EFFECT OF DIFFERENT SAWDUST ON THE GROWTH, YIELD AND PROXIMATE COMPOSITION OF *Ganoderma lucidium*

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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 *Ganoderma lucidium.***" 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 **Natasha Habib,** Registration No. **08-3134** 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.

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The Author

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EFFECT OF DIFFERENT SAWDUST ON THE GROWTH, YIELD AND PROXIMATE COMPOSITION OF REISHI MUSHROOM *(Ganoderma lucidum)*

ABSTRACT

The study was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka to study the effect of different sawdust on the growth, yield and proximate composition of Reishi mushroom (*Ganoderma lucidum*).Six different types of sawdust viz: jackfruit (*Artocarpus heterophyllus) sawdust*, mango (*Mangifera indica*) sawdust, rain tree *(Albizia saman*) sawdust, shegun *(Tectona grandis*) sawdust, mahagony *(Swietenia mahagony*) sawdust and mixture of all five supplemented sawdust (jackfruit, mango, rain tree, shegun, mahagony) with 30% wheat bran and 1% lime as basal substrates were selected for research. Data on different growth, yield and nutrient composition and mineral content were recorded and significant variation was found for different studied parameter. Mycelium running rate in spawn packet and highest average number of primordia/packet was found to be differed due to different sawdust used. The highest average number of fruiting body per packet was obtained 10.00 with mango sawdust. The highest biological yield (97.3 g), economic yield (89.2 g), biological efficiency (55.60%), cost benefit ratio (4.89) was counted under mango sawdust with 30% wheat bran and 1% lime. Among the chemical characteristics highest content of carbohydrate (74.15%), protein (12.25%), ash (1.36%), lipid (3.23%) was found with mango sawdust treatment. Among the minerals highest amount of nitrogen (1.96%), potassium (2.65%), calcium (1.92%) was found with mango sawdust with 30% wheat bran and 1% lime treatment and the lowest amount of magnesium (0.673mg), zinc (15.15mg) was found with jackfruit sawdust with 30% wheat bran and 1% lime treatment. Therefore it can be concluded that mango sawdust with 30% wheat bran and 1% lime contributed significantly on different growth, yield and nutrient composition and mineral content of Reishi mushroom (*Ganoderma lucidum*).

Chapter 1

Introduction

Mushrooms are large reproductive structures of edible fungi belong to the class of Basidiomycetes or Ascomycetes, having approximately 300 thousands varieties. Among them which are fully edible and have no toxic effect are to be considered as edible mushroom. Out of 2000 species of prime edible mushrooms about 80 have been grown experimentally, 20 cultivated commercially and 4-5 produced on industrial scale throughout the world (Chang and Miles, 1988). 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. Mushroom is a highly nutritious, delicious, medicinal and economically potential vegetable. Mushroom can play an important role to meet up the nutritional requirements of the population of our country. Mushroom has qualities like lowering the blood cholesterol level, warding against cancer and invigorating hair growth. 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.Mushrooms have been considered as a special kind of food since the earliest time. The Greeks believed that mushrooms provided strength for warriors in battle. The Pharoaphs prized mushrooms as a delicacy and the Romans regarded mushrooms 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). Edible mushroomsare recommendedby theFAOasfood,tomeetprotein requirement of developingcountries, thelarge proportion of which dependsmainlyon cereals (World Bank, 2004).The history of mushroom cultivation is very recent in Bangladesh; its consumption is increasing rapidly in this country.The vitamins of mushrooms are not destroyed by cooking, drying and freezing. It has been used as a food and medicine by different civilizations since immemorial time due to its delicious taste and dietetic qualities. But technology for artificial cultivation of mushroom is recent innovation.Mushroom reduces the diabetic on regular feeding. It also reduces the serum cholesterol in human bodies which reduces hypertension (Suzuki and Oshima, 1979). Mushroom inhibits the growth of tumor and cancer (Mori, 1986). Mushrooms are source of Niacin (0.3 g) and

Riboflavin (0.4 mg). Mushroom is a good source of trypsin enzyme. It is also rich in iron, copper, calcium, potassium, vitamin D and folic acid. Mushrooms are valuable health food which are low in calories, high in vegetable proteins, zinc, chitin, fiber, vitamins and minerals (Alam and Saboohi, 2001).Reishi mushroom is a purplish-brown fungus with a long stalk, brown spores, and a fan-shaped cap with a shiny, varnish-coated appearance with bitter taste for this it is not used as edible mushroom.it is used in modern medicine for their medicinal values (Kovfeen, 2004). *Ganodermalucidum* is a member of fungal group Basidiomycetes which belongs to Polyporaceae (Ganodermaceae) of Aphyllophorales. Its fruiting body is named as "Reishi" in Japanese and "Lingzhi" in Chinese. World-wide Reishi occupies a major source of medicine that has been used for more than 2000 years. Commercial *G. lucidum* products are available in various forms, such as powders, dietary supplements, and tea which are farmed from different parts of the mushroom, including mycelia, spores, and fruit body. *G. lucidum*has been used in Traditional Chinese Medicine (TCM) as a remedy to treat more than 20 different illnesses which include migraine and headache, hypertension, arthritis, bronchitis, asthma, anorexia, gastritis hemorrhoids, hyper-cholesterolaemia, nephritis, dysmenorrhoea, constipation, lupus erythematosis, hepatitis, leucopoenia, cardiovascular problems and cancer. Besides, reishi or lingzhi also attribute some health benefits which principally include the control of blood glucose levels, modulation of the immune system, hepato-protection, and bacteriostasis. Recent studies on lingzhi have demonstrated numerous biological activities amongst this type of mushroom, including anti-tumor,anti-inflammatory,hepato-protective,anti-microbial, hypotensive, anti-diabetic and hypolipodemic effects. To meet the gradually increasing demand for *G. lucidum* as a natural medicine, commercial cultivation of American Journal of BioScience 2015; mushroom has been initiated worldwide, especially in the tropical Asian countries. As different members of the Ganoderma genus seek different conditions for growth and cultivation, and the traditional cultivation technique takes several months for fruiting body development, artificial cultivation of *G. lucidum* has been implemented using the available substrates such as grain, sawdust, wood logs and cork residues. Several substrates have been investigated worldwide for the cultivation of *G. lucidum* till date. Usually in Bangladesh the summer season is considered as the best time for the cultivation of mushroom. Different environmental factors, oxygen level, and calcium ion concentration, etc. are also important for the cultivation.

Bangladesh Council of Scientific and Industrial Research (BCSIR) investigated the efficacy of sawdust supplemented with rice or wheat bran as substrate, and found the 9:1 ratio of sawdust and rice bran/wheat bran to be effective for the cultivation of *G. lucidum* with elevated production, even in the large scale. Although extensively used in several Asian and tropical countries, unfortunately its application as medicine in Bangladesh is still in scarce. Commercial-based cultivation of such mushroom in this country is thus critical to confer its extended medicinal use as it could be a suitable alternative to synthetic drugs with less adverse effects. Along these lines, the present study assessed the best cultivation media for achieving high yield, biological efficiency, and growth (mycelial, primordial and fruiting body) rate of *G.lucidum.* The substrates on which mushroom spawn (Merely vegetative seed materials) is grown, affects the mushroom production (Klingman, 1950).Reishi mushroom can grow on sawdust, wheat and paddy straw, banana leaves, sugarcane bagasse and leaves, wheat barn, rice husk etc. and their culture can be concentrated within a relatively small space. In the present study five different sawdust viz: Jackfruit(*Artocarpusheterophyllus*) sawdust ,Mango(*Mangiferaindica*)sawdust, Rain tree(*Albiziasaman*) sawdust, Shegun(*Tectona grandis)* sawdust ,Mahagony (*Swieteniamahagony*) sawdust and Mixture of all five supplemented sawdust (Jackfruit, Mango, Rain tree, Shegun, Mahagony) with 30% wheat bran and 1%lime as basal substrates were selected for studied their performance on growth,yield and nutritional composition of*Ganodermalucidium.* If different sawdust can be used in mushroom production then low price and easily available sawdust could be select and which one is better and also best for mushroom production can be identified. So, the investivation is undertaken to fulfill the following objectives:

1. To improve the yield of reishimushroom(*Ganodermalucidum).*
2. To observephysio-chemical characteristics of reishimushroom(*Ganodermalucidum)*
3. To find out benefit cost ratio of the sawdust based spawn packet.
4. To find out the mineral content of reishi Mushroom.

Chapter 2

Review of Literature

A number of literatures relating to the performance of different substrate on mushroom cultivation are available but performances on same substrate with same supplements in different level are not available. The review of literature given below is based on the present information about the performance of *Ganoderma lucidum* and the effect of different kinds of substrate on mushroom cultivation. The review includes report of several investigators which appear pertinent in understanding the problem and which may lead to the explanation and interpretation of results of the present investigation.

Ribeiro *et al.* (2006) found that edible mushrooms also contain various polyphenolic compounds recognized as an excellent antioxidant due to their ability to scavenging free radicals by single-electron transfer.

Fu *et al*. (2002) reported that several cultivated edible mushrooms such as *Agaricus bisporus, Hericiumerinaceus, Flammulina velutipes, Lentinus edodes,Pleurotus eryngii* and *Pleurotus ostreatus* had significant antioxidant and free radical scavenging activities which have been also found in medicinal mushrooms including *Agaricus blazei, Sparassiscrispa, Phellinus linetus, Ganoderma lucidum, and Inonotus obliquus*.

Paterson *et al*. (1981) have assessed the minerals and heavy metals content in *Ganoderma spp*.

Kim *et al*. (2008). Detected that twenty-eight of 30 phenolic compounds in mushroom species *Agaricus bisporus, Flammulina velutipes, Pleurotus erygii, Agaricus blazei, Sparassis crispa, Ganoderma lucidum, Innotus obliquus, Phellinus linteus Ganoderma lucidum* is not only found to be medicinally effective as antitumor, antibacterial, antiviral and haematologicalagents and in immunomodulating treatments.

Wasserand Weis (1999) found that *Ganoderma* possess significant antioxidant capacity and these mushrooms can be used both as a food ingredient and in pharmaceutical industry.

Lindequist *et al*. (2005) reported that reishi mushroom has antiviral activity with specific action on HSV-1 and HSV-2, Influenza virus, Vessicular stomatitis and HIV type1 or remedy for maladies Although these reports were mostly based on crude or hot water extracts, purification and specific identification of few chemicals, there is yet, the need to analyze the chemical compositions using various organic solvents soluble fractions from the extracts of the of the wild mushroom-*G.lucidum* (Mizuno, 2011) to assess its chemical composition.

Ko *et al.* (2008) who reported that steroids found from *G.lucidum* includes 0.3-0.4% which has anti-inflammatory activity. These steroids are also precursors of ganoderic acid and protease inhibitors (Wikipedia, 2012).

Idowu *et al.* (2003) found that the presence of alkaloids in the mushroom powder explains its anti-bacterial activity, since this phytochemical is reported to have anti-bacterial activity However, this property may be lost during fractionation with Methanol, Ethyl acetate and N-butanol which showed absence of this phytochemical.

Saikumar *et al.* (2010) conducted an experiment that polyphenol, flavonoids found in weak concentration in reishi mushroom are known to be source of plant based antioxidants which can protect the nerves, heart, liver and other organs and tissues. This anti-oxidant property may be responsible for reduction of hepatic damage Lakshmi *et al*. (2006)

Guo *et al*. (2003) found that carbohydrates or high fiber fraction of diets which are broadly classified as polysaccharides or indigestible carbohydrates are known to inhibit colonization of pathogenic microbial flora in the intestines, hence, the elimination of these pathogens from the gut system accompanied by improved immunity.

