

**EFFECT OF SUBSTRATE AMOUNT AND MOTHER CULTURE  
(INOCULA) ON YEILD ATTRIBUTES AND YEILD OF REISHI  
MUSHROOM**

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MUSHROOM**

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**CERTIFICATE**

This is to certify that the thesis entitled " EFFECT OF SUBSTRATE AMOUNT AND MOTHER CULTURE (INOCULA) ON YEILD ATTRIBUTES AND YIELD OF REISHI MUSHROOM" submitted to the **DEPARTMENT OF HORTICULTURE**, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (M.S.) in HORTICULTURE, embodies the results of a piece of bona fide research work carried out by **JASMIN AREA**, Registration. No. 15-06821, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in any other institution.

I further certify that any help or sources of information received during the course of this investigation have been duly acknowledged.

Date: June, 2022  
Place: Dhaka, Bangladesh

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**DEDICATED TO**  
**MY**  
**BELOVED PARENTS**

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**The Author**

# **EFFECT OF SUBSTRATE AMOUNT AND MOTHER CULTURE (INOCULA) ON YEILD ATTRIBUTES AND YIELD OF REISHI MUSHROOM**

## **ABSTRACT**

The experiment was conducted at the laboratory and culture house of Mushroom Development and Extension Programme, Mushroom Development Institute, Sobhanbang, Savar, Dhaka during the period from March to August 2021 to study the effect of different substrate amount and mother culture (inocula) on yield and yield attributes of Reishi mushroom. The experiment consists of two factors viz.: factor A: different amount of substrates; B<sub>1</sub> = 250 g, B<sub>2</sub> = 500 g, B<sub>3</sub> = 750 g, B<sub>4</sub> = 1000 g, B<sub>5</sub> = 1250 g, B<sub>6</sub> = 1500 g and factor B: Types of mother culture; T<sub>1</sub> = Bamboo stick, T<sub>2</sub> = Sawdust, T<sub>3</sub> = Wheat grain. The experiment was laid out in two factors Completely Randomized Design with six replications. Data were gathered on several growth and yield parameters and it was discovered that the analyzed parameters varied significantly. In case of different substrate amount, yield contributing characters such as maximum number of fruiting body per packet (4.92), maximum single weight of fruiting body (3.18 g), longest length of stalk (2.50 cm), highest diameter of stalk (1.60 cm), diameter of pileus (6.92 cm), thickness of pileus (1.73 cm), biological yield (97.52 g), economic yield (88.24 g), dry yield (61.27 g) was observed from B<sub>4</sub> treatment. In case of mother culture (inocula), maximum number of fruiting body per packet (4.67), weight of individual fruiting body (3.12 g), length of stalk (2.27 cm), diameter of stalk (1.56 cm), thickness of pileus (1.42 cm), highest biological yield (84.74 g), highest economic yield (73.24) and highest dry yield (54.39 g) was recorded from T<sub>2</sub> treatment. In case of combined effect, maximum number of fruiting body (4.82), highest single weight of fruiting body (3.59 g), longest length of stalk (2.77 cm), diameter of stalk (1.83 cm), diameter of pileus (7.25 cm), thickness of pileus (1.72 cm), maximum biological yield (98.20 g), maximum economic yield (88.65 g) and maximum dry yield (62.35 g) was recorded from the combination of B<sub>4</sub>T<sub>2</sub> treatment. Among the treatments B<sub>4</sub> (1000 g) substrate with T<sub>2</sub> (Sawdust) treatment combination exhibited better growth and yield of Reishi mushroom.

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## LIST OF ABBREVIATIONS

### **Abbreviation : Full word**

%	: Percent
@	: At the rate of
°C	: Degree centigrade
BAU	: Bangladesh Agricultural University
BCSIR	: Bangladesh Council of Scientific and Industrial Research
CV	: Coefficient of Variance
DEP	: Direct Explant Planting
DMRT	: Duncan's Multiple Range Test
e.g.	: (For example) <i>exampoli gratia</i>
et al.	: (And others) <i>et alibi</i>
etc.	: Etcetera
g	: Gram
hr	: Hour (s)
kg	: Kilogram
LSD	: Least Significant Difference
no.	: Number
PMC	: Pure Mycelium Culture
SAU	: Sher-e-Bangla Agricultural University
wt.	: Weight
BE	: Biological efficiency
MRR	: Mycelium Running Rate
mg	: Milligram
SE	: Standard error
Conc.	: Concentration



**Chapter I**

**Introduction**

## Chapter I

### INTRODUCTION

Reishi mushroom (*Ganoderma lucidum*) is a member of fungal group Basidiomycetes which belongs to Polyporaceae (Ganodermataceae) of Aphyllophorales. Its fruiting body is named as “Reishi” in Japanese and “Lingzhi” in Chinese. 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. 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. *Ganoderma lucidum* contains a variety of active compounds, including triterpenes, polysaccharides, and sterols, which are believed to be responsible for its medicinal properties. Today, *Ganoderma lucidum* is often consumed as a dietary supplement in the form of capsules, extracts, or teas. It is also used in some cosmetic and skincare products due to its antioxidant and anti-inflammatory properties. 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.

Depending on a number of variables, including the type of mushroom being grown, the substrate being used, and the ambient circumstances, the impact of different substrate amount on mushroom development might vary. Larger bags typically offer greater room for mycelium colonization and fruiting, which may lead to higher yields of mushrooms. This is due to the fact that larger bags can hold more substrate and permit better airflow, both of which can encourage growth and development. Larger bags, on the other hand, would need more materials, including substrate and spawn, and they might be more challenging to

handle and manage in terms of temperature and humidity control. On the other hand, smaller bags could be less demanding on resources and easier to handle and monitor. Smaller bags, however, can restrict the area accessible for mycelium colonization and fruiting, which might lead to lower mushroom harvests. The type and quantity of substrate being utilized, and the environmental conditions in the growing region will all have an impact on mushroom production. Bamboo is a rapidly growing and highly renewable resource, making it a more environmentally friendly choice than other substrate materials. It contains a lot of cellulose and lignin, which can be used to feed mushroom mycelium. This can lead to faster colonization and potentially higher mushroom yields. Bamboo stick's shape and structure can provide support for mushroom fruiting bodies, reducing the risk of collapse or damage to the mushrooms. Bamboo sticks are challenging to prepare for mushroom cultivation because they must be cut, stripped of leaves and branches, and sterilized before use. It has the ability to absorb and retain moisture, resulting in a substrate that is too wet for optimal mushroom growth. This increases the possibility of contamination and lowers yields. Sawdust is a waste product from the lumber industry and is a common and reasonably priced substrate material. To give mushroom mycelium a robust food source, sawdust can be mixed with extra nutrients like wheat bran or soybean meal. This may lead to quicker colonization and maybe larger mushroom harvests. Sawdust is a practical substrate material for mushroom growing since it is simple to sterilize using steam or heat treatment. Sawdust has a low capacity to retain moisture, which can cause a substrate that is too dry for the best growth of mushrooms. Reduced yields and a slower rate of colonization may result. If the substrate is not properly sterilized, competing fungus or bacteria may be more likely to contaminate sawdust. For mushroom production, wheat bran can be utilized as a mother culture substrate, where mycelium can colonize before being moved to the fruiting substrate. A great supply of vitamins, proteins, and carbs are found in wheat bran. Fast mycelial development and robust colonization may be supported by this. Wheat bran may be sterilized readily using steam or heat treatment, making it a useful substrate material for

mother culture preparation. Compared to certain other substrate materials, properly sterilized wheat bran has a lesser danger of contamination from rival fungus or bacteria. Due to its affordability when compared to some other substrate materials, wheat bran is an economical choice for the cultivation of mushrooms. While too little moisture can prevent mycelial growth, too much moisture can result in bacterial infection.

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. 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. If different bag size and suitable substrate for mother culture can be used in mushroom cultivation, higher production can be achieved. So, the investigation is undertaken to fulfill the following objectives.

#### **OBJECTIVES:**

- To identify the suitable amount of substrate for optimum growth and yield of Reishi mushroom;
- To identify the suitable mother culture for optimum growth and yield of Reishi mushrooms; and
- To identify the suitable combination of substrate amount and mother culture for optimum growth and maximum yield of Reishi mushroom.



**Chapter II**

**Review of Literature**

## Chapter II

### 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 mother 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.

Deke (1994) reported on present invention relates to a culture method of edible fungi and its processing method and utilization, belonging to the field of fungus culture and food processing. Firstly, said invention uses "DK-5 culture medium" to produce the mother seed of edible fungus, in particular that of rare edible fungus, then uses "DK-6 culture medium" to produce edible (medicinal) mycelia, and then those mycelia can be made into various foods or beverages. Its advantages are as follows: the quality and output of the edible fungi are raised, and several rare fungus seeds which are not cultured so far can be produced in plant-scale, and its raw material is rich, and its cost can be reduced.

Bing *et al.* (1998) provided a technique for cultivating bailing mushroom includes the components and preparation of the culture medium for mother spawn, the preparation of the culture medium for foundation stock and cultivated species, the sterilization method for cultivating material, and features that the powdered stem of an umbellifer is used as a part of culture medium for promoting the health and rapid growth of mycelia, as well as a complex sterilization using fermentation before using ordinary-pressure steam sterilization, are all used. Its advantages are high-quality and rapid growth, high biological efficiency and high yield.

Wenli *et al.* (1989) made a statement on present invention relates to the development and utilization of edible fungus, in particular mushroom is made of

solid culture nutrient solution process, characterized in that the mushroom mother stock allograft expansion for incubated cultivar (original species and cultivars of wheat are medium the main raw material) to be through long hyphae, in large quantities after the discharge primordia extraction enzymatic saccharification filtered and concentrated. This production process short production cycle, low cost, the extraction rate, rich nutrition finished products. This production process preparation of nutrient solution contains 18 kinds of amino acids, vitamins and trace elements, the development prospects in terms of nutrition, health food, pharmaceuticals, biochemical reagents considerable.

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.

Yang (2008) aimed to enhance the yield and quality of cultivated *Ganoderma lucidum* and lay a foundation for further researching and exploiting *G. lucidum*. With Japanese *G. lucidum* as material, the mother culture, original seed and cultivation material media of *G. lucidum* were screened out, and the screening experiment was conducted on the addition amount of wheat bran in the medium with cottonseed shell as main material so as to confirm the optimum media for the growth of *G. lucidum*. The growth velocity of *G. lucidum* on the mother culture medium of cottonseed shell was faster and its difference from that on the other media reached extremely significant level. The original seed grew fast on the corn grain medium and had thick hypha. *G. lucidum* cultured on the medium of cottonseed shell added sawdust had highest yield and good quality. The *G. lucidum* yield was highest when the addition amount of wheat bran was 20 % in cottonseed shell medium, but had no significant difference from the yield of adding 15 % wheat bran into cottonseed shell medium. Comprehensively considering, the optimum mother culture medium was cottonseed shell, the optimum original seed medium was corn grain, the optimum cultivation material

medium was cottonseed shell added sawdust and the optimum addition amount of wheat bran was 15 % for the growth of *G. lucidum*.

Mayzumi *et al.* (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).

Hongmei *et al.* (2013) carried an experiment to find suitable culture medium for different *Ganoderma lucidum* varieties. Mycelium and fruiting body were gathered by culturing number 5 and 4-3 strains with coconut husk substitute cultivation and sawdust. Ganoderic acids was determined by spectrophotometric method using glacial acetic acid and vanillin sulfuric acid as color developer. The results showed total ganoderic acid of fruiting body in number 5 strain, which was cultured with sawdust, was 0.98% more than that of three levels of mycelium; coconut husk was 1.20% more than that of three levels of mycelium and ganoderic acid of body fruiting with culture of sawdust was 0.27% lower than that of coconut husk. Ganoderic acid of fruiting body cultivated by sawdust was 1.24% more than that of three levels of mycelium, and by coconut was more than 1.13%, while ganoderic acid of fruiting body with culture of sawdust was 0.20% more than that of coconut husk. The ganoderic acid content is related with cultivars and culture medium.

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

Magday *et al.* (2014) optimized growth conditions and fruiting body production of *G. lucidum* on different culture media, physical parameters like pH, aeration, illumination and temperature, spawn materials and rice straw– sawdust based substrate formulations. After 5 days of incubation, coconut water gelatin of pH 6.0 and in sealed and lighted conditions at room temperature (32<sup>o</sup> C) yielded the most efficient mycelial growth. Among the grains evaluated, corn grit produced a luxuriant mycelial growth in the

shortest period of 5 days. Substrates having 70% rice straw and 30% sawdust recorded the shortest incubation period of 17.33 days for fructification. Basidiospores were germinated efficiently in coconut water gelatin after 72 hours of incubation. The basidiospores have a typical type of germination wherein the sporoderm produced a single germ tube, elongated, septated into a hypha, and branched to become monokaryotic primary mycelia. Mycelial coat hardening, primordial initiation, antler-like formation and basidiocarp maturation and spore liberation were observed as the sequence of fruit body development.

Lingzhi (2006) did a comparative cultivation tests of *G. lucidum* Karst and *G. lucidum* H.G that were prepared by using four culture media at different contents of cottonseed hull, bran, sawdust and amino acid. Control composition was cottonseed hull 98%, gypsum powder 1% and sucrose 1%. Experimental results show that medium 2 is suitable for both *G. lucidum* Karst and *G. lucidum* H.G in high yielding and good quality. There are slightly different formulations to media and high bran content medium is better for cultivating *G. lucidum* H.G.

Kumari (2017) carried an investigation to provide a more effective, affordable in-vitro culture procedure for mycelium growth on a wide scale that can bear fruiting bodies, which is necessary for the artificial cultivation of *Ganoderma lucidum*. In the suggested investigation, *G. lucidum* pure culture was propagated in vitro on slants of potato dextrose (PDA), oatmeal agar, and wheat extract agar. Four types of *Ganoderma lucidum* cultures were created for each media in order to increase economic viability. These groups were vaccinated using a mother culture sample that was procured from NRCM, Solan. The produced culture tubes were subjected to incubation for 10 to 14 days in aseptic conditions at 32<sup>0</sup>-35<sup>0</sup> C to achieve a good mycelial growth. It was found that the group-4 culture could produce a fruiting body after transplantation on sterilized substrate prepared for artificial *G. lucidum* cultivation. Further, PDA was observed to be the most suitable culture media for the propagation of *Ganoderma lucidum*. After experiment on the different groups and evaluations based on the standard metrics,

spore of different strains has been found most suitable for commercial viable production of *G. lucidum* fruiting body.

