UNIVERSITI PUTRA MALAYSIA

CULTURING CONDITION FOR PRODUCTION OF GANODERMA LUCIDUM (LEYSS.: FR.) KARST

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CULTURING CONDITION FOR PRODUCTION OF *GANODERMA LUCIDUM* (LEYSS.: FR.) KARST

By

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A Project Report Submitted In Partial Fulfillment of the Requirement for the
Bachelor of Science Bioindustry In The
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Campus

2007

TO MY BELOVED FAMILY AND FRIENDS

ABSTRACT

Growth and production of the Ling zhi mushroom, *Ganoderma lucidum* on several agricultural organic wastes such as oil palm mesocarp fiber (MF), rubber wood chips (RWC), mix wood sawdust (MWS) and shredded newspaper (SN), in the laboratory were evaluated. The MF substrate gave the fastest spawn run (15 days), shortest time to primordia formation (26 days), best fructification and highest biological efficiency (5.58%) when compared to all the other substrates. Addition of nutrient supplementations such as paddy bran (PB) and urea (U) further enhanced the growth and production on all substrates. The best combination was MF + 10% PB + 0.01% U. Evaluation of grains such as barley, wheat, soybean, paddy seeds and mungbean for spawn production were also done. Barley produced the fastest mycelia growth while wheat was found to be the most suitable grain substrate for spawn production. Chemical analysis of the fruiting bodies indicated the presence of quantities of Ca (113.52 - 385.43 µg/g), Mg (1142.94 - 2120.28 µg/g), Mn(12.64 - 21.65 µg/g) and Zn (62.86 - 122.55 µg/g), and negligible or trace quantities of heavy metals such as Pb (< 4.34 µg/g), Cd (< 2.19 µg/g) and Cu (< 3.52 µg/g). Problem encountered during the cultivation of *G. lucidum* include infestation by common pests such as phorid flies and mites, and the green mold disease cause by *Trichoderma* sp.

ABSTRAK

Pertumbuhan dan pengeluaran cendawan Ling Zhi, *Ganoderma lucidium* dengan bahan buangan organik seperti gentian mesokarp kelapa sawit (MF), serpihan kayu getah (RWC), campuran habuk kayu (MWS) dan cerbisan suratkhabar (SN) dikaji di dalam makmal. Substrat MF memberikan masa larian miselia yang paling pantas (15 hari), masa tersingkat untuk pembentukan primodia (26 hari), pembuahan terbaik dan kecekapan biologi tertinggi (5.58%) apabila dibandingkan dengan substrat lain. Penambahan bahan tambahan seperti habuk padi (PB) dan urea (U) akan meningkatkan pertumbuhan dan pengeluaran pada semua substrat. Kombinasi terbaik adalah MF + 10% PB + 0.01% U. Kajian terhadap bijirin seperti barli, gandum, kacang soya, benih padi, dan kacang hijau untuk pembentukan miselia juga dilakukan. Barli menunjukkan pertumbuhan miselia terpantas manakala gandum didapati substrat paling sesuai untuk pembentukan miselia. Analisis kimia bagi pembuahan kulat menunjukkan terdapat sedikit kuantiti Ca (113.52 - 385.43 μg/g), Mg (1142.94 - 2120.28 μg/g), Mn(12.64 - 21.65 μg/g) dan Zn (62.86 - 122.55 μg/g). Terdapat kuantiti logam berat yang sedikit iaitu Pb (< 4.34 μg/g), Cd (< 2.19 μg/g) dan Cu (< 3.52 μg/g). Masalah yang seringkali wujud semasa kultivasi *G. lucidium* merangkumi perosak umum seperti lalat phorid, hamama dan penyakit kulat hijau yang disebabkan oleh *Trichoderma* sp.

ACKNOWLEDGEMENTS

In the preparation of this final year project, the writer received help in a number of ways. A sincere note of thanks is due to a number of people who have assisted in the successful completion of this project.

I would like to give a special sincere gratitude to my supervisor, Associate Professor Dr. Joseph Bong Choon Fah for his advice and guidance through out this project. This gratitude also extends to UPM, Serdang for Associate Professor Dr. Tan Yee How for his mushroom strain - *Ganoderma lucidum* (G 902) and invaluable advice.

My sincere appreciation to all lecturers and staff at the Department of Crop Science and my laboratory mates from Entomology and Pathology who have assisted and given concern to me through out this project.

Finally, I would like thank my family members and friends who have supported and shared the happiness or sadness along this critical period.

I certify that this research project report entitled "Culturing Condition For Production Of *Ganoderma Lucidum* (Leyss.: Fr.) Karst ." has been examined and approved as a partial fulfillment of the requirement for degree of Bachelor of Science Bioindustry in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

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Date: 4/5/07

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LIST OF ABBREVIATIONS

AAS	: Atomic Absorption Spectrophotometer
AI	: Adequate Intake
ANOVA	: Analysis Of Variance
Ca	: Calcium
$CaCO_3$	: Calcium Carbonate (Lime)
$CaSO_4$	: Calcium Sulphate (Gypsum)
Cd	: Cadmium
Cu	: Copper
Cm	: Centimeter
CRD	: Completely Randomize Design
DF	: Degree Of Freedom
DNMRT	: Duncan's New Multiple Range Test
ESADDI	: Estimated Safe and Adequate Daily Dietary Intake
FAO	: Food and Agriculture Organization
FNB	: Food and Nutrition Board
g	: Gram
G. lucidum	: *Ganoderma Lucidum*
kg	: Kilogram
MF	: Oil Palm Mesocarp Fiber
Mg	: Magnesium
ml	: Milliliter
mm	: Millimeter
Mn	: Manganese
MWS	: Mix Wood Sawdust
MSE	: Mean Square Error
PB	: Paddy Bran
PDA	: Potato Dextrose Agar
pp	: Polypropylene
ppm	: Parts Per Million
Pb	: Lead
Pr	: Probability
p.s.i	: Pounds Per Square Inch
PVC	: Polyvinyl Chloride
R^2	: Goodness of Fit
RDA	: Recommended Dietary Allowance
Rep.	: Replicate
RWC	: Rubber Wood Chips
SAS	: Statistical Analysis System
SD	: Standard Deviation
SN	: Shredded Newspaper
Sp.	: Species
Sq.	: Square
SS	: Sum of Square
U	: Urea

US	: United State
w/w	: Weight by Weight
WHO	: World Health Organization
Zn	: Zinc
%	: Percentage
^{o}C	: Degree Celsius
µg	: Microgram
µm	: Micrometer
<	: Less Than
>	: More Than
=	: Equation

CHAPTER I

INTRODUCTION

Background

Ganoderma lucidum (Leyss.: Fr.) Karst, is a basidiomycete belonging to the family Polyporaceae (Stamets, 1993). It is also known as Reishi (divine or spiritual mushroom); Mannentake (10,000-Year Mushroom); Sarunouchitake ("Monkey's Seat") or Saiwai-take (Good-Fortune Mushroom) from the Japanese; Ling Chi, Ling Chih or Ling Zhi as mushroom of immortality from the Chinese (Chan *et al.*, 2005) and Youngzi from the Korean (Mayzumi *et al.*, 1997).

Ganoderma lucidum have been widely used as a traditional medicine in China for more than 2000 years. The basidiocarp (fruiting body) of *G. lucidum* produces polysaccharides and terpenoids, which might be responsible for some of the medicinal properties which can be used to treat ailments such as hepatopathy, chronic hepatitis, nephritis, hypertension, hyperlipemia, arthritis, neurasthenia, insomnia, bronchitis, asthma, gastric ulcer, arteriosclerosis, leukopenia, diabetes, anorexia and cancer (Wagner *et al.*, 2003).

Artificial cultivation of this mushroom was successfully done to replace the wild *Ganoderma lucidum.* This involves the preparation of the pure culture, spawn and growth substrates for mushroom cultivation. The most common method of commercial production is the use of sawdust in sterilized polypropylene (pp) bag. Mycelia from the spawn will spread through the substrate and fully colonize it. During the mycelium

development enzymes will be secreted into the substrate and break down the food sources to become soluble organic compounds to be used by the hyphae for growth of the fungus. Fruiting bodies will be produced a few weeks after mycelia fully colonize the sawdust in the pp bags.

The control of environmental factors is important for the fructification stage of mushroom. Normally the optimum temperature for fructification will be lower than the mycelia growth stage (Kurtzman, 1979). The supply of carbon dioxide and oxygen will affect the morphogenesis of the mushroom. Lighting assists the fruiting stage. Suitable relative humidity both in substrates and air are important during the cultivation. The pH of the substrates is normally adjusted by the adding lime. Nutrients are obtained from the break down of lignin, protein, cellulose and hemicelluloses in the substrates.

The importance of *Ganoderma lucidum* as a health food has made it a potential commercial crop for the local industry. The ability of *G. lucidum* to be grown on several substrates has made it a rather versatile fungus to be cultivated. Recently with the rapid development of the oil palm industry in Sarawak, a lot of agricultural waste such as empty fruit bunches and oil palm mesocarp fibers are being produced by the mill. This study was carried out to evaluate the use of some of these agricultural wastes for the production of *G. lucidum.* Since *G. lucidum* or Ling Zhi is a much sought after health food, there is a need to find local suitable substrates for possible mass production of the mushroom.

Problem Statement

This study was conducted to evaluate the growth and sporulation of *Ganoderma lucidum* on different local substrates.

Objectives

1. To find the most suitable local grain substrates for *Ganoderma lucidum* spawn production.
2. To evaluated the effect of different local substrates on the growth and sporulation of *Ganoderma lucidum*.
3. To determine the mineral nutrient contents of *Ganoderma lucidum* grown on different substrates.