Khalil and Eladawy (1994) reported that saponins in free forms have hemolytic activity, the bitter taste of reishi mushroom extract can be attributed to the Saponin-triterpenoid complex.

Ray Sahelian (2012) reported that oral administration of saponins leads to hydrolysis of glycosides from terpenoids, hence reducing toxicity associated with intact glycoside molecule. (Wikipedia, 2012), Saponins are also reported to have anti-inflammatory, expectorant and immune stimulating effects.

Ogbe *et al*. (2012) examined that low fats content (2.60%) found in *Ganoderma spp* that shows the health benefits of reishi mushroom and stressing its nutritional value.

Berger *et al.* (2004) conducted an experiment to evaluate the extract from reishi mushroom.And found that it has cholesterol lowering properties in Hamsters and Minipigs hence , its antihypertensive potentials in humans too.

Saikumar *et al.* (2010) researched that polyphenol, flavonoids, found in weak concentration in reishi mushrooms are known to be source of plant based antioxidants which can protect the nerves, heart, liver and other organs and tissues. This anti-oxidant property may be responsible for reduction of hepatic damage.

Riu *et al.* (1997) carried out an experiment to find out the easy cultivation procedure of *Ganoderma spp.*They have normally been cultivated in solid substrates such as grain or other lignocellulosic materials such as straw, sawdust and supplements. Supplements such as sucrose, wheat and rice bran are generally added to the mix (Chen, 1998).

Gonzalez-Matute *et al.* (2002) reported that sunflower seed hull can be used as main energy and nutritional sources in the formulation of a substrate for cultivation of *G. lucidum* in synthetic logs with an acceptable mushroom production rate, and the addition of 5% malt to sunflower seed hulls were significantly improved the mushroom productivity.

In a study conducted by Tang and Zhong (2002), it was found that biomass productivity was higher on maltose but lactose was the best sugar for both cell growth and production of compenents such as IPS (intracelllar polysaccharide) and ganoderic acid in reishi mushroom Wagner *et al.* (2003).

Hsieh *et al.* (2005) found that while molasses addition promoted higher mycelia growth rate and cell concentration, the polysaccharide production was lower than with glucose in *Ganoderma lucidum.*

Yang *et al*. (2003) used substrates were filled into the polypropylene bags with 1.0 kg per bag. Substrates were wetted to increase moisture content approximately to 60 - 70%. The wetted bags were plugged with cotton plug by using PVC ring and autoclaved at 121oC for 1.5 h.And about 200g of reishi mushroom per kg.

Wasser, S.P. 2005 used wood log, short wood segment, tree stump, sawdust bag and bottle procedures for reishi mushroom production.

Staments, P. (2000) and Chen,A.W. (1999) found that for the cultivation of most medicinal mushrooms the basic substrate is hardwood sawdust (a mixture of fine and coarse sawdust to ensure good aeration) 75-80%, supplemented with wheat bran 20%, gypsum 1%, sucrose 1%, moisture content 60-65% and pH 5.5-6.5.

Tea waste was investigated by Peksen *et al.* 2009 and it was concluded that tea waste can be used as a supplement for substrate preparation in *G. lucidum* cultivation.

Erkel (2009) found that the yield from the *Shorea robusta* sawdust was inconsistent than the *Dalbergia sisoo* and *Shorea robusta* sawdust.

Triratana *et al.* (1991) investigated the suitability of rice bran, rice husks, coconut fiber, peanut hulls, corn, sorghum and sugarcane bagasse as supplements for the substrate mixture for the artificial cultivation of Ganoderma and rice bran, ground corn and ground sorghum were found good supplements compared to some other agricultural residues such as rice husk, coconut fiber, peanut hull and sugarcane bagasse.

Malarvizhi *et al*. (2003) tested the different agricultural wastes for the production of xylanase by Ganoderma lucidum on liquid and solid state culture by and among the different agricultural wastes used, wheat bran was found to be the best substrate for the test fungus for the production of xylanase compared to sugarcane bagasse and rice bran in solid-state fermentation.

Wasser (2005); Olei, P. (2003) reported that Ganoderma can be cultivated on the sawdust which may originate from different kinds of trees.

Yang *et al*. (2003) pointed out that the higher levels of ground rice tended to decrease the growth rate, in contrast, stillage grain and wheat bran were found to be suitable for mycelial growth.

Triratana *et al.* (1991) stated that rice bran, ground corn and ground sorghum have provided the best mycelial growth and yield.

Royse *et al.* (1996) used at the 10-15% ratio of rice bran in the mixture as a growing medium for *Ganoderma.*

Smith *et al*. (2002) found that the cultivation of medicinal reishi mushrooms largely increased due to the use of different sizes of polypropylene bags or containers.

Wasser (2005) found that the methods most widely adopted for commercial production are the wood log, short wood segment, tree stump,sawdust bag and bottle procedures for reishi mushroom cultivation.

Erkel (2009) investigated the effect of three kinds of saw dusts (poplar,oak and beech) and brans (Wheat, rice and corn) on the yield of G. lucidum. The highest yield and biological efficiency were obtained from oak sawdust compared to the other sawdusts and also from wheat bran compared to the other supplements.

H.Y. Lee (1999) examined that Supplementation showed positive role in mycelia growth and yield of mushroom.

Chen and Chao (1997) repoted about a successful artificial cultivation of Ganoderma lucidum has been reported on most broad-leaf hardwood trees and commonly used species include oak, pecan, elder, choke cherry, and plum.

Paterrson-Beedle *et.al*. (2002) used the approximate composition of molasses as a growing medium for Ganoderma spp is: 17- 25% of water, 30-40% of sucrose, 4 - 9% of glucose, 4 -12% of fructose, 2 - 5% of starch, 7 - 15% ash, 2.5 -4.5% nitrogen compounds, 0.5 - 4.5% of protein and 1.5 - 6% non-nitrogenous acids with varying amounts of vitamins.

In Nepal, G. lucidum had been cultivated in 2004 AD in Nepal Agricultural Research Council taking four different types of substrate composition (sample A (sawdust 90%, rice bran 10%), sample B (sawdust 72% , Corn meal 20%, rice bran 7.8 %, CaCO3 0.2%), sample C (sawdust90%, Wheat bran 12%) and sample D(sawdust 90% , wheat bran 10%). 65-70 %moisture was adjusted in all four samples.The result showed that the above mentionedcomposition can be substrate for cultivationof G. lucidum (Plant Pathology Division,2007). Similarly, paddy straw was used in Plant Pathology Division Nepal Agricultural Research Council) and the result showed that paddy straw is also one of the substrate for cultivation of Ganoderma lucidum.

Erkel (2009) found that Gram flour showed significance difference with Wheat bran and Corn flour in yield statistically.Although yield showed different among the supplements, gram flour was not different from Rice bran statistically.

P.G. Miles (2004) found that Alnus nepalensis sawdust supplemented with gram flour showed highest yield among all treatments.There was no significant difference between gram flour and rice bransupplement.

Okhuoya *et al.* (1998) who reported that mushroom could not grow well on sawdust of some tree species like *Borasus flabellifer* because of its thin layer of porus system.

Kacar, B. (1994) carried out an experiment for the proximate analysis of air dried reishi mushroom.The harvested *Ganoderma lucidum* was air dried at 370C and grinded to powder; this was preliminary analyzed for proximate contents,

phytochemical constituents. The crude powder was subjected to soxhlet extraction at 400C using Methanol, Ethylacetate and N-butanol to obtain different organic solvent fractions, these were then concentrated in vacuo at 240C for 48 hours to obtain different solvent extract fractions. These extracts were then analyzed for phytochemical contents using standards methods.Analysis for proximate constituent showed Moisture contents was 10.54%, Total ash 5.93%, Protein 17.55%, Crude Fats 2.60%, Crude Fiber 30.25%, Carbohydrates 33.13%, and Nitrogen 23.52%.

Bangladesh Council of Scientific and Industrial Research (BCSIR) investigated the efficacy of sawdust supplemented with rice or wheat bran as substrate, and found the 9:1 ratio of sawdust and rice bran/wheat bran to be effective for the cultivation of G. lucidum with elevated production, even in the large scale.

Chen (1999) examined that hardwood sawdust is the basic substrate for the cultivation of most medicinal mushrooms.

Ayodele (2007) investigated that the growth and development of reishi mushroom varied from one tree species to another.

Peksen and Yakupoglu, (2009) and Yang et al. (2003) used hardwood sawdust and wheat bran mixture in production of Ganoderma lucidum and the highest yield obtained 63.66g per kg and 18.63% biological efficiency were obtained.

Wasser, (2005) reported that more than 100 polysaccharides are found in G. lucidum and these polysaccharides are considered to contribute to the bioactivity of the mushroom. The polysaccharide is also known to potentiate immunefunction by binding to leukocyte surfaces or serum specific proteins leading to activation of macrophages, T-helper cell, Natural killer (NK) cells and other effector cells, and this can be the basis for its anti inflammatory effect.

Mueller *et al.* (2000); Stavinoha (2011) evaluated the high protein content suggest its importance in cellular function and tissue regeneration. It has been reported that protein isolate (LZ-8) from this mushroom can suppress bovine serum albumin

induced anaphylaxis and an important means of managing histamine mediated allergic responses.

Erkel (2009) found the highest yield (63.66 g/kg) and biological efficiency (18.63%) using oak sawdust where he used wheat bran as supplement.

Tham *et al.* (1999) conducted an experiment to investigate the chemical characteristics of *Ganoderma lucidum*.Results showed high concentration of calcium (322.6mg/kg), potassium (317.1mg/kg), phosphorus (197.1mg/kg) and sodium (192.5mg/kg). Moderate presence of carbon (68.2mg/kg), iron (44.6mg/kg) and zinc (14.65mg/kg) were observed. However, other elements such as magnesium (8.7mg/kg), silicon (4.10mg/kg), arsenite (1.23mg/kg) and manganese (1.03mg/kg) were found in moderately slight concentrations. Elements such as copper (0.843mg/kg), aluminum (0.20), chromium, (0.140mg/kg), lead, (0.106), molybdenum nickel (0.095mg/kg), (0.090mg/kg), cobalt (0.026mg/kg) and fluorine (0.0039mg/kg), were found in this study, to be in trace quantity, but selenium (0.00mg/kg) was totally absent.

Wasser (2005) reported that the nitrogenous component consists of 23.52% in *Ganoderma* Spp.This provide essential requirement for nucleotides and nucleosides formation in the body, these amino acids, are important components of DNA and RNA that are useful in cellular function and cell differentiation, thus, its mitogenic capacity

Idowu *et al.* (2003) Examined that the presence of alkaloids in the reishi mushroom powder explains its anti-bacterial activity, since this phytochemical is reported to have anti-bacterial activity. However, this property may be lost during fractionation with Methanol, Ethyl acetate and N-butanol which showed absence of this phytochemical.

M. Azizi *et al*. (2012) used Potato Dextrose Agar (PDA) culture to obtain the pure culture of *G. lucidum*.

S. Singh (2014) used for the production of reishi mushroom to achieve 3.5 kg dry weight of substrate (35% of the mixture), and sawdust and supplements ratio of 9:1,

3.1 kg (90%) of sawdust was mixed with 0.3 kg (8%) rice bran or wheat bran and 0.1 kg (2%) of CaCO3.

Karma and Bhatt (2013) in India noticed primordial initiation in reishi mushroom after 35 days using sawdust as substrate supplemented with rice and wheat brans, maize flour, and bagasse.

Azizi *et al.* (2012) reported the yield of 102.58 g/kg with biological efficiency of 12.89% using hornbeam saw dust supplemented with 5% malt extract and 10% wheat bran in reishi mushroom.

