy straw, banana leaves, sugarcane baggage, water hyacinth, betel nut husk and he found moisture content varied from 88.15 to 91.64%. Bhuyan (2008) found no significant differences among the mushrooms produced in sawdust supplemented with wheat bran.

4.2.1.2 Dry matter

Different sawdust showed statistically significant variation in terms of dry matter content (Table 5). The lowest dry matter content was obtained from MT (1.23%), which was followed by JFT (23.89%), whereas the highest dry matter content was recorded in GT (32.24%) which was statistically similar with ST (16.66%). The result of the present study matches with the findings of previous one that reported by Hossain *et al.* (2010), they revealed that the dry matter percentage of the fruiting body was ranged from 9.40 to 9.98 due to sawdust supplemented with different levels of cow dung. Bhuyan (2008) found no significant differences among the treatments when cow dung used as supplement. But in this study there was significant differences found among the treatments. This might be due to different levels of cultural practices.

4.2.1.3 Protein content

Different sawdust showed statistically significant variation in terms of protein content (Table 5). All the treatment contains a considerable amount of protein. The highest protein content was recorded from MT (35.23%), followed by KT (26.67%), while the lowest protein content observed in GT (15.78%) followed by ST (23.43%). The results of the present study was supported by the findings of previous workers (Chan *et al.*, 2001;Hossain *et al.*, 2010 and Zhang-Ruihong *et al.*, 1998). Chan *et al.* (2001) reported that the fruiting bodies of mushrooms contained 26.6-34.1% crude protein. Hossain *et al.* (2010) cultivated the shiitake mushroom (*Lentinus edudes*) and found that the percentage of crude protein varied from 18.46 to 27.78% respectively. Zhang-Ruihong *et al.* (1998) found the protein content of mushroom was 27.2% on an average.

4.2.1.4 Lipid content

Significant difference was observed in terms of lipid content of *Lentinus edudes* mushroom due to different sawdust (Table 5). The highest lipid content was found from MT (4.03%), followed by KT (3.93%), the lowest lipid content was recorded in GT (2.78%) followed by ST (3.53%). The results of the present study was found more or less similar with the findings of Hossain *et al.* (2010) who reported 4.30 to 4.41% lipids in oyster mushroom grown on different substrates. Kulsum *et al.* (2009) also found that lipid content was ranged from 3.44 to 5.43% due to sawdust supplemented with different levels of cow dung which is more or less similar to the present study.

4.2.1.5 Carbohydrate

Different amount of carbohydrate content was recorded under the present trial (Table 5). The highest carbohydrate was observed from MT (54.32%), followed by ST (48.65%), whereas the lowest carbohydrate content was observed in KT (41.21%) followed by GT (46.44%). The finding of the present study does not match with the study of Chan *et al.* (2001) reported that the fruiting bodies of mushrooms contained 40.30-50.7% carbohydrates. But it was supported by Hossain *et al*. (2010) who found 39.82 to 42.83% of carbohydrates in *Lentinus edudes.* The findings of the present study are supported by the study of Kulsum *et al.* (2009) who found that carbohydrate content was ranged from 32.85 to 56.38 % due to sawdust supplemented with different levels of cow dung.

4.2.1.6 Crude fiber

Statistically significant variation was recorded in term of crude fiber content showed due to different sawdust (Table 5). The highest crude fiber was recorded from MT (29.68%), which was statistically similar with KT (25.65%). On the other hand, the lowest crude fiber content was found in GT (15.45%). The findings of the present study corroborate with the study Hossain *et al*. (2010) reported 22.87g/100g to 23.29g/100g of fiber in *Lentinus edudes.*

4.2.1.7 Ash

Statistically significant variation was recorded in term of ash content showed due to different sawdust (Table 5). The highest ash content was recorded from MT (8.90%), which was statistically similar to KT (7.93%). On the other hand, the lowest ash content was found in GT (7.16%) followed by ST (7.55%). The findings of the present study was supported by the study of Kulsum *et al.* (2009) who found that ash content was ranged from 6.58 to 8.41% due to sawdust supplemented with different levels of cow dung. Khlood and Ahmad (2005) reported that ash contents were moderate in the fruiting bodies. Hossain *et al.* (2010) reported 8.28 to 9.02% ash in *Lentinus edudes.*