CHAPTER II

LITERATURE REVIEW

Biology and Uses of *Ganoderma lucidum*

The classification of *Ganoderma lucidum* is as follows:

KINGDOM : Fungi

PHYLUM : Basidiomycota

CLASS : Homobasidiomycetes

ORDER : Polyporales

FAMILY : Ganodermataceae (Polyporaceae)

GENUS : *Ganoderma*

SPECIES : *Ganoderma lucidum*

Ganoderma lucidum is conk or kidney shape-like, woody textured mushroom (basidiocarp), 5 - 20 cm in diameter, long stem (stipe) and a lotus leaf shape of red to reddish brown cap. Spore size for *G. lucidum* is in the range of 13 - 17 μm in length by 7.5 - 10 μm. The color of spore is brown and the shape is oval or dimpled, rounded at the base and narrowly rounded at the top (Stamets, 2000). The spores are produced by the basidia on the underside of the basidiocarp. The airborne haploid spores will germinate when they reach a suitable substrate with the right condition. The mycelia will colonize the new substrate and subsequently produce new basidiocarp with spores.

Various records in both Chinese and Japanese indicated that *Ganoderma lucidum* had been used as a traditional medicine in the orient for more than 2000 years (Stamets, 1993). The basidiocarp of *G. lucidum* was still used to treat conditions like gastric ulcer, chronic hepatitis, nephritis, hypertension, hyperlipemia, arthritis, insomnia, bronchitis, asthma, arteriosclerosis, leukopenia, diabetes, anorexia and cancer (Stamets, 1993). It was also for health invigorating effects, increasing longevity, treatment and resistance of cancer and recovery from diseases.

Ganoderma lucidum, like many of the common mushroom could be grown on suitable substrate. Bong (1987) reported that *G. lucidum, Pleurotus, Volvariella volvaceae, Lentinus edodes and Auricularia* sp. could be cultivated by using agricultural wastes and industrial by-products. These fungi were able to degrade the substrate and released nutrients for growth and sporulation.

Cultivation of *Ganoderma lucidum*

The wild *Ganoderma lucidum* grew in the natural forests and was distributed in temperate, subtropical and tropical regions, especially in China. Because of the limitation in the supply of wild mushrooms and difficulties in the quality control of fruit bodies, artificial cultivation of *G. lucidum* were commercialized where the fungus was grown in a controlled environment. Since the early 1970s, China had applied the method of artificial cultivation of *G. lucidum* and this had led to rapid production of the mushroom. *Ganoderma lucidum* could be cultivated on wood log, short wood segment, tree stump, sawdust bag and pots (Stamets, 2000).

There were two stages in mushroom cultivation. The first stage was the preparation of fungal stock culture and spawn. The second stage was the preparation of growing substrate for mushroom.

Nutritional Requirement

Hayes (1972) reported that primordia formation required the supply of nutrients. The basic growth requirements for most mushrooms were carbon, nitrogen, essential elements and vitamins.

Carbon

Carbon contributed to the structural and energy need of the fungal cells. It was needed for the formation of sugar or other compounds such as lipids, amino acids and protein. Natural plant cell wall which contained the polymers such as polysaccharides, cellulose and hemicelluloses were good carbon source and these can be found in sawdust or others vegetable substrates. The hyphae from the fungus broke down and utilized the plant substrate. Increase in cellulose supply enhanced the supply of carbohydrate in mushroom fruit body formation (Chang and Philip, 2004).

Nitrogen

All fungi required nitrogen for the growth. Three types of nitrogenous compounds, namely protein, glucosamine and nucleic acids were important for development of mushroom growth.

Reishi (*Ganoderma lucidum*) grew on hardwood substrates which could be alternated by the sawdust or wood chips mixture and together with a nitrogen-rich supplement (Hamlyn and Temple, 1997). Ishikawa (1967) reported that the optimum nitrogen concentration for hyphal growth according to the nitrogen sources were 0.03 % ammonium sulfate or 0.06 % ammonium tartrate. However, Tokimoto and Komatsu (1978) reported that high concentration of nitrogen (> 0.02 %) inhibited the formation of fruit body primordial in *Lentinus edodes.*

Essential Elements

Essential elements which were required for mushroom growing were normally in the form of inorganic compounds. These elements were divided into 2 groups namely, the macronutrients of which approximately 10^{-3} mol was needed, and micronutrients (mineral nutrients) of which 10^{-6} mol or less was required. Ishikawa (1967) reported that under suitable concentration of iron, zinc and manganese, the mixture of copper, molybdenum and cobalt will promote the growth in the mushroom, *Lentinus edodes*.

Vitamins and Oils

Vitamins acted as growth factors, sources of energy or structural material of protoplasm during mushroom growth. Wardle *et al.* (1969) reported that the commercial vegetable oils and esters of oleic and linoleic acid would stimulate growth in mushroom, *Agaricus bisporus*. Lehrian *et al.* (1976) reported that the addition of small quantities of sodium acetate or linoleic acid increased the fungal dry matter yield.

Environmental Factor Inducing Mycelia Growth and Fructification

Temperature

The optimum temperature for mycelia growth and fruiting body formation were different for each species of mushroom. Different temperature range was important to promote certain stage of the life cycle in mushroom. The normal temperature range for mushroom growing was about 21 - 30 ^{o}C. The optimum temperature for fruiting normally was lower than the optimum temperature for vegetative growing. According to Flegg and Wood (1985) the optimum temperature for mycelia growth was 24 ^{o}C while that for fruiting body was 22 - 24 ^{o}C. When the temperature was above 28 ^{o}C, the growth rate of mushroom decreased rapidly and death of mycelia occurred at 32 ^{o}C and above (Flegg and Wood, 1985). Ishikawa (1967) reported that the optimum temperature for spawn run was 22 ^{o}C - 28 ^{o}C for *Lentinus edodes*. Tokimoto and Komatsu (1978) reported that the mycelia growth would be inhibited at below 5 ^{o}C and above 35 ^{o}C. According to Stamets (2000), the optimum temperature for incubation and fruiting for *Ganoderma lucidum* was 21 - 27 ^{o}C.

pH of the substrates

The pH levels affected morphological development of mushroom. Most fungi grew at pH 4 - 8. Optimum pH for mycelia developments of *Ganoderma lucidum* was 6 - 7.5 (Stamets, 2000).

Relative Humidity

High humidity was maintained from the spawn run stage until primordia formation for *Ganoderma lucidum,* reaching 90 - 95 % during the fruiting stage (Stamets, 2000).

Light

Kitamoto *et al.* (1972) reported that light was essential for the formation and maturation of reproductive structures in various species of wood rotting basidiomycetes, *Favolus arcularius*. Light was required for the initial fruiting and development in mushroom, *Agaricus*. Light irradiation played a role in fruit body development, especially gill and spore formation. Eger (1978) reported that the quantity, quality, duration and the intensity of light were important during the formation and maturation processes of *Pleurotus* sp. The inhibitory effects of light was apparent when light density was greater than 50 lux during the spawn run and this condition inhibit primordia formation for shiitake mushroom (Ishikawa, 1967). According to Stamets (2000), for *Ganoderma lucidum* light requirement was nil during the spawn run while a 750 - 15000 lux light was needed during fruiting stage. Phototrophic response was reported by San Antonio and Fordyce (1972) whereby at least 15 minutes of full sunlight was required for fruit body initiation and the stalked mushrooms would become long when the light level was low.

Aeration

Many researchers have shown that the stalk of mushrooms would become long when the level of carbon dioxide was high. Kurtzman *et al.* (1982) reported that the

mushroom *Pleurotus* would have elongation and branching of the stipes during the fruiting stage when the concentration of carbon dioxide was high. San Antinio *et al.* (1972) reported the mycelia growth of mushroom was increased when the oxygen concentration there was 0.1 - 0.5 %. In the absence of carbon dioxide there was no mycelia growth (Stamets, 2000). A carbon dioxide concentration of less than 5 % would stimulate stipe elongation and suppress pileus expansion. Stamets (2000) noted that suitable aeration was when concentration of carbon dioxide was > 50,000 ppm during the spawn run stage; 20,000 - 40,000 ppm for primordia (antler) formation stage; 5,000 - 2,000 ppm for primordia (young conk) formation stage and < 2000 ppm for fruiting stage for *Ganoderma lucidum*. Fresh air exchange was required all the time during cultivation.

Efficacy of Grains for Spawn Culture and Production

Spawn was defined as the mycelia of mushroom. The 'seed' or spawn (propagating unit) was used to cultivate the mushroom.

The function of grain spawn was for development and expansion of the mycelia mass. Size of grain affected spawn run because grain was functioning as a nutritional supplement for mycelia. Smaller kernels provided more surface area for inoculation of spawn. Cereal grains were normally used in spawn making. If the grain was dry, growth would be retarded. If the moisture content was high, bacterial contamination would be promoted. The best moisture content was 50 % (Stamets, 2000).

Different types of grains have been used for mushroom spawn production, such as barley for oyster mushroom (Mandeel *et al.,* 2005), and paddy seeds for oyster mushroom (Siti Haijijah and Bong, 1993). Nwanze *et al.* (2005) cultivated *Lentinus squarrosulus* with different spawn grains such as corn, millet and wheat.

Substrates for Mushroom Cultivation

Spawn run and fruiting body could be developed on lignin and cellulose materials such as corn cobs, all cereal straws, paper, wood shavings, sawdust, nutshells and vegetable wastes as well as food industry wastes (Baysal *et al.,* 2003). Mandeel *et al.* (2005) cultivated the oyster mushroom by using untreated organic wastes which included chopped office papers, cardboard, sawdust and plant fibers.