Karma and Bhatt (2013) tested different substrates such as sawdust, sugarcane bagasse, rice straw, wheat straw and waste paper for the production of reishi mushroom in polypropylene bag. Different substrates significantly affected the number of primordia, number of fruiting bodies and amount of fresh weight or yield. This experiment revealed that the highest number of primordia and fruiting bodies were found in wheat straw 31.00.The highest amount of fresh weight was found waste 44.25g.

Mayzumi ;Okamoto and Mizuno (1997) examined and then reported that different members of the Ganoderma genus need different conditions for growth and cultivation. Different culture conditions and medium compositions have also been reported to strongly influence mycelial growth and the production of biopolymers (e.g., polysaccharides) that are extruded from the cell (exopolysaccharides).

Chang and Buswell (1999) reported that the artificial cultivation of *G. lucidum* has been achieved using substrates such as grain, sawdust, wood logs, and cork residues (Riu, Roig, and Sancho 1997).

Yang and Liau (1998) reported that polysaccharide production by fermenter-grown mycelia of G. lucidum was optimum at 30°C–35°C and a pH of 4–4.5, and the addition of supplements such as fatty acids was found to accelerate mycelial growth and the production of bioactive components.

Kim, Park, and Yun (2006) reported that pH-control strategy, developed to maximize mycelial biomass and EPS production, revealed that culture pH had a significant effect on EPS yield, chemical composition and molecular weight, and mycelial morphology of reishi mushroom.

Zhang and Tang (2008) reported that a novel three-stage light irradiation strategy has been developed in submerged cultures of G. lucidum for the efficient production of polysaccharides and one of the triterpene components, ganoderic acid.

Upton (2000) examined that the reishi mushroom consists of a matrix of the polysaccharide chitin, which is largely indigestible by the human body and is partly responsible for the physical hardness of the mushroom.

Guo *et al.* (2003) examined that carbohydrates or high fiber fraction found in Ganoderma which are broadly classified as polysaccharides or indigestible carbohydrates are known to inhibit colonization of pathogenic microbial flora in the intestines, hence, the elimination of these pathogens from the gut system accompanied by improved immunity.

Dei *et al.* (2007) obtained that the presence of tannins in both the powdered and extract fractions of methanol and n-butanol which can complex with the metal ions and macromolecules such as proteins and carbohydrates in the powdered sample of Ganoderma can be utilized in weight reduction management.

Ray Sahelian, (2012) examined that saponins found in Ganoderma and oral administration of Saponins leads to hydrolysis of glycosides from Terpenoids, hence reducing toxicity associated with intact glycoside molecule.(Wikipedia, 2012), Saponins are also reported to have anti-inflammatory, expectorant and immune stimulating effects.

Wagner, R.,Mitchell, D.A., Sassaki, G.L.,Amazonas, M.A.L.A. and Berovic, M. (2003)found that the yield and biological efficiency of Ganoderma lucidum varied widely, depending on the kind of sawdust, bran and their combinations. Therefore, it is important to use the proper combination of substrate formulations for the commercial production of G. Lucidum.

Ames *et al.,* (1993); Weisburger (1999) report that G. lucidum was found to contain high both total free and bound phenolic compounds which is different from the other mushrooms significant amount of phenolic compounds which are major contributors to the antioxidant capacity and give protection against the risks for chronic angiogenic diseases, such as cardiovascular diseases, arthritis, chronic inflammation and cancers.

Ko *et al.,* (2008) who reported that steroids found from G.lucidum includes 0.3-0.4% which has anti-inflammatory activity. These steroids are also precursors of ganoderic acid and protease inhibitors (Wikipedia, 2012).

Ogbe *et al*. (2012) conducted an experiment for the elemental analysis of the G.lucidum powder and found calcium has 322.6mg/kg, potassium 317.1mg/kg, phosphorus 197.1mg/kg sodium, 193mg/kg, these elements are found in high concentration while carbon 68.2mg/kg, iron 44.6mg/kg, zinc 14.65mg/kg and magnesium 8.7mg/kg, are found to be in moderate concentration when compared to other elements. The above observed elements have physiological importance and maintenance of cellular enzymatic functions; these elements are required for normal growth, muscular activity and skeletal muscle development, especially calcium.

Dei *et al*. (2007) examined that saponins found in Ganoderma as secondary metabolites can be found as hydrophilic glycoside moiety combined with a lipophyllic triterpene derivative to form a therapeutically cardio-active agent in form of steroid-saponins and triterpenoid saponins when found in high concentration they can cause hypercholesterolemia by binding to cholesterol, thereby making it unavailable for absorption (Soetan and Oyewole, 2009).

Muhammad *et al.* (2011) reported that Sodium and potassium found in Ganoderma are required for the maintenance of fluid balance, while potassium and calcium are important in stimulating action potential across nerve endings, and also to enhance heart contractile rate. Iron is highly required physiologically for heme formation and to enhance oxygen carrying capacity of red blood cells. Zinc is an important requirement in protein synthesis, normal body development and recovery from illnesses. it is a co-factor in the function of the enzyme carbonic anhydrase required

for carbon dioxide transport and as part of peptidases needed for protein digestion it is also a necessary part of DNA, for cell division and synthesis hence its importance in wound healing (innvista.com/health/elements.htm, 2012).

Crisan and Sands (1978); Bano *et al.*, (1988) conducted a study to determine the carbohydrate percentage in reishi mushroom.And reported that the carbohydrate contents varied 67.5-78% on a dry weight basis.

Smith, J.E., Rowan, N.J. and Sullivan, R. (2002) stated that sawdust is the most preference main ingredient used in substrate mixtures for Ganoderma lucidum cultivation. To investigate the feasibility of using three kinds of sawdusts as basal substrates, poplar, beech and oak sawdust were tested with three kinds of brans as supplements. The highest yield of 60.24 g kg-1 and BE of 17.48% were obtained from oak sawdust, and followed by BS (53.24 g kg-1 and 15.94%). These findings are in conformity with some authors who reported hardwood sawdust have been preferred for the commercial production.

Chapter 3

MATERIALS AND METHODS

The study was conducted during the period from January to July 2014 to study the effect of different sawdust on the growth, yield and proximate composition of *Ganoderma lucidium.* The chapter includes a brief description of the location of experiment, soil and climate condition, materials used for the experiment, design of the experiment, preparation of substrates, preparation of packets, cultivation of spawn packet, collection of produced mushrooms, proximate analysis of the mushrooms, data collection and data analysis procedure.

3.1. Location of Experiment

The experiment was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, and Dhaka. Details of the meteorological data during the period of the experiment was collected from the Bangladesh Meteorological Department, Aragon, Dhaka and presented in Appendix I.

3.2. Experimental materials

Mother culture of *Ganoderma lucidium* was collected from National Mushroom Development and Extension Center (NAMDEC), Saver, Dhaka.

3.3. Varietal characteristics of *Ganoderma lucidium*

Ganoderma lucidium mushroom is characterized by basidiocarps that are large, perennial, woody brackets also called "conks". They are lignicolous and leathery either with or without a stem. The fruit bodies typically grow in a fan-like or hoof-like form on the trunks of living or dead trees. They have double-walled, truncate spores with yellow to brown ornamented inner layers. Bitter taste with a high medicinal value Reishi is used as medicine and not as food because it is bitter and corky hard.

3.4 Treatments of the experiments

Two different experiments with six treatments with five replications were conducted to achieve the desired objectives. The experiments were as follows:

Experiment 1: Effect of different sawdust substrates on yield contributing character and proximate composition analysis of *Ganoderma lucidium* mushroom.

Treatments used:

T_1: Controlled (Mixture of sawdust) supplemented with 30% wheat bran and 1% lime

T_2: Mango (*Mangifera indica*) sawdust supplemented with 30% wheat bran and 1% lime

T_3: Mahogony (*Swietenia mahagony*) sawdust supplemented with 30% wheat bran and 1% lime

T_4: Jackfruit (*Artocarpus heterophyllus*) sawdust supplemented with 30% wheat bran and 1% lime

T_5: Teak tree (*Tectona grandis*) sawdust supplemented with 30% wheat bran and 1% lime

T_6:Rain tree (*Albizia saman*) sawdust supplemented with 30% wheat bran and 1% lime

3.5. Design and layout of the experiment

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

3.6. Sterilization procedure

In the laboratory, all instruments, glassware and culture media were sterilized by autoclaving strictly for maintaining sterility.

3.6.1. Sterilization of culture media

The bottles containing the media and also spawn packets were autoclaved with 15 PSI at 121^0C for 1-2 hours. The culture media were allowed to be cold under normal condition after autoclaving.

3.6.2. Sterilization of glassware and instruments

Beakers, test tubes, conical flasks, measuring cylinders flat bottles pipettes, metallic instruments like forceps, scalpels, needles and spatulas, petridishes, culture tubes,

nanoabsorbent cotton and brown paper were sterilized in the autoclave at 121^0C for 1 hours at 1.5 kg/cm^2 pressure.

3.6.3. Sterilization of culture room and transfer area

The culture room of the laboratory was cleaned by gently washing with detergent followed by 70% ethyl alcohol regularly. Before inoculation, laminar airflow cabinet was sterilized using ultra violet light for 30 min keeping blower active.

3.6.4. Precautions to ensure sterile condition

All inoculation measures were carried out in the laminar airflow cabinet to avoid contamination. The cabinet was exposing on the UV light for 30 min before use. All the instruments and equipments used were sterilized with alcohol before use.

3.7. Production of *Ganoderma lucidium* mushroom

To produce *Ganoderma lucidium* mushroom following steps were undertaken.

3.7.1. Preparation of PDA media:

At first, 250 g potatoes were washed, peeled and slice to prepare 1000ml PDA media. Then peeled and slice potatoes were boiled in water to make them soft and also filtered through a cheese cloth and further water was added to get 1000 ml media. After adding 18 g agar and 20 g dextrose, it was heated and stirred for about 45 min. Then 10 ml media was taken into each of each of test tubes and mouths of test tube and mouths of the test tubes were plugged with cotton and brown paper. After that all the test tubes were sterilized in an autoclave for 20 minutes at 121^0C for 1 hour at 1.5 kg/cm^2 pressure and kept in slanting position for having maximum space for the organism in pure culture to proliferate.

3.7.2. Tissue culture

To obtain pure culture, a small piece of tissue was collected from the fruiting body of *Ganoderma lucidium* mushroom and placed on the sterilized PDA medium under aseptic condition in a laminar air flow cabinet. It was then kept for 7-10 days in an incubator under 25^0C for sufficient mycelial growth. These pure cultures were used for the entire experiment.

3.7.3. Preparation of mother spawn

Mother culture substrate was prepared by using sawdust. Sawdust was sieved and sun dried. The mother culture substrate was prepared by sawdust and wheat bran in 2:1 ratio with 0.1% calcium carbonate (Ruhul Amin, 2007). Then it was mixed thoroughly with hands and maintained 55% moisture content by adding sufficient water. Then 200 gm of mixture was packed tightly 10*12 inch polypropylene (PP) bag. Each of the bags was 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. The packets were sterilized for 1 hour at 121^0C for 1 hour at 1.5 kg/cm^2 pressure in an autoclave and kept them for cooling. Then inoculums from pure culture were placed aseptically to the mother spawn packets. The packets after inoculation were again plugged with cotton and were kept at 20-22^0C for spawn run. The whole packet containing substrate became white due to fungal mycelia proliferation within 15-20 days and thus ready for spawning the substrate.

3.7.4. Preparation of substrates

Spawn packets using different sawdust, wheat bran, $CaCo_3$ in ratio 69:30:1 respectively and moisture should be maintained. 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 65%.