ST: Teak tree (*Tectona grandis*) sawdust supplemented with 12% wheat bran and 1% lime

MT: Mango tree (*Mangifera indica*) sawdust supplemented with 12% wheat bran and 1% lime

KT: White siris (*Albizia procer)* sawdust supplemented with 12% wheat bran and 1%

JFT: Jackfruit (*Artocarpus heterophyllus*) sawdust supplemented with 12% wheat bran and 1% lime

GT: Timber tree (*Anogeissus latifolia*) sawdust supplemented with 12% wheat bran and 1%

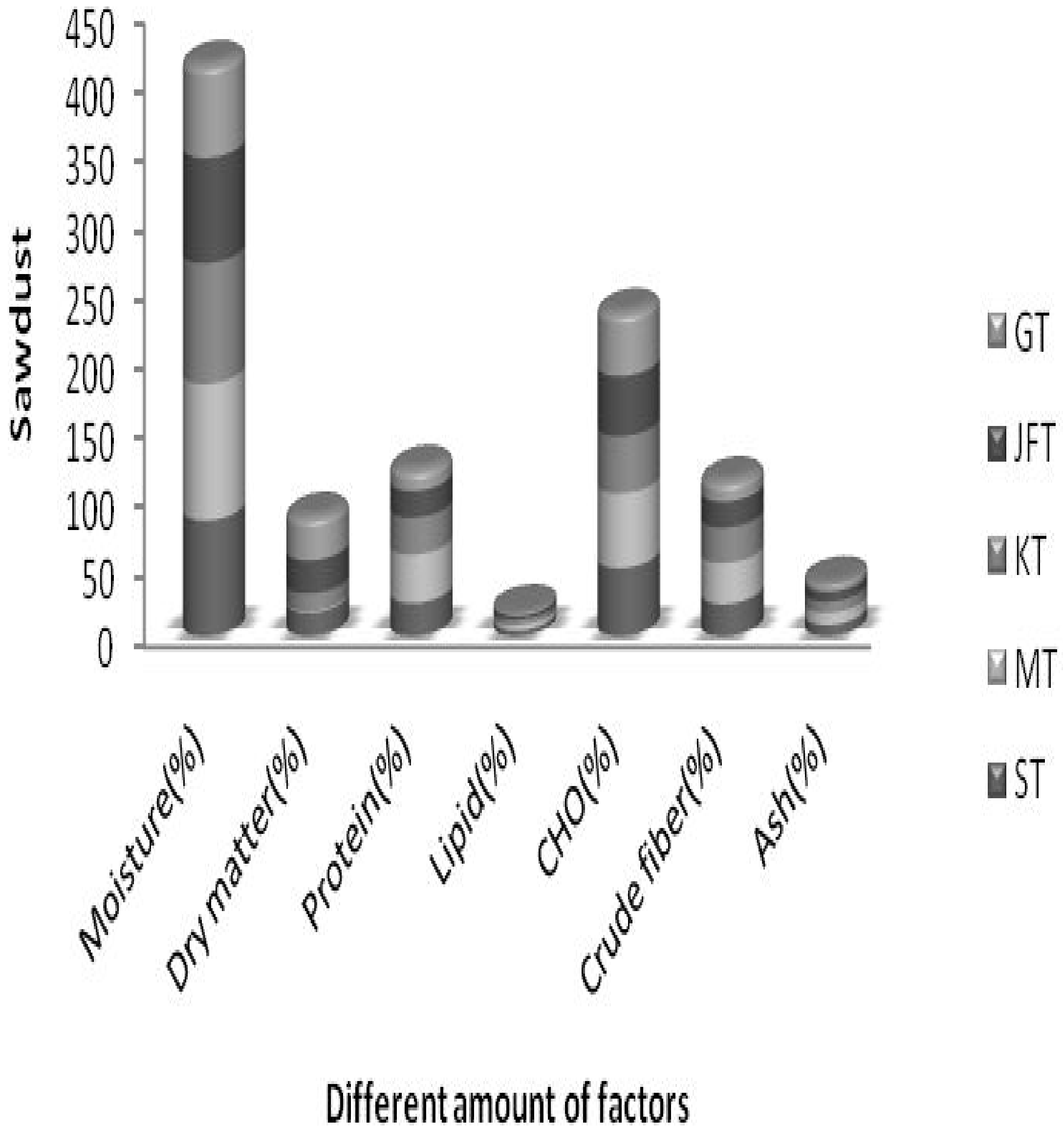

Fig.12 Effect of different sawdust substrates on proximate composition analysis of different amount of *Lentinus edudes.*

4.2.2 Effect on major mineral content

4.2.2.1 Nitrogen (N) content

Statistically significant variation was recorded in terms of Nitrogen content due to different sawdust used (Table 6). The highest amount of nitrogen content was recorded in MT (6.78%) which was followed by GT (6.43%), whereas the lowest in ST 5.82%) wich was statistically similar with JFT (6.21%). The findings of the present study matches with the study of Hossain *et a* .(2010) who analyzed for various nutritional parameters and found 4.22 to 5.59 % of nitrogen on dry matter basis in fruiting bodies of shiitake mushroom.

4.2.2.2 Phosphorus (P) content

Statistically significant variation was recorded in terms of phosphorus content due to different sawdust used (Table 6). The highest amount of phosphorus content was recorded in MT (1.98%) which was followed by ST (1.53%), whereas the lowest in GT (1.06%) which was statistically similar with KT (1.23%). The findings of the present study match with the study of Moni *et al.* (2004) who found 0.97% phosphorus in shiitake mushroom grown on sawdust based substrates. Kulsum *et al.* (2009) also found that phosphorus content was ranged from 0.84 to 0.92% due to sawdust supplemented with different levels of cow dung.

4.2.2.3 Potassium (K) content