Hu *et al.* (2018) aimed to develop an alternative way to produce useful triterpenoids from *G. lucidum*. We cultured the strain using a two-stage liquid culture strategy and investigated the effects of nitrogen limitation, carbon supply, static culture volume and air supply in the static culture stage on the accumulation of five triterpenoids (GA-P, GA-Q, GA-T, GA-S, GA-R). Our results showed that, under optimized condition, the total yield of the five triterpenoids reached 963 mg/L (as determined by HPLC). Among the five triterpenoids, GA-T accounted for about 75% of the total yield. Besides, a bioreactor suitable for fungal liquid static culture with a 10 L extensible plastic bag shaped culture unit was designed and in which the maximum total yield of the five GAs reached 856.8 mg/L, and the GAs content reached 5.99%. Our results demonstrate the potential of industrial application of *G. lucidum* culture for the production of triterpenoids, especially GA-T. Air supply significantly improved the accumulation of triterpenoids, and this will provide important clues to understand why more triterpenoids are produced in the mycelia mat under static liquid culture conditions.

Azizi *et al.* (2012) studied the effect of sawdust, malt extract, and wheat bran on yield, biological efficiency (BE), and mycelia growth of *Ganoderma lucidum*. Three kinds of sawdust (beech, poplar, and hornbeam) as basal medium were mixed with two levels of wheat bran (5% and 10% w/w) and malt extract (2.5% and 5% w/w) as medium supplement for production of *G. lucidum* in factorial experiments on the basis of completely randomized design with three replications. The highest fruiting body yield and BE (102.58 g/kg and 12.89%, respectively) were found using hornbeam sawdust. The beech sawdust promotes the mycelia growth rate more than other sawdust. A final comparison of the different formulae indicated that the best combinations for high yield (142.44 g/kg) and BE (18.68%) were obtained in a combination of poplar sawdust with 5% malt extract and 10% wheat bran. The highest mycelia growth rate (10.6

mm/day) was obtained in a combination of beech sawdust with 2.5% malt extract and 10% wheat bran.

Shah *et al.* (2018) carried out a study to optimize the mycelial biomass production of *G. lucidum* by submerged fermentation. Conventional one factor at a time was used as an initial screening process, i.e., one factor was varied, while keeping all the others constant. Different factors like pH, temperature, carbon source, nitrogen source and inoculum size were selected for the optimization process. The optimum pH and temperature of the medium was found to be 5 and 30°C respectively. Different carbon and nitrogen sources were screened for optimum mycelial biomass production. Glucose at a concentration of 1.5% w/v and yeast extract at a concentration of 0.25% w/v was found to be the most effective for the mycelial biomass production. Further, an inoculum size of 6% (v/v) was found to be the best for mycelial biomass production of *G. lucidum*. was found to be 368±3.71 mg/100 mL.

Jeewanthi *et al.* (2017) conducted research with a view to popularize the cultivation of *G. lucidum*, a study was done to identify an appropriate substrate from local raw materials for its artificial cultivation in polypropylene bags. Sawdust of four woods viz. rubber (*Hevea brasiliensis*), mango (*Mangifera indica*), jack (*Artocarpus heterophyllus*) and lunumidella (*Melia dubia*) were used as substrates after mixing with other general ingredients. The mushroom was grown in 100% rubber, 100% mango, 100% jack, 100% lunumidella, 50% rubber + 50% mango, 50% rubber + 50% jack, and 50% rubber + 50% lunumidella sawdust combinations. The number of days taken for colonization and primordial formation, number of fruiting bodies per bag, yield per bag and biological efficiency (BE) were recorded. Substrates with mango (100%), rubber (100%), rubber + jack (50%:50%) and rubber + lunumidella (50%:50%) sawdust resulted in equally high yield per bag (49.3 g, 42.5 g, 45.7 g and 43.7 g, respectively) within 1 month after primordia formation, and higher BE of 5.4%, 5.1%, 5.3% and 5.7%, respectively. Higher predicted total yields for a duration of 3 months were resulted by 100% mango sawdust (541.91 g/bag), rubber + mango sawdust (502.37 g/bag), and rubber + lunumidella sawdust (480.92 g/bag), followed by rubber (467.39 g/bag) substrates while the minimum yield (289.08 g/bag) was given by rubber

+ jack sawdust mixture. Considering the scarcity of rubber and mango wood as a sole source for substrate, the mixture of rubber and mango (50%:50%) or rubber and lunumidella (50%:50%) could be successfully used for cultivation of *G. lucidum*.

Veena et. al. (2011) made an attempt to use paddy straw as a substrate to cultivate *G. lucidum*. Different proportions of paddy straw were mixed with 0, 22.5%, 45%, and 67.5% sawdust and 10% rice bran. Spawn run period, fruiting initiation period, yield, moisture content, dry recovery and fruiting body characteristics were recorded and compared. Fructification was observed with all the substrate formulations and they did not show any significant difference in yield. The highest biological efficiency (BE) (29.9%) was observed with the combination sawdust: paddy straw: rice bran 22.5:67.5:10, followed by saw dust: paddy straw: rice bran 45:45:10 with BE 27.3%. The study demonstrated for the first time that the cultivation of *G. lucidum* is possible with paddy straw as the base substrate and indicated the enormous potential of paddy straw for the cultivation of *G. lucidum*.

Erkel (2009) studied the effects of various kinds of sawdust and bran on the yield of *Ganoderma lucidum* were investigated in artificial cultivation. Three types of bran (wheat, rice, and corn) and three types of sawdust (poplar, oak, and beech) were utilized as substrate medium in the culture of *Ganoderma lucidum*. Significant differences ( $P < 0.01$ ) were found among varieties of sawdust, bran and mixtures both in yield and biological efficiency (BE). The highest yield and BE were obtained from oak sawdust (OS) compared to the other sawdusts and also from wheat bran (WB) compared to the other bran. Yield and BE of rice bran (RB) at whole combinations, especially combination of OS: RB, were lower than in the other treatments, while substrates containing WB gave the highest yield.

Smith et al. (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 sawdust 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<sup>-1</sup> and BE of 17.48% were obtained from oak sawdust, and followed by BS (53.24 g kg<sup>-1</sup> and 15.94%). These results are consistent with those of some authors who claimed that hardwood sawdust was preferable for industrial manufacturing.

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*. In comparison to other agricultural wastes such rice husk, coconut fiber, peanut hull, and sugarcane bagasse, it was discovered that rice bran, crushed maize, and ground sorghum were good supplements.

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 *Ganoderma 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.

Riu *et al.* (1997) carried out an experiment to find out the easy cultivation procedure of *Ganoderma spp.* Typically, they have been grown in solid substrates like grain or other lignocellulosic materials including straw, sawdust, and additives. Supplements such as sucrose, wheat and rice bran are generally added to the mixture (Chen, 1999).

Wasser (2005) used wood log, short wood segment, tree stump, sawdust bag and bottle procedures for Reishi mushroom production.

Olei (2003) reported that *Ganoderma* can be cultivated on the sawdust which may originate from different kinds of trees.

Gurung *et al.* (2013) examined how different types of sawdust and additives affected the amount of *Ganoderma lucidum* that could be grown artificially. *Ganoderma lucidum* was grown on *Alnus nepalensis*, *Shorea robusta*, and *Dalbergia sisoo* sawdust as well as rice bran, wheat bran, maize flour, and gram flour supplements. In the control, the cultivation's substrate media consisted just of sawdust. Significant differences ( $P < 0.05$ ) were found among varieties of

sawdusts and supplements. Similar substantial differences ( $P < 0.05$ ) in yield and biological effectiveness were seen between sawdust with and without additives. *Dalbergia sisoo* sawdust could not give yield in ambient condition; Very little yield and biological efficiency were produced by *Shorea robusta*. *Alnus nepalensis* gave good yield and biological efficiency compared to the other sawdust. Compared to the other bran, gram flour had the best yield and biological efficiency. Among all treatments, *Alnus nepalensis* sawdust combined with gram flour demonstrated the highest output. Supplementation had a beneficial effect on the growth and yield of the mushroom's mycelia.

Rashid *et al.* (2008) conducted an experiment to investigate the effect of different sizes of bags on the yield of oyster mushroom. The bags of different sizes were used (6x12"; 7x14"; 8x12"; and 9x14"). Spawn running, pin head, fruit body formation, and yield of oyster mushroom (*Pleurotus ostreatus*) were determined by using growth media of cotton waste supplemented with lime and wheat bran 4% each. Results showed that bag size in the (8x12") and (9x14") significantly affect the yield. It takes 30 days for the quicker spawn to mature in the bag-sized (8x12") container. The bag's (8x12") maximum pin head formation produced 48.8 numbers. The bag measuring 7x14" yielded the most ripe mushrooms at 15.4 number. The bag measuring 6x12" had the highest total yield of 69.2 g.

Young *et al.* (2014) experimented the media composition in bag culture and determined the possibility of artificial cultivation of *Auricularia auricula*. Sawdust spawn of media composition for optimal growth were found to be oak sawdust 80% combination of 20% poplar-sawdust were the best of the optimal combination. The most effective substrate combination was discovered to be cotton-seed meal combined with 10% wheat bran and 5% mixed. The duration of spawn run period and primordial formation period on bag (1.2 kg) were 50 days and 7 days, respectively. The weight of fruiting body and the yield of fresh fruit-body were 24 g and 450.00 g, respectively.

Bisariaa *et al.* (2003) studied the cultivation of the oyster mushroom *Pleurotus sajor-caju* (Fr.) carried out in cylindrical wire-mesh structures to ascertain how structural variation affects its yield. The yield of the mushroom, which was discovered to be 1.32 kg/kg dry paddy straw, was best when an area per unit volume of 0.18 cm<sup>2</sup>/cm<sup>3</sup> was used in cylindrical constructions of various diameters.

Jiangong *et al.* (2014) related to a cultivation method for fungi, especially to an artificial bag cultivation process for *Phellinus igniarius*. The process comprises the following steps: (1) purifying a strain isolated from wild *Phellinus igniarius* sporocarp, then inoculating the purified strain to a mother culture medium for cultivation so as to obtain a mother *Phellinus igniarius* strain, inoculating the mother *Phellinus igniarius* strain to a stock culture medium for cultivation so as to obtain a *Phellinus igniarius* stock and inoculating the *Phellinus igniarius* stock to a cultivar culture medium for cultivation so as to obtain a *Phellinus igniarius* cultivar; (2) inoculating the *Phellinus igniarius* cultivar to a mushroom-stick and carrying out cultivation for *Ganoderma* germination; and (3) managing germinated *Ganoderma*. According to results of considerable cultivation practice, it is proved that the artificial bag cultivation process for *Phellinus igniarius* in the invention can realize artificial cultivation of real *Phellinus igniarius*; moreover, the size of individual sporocarp can be artificially controlled by controlling the size of an opening. Through artificial bag cultivation of *Phellinus igniarius*, artificial short-term regeneration of wild rare resources and resources needing a long regeneration period is realized.

Kamra and Bhatt (2013) attempted to make a study to develop organic cultivation technique of *Ganoderma lucidum* on polypropylene bags under subtropical habitat. Mother culture was procured from NRCM, Solan. Locally available substrate (Sawdust, wheat bran, rice bran, maize flour, and bagasse) was used for artificial cultivation. Grain master was prepared with fresh unbroken grains. The moistened sawdust powder was blended with dry rice bran, dry wheat bran, maize flour and bagasse for organic cultivation. The heat

resistant polypropylene plastic bags were filled with the substrate. These bags were sterilized and then cooled overnight and aseptically spawned with the grain master and immediately transferred to the workstation. The spawn run was completed in 51 days. Then spawn bags were exposed to  $30\pm 1^{\circ}\text{C}$  and 90-95% RH. The first primordial initiation was noticed after 35 days. Vegetative and fruiting phase were completed in 67 days and 92 days respectively. Interval between two flushes was 65 days and total period of crop was recorded as 224 days. The total yield was 570 gm. No pest attack was seen during cultivation.

Subarna *et al.* (2015) studied to implement a practical technique for *G. lucidum* artificial culture in polypropylene bags using a range of affordable and easily accessible substrates. As substrates, calcium carbonate was added to the sawdust of five different types of wood: *Swietenia mahagoni*, *Dipterocarpus turbinatus*, *Tectona grandis*, *Gmelina arborea*, and *Michelia champaca* ( $\text{CaCO}_3$ ) and either rice or wheat bran for cultivation. *T. grandis*, *G. arborea* and *M. champaca* were not found to encourage the continued extension of mycelial growth, which restricted the growth. On the contrary, *S. mahagoni* and *D. turbinatus* were observed to impart a biological efficiency and rather good output. It was discovered that wheat bran performed better as a supplement than rice bran. However, *S. mahagoni* supplemented with wheat bran generated the highest output of mushrooms among the substrates, with subsequent yields of 235.2 g/kg and biological efficiency of 7.6 percent after mycelial growth, primordial formation, and harvesting, respectively, took 6 days, 33 days, and 60 days.

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.