3.7.5. Preparation of spawn packets

The mixed substrates were filled into 10×12 inch polypropylene bag @ 500 g. The filled polypropylene bags were prepared by using plastic neck and plugged the neck with cotton and covered with brown paper placing rubber band to hold it tightly in place.

3.7.6. Sterilization, inoculation and mycelium running in spawn packets

The spawn packets were sterilized about 1 hour and then these were kept for cooling. After cooling, 5 g mother spawn was inoculated into the packets in the laminar airflow cabinet and the packets were kept at 20-22^0C 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. Then these spawn packets were transferred to the culture house.

3.7.7. 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 by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a tea spoon for removing the thin whitish mycelial 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 newspaper. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3 times a day. The light around 300-500 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22^0C to 25^0C. 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.

3.7.8. Harvesting of mushrooms

Ganoderma lucidium mushrooms matured within 6-7 days after primordia initiation. The matured fruiting body was identified by curial margin of the cap, as described by Amin (2002). Mushrooms were harvested by twisting to uproot from the base.

3.8. Data collection

3.8.1. Mycelial growth (%):

Mycelial growth was counted by taking the full packet as a full unit and generally the data was taken at every two days intervals.

3.8.2. Mycelium running rate in spawn packet

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

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

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

N= Number of days

3.8.3. Days required for completing mycelium running

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

3.8.4. Time from stimulation to primordial initiation (days):

Time required from stimulation to primordial initiation (days) were recorded.

3.8.5. Time from stimulation to primordial initiation to harvest (days):

Time required from stimulation to primordial initiation to harvest (days) were recorded.

3.8.6. Average number of primordia per packet:

Number of primordial per packet was recorded.

3.8.7 Average number of fruiting body per packet:

Number of well-developed fruiting body was recorded. Dry and pinheaded fruiting bodies were discarded but tiny fruiting bodies were included in counting.

3.8.8. Average number of effective fruiting body per packet:

Number of well-developed fruiting body was recorded. Tiny fruiting bodies were discarded from counting.

3.8.9. Average 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.

3.8.10. Dimension of fruiting body (stipe and pileus)

Thickness of the pileus of three randomly selected fruiting bodies was measured using a slide calipers. Diameter of stipe, length and diameter of stripe were also measured

a. Diameter of pilus(cm)
b. Length of pileus (cm)
c. Thickness of stalk
d. Length of stripe (cm)

3.8.11. Biological yield

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

3.8.12. Economic yield

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

3.8.13. Drying of mushrooms

The collected fruiting bodies of the mushroom were transferred to the laboratory. Then data were collected on different parameter. After collection of the data the fruiting bodies were dried in the sun separately as per treatment. In the time of drying the stipe and the pileus were separated for better drying.

3.8.14. 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):

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

3.8.15. Cultural operations for subsequent flushes

After completing the first harvest again the packets were scraped at the place where the 'D' shaped cut had been done and were soaked in a bucket for five minutes and then were placed in the culture house and water was sprayed regularly. The primordia appeared 9-10 days after first harvest and 7-8 days after second harvest. Water spraying was continued until the mushrooms were ready to be harvested.

3.8.16. Biological efficiency

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

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

3.8.17. 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 (Sarker, 2004).

3.9. Proximate analysis of the mushrooms

3.9.1. 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.

3.9.2. Determination of 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.

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

3.9.3. Determination of 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

3.9.4. Grinding

The dried plant materials were cut into small pieces with a knife or scissor. The sample was grinded in a plant grinder fitted with a suitable screen. If the grinding takes a long time, the sample will absorb moisture and it is necessary to dry the sample again in the oven at 105^0C overnight.

3.9.5 Determination of total ash

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 (Raghuramulu *et al.*, 2003)

3.9.6 Determination of crude fiber

Ten gram of moisture and fat-free sample was taken in a beaker and 200 ml of boiling 0.255 N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a Moslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker and 200 ml of boiling 0.313 N NaOH was added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a Moslin cloth and the residue was washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100°C and weighed (Us) in an electric balance *(KEY: JY-2003; China).* The crucible was heated in a muffle furnace *(Nebertherm: Mod-L9/11/c6; Germany)* at 600°C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber.

Therefore,

Crude fiber (g/l00 g sample) = [100-(moisture + fat)] x (We-Wa)/Wt. of sample.

(Raghuramulu *et al.*, 2003).

3.9.7 Total 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] (Raghuramulu *et al.*, 2003).

3.9.8. Total Fat estimation

Fat was estimated as crude by the ethereal extraction of the dried mushroom using the method that reported by (Raghuramulu *et al.,* 2003). The dried sample (about 5g) was weighted into a conical flask and plugged with fat free cotton. The flask was then placed in an electric shaker and extracted with anhydrous ether for about 16 hours. The ether extract was filtered into another weighted conical flask. The flask containing the original ether extract was washed 4 to 5 times with small quantities of ether and then the washings were also transferred to the filter paper. The ether in the conical flask was then removed by evaporation and the flask with the residual was dried in an oven at 80°C too 100°C, cooled in a desiccators and weighted. The result was expressed as follows:

Fat contents (g) per 100g of dried sample=

$$\frac{\text{Weight of ether extract x Percentage of dried sample}}{\text{weight of the dried sample taken}}$$

3.10. Determination of approximate composition of mineral content

3.10.1. The following Equipments were used

Electric balance, Muffle furnace, Oven, desiccator, Atomic Absorption Spectrophotometer (AAS), Grinding machine, Porcelain crucible, Beaker and Flame photometer etc.

3.10.2. 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.

3.10.2.1. Reagents

a) Concentrated sulfuric acid

98% H_2SO_4, specific gravity 1.84.

b) Catalyst Mixture

Crush and mixed 100g potassium sulphate (K_2SO_4) and 100g Copper (II) sulphate ($CuSO_4.5H_2O$) in a motor.

c) 33% Sodium hydroxide

Can be procured as a solution or prepared by dissolving 16.67g NaOH in water in a 5 I volumetric flask. After complete dissolution, the flask is filled to volume with water and the content is mixed.

d) 0.0500 M Sodium hydroxide

Transfer the content of 1 vial sodium hydroxide (4.0g) to a 2 L volumetric flask filled to with water and mixed.

e) 0.0500M hydrochloric acid

Transfer the content of 1 vial hydrochloric acid (3.645 g) to a 2 L volumetric flask filled to with water and mixed.

f) Methyl red- methylene blue indicator solution

Dissolve 0.667 g methyl red in 500ml 96% ethanol. Also dissolve 0.625 g methylene blue in 500 ml 96% ethanol. Mixed equal volumes of the two solutions.

3.10.2.2. Digestion

Step 1: The digester was turned on to reach the digestion temperature (390°C) by the time the samples were ready for digestion.

Step 2: 20 clean and dry digestion tubes were placed in the digestion rack. Every 0.20 g sample was taken in each of 18 tubes. The 2 remaining tubes served as blanks.

Step 3: 1g catalyst mixture and 5ml conc. H_2SO_4 were added to each tube included the blanks.

Step 4: The rack with the tubes was put beside the digester and place the exhaust manifold on the tubes. All the stoppers were properly inserted into the tubes. Then the exhaust pump were started to open the regulating valve fully.

Step 5: The tubes were placed in the digester at 390°C. After about 5 min the suction rate was reduced by almost closing the regulating valve. Digestion was continued for 2 hours.

Step 6: Turning off the digester, the rack with the tubes was removed and placed it besides the digester for cooling. Suction was continued for 5 min; the exhaust manifolds were removed from the tubes and turned off the exhaust pump.

3.10.2.3. Distillation

Some water was distilled following the appropriate distillation procedure and distilled was received in a conical flask for further use 20.00 mi .0500 M HCl was taken from a burette into a conical flask and placed the flask on the platform in the distilled. 25 ml water was added carefully to one of the digestion tubes from the digestion rack. The addition of water was done carefully as the mixture become very hot. The tube was placed in the left hand side of the distilled around the plastic tube. To touch the plastic tube with the hands was avoided. The tube was tightening properly against the upper rubber adapter and then the safety door was closed. 25 ml 33% NaOH was dispensed gently into the digestion tube by pulling the alkali handle to it's down position and was released it. The steam valve was opened by pulling it to its down position and the timer was set to 3 min. When the alarm sounds, the distillation was continued for about 30 sec by lowering the platform with the receiver flask. Closing the steam valve by pushing it up, the safety door was opened and the receiver flask was replaced with another flask containing 20.00 ml 0.05 M HCl. The platform was then pushed up. The content in the flask which was removed from the distilled titrated with 0.05 M NaOH as described below. Following the above procedure all of the digestion tubes were distilled.

3.10.2.4. Titration

4 drops of indicator solution were added to the content in the flask and titrate with 0.05 NaOH until the color was changed from violet to green.

3.10.2.5. Calculation

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

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

3.10.3. Determination of total protein:

The total protein was estimated by multiplying total nitrogen with 6.25 (Reff. Standard Micro-Kjeldhal procedure of AOAC,1975).

3.10.4. Determination of Ca, Mg, K, Fe, Zn, Co, Mo, Se and P

The sample was digested with nitric acid to release of Ca, Mg, K, Fe, Zn, Co, Mo, Se and P. Ca, Mg, Fe, Co, Mo, Se and Zn were determined by atomic absorption spectrophotometer, K was determined by flame photometry and P by spectrophotometer using the following formula.

3.10.4.1. Digestion

Step 1: 0.500 g of dried sample was taken into each of 18 nitrogen digestion tubes. The two remaining tubes were kept blanks. 5 ml nitric acid was added to each of all 20 tubes. The tubes were left overnight mixing the contents in the tubes. Covering with the exhaust manifold, the tubes were placed in the digester and the temperature was set to 125℃, turning on the digester, the digestion was continued for 4 hours after boiling started. Every tube was observed to avoid drying.

Step2: After cooling, the digestion mixture was transferred with distilled water to a 100 ml volumetric flask. Water was added to the flask to make the volume up to the mark.

Step 3. Filtration was performed on a dry filter into a dry bottle, which could be closed with a screw cap. Keeping the filtrate in the closed bottle Ca, Mg, K, Fe, Mn, Zn, S, Cu and P were determined in the filtrate.

3.10.4.2 Estimation of Ca

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 was measured by atomic absorption spectrophotometer (AAS).

310.4.3 Estimation of Mg

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 Mg was measured by atomic absorption spectrophotometer (AAS).

3.10.4.4 Estimation of K

10 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette to volume with water and mixed. The content of K was measure by flame photometer.

3.10.4.5 Estimation of P

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.

3.10.4.6 Estimation of Fe, Zn, Co, Mo and Se

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

3.10.4.7 Calculations

For Ca, Mg, K, P

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

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, Mo, Co, Se

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

Zn and Fe measured on atomic absorption spectrophotometer

c = g sample weighed into the digestion tube

3.11. 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 (Gomez and Gomez, 1984).

Chapter 4

RESULTS AND DISCUSSIONS

The study was conducted to find out the effect of different sawdust of the growth, yield and proximate analysis of *Ganoderma lucidum*. Data on different growth, yield contributing characters, proximate composition of mushroom were recorded. The results have been presented and discussed with the help of table, graphs and possible interpretations given under the following headings:

4.1 Effect of different sawdust substrates on mycelia growth and yield contributing characters of *Ganoderma lucidum*

4.1.1 Effect on mycelium running rate in spawn packet (cm)

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. Mycelium running rate in spawn packet was found to be different due to different sawdust used. The highest running rate was observed in T_2 (0.72 cm) followed by T_1 (0.69 cm) and the lowest mycelium running rate was observed in T_6 (0.55cm). Different sawdust showed different mycelium running because of different carbohydrate based on availability and the environment of the spawn. Mycelium running rate varied due to use of different saw dust. (Table 1). The present findings found more or less similar with the previous workers. Peksen and Yakupoglu, 2009 and Yang *et al.*, 2003 amended with different organic supplement like wheat chaff, wheat bran, paddy straw, cotton waste etc. provided suitable condition for spawn running.Wasser, S. P. 2005.found that the mycelium running rate of Reishi mushroom greatly influenced with the supplement of wheat barns in different levels.N. J. & Sullivan 2002 also found similar result as found in the present experiment.