Statistically significant variation was recorded in term of Potassium content showed due to different sawdust (Table 6). The highest amount of potassium was attained from MT (1.98%) which was statistically similar with ST (1.91%), the lowest potassium content was found in GT (1.68%) followed by JFT (1.75%). The findings of the present study similar with the study of Chan et al. (2001) who reported that the fruiting bodies of Potassium contained 1.432 to 1.88 mg/g of K on dry weight basis. Sarker *et al.* (2007) also found 1.3% potassium in oyster mushroom grown on sawdust based substrates.

4.2.2.4 Calcium (Ca) content

Statistically significant variation showed due to different sawdust used under the present trial (Table 6). The highest amount of calcium was observed from MT (2.38%) which was followed by KT (2.32%), whereas the lowest calcium content was observed in GT (2.18%) which was followed by ST (2.24%). Hossain *et al.* (2010) who found 22.15 to 33.7 mg/100 g calcium in different shiitake mushroom varieties. Sarker *et al.* (2007) also found 2400 ppm calcium in oyster mushroom grown on sawdust based substrates.

4.2.2.5 Magnesium (Mg) content

Variation observed in terms of magnesium content due to different sawdust under the present trial (Table 6).The highest amount of magnesium was attained from MT (0.976%) which was followed by ST (0.912%).On the other hand, the lowest magnesium content was found in JFT (0.823%) which was followed by GT (0.843%). *Sarker et al.* (2004) also found 0.21% magnesium in shiitake mushroom grown on sawdust based substrates.

4.2.2.6 Iron (Fe) content

Iron content showed statistically significant variation due to use of different sawdust under the present trial (Table 6). The highest amount of iron was recorded from MT (625.23ppm) which was followed by ST (617.56ppm), whereas the lowest iron content was observed in GT (608.76 ppm) which was followed by JFT (612.45ppm) .The result of the present study found iron higher than the value found by Hossain *et al.* (2010) who found that iron content of different shiitake mushroom varieties ranged from 33.45 to 43.2 mg/100g. Sarker *et al.* (2007) found 92.09 ppm to 118.40 ppm iron in shiitake mushroom grown on sawdust based substrates.