Stamets (2000) formulated spawn substrates from 50 : 50 hardwood sawdust or wood chip mixture incubated in polyethylene space bags. The mixture substrate was soaked for 3 to 4 days in molasses enriched water (50 ml molasses or 20 liters water) before being filled in bag and sterilized for 2 hours at 15 p.s.i. After cooling the bags were inoculated, heat sealed and placed inside a clean incubation room. The bag culture method was first developed in Taiwan with the cultivation of *Auricularia* in early 1970's (FAO, 1990). It was most popular for the cultivation of button mushroom. This was inexpensive, portable and disposable using containers and plastic bags.

Effects of Pests and Diseases on Mycelia Growth and Fructification

Ganoderma lucidum may be attacked by pests and diseases. Keil (2002) reported major insect pests in mushroom cultivation such as sciarid fly, phorid fly, cecid fly and their larvae. Coles and Barber (2002) reported several common fungal pathogens in mushroom cultivation which included *Verticillium*, *Trichoderma harzianum* and *Cladobotryum.* The Asian Shiitake mushroom, *Lentinula edodes* which was cultivated on log faced problem from competing microorganism, especially *Trichoderma* spp. that contaminated the log.

Tibbles *et al.* (2005) studied the behavioural responses of adult female phorid [*Megaselia halterata* (Wood) (Diptera: Phoridae)] and sciarid [*Lycoriella castanescens* (Lengersdorf) (Diptera: Sciaridae)] flies to the commercial white mushroom, *Agaricus bisporus* (Lange) Imbach, grown on a standard pasteurised composted substrate. The result showed that yield of *L. castanescens* was highest from the uncolonised composted substrate, and there was a negative relationship between emergence and the amount of mycelium in the composted substrate. The cultivated mushroom (*Agaricus bisporus*) in Shanghai was infested by mites, *Brennandania lambi*, through contaminated spawn (Wu *et al.,* 2004). Bussaman *et al.* (2006) reported that the mushroom mite, *Luciaphorus* sp. was a serious pest of tropical mushrooms.

The Mineral Nutrients for Human Consumption

Mushrooms have a relatively high nutritional status: the protein content of certain mushroom species is known to be higher than in vegetables apart from spinach and soybean (Alexopoulos *et al.* 1996). They are also a good source of valuable vitamins, such as vitamin D (Mattila *et al.* 2000), and several essential minerals (Souci *et al.* 1981, Alexopoulos *et al.* 1996). Mineral nutrients are important for human body to maintain the daily function (WHO, 1996). There are divided into macro elements and micro elements. Macro elements such as sodium, potassium, magnesium, calcium and phosphorus are required by human body in amounts more than 100 mg/day. Micro elements such as iron, copper, zinc and manganese are required in amounts less than 100 mg/day (Murray *et al.,* 2000).

Mineral nutrients essential for life generally occur in the body in microgram per gram of tissue. Our body require in amounts of milligrams per day for copper, Iron, manganese, zinc, manganese and boron. On the Recommended Dietary Allowance (RDA), Estimated Safe and Adequate Daily Dietary Intake (ESADDI) or Adequate Intake (AI) from the US Food and Nutrition Board, FNB (1986) recommended the daily dietary intake of mineral nutrients for human to avoid excessive intake that would cause malabsorption and excessive excretion for human.

ESADDI for copper (Cu) daily intake from the normal adult diet is between 1.5 - 3 mg and Manganese (Mn) is 2 - 5 mg. RDA for Zinc (Zn) daily intake from the normal adult diet is between 12 - 15 mg; Magnesium (Mg) is 2 - 5 mg and Calcium (Ca) is 800 -

1200 mg (pregnant and young adult). Based on the provisional tolerable weekly Intake (PTWI) value last set by the joint FAO/WHO Expert Committee for Additives and Contaminants (JECFA), the heavy metal such as Cadmium (Cd) is 0.057 - 0.071 mg/kg and Lead (Pb) is less than 0.025 mg/kg of body weight.

Kalac *et al.* (2000) reported that most of the wild mushroom species were able to accumulated high concentration of cadmium, mercury, lead and copper in fruiting bodies. There would be higher metal concentrations in younger fruiting bodies due to the transport of a metal from mycelium to the fruiting body during the start of fructification. Most of the elements were distributed unevenly within a fruiting body. The highest levels were observed in the sporophore (but not in spores), less in the rest of the cap and the lowest in stipe.

There were a few studies on mushroom uptake of mineral nutrients. Racz *et al.* (1996) reported that the cultivated *G. lucidum* contained 0.01-0.1μg/g for Cd, 1 - 5 μg/g for Pb, 10 - 120 μg/g for Mn, Cu and Zn. Akindahunsi and Oyetayo (2005) reported that the *Pleurotus* contained 1.2 - 2.9 mg/g of Ca, 0.02 - 0.04 mg/g of Mg, 0.002 - 0.003 mg/g of Cu, 0.02 - 0.05 mg/g of Zn. Sesli *et al.* (1999) reported that *Lactarius piperatus* contained 0.75 mg/g of Cd, *Pleurotus ostreatus* contained 0.17 mg/g of Pb and *Agaricus bisporus* contained 3.61 mg/g of Mn. The concentrations of mineral nutrient in mushroom could be reduced during the preservation process of drying, freezing or sterilization, and with different culinary treatments (Kalac *et al.*, 2004).

CHAPTER III

METHODOLOGY

Source of Culture

The stock culture of *Ganoderma lucidum* strain 902 was originally obtained from Prof Madya Dr Tan Yee How of the Plant Protection Department, Faculty of Agriculture, Universiti Putra Malaysia, Serdang. This culture was maintained on PDA until use.

Source of Grain for Spawn Production

Five types of grains were used in the experiment (Figure 3.1). These were barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), soybean (*Glycine max*), paddy seed (*Oryza sativa*) and mungbean (*Phaseolus aureus*). These grains were obtained from a local grocery shop.

Source of Substrates

Four types of substrate were used in the experiment (Figure 3.2). These were shredded newspaper, rubber wood (*Hevea brasiliensis*) chips, mix wood sawdust and oil palm mesocarp fiber. The fresh rubber tree trunks where collected from the campus rubber garden and converted into wood chips by using a wood chipping machine. The newspapers were collected locally on campus and shredded manually before use. Mix wood sawdust was obtained from a local sawmill. The oil palm mesocarp fiber was obtained from Suburmas Palm Oil Mill in Bintulu.

Figure 3.1: Type of grains for spawn production.

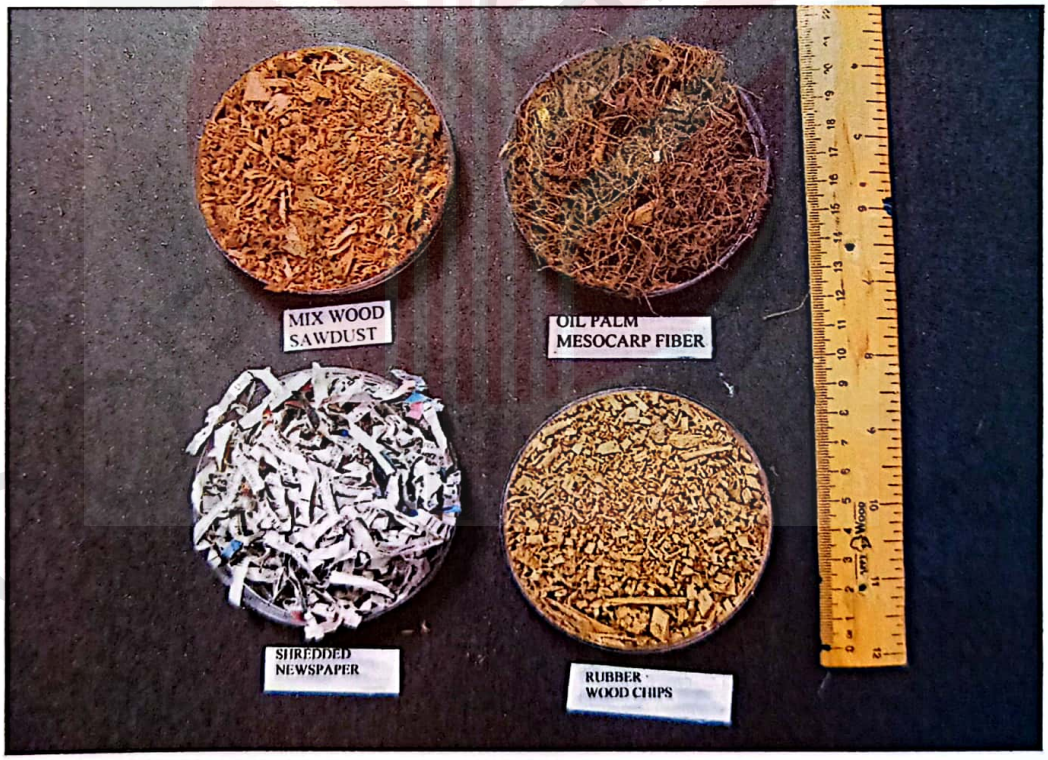

Figure 3.2: The four types of substrates used for *Ganoderma lucidum* production.

Containers and Supplements for Experiments

The containers and supplements use for the experiment were as follow:

a. Polypropylene (pp) bags with size 9 cm x 38 cm x 0.04 cm.

b. Necks (4 cm x 4 cm) for the pp bags made from cutting the PVC pipe.

c. Cap for the pp bags were replaced by using cotton wool and aluminum foil.

d. Calcium carbonate and calcium sulphate.

e. Paddy bran was obtained from a local rice mill.

Preparation of *Ganoderma lucidum* Culture

The most common agar medium, which is potato dextrose agar (PDA) at 39 gram per liter of distilled water, was used to grow the *Ganoderma lucidum* culture. The PDA was dissolved in hot distilled water before being autoclaved at 121 ^{o}C and 15 p.s.i. The cooling autoclaved PDA was poured into the petri dishes at 12 – 15 ml per dish and left to solidity in a laminar flow chamber before being inoculated.