4.1.2 Effect on time from stimulation to primordial initiation (Days)

The time from stimulation to primordial initiation range from 20 days to 10 days. The highest time from stimulation to primordial initiation was observed in T_4 (20.75 days). Statistically similar lowest stimulation to primordial initiation was shown in T_2 (10.64

days).The other treatments were statistically similar (Table 2). The result of present findings keeps in with the findings of previous scientists (Chada and Sharma, 1998). Sharma found that the fruiting bodies appeared 12-15 days after the packet cutting and the first crop was harvested 7-8 days later on mango saw dust, mahogony sawdust, raintree sawdust, teak sawdust, jackfruit sawdust and mixed sawdust packet.

4.1.3 Effect on time from primordial initiation to harvest (Days)

Numerically the lowest time from primordial initiation to harvest was in the treatment T_5 (7.68 days)followed by T_1 (8.33 days) and the highest time from primordial initiation to harvest was observed in the treatment T_4 (12.50 days).The result of present findings keeps in with the findings of previous scientists (Basunia *et al.*2007, Rahman *et al.*2007,And Gomez (2002). Basunia *et al*. (2007) reported that after spwan running pinhead formation took 12-15 days and fruiting body formed after 7-8 days, sporocarps may be harvested after 10-12 days. Rahman *et al.*2007 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 42-43.50 days depending on different substrates. Gomez (2002) found as the spawn rate increased the number of days to production decreased.

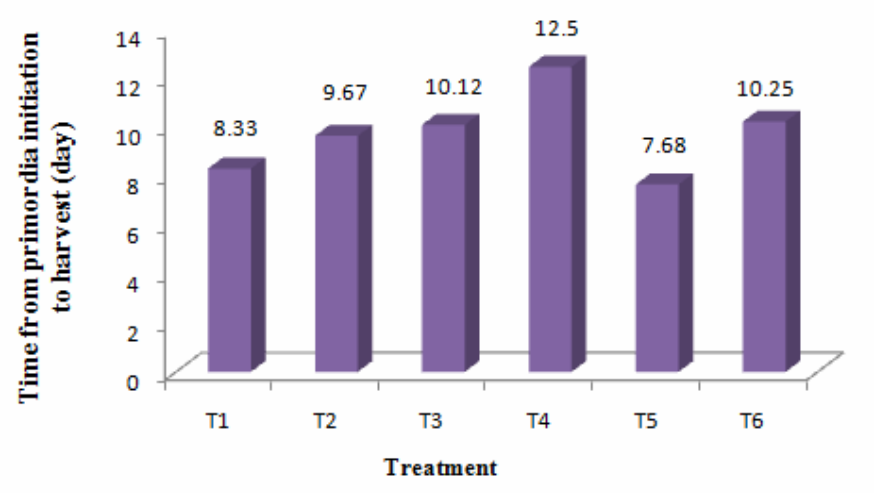

Figure 1: Effect of different sawdust on time from primordia initiation to harvest of Reishi mushroom

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree sawdust with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime.

4.1.4 Effect on Average number of fruiting body per packet : Different sawdust spawn packet showed statistically significant differences in terms of fruiting body per packet of Reishi mushroom (Table 1). The highest average number of fruiting body per packet was observed in T_2 (10.00) which was statistically similar with T_1 (9.60) and T_6 (9.20).whereas the lowest average number of fruiting body per packet was found in T_4 (5.19) treatment.

4.1.5 Effect on Average weight of individual fruiting body: Significant variation was observed in terms of average weight of individual fruiting body of Reishi mushroom due to different sawdust packet (Table 1). The highest average weight of individual fruiting body was attained in T_4 (12.62 g) treatment and the second highest average weight was T_3 (10.45 g) which was statistically similar with T_5 (9.60 g) and On the other hand, the lowest average weight of individual fruiting body was found in T_2 (8.9 g) treatment which was statistically similar with T_1 (9.0 g) treatment.

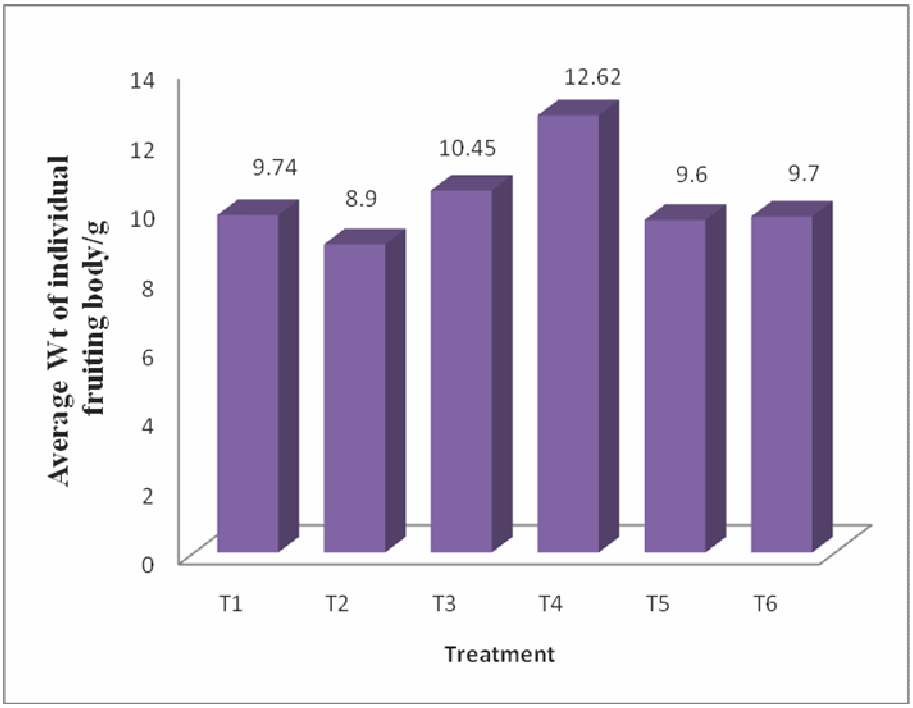

Figure 2 : Effect of different Sawdust on average weight of individual fruiting body of Reishi mushroom

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree sawdust with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime.

Table 1. Effect of sawdust of different tree species on mycelia growth and yield contributing characters of *Ganoderma lucidum*

Treatments	Mycelium growth rate in spawn packet (cm/day)	Time from stimulation to primordia initiation (days)	Time from primordia initiation to harvest (days)	Average number of fruiting body/ packet	Average weight of individual fruiting body/g
T_1	0.69 ab	12.00 de	8.33 c	9.60 b	9.74 d
T_2	0.72 a	10.64 e	9.67 b	10.00 a	8.90 e
T_3	0.62 bc	15.25 c	10.12 b	7.40 d	10.45 ab
T_4	0.59 c	20.75 a	12.50 a	5.19 e	12.62 a
T_5	0.63 bc	13.67 cd	7.68 c	8.40 c	9.60 cd
T_6	0.55 c	17.38 b	10.25 b	9.20 b	9.70 d
LSD (0.05)	0. 0788	1.744	1.148	1.793	0.8347
CV(%)	1.66%	4.75%	4.73%	5.14%	8.7%

In a column means having similar letter(s) are statistically similar and those having dissimilar letter differ significantly at 0.01 level of probability

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree sawdust with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime.

4.2 Effect of different sawdust substrate on the development and size of fruiting body

4.2.1 Effect on antler initiation

The reproductive growth of *Ganoderma lucidum* varied from one wood species to another (Table 3). The first antler initiation (from opening to primordia), within

3.75days, was recorded on Mango sawdust which was statistically similar with Mixed sawdust (4.00days),Mahogony sawdust (6.50). Except jackfruit sawdust and Teak Sawdust cause jackfruit sawdust takes the longest time (11.75 days).

4.2.2 Effect on conk formation

The lowest time required from opening to conk development (10.25 days) was recorded both in *Mangifera indica* and mixed sawdust. The highest period of conk formation (20.00 days) was Observed in jackfruit sawdust which was significantly higher to all the treatments. Number of fruiting bodies did not differ significantly in the sawdust of different tree species.

4.2.3 Effect on Length of stalk (cm)

Length of stalk of Reishi mushroom varied significantly due to different sawdust (Table 3). The highest length of stalk was observed in T2 (2.68 cm) treatment which was statistically similar with T3 (2.35 cm) and closely followed by T5 (2.33 cm). whereas the lowest length of stalk was found in T4 (1.10 cm) which was closely followed by T6 (1.23 cm) Malarvizhi et al. (2003) reported that length of stalk ranged from 1.80 to 2.79 cm which was similar to the findings of this experiment.

4.2.4 Effect on Diameter of stalk

Statistically significant variation was recorded in terms of diameter of stalk of Reishi mushroom due to different sawdust treatment (Table3). The highest diameter of stalk was found in T_1 (1.33 cm) treatment was statistically similar with T_3(1.30cm) and T_5 (1.20cm) treatment. While the lowest diameter of stalk was attained in T_4 (0.95 cm) treatment which was statistically similar with T_2 (1.13 cm) and closely followed by T_5 (1.20cm) treatment. Chen (1998) reported significant effects of various substrates on diameter of stalk. Muhammad (2011) found that stalk of reishi mushroom on different sawdust varied from 0.99 cm to 2cm.

4.2.5 Effect on Diameter of pileus (cm)

Different sawdust method showed statistically significant variation in terms of diameter of pileus of Reishi mushroom (Table 3). The highest diameter of pileus was recorded in T1 (6.05cm) treatment which was statistically similar with T5 (5.73 cm) and T3 (5.60cm) .whereas the lowest diameter of pileus was observed in T4(4.00 cm)

treatment which was closely followed by T6 (4.58 cm) treatment. Paterson (2006) reported the diameter pileus ranged from 5.66 to 7.44 cm. The highest diameter of pileus (7.44 cm) was found in autoclaved sawdust mixed with rice & wheat bran.

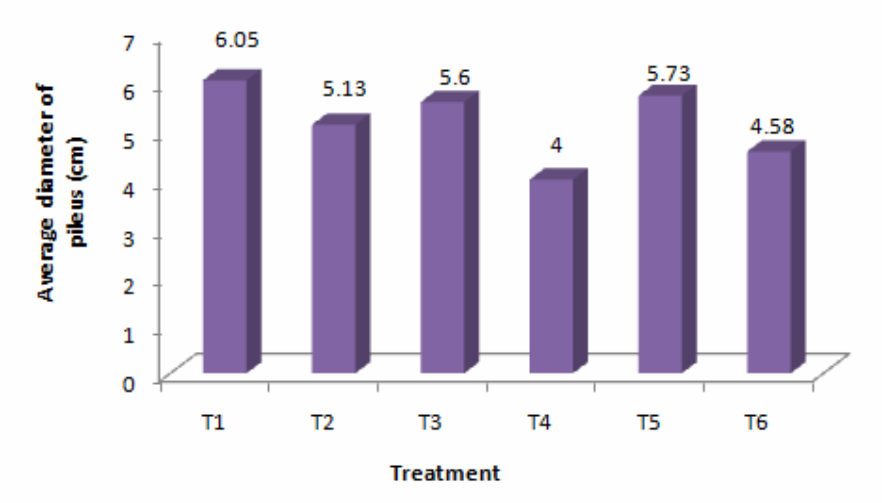

Figure 3 : Effect of different Sawdust on average diameter of pileus of Reishi mushroom

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2:Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with + 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime

4.2.6 Effect on Thickness of pileus

Significant difference was recorded in terms of thickness of pileus of Reishi mushroom due to different sawdust packet (Table 3). The highest thickness of pileus was observed in T_3 (1.53 cm) treatment which was closely followed with T_1(1.50cm) and T_6 (1.50 cm) treatment. On the other hand, the lowest thickness of pileus was found in T_5 (1.28cm) treatment which was closely followed by T_4 (1.38cm). Muhammad (2011) reported the thickness of pileus ranged from 0.47 to 1.55 cm respectively and the highest was found in mixed sawdust with the mixture of rice and wheat bran.