4.2.2.7 Zinc (Zn) content

Different sawdust showed statistically significant variation in terms of zinc content (Table 6). The highest amount of zinc was obtained from KT (9.67%) which was followed by MT (7.89%), whereas the lowest zinc content was recorded in GT (6.24%) which was followed by JFT (6.95%). The results of the present study have the similarity with the study of Alam *et al.* (2007) found from their earlier experiment that zinc content of different shiitake mushroom ranged from 16 to 20.9%. Hossain *et al.* (2010) found 30.92 ppm zinc in shiitake mushroom grown on sawdust based substrates.

4.2.2.8 Sulphur (S) content

Statistically significant variation was recorded in terms of S content due to different sawdust substrates (Table 6). The highest S content was found in MT (0.456%) which was statistically identical with ST (0.334%), whereas the lowest S content was recorded in JFT (0.345%) treatment which was statistically similar with GT (0.378%). The findings of the present study were supported with the findings of Hossain *et al.* (2010) who recorded 0.238 to 0.321% of sulphur from their earlier study in shiitake mushroom varieties.

Table 6. Effect of sawdust substrate on mineral contents of shiitake mushroom (*Lentinus edudes*)

Treatment	N (%)	P (%)	K (%)	Ca(%)	Mg(%)	Fe (mg/100 mg)	Zn (mg/100mg)	S (mg/100mg)
ST	5.82b	1.53a	1.91ab	2.24b	0.912ad	617.5bc	7.06a	0.334a
MT	6.78a	1.98a	1.98a	2.38c	0.976b	625.23a	7.89b	0.456a
KT	6.02b	1.23bc	1.84b	2.32bc	0.875c	614.43c	9.67a	0.421b
JFT	6.21c	1.11d	1.75c	2.26d	0.823a	612.45d	6.95b	0.345b
GT	6.43c	1.06c	1.68a	2.18a	0.843b	608.76b	6.24b	0.323 c
CV (%)	2.3%	2.03%	3.34%	1.54%	2.13%	0.24%	0.17%	1.63%
LSD (0.05)	1.05	0.168	0.086	0.342	0.237	1.56	0.056	0.012

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

ST: Teak tree (*Tectona grandis*) sawdust supplemented with 12% wheat bran and 1% lime

MT: Mango tree (*Mangifera indica*) sawdust supplemented with 12% wheat bran and 1% lime

KT: White siris (*Albizia procer)* sawdust supplemented with 12% wheat bran and 1%

JFT: Jackfruit (*Artocarpus heterophyllus*) sawdust supplemented with 12% wheat bran and 1% lime

GT: Timber tree (*Anogeissus latifolia*) sawdust supplemented with 12% wheat bran and 1%

CHAPTER V

SUMMARY AND RECOMMENDATION

The study was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka during the period from October 2016 to March 2017 to evaluate the performance of different sawdust on the growth, yield and proximate composition of *Lentinus edudes*. The experiment consists of five different type of sawdust as- MT: Mango tree (*Mangifera indica*) sawdust supplemented with 12% wheat bran and 1% lime; ST: Teak tree (*Tectona grandis*) sawdust supplemented with 12% wheat bran and 1% lime; JFT: Jackfruit (*Artocarpus heterophyllus*) sawdust supplemented with 12% wheat bran and 1% lime; KT: White siris (*Albizia procer)* sawdust supplemented with 12% wheat bran and 1%; GT: Timber tree (*Anogeissus latifolia*) sawdust supplemented with 12% wheat bran and 1%. 12% wheat bran was taken as basal substrate. The experiment was laid out in single factor Completely Randomized Design. Data on different growth, yield and nutrient composition and mineral content were recorded and significant variation was recorded for different studied parameter.