Alam and Singh (2020) conducted a research work to investigate the efficacy of different mother culture media viz., rice straw ( $T_1$ ), rice straw and rice bran ( $T_2$ ), rice husk ( $T_3$ ), rice grain ( $T_4$ ), maize grain ( $T_5$ ), and rice straw and wheat bran ( $T_6$ ) and the impact of age of mother culture and substrate sterilization techniques viz., sun dried for 8 hours covering with transparent polythene ( $A_1$ ), black polythene ( $A_2$ ), blue polythene ( $A_3$ ) sheet, autoclave for two hours at  $121^{\circ}\text{C}$  ( $A_4$ ),

and hot water for one hour (A<sub>5</sub>) for the commercial cultivation of *Volvariella volvacea* (Bull.). The maximum mycelium run rate and minimum days required for completing the mother culture were recorded in T<sub>4</sub>. The lowest days required for primordial initiation (DRFPI) was 6 in T<sub>1</sub> and T<sub>2</sub>, whereas highest DRFPI was recorded in T<sub>3</sub>. The maximum (13.33) days required for first harvest (DRFFH) and lowest (109) number of effective fruiting bodies (NEFB) were recorded in T<sub>3</sub>. The minimum (10.67 days) DRFFH was found in T<sub>2</sub> and maximum (239.30) NEFB was recorded in T<sub>1</sub>. The lowest length and diameter (LFB and DFB) were recorded in T<sub>5</sub> (3.03 cm) and T<sub>1</sub> (1.66 cm), while highest LFB and DFB were observed in T<sub>3</sub> (3.20 and 2.39 cm). Maximum biological yield and efficiency were observed in rice straw and wheat bran materials. The highest NEFB, DFB, biological yield and efficiency were recorded in 30 days old of mother culture. The results revealed that combined rice straw and wheat bran were the excellent mother culture medium and 30 days old was the best age for the commercial production of paddy straw mushroom. Considering the experimental results on the sterilization techniques it may be suggested that hot water sterilization of rice straw substrate was the best sterilization technique for the commercial production and yield improvement of *V. volvacea*.

Mubasshira *et al.* (2020) conducted a study to compare the performance of different substrates and mother culture materials on yield and yield parameters of oyster mushroom (*Pleurotus ostreatus*). Accordingly, three substrates (sawdust, rice straw, sawdust + rice straw (1:1)) and three mother cultures (rice, maize, sawdust) were used in oyster mushroom cultivation. Among the substrates and mother culture components, using rice straw and sawdust mother spawn, the maximum length of stipe was recorded (23.27 mm and 24.29 mm, respectively). Applying sawdust + rice straw (1:1) and maize mother spawn, the peak diameter of stipe was calculated (9.90 mm and 10.01 mm, respectively). The maximal diameter of pileus was observed in sawdust + rice straw (1:1) and rice mother spawn (72.90 mm and 67.57mm, respectively). With the application of rice straw and maize mother spawn, thickest pileus was viewed (5.60 mm and 5.47 mm respectively). The sawdust and sawdust mother spawn delivered peak

number of fruiting body (6.67 and 7.33, respectively). Among the substrates, rice straw gave the highest biological yield (44.40 g/packet) and sawdust gave the lowest (41.73 g/packet). Among the mother spawn, sawdust mother spawn presented the highest biological yield (45.47 g/packet) and maize mother spawn gave the lowest (39.16 g/packet). In the comparison of combined effect of substrates and mother spawn, sawdust mother spawn performed best in the biological yield (50.80 g/packet) with rice straw as substrate material and maize mother spawn showed comparatively lower biological yield (37.60 g/packet) with both sawdust and rice straw as substrate material. Rice straw and sawdust mother spawn can be recommended for its suitability in oyster mushroom (*Pleurotus ostreatus*) cultivation.

Pei-da *et al.* (2015) used spent mushroom substrate (SMS) from *Pleurotus ostreatus*, *Lentinus edodes*, *Ganoderma lucidum*, *Hypsizygus marmoreus*, *Tremella fuciformis*, *Pleurotus eryngii* and *Flammulina velutipes* for the materials selection in producing mother culture media for the cultivation of *D. indusiata* mycelium. Water soluble substance of SMS that can promote the rapid growth of *D. indusiata* mycelium were extracted. Then, culture media added with different ratio of water-soluble extracts were used in growing mycelium of *D. indusiata* and then the appropriate dose of SMS extracts were screened in order to get a new formula of mother culture media of *D. indusiata*, compared with general PDA media. The results showed that water-soluble extracts from SMS of *G. lucidum* performed the best effect in growth of *D. indusiata* mycelium, and the latter grew faster compared with other spent mushroom substrates (P0.01). *D. indusiata* mycelium grew faster and showed better quality while 6g extracts from SMS *G. lucidum* were added to 1L mother media.

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

Indira *et al.* (2010) undertaken an experiment to prepare mother cultures, to compare the development and yield of mushrooms grown on supplemented and unsupplemented paddy straw, to determine the impact of agnihotra ash on the

growth of spawn, to choose the best culture medium for abundant mycelial growth. The following is a presentation and discussion of the findings of the current study on the evaluation of several spawn supplements and culture supplements of *Pleurotus sajor-caju*. The straw substrate was supplemented with agnihotra ash at a rate of 97.5 percent and cotton seed powder at a rate of 90 percent (B. E), respectively, to achieve the highest yield. The best maximum yield (235 g) and biological efficiency (78.3%) of fruit bodies from the three flushes were determined to be paddy straw soaked for 18 hours. The smallest yield (145 g) and biological efficiency (48.3 percent) of fruiting bodies were obtained after 10 hours of soaking. *Pleurotus sajor-caju* gave shorter spawn runs on paddy straw supplemented with agnihotra, according to the findings of the current study.

Sahib *et al.* (2022) researched to determine the optimal conditions of developing a local culture medium fit for mother cultures of the wild shiitake Iraqi strain mushroom *Lentinula edodes* RSR using various agro-natural wastes, with various natural materials made of wheat, oats, barley, corn cobs, green peas, chickpeas, wheat bran, rice bran, barley bran, bumper leaves, moringa leaves, sawdust, and whey cheese. In addition to potato dextrose agar (PDA), which was utilized as a comparison at a concentration of 10 to 40 g/L. The best pH for this strain's mycelium development was 6.5 at 23<sup>0</sup> C in the dark, and the ideal concentration of the medium made was 20 g/L. On wheat flour agar media, the best mycelial growth and density were discovered.

Bich *et al.* (2018) carried on a study to investigate the optimal culture conditions including pH level, temperature, media and substrate mixtures for the mycelium growth and cultivation of Monkey head mushroom (*Hericium erinaceus*) strain He-2. Results of the study revealed that the optimal conditions for mycelial growth were observed at 25 ± 1<sup>0</sup> C and pH 8.0. *H. erinaceus* was cultured on five different types of culture media: Czapek, Raper, PGA (potato, glucose, agar), PGA supplemented with rice bran, and PGA supplemented with fresh mushrooms. PGA supplemented with fresh mushrooms was found to be the best medium for the growth of mycelia. A media containing 99% grain of rice + 1%

CaCO<sub>3</sub> was considered as the best mother spawn media for mycelial growth. Among various culture media, the highest mycelium growth rate and biological efficiency of *H. erinaceus* were obtained when grown on a treatment of 87% sawdust + 4% corn bran + 8% rice bran + 1% CaCO<sub>3</sub>.

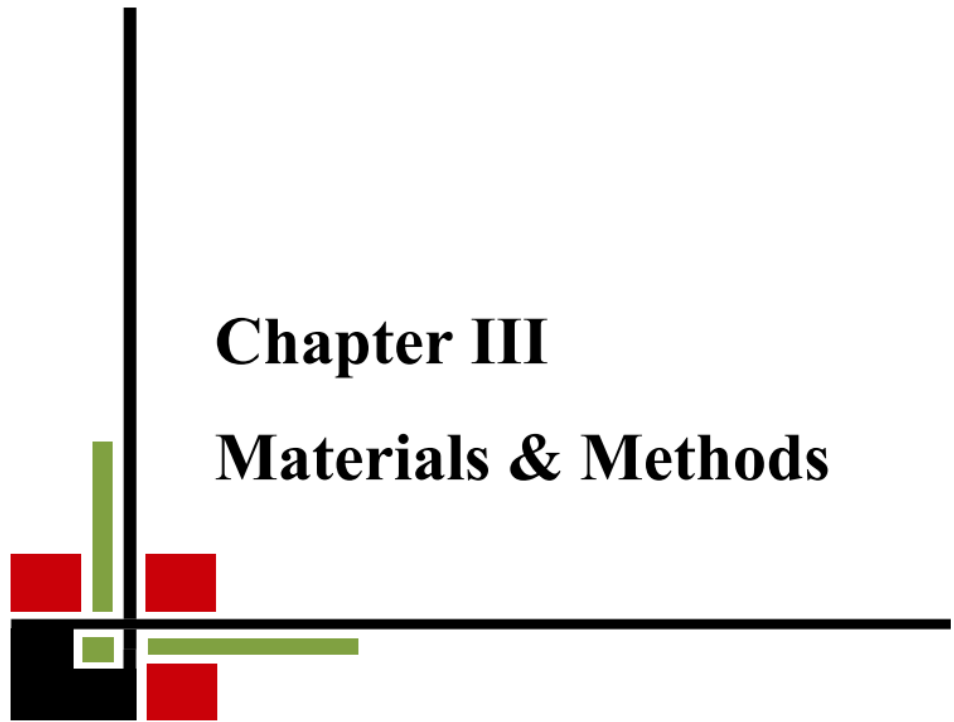
Kun *et al.* (2007) reported about the domestication of *Armillariella mellea*, some culture media have been selected such as gelose medium, grain medium, straw medium and liquid medium. A few of primary physical factors have been tested by growing mother fungi in gelose medium. The mushroom can grow within a temperature range of 10°C-35°C, and the appropriate temperature for its growth is 25°C-30°C. It cannot grow in culture medium which have acid value beyond pH 4-10 (before sterilization), and the proper scale is pH 6.0-7.0.

Zheng *et al.* (2014) screened *Pleurotus eryngii* strain suitable for shaking flask fermentation, and the best culture conditions was optimized. The fermentation culture medium and fermentation conditions were optimized by the single factor method, the optimum medium formula was obtained by orthogonal experiment, the three key factors (temperature, speed and fermentation cycle) were optimized by Box-Behnken design principles, and the experimental data were analyzed by response surface analysis. The result showed that glucose 3.50 g/100 mL, tryptone 0.50 g/100 mL, monopotassium 0.30 g/100 mL, magnesium sulfate 0.20 g/100 mL were the best medium, pH naturally. Under the conditions of 25°C, 170 r/min and 7.5 d (180 h), the mycelium biomass reached 1.687 g/100 mL. The optimal fermentation culture conditions were screened by single factor, orthogonal and response surface experimental methods.

Xiangying *et al.* (2002) reported on kind of deep fermentation process of culturing liquid xianggu mushroom seed and its culture medium, and aims at providing one liquid xianggu mushroom seed process superior to available technology. The culture process includes the steps of: mother seed culture in mother seed culturing medium; dark transitional culture of the obtained mycelium in sawdust culture medium at 22-27 °C for 12-20 days; the first stage of shaking culture of the crushed sawdust seed inside liquid culture medium for

5-7 days; the second stage of shaking amplification culture in fresh liquid culture medium; and fermentation treatment of the shaking cultured mushroom liquid to obtain liquid xianggu mushroom seed product. The relevant culture medium includes culture medium for xianggu mushroom mother seed, sawdust culture medium and deep fermenting culture medium.

Min (2014) undertook *Cordyceps sinensis*, *Pleurotus cornucopiae*, *Auricularia auricula*, *Stropharia rugoso-annulata*, *Pleurotus nebrodensis*, *Agrocybe aegerita*, *Pleurotus eryngii* as materials and studied the effect of medium with different concentrations gradient of *Pholiota nameko* mushroom residue and potato and PDA medium on the growth of them, in order to select the best medium for them. The results showed that *Pholiota nameko* mushroom residue had inhibition effect on growth of mentioned materials; while it had a promoting effect on mycelial growth of *Pleurotus eryngii* and *Pleurotus cornucopiae*. *Pleurotus eryngii* was suitable for the ratio of *Pholiota nameko* mushroom bran 15% and 5% potato recipe as medium. *Pleurotus cornucopiae* was suitable for the ratio of 5% *Pholiota nameko* mushroom bran and 15% potato recipe as medium.



# **Chapter III**

## **Materials & Methods**

## Chapter III

### MATERIALS AND METHODS

The experiment was conducted during the period from march to august 2021 to study the effect of different amount of substrate and mother culture (inocula) on the growth and yield of *Ganoderma lucidum*. 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, analysis of the mushrooms, data collection and data analysis procedure.

#### 3.1 Experimental site

The experiment was conducted at the Laboratory and Culture house of Mushroom Development and Extension Programme, Mushroom Development Institute, Sobhanbang, Savar, Dhaka during March to August, 2021.

#### 3.2. Experimental materials

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

#### 3.3. Varietal characteristics of *Ganoderma lucidum*

*Ganoderma lucidum* 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 fanlike 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. The cap of *Ganoderma lucidum* is typically kidney-shaped or fan-shaped, and can range in size from 5 to 25 cm in diameter. The cap surface is smooth and shiny, with a reddish-brown to dark brown color. It may have concentric rings or bands of different colors. The stem of *Ganoderma lucidum* is usually short and stubby, and often off-center on the cap. It is usually thicker at the base and tapering towards the top. The stem is typically the same color as the cap or slightly lighter. The underside of the

*Ganoderma lucidum* cap is covered with tiny, closely-packed pores instead of gills. The pores are typically white when the mushroom is young, but can turn brownish with age. The spores of *Ganoderma lucidum* are ellipsoid or cylindrical in shape, and measure 5-10 micrometers long by 3-5 micrometers wide. They are typically smooth and hyaline (translucent), and appear white or pale yellow. The flesh of *Ganoderma lucidum* is woody and tough, with a white to brownish color. It has a bitter taste and a slightly woody, mushroomy aroma. Overall, *Ganoderma lucidum* is a distinctive mushroom with a characteristic reddish-brown, shiny cap and white pores on the underside. Its stem is short and off-center, and its flesh is woody and tough.