Table 2: Effect of different sawdust on the development and size of fruiting bodies of *Ganoderma lucidum*

Treat ments	Days required for antler initiation	Days required for conk formation	Average Length of stalk (cm)	Average diameter of stalk (cm)	Average Thickness of pileus (cm)	Diameter of pileus (cm)
T_1	4.00 d	10.25 d	1.95 c	1.33 a	1.50 ab	6.05 a
T_2	3.75 d	10.25 d	2.68 a	1.13 b	1.48 ab	5.13 ab
T_3	6.50 c	13.00 c	2.35 b	1.30 a	1.53 a	5.60 ab
T_4	11.75 a	20.50 a	1.10 e	0.95 c	1.38 bc	4.00 c
T_5	9.75 b	16.33 b	2.33 b	1.20 ab	1.28 c	5.73 a
T_6	5.75 c	12.25 cd	1.23 d	1.25 ab	1.50 ab	4.58 bc
CV(%)	5.93	5.93	2.19	4.89	4.04	7.92
LSD(0.05)	1.022	2.037	0.1115	0.138	0.1366	1.022

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.01 level of probability.

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with + 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime

4.3 Effect of different sawdust on the yield, biological efficiency and benefit cost ratio of Reishi mushroom

4.3.1 Effect of different sawdust substrates on biological yield

Different sawdust substrates had great effect on biological yield. The highest biological yield was recorded from T_2 (97.30 g), which was statistically similar with T_1 (92.75 g) and T_6 (90.82 g) while the lowest biological yield was recorded in T_4 (73.68 g). The result of the present study found similar with the of previous studies (Smith *et al.,* 2002; Wasser *et al.,* 2005 and Yang *et al.,* 2003). Erkel *et al.* (2009) found the highest biological yield 87.3 g/packet. Smith *et al.* (2002) examined the

effects of adding various lime percentage and temperature fluctuation gave the highest yield of Reishi mushroom. Wasser *et al.* (2005) found significant effect of different agro-wastes on yield of Reishi mushroom. Erkel *et al.* (2009) found the highest yield of Reishi mushroom with the substrate composed of 20% rice husk in weigh.

4.3.2 Effect of different sawdust substrates on economic yield

Economic yield of *Ganoderma lucidum* grown on different sawdust showed statistically significant variation (Table 4). The highest economic yield was recorded from T_2 (89.20 g), which was statistically similar with T_1 (86.55 g) and followed by T_6(83.48 g). whereas the lowest economic yield was observed in T_4 (65.50 g) which was followed by T_3 (77.34 g). The findings of this experiment also supported by the earlier findings of Paterson, *et al.* (2002) and Amin *et al.* (2007). Amin *et al.* (2007) found that the trend of economic yield corresponded with different supplements at different level. paterson *et al.* (2002) found the highest yield of Reishi mushroom with the substrate composed of 20% rice husk in weight.

4.3.3 Effect of different sawdust substrates on Dry yield

The highest dry yield was observed from T_2 (60.05 g), which was statistically similar with T_1 (56.36g) and followed by T_6 (55.77 g) On the other hand, the lowest dry yield was attained in T_4 (42.65 g) which was statistically similar with T_5(52.95g). Chen&Fang (1998) observed that the diameter of pileus increased the quality and yield mushroom and highest dry yield from mango sawdust.

4.3.4 Effect of different sawdust substrates on Biological efficiency

The highest biological efficiency was recorded from T_2 (55.60%), which was statistically similar with T_1 (53.00%) and followed by T_5 (50.57%) . And the lowest biological efficiency was observed in T_4 (42.17%) which was statistically similar with T_3(48.46%).

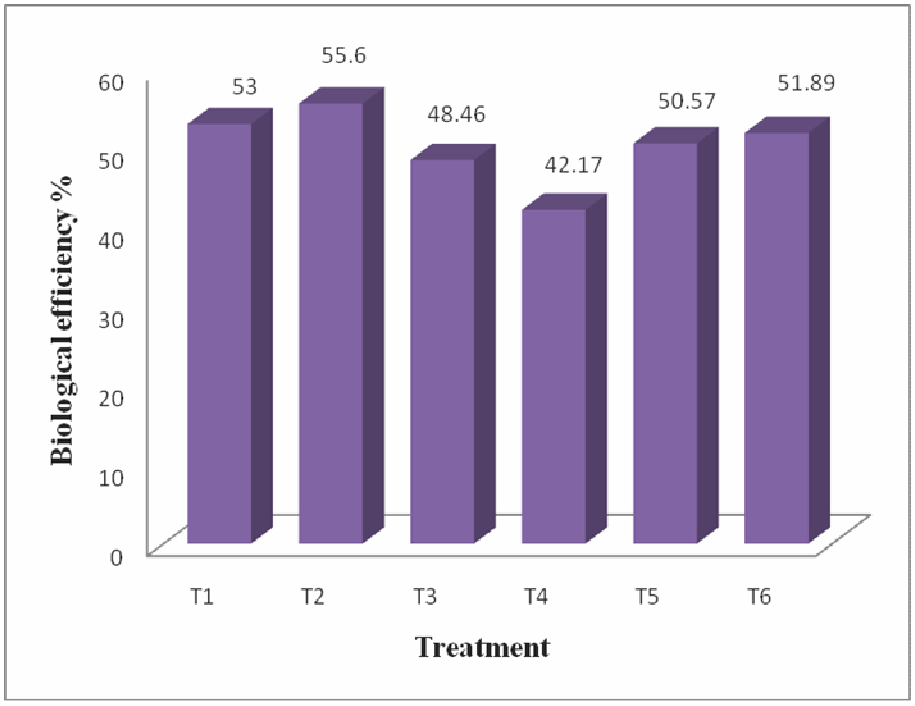

Figure 4 : Effect of different Sawdust on biological efficiency of Reishi mushroom

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with + 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime

4.3.5 Effect of different sawdust substrates on Benefit cost ratio

The highest benefit cost ratio was found from T_3(5.10), which was statistically similar with T_6 (4.92) and followed by T_2 (4.89) On the other hand, the lowest benefit cost ratio was recorded in T_4 (4.14) which was statistically similar with T_5(4.56). The present findings found similar with the findings of previous research. Lim *et al.* (1997) analyzed the cost and return of *different* species of *Ganoderma* mushroom production and found the BCR of 8.9 and 5.1.Muhammad (2011) also observed the benefit cost ratio of 7.32, 23.78 and 16.23 in case of *Ganoderma* .The cause of these variations between the results of this study might be due to consideration of other

costs involved in the production of Reishi mushroom or might be due to measuring system. Sarker *et al*, (2007) 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 bran.

Table 3. Effect of sawdust of different tree species on the yield, biological efficiency and benefit cost ratio of *Ganoderma lucidum*

Treatments	Biological yield (g/packet)	Economic yield (g/packet)	Dry yield (g/packet)	Biological Efficiency (%)	Benefit cost Ratio
T_1	92.75 b	86.55 b	56.36 ab	53.00 ab	4.76 c
T_2	97.30 a	89.20 a	60.05 a	55.60 a	4.89 b
T_3	84.82 cd	77.34 cd	53.86 bc	48.46 cd	5.10 a
T_4	73.68 d	65.50 d	42.65 cd	42.17 d	4.14 e
T_5	88.50 c	80.68 c	52.95 c	50.57 c	4.56 d
T_6	90.82 b	83.48 b	55.77 ab	51.89 bc	4.92 b
CV%	0.03%	0.03%	0.04%	0.06%	0.85%
LSD (0.05)	0.176	0.176	0.024	0.115	0.115

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with + 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6:Rain tree sawdust with 30% wheat bran and 1% lime

4.4 Effect of different sawdust substrates on Proximate analysis of *Ganoderma lucidum*

4.4.1 Effect on Moisture content Moisture

Statistically significant variation was recorded in terms of moisture content of reishi mushroom due to different sawdust treatment (Table 4). The highest moisture content was found in T_3 (69.65%) treatment which was statistically identical with T_2 (67.33%) and T_6 (66.81) treatment, while the lowest moisture content was recorded in T_4 (65.12%) and T_1 (65.12%) treatment which was statistically similar with T_5 (65.63%) Treatment . The findings of the present experiment corroborate with the Mohammad *et al.* (2011) cultivated the Reishi mushroom on paddy straw, banana leaves, sugarcane bagasse, water hyacinth, betel nut husk and he found moisture content varied from 58.15 to 71.64%. Yung (2008) found no significant differences among the mushrooms produced in sawdust. supplemented with wheat bran.

4.4.2 Effect on Dry matter

Different sawdust treatment varied significantly in terms of dry matter content of Reishi mushroom (Table 4). The highest dry matter content was found from T_1 (34.88%) and T_4 treatment which was statistically identical with T_5 (34.37%), T_6 (33.19%) treatment, whereas the lowest dry matter content was recorded in T_3 (30.35%) treatment which is nearly similar to T_2 (32.67). The result of the present study matches with the findings of previous one that reported by Mueller,*et al.* (2009), 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 in oyster Mushroom. Gottlieb et al. (1998) 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 Species of mushroom.

4.4.3 Effect on Protein content

All the treatment contains a considerable amount of protein.(table 4). The highest protein content was recorded in T_1 (12.25%) treatment which was statistically identical with T_2 (11.88%) , T_3 (11.43%), T_6 (11.18%), T_5 (10.69%)treatment. On the other hand, the lowest protein content was observed in T_4 (7.43%) treatment.

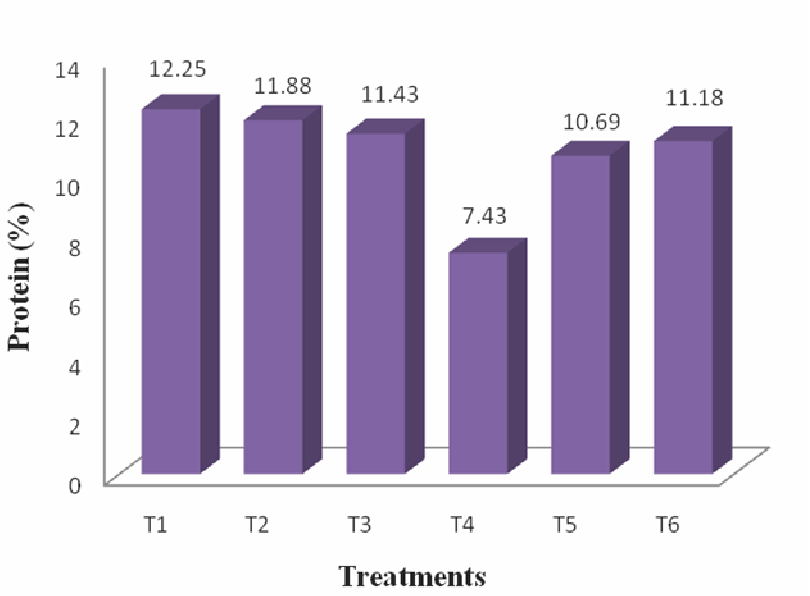

Figure 5 : Effect of different Sawdust on protein percentage of Reishi mushroom

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with + 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6:Rain tree sawdust with 30% wheat bran and 1% lime

4.4.4 Effect on Lipid content

Statistically significant variation was recorded in terms of lipid content of *Ganoderma* mushroom due to different sawdust treatment (Table 4). The highest lipid content was observed from T_2 (3.23%) treatment which was statistically identical with T_4 (2.94%) and T_1 (2.91%) treatment, whereas the lowest lipid content was obtained in T_3 (2.77%) which was statistically similar with T_6 (2.83%) and T_5 (2.84%) treatment. The result of the present study was found more or less similar with the findings of Alam *et al.* (2007) who reported 4.30 to 4.41% lipids in Reishi mushroom.