Spawning and Growth Rate Assessment

Measurement of spawn run was done to compare the growth of mycelia on five types of grains and four types of substrates.

Visual Observation on the Denseness of Mycelium

Denseness of mycelium growth was observed during the spawn run on grains and substrates. Scoring system was:

1 Very dense

2 Dense

3 Moderately dense

4 Thin mycelia

5 Sparse

Spawn Run on Different Types of Grains

Grain Preparation

Cereal grains were used for preparation of spawn (FAO, 1990). Grains were washed and pre-wetted by boiling it in water to increase the moisture content from 10 % to 40 % by weight. Boiling time for each grain varied and was shown in table 3.1.

Table 3.1: Boiling time for five types of grains for spawn run experiment.

Grains	Boiling time (minutes)
Barley	15
Wheat	40
Paddy Seed	60
Soya Bean	60
Mungbean	45

After boiling, the grains were dried and cooled. Lime ($CaCO_3$), gypsum ($CaSO_4$) and paddy bran were added in the ration of 1:1:3. Each type of grain was filled to ¾ full in scottle bottle (500 ml) and covered with cotton wool and aluminum foil with five replications per treatment (Figure 3.3). The grains were then autoclaved for 1 hour at 121 ^oC and 15 p.s.i (FAO, 1990). This was to kill the thermophilic bacteria, which will cause the souring of cooked grain. The bottles were then allowed to cool down before being inoculated with *Ganoderma lucidum* culture.

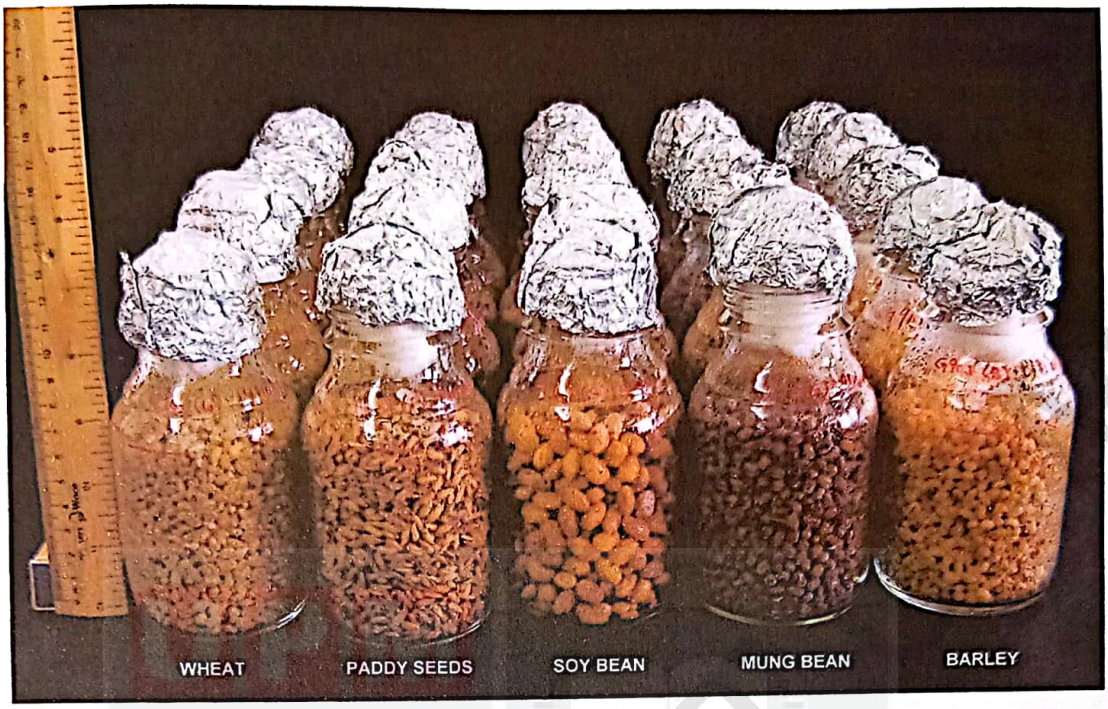

Figure 3.3: The five types of grains in culture bottles.

Inoculation and Spawn Run

Petri dish culture of *Ganoderma lucidum* of less than 2 weeks old was used. A metal bore was used to cut 8 mm mycelia disc from the culture. Inoculation of the grain was done aseptically by placing one mycelia disc in each bottle.

The bottle were arranged in a completely randomized design (CRD) and incubated at 25 - 27 ^{o}C under a light regime of 14:10 day light. The data was analyzed by ANOVA. Spawn run for each grain was monitored for mycelia growth and denseness and contaminating agents such as yeast, bacterial, mites and others agents.

Substrate Preparation

Substrates were mixed with lime ($CaCO_3$), paddy bran and water. Water was added into the substrate until 60 – 70 % moisture. The wetness was determined by pressing a handful of the mixture. If no water ran off between the fingers and the form of the mass remained unchanged after pressure was released, the mixture contained more or less 45 - 60 % of moisture (FAO, 1990).

There were three treatments based on varying combinations of supplement ingredients for this experiment to test the efficiency of spawn run with twelve replications per treatment. The composition for each treatment is given below (table 3.2):

Table 3.2: Concentration of supplementation for three different treatments.

Supplements	Supplement ingredients for substrate		
	A	B	C
Lime	2 %	2 %	2 %
Water	60-70 %	60-70 %	60-70 %
Paddy Bran	5 %	10 %	10 %
Urea	0	0	0.01 %

Each pp bag was filled with 400 g of substrate. The substrate was compacted by pressing to reach a height of about 120 - 130 mm (Figure 3.4). A PVC ring was pushed through the opening of each bag with the protruding plastic turned over the ring to allow a standard size opening for each bag. Each bag was then covered with cotton wool and aluminum foil. Materials were then autoclaved for 1 hour at 121°C and 15 p.s.i. (FAO, 1990), and later cooled to room temperature before inoculation.

Figure 3.4: Four types of substrates to compare spawn run and denseness of mycelia. From left: mix wood sawdust, rubber wood chips, shredded newspaper and oil palm mesocarp fiber.

Inoculation of Substrate Bags and Incubation

The experiments were done to get the most efficient cultivation media and supplements for production of *G. lucidum*. There were compared among four types of substrates and three different concentrations of supplements for *G. lucidum* cultivation at 24 - 27 ^{o}C.

Spawn of less than 2 weeks old with thick mycelia growth were selected and aseptically transferred into the substrate bags. An inoculation rate of 3 - 5 % w/w spawn per substrate bag was used. The inoculated bags were then incubated at 24 - 27 ^{o}C and 80 % relative humidity in a clean room. A few open containers with clean water were placed in the room plus spraying water two times per day to maintain the high humidity with twelve hours daylight and ample aeration.

Cumulative vertical growth of the mycelia was measured every three days until spawn run was complete. A split plot design was used for this experiment and the results were analyzed by ANOVA using SAS. Treatment means were separated by DNMRT at P = 0.05.

Fructification

The covers for fruiting bags were opened when spawn run was complete with the substrate fully colonized. High humidity was maintained during the fructification period.

The day and number of primodium initiation from fruiting bags was recorded. The biological efficiency and related data were recorded. Biological efficiency referred to ratio of mushroom's fresh weight to the dry weight of substrates used. A Completely Randomized Design (CRD) was used for this experiment and the results were analyzed by ANOVA using SAS. Treatment means were separated by DNMRT at P = 0.05.

Method of Sample Preparation for Atomic Absorption Spectrophotometer (AAS)

The *Ganoderma lucidum* fruit body was cleaned and dried in an oven at 40 ^{o}C for 24 hours. The sample of dried mushroom was grinded into a powdered form. A 0.2 g sample of mushroom was placed into a porcelain crucible and ashed in a furnace at 500 ^{o}C for 5 - 8 hours until a white or grey ash residue was obtained. The ash residue was then wet digested with 6 ml of concentrated nitric acid in closed fume cup board. The digested samples were further diluted to 10ml using distilled water (Falandysz *et al.*, 2001).

Analysis by Atomic Absorption Spectrophotometer (AAS)

The digested samples were analyzed using the AAS. Detection limit values of elements as part per million (ppm) in flame AAS were found to be 6.0 for Ca, 0.6 for Mg, 3.0 for Mn, 3.0 for Cu, 5.0 for Zn, 5.0 for Pb and 3.0 for Cd. A Completely Randomized Design was used for this experiment and the results were analyzed by ANOVA using SAS. Treatment means were separated by DNMRT at $P = 0.05$.

CHAPTER IV

RESULTS

Effect of Selected Grains on Mycelia Growth for Spawn Production

The daily growth rate of mycelia was measured to compare the spawn run efficiency among the five types of grains (Figure 4.1 and 4.2). Table 4.1 showed the daily growth rate (mm) and mycelial denseness scoring (Figure 4.1) of *G. lucidum* on five types of grains incubated in the dark at 24 - 27 ^{o}C.

There were significant differences in mycelia growth among the five types of grain used for spawn production. Barley exhibited the fastest mycelia growth and completed the spawn run in 12 days. This was followed by the wheat and soybean where the mycelial took 15 days to fully colonize the grains. The paddy seeds were fully colonized in 18 days while the slowest was mungbean which took 27 days to complete the spawn run. In terms of denseness of the mycelia, barley, wheat and soybean had dense mycelia growth while that of paddy seeds and mungbean was thin to sparse.