The highest mycelium growth rate (4.23 mm/day) was recorded in Le 11 with 1000g of spawn packet; the lowest mycelium growth rate (2.60 mm/day) was recorded in Le 8 with 500g weight of spawn packet. The highest time (114.80 days) required to completion of mycelium running was obtained from the treatment completion of strain Le 12 with 1000g spawn packet; The lowest time (33.25 days) required to completion of mycelium running was obtained from the strain Le 11 with 300g spawn packet. The highest time (149.30 days) required for bump formation was obtained from the treatment combination of strain Le 11 with 1000g spawn packet; the lowest time (79.50 days) required for bump formation was obtained from the strain Le 8 with 300g spawn packet. The highest time (16.50 days) required from opening to first harvest was obtained from the treatment combination of strain Le 12 with 1000g spawn packet; The lowest time (4.00 days) required from opening to first harvest was obtained from the strain Le 16 with 300g spawn packet. The highest time (160.00 days) required for harvest was recorded from the treatment combination of Le 11 with 1000g weight of spawn packet; The lowest time (84.00 days) required for harvest was found from the treatment combination of Le 8 with 300g weight of spawn packet. The highest length (7.18 cm) of stalk was found from the treatment combination of Le 8 with 750g weight of spawn packet; the lowest length (3.38 cm) of stalk was recorded from the treatment combination of Le 11 with 750g weight of spawn packet. The highest diameter (10.25 cm) of stalk was observed from the treatment combination of Le 12 with 1000g weight of spawn packet; The lowest diameter (0.80 cm) of stalk was observed from the treatment combination of Le 11 with 1000g weight of spawn packet. The highest diameter (10.38 cm) of pileus was recorded from the treatment combination of Le 8 with 1000g weight of spawn packet; The lowest diameter (1.28 cm) of pileus was recorded from the treatment combination of Le 12 with 1000g weight of spawn packet. The highest thickness (2.33 cm) of pileus was recorded from the treatment combination of Le 8 with 1000g weight of spawn packet; The lowest thickness (0.80 cm) of pileus was recorded from the treatment combination of Le 12 with 500g weight of spawn packet. The highest yield (135.80g) was recorded from the treatment combination of Le 12 with 500g weight of spawn packet; The lowest yield (24.50g) was recorded from the treatment combination of Le 12 with 750g weight of spawn packet.

The highest average number of primordial per packet was recorded from MT (66.45); the lowest average number of primordial per packet was observed from GT (53.24). The highest average number of fruiting body per packet was recorded from ST (70.23); the lowest average number of fruiting body per packet was observed from GT (42.89). The highest average weight of individual fruiting body was recorded from MT (3.94); the lowest average weight of individual fruiting body was observed from GT (3.28). The highest average length of stripe was recorded from MT (1.60); the lowest average length of stripe was observed from GT (0.96). The highest average thickness of pileus was recorded from KT (0.70); the lowest average thickness of pileus was observed from GT (0.55).

The highest biological yield was recorded from MT (211.78 g); the lowest biological yield was recorded in GT (169.76 g). The highest economic yield was recorded from MT (245.38 g); the lowest economic yield was observed in GT (172.32 g). The highest dry yield was observed from MT (25.11 g); the lowest dry yield was attainedin GT (15.1 g) which was statistically similar with ST (19.79 g). The maximum biological efficiency was recorded from MT (123.21%); the lowest biological efficiency was observed in GT (92.9%). The highest benefit cost ratio was found from MT (5.91); the lowest benefit cost ratio was recorded in GT (3.93).

The highest moisture content was observed from MT (98.76%); the lowest moisture content was found in GT (67.76%). The highest dry matter content was recorded in GT (32.24%); lowest dry matter content was obtained from MT (1.23%). The highest protein content was recorded from MT (35.23%); the lowest protein content observed in GT (15.78%). The highest lipid content was found from MT (4.03%); the lowest lipid content was recorded in GT (2.78%). The highest carbohydrate was observed from MT (54.32%); the lowest carbohydrate content was observed in KT (41.21%). The highest crude fiber was recorded from MT (29.68%); the lowest crude fiber content was found in GT (15.45%). The highest ash content was recorded from MT (8.90%); the lowest ash content was found in GT (7.16%).