4.4.5 Effect on Carbohydrate

Statistically significant variation was recorded in terms of carbohydrate content of reishi mushroom due to different sawdust treatment (Table 4). The highest carbohydrate content was recorded in T_2 (74.15%) treatment which was statistically identical with T_1 (73.28%) and T_3 (72.12%) treatment, whereas the lowest was observed in T_6 (64.33%) treatment which was closely followed with T_5 (66.6%) treatment. The findings of the present study were not supported by the study of Chang yung *et al.* (2009) who found that carbohydrate content was ranged from 32.85 to 56.38% which showed a high rate of variation.

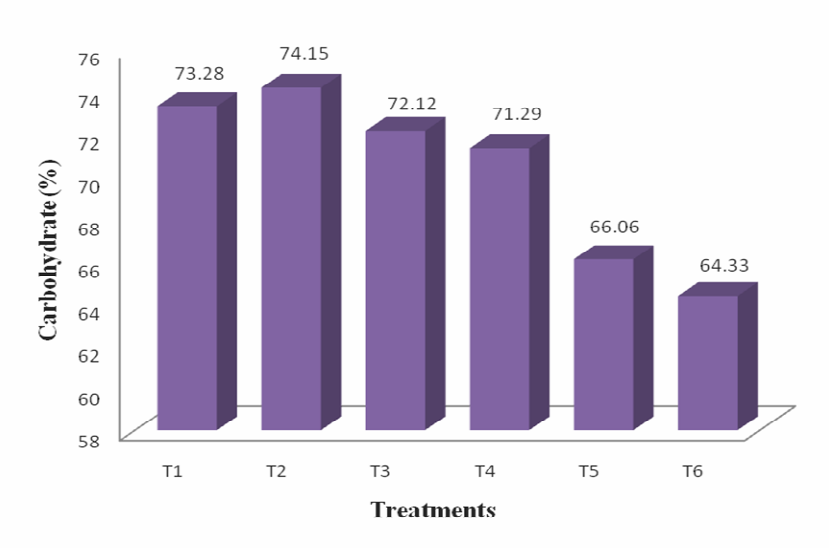

Figure 6 : Effect of different Sawdust on carbohydrate percentage of Reishi mushroom

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with + 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6:Rain tree sawdust with 30% wheat bran and 1% lime

4.4.6 Effect on Crude fiber

Statistically significant variation was recorded in term of crude fiber content showed due to different sawdust (Table 4). The highest crude fiber content was found in T_6 (20.82%) treatment which was statistically identical with T_5 (19.46%) and T_4 (17.30%) and treatment, while the lowest crude content was obtained in T_2 (9.38%) treatment which was statistically similar with T_1 (10.23%) treatment. The findings of the present study corroborate with the study Alam *et al*. (2007) reported 22.87g/100g to 23.29g/100g of fiber in *Ganoderma spp.* Gottlieb *et al.* (1998) reported that on an average, a serving (100 g) of mushroom will supply 9 to 40% of the recommended of dietary fiber which was also differ from the present study.

4.4.7 Effect on Ash

Different sawdust treatment varied significantly in terms of ash content of Reishi mushroom under the present trial (Table 4). The highest ash content was found in T_2 (1.36%) treatment which was statistically identical with T_1 (1.33%), T_3 (1.15%) T_4 (1.04) and the lowest ash content was recorded in T_6 (0.84%) treatment which was statistically similar with T_5 (0.95%) treatment.

Table 4 Effect of different sawdust on proximate nutrient composition of *Ganoderma lucidum*

Treatment	Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	Carbo hydrate (%)	Crude fiber (%)
T_1	65.12 c	34.88 a	12.25 a	2.91bc	1.33b	73.28b	10.23 e
T_2	67.33 ab	32.67 ab	11.88 b	3.23a	1.36a	74.15a	9.38 f
T_3	69.65 a	30.35 d	11.43 cd	2.77e	1.15 c	72.12 c	12.53 d
T_4	65.12 c	34.88 cd	7.43 e	2.94 c	1.04 d	71.29 d	17.30 c
T_5	65.63 c	34.37a	10.69d	2.84 cd	0.95 bc	66.06 e	19.46 b
T_6	66.81bc	33.19 c	11.18cd	2.83 d	0.84f	64.33 f	20.82 a
LSD(0.01)	2.107	0.6956	0.6006	0.0788	0.0249	0.0078	0.2732
CV(%)	0.98	2.14	1.01	0.51	0.15	0.03	0.45

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

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with + 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1%

4.5 Effect of different sawdust on the Mineral content of *Ganoderma lucidum*

4.5.1 Effect on Nitrogen (N)

Statistically significant variation was recorded in terms of N content of Reishi mushroom due to different sawdust method (Table 6). The highest N content was recorded in T_1 (1.96%) treatment which was statistically identical with T_2 (1.90%), T_3 (1.83%) and T_6 (1.79%) treatment. On the other hand, while the lowest N content was observed in T_4 (1.19%) treatment which was statistically similar with T_5 (1.71%) treatment.

4.5.2 Effect on Phosphorus (P)

Different sawdust treatment showed significant differences in terms of P content of Reishi mushroom (Table 6). The highest P content was observed in T_2 (1.47%) treatment which was statistically identical with T_1 (1.45%), T_3 (1.43%), T_5 (1.43%) and T_6 (1.41%) treatment, while the lowest P content was found in T_4 (1.28%) treatment. Muhammad *et al.* (2011) also found that phosphorus content was ranged from 0.84 to 0.92% which was smaller than the findings of this experiment.

4.5.3 Effect on Potassium (K)

Significant variation was recorded in terms of K content of Reishi mushroom due to different treatment (Table 6). The highest K content was recorded in T_2 (2.65%) treatment which was statistically identical with T_3 (2.63%), T_1 (2.58%) and T_4 (2.55%) treatment, whereas the lowest K content was observed in T_6 (2.40%) treatment which was statistically similar with T_6 (2.44%) treatment. The findings of

the present study similar with the study of Chang *et al.* (1981) who reported that the fruiting bodies of *Ganoderma* contained 1.43 to 1.88 g of K on dry weight basis. Sarker *et al.* (2004) also found 1.3% potassium in Reishi mushroom which was smaller than the findings of present study.

4.5.4 Effect on Calcium (Ca)

Different treatment varied significantly in terms of Ca content of Reishi mushroom (Table 6). The highest Ca content was observed in T_3 (1.96%) treatment which was statistically identical with T_2 (1.92%), T_5 (1.91%), T_6 (1.91%) and T_1 (1.90%) treatment and the lowest Ca content was recorded in T_4 (1.73%) treatment.

4.5.5 Effect on Magnesium (Mg)

Statistically significant variation was recorded in terms of Mg content of Reishi mushroom (Table 6). The highest Mg content was found in T_2 (0.727%) treatment which was statistically identical with T_5 (0.721%), whereas the lowest Mg content was recorded in T_4 (0.673%) treatment which was closely followed by T_6 (0.708%) and T_3 (0.713%) treatment and they were statistically identical. Sharma *et al.* (2004) also found 0.21% magnesium in Reishi mushroom which was smaller than the findings of this experiment.

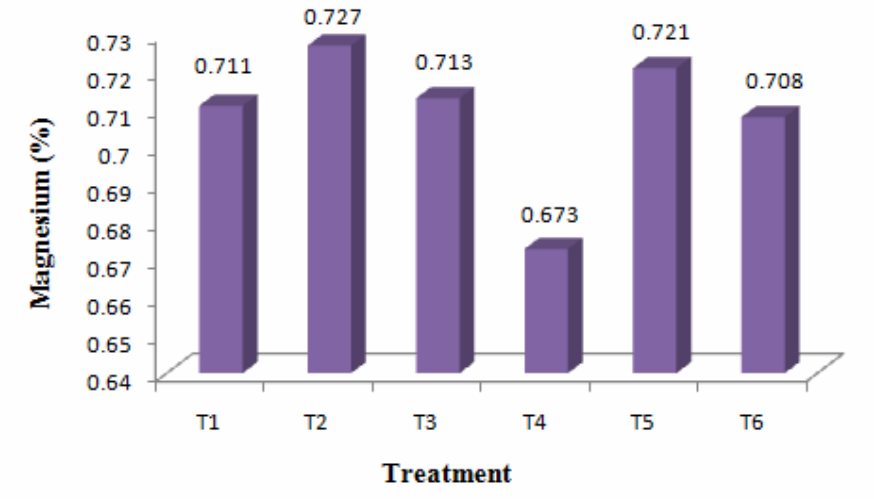

Figure 7 : Effect of different Sawdust on Magnesium percentage of Reishi mushroom

T_1: Controlled(Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with + 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime

4.5.6 Effect on the Iron (Fe)

Fe content of Reishi mushroom showed statistically significant variation.(Table 6). The highest Fe content was recorded in T_2 (515.48 ppm) treatment which was statistically identical with T_1 (510.08 ppm), T_4 (503.12 ppm) and T_6 (498.86 ppm) treatment. On the other hand, the lowest Fe content was observed in T_3 (494.89 ppm) treatment which was statistically similar with T_5 (495.23 ppm) treatment. Thangamuthu (1990) reported that content of Fe in the mushroom grown on different substrates varied from 92.09 ppm to 118.40 ppm. The result of the present study found iron higher than the value found by Alam *et al.* (2007) who found that iron content of different Reishi mushroom varieties ranged from 33.45 to 43.2 ppm.

4.5.7 Effect on the Sculpture (S)

Statistically significant variation was recorded in terms of S content of Reishi mushroom due to different sawdust treatment (Table 6). The highest S content was found in T_3 (0.323%), treatment which was statistically identical with T_2 (0.315%), T_6 (0.313%) and T_1 (0.312%) treatment, whereas the lowest S content was recorded in T_5 (0.281%) treatment which was statistically similar with T_4 (0.299%) treatment. The findings of the present study were supported with the findings of Gottlieb *et al.* (1998) who recorded 0.238 to 0.321% of sulphur from their earlier study in reishi mushroom varieties.

4.5.8 Effect on the Zinc (Zn)

Statistically significant variation was recorded in terms of Zn content of Reishi mushroom due to different sawdust treatment (Table 6). The highest Zn content was

observed in T_5 (15.54%) treatment which was statistically identical with T_2 (15.49%), T_3 (15.36%), T_1 (15.32%), and T_4 (15.15%) treatment and the lowest Zn content in T_6 (14.02%) treatment. 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 Reishi mushroom ranged from 16 to 20 .09.