### **3.4 Treatment of the experiment**

The experiment consisted of two factors:

#### **Factor A: Different amount of substrate**

B<sub>1</sub> = 250 g (6 inch x 8 inch)

B<sub>2</sub> = 500 g (7 inch x 10 inch)

B<sub>3</sub> = 750 g (7 inch x 12 inch)

B<sub>4</sub> = 1000 g (8 inch x 14 inch)

B<sub>5</sub> = 1250 g (10 inch x 14 inch)

B<sub>6</sub> = 1500 g (12 inch x 16 inch)

#### **Factor B: Types of mother culture**

T<sub>1</sub> = Bamboo stick

T<sub>2</sub> = Sawdust

T<sub>3</sub> = Wheat grain

### **3.5 Treatment combination**

There were total 18 treatment combination: B<sub>1</sub>T<sub>1</sub>, B<sub>1</sub>T<sub>2</sub>, B<sub>1</sub>T<sub>3</sub>, B<sub>2</sub>T<sub>1</sub>, B<sub>2</sub>T<sub>2</sub>, B<sub>2</sub>T<sub>3</sub>, B<sub>3</sub>T<sub>1</sub>, B<sub>3</sub>T<sub>2</sub>, B<sub>3</sub>T<sub>3</sub>, B<sub>4</sub>T<sub>1</sub>, B<sub>4</sub>T<sub>2</sub>, B<sub>4</sub>T<sub>3</sub>, B<sub>5</sub>T<sub>1</sub>, B<sub>5</sub>T<sub>2</sub>, B<sub>5</sub>T<sub>3</sub>, B<sub>6</sub>T<sub>1</sub>, B<sub>6</sub>T<sub>2</sub>, B<sub>6</sub>T<sub>3</sub>.

### **3.6 Design and layout of the experiment**

The experiment was laid out in two factors Completely Randomized Design (CRD). The experiment included 18 (6 × 3) treatments with six replications.

### **3.7. Sterilization procedure**

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

#### **3.7.1. Sterilization of culture media**

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

#### **3.7.2. Sterilization of glassware and instruments**

Beakers, test tubes, conical flasks, measuring cylinders flat bottles pipettes, metallic instruments like forceps, scalpels, needles and spatulas, Petri dishes, culture tubes, nano absorbent cotton and brown paper were sterilized in the autoclave at 121<sup>0</sup> C for 1 hours at 1.5 kg/cm<sup>2</sup> pressure.

### **3.7 Preparation of mother culture**

#### **3.7.1 Preparation of mother culture: Bamboo stick**

The media used in mushroom growth is bamboo stick. Bamboo is split into several pieces and then cut into different size pieces, soaked into the water and dried in the shade to dry. The sticks are placed in the bag according to their size. Then sterilized in an autoclave at 121<sup>0</sup>C for two hours. The master mother of *G. lucidum* was poured aseptically at 10% in the opening of bamboo stick containing mother culture packets and substrate media was covered by whitish mycelium within 12-30 days according to variety after inoculation into polypropylene bags of different sizes according to the treatment. After that, the media is ready to inoculate with the fungus on the incubation chamber and continued with the maintenance of the fungus. The whole packet containing substrate became white due to fungal mycelia proliferation within 15-20 days and thus ready for spawning the substrate.

#### **3.7.2 Preparation of mother culture: Sawdust**

To prepare mother culture, saw dust was used as media of mother culture by mixing sawdust and wheat bran at the ratio of 2:1. Calcium carbonate was used at the rate of 0.2% of the mixture. The moisture level of the mixture was

maintained at 65% by adding tap water. Polypropylene bags of different sizes were filled according to the treatment with above mentioned mixture and packed tightly. The substrate in bags was sterilized in an autoclave for 2 h at 121<sup>0</sup> C under 1.1 kg / cm<sup>2</sup> pressures and allowed to cool for 24 h. Then the master mother was poured aseptically at 10% in the opening of saw dust containing mother culture packets and substrate media was covered by whitish mycelium within 12-30 days according to variety after inoculation. The fully colonized packets were used as mother culture for spawning. Then the packets were sterilization, inoculation and incubation in the same process. The whole packet containing substrate became white due to fungal mycelia proliferation within 15-20 days and thus ready for spawning the substrate.

### **3.7.3 Preparation of mother culture: Wheat grain**

To prepare mother culture, wheat grains were used as media of mother culture. At first grains collected which was free from diseases and not broken, old, and insect damaged. The grains were thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses, weeds, etc. Washed grains were then soaked in sufficient water for 7-8 hours and boiled in a container for 10-15 minutes before the skin started to crack. Excess water from the boiled grains was removed by stirring and heating. Then the grains were thoroughly mixed with calcium carbonate at 0.1% and gypsum at 0.2% so that the pH of the grains was around 7.0 to 7.8. This mixing was done on the same container after wearing gloves. The substrate was poured into polypropylene bags of different sizes according to the treatment. The neck of the bags was heat resistant plastic and the neck was plugged with cotton wool, covered with paper piece and then tied together by a rubber band. The substrate in bags was sterilized in an autoclave for 2 h at 121<sup>0</sup> C under 1.1 kg / cm<sup>2</sup> pressures and allowed to cool for 24 h. Then the master mother was poured aseptically at 10% in the opening of wheat grain containing mother culture packets and substrate grain was covered by whitish mycelium within 12-30 days according to variety after inoculation. As Reishi is a cellulose loving mushroom, so it's impossible to prepare mother culture from wheat grain, because it contains

very low (<5%) amount of cellulose, so it gets rotten and no mother packet was prepared as well as spawn packet for wheat grain and no result obtained from this factor.

#### **3.7.4. Preparation of substrates**

Spawn packets using different sawdust, wheat bran, CaCO<sub>3</sub> 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 different sized polybags. 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<sup>0</sup> C temperature until the packets become white with the mushroom mycelium. After completion of the mycelium running the rubber band, brown paper, cotton plug and plastic neck of the mouth of the 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<sup>0</sup> C to 25<sup>0</sup> C. The first primordia appeared 2-4 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

### **3.7.8. Harvesting of mushrooms**

*Ganoderma lucidum* mushrooms matured within 45-65 days after primordia initiation. The matured fruiting body was identified by curial margin of the cap. Mushrooms were harvested by twisting to uproot from the base of the stem.

## **3.8 Data Collection**

### **3.8.1 Mycelia growth**

Mycelia's 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 or 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:

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

Where L = Average length of mycelium running (cm)

N= Number of days

### **3.8.2 Days to require from opening to primordial initiation**

Days required from opening to primordial initiation were recorded.

### **3.8.3 Days to require from opening to harvest**

Days required from opening to harvest were recorded.

### **3.8.4 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.

### **3.8.5 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.

### **3.8.6 Dimension of fruiting body (stipe and pileus)**

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

- a. Length of stipe (cm)
- b. Diameter of stipe (cm)
- c. Diameter of pileus (cm)
- d. Thickness of pileus (cm)

### **3.8.7 Biological yield**

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

$$\text{Biological yield (g/ packet)} = \frac{\textit{The weight of Biomass (g)}}{\textit{Amount of packet (g)}}$$

### **3.8.8 Economic yield**

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

### **3.8.9 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 stalk and the pileus were separated for better drying.

### **3.7810 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<sup>0</sup> C 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):

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

### **3.9 Statistical analysis**

The data obtained for different parameters were statistically analyzed to find out the significance of the difference among the treatment. 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 IV**

## **Results and Discussion**

## Chapter IV

### RESULTS AND DISCUSSION

The experiment was conducted to find out the effect of different amount of substrate and mother culture of the growth and yield of *Ganoderma lucidum*. Data on different growth, yield contributing characters of mushroom were recorded. The results have been presented and discussed with the help of table, graphs and possible interpretations given under the following heads:

#### **4.1 Effect on mycelia growth and yield contributing characters of *Ganoderma lucidum***

##### **4.1.1 Effect on mycelium running rate**

Mycelium running rate of Reishi mushroom showed statistically significant result due to different amount of substrate under the present trial (Appendix II). The highest mycelium running rate was recorded from B<sub>1</sub> (0.67 cm), followed by B<sub>2</sub> (0.65 cm), while the lowest mycelium running rate was observed in B<sub>5</sub> (0.53 cm) closely associated with B<sub>6</sub> (0.56 cm) (Table 4.1). This may be due to the optimum amount of substrate can influence the availability of nutrients, oxygen and moisture within the bag that significantly affected the mycelium running rate. 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.

Mycelium running rate of Reishi mushroom showed statistically significant variation due to different mother culture (Appendix II). The lowest mycelium running rate was recorded from T<sub>1</sub> (0.56 cm), while the highest mycelium running rate was observed in followed T<sub>2</sub> (0.63 cm) (Table 4.2). Gurung *et al.* (2013) and Azizi *et al.* (2012) showed similar results on sawdust mother culture showing positive mycelium growth and better yield performance.

Significant combined effects of different amount of substrate and mother culture on mycelium running rate of Reishi mushroom was observed (Appendix II). The highest mycelium running rate was recorded from B<sub>1</sub>T<sub>2</sub> (0.67 cm). Statistically

similar result was observed from B<sub>2</sub>T<sub>2</sub>(0.62 cm). On the other hand, the lowest mycelium running rate was observed in B<sub>6</sub>T<sub>1</sub> (0.44 cm) (Table 4.3).

**Table 4.1. Effect of different amount of substrate on mycelium growth, days required for primordial initiation, days required from opening to harvest**

Substrate amount	Mycelium running rate(cm/day)	Days required for primordial initiation	Days required from opening to harvest
<b>B<sub>1</sub></b>	0.67 a	16.00 f	42.23 f
<b>B<sub>2</sub></b>	0.65 b	17.16 e	44.72 e
<b>B<sub>3</sub></b>	0.61 c	17.93 d	45.05 d
<b>B<sub>4</sub></b>	0.59 d	18.16 c	48.16 c
<b>B<sub>5</sub></b>	0.53 f	19.83 b	52.42 b
<b>B<sub>6</sub></b>	0.56 e	20.00 a	57.00 a
<b>Level of significance</b>	**	**	**
<b>CV (%)</b>	2.05	2.23	1.42

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

[Here, B<sub>1</sub>= 250 g, B<sub>2</sub>= 500 g, B<sub>3</sub>= 750 g, B<sub>4</sub>= 1000 g, B<sub>5</sub> = 1250 g, B<sub>6</sub>= 1500 g]

#### **4.1.2 Effect on days required to primordial initiation**

There was significant variation in terms of days to require from opening to primordial initiation of Reishi mushroom due to different amount of substrate (Appendix II). The maximum days to require from opening to primordial initiation was found from B<sub>6</sub> (20.16 days) closely associated with B<sub>5</sub> (19.83 days), whereas the minimum was recorded in B<sub>1</sub> (16.00 days) (Table 4.1).

Days to require from opening to primordial initiation showed statistically significant results due to mother culture (Appendix II). The maximum days to require from stimulation to primordial initiation was recorded from T<sub>1</sub> (18.75 days), while the minimum days to required was observed in T<sub>2</sub> (17.22 days) (Table 4.2). Magday *et al.* (2014) found similar types of result that stated, the mycelial coat hardening, primordial initiation, antler-like formation and basidiocarp maturation and spore liberation were observed from higher percentage of sawdust mother culture as the sequence of fruit body development.

Significant combined effects of different amount of substrate and mother culture on days to require from opening to primordial initiation was observed (Appendix II). The maximum days to require from opening to primordial initiation was recorded from B<sub>6</sub>T<sub>1</sub> (21.5 days) (Table 4.3). On the other hand, the minimum days to require from opening to primordial initiation was observed in B<sub>1</sub>T<sub>2</sub> (12.83 days).

**Table 4.2: Effect of different mother culture on mycelium growth, days required for primordial initiation, days required from opening to harvest**

<b>Mother Culture</b>	<b>Mycelium running rate(cm/day)</b>	<b>Days required for primordial initiation</b>	<b>Days required from opening to harvest</b>
<b>T<sub>1</sub></b>	0.56 b	18.75 a	51.69 a
<b>T<sub>2</sub></b>	0.63 a	17.22 b	47.05 b
<b>T<sub>3</sub></b>	0	0	0
<b>Level of significance</b>	**	*	*
<b>CV (%)</b>	2.05%	2.23%	1.42%

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

[T<sub>1</sub> = Bamboo stick      T<sub>2</sub> = Sawdust      T<sub>3</sub> = Wheat grain]

### 4.1.3 Effect on days required from opening to harvest

There was significant variation in terms of days to require from opening to harvest of Reishi mushroom due to different amount of substrate (Appendix II).

**Table 4.3: Combined Effect of different amount of substrate and mother culture on mycelium growth, days required for primordial initiation, days required from primordial initiation to harvest**

Treatments	Mycelium running rate(cm/day)	Days required for primordial initiation	Days required from opening to harvest
B <sub>1</sub> T <sub>1</sub>	0.61 bc	15.01 j	43.20 kl
B <sub>1</sub> T <sub>2</sub>	0.67 a	14.86 jk	42.16 l
B <sub>1</sub> T <sub>3</sub>	0	0	0
B <sub>2</sub> T <sub>1</sub>	0.59 d	17.36 h	45.66 gh
B <sub>2</sub> T <sub>2</sub>	0.62 b	16.00 i	43.97 jk
B <sub>2</sub> T <sub>3</sub>	0	0	0
B <sub>3</sub> T <sub>1</sub>	0.56 ef	18.89 ef	47.02 fg
B <sub>3</sub> T <sub>2</sub>	0.58 de	18.16 g	45.33 hi
B <sub>3</sub> T <sub>3</sub>	0	0	0
B <sub>4</sub> T <sub>1</sub>	0.57 def	19.63 d	51.29 e
B <sub>4</sub> T <sub>2</sub>	0.55 fg	19.13 e	48.23 f
B <sub>4</sub> T <sub>3</sub>	0	0	0
B <sub>5</sub> T <sub>1</sub>	0.52h	19.92 c	54.30 b
B <sub>5</sub> T <sub>2</sub>	0.55 fg	20.04 bc	52.89 de
B <sub>5</sub> T <sub>3</sub>	0	0	0
B <sub>6</sub> T <sub>1</sub>	0.44 ij	21.04 a	57.83 a
B <sub>6</sub> T <sub>2</sub>	0.46 i	20.67 b	53.33 c
B <sub>6</sub> T <sub>3</sub>	0	0	0
<b>Level of significance</b>	*	**	**
<b>CV (%)</b>	2.05	2.23	1.42

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

[Here, B<sub>1</sub>= 250 g, B<sub>2</sub>= 500 g, B<sub>3</sub>= 750 g, B<sub>4</sub>= 1000 g, B<sub>5</sub>= 1250 g, B<sub>6</sub>= 1500 g

T<sub>1</sub>= Bamboo stick      T<sub>2</sub>= Sawdust      T<sub>3</sub>= Wheat grain]

The maximum days to require from opening to harvest was found from B<sub>6</sub> (57.0 days), whereas the minimum was recorded in B<sub>1</sub> (42.23 days) (Table 4.1).