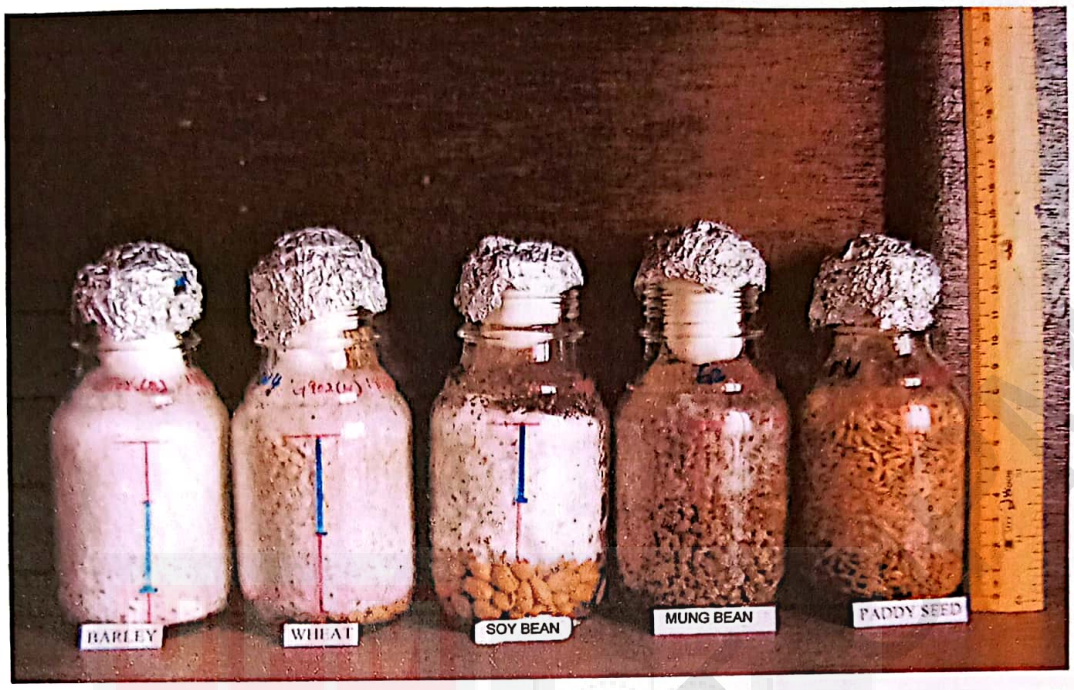

Figure 4.1: Spawn run in different grain substrates. From (left): barley, wheat, soybean, mungbean and paddy seeds.

Table 4.1: Days required to fully colonize the substrate, means of vertical growth rate (mm) and mycelia denseness scoring of *Ganoderma lucidum* on five types of grains incubated in the dark at 24 - 27°C.

Grains	Fully colonized (days)	Mean of vertical growth rate (mm) [1]	Dense Scoring [2]
Barley	12	8.44 **a**	1
Wheat	15	6.31 **b**	2
Paddy Seeds	18	5.28 **c**	5
Soybean	15	6.36 **b**	1
Mungbean	27	3.23 **d**	5

[1] Means with the similar letters in the same column are not significantly different at P = 0.05 by Duncan's New Multiple Range Test (DNMRT).

[2] Scoring of mycelia denseness are:
1 = very dense mycelia; 2 = dense mycelia; 3 = moderately dense; 4= thin mycelia and 5 = sparse.

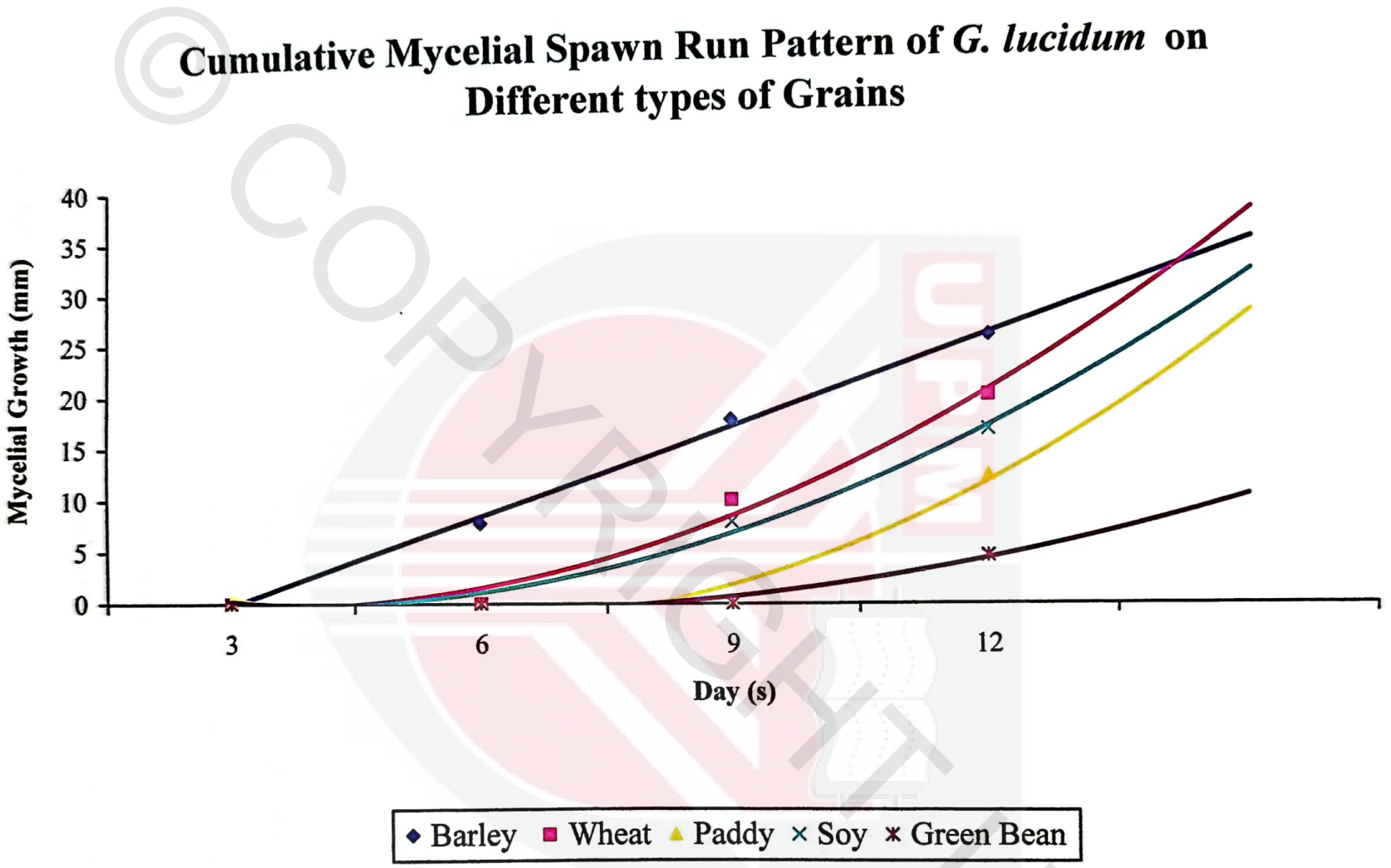

Figure 4.2: Mycelia spawn run of *Ganoderma lucidum* in five types of grain.

Table 4.2 showed the regression equation for cumulative vertical mycelia spawn run of *Ganoderma lucidum* on five types of grains. Barley showed the highest R^2 for the best fit polynomial line to the original data plot. The result was followed by the soybean, wheat, paddy seeds and mungbean in descending order.

Table 4.2: Regression equations for mycelia spawn run of *Ganoderma lucidum* on five types of grains.

Treatments	Regression Equation	R^2
Barley	$Y = 0.0014 X^2 + 8.6267 X - 8.8444$	0.9975
Wheat	$Y = 2.4444 X^2 - 5.28 X + 2.3222$	0.9805
Soybean	$Y = 2.1611 X^2 - 5.0478 X + 2.5278$	0.9862
Paddy Seeds	$Y = 3.0611 X^2 - 11.632 X + 9.1833$	0.9333
Mungbean	$Y = 1.1389 X^2 - 4.3278 X + 3.4167$	0.9333

Mycelia Growth Response of *Ganoderma lucidum* to Supplementations on Different Substrates

The results of mycelia growth response to supplementation on different substrates were presented in Tables 4.3 and 4.4. Colonization was completed within 15 to 24 days in all treatments. Among the substrates and supplementations, oil palm mesocarp fiber (MF) with 10 % paddy bran (PB) and 0.01 % urea (U) had the fastest colonization in 15 days (Table 4.3) and the best daily growth rate of 8.94 mm per day (Table 4.4). This was followed by mixed wood sawdust (MWS) and shredded newspaper (SN), with rubber wood chips (RWC) taking the longest time to be fully colonized as well as showing the slowest growth rate. When only supplementation was added, *G. lucidum* grew best with 10 % PB plus 0.01 % U, followed by the 10% PB and 5 % PB. The results also indicated that *G. lucidum* grew better with increasing quantity of PB and that addition of urea further enhanced the growth.

Table 4.3: Days to full colonization of different substrates with varying concentrations of supplements.

Media [1]	Days to full colonization of substrate [2]		
	5% PB	10% PB	10% PB + 0.01% U
SN	24 **a**	21 **b**	18 **b**
RWC	24 **a**	21 **b**	21 **b**
MWS	21 **b**	21 **b**	18 **c**
MF	18 **c**	18 **c**	15 **d**

[1] MF = Oil palm mesocarp fiber; MWS = Mix wood sawdust; RWC = Rubber wood; SN = Shredded newspapers; PB = Paddy bran and U = Urea.

[2] Means with the similar letters in the same column are not significantly different at P = 0.05 by Duncan's New Multiple Range Test (DNMRT).

Table 4.4: Effects of supplementations on different substrates to daily mycelia growth of *Ganoderma lucidum*.