The highest amount of nitrogen content was recorded in MT (6.78%), the lowest in ST (5.82%). The highest amount of phosphorus content was recorded in MT (1.98%), the lowest phosphorus content was found in GT (1.68%) %). The highest amount of potassium was attained from MT (1.98%), the lowest potassium content was found in GT (1.68%). The highest amount of calcium was observed from MT (2.38%), whereas the lowest calcium content was observed in GT (2.18%). The highest amount of magnesium was attained from MT (0.976%), the lowest magnesium content was found in JFT (0.823%). The highest amount of iron was recorded from MT (625.23ppm), whereas the lowest iron content was observed in GT (608.76 ppm). The highest amount of zinc was obtained from KT (9.67%), whereas the lowest zinc content was recorded in GT (6.24%). The highest S content was found in MT (0.456%), whereas the lowest S content was recorded in JFT (0.345%).

Recommendations

In this experiment, MT: Mango sawdust supplemented with 12% wheat bran and 1% lime performed better in respect of different growth, yield and nutrient composition and mineral content of *Lentinus edudes*. Therefore, MT: Mango sawdust supplemented with 12% wheat bran and 1% lime can be recommended for farmer level *Lentinus edudes* mushroom cultivation.

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APPENDICES

Appendix I. Monthly record of air temperature, relative humidity, rainfall, and sunshine (average) of the experimental site during the period from Octaber 2016 to February 2017

Month (2014)	Air temperature (^{0}c)		Relative humidity (%)	Rainfall (mm)	Sunshine (hr)
	Maximum	Minimum			
October	26.5	19.4	81	22	6.9
November	25.8	16.0	78	00	6.8
December	22.4	13.5	74	00	6.3
January	19.7	8.4	71	00	6.0
February	20.6	10.1	73	9	6.2

Source: Bangladesh Meteorological Department (Climate & weather division) Agargoan, Dhaka–1212.

Appendix II. Effect of sawdust substrate on mushroom on the yield attributes Of shiitake mushroom (*Lentinus edudes*)

Amount of substrates	Number of primordial/packet	Number of fruiting body/packet	Number of effective fruiting body/packet	Weight of individual fruiting body (g)
300g	167 a	24.00 c	17.75 bc	3.21 a
500g	186 d	81.50 a	53.00 a	4.54 d
750g	198 a	11.00 e	6.25 d	5.43 b
1000g	212 a	6.00 f	3.75 de	6.12 c
LSD(0.05)	1.941	8.51	4.03	0.109
CV(%)	0.51	34.32	23.78	1.30

Appendix III. Effect of different sawdust on proximate nutrient composition of Shiitake mushroom (*Lentinus edudes*)

Treat-ments	**Mois-ture** (%)	**Dry matter** (%)	**Protein** (%)	**Lipid** (%)	**Carbo-hydrate** (%)	**Crude fiber** (%)	**Ash** (%)
ST	83.34 b	16.66 c	23.43 b	3.53 c	48.65	22.76 b	7.55 a
MT	98.76 c	1.23 a	35.23 a	4.03 c	54.32	29.68 a	8.90 d
KT	87.46 b	12.54 b	26.67 b	3.93 b	41.21	25.65 b	7.93 b
JFT	76.21 c	23.89 a	19.45 c	2.99 d	43.51	18.83 a	7.32 c
GT	67.76 a	32.24 d	15.78 d	2.78 d	46.44	15.45 a	7.16 c
CV (%)	0.07%	0.43%	0.1%	1.79%	1.32%	0.80%	0.98%
LSD (0.05)	0.124	0.113	0.087	0.167	0.214	0.275	0.179

Plate 1: preparation of sawdust substrates

Plate 2: Prepared packet in mushroom house

Plate 3: Nursering mycelium growth media packets

Plate 4: Mycelium growth in spawn packet