Days to require from opening to harvest showed statistically significant variation due to different mother culture (Appendix II). The maximum days to require from opening to harvest was recorded from T<sub>1</sub> (51.69 days), while the minimum days to require from opening to harvest was observed in T<sub>2</sub> (47.05 days) (Table 4.2).

Significant combined effects of different amount of substrate and mother culture on days to require from opening to harvest was observed (Appendix II). The maximum days to require from opening to harvest was recorded from B<sub>6</sub>T<sub>1</sub> (57.83 days). On the other hand, the minimum days to require from opening to harvest was observed in B<sub>1</sub>T<sub>2</sub> (42.16 days) followed by B<sub>2</sub>T<sub>2</sub> (43.97 days) (Table 4.3).

#### **4.1.4 Number of fruiting body per packet**

Number of fruiting body per packet of Reishi mushroom varied significantly due to different amount of substrate under the present trial (Appendix III). The highest number of fruiting per packet was observed from B<sub>4</sub> (4.92), which was followed by B<sub>6</sub> (4.28), again the lowest number of fruiting body per packet was found in B<sub>1</sub> (2.61) (Table 4.4). Average number of fruiting body was highest in T<sub>2</sub> (4.67) and lowest number of fruiting body found in T<sub>1</sub> (3.91) due to the effects of different mother culture (Table 4.5).

Combined effect showed significant variation in respect of no. of fruiting body (Appendix III). The highest no of fruiting body (4.82) was recorded from the combination of B<sub>4</sub>T<sub>2</sub> treatment whereas, the lowest was recorded from the combination of B<sub>1</sub>T<sub>1</sub> (2.16) treatment. Similar result was recorded by Smith *et al.* (2002) found that the cultivation of medicinal Reishi mushrooms largely increased in different parameters like no. of fruiting body, weight of the fruiting body due to the use of different sizes of polypropylene bags or containers.

**Table 4.4: Effect of different amount of substrate on number of fruiting body per packet, weight of individual fruiting body and diameter of the pileus**

Substrate amount	No. of Fruiting body per packet	Weight of the individual fruiting body per packet	Diameter of pileus
<b>B<sub>1</sub></b>	2.61 f	2.21 e	4.20 f
<b>B<sub>2</sub></b>	2.00 e	2.17 ef	4.78 ef
<b>B<sub>3</sub></b>	3.33 c	2.29 d	5.16 d
<b>B<sub>4</sub></b>	4.92 a	3.18 a	6.92 a
<b>B<sub>5</sub></b>	3.66 d	2.74 c	5.99 c
<b>B<sub>6</sub></b>	4.28 b	3.13 ab	6.25 b
<b>Level of significance</b>	**	*	**
<b>CV (%)</b>	3.32	2.05	2.02

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

[Here, B<sub>1</sub>= 250 g, B<sub>2</sub>= 500 g, B<sub>3</sub>= 750 g, B<sub>4</sub>= 1000 g, B<sub>5</sub> = 1250 g, B<sub>6</sub>= 1500 g]

#### **4.1.5 Effect on average weight of individual fruiting body**

Statistically variation was observed in case of single weight of fruiting body of Reishi mushroom for different amount of substrate under the present trial (Appendix III). The maximum single weight of fruiting body was found from B<sub>4</sub> (3.18 g), which was followed by B<sub>6</sub> (3.13 g). On the other hand, the lowest single weight of fruiting body was found in B<sub>2</sub> (2.17 g) which was statistically similar to B<sub>1</sub> (2.21 g) (Table 4.4).

Single weight of fruiting body was significantly influenced by different mother culture (Appendix III).

**Table 4.5: Effect of mother culture (inocula) on number of fruiting body per packet, weight of individual fruiting body and diameter of the pileus**

<b>Mother Culture</b>	<b>No. of fruiting body per packet</b>	<b>Weight of the individual fruiting body per packet</b>	<b>Diameter of pileus</b>
<b>T<sub>1</sub></b>	3.91 b	2.52 b	1.23 b
<b>T<sub>2</sub></b>	4.67 a	3.12 a	1.56 a
<b>T<sub>3</sub></b>	0	0	0
<b>Level of significance</b>	*	*	**
<b>CV (%)</b>	3.32	2.05	1.37

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

[T<sub>1</sub> = Bamboo stick    T<sub>2</sub> = Sawdust    T<sub>3</sub> = Wheat grain]

The lowest single weight of fruiting body (2.52 g) was produced from T<sub>1</sub>, whereas the highest 3.12 g was counted from T<sub>2</sub>. (Table 4.5). This may be due to the fact that bamboo stick mother culture is generally slower to colonize and produce smaller mushroom whereas sawdust tends to produce larger and more uniform mushrooms due to its balanced nutritional effect and good moisture for growing Reishi mushroom.

Combined effect showed significant variation in respect of single weight of fruiting body (Appendix III). The highest single weight of fruiting body (3.59 g) was recorded from the combination of B<sub>4</sub>T<sub>2</sub> treatment followed by B<sub>5</sub>T<sub>2</sub> (3.30) and B<sub>6</sub>T<sub>2</sub> (3.19) whereas, the lowest (2.01 g) was recorded from the combination of (B<sub>1</sub>T<sub>1</sub>) treatment followed by B<sub>2</sub>T<sub>1</sub> (2.08 g).

**Table 4.6: Combined Effect of different substrate amount and mother culture no. of fruiting body, weight of individual fruiting body and diameter of pileus**

<b>Treatments</b>	<b>Number of fruiting body</b>	<b>Weight of individual fruiting body (g)</b>	<b>Diameter of pileus (cm)</b>
<b>B<sub>1</sub>T<sub>1</sub></b>	2.16 l	2.01 h	3.97 j
<b>B<sub>1</sub>T<sub>2</sub></b>	3.93 h	2.28 fg	4.30 ij
<b>B<sub>1</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>2</sub>T<sub>1</sub></b>	2.83 kl	2.08 gh	4.41 hi
<b>B<sub>2</sub>T<sub>2</sub></b>	4.17 fg	2.39 efg	4.84 gh
<b>B<sub>2</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>3</sub>T<sub>1</sub></b>	3.30 j	2.79 d	5.82 f
<b>B<sub>3</sub>T<sub>2</sub></b>	4.71 b	3.34 b	6.52 bcd
<b>B<sub>3</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>4</sub>T<sub>1</sub></b>	3.73 hi	2.76 de	6.48 cd
<b>B<sub>4</sub>T<sub>2</sub></b>	4.82 a	3.59 a	7.25 a
<b>B<sub>4</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>5</sub>T<sub>1</sub></b>	4.27 ef	2.41 e	6.10 e
<b>B<sub>5</sub>T<sub>2</sub></b>	4.59 cd	3.30 b	6.61 bc
<b>B<sub>5</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>6</sub>T<sub>1</sub></b>	4.33 e	2.96 cd	5.91 ef
<b>B<sub>6</sub>T<sub>2</sub></b>	4.51 d	3.19 c	6.66 b
<b>B<sub>6</sub>T<sub>3</sub></b>	0	0	0
<b>Level of significance</b>	*	*	*
<b>CV (%)</b>	1.42	3.32	1.37

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

[ B<sub>1</sub>= 250 g, B<sub>2</sub>= 500 g, B<sub>3</sub>= 750 g, B<sub>4</sub>= 1000 g, B<sub>5</sub> = 1250 g, B<sub>6</sub>= 1500 g  
T<sub>1</sub> = Bamboo stick      T<sub>2</sub> = Sawdust      T<sub>3</sub> = Wheat grain]

## **4.2 Effect on the development and size of fruiting body**

### **4.2.1 Effect on length of stalk**

Length of stalk of Reishi mushroom showed statistically significant variation and that might be due to different amount of substrate under the present trial (Appendix IV). The longest length of stalk (2.50 cm) was recorded from B<sub>4</sub>. On the other hand, the shortest length of stalk (1.78 cm) was found in B<sub>1</sub> (Table 4.6). A larger amount of substrate with balanced amount of nutrients provides more space for the reishi mushroom to grow and expand, potentially resulting in longer stalks. Malarvizhi *et al.* (2003) reported that length of stalk ranged from 1.40 to 2.79 cm which was similar to the findings of this experiment. Again substrate amount preferred in case of proper aeration and distribution for mushroom growth including fruiting body, stalk length, diameter, pileus length, diameter etc.

Length of stalk of Reishi mushroom significantly influenced by different mother culture under this present experiment (Appendix IV). The longest length of stalk (2.27 cm) was recorded from T<sub>2</sub>. On the other hand, the shortest length of stalk (1.94 cm) was observed from T<sub>1</sub> (Table 4.7). Sawdust-based cultures can result in longer mushroom stalks because the culture tends to be more nutrient-rich and retains moisture well, promoting longer stem growth. The results of this experiment supported by the finding of previous Mubasshira *et al.* (2020). It was found that among the substrates and mother culture components, using rice straw and sawdust mother spawn, the maximum length of stipe was recorded (23.27 mm and 24.29 mm, respectively).

Combined effect of different amount of substrate and mother culture showed significant variation in respect of length of stalk during the experiment (Appendix IV). The longest length of stalk (2.77 cm) was recorded from the combination of (B<sub>4</sub>T<sub>2</sub>) treatment. Statistically similar result observed from B<sub>5</sub>T<sub>2</sub> (2.24 cm) and B<sub>6</sub>T<sub>2</sub> (2.47 cm) whereas, the shortest length of stalk (1.42 cm) was

observed from the combination of (B<sub>2</sub>T<sub>1</sub>) treatment followed by B<sub>1</sub>T<sub>1</sub> (1.56 cm) (Table 4.8).

**Table 4.7. Effect of different amount of substrate on length of stalk, diameter of stalk and thickness of pileus of Reishi mushroom**

Substrate amount	Length of stalk (cm)	Diameter of stalk (cm)	Thickness of pileus (cm)
<b>B<sub>1</sub></b>	1.78 e	1.27 d	1.05 d
<b>B<sub>2</sub></b>	1.94 d	1.27 cd	1.18 cd
<b>B<sub>3</sub></b>	1.92 d	1.29 c	1.29 c
<b>B<sub>4</sub></b>	2.50 a	1.60 a	1.73 a
<b>B<sub>5</sub></b>	2.17 c	1.53 ab	1.55 b
<b>B<sub>6</sub></b>	2.23 b	1.46 bc	1.61 ab
<b>Level of Significance</b>	**	**	**
<b>CV (%)</b>	2.74	1.37	1.11

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

[Here, B<sub>1</sub>= 250 g, B<sub>2</sub>= 500 g, B<sub>3</sub>= 750 g, B<sub>4</sub>= 1000 g, B<sub>5</sub>= 1250 g, B<sub>6</sub>= 1500 g.]

#### 4.2.2 Effect on diameter of stalk

Different amount of substrate showed significant differences in terms of diameter of stalk of Reishi mushroom (Appendix IV). The highest diameter of stalk was found from B<sub>4</sub> (1.60 cm) followed by B<sub>5</sub> (1.53 cm), whereas the lowest diameter of stalk was recorded in B<sub>1</sub> (1.27 cm) closely associated with B<sub>2</sub> treatment (1.27 cm). (Table 4.6). Different mother culture had significant effect on diameter of stalk (Appendix IV). The highest diameter of stalk (1.56 cm) was found in T<sub>2</sub> and the lowest (1.23 cm) was found from T<sub>1</sub> (Table 4.7).

It was found that diameter of stalk was affected significantly due to the combined of different amount of substrate and mother culture (Appendix IV). The highest diameter of stalk (1.83 cm) was recorded from B<sub>4</sub>T<sub>2</sub>. Nearly Similar result

observed from B<sub>5</sub>T<sub>2</sub> (1.71 cm) treatment. On the other hand, the lowest diameter of stalk (0.99 cm) was found from B<sub>1</sub>T<sub>1</sub> (Table 4.8).

**Table 4.8. Effect of different mother culture on length of stalk (cm), diameter of stalk (cm) and thickness of pileus (cm)**

Mother culture	Length of stalk (cm)	Diameter of stalk (cm)	Thickness of pileus (cm)
T <sub>1</sub>	1.94 b	1.23 b	1.29 b
T <sub>2</sub>	2.27 a	1.56 a	1.42 a
T <sub>3</sub>	0	0	0
Level of Significance	*	**	*
CV (%)	2.74	1.37	1.11

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

[T<sub>1</sub> = Bamboo stick    T<sub>2</sub> = Sawdust    T<sub>3</sub> = Wheat grain]

#### 4.2.3 Effect on diameter of pileus

Diameter of pileus of Reishi mushroom varied significantly due to different amount of substrate under the present trial (Appendix IV). The highest diameter of pileus was recorded from B<sub>4</sub> (6.92 cm), followed by B<sub>6</sub> (6.25 cm) and B<sub>5</sub> (5.99 cm), again the lowest diameter of pileus was found in B<sub>1</sub> (4.20 cm) (Table 4.4). Result indicated that larger amount of substrate can provide more space for the Reishi mushroom to grow and expand, potentially resulting in a larger cap diameter.

Different mother culture had significant effect on diameter of pileus (Appendix IV). The highest diameter of pileus (5.56 cm) was found in T<sub>2</sub> and the lowest (5.11 cm) was found from T<sub>1</sub> (Table 4.5). It was observed that sawdust-based cultures can promote larger cap diameter because it's tendency to be more

nutrient-rich and retains moisture well, which can provide the Reishi mushroom with the resources it needs to produce a larger cap.

It was found that diameter of pileus was affected significantly due to the combined effect of different amount of substrate and mother culture (Appendix IV). The highest diameter of pileus (7.25 cm) was recorded from B<sub>4</sub>T<sub>2</sub>. On the other hand, the lowest diameter of pileus (3.97 cm) was found from B<sub>1</sub>T<sub>1</sub>. Previous finding reports statement was, the peak diameter of stipe was calculated (9.90 cm and 10.01 cm, respectively). The maximum diameter of pileus was observed in combination of sawdust with relatively large amount of substrate that provided proper spacing for forming the fruiting body with higher diameter of pileus.