Media [1]	Means daily mycelia growth (mm) [2]		
	5 % PB	10 % PB	10 % PB + 0.01 % U
SN	5.28 **e**	5.8 **cd**	7.28 **a**
RWC	5.17 **i**	5.94 **c**	6.33 **b**
MWS	5.84 **cd**	6.63 **h**	7.93 **g**
MF	6.37 **b**	7.18 **a**	8.94 **f**

[1] MF = Oil palm mesocarp fiber; MWS = Mix wood sawdust; RWC = Rubber wood; SN = Shredded newspapers; PB = Paddy bran and U = Urea.

[2] Means with the similar letters are not significantly different at P = 0.05 by Duncan's New Multiple Range Test (DNMRT).

Fructification

The primordia began to emerge from the production bags after completion of spawn run (Figure 4.3). This was followed by the elongation of the stipe (Figures 4.4 and 4.5) and formation of the fruiting bodies (Figures 4.6). Table 4.5 showed the results of primordia formation, number of primordia after the cover was removed, weight of fruit bodies per bag and biological efficiency of *Ganoderma lucidum* on different substrates.

Figure 4.3: Formation of primordia from production bags.

Figure 4.4: Fructification of *Ganoderma lucidum* from SN + 10 % PB and 0.01 % U after 87 days primordia formation.

'igure 4.5: Fructification of *Ganoderma lucidum* from MF + 10 % PB + 0.01 % U after 87 days primordia formation.

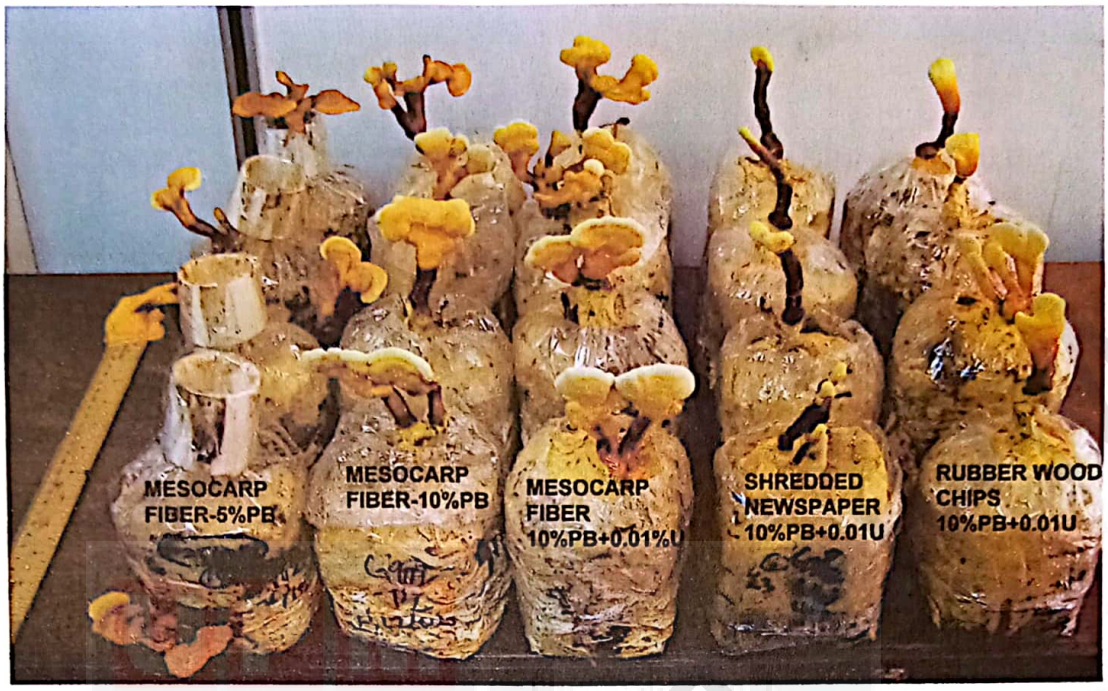

Figure 4.6: Fructification of *Ganoderma lucidum* on different substrates at 133 days (MF + 5 % PB), 88 days (MF + 10 % PB) and 87 days (MF, SN and RWC, + 10 % PB + 0.01 % U).

Oil palm mesocarp fiber gave good results especially when 10 % PB + 0.01 % U were added with the fastest primordia formation (27.7 days), highest number of primordia formed (2.2), most number of fruits bodies (1.3), the heaviest fresh weight (13.942 g) and the best biological efficiency for any composition of supplements when compared to other substrates (Table 4.5).

Table 4.5: Days to primordia formation, number of primordia formation and the biological efficiency of *Ganoderma lucidum* on different substrates.

Substrates with supplements [1]	Means [2]				
	Days to Primordia formation	Number of primordia	Number of fruiting bodies	Fresh weight (g)	Biological efficiency (%)
MF + 5 % PB	36.5 **a**	1.3 **a**	1.2 **a**	13.89 **a**	5.56 **a**
MF + 10 % PB	35.3 **a**	1.3 **a**	1.2 **a**	12.45 **a**	4.98 **ba**
MF + 10 % PB + 0.01 % U	27.7 **b**	2.2 **b**	1.3 **a**	13.94 **a**	5.58 **a**
SN + 10 % PB + 0.01 % U	31.7 **c**	2.2 **b**	1.3**a**	6.04 **b**	3.62 **b**
RWC + 10% PB + 0.01 % U	31.3 **c**	0.7 **c**	0.7 **a**	4.82 **b**	1.93 **c**

[1] MF = Oil palm mesocarp fiber; SN = Shredded newspaper; RWC = Rubber wood sawdust; PB = Paddy bran; U = Urea.

[2] Means with the similar letters in the same columns are not significantly at P = 0.05 by Duncan's New Multiple Range Test (DNMRT).

Pest Infestation and Fungal Contamination

Some pest infestations occurred during the incubation and fructification period of *Ganoderma lucidum.* The major pests were larvae of the phorid fly (Figure 4.7, 4.8 and 4.9), mites (Figure 4.10 and 4.11) and *Trichoderma* sp. (Figure 4.12 and 4.13).

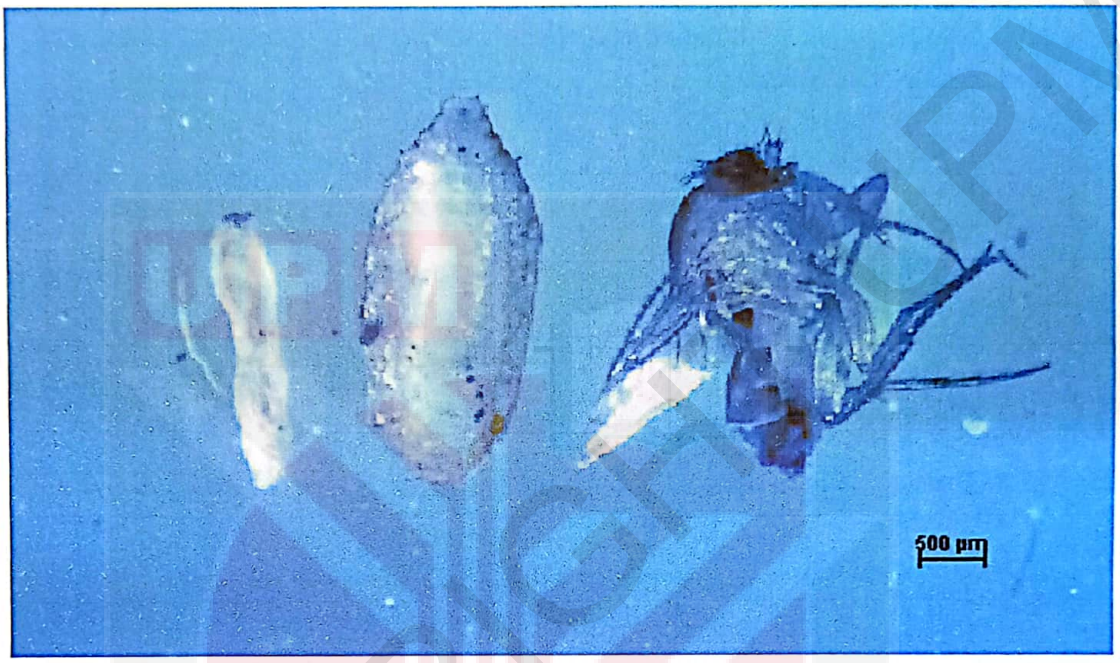

Figure 4.7: Phorid fly, from left: larvae, pupa and adult fly.

Figure 4.8: Phorid fly's larvae found in shredded newspaper with barleys spawn.

Figure 4.9: Phorid fly's larvae found in oil palm mesocarp fiber substrate.

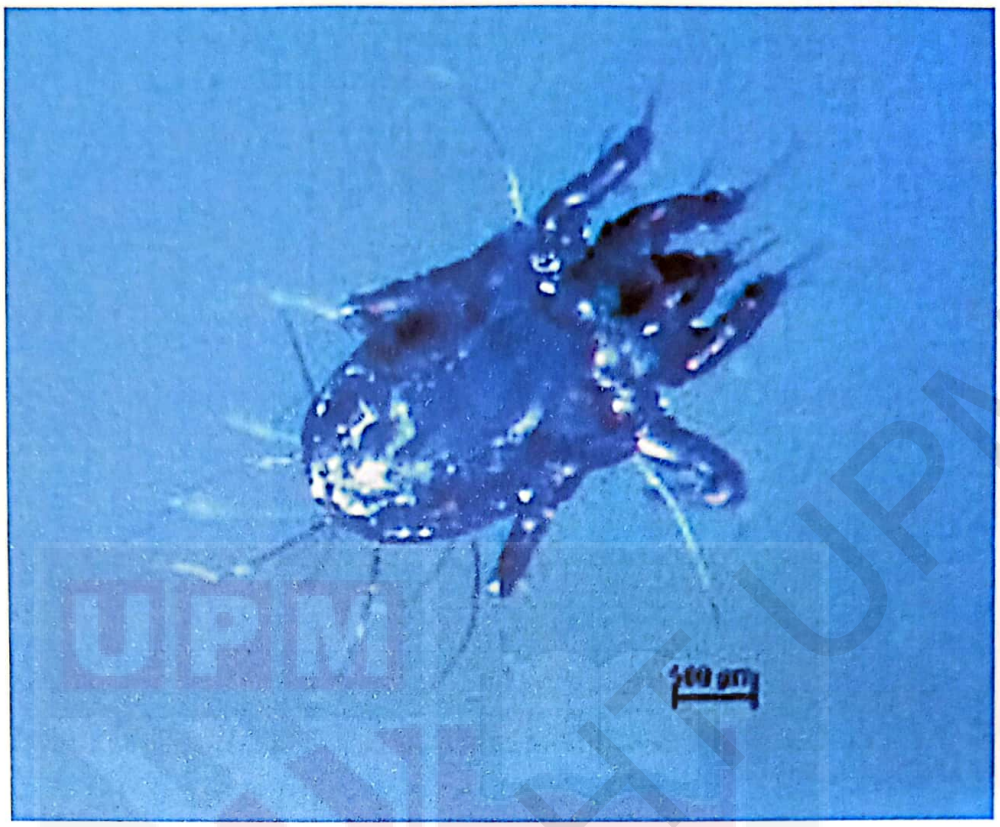

Figure 4.10: Mite from fully colonized production bags.