Table 5. Effect of different sawdust on major mineral contents of *Ganoderma lucidum*

Treatments	N (%)	P (%)	K (%)	Ca mg/100gm	Mg mg/100g	Fe mg/100g	S mg/100g	Zn mg/100g
T_1	1.96 a	1.45 b	2.58ab	1.90 a	0.711 b	510.0 b	0.312 b	15.32 d
T_2	1.90 b	1.47 a	2.65 a	1.92 a	0.727 a	515.5 a	0.315 b	15.49 b
T_3	1.83 c	1.43 c	2.63ab	1.96 a	0.713 b	495.2 e	0.383 a	15.36 c
T_4	1.19 f	1.28 e	2.50bc	1.73 b	0.673 c	503.1 c	0.299 c	15.15 e
T_5	1.71 e	1.43 c	2.40 c	1.91 a	0.721 a	494.9 f	0.281 d	15.54 a
T_6	1.79 d	1.41 d	2.44 c	1.91 a	0.708 b	498.9d	0.313 b	14.02 f
LSD(0.01)	0.0078	0.0078	0.1366	0.4461	0.007	0.008	0.007	0.008
CV(%)	0.26	0.71	2.30	9.69	0.14	0.001	0.33	0.07

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: Controlled (Mixture of sawdust) with 30% wheat bran and 1% lime

T_2: Mango sawdust with 30% wheat bran and 1% lime

T_3: Mahogony sawdust with + 30% wheat bran and 1% lime

T_4: Jackfruit sawdust with 30% wheat bran and 1% lime

T_5: Teak tree with 30% wheat bran and 1% lime

T_6: Rain tree sawdust with 30% wheat bran and 1% lime

Chapter 5

SUMMARY AND CONCLUSION

The study was conducted at the Biochemistry laboratory and Mushroom Culture House (MCH) of the Department of Biochemistry, Sher-e-Bangla Agricultural University, and Dhaka during the period from August to December, 2014 to evaluate the performance of different sawdust on the growth, yield and proximate composition of Reishi mushroom (*Ganoderma lucidum*). The experiment consists of six different type of sawdust as- T_1: Mixed sawdust (Jackfruit, mango, mahogony, teak and raintree) + 30% wheat bran, T_2: Mango sawdust + 30% wheat bran; T_3: Mahogony sawdust + 30% wheat bran; T_4: Jackfruit sawdust + 30% wheat bran; T_5: Teak sawdust + 30% wheat bran and T_6: Raintree sawdust + 30% 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 running rate (0.72 cm) was recorded from T_2, while the lowest mycelium running rate (0.55 cm) was observed in T_4. The highest time from stimulation to primordial initiation (20.75days) was found from T_4, whereas the lowest time from stimulation to primordial initiation (10.64days) was recorded in T_2. The highest time from primordial initiation to harvest (12.50 days) was attained from T_4 and the lowest time from primordial initiation to harvest (7.68 days) was found in T_5. The maximum average number of fruiting body/packet (10.00) was observed from T_2, again the minimum average number of fruiting body /packet (05.19) was found in T_4.The highest average weight of individual fruiting body (12.62 g) was attained from T_4 and the lowest average weight of individual fruiting body (8.90 g) was found in T_2. The longest length of stalk (2.68cm) was recorded from T_2, while the shortest length of stalk (1.10cm) was found in T_4. The highest diameter of stalk (1.33 cm) was found from T_1, whereas the lowest diameter of stalk (0.95 cm) was recorded in T_4. The highest diameter of pileus (6.05 cm) was recorded from T_1, again the lowest diameter of pileus (4.00 cm) was found in T_4. The highest thickness of pileus (1.53 cm) was observed from T_3, and the lowest thickness of pileus (1.28cm) was found in T_5. The highest biological yield (97.30g) was attained from T_2, while the lowest biological

yield (73.68 g) was recorded in T_4. The highest economic yield (89.20 g) was recorded from T_2, whereas the lowest economic yield (65.50 g) was observed in T_4.The maximum biological efficiency (55.60%) was recorded from T_2, again the lowest biological efficiency (42.17%) was observed in T_4. The highest benefit cost ratio (5.10) was found from T_3, and the lowest benefit cost ratio (4.14) was attained in T_4.

The highest moisture content (69.65%) was observed from T_3, while the lowest moisture content (65.63%) was found in T_5. The lowest dry matter content (30.35%) was found from T_3, whereas the highest dry matter content (34.88%) was recorded in T_1. The highest protein content (12.25%) was recorded from T_1, while the lowest protein content (7.43%) was observed in T_4. The highest lipid content (3.23%) was found from T_2, again the lowest ash content (0.84%) was recorded in T_6. The highest ash content (1.36%) was recorded from T_2.The highest carbohydrate (74.15%) was observed from T_2, whereas the lowest carbohydrate content (64.33%) was observed in T_6. The highest crude fiber (20.82%) was recorded from T_6, and the lowest crude fiber content (9.38%) was found in T_2.

The highest amount of phosphorus content (1.47%) was attained from T_2, whereas the lowest phosphorus content (1.28%) was found in T_4. The highest amount of potassium (2.65%) was attained from T_2, again the lowest potassium content (2.40%) was found in T_5. The highest amount of calcium (1.96mg) was observed from T_3, whereas the lowest calcium content (1.73mg) was observed in T_4. The highest amount of magnesium (0.727mg) was attained from T_2 and the lowest magnesium content (0.673mg) was found in T_4. The highest amount of iron (515.48 mg) was attained from T_2, whereas the lowest iron content (494.9 mg) was observed in T_5. The highest amount of sulphur (0.383mg) was found from T_3, while the lowest sulphur content (0.281mg) was attained in T_5. The highest amount of zinc (15.54%) was observed from T_5, whereas the lowest zinc content (14.22%) was recorded in T_6.

Conclusion

The effect of various kinds of sawdust and supplement on the yield of *Ganoderma lucidum* was investigated in this study. As described above yield of *G. lucidum varied*

widely depending on the kind of sawdust and supplement. it was observed that treatment T_2 Mango sawdust + 30% wheat bran and Mixed sawdust (jackfruit,mango,teak,mahogony and rain tree) + 30% wheat bran, among the treatments performed significantly better on growth, yield, nutrient and mineral content of Reishi mushroom (*Ganoderma lucidum*). As wheat bran is the industrial by product and economically cheaper than other like gram flour, corn flour etc. the above discussion,

Recommendations

In this experiment, Mango sawdust with 30% wheat bran performed better in respect of different growth, yield and nutrient composition and mineral content of Reishimushroom. Therefore, Mango sawdust with 30% wheat bran substrate can be recommended for wide range cultivation of reishi mushroom.

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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 August to December 2014

Month (2014)	Air temperature (^{0}c)		Relative humidity (%)	Rainfall (mm)	Sunshine (hr)
	Maximum	Minimum			
August	36.0	23.6	81	319	4.0
September	34.8	24.4	81	279	4.4
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

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

Appendix II. Analysis of variance on data with the effect of sawdust substrates on mycelium growth of *Ganoderma lucidum*

Source of variation	Degrees of freedom	Mean square of				
		Mycelium running rate in spawn packets(cm)	Time from stimulation to primordial initiation (days)	Time from primordial initiation to harvest (days)	Average number of fruiting body/ packet	Average weight of individual fruiting body g
Replication	5	0.012	42.21	8.595	48.06	1.037
Treatment	12	0.001	0.498	0.212	0.517	0.112

Significant at 1% level of probability;

Appendix III. Analysis of variance on data with the effect of sawdust substrates on development and size of fruiting bodies of *Ganoderma lucidum*

Source of variation	Degrees of freedom	Mean square of					
		Days required for antler initiation	**Days required for conk formation**	**Average Length of stalk (cm)**	**Averae diameter of stalk (cm)**	**Average Thickness of pileus (cm)**	**Diametr of pileus (cm)**
Replication	5	30.875	47.718	1.264	0.058	0.027	1.794
Treatment	12	0.168	0.667	0.002	0.003	0.003	0.168

Significant at 1% level of probability;

Appendix IV. Analysis of variance on data with the effect of sawdust substrates on the yield, biological efficiency and cost benefit ratio of *Ganoderma lucidum*

Source of variation	Degrees of freedom	Mean square of				
		Biological yield (g)	Economic yield (g)	Dry yield (g)	Biological efficiency (%)	Benefit cost ratio
Replication	5	782.96	654.54	19.33	264.29	0.346
Treatment	12	0.005	0.005	0.0340	0.002	0.002

Significant at 1% level of probability;

Appendix V. Analysis of variance on data with the effect of sawdust substrates on proximate composition of *Ganoderma lucidum*

Source of variation	Degrees of freedom	Mean square of						
		Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	CHO (%)	Crud fiber (%)	Ash (%)
Replication	5	7.375	7.260	3.242	0.093	0.130	8.868	1.372
Treatment	12	0.714	0.078	0.058	0.001	0.970	0.900	0.012

Significant at 1% level of probability;

Appendix VI. Analysis of variance on data with the effect of sawdust substrates on minerals content of *Ganoderma lucidum*

Source of variation	Degrees of freedom	Mean square of							
		N(%)	P(%)	K (%)	Ca(%)	Mg(%)	Fe(ppm)	Zn(%)	S (%)
Replication	5	0.095	0.014	0.032	0.060	0.001	209.49	0.001	0.971
Treatment	12	0.560	0.080	0.003	0.032	0.003	0.050	0.093	0.073

Significant at 1% level of probability;

LIST OF PLATES

Plate 1: Preparation and mixing of sawdust substrates

Plate 2: Preparation of sawdust substrates packet of prescribed quantity

Plate 3: Prepared packet

Plate 4 :Laminar flow cabinet for safe culturing in spawn packet

Plate 5 :Mushroom Spawn packets in cold environment for primordial initiation

Plate 6 : Watering the spawn packets

Plate 7 : ‘D’ Cut in the spawn packet and pin head formation

Plate 8 :Mycelium growth and young fruiting body

Plate 9 :Matured fruiting body in the packet

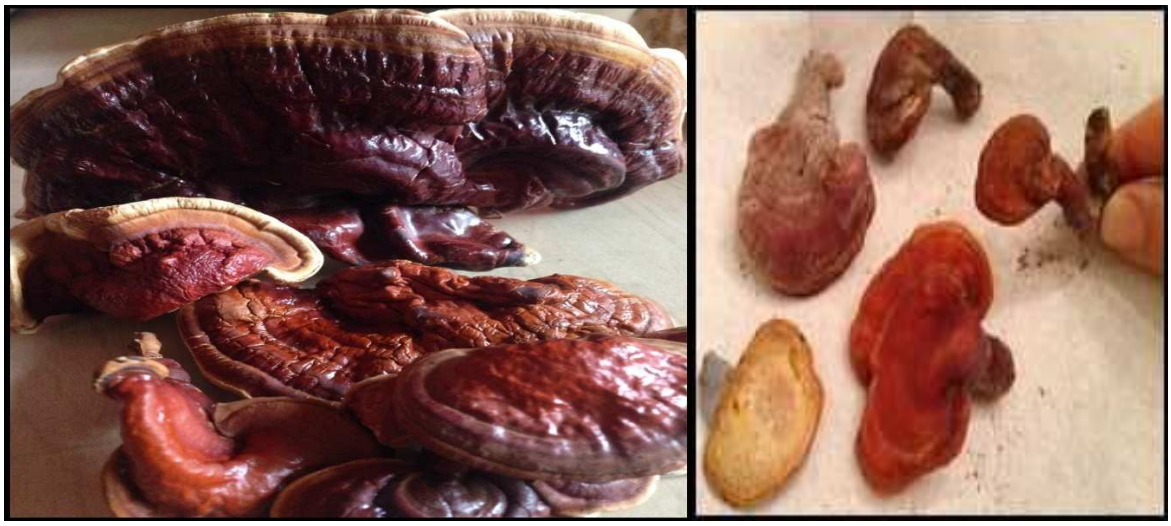

Plate 10 :Dried Mushrooms

Plate 11 :Autoclave used in sterilization plate