#### **4.2.4 Effect on Thickness of pileus**

There was significant variation in terms of thickness of pileus of reishi mushroom due to different amount of substrate (Appendix IV). In numerically, the highest thickness of pileus was observed from B<sub>4</sub> (1.73 cm) followed by B<sub>6</sub> (1.61 cm). On the other hand, the lowest thickness of pileus was found in B<sub>1</sub> (1.05 cm), followed by B<sub>2</sub> (1.18 cm). (Table 4.6). Chang *et al.* (2006) stated that a larger bag size may result in a more challenging environment to maintain optimal humidity and air exchange, potentially impacting pileus thickness.

Thickness of pileus of Reishi mushroom significantly influenced by different mother culture (Appendix IV). The highest thickness of pileus (1.42 cm) was recorded from T<sub>2</sub>. On the other hand, the lowest thickness of pileus (1.29 cm) was observed from T<sub>1</sub> (Table 4.7). Present study showed that thickness of pileus increased when increased the sawdust percentage.

Combined effect of different amount of substrate and mother culture showed significant variation in respect of thickness of pileus (Appendix IV). The highest thickness of pileus (1.72 cm) was recorded from the combination of B<sub>4</sub>T<sub>2</sub> treatment whereas, the shortest (0.94 cm) was observed from the combination of B<sub>1</sub>T<sub>1</sub> treatment (Table 4.8).

**Table 4.9. Combined effect of different substrate amount and mother culture (inocula) on length of stalk, diameter of stalk and thickness of pileus of Reishi mushroom**

<b>Treatments</b>	<b>Length of stalk (cm)</b>	<b>Diameter of stalk (cm)</b>	<b>Thickness of pileus (cm)</b>
<b>B<sub>1</sub>T<sub>1</sub></b>	1.56 ghi	0.99 h	0.94 j
<b>B<sub>1</sub>T<sub>2</sub></b>	1.84 g	1.18 g	1.12 hi
<b>B<sub>1</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>2</sub>T<sub>1</sub></b>	1.42 j	1.10 gh	1.08 hij
<b>B<sub>2</sub>T<sub>2</sub></b>	1.91 efg	1.22 ef	1.40 fg
<b>B<sub>2</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>3</sub>T<sub>1</sub></b>	1.67 gh	1.30 de	1.15 h
<b>B<sub>3</sub>T<sub>2</sub></b>	1.99 ef	1.39 d	1.45 efg
<b>B<sub>3</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>4</sub>T<sub>1</sub></b>	2.10 cd	1.22 ef	1.54 d
<b>B<sub>4</sub>T<sub>2</sub></b>	2.77 a	1.83 a	1.72 a
<b>B<sub>4</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>5</sub>T<sub>1</sub></b>	2.02 e	1.48 cd	1.57 cd
<b>B<sub>5</sub>T<sub>2</sub></b>	2.24 c	1.71 ab	1.65 b
<b>B<sub>5</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>6</sub>T<sub>1</sub></b>	2.09 de	1.23 ef	1.47 ef
<b>B<sub>6</sub>T<sub>2</sub></b>	2.47 b	1.55 c	1.58 bc
<b>B<sub>6</sub>T<sub>3</sub></b>	0	0	0
<b>Level of significance</b>	**	*	*
<b>CV (%)</b>	2.74	1.37	1.11

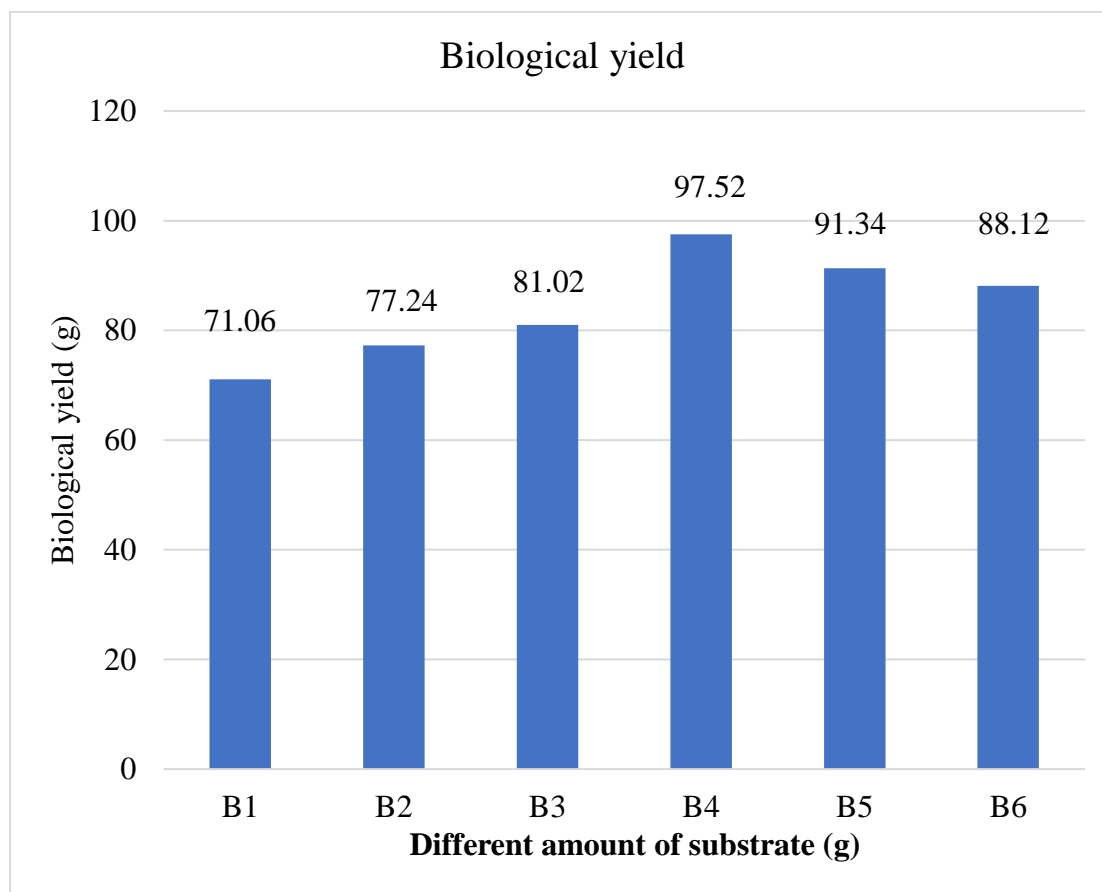
In a column means having similar letter (s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability. [Here, B<sub>1</sub>= 250 g, B<sub>2</sub>= 500 g, B<sub>3</sub>= 750 g, B<sub>4</sub>= 1000 g, B<sub>5</sub>= 1250 g, B<sub>6</sub>= 1500 g

T<sub>1</sub> = Bamboo stick      T<sub>2</sub> = Sawdust      T<sub>3</sub> = Wheat grain]

### 4.3 Effect on the yield

#### 4.3.1 Effects on biological yield

Biological yield of Reishi mushroom showed statistically significant variation due to different amount of substrate under the present trial (Appendix V). The highest biological yield (97.52 g) was recorded from B<sub>4</sub>, followed by B<sub>5</sub> (91.34 g), while the lowest biological yield (71.06 g) was recorded in B<sub>1</sub> (Figure 1).



**Figure 1: Effect of different amount of substrate on biological yield**

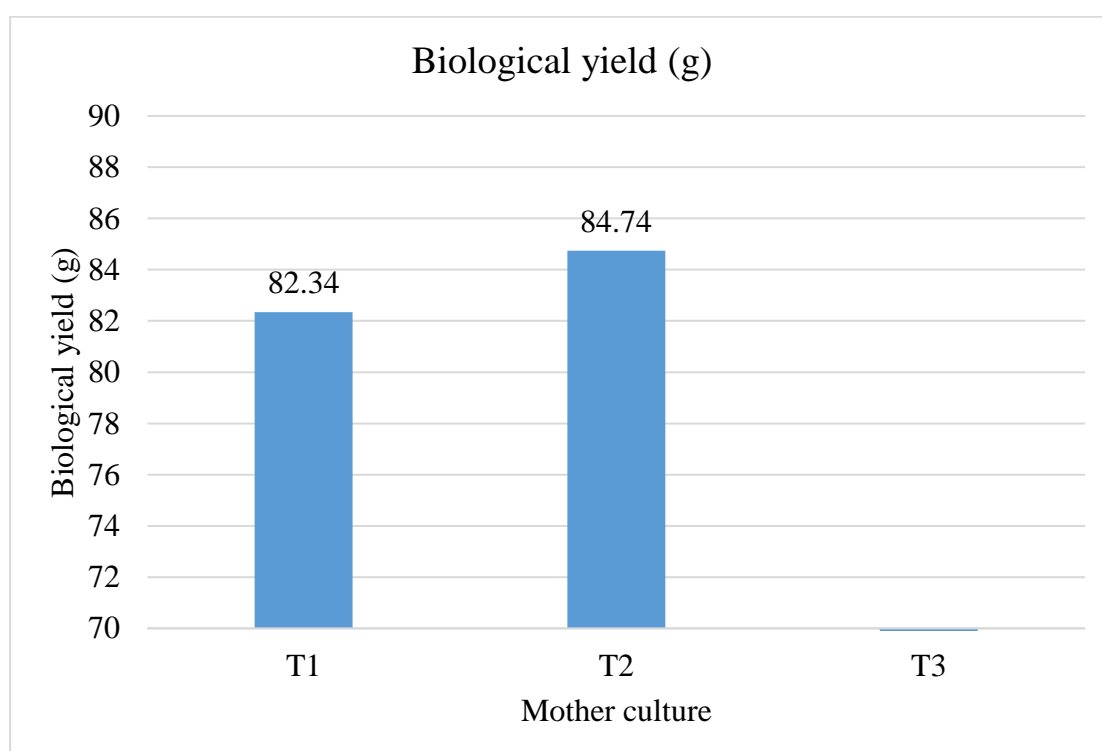
In a column means having similar letter (s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

[Here, B<sub>1</sub>= 250 g, B<sub>2</sub>= 500 g, B<sub>3</sub>= 750 g, B<sub>4</sub>= 1000 g, B<sub>5</sub>= 1250 g, B<sub>6</sub>= 1500 g.]

Biological yield of Reishi mushroom showed statistically significant variation due to different mother culture under the present trial (Appendix V). The highest biological yield (84.74 g) was recorded from T<sub>2</sub>, while the lowest biological yield (82.34 g) was recorded in T<sub>1</sub>(Figure 2).

It was found that biological yield was affected significantly due to the combined of different amount of substrate and mother culture (Appendix V). The highest biological yield (98.20 g) was recorded from B<sub>4</sub>T<sub>2</sub> which statistically similar to B<sub>6</sub>T<sub>2</sub>(97.56). On the other hand, the lowest biological yield (65.05 g) was found from B<sub>1</sub>T<sub>2</sub> (Table 4.10).

The result of the present study found similar with the of previous studies of Mubasshira *et al.* (2020), reported that among the mother spawn, sawdust mother spawn presented the highest biological yield. Suitable amount of substrate led to the proper growth and development of mushroom fruiting body which was significantly better than other combination of treatments. Azizi *et al.* (2012) reported the best combinations for high yield and biological efficiency (BE) were obtained culture medium with the combination of poplar sawdust with 5% malt extract.



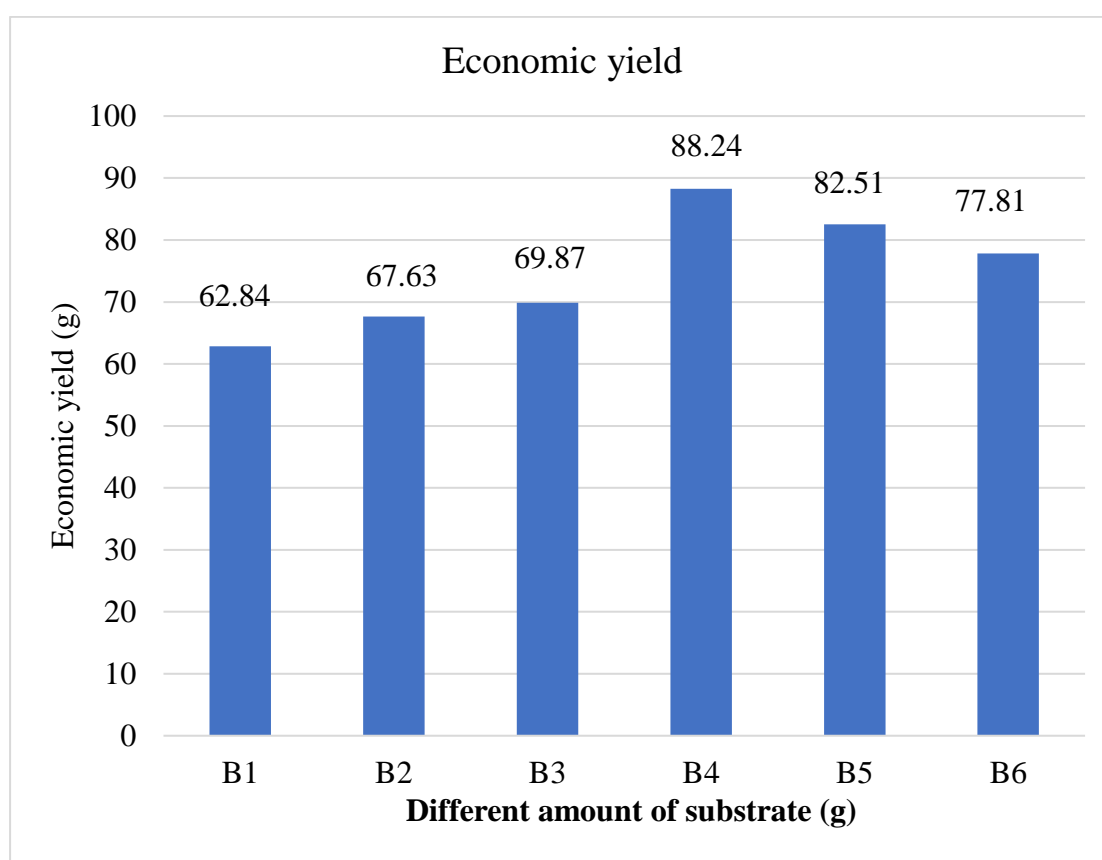
**Figure 2: Effect of mother culture on biological yield**

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

[T<sub>1</sub> = Bamboo stick      T<sub>2</sub> = Sawdust      T<sub>3</sub> = Wheat grain]

### 4.3.2 Effects on economic yield

Economic yield of Reishi mushroom grown on different amount of substrate showed statistically significant variation under this present trail (Appendix V). The maximum economic yield (88.24 g) was recorded from B<sub>4</sub> treatment, whereas the minimum economic yield (62.84 g) was observed in B<sub>1</sub>(Figure 3). Result indicated that comparatively larger bag sizes can provide more substrate for the Reishi mushroom with proper distribution of nutrients and enhance good moisture to grow on, which can potentially result in higher yields per bag that lead to higher economic yield of Reishi mushroom.