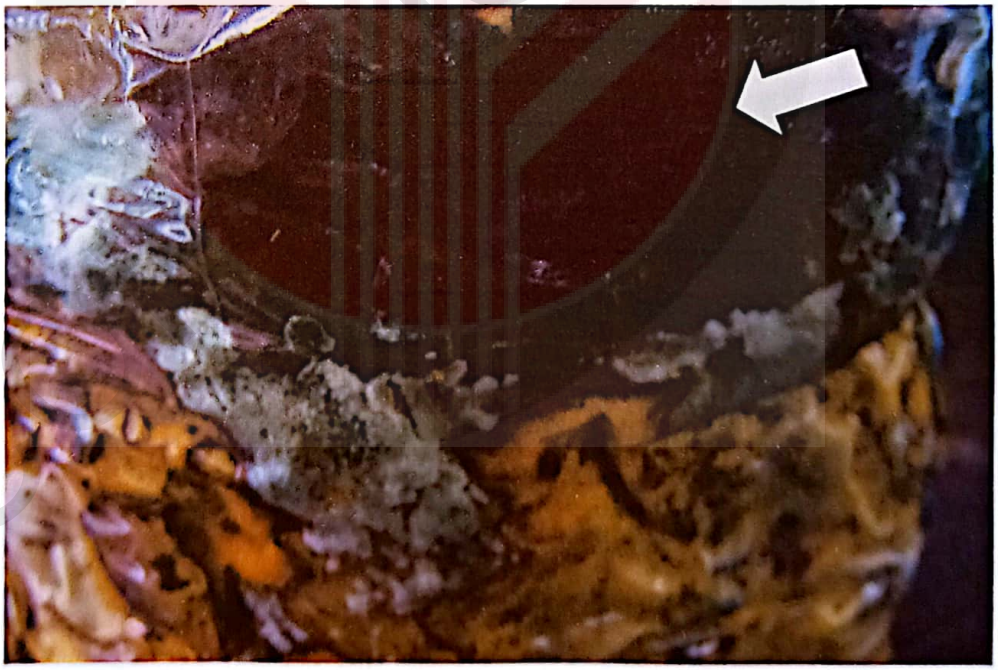

Figure 4.11: Infected production bag showing infestation by mites and green mold disease.

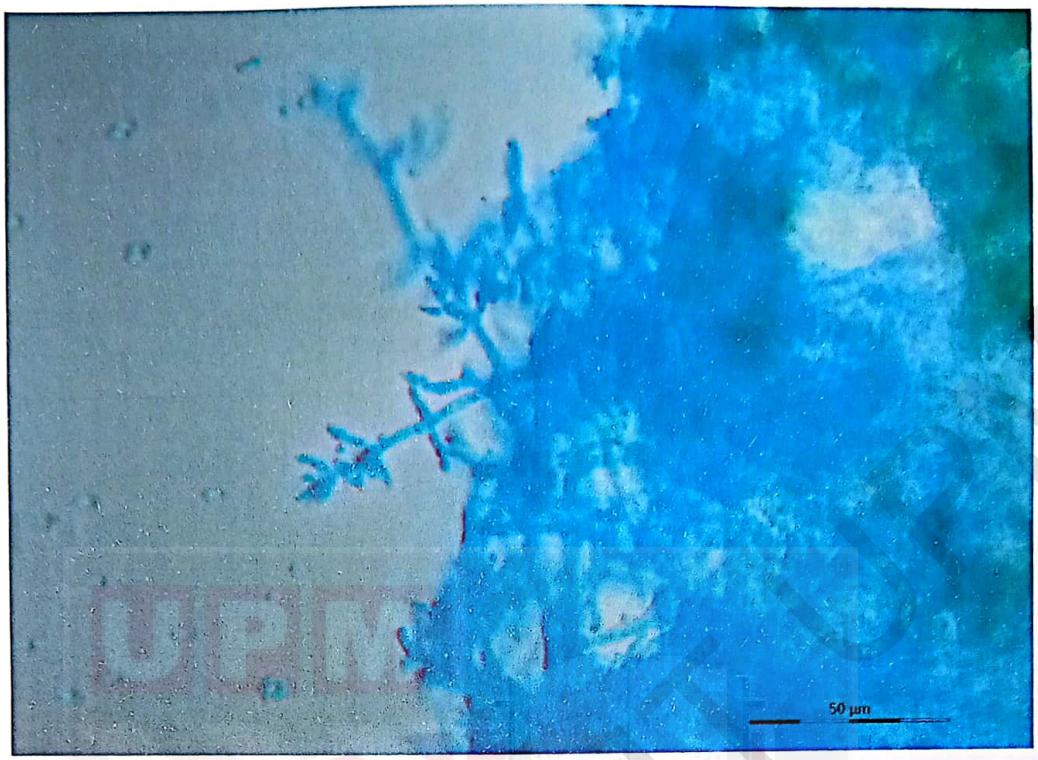

Figure 4.12: *Trichoderma* sp. from fully colonized production bags.

Figure 4.13: Production bag contaminated by green mold disease.

Atomic Absorption Spectrophotometer (AAS) Determination of Mineral Nutrients

The fruit bodies of *Ganoderma lucidum* were analyzed to test the contents of mineral nutrients. The results were given in Table 4.6.

Ganoderma lucidum grown on oil palm mesocarp fiber had the least amount of Ca (113.52 µg/g) when compared to shredded newspaper (269.93 µg/g) or rubber wood chips (385.43 µg/g). In terms of the contents for Mg and Mn, there were no significant differences among mushroom from the three substrates. Mushroom from rubber wood chip had significantly higher concentration of zinc (122.55 µg/g) when compared to shredded newspaper and oil palm mesocarp fiber. Only quantities of Cu (< 3.52 µg/g), Pb (< 4.34 µg/g) and Cd (< 2.19 µg/g) were found in the fruit bodies of *G. lucidum* from each substrate.

Table 4.6: The mineral nutrient contents for *Ganoderma lucidum* harvested from different substrates under 10 % PB + 0.01 % U of supplements.

Mineral nutrients	Means [1] (µg/g)		
	MF [2]	SN [2]	RWC [2]
Calcium (Ca)	113.52 **b**	269.93 **ab**	385.43 **a**
Magnesium (Mg)	2120.28 **a**	1432.29 **a**	1142.94 **a**
Manganese (Mn)	21.65 **a**	12.64 **a**	20.43 **a**
Zinc (Zn)	90.18 **a**	62.86 **a**	122.55 **b**
Copper (Cu)	3.52 **a**	1.01 **b**	1.59 **c**
Lead (Pb)	4.34 **a**	3.52 **a**	0 **a**
Cadmium (Cd)	2.19 **a**	2.30 **a**	2.39 **a**

[1] Means with the same letters in the same rows are not significantly different at P = 0.05 by Duncan's New Multiple Range Test (DNMRT).

[2] MF = Oil palm mesocarp fiber fiber; SN = Shredded newspaper; RWC = Rubber wood sawdust.

CHAPTER V

DISCUSSION

Effect of Selected Grains on Mycelia Growth for Spawn Production

One important step in mushroom production is the preparation of spawn to be used for inoculation of the production substrate. A common material used in spawn production is the grain. Grains provide high content of nutrients source for mushroom mycelia establishment (Stamets, 2000). Among the five selected grains (barley, wheat, soybean, paddy seeds and mungbean) used in the study, barley was found to give the fastest mycelia growth, completing the spawn run in 12 days. Moyson and Verachtert (1991) reported that the spawn done by barley grains was good and could be homogenized into the substrate. The best growth rate on barley was due to the "wet" condition for the surface of grains after sterilization. Suitable "wet" condition assisted the mycelia to spread faster and homogenized on the surface of barley grains. Spawn run on paddy seeds and mungbean were the slowest with sparse mycelia on the grain when compared with others. This could be because these grains were rather dry.

There were also differences in the denseness of mycelia growth among the grain used. Barley, soybean and wheat have dense mycelia growth, followed by the mungbean and paddy seeds when compared 12 days after inoculation. The denseness of mycelia is related to the water content of the grain. The optimum water content of the grain is between 55 - 70 %. The sparse mycelia in mungbean and paddy seeds were probably due to the low water content of grain. Flegg and Wood (1985) reported that mycelia growth in "wet" condition was better when compared to "dry" condition due to the aeration in wet condition. However, if the mungbean and paddy seeds were allowed to

spawn run for a longer period of time, thicker mycelia could be generated. The long time taken may make them unsuitable to be used for commercial spawn production.