**Figure 3: Effect of different amount of substrate on economic yield**

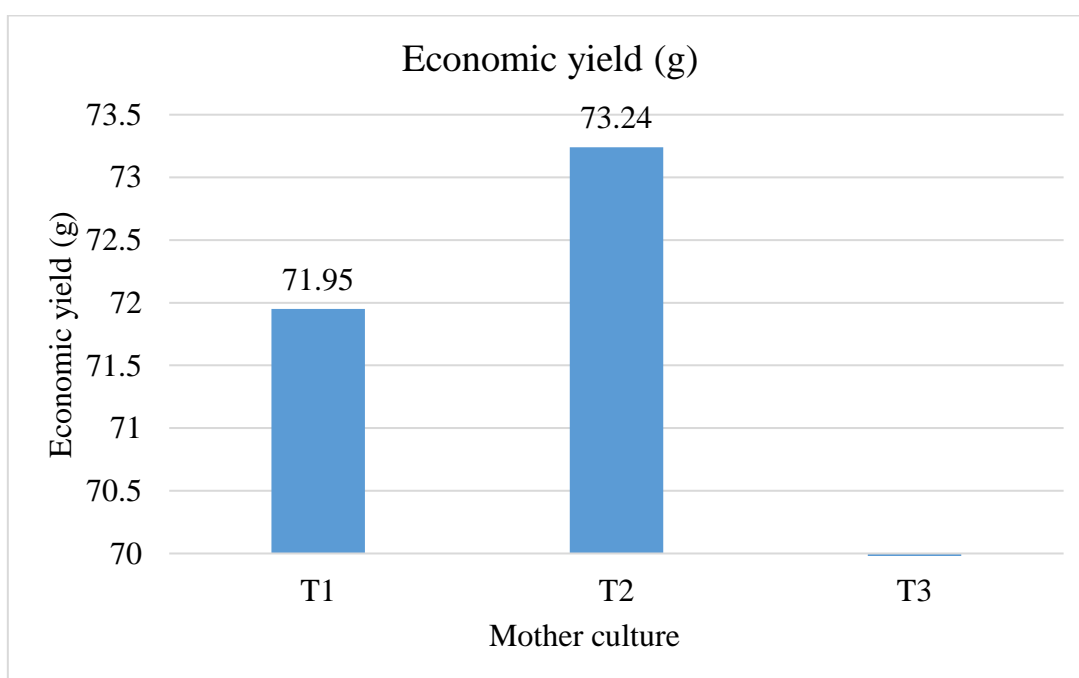
In a column means having similar letter (s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

[Here, B<sub>1</sub>= 250 g, B<sub>2</sub>= 500 g, B<sub>3</sub>= 750 g, B<sub>4</sub>= 1000 g, B<sub>5</sub>= 1250 g, B<sub>6</sub>= 1500 g.]

Significant variation was observed at different levels of supplements under different mother culture substrate combinations (Appendix V). Under the present trial the maximum economic yield (73.24 g) was recorded from T<sub>2</sub>, while the

minimum economic yield (71.95 g) was found from T<sub>1</sub> (Figure 4). It was observed that the quality of the sawdust mother inoculum affected the rate and extent of colonization of the substrate, which impacted in higher yield and quality of the fruiting bodies produced, resulted in higher economic yield.

It was found that economic yield was affected significantly due to the combined effect of different amount of substrate and mother culture (Appendix V). The maximum economic yield (88.65 g) was recorded from B<sub>4</sub>T<sub>2</sub> which was statistically similar to B<sub>6</sub>T<sub>2</sub> (86.82 g). On the other hand, the minimum economic yield (53.12 g) was found from B<sub>1</sub>T<sub>2</sub>(Table 4.10). 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.



**Figure 4: Effect of mother culture on economic yield**

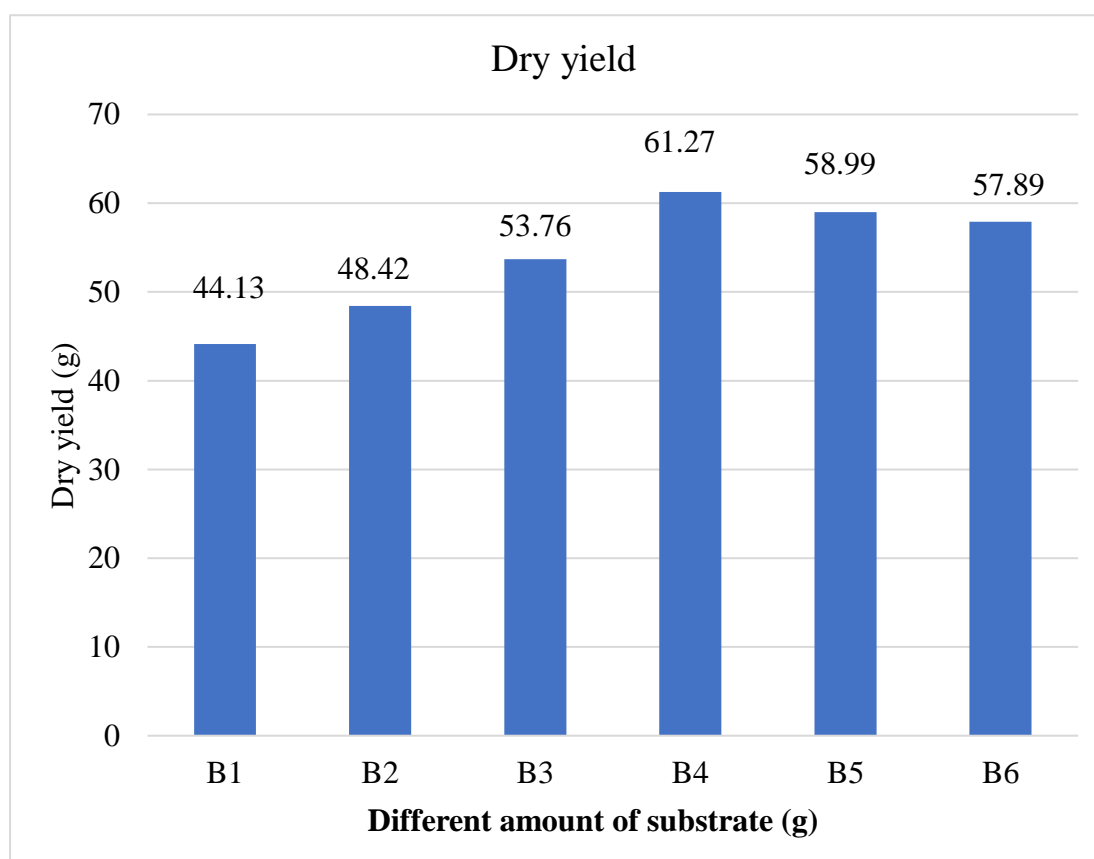
In a column means having similar letter (s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

[T<sub>1</sub> = Bamboo stick      T<sub>2</sub> = Sawdust      T<sub>3</sub> = Wheat grain]

### 4.3.3 Effects on Dry yield

Significant variation was recorded in terms of dry yield of Reishi mushroom due to different amount of substrate (Appendix V). The highest dry yield (61.27 g) was observed from B<sub>4</sub> treatment. On the other hand, the lowest dry yield was attained in B<sub>1</sub> (44.13 g) treatment (Figure 5). The result of this findings is conincided with the previous study of Rashid and Hanif (2008) who reported that the yield was maximum in the bag sized (6x12") 69.2 g.

Dry yield of Reishi mushroom showed statistically significant variation due to different mother culture under the present trial (Appendix V). The highest dry yield (54.39 g) was recorded from T<sub>2</sub> treatment while the lowest dry yield (52.79 g) was recorded in T<sub>1</sub> treatment application (Figure 6). Previous report of Shah *et al.* (2018) stated that the maximum dry mycelial biomass obtained after combining these optimum conditions of mother culture and suitable bag size.

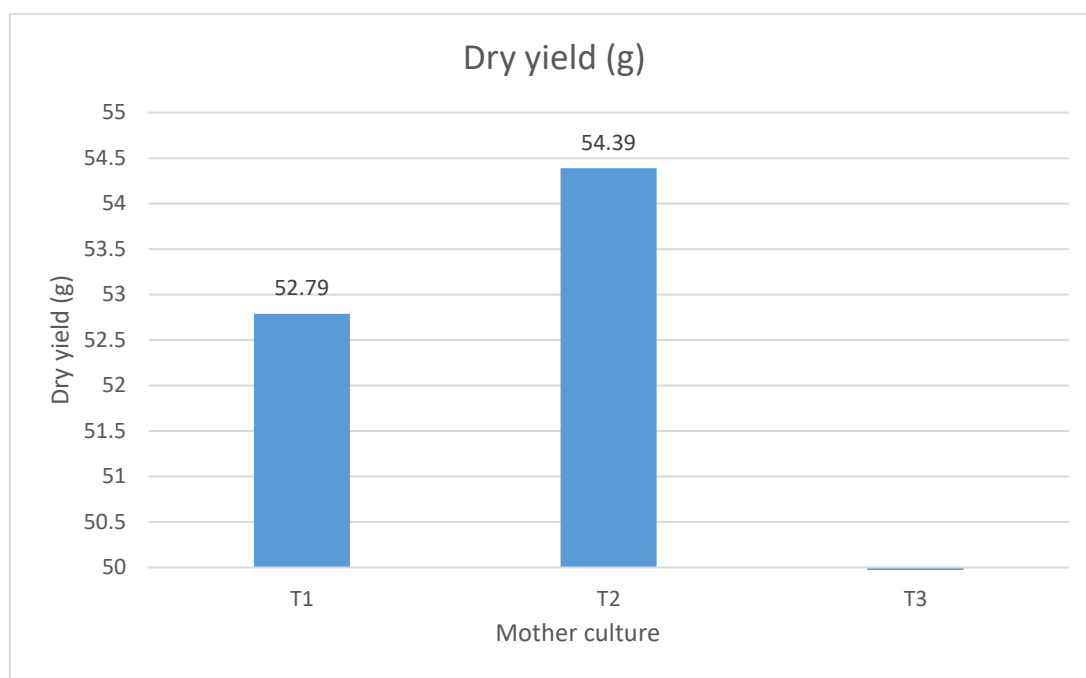


**Figure 5: Effect of different amount of substrate on dry yield**

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

[Here, B<sub>1</sub>= 250 g, B<sub>2</sub>= 500 g, B<sub>3</sub>= 750 g, B<sub>4</sub>= 1000 g, B<sub>5</sub>= 1250 g, B<sub>6</sub>= 1500 g.]

Combined effects of those factor showed significant result in case of dry yield of Reishi mushroom (Appendix V). The maximum dry yield (62.35 g) was recorded from B<sub>4</sub>T<sub>2</sub> which was statistically similar to B<sub>6</sub>T<sub>2</sub> (61.07 g). On the other hand, the minimum economic yield (45.26 g) was found from B<sub>1</sub>T<sub>1</sub> (Table 4.10).



**Figure 6: Effect of mother culture on dry yield**

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

[T<sub>1</sub> = Bamboo stick    T<sub>2</sub> = Sawdust    T<sub>3</sub> = Wheat grain]

**Table 4.10: Combined effect of different amount of substrate and mother culture (inocula) on biological yield, economic yield and dry yield of Reishi mushroom.**

<b>Treatments</b>	<b>Biological yield (g)</b>	<b>Economic yield (g)</b>	<b>Dry yield (g)</b>
<b>B<sub>1</sub>T<sub>1</sub></b>	65.05 ij	53.12 j	45.26 ij
<b>B<sub>1</sub>T<sub>2</sub></b>	67.58 i	55.32 i	46.12 hi
<b>B<sub>1</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>2</sub>T<sub>1</sub></b>	75.42 gh	64.29 h	47.92 h
<b>B<sub>2</sub>T<sub>2</sub></b>	78.26 fg	65.69 gh	51.94 fg
<b>B<sub>2</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>3</sub>T<sub>1</sub></b>	77.58 fgh	76.67 f	52.67 f
<b>B<sub>3</sub>T<sub>2</sub></b>	84.03 e	78.21 ef	55.08 e
<b>B<sub>3</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>4</sub>T<sub>1</sub></b>	95.26 bc	85.85 bc	61.83 ab
<b>B<sub>4</sub>T<sub>2</sub></b>	98.20 a	88.65 a	62.35 a
<b>B<sub>4</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>5</sub>T<sub>1</sub></b>	89.41 cd	79.26 e	59.67 cd
<b>B<sub>5</sub>T<sub>2</sub></b>	93.41 c	85.31 bcd	60.31 bc
<b>B<sub>5</sub>T<sub>3</sub></b>	0	0	0
<b>B<sub>6</sub>T<sub>1</sub></b>	88.58 de	84.29 d	58.24 cd
<b>B<sub>6</sub>T<sub>2</sub></b>	97.56 ab	86.82 ab	61.07 ab
<b>B<sub>6</sub>T<sub>3</sub></b>	0	0	0
<b>Level of significance</b>	*	*	**
<b>CV (%)</b>	1.11	6.54	6.10

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

[Here, B<sub>1</sub>= 250 g, B<sub>2</sub>= 500 g, B<sub>3</sub>= 750 g, B<sub>4</sub>= 1000 g, B<sub>5</sub> = 1250 g, B<sub>6</sub>= 1500 g

T<sub>1</sub> = Bamboo stick      T<sub>2</sub> = Sawdust      T<sub>3</sub> = Wheat grain]



**Chapter V**

**Summary and Conclusion**

## Chapter V

### SUMMARY AND CONCLUSION

The experiment was conducted at the Laboratory and Culture house of Mushroom Development and Extension Programme, Mushroom Development Institute, Sobhanbang, Savar, Dhaka during March-August, 2021 to evaluate the performance of different amount of substrate and mother culture on the growth, yield and proximate composition of Reishi mushroom (*Ganoderma lucidum*). Mother culture of Reishi mushroom was collected from Mushroom Development Institute (MDI), Savar, Dhaka. The experiment consists of two factors design with six replications. Factor A: Different amount of substrate B<sub>1</sub> = 250 g, B<sub>2</sub> = 500 g, B<sub>3</sub> = 750 g, B<sub>4</sub> = 1000 g, B<sub>5</sub> = 1250 g, B<sub>6</sub> = 1500 g and Factor B: Types of mother culture T<sub>1</sub> = Bamboo stick, T<sub>2</sub> = Sawdust, T<sub>3</sub> = Wheat grain. The experiment was laid out in two factors Completely Randomized Design (CRD).

There was significant variation in terms of mycelium running rate, days to require from opening to primordial initiation, days to require from primordial initiation to harvest, number of fruiting body per packet, single weight of fruiting body, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus biological yield, economic yield and dry yield in term of Reishi mushroom due to different amount of substrate. The highest mycelium running rate was (0.67 cm), minimum days from opening to primordial initiation (16.00 days), minimum days to require from opening to primordial initiation to harvest (42.23 days), lowest number of fruiting body per packet was (.61), lowest diameter of pileus (4.20 cm), lowest length of stalk (1.78 cm), lowest diameter of stalk (1.27 cm), lowest thickness of pileus (1.05 cm) lowest biological yield (71.06 g), economic yield (62.84 g), dry yield (44.13 g) was recorded from B<sub>1</sub> treatment. Again the lowest mycelium running rate 0.56 cm found in B<sub>5</sub> treatment. Maximum days from opening to primordial initiation (20.14 days), maximum days to require from opening to harvest (57.00 days) was recorded from B<sub>6</sub>. The maximum number of fruiting body per packet (4.92), maximum single weight of

fruiting body (3.18 g), longest length of stalk (2.50 cm), highest diameter of stalk (1.60 cm), diameter of pileus (6.92 cm), thickness of pileus (1.73 cm), biological yield (97.52 g), economic yield (88.24 g), dry yield (61.27 g) was observed from B<sub>4</sub>.

There was statistically significant variation in terms of mycelium running rate, days to require from opening to primordial initiation, days to require from opening to harvest, number of fruiting body per packet, number of effective fruiting body per packet, single weight of fruiting body, length of stalk, diameter of stalk, length of pileus, diameter of pileus, biological yield, economic yield and dry yield of Reishi mushroom due to different types of mother culture. The highest mycelium running rate (0.63 cm), minimum days require for primordial initiation (17.22 days), minimum days required to harvest (47.05 days), number of fruiting body per packet (4.67), weight of individual fruiting body (3.12 g), length of stalk (2.27 cm), diameter of stalk (1.56 cm), diameter of pileus (1.56 cm), thickness of pileus (1.42), highest biological yield (84.74 g), highest economic yield (73.24), highest dry yield (54.39 g), was recorded from T<sub>2</sub> treatment. While the maximum days for primordial initiation (18.75 days) and days required to harvest (51.69 days) was recorded from T<sub>1</sub> treatment.

Significant Interaction effects of different amount of substrate and different types of mother culture on mycelium running rate, days to require from opening to primordial initiation, days to require from opening to harvest, number of fruiting body per packet, number of effective fruiting body per packet, single weight of fruiting body, length of stalk, diameter of stalk, length of pileus, diameter of pileus, biological yield, economic yield and dry yield of Reishi mushroom was observed in this experiment. The highest mycelium running rate 0.67 cm, minimum days for primordial initiation 14.86 days, minimum days for harvesting 42.16 days was recorded from B<sub>1</sub>T<sub>2</sub> treatment. While lowest mycelium running rate 0.44 cm, maximum days for primordial initiation 21.04 days, maximum days for harvesting 57.83 days recorded from B<sub>6</sub>T<sub>1</sub> treatment combination. Maximum number of fruiting body 4.82, highest single weight of

fruiting body 3.59 g, longest length of stalk 2.77 cm, diameter of stalk 1.83 cm, diameter of pileus 7.25 cm, thickness of pileus 1.72 cm, maximum biological yield (98.20 g), maximum economic yield (88.65 g) and maximum dry yield (62.35 g) was recorded from the combination of B<sub>4</sub>T<sub>2</sub>.

In this experiment, due to the interaction between different amount of substrates and types of mother culture, results observed with many significant variations. It has been observed that bags of B<sub>4</sub> = 1000 g and their interaction with T<sub>2</sub> = Sawdust combinely performed better in most of the cases. This may be due to the suitable spacing of the bag and proper distribution of the mother culture surrounding the bags.

### **Conclusion**

1. From the above study it can be concluded that both amount of substrate and mother culture had significant effect on most of the growth and yield contributing parameters of Reishi mushroom.
2. For substrate amount, B<sub>4</sub> (1000g) showed better performance in respect to yield and yield contributing characters of mushroom.
3. On the other hand, for different mother culture T<sub>2</sub> (sawdust) showed higher yield and promising results on growth parameters of mushroom.
4. Overall in case of interactive effect, B<sub>4</sub>T<sub>2</sub> (B<sub>4</sub>=1000g and T<sub>2</sub>=sawdust) combination was comparatively better than other treatment combination for giving higher yield of mushroom.



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# Appendices

## APPENDICES

**Appendix I: Monthly record of air temperature, relative humidity, rainfall, and sunshine (average) of the experimental site during the period from march to august 2021**

Month	Average air temperature(C)			Average relative humidity	Average rainfall (mm)	Average sunshine per day (hrs)
	Maximum	Minimum	Mean			
<b>March</b>	36.0	23.6	29.8	81	145	5.6
<b>April</b>	35.0	22.0	29.3	86	291	3.9
<b>May</b>	36.5	24.1	30.3	85	284	3.7
<b>June</b>	35	23.2	29.1	85	254	4.2
<b>July</b>	32	21.5	26.75	82	235	5.5
<b>August</b>	29	19.4	22.95	78	185	6.9

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

**Appendix II: Analysis of variance on different mycelium running rate, days required from opening to primordial initiation and days required from primordial initiation to harvest with the effect of substrate amount and mother culture on Reishi mushroom.**

Sources of variation	Degrees of freedom	Mean square value		
		Mycelium running rate	Days required from opening to primordial initiation	Days required from primordial initiation to harvest
<b>Substrate amount (A)</b>	5	0.01538*	89.88**	28.614**
<b>Mother culture (B)</b>	2	0.0184*	8.77*	22.842*
<b>Interaction (A x B)</b>	10	0.0096	10.377**	25.142**
<b>Error</b>	90	0.00523	2.824	5.503

\*\* Significant at 1% level

\* Significant at 5% level

**Appendix III: Analysis of variance on no. of fruiting body per packet and weight of individual fruiting body per packet with the effect of substrate amount and mother culture on Reishi mushroom**

Sources of variation	Degrees of freedom	Mean square value	
		No. of fruiting body per packet	Weight of individual fruiting body per packet (g)
<b>Substrate amount (A)</b>	5	166.053**	3.93**
<b>Mother culture (B)</b>	2	21.231*	0.320*
<b>Interaction (A x B)</b>	10	12.253*	0.379*
<b>Error</b>	90	6.179	0.169

\*\* Significant at 1% level

\* Significant at 5% level

**Appendix IV: Analysis of variance on length of stipe, diameter of stipe, diameter of pileus, thickness of pileus with the effect of substrate amount and mother culture of Reishi mushroom**

Sources of Variation	Degrees of freedom	Mean square value			
		Length of stipe (cm)	Diameter of stipe (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
<b>Substrate amount (A)</b>	5	1.208**	0.393**	29.746**	1.311**
<b>Mother culture (B)</b>	2	1.011*	0.681**	1.345*	0.171*
<b>Interaction (A x B)</b>	10	0.664*	0.113*	1.177*	0.111*
<b>Error</b>	90	0.237	0.055	0.495	0.054

\*\* Significant at 1% level

\* Significant at 5% level

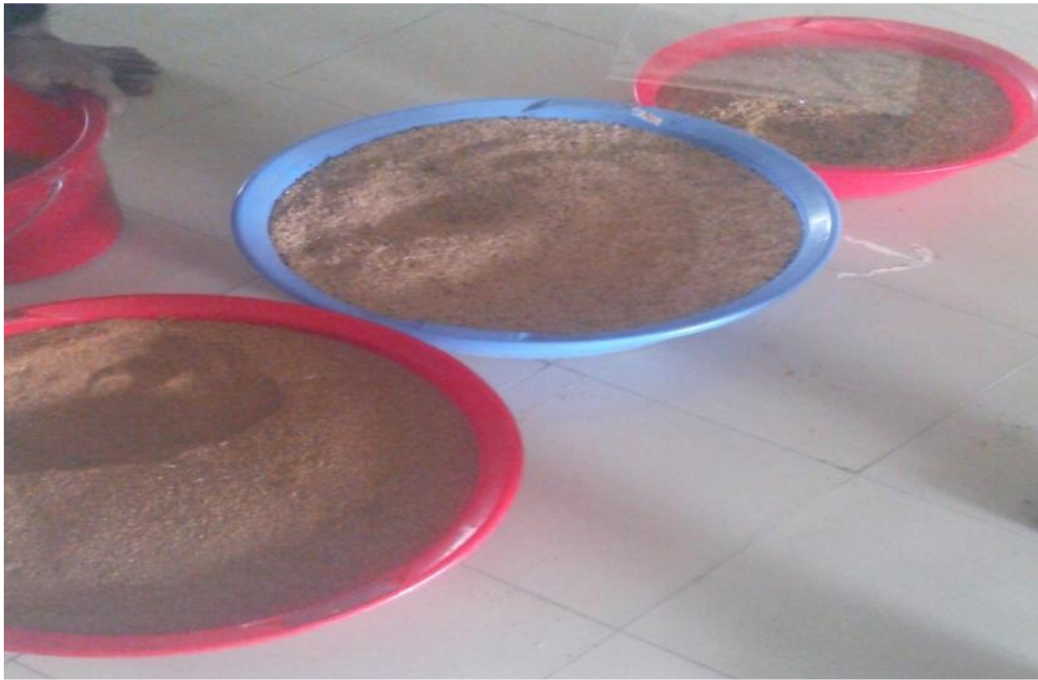
**Appendix V: Analysis of variance on biological, economical, dry yield with the effect of substrate amount and mother culture of Reishi mushroom**

Sources of variation	Degrees of freedom	Mean square value		
		Biological yield (g)	Economic yield (g)	Dry yield (g)
<b>Substrate amount (A)</b>	5	2263.37**	2566.722**	952.436**
<b>Mother culture (B)</b>	2	57.590*	100.611*	32.413*
<b>Interaction (A x B)</b>	10	42.107*	44.5050*	21.338*
<b>Error</b>	90	30.140	22.423	7.012

\*\* Significant at 1% level

\* Significant at 5% level

## PLATES



**Plate 1: Preparation of substrate**



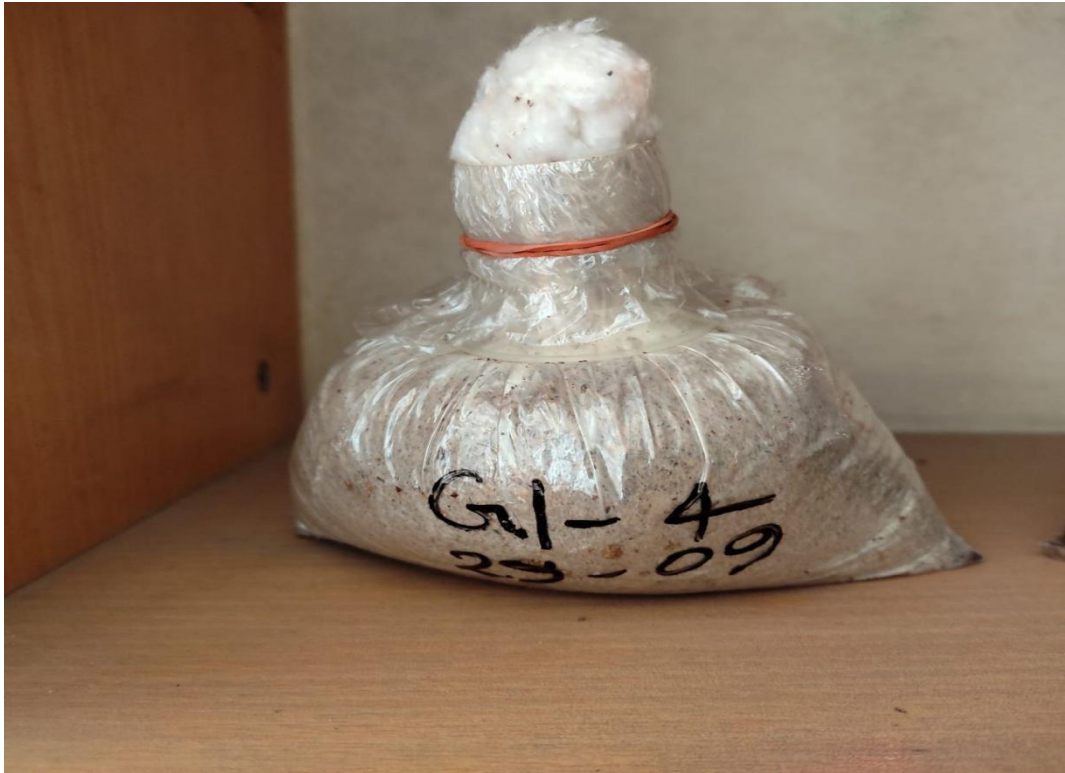
**Plate 2: Preparation of spawn packet**



**Plate 3: Prepared Spawn Packets**



**Plate 4: Prepared packet in mushroom house**



**Plate 5: Mushroom mother culture packet**



**Plate 6: Fully matured mushroom for harvest**



**Plate 7: Measuring weight of harvested mushroom**



**Plate 8: Dried Reishi mushroom**