Although barley has the fastest growth rate and dense mycelia, it is not recommended because the high starchy and wet conditions after sterilization appear to attract more pest and disease problem during incubation and fructification stages. Spawn on wheat, on the other hand grew very well with thick mycelia, less moist and with less pest and disease problem. Wheat could be recommended for spawn production.

Mycelia Growth Response of *Ganoderma lucidum* to Supplementations on Different Substrates

Aziz *et al.* (2002) report that the oil palm press fiber or mesocarp fiber (MF) contains holocellulose (cellulose and hemicellulose) and lignin. Thus, the MF contains more cellulose and lignin as the carbon source which are required for mycelia growth. When enough urea (0.01 % U) was added into MF, the nitrogen source promoted mycelia growth of the mushroom. As a result, MF + 10 % PB + 0.01 % U was the superior combination for cultivation of *Ganoderma lucidum*.

Mix wood sawdust (MWS) gave the second fastest mycelia growth. However the mycelium was sparse indicating that the variable source of wood sawdust may not be suitable for *G. lucidum* cultivation. Mycelia growth was fast or on shredded newspaper (SN) when compared to rubber wood chips (RWC). The reason might be due to the shredded and pressed condition of SN which promoted the better environment for mycelia to spread on it. On the other hand the sizes of RWC created bigger air space

that may slow the spreading of mycelia. The RWC showed better mycelia growth when given supplements of 10 % PB or 10 % PB + 0.01 % U. This was probably because the paddy bran filled up the air spaces between wood chips thus enabling the mycelia to have uninterrupted growth. Ohga (1990) reported that when the particle size of substrate decreased, the radial mycelia extension rate increased while mycelia biomass decreased. This is because oxygen depletion may reduce mycelia biomass development in the substrates.

There was a general trend that an increase in percent of paddy bran increased the growth of *G. lucidum.* Silva *et al.* (2005) reported that the increased percentage of rice bran (paddy bran) can increase the specific growth of *Lentinula edodes.* Baysal *et al.* (2003) showed that the addition of paddy bran to waste paper significantly increased mycelia development, primordia formation, fructification and mushroom yield for *P. ostreatus* cultivation. Further more the addition of urea (U) as one of the nitrogenous sources for protein increased mycelia growth. Babitskaya *et al.* (2005) reported that ammonium sulfate was the source of nitrogen for polysaccharide production by *G. lucidum* and could regulate the growth and metabolism. Hsieh *et al.* (2006) reported that the lowest molecular weight of polysaccharides was found under nitrogen-source limitation. Thus, the present study indicated that a nitrogen source is required to assist mycelia growth and metabolism. The addition of 0.01 % urea to each substrate enhanced growth where the mycelium was dense and high daily mycelia growth required less day to complete spawn run.

Fructification

The small whitish primordia of *Ganoderma lucidum* appeared only when the condition were optimum to allow them to develop into a fruit body. The fruit body or basidiocarp of *G. lucidum* was whitish colour initially which gradually changed to yellowish-orange and finally to dull red to reddish brown. The stipe of *G. lucidum* was long and blackish-brown in colour.

Ganoderma lucidum cultivatied in SN + 10 % PB + 0.01 % U formed long stipe with the basidiocarp formation being significantly slower than that of other substrates.

Flegg and Wood (1985) reported that the number of primordia formed depended on the quality of mycelia and the type of nutrient medium. In the present study, *G. lucidum* formed primordia and fruit bodies in most of the substrates used. Primordia can form in any corner of the production bag as long as there is enough space for them to grow. Not all the primordia are able to develop into fruit body. Only the dominant one can grow into a fruit body.

Fructification of *G. lucidum* is best on oil palm mesocarp fiber substrate (MF), where the largest or longest fruit bodies were found. This was probably due to the high nutrient content of the MF. Aziz *et al.* (2002) reported that the oil palm press fiber (mesocarp fiber) contained a high percentage of holocellulose (cellulose and hemicellulose) and lignin which was similar to lignocellulosic or woody materials. High content of holocellulose contained sugars (glucose and xylose) and pentose which were

required to promote the fructification of mushroom. The supplements added to the substrates did affect the mushroom yield and biological efficiencies for each type of substrates. This showed that the fruiting of mushroom depended on the original starting nutrient content of the growth medium as contrived by Chang and Philip (2004).

Pest Infestation and Fungal Contamination

Pest Infestation

Phorid fly

Phorid fly is one of the pests in mushroom cultivation. *Megaselia halterata* was the phorid species that was found at mushroom compost production sites (Jess *et al.*, 2007).

Phorid flies that were found during the cultivation of *G. lucidum* were similar to that reported by Keil (2002); the flies were small and adult flies were 2 - 3 mm in length, with a humpback appearance and very small antennae. The larvae were creamy-white maggots no longer than 4 mm. The pupa was creamy-yellow in colour. Keil (2002) also reported that the phorid larvae fed on mycelium at warm compost temperatures of 24 – 27 ^{o}C and that they were involved in transmitting fungal spores. In the study the MF substrate with high starchy spawn grains were easily infested by phorid flies. The high nutrients and oil from MF and the starchy condition from spawn grain were the best conditions for phorid flies to lay their eggs. Once the production bags were infected by phorid fly and their larvae, the production bags would become contaminated and produced bad odour. Infested bags were destroyed immediately to prevent spread of the pest.

Mites

Pediculaster flechtmann (Wicht) (Acari: Pygmephoroidea) is the common mite pest in the cultivation of mushroom (Cross and Kaliszewski, 1988). This species of mites found in mushroom can cause qualitative and quantitative losses of mineral element (Kheradmand *et al.*, 2006). Mites found during the fructification phase were very tiny and difficult to detect visually. The mites were detected inside the production bags when the mycelia of *G. lucidum* became dark in colour and the surrounding area contaminated by green mold disease. It was believed that the mites found in the production bags would either feed on *Trichoderma* spores or mushroom mycelia and transmitted the green mold disease. The mites also damaged the fruiting bodies of *G. lucidum* by pitting them.

Fungal Contamination

Trichoderma harzianum biotype Th2 was responsible for green mold disease. This disease was responsible for important economic losses of mushroom (Savoie *et al.*, 2001). There was a strong possibility that the production bags that were infected by green mold disease were the result of inadequate sterilization. Insects or flies could carry the fungal spores. When the production bags were contaminated by *Trichoderma* sp. green patches appeared on the substrate with large number of mites. The *Trichoderma* mycelia were gray initially, changed to whitish dense mycelia and later became dark green in color. Once the green mold germinated, the mycelia would rapidly colonize the substrate and over ran the mushroom mycelia. As a result the mushroom mycelia could not grow on infected part (FA0, 1990).

Atomic Absorption Spectrophotometer (AAS) Determination of Mineral Nutrients

Chang and Philip (2004) reported that the minerals present in the substrate will be taken up by mycelia and translocated to the sporophores, and thus the mushrooms are a good source of mineral. Falandysz and Bielawski (2001) reported that the magnitudes of substrate (soil) can determine elements content in the fruiting bodies of higher mushrooms. Vetter *et al.* (2005) found that when the element contents of substrate increase, the concentration in fruiting bodies also will increase. This is important in the consideration for substrate for *G. lucidum* cultivation. Analysis of fruit bodies from this study showed trace contents of Cd, Cu and Pb (heavy metals) in this *G. lucidum.*

The low concentration of Cd and Cu may be reduced to that Cd intake helps Cu intake for mushroom metabolism (Tham *et al.*, 1999) and this low Cd would lead to similarly low Cu uptake. Adaskaveg *et al.* (1990) reported that there is a high tolerance of *G. lucidum* to Cu, Mn and Zn uptake due to its wood-decaying ability, particularly with the functions of ligninocellulolytic enzymes. This may help explain the low concentrations of Cu, Mn and Zn found in the *G. lucidum* in this study.

CONCLUSION

It is possible to produce *Ganoderma lucidum* spawn by using barley, wheat, soybean, paddy seeds and mungbean. Barley gave the fastest spawn production in 12 days while mungbean took 27 days. Dense mycelia were produced on barley, wheat and soybean but mycelia on paddy seeds and mungbean were sparse initially and took much longer time to densely colonize the spawn substrate. In this study, it is clearly indicated that substrates such as oil palm mesocarp fiber (MF), shredded newspaper (SN), rubber wood chips (RWC) and mix wood sawdust (MWS) were able to promote the mycelia growth and produced the fruit bodies of *G. lucidum.* The oil palm mesocarp fiber gave the best performance among all the substrates with the fastest colonization in 15 days and biological efficiency (5.58 %). Increasing percentage of paddy bran (PB) in all substrates accelerated mycelia development, primordia formation and production of *G. lucidum*. The addition of 0.01 % urea (U) further enhanced *G. lucidum* production.

Analysis for mineral contents showed that fruit bodies from RWC + 10 % PB + 0.01 % U had the highest content of Ca (385.43 µg/g) and Zn (122.55 µg/g) among the substrates tested. There was no difference in the contents for Mg and Mn among the substrates. The concentrations of heavy metals such as Cu (< 3.52 µg/g), Cd (< 2.39 µg/g) and Pb (4.34 µg/g) in the fruit bodies were negligible in all the substrate. This indicated that the waste materials used were safe for the cultivation of *G. lucidum*.

Some pests and disease problem encountered in the study indicated the need for though sterilization of the substrates and a clean environment for the cultivation of *G. lucidum*.

Pests such as phorid fly and mites, and the green mold disease caused by *Trichoderma* sp. could reduce yield of the mushroom.

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitle "Culturing Condition for Production of *Ganoderma lucidum* (Leyss.: Fr.) Karst" by the supervisor in a joint authorship. Ever how, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in the form approved by the faculty.

EMILY SEAH SOOH MEI

Date: 4/5